

Effect of fatty acid modification on prostaglandin production by cultured 3T3 cells

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Abstract We have investigated the extent to which modifications in the essential fatty acid content of mammalian cells can affect prostaglandin production. Swiss mouse 3T3 cells stimulated with the calcium ionophore A23187 produced 1.7 to 7 times more prostaglandin E₂ (PGE₂) when the cultures were supplemented with linoleic acid. Increases in PGE₂ production as a result of linoleic acid supplementation occurred under all culture conditions except during the first 24 hr after attachment, when prostaglandin production was very high. Arachidonic acid supplementation produced a similar enhancement in the capacity of the cells to produce PGE₂, but no appreciable increase occurred when the cultures were supplemented with oleic acid. The phospholipids of the cells exposed to the linoleate-enriched medium contained 4 times more arachidonic acid and twice as much linoleic acid as compared with the corresponding controls. The choline phosphoglycerides were most highly enriched in arachidonic acid, but 2- to 3-fold increases also occurred in the inositol and ethanolamine phosphoglycerides. When cultures initially enriched with linoleic acid were transferred to an unsupplemented medium, the fatty acid composition as well as the capacity of the cells to produce PGE₂ reverted almost to control values. The amount of exogenous arachidonic acid converted to PGE₂ as measured by radioimmunoassay also was greater when the cells were enriched with linoleic acid. Studies with radioactive arachidonic acid indicated that the distribution of prostaglandin metabolites was not affected appreciably by linoleic acid enrichment. These findings suggest that at least two factors contribute to the increased capacity of the cultures supplemented with linoleate to produce PGE₂. One is enrichment of the phospholipid substrate pools with arachidonic acid. The other is an increased ability of the cells to synthesize PGE₂ from unesterified arachidonic acid, perhaps because the prostaglandin-forming enzymes are more active.—Denning, G. M., P. H. Figard, and A. A. Spector. Effect of fatty acid modification on prostaglandin production by cultured 3T3 cells. *J. Lipid Res.* 1982. 23: 584–596.

Supplementary key words prostaglandin E₂ • linoleic acid • arachidonic acid • phospholipids • calcium ionophore • polyunsaturated fatty acids • essential fatty acids

Most of the prostaglandins produced by mammalian tissues are dienoic and are derived from arachidonic acid (20:4 n-6)¹, a member of the n-6 series of essential polyunsaturated fatty acids. Linoleic acid (18:2 n-6), the most abundant n-6 polyunsaturate contained in the diet and blood plasma under ordinary circumstances, is the precursor of arachidonic acid. Linoleic acid deficiency in

animals reduces the capacity of many tissues to synthesize prostaglandins, whereas dietary supplementation with linoleic acid enhances prostaglandin production (1). These effects are thought to occur through changes in the arachidonic acid content of the tissue phospholipids (1).

In an attempt to determine whether enrichment with essential polyunsaturates might protect against thrombosis, we investigated the effect of linoleic acid supplementation on the capacity of cultured human endothelial cells to produce prostaglandin I₂ (PGI₂).² As opposed to the results obtained by dietary supplementation of animals (1), we found that enrichment of the culture medium with unesterified linoleic acid reduced the capacity of the cells to produce PGI₂ in response to either thrombin or the calcium ionophore A23187 (2). Linoleate supplementation of the endothelial cultures produced a decrease in the arachidonic acid content of the cellular phospholipids, in agreement with our previous findings with cultured human skin fibroblasts (3). In addition, an accumulation of linoleic and eicosadienoic (20:2 n-6) acids occurred in the cellular phospholipids (2). Studies with other cultured cells indicated that a reduction in the arachidonic acid content of the cellular lipids is associated with a decrease in prostaglandin production (4). Furthermore, prostaglandin synthesis in tissue homogenates is inhibited by linoleic acid as well as other polyunsaturates, presumably through competitive inhibition for binding to cyclooxygenase (5, 6). Based upon this information, we attributed the decreased capacity of the endothelial cultures to produce PGI₂ to these two factors (2).

The inhibitory effect of linoleic acid supplementation on the capacity of endothelial cells to produce PGI₂ was

¹ The fatty acids are abbreviated as number of carbon atoms:number of double bonds. Thus, 20:4 signifies a 20 carbon atom fatty acid containing four double bonds. The notation n-6 indicates that the first double bond is located six carbon atoms removed from the methyl terminus of the acyl chain.

