The Effects of *trans* Fatty Acids on Fatty Acyl Δ5 Desaturation by Human Skin Fibroblasts

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ABSTRACT

The effectiveness of different fatty acids as inhibitors of fatty acyl Δ5 desaturation activity in human skin fibroblasts has been investigated. When incubated with 2.25 μM [^{14}C] eicosatrienoate (20:3ω6) in otherwise lipid-free medium, these cells rapidly incorporate the radiolabeled fatty acid into cellular glycerolipids and desaturate it to produce both [^{14}C]arachidonate and [^{14}C]docosatetraenoate. The Δ5 desaturation activity can be enhanced by prior growth of the cells without serum lipids. Elaidate (9t-18:1) is a potent inhibitor of Δ5 desaturation while *trans*-vaccenate (11t-18:1) is virtually without effect. Oleate and linoleate are only mildly inhibitory. Linoelaidate (9t,12t-18:2) is more inhibitory than linoleate but significantly less effective than elaidate. The effects of elaidate can be readily overcome by increasing the concentration of exogenous eicosatrienoate. Studies with a variety of *trans* monounsaturates of differing chain lengths indicate that the ω9 *trans* fatty acids are potent inhibitors of Δ5 desaturation, while ω7 *trans* fatty acids are relatively ineffective. Intact human fibroblasts could thus be important in characterizing novel fatty acids as selective inhibitors of arachidonate synthesis in vivo.

Lipids 19:869-874, 1984.

INTRODUCTION

Mammalian cells contain a series of microsomal enzymes which catalyze the desaturation and elongation of dietary linoleate (9c,12c-18:2) to produce longer chain 20- and 22-carbon polyunsaturated fatty acids. Fatty acyl Δ5 desaturase is of special interest in that it converts the CoA ester of 8c,11c,14c-eicosatrienoate to arachidonate (5c,8c,11c,14c-eicosatetraenoate) (1). In addition to being major components of cellular phospholipids, these two fatty acids are the precursors of the 1- and 2-series prostaglandins (2). In most tissues arachidonate normally predominates over 20:3 as a component of membrane phospholipids (3) and as a substrate for the cyclooxygenase and lipoxygenase enzymes. Inhibition of fatty acyl Δ5 desaturation could thus shift the synthesis of prostaglandins, resulting in an increase in PG_1's relative to PG_2's (4). Regulation of enzyme activity may be of particular importance in humans, where the level of Δ5 desaturase activity appears to be less than in the mouse or rat (5).

The mixtures of *trans* fatty acids present in partially hydrogenated vegetable oils have been shown to suppress synthesis of arachidonic acid in vivo and to exacerbate symptoms of essential fatty acid deficiency (6,7). Although in vivo studies have demonstrated inhibition of the initial Δ6 desaturation of linoleate by *trans* monounsaturates (8) and linoelaidate (9t,12t-18:2) (9), they provide only indirect data on Δ5 desaturation activity. In vitro studies using liver microsomal preparations have shown that a variety of isomeric *cis*- and *trans*-octadecenoic acids directly inhibit the desaturation of 8,11,14-eicosatrienoyl CoA (10,11)

Our laboratory has developed a protocol for investigating fatty acyl desaturation activities in intact human cells (12). Human skin fibroblasts readily incorporate exogenous free fatty acids into cellular phospholipids and triacylglycerol, thus facilitating nutritional modification of cellular composition. We have shown that both linoelaidate and elaidate are potent inhibitors of Δ6 desaturation in these cells. By contrast, the Δ9 desaturation of [^{14}C]stearate is inhibited by *cis* but not by *trans* fatty acids. Interestingly, the relative effectiveness of various fatty acids in the intact cell system is different from that found in microsomal studies. The cell culture system thus appears to be a useful tool for evaluating the physiological effects of isomeric fatty acids on cellular metabolic processes.

The present study uses this cell culture system to examine fatty acyl Δ5 desaturation in human cells. In particular we have investigated the effects of medium supplementation with individual *trans* fatty acids on the synthesis of arachidonate from [^{14}C]eicosatrienoate.