² The prostaglandins are abbreviated as PG, followed by the letter designating ring structure and subscript designating the number of unsaturated bonds contained in the aliphatic chains.

unexpected. As part of an assessment of the potential importance of this finding, we wished to determine whether linoleate enrichment would have a similar effect on other types of prostaglandin-producing cells. The present report describes results obtained with Swiss mouse 3T3 cells, a widely studied cultured cell line whose main prostaglandin product is PGE₂ (7, 8). Previous work has shown that the fatty acid composition of 3T3 cells can be modified extensively in culture (9). Our findings indicate that, as opposed to the endothelial cultures (2), prostaglandin production by 3T3 cells under most conditions is stimulated greatly as a result of linoleic acid supplementation.

MATERIALS AND METHODS

Reagents

Dulbecco's modified Eagle's medium, fetal bovine serum, and glutamine were obtained from Grand Island Biological Co. (Grand Island, NY). Fatty acids were obtained from Nu-Chek-Prep (Elysian, MN). Bovine albumin (Fraction V, fatty acid-free) was purchased from Miles Laboratories, Inc. (Elkhart, IN). The calcium ionophore A23187 was supplied by Calbiochem-Behring (La Jolla, CA). Bovine γ -globulin (Cohn fraction II) and prostaglandins were purchased from Sigma Chemical Co. (St. Louis, MO). Anti-Prostaglandin E₂ was supplied by Boehringer/Mannheim GmbH (Mannheim, Germany). This antibody exhibited 3.2% cross-reactivity against PGE₁ and less than 0.2% cross-reactivity against PGA₁, PGA₂, PGB₁, PGB₂, PGF_{1 α} and PGF_{2 α} . [¹⁴C]Arachidonic acid (47 mCi/mmol) and [³H]prostaglandin E₂ (165 Ci/mmol) were purchased from New England Nuclear (Boston, MA). All other chemicals were commercial reagent grade quality.

Cell culture

Swiss mouse 3T3 fibroblasts (ATCC CCL 92) obtained from American Type Culture Collection (Camden, NJ) were grown in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, 100 units/ml penicillin, and 100 μ g/ml streptomycin at 37°C in an atmosphere of 95% air and 5% CO₂. Seeding was performed following detachment of cells from flasks with 1% trypsin and 0.02% EDTA in a solution containing 150 mM NaCl, 5 mM KCl, 8 mM Na₂HPO₄, and 2 mM KH₂PO₄, pH 7.4, (buffer solution) and suspension in the Dulbecco medium containing 10% fetal bovine serum.

Cells were seeded at a concentration of 3–5 \times 10³ cells/cm² in P60 dishes for radioimmunoassay studies and in T25 or T75 flasks for lipid analytical studies.

The cultures were allowed to become confluent (3–5 days) and then were fed the Dulbecco medium containing either unmodified 10% fetal bovine serum or 10% fetal bovine serum supplemented with fatty acid. Fatty acids were added as the sodium salt (3) and, unless otherwise specified, the concentration of the supplemental fatty acid was 100 μ M. In one series of experiments, the cultures were supplemented with linoleic acid during active growth. The cells were seeded at a density of 1–2 \times 10³ per cm² in P-60 dishes. After allowing 24 hr for attachment, the cultures were fed Dulbecco's modified Eagle's medium containing either unmodified 10% fetal bovine serum or the serum supplemented with 100 μ M linoleic acid. All cultures were given fresh medium at 3-day intervals.

For prostaglandin measurements, cell monolayers were washed with 2 ml of the buffer solution containing 0.125 M albumin, and this was followed by two washings with 2 ml of the buffer solution alone. The cells were then incubated for 5 min at 37°C with buffer solution containing 2 mg/ml bovine γ -globulin and 10 μ M of the calcium ionophore A23187. The medium was removed and analyzed for PGE₂ by radioimmunoassay. After washing the cells three times with 2 ml of the buffer solution, they were scraped and assayed for protein by a modification of the Lowry method (10).

Prostaglandin formation from exogenous arachidonic acid was also measured by radioimmunoassay. Cell monolayers were washed with the albumin and buffer solutions as described above and then were incubated 5 min at 37°C with varying concentrations of the sodium salt of arachidonic acid dissolved in 2.0 ml of buffer solution containing 2 mg/ml bovine γ -globulin. Corresponding control incubations were done in the absence of cells.