MATERIALS AND METHODS

Cell Culture

Normal human skin fibroblasts derived from a 3-month fetus (GM-10) were obtained from the NIGMS Human Genetic Mutant Cell Repos-

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itory (Camden, New Jersey). Cells were propagated in Eagle's Minimum Essential Medium containing 10% Fetal Bovine Serum (Gibco, Grand Island, New York) as described previously (12,13). Each experiment used replicate flasks of subconfluent, actively mitotic cells with approximately 600 μg cellular protein/25cm^2 flask. Delipidized calf serum was prepared by acetone:ethanol (1:1 v/v) extraction (14) and reconstituted in Earle's Balanced Salt Solution. The delipidized serum was used to replace complete serum for 4 days prior to each experiment as well as during fatty acid supplementation.

Fatty Acid Supplementation

(8c,11c,14c)-[1-^{14}C]Eicosatrienoate (54.9 mCi/mmol) was obtained from New England Nuclear Corp. (Boston, Massachusetts). The *trans* fatty acids 9t-16:1, 10t-17:1, 10t-19:1 and 11t-20:1 were obtained from NuChek Prep, Inc. (Elysian, Minnesota), and other fatty acids were from Sigma (St. Louis, Missouri). The fatty acids were stored in hexane at -20 C under a nitrogen atmosphere. Concentrations and purity of the free fatty acids were confirmed by gas liquid chromatography (GLC) after methylation with BCl_3 in methanol using internal standards (12). For each experiment, aliquots of fatty acid solutions were evaporated to dryness under N_2 and resuspended in 95% EtOH. The fatty acids were then transferred quantitatively to reconstituted delipidized serum and diluted with culture medium (15).

Lipid Extraction and Analysis

The cells were harvested by trypsinization and cellular lipids extracted in a 2:1:1 mixture of ethyl acetate/acetone/cell suspension (16). Fatty acid methyl esters were prepared from cellular lipids using methanolic base. Gas-liquid chromatography was performed on a Packard 427 chromatograph (Downers Grove, Illinois) with flame ionization detection using a 6-ft glass column packed with 10% SP-2330 on 100/120 Chromasorb W AW (Supelco, Bellefonte, Pennsylvania). After a 3 min initial hold, the oven was programmed from 160 to 235 C at 2 degrees/min. The distribution of [^{14}C] fatty acid methyl esters was determined with a Packard 894 Gas Flow Proportional Counter interfaced to the chromatograph. A Linear dual-pen recorder was used to obtain simultaneous radioactivity and mass tracings of each chromatographic separation; a Spectra-Physics (Santa Clara, California) Minigrator was used to integrate radioactivity peak areas. Thin layer chromatography (TLC) to separate neutral lipids and the major classes of phospholipids was performed as described previously (13).

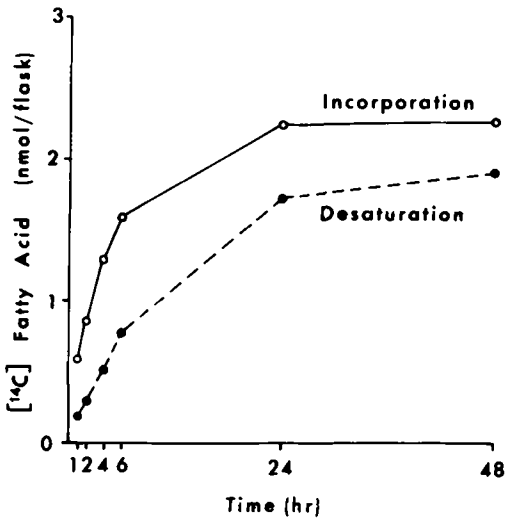

FIG. 1. Time course of incorporation of [1-^{14}C] eicosatrienoate into cellular lipids and its desaturation to arachidonate plus docosatetraenoate. GM-10 cells were grown for 4 days in medium without serum lipids. They were then incubated with 0.25 μCi [^{14}C] eicosatrienoate in 4ml Eagle's minimum essential medium plus delipidized serum protein as described in Materials and Methods. At each time point, 2 replicate flasks were harvested and cellular lipids analyzed. o – o, total incorporation into cellular lipids, of which >96% is glycerolipids; • - - •, percentage desaturation calculated from [^{14}C] arachidonate plus [^{14}C] docosatetetraenoate (22:4) divided by total cellular ^{14}C-fatty acids.