PGE₂ radioimmunoassay

All solutions were made up in 10 mM K₂HPO₄ adjusted to pH 7.4, containing 150 mM NaCl and 2 mg/ml bovine γ -globulin. 100 μ l of buffer (0% control), standard, or sample were mixed with 50 μ l of antiserum at a dilution that bound 50% of the radioactivity in the absence of standard. After vigorous mixing, samples were incubated at room temperature for 30 min. A 100% control contained 150 μ l of buffer before addition of radioisotope. One hundred μ l of [³H]PGE₂ solution (200,000 dpm/ml) was added to each tube with mixing, and the reaction mixtures were incubated at 4°C for 1 hr. After addition of 250 μ l of cold 25% polyethylene glycol 6000 and vigorous mixing, the samples were centrifuged at 4°C for 2 min in a Beckman microfuge. A 400- μ l aliquot of the supernatant solution was added to 5 ml of LSC cocktail for radioimmunoassay (J. T. Baker Chemical Co., Phillipsburg, NJ), and the radioactivity

was measured in a Beckman LS7000 liquid scintillation spectrometer. Quenching was monitored with the external standard.

Formation of radioactive prostaglandins

Cell monolayers were incubated 5 min at 37°C with 2.0 ml of the buffer solution containing 0.5–5.0 μM [^{14}C]arachidonic acid (47 Ci/mol) added as the sodium salt. Corresponding controls were incubated for 20 min with 2 ml of the buffer solution containing 1 mM aspirin before addition of the labeled arachidonic acid. After incubation, the medium was removed, acidified by addition of 1 drop of 1 M citric acid and, after saturation with solid NaCl, extracted with 2 volumes of ethyl acetate. The organic extract was chromatographed on Silica Gel G plates (Analabs, Inc., North Haven, CT) developed twice with the organic phase of a mixture containing ethyl acetate–acetic acid–2,2,4-trimethyl pentane–water 110:20:50:100 (v/v) (11). Prostaglandin standards were added to each chromatogram. After staining with I_2 vapor and sublimation, the bands of silica gel containing the various prostaglandins were scraped into counting vials containing 10 ml of Budget-Solve, and radioactivity was measured in the liquid scintillation spectrometer. The radioactivity recovered from the control cultures treated with aspirin was subtracted from the experimental values.

Lipid analyses

Cell monolayers were washed three times with ice-cold buffer solution, scraped into conical tubes, and sedimented at 1500 rpm for 15 min. The cell pellet was resuspended in 1 ml of buffer solution, and aliquots were taken for measurement of protein content (10). Cellular lipids were extracted with chloroform–methanol 2:1 (v/v) (12). Aliquots of the separated chloroform phase were taken for measurement of the phospholipid (13), cholesterol, and cholesteryl ester content (14). The triglyceride content of the cell lipid extract was determined by a modification of a fluorometric method that utilizes sodium periodate and acetyl acetone (15).

Gas–liquid chromatography was employed to analyze the fatty acid composition of the lipid extract (3). Phospholipid and neutral lipids were separated by thin-layer chromatography on silica gel G (3). The chromatograms were developed in a solvent system of hexane–diethyl ether–acetic acid–methanol 170:40:4:4 (v/v), and the lipid classes were eluted from the gel with chloroform–methanol 1:1 (v/v). Individual phospholipid classes were separated by thin-layer chromatography on silica gel H plates (Applied Science Laboratories, Inc., State College, PA) that were predeveloped in a solvent system containing chloroform–methanol–acetic acid–water 100:50:16:7 (v/v) and activated at 110°C for 1 hr. The chromato-

grams were developed in this solvent system, and the samples were eluted from the gel by stepwise extractions with this solvent mixture followed by methanol and then methanol–acetic acid–water 94:1:5 (v/v) (16). All eluted fractions were saponified for 45 min at 70°C in 2.5 ml of 95% ethanol and 0.05 ml of 33% KOH. The nonsaponifiable components were removed by extraction with n-heptane, the solution was acidified, and the fatty acids were extracted into n-heptane. After the fatty acids were methylated with 14% BF_3 in methanol for 10 min at 95°C, the methyl esters were extracted into n-heptane (3). The fatty acid methyl esters were separated with a gas–liquid chromatograph containing a glass column of 2 mm inside diameter \times 1.8 m, packed with 10% SP2330 on 100/120 mesh Chromosorb WAW (Supelco, Inc., Bellefonte, PA) (3). Peaks were identified by chromatography of purified fatty acid methyl ester standards obtained from either Supelco, Inc. or Applied Science Laboratories, and the area contained under each peak was measured with a Hewlett Packard 3380S integrator.

Since mechanical manipulation such as scraping of cells into a buffer solution can stimulate prostaglandin release (17, 18), we determined whether our procedure for isolating the cells might reduce the arachidonic acid content through such an artifact. Cultures were harvested by scraping as described above, by addition of 5% trichloroacetic acid, or by direct extraction of the attached cells with the chloroform–methanol mixture. In cultures that were not supplemented with fatty acid, arachidonic acid accounted for 7.0 ± 0.2 , 5.6 ± 0.2 , and $7.2 \pm 0.1\%$ of the cell phospholipid fatty acids, respectively, by these three procedures. When the cells were supplemented with linoleic acid, the arachidonic acid values were 15.5 ± 0.2 , 17.0 ± 1.0 , and $15.1 \pm 0.2\%$, respectively ($n = 4$). Because scraping did not lead to any appreciable reduction in the arachidonic acid content of the cell phospholipids, this method was employed subsequently for the lipid analytical studies.

RESULTS

Linoleic acid enrichment and prostaglandin production

When 3T3 cells were incubated with 2.5 μM [^{14}C]arachidonic acid and the labeled prostaglandin products were separated by thin-layer chromatography, $51 \pm 4\%$ of the radioactivity converted into prostaglandins was recovered in PGE_2 in the cultures supplemented with linoleic acid as compared with $50 \pm 0.5\%$ in corresponding unsupplemented cultures. With 5 μM [^{14}C]arachidonic acid, the values were $44 \pm 2\%$ for the control cultures and $59 \pm 5\%$ for the cultures supple-

mented with linoleate ($n = 4$). These findings are in agreement with the results obtained by others, indicating that PGE₂ is the predominant labeled prostaglandin product formed by 3T3 cells (7, 8). PGF_{2 α} accounted for about 5% of the radioactivity in both the control cultures and those supplemented with linoleate, and an unidentified metabolite that co-chromatographed with PGD₂ accounted for 22% and 16%, respectively. In every case, more [1-¹⁴C]arachidonic acid was incorporated into PGE₂, PGF_{2 α} , the substance migrating as PGD₂, and all of the other prostaglandin fractions by the cells enriched with linoleic acid as compared with the corresponding unsupplemented cultures.

To more thoroughly evaluate this difference, we compared prostaglandin production by cells enriched with linoleic acid with either unsupplemented cultures or those supplemented with oleic acid (18:1 n-9). The cultures initially were grown to confluence in an unsupplemented medium and then were exposed to medium containing 100 μ M of these supplemental fatty acids for an additional 48 hr. After removal of the medium and washing with albumin and buffer solutions, the cells were exposed to the calcium ionophore A23187 for 5 min, and PGE₂ was measured by the radioimmunoassay. PGE₂ production in each of four separate experiments was greater in the cells enriched with linoleic acid as compared with the control cultures. The increase ranged from 1.7- to 7.0-fold. By contrast, no increase was observed in the cultures supplemented with oleic acid and, in two cases, these cells released less PGE₂ than the control cultures. There was considerable variability from one experiment to the next in the absolute amount of PGE₂ produced and the extent of the increase resulting

TABLE 1. Spontaneous release of PGE₂ into the culture medium

Time in culture hr	PGE ₂ Released	
	Control ^a	Linoleic ^b
	pmol/mg protein	
Serum ^c 48	68 \pm 6	175 \pm 14
Serum-free ^d 1	<0.1	12 \pm 2
2	0.5 \pm 0.08	24 \pm 9

^a The culture medium was not supplemented with fatty acid.

^b The culture medium was supplemented with 100 μ M linoleic acid.

^c PGE₂ production was measured in these cultures over a 48-hr period, during which time the medium contained Dulbecco's minimum essential medium and 10% fetal bovine serum. Each value is the mean \pm SE of six separate cultures.