RESULTS AND DISCUSSION

Figure 1 shows the results obtained when subconfluent, actively mitotic human skin fibroblasts are incubated with 2.3 μM [1-^{14}C] eicosatrienoate in medium with delipidized serum. Initial incorporation of the exogenous [^{14}C] 20:3 into cellular lipids is quite rapid, reaching 48% in 6 hr. Active desaturation of the incorporated [^{14}C] 20:3 is seen; within 1 hr 33% of the cellular ^{14}C-acyl groups consist of arachidonate (20:4) plus docosatrienoate (22:4). Δ5 desaturation activity continues after incorporation has slowed considerably, and the percentage desaturated rises to 85% in 48 hr. Throughout this process, most of the radioactivity in cellular lipids was in phospholipids. For example, at 6 hr 86.8% of the cellular ^{14}C-acyl groups were esterified in phospholipids, with 9.5% in triacylglycerol and 3.0% in diacylglycerol.

The data shown in Figure 1 was obtained with cells which had been grown for 4 days in lipid-free medium prior to the experiment. A comparison was made between these cells and replicate flasks grown with 10% fetal bovine serum. The incubations with [^{14}C] 20:3 were performed under identical conditions. Although

total incorporation at 6 and 24 hr was similar for both groups, the cells grown with fetal bovine serum exhibited 40% less desaturation of the incorporated [^{14}C]20:3 (data not shown). Previous studies in our laboratory (12) have demonstrated a similar enhancement of both fatty acyl Δ9 and Δ6 desaturation activities in human skin fibroblasts after growth without serum lipids. Regulation of fatty acid synthesis and fatty acyl Δ9 and Δ6 desaturation in fibroblasts are thus similar to what is observed in vivo. Dietary regulation of Δ5 desaturase activity in vivo has not, however, been completely elucidated. One recent study (17) found that enzyme activity in liver microsomes decreased in response to a fat-free diet. De novo enzyme synthesis was enhanced by dietary supplementation with linoleate but not palmitate. By contrast, De Schrijver and Privett (18) report that addition of safflower oil to a fat-free diet depressed Δ5 desaturase activity. This latter study appears to have induced a greater degree of essential fatty acid deficiency in its controls. Growth of human skin fibroblasts without serum lipids results in a progressive depletion of ω6 polyunsaturated fatty acids and the consequent synthesis of 20:3ω9 (13). Further studies with the cell culture system may help elucidate the apparently complex long term effects of dietary lipids on Δ5 desaturase activity.

The present study focuses on short term effects of exogenous fatty acids on Δ5 desaturation activity. All subsequent experiments thus used a 6-hr incubation period. In all cases, the fibroblast cells were previously grown for 4 days in lipid-free medium to enhance their desaturation of [^{14}C] eicosatrienoate.

Effects of Exogenous Fatty Acids

The effects of medium supplementation with free fatty acids on the metabolism of [^{14}C] eicosatrienoate are shown in Table 1. We find that total incorporation of the [^{14}C] 20:3 varies less than 15% with the addition of different fatty acids. Palmitate (16:0) does not significantly affect total Δ5 desaturation, although it appears to inhibit the elongation of [^{14}C] arachidonate to [^{14}C]22:4. Mild inhibition is seen with oleate (9c-18:1) and linoleate (9c, 12c-18:2), while inhibition with arachidonate (5c, 8c, 11c, 14c-20:4) is greater than 50%. Interestingly, the 2 *trans* fatty acids, elaidate (9t-18:1) and linoelaidate (9t,12t-18:2) are more inhibitory than their *cis* isomers. Elaidate is even more potent an inhibitor of Δ5 desaturation activity than is arachidonate, the product of the reaction.

Increased concentrations of free fatty acids in the culture medium have been shown to result in intracellular accumulation of triacylglycerol (15). In this study, we found that when the cells were incubated with either 40 μM elaidate or 40 μM oleate along with the 2.5 μM[^{14}C] eicosatrienoate, the percentage of ^{14}C-acyl groups esterified in triacylglycerol increased from 9.5% to 36% and 50% respectively. Both [^{14}C] 20:3 and the products of its desaturation were present in both phospholipids and triacylglycerol (data not shown). Consistent with previous findings in this laboratory (13), relatively more arachidonate and [^{14}C] 22:4 were esterified in phospholipids.

Figure 2 shows the effects of different concentrations of *trans* fatty acids on Δ5 desaturation. Inhibition by elaidate is seen at medium

TABLE 1

The Effects of Exogenous Fatty Acids on Incorporation and Desaturation of [^{14}C] Eicosatrienoate[a]

Fatty acid	[^{14}C] Incorporated[b] (n mol/flask)	^{14}C-Fatty acids 20:3	20:4	22:4	Desaturation[c] (%)
Control (no fatty acid)	5.22 ± 0.06	2.26	2.34	0.52	54.8
Palmitate (16:0)	5.12 ± 0.12	2.12	2.82	0.18	58.6
Oleate (9c-18:1)	5.68 ± 0.24	3.04	2.48	0.14	46.2
Linoleate (9c,12c-18:2)	4.90 ± 0.10	2.88	1.88	0.10	40.4
Arachidonate (5c,8c,11c,14c-20:4)	5.02 ± 0.14	3.52	1.14	0.30	28.7
Elaidate (9t-18:1)	5.36 ± 0.11	4.18	1.16	–	21.6
Linoelaidate (9t,12t-18:2)	5.28 ± 0.08	3.12	1.96	–	37.2

[a]Replicate flasks of GM-10 cells were established in lipid-free medium as in Figure 1. They were then preincubated with 3ml of medium containing delipidized serum and 40 μM fatty acid. After 2 hr, 1 ml of medium containing 0.5 μ Ci [1-^{14}C]eicosatrienoate was added to each flask. Final concentrations were 2.25 μM [^{14}C]eicosatrienoate and 30 μM non-radioactive fatty acid. The cells were harvested after a 6-hr incubation and their lipids analyzed as described in Materials and Methods.

[b]Values are means ± S.D. from analyses of 3 separate flasks from one of 2 similar experiments.

[c]Percentage desaturation calculated from Σ20:4 + 22:4/Total Incorporation.

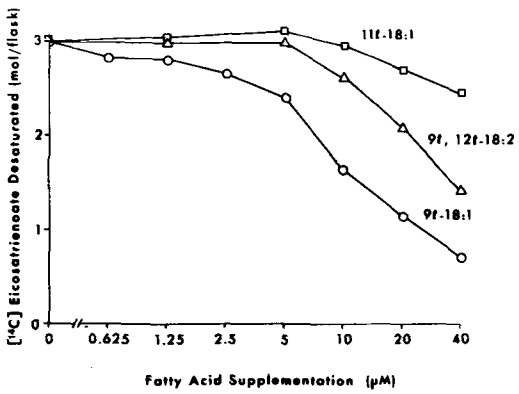

FIG. 2. The effect of concentration of exogenous *trans* fatty acids on the desaturation of [^{14}C]eicosatrienoate. As described in Table 1, replicate flasks were preincubated for 2 hr with *trans* fatty acids before addition of [^{14}C]20:3. ○ – ○, elaidate; □ – □, *trans* vaccenate, and △ – △, linoelaidate.

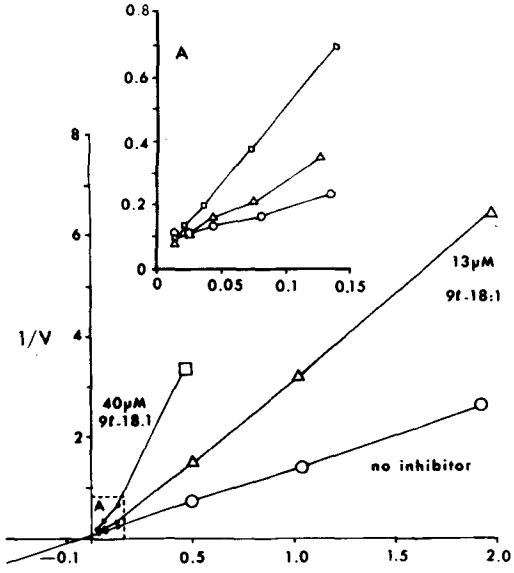

FIG. 3. The effects of varied substrate concentration on the desaturation of [^{14}C]eicosatrienoate in the presence and absence of elaidate. Replicate flasks were incubated for 6 hr with 0.2-30 μM [^{14}C]eicosatrienoate; concentrations above 4.5 μM 20:3 were obtained by addition of non-radioactive fatty acid. For transformation to the double reciprocal plot, the total incorporation of [^{14}C]20:3 nmol/flask was taken as substrate concentration, [S]. Velocity of the reaction was expressed as nmol 20:3 desaturated during the 6-hr incubation. The area with high substrate concentrations (1/[S] < 0.15), corresponding to 3.3-30 μM exogenous [^{14}C]eicosatrienoate, is magnified in the insert. ○ – ○, no elaidate; △ – △, 13 μM elaidate, and □ – □, 40 μM elaidate.