^d These cultures were incubated for 48 hr with either control or linoleate-supplemented medium. Following this period, the medium was removed and the cultures were washed. They then were incubated with Dulbecco's modified Eagle's medium during the 1- or 2-hr assay period. The latter medium contained neither serum nor supplemental fatty acid. Each value is the mean \pm SE of three separate cultures.

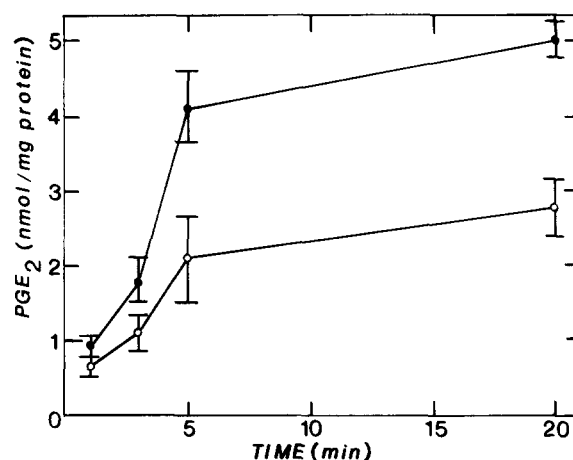


Fig. 1. Effect of time of exposure to the calcium ionophore on PGE₂ release. Cultures were exposed for 48 hr to medium without added fatty acid (○) or medium supplemented with 100 μ M linoleic acid (●). The cells were then washed and incubated with 10 μ M calcium ionophore A23187 for the indicated times. PGE₂ release was measured by radioimmunoassay. Each point represents the mean \pm SE of the values obtained from three separate cultures.

from linoleic acid enrichment. This variability persisted in spite of our efforts to control the seeding density, culture conditions, length of time of the cells in culture, and assay conditions.

As shown in **Table 1**, enrichment of the cultures with linoleic acid also increased the amount of PGE₂ released spontaneously by the cells. During the 48-hr modification period in medium containing 10% fetal bovine serum, the cells exposed to supplemental linoleic acid released 2.6 times more PGE₂ than the corresponding control cultures. In addition, cultures enriched with linoleate released a small quantity of PGE₂ during a subsequent 2-hr period of culture in a serum-free medium. By contrast, the unsupplemented cultures released only a trace quantity of PGE₂ under these conditions.

Several additional variables were examined in order to more completely assess the difference between the linoleic acid supplemented and unsupplemented cultures. One of these was the length of time that the cells were exposed to the calcium ionophore. As seen in **Fig. 1**, the cultures enriched with linoleic acid released more PGE₂ than the corresponding unsupplemented cultures at each time point tested between 1 and 20 min. In both cases almost all of the PGE₂ release occurred during the first 5 min of exposure to the ionophore. In a second experiment (not shown), the cultures were exposed to four repeated pulses of the calcium ionophore, each pulse being for 5 min. After each 5-min period, the medium was removed and fresh medium was added. Both types of cultures released smaller amounts of PGE₂ with each succeeding pulse, and almost 50% of the total PGE₂ release occurred in response to the first pulse. During each

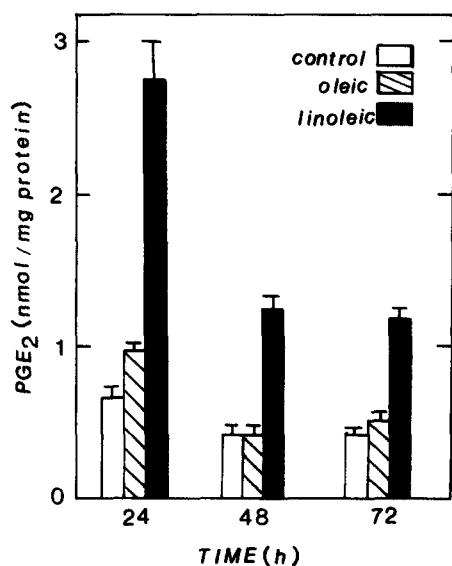


Fig. 2. PGE₂ production relative to the time of exposure to supplemental fatty acid. Cells were grown to confluence (5 days) and then exposed to control medium (no added fatty acid) or medium supplemented with 100 μ M oleic or linoleic acid. The media were removed at 24, 48, or 72 hr. After the cells were washed, they were incubated with 10 μ M calcium ionophore A23187 for 5 min. PGE₂ release was measured by radioimmunoassay. Each bar represents the mean \pm SE of the values obtained from three separate cultures. All of the values for the cells enriched with linoleic acid are significantly different from the control values ($P < 0.01$). With oleic acid supplementation, only the 24-hr value is significantly different from the control values ($P < 0.01$).

of the four pulses, more PGE₂ was released by the cells exposed to supplemental linoleic acid. The summated value for the cultures supplemented with linoleate (9.85 nmol/mg cell protein) was 3.5 times greater than that for the unsupplemented cultures (2.85 nmol).

The effect on PGE₂ production of the length of time to which the cultures were exposed to the supplemented medium also was examined. In every case the cultures first were grown to confluency in an unsupplemented medium, and then either fresh unsupplemented medium or medium enriched with 100 μ M linoleic acid was added. As shown in **Fig. 2**, PGE₂ release from the cells exposed to supplemental linoleate for 24, 48, or 72 hr was considerably higher than from either the unsupplemented cultures or those enriched with oleic acid. In these experiments, a 5-min exposure to the calcium ionophore was the stimulus for prostaglandin production.

Cellular lipid composition

The cellular fatty acid composition was assessed after 48 hr of exposure of confluent cultures to either the medium supplemented with 100 μ M oleic or linoleic acids, as compared with unsupplemented medium. **Table 2** contains the results for the total phospholipid and neutral lipid fractions. In the phospholipids, little change

in fatty acid composition was produced by exposure to oleic acid. By contrast, there was a 12% increase in saturated fatty acid, a 40% increase in polyenoic fatty acid, and a 38% decrease in monoenoic fatty acid content of the cellular phospholipids in the cultures supplemented with linoleic acid as compared with those from the unsupplemented controls. This was accounted for primarily by increases in the 18:2, 20:4, and 22:4 contents and a decrease in the 18:1 content of the phospholipids from the cells exposed to linoleic acid. As opposed to the phospholipids, there was little change in the neutral lipid fatty acid composition when the cultures were exposed to media containing supplemental fatty acid. Both of these findings differ from those observed when either human skin fibroblasts or endothelial cells are cultured in media containing supplemental linoleic acid. In both of these cases, large changes in fatty acid composition were noted in the neutral lipid as well as the phospholipid fractions (2, 3, 19). Furthermore, in these cases, supplementation with linoleic acid produced a decrease in the 20:4 content of the phospholipids (2, 3, 19), whereas the 20:4 percentage increased in the 3T3 cell phospholipids.

Values for the contents of various lipids present in the 3T3 cells are listed in **Table 3**. There was no statistically significant difference in the phospholipid content of the supplemented and control cultures. Therefore, the 4-fold percentage increase in the 20:4 content of the cells enriched with linoleic acid represents an actual increase in the quantity of arachidonic acid contained in the normal amount of cellular phospholipids. Likewise, there was no significant difference in the cholesterol content of the three types of cultures. A 50% increase was observed in the triglyceride content of the cultures exposed to media containing supplemental fatty acid, but there was no difference in triglyceride content of the cells exposed to oleate as compared with linoleate.

Table 4 shows the fatty acid compositions of the four main glycerophospholipid fractions of the control and fatty acid supplemented cultures. The largest changes were observed in the choline fraction, whereas the serine fraction was most resistant to change. There was a 7-fold increase in the 20:4 content of the choline fraction of the cells exposed to supplemental linoleate, as compared with either the unsupplemented control or the oleate-enriched cultures. By contrast, the increase in 20:4 was only about 2-fold in the ethanolamine and inositol glycerophospholipid fractions of the linoleate-enriched cells, and no significant increase was noted in the serine glycerophospholipids. Large increases in 18:0 and 18:2, and decreases in 16:1, 18:1 and 20:3 occurred in the choline and ethanolamine phosphoglycerides of the linoleate-enriched cells. Certain of these changes also occurred in the inositol fraction, but to a lesser extent.