concentrations as low as 0.63 μM, one-fourth that of the [^{14}C]eicosatrienoate. Increased elaidate concentrations have a dose-dependent effect on Δ5 desaturation. By contrast, *trans* vaccenate (11t-18:1), a positional isomer of elaidate, has virtually no effect on the synthesis of arachidonate from [^{14}C]20:3. Although linoelaidate acts in an intermediate manner at 10-40 μM, lower concentrations are without measurable effect.

Our previous studies (12) have shown that *trans* vaccenate is readily incorporated into the glycerolipids of human skin fibroblasts. In addition, *trans* vaccenate acts similarly to elaidate in both its inhibition of de novo fatty acid synthesis and promotion of triacylglycerol accumulation. This seems to indicate that the markedly greater effects of elaidate on the desaturation of both [^{14}C]eicosatrienoate and [^{14}C]linoleate by human skin fibroblasts appear to be specific to the desaturation reactions. We also have performed similar experiments using human endothelial cells derived from umbilical vein (19). When these cells are incubated with [^{14}C]eicosatrienoate, the inhibition of Δ5 desaturation by elaidate is 2-3 fold that of linoelaidate; *trans* vaccenate has virtually no effect. Thus the relative effectiveness of the 3 *trans* fatty acids as inhibitors of Δ5 desaturation activity of human cells in culture is similar for 2 markedly different cell types.

There are 2 interesting differences in the effects of *trans* fatty acids on Δ6 and Δ5 desaturation activities of human skin fibroblasts. First, elaidate is a far more potent inhibitor of Δ5 desaturation than is linoelaidate, and its effects are seen at much lower inhibitor concentrations. The reverse relationship occurs for Δ6 desaturation. Second, the relative effectiveness of 9t-18:1 and 11t-18:1 on Δ5 desaturation in these cells is similar to that found in studies with rat liver microsomal preparations (10). The effects on Δ6 desaturation are quite different, with 11t-18:1 being more inhibitory than 9t-18:1 in microsomal studies and virtually without effect in the cell culture system.

Kinetics of Inhibition by Elaidate

We have examined the effects of elaidate on cells incubated with varied concentrations of [^{14}C]eicosatrienoate. In the absence of inhibitor, a double reciprocal plot of Δ5 desaturation activity vs. total incorporation gives a linear result (Fig. 3). Elaidate is inhibitory in a dose-dependent manner over a wide range of eicosatrienoate concentrations. As seen in Figure 3, the inhibitory effect of elaidate can be overcome at high substrate concentrations.

Mahfouz et al. (10) have pointed out that since both substrate and products are incorporated into a variety of glycerolipids, a Lineweaver-Burk plot should not strictly apply for the microsomal fatty acyl desaturase reactions. They found, however, that the microsomal system did give linear double reciprocal plots for fatty acyl Δ5 desaturation indicating that the desaturation step is rate limiting. In their assays *trans* octadecenoates acted as classic competitive inhibitors. Use of exogenous fatty acids by intact cells is clearly more complex than a microsomal assay, and the incubation times are longer. The convergent lines obtained with intact cells (Fig. 3) are suggestive of competitive inhibition. However, examination of the data for high substrate concentrations (Fig. 3A) indicates that the lines do indeed converge at a finite substrate concentration and actually are curves at low 1/[s] values. This may be due to the increased importance of other factors such as relative scarcity of CoA or accumulation of neutral lipid. Alternatively, our assay may actually measure 2 reactions, both the conventional eicosatrienoyl CoA desaturase and the eicosatrienoyl lecithin desaturase described by Pugh and Kates (20).