TABLE 2. Effect of fatty acid supplementation on composition of 3T3 cell lipids

Fatty Acid	Composition ^a					
	Phospholipids			Neutral Lipids		
	Control ^b	Oleic	Linoleic	Control	Oleic	Linoleic
	%					
Saturation class						
Saturated	35.9	35.5	40.3	44.9	45.9	49.2
Monoenoic	38.6	40.1	24.0	26.9	33.8	28.1
Polyenoic	25.5	24.4	35.7	28.2	20.3	22.7
Individual acids ^c						
16:0	18.4 ± 1.2	17.3 ± 0.6	21.0 ± 0.4	25.0 ± 0.9	24.6 ± 1.5	24.2 ± 1.9
16:1	6.1 ± 0.6	5.6 ± 0.4	4.9 ± 0.5	5.5 ± 0.6	5.7 ± 0.4	4.8 ± 0.5
18:0	15.3 ± 0.7	17.1 ± 0.2	17.2 ± 0.9	17.1 ± 0.8	19.3 ± 1.4	19.7 ± 1.8
18:1	31.4 ± 1.3	33.4 ± 1.1	18.6 ± 0.5 ^d	21.4 ± 1.8	27.0 ± 1.0	22.5 ± 1.4
18:2	2.3 ± 0.2	2.3 ± 0.1	5.0 ± 0.1 ^d	2.8 ± 0.4	3.6 ± 0.2	3.1 ± 0.3
20:3	4.7 ± 0.2	5.9 ± 0.4	1.8 ± 0.3 ^d	N.D. ^f	N.D.	N.D.
20:4	3.7 ± 0.3	4.3 ± 0.2	14.6 ± 0.9 ^d	2.5 ± 0.7	0.8 ± 0.1	2.2 ± 0.2
22:4	1.0 ± 0.1	1.2 ± 0.2	3.5 ± 0.6 ^e	6.0 ± 1.6	1.7 ± 0.6	3.6 ± 0.9
22:5	1.1 ± 0.3	1.0 ± 0.3	1.4 ± 0.6	N.D.	N.D.	N.D.
22:6	1.2 ± 0.2	1.4 ± 0.2	1.5 ± 0.4	N.D.	N.D.	N.D.

^a Values are the mean ± SE of six separate cultures.

^b Medium in which the cells were cultured after becoming confluent: control, no added fatty acid; 100 μM oleic or linoleic acid added as a supplement. The cells were exposed to the medium containing supplemental fatty acid for 48 hr.

^c Only the major individual acids are listed, and these percentages do not total to 100%.

^d Significantly different from the control value, *P* < 0.001.

^e Significantly different from the control value, *P* < 0.01.

^f Not detected (<0.1%).

Supplemental fatty acid concentration

To more completely assess the enhancement of prostaglandin formation produced by linoleic acid, we investigated the effect of supplemental fatty acid concentration in the medium during the 48-hr culture period. As shown in Fig. 3 (left side), increased PGE₂ production in response to stimulation with the calcium ionophore was observed when the medium was supplemented with as little as 25 μM linoleic acid. PGE₂ production increased progressively as the linoleic acid concentration was raised to 100 μM but decreased sharply when it was raised further to 150 μM. The changes in PGE₂ production were paralleled closely by the changes in the 20:4 content of the cellular phospholipids. In addition, the amount of 18:2 increased and 18:1 decreased in phospholipids as the linoleic acid concentration of the culture medium was raised over this range.

Of six other fatty acids tested, only supplemental arachidonic acid produced a large increase in the capacity of the 3T3 cells to make PGE₂. The concentration dependence of the arachidonic acid effect is shown in the right side of Fig. 3. PGE₂ production in response to the calcium ionophore increased as the arachidonic acid concentration was raised, with a maximum release occurring when the supplemental concentration was 75 μM. At higher concentrations, there was a small decrease in PGE₂ release. Arachidonic acid supplementation led to

a large increase in the 20:4 content of the cellular phospholipids. An accumulation of 22:4 n-6, the elongation product of arachidonic acid, also occurred, and this was especially pronounced when the supplemental arachidonic acid concentration was 100 μM or higher. These increases were accompanied by a large reduction in the 18:1 content of the cellular phospholipids.

Short-term exposure to linoleic acid

In cultured endothelial cells, the effects of linoleic acid supplementation on prostaglandin production in response to stimulation with the calcium ionophore were

TABLE 3. Lipid content of 3T3 cells

Lipid Fraction	Amount ^a		
	Control ^b	Oleic	Linoleic
	μg/mg protein		
Phospholipids	402 ± 15	375 ± 19	377 ± 25
Cholesterol	61 ± 0.9 (7) ^c	65 ± 1.9 (7)	57 ± 2.2 (9)
Triglycerides	27 ± 0.9	40 ± 2.6 ^d	41 ± 3.3 ^e

^a Values obtained are the mean ± SE of six separate cultures.