Influence of Position of the *Trans* Double Bond

The different results obtained with elaidate and *trans* vaccenate (Fig.2) would seem to indicate that the position of the *trans* double bond is important in determining the effects of the fatty acid on Δ5 desaturation. To investigate this further we examined the effects of a series of commercially available *trans* monounsaturated fatty acids. As seen in Table 2, there is no correlation between the position of the *trans* double bond as counted from the carboxyl carbon (9t, 10t, or 11t) and effectiveness as an inhibitor. Thus palmitoleate (9t-16:1) does not inhibit the desaturation of [^{14}C] eicosatrienoate, while elaidate (9t-18:1) does. When the *trans* fatty acids are grouped by the position of the *trans* double bond from the methyl carbon, a definite pattern is seen. All the ω9 *trans* fatty acids tested are potent inhibitors of Δ5 desaturation activity in human skin fibroblasts; the ω7 *trans* fatty acids are not particularly inhibitory.

This selectivity in the inhibitory effects of *trans* monoenoic fatty acids is quite puzzling. Substrate specificity of mammalian desaturases appears to involve recognition of the carboxyl end of the molecule (21). The major substrate requirements of the Δ5 desaturase involve 11c and 14c double bonds, while the 8c bond enhances reactivity. Pollard et al. (22) have shown very low levels of Δ5 desaturation of 9t-20:1 but not 11t-18:1 by rat liver microsomes. They found, however, that desaturation of 9t-20:1 was 7-fold greater than 9t-18:1 Our data (Table 2) indicates that 9t-20:1 is not as effective an inhibitor as is 9t-18:1. Thus, there is no correlation between the extent of Δ5 desaturation of a *trans* monoenoate in the microsomal assays

TABLE 2

The Effects of Different *trans* Monoenoic Fatty Acids on the Incorporation and Desaturation of [^{14}C] Eicosatrienoate[a]

Fatty acid	Concentration	Incorporation of [^{14}C] 20:3 (n mol/flask)	Desaturation (%)
Control (no fatty acid)	–	5.62 ± 0.26	39.2
9t-16:1 (ω7)	13 μ M	4.68 ± 0.10	46.2
10t-17:1 (ω7)	13 μ M	5.40 ± 0.14	41.8
11t-18:1 (ω7)	13 μ M	6.00 ± 0.08	39.0
9t-18:1 (ω9)	13 μ M	5.66 ± 0.08	24.8
10t-18:1 (ω9)	13 μ M	6.12 ± 0.02	31.3
11t-20:1 (ω9)	13 μ M	6.32 ± 0.03	34.6
9t-16:1 (ω7)	40 μ M	5.42 ± 0.16	43.7
10t-17:1 (ω7)	40 μ M	5.94 ± 0.11	34.7
11t-18:1 (ω7)	40 μ M	6.54 ± 0.08	29.4
9t-18:1 (ω9)	40 μ M	6.62 ± 0.13	14.7
10t-19:1 (ω9)	40 μ M	6.50 ± 0.20	13.7
11t-20:1 (ω9)	40 μ M	6.86 ± 0.22	19.5

[a]Replicate flasks of GM-10 cells were preincubated with *trans* fatty acids for 2 hr as described in Table 1. 0.5 μ CI [1-^{14}C] Eicosatrienoate/flask was then added for the 6-hr incubation. Final *trans* fatty acid concentrations are indicated above; other procedures as in Table 1.

and its inhibition of eicosatrienoate desaturation activity in intact cells. If these *trans* monosaturates do indeed act as competitive inhibitors, (10), elucidation of their specificity may contribute to our understanding of the structural configuration of the active site.

A decrease in liver microsomal Δ5 desaturase activity has been observed in rats fed partially hydrogenated marine oils but not partially hydrogenated peanut oil (21,22). Svensson (23) has suggested that this effect may be due to *trans* eicosenoic and docosenoic fatty acids in the marine oil products. In our system 11t-20:1 is only slightly less effective than elaidate as an inhibitor of the desaturation of [^{14}C]eicosatrienoate. 11t-Eicosatrienoate is, however, significantly less effective than elaidate as an inhibitor of the desaturation of [^{14}C]linoleate (Rosenthal, M.D.; Doloresco, M.A., and Banerjee, N., unpublished observations). It would be of interest to examine the effects of other isomeric *trans* eicosenoates. A *trans* fatty acid or fatty acid analogue which is still more selective in its effects on Δ5 desaturation might have potentially useful effects on the metabolism of eicosatrienoate and arachidonate in vivo.

ACKNOWLEDGMENTS

This work was supported by a grant from the National Institutes of Health and a Grant-in-Aid from the American Heart Association, with funds contributed in part by the AHA, Virginia Affiliate.

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[Received February 17, 1984]