^b Medium in which the cells were cultured for 48 hr after becoming confluent.

^c Percent of the cholesterol content present as cholesteryl esters.

^d Significantly different from the control value, *P* < 0.001.

^e Significantly different from the control value, *P* < 0.01.

TABLE 4. Fatty acid composition of

Fatty Acid ^b	Composition ^d					
	Choline Glycerophospholipids			Ethanolamine Glycerophospholipids		
	Control ^c	Oleic	Linoleic	Control	Oleic	Linoleic
	%					
Saturation class						
Saturated	34.5	42.5	42.1	33.6	36.2	40.2
Monoenoic	48.3	46.2	25.4	39.2	32.7	20.7
Polyenoic	17.2	11.3	32.5	27.2	31.1	39.1
Individual acids						
16:0	18.3 ± 2.5	23.2 ± 0.7	14.4 ± 4.0	11.9 ± 1.2	11.0 ± 1.6	13.1 ± 3.0
16:1	7.4 ± 2.1	6.7 ± 0.3	1.7 ± 1.0	2.4 ± 0.2	1.8 ± 0.1	1.7 ± 0.5
18:0	14.7 ± 1.4	18.1 ± 1.7	26.5 ± 4.0 ^d	21.5 ± 0.9	24.5 ± 0.4	26.2 ± 1.8
18:1	39.3 ± 6.4	38.0 ± 1.8	22.6 ± 1.5	35.1 ± 0.7	29.1 ± 0.6 ^e	18.4 ± 1.3 ^f
18:2	2.8 ± 0.6	2.0 ± 0.5	5.6 ± 0.5	2.8 ± 0.4	3.1 ± 0.1	4.0 ± 0.4
20:3	3.0 ± 0.1	3.8 ± 0.6	1.2 ± 0.8	7.3 ± 0.8	8.1 ± 0.4	2.3 ± 0.5 ^e
20:4	2.3 ± 0.5	2.1 ± 0.1	16.0 ± 2.2 ^e	6.3 ± 0.3	6.7 ± 0.1	16.5 ± 2.1 ^e
22:4	0.2 ± 0.1	N.D. ^g	1.1 ± 0.7	3.0 ± 0.3	1.8 ± 0.4	6.4 ± 0.7 ^d
22:5	N.D.	N.D.	N.D.	1.2 ± 0.1	1.4 ± 0.4	1.3 ± 0.4
22:6	0.1 ± 0.1	N.D.	N.D.	1.4 ± 0.2	1.4 ± 0.3	1.2 ± 0.4

^a Values are the mean ± SE of three separate cultures.

^b Since only the major individual acids are listed, these percentages do not add up to 100%.

^c Medium in which the cells were cultured for 48 hr after becoming confluent.

^d Significantly different from the control value, $P < 0.05$

^e Significantly different from the control value, $P < 0.01$.

^f Significantly different from the control value, $P < 0.001$.

^g Not detected (<0.1%).

evident within 3 hr of exposure (2). We wished to determine whether similar short-term effects occurred in the 3T3 cells. As seen in Fig. 4, consistent differences in PGE₂ release as compared with corresponding un-supplemented control cultures were not observed during the first 6 hr of exposure. PGE₂ release during these early times was stimulated when either the un-supplemented or the linoleate-enriched medium was added, the maximum stimulation occurring within 1 to 2 hr. This stimulation probably is due to the introduction of fresh medium containing 10% fetal bovine serum, for serum has been shown to stimulate prostaglandin production in a number of different types of cells (7, 18, 20, 21). By 4 to 6 hr, the values returned toward the baseline range. Because the overall effect during the initial period of exposure was generally similar to that observed with un-supplemented control cultures, we did not examine whether the differences noted at 1 and 2 hr were reproducible.

The phospholipid fatty acid compositions also were measured during these early time points. Table 5 shows that very little change in composition occurred in the un-supplemented controls during the initial 6 hr. Therefore, it appears that factors other than fatty acid compositional changes are responsible for the PGE₂ responses which occurred during the first 6 hr. Fatty acid compositional changes were noted, however, in the cells exposed to supplemental linoleic acid. They were man-

ifest within 2 hr, the earliest time tested, and became more pronounced at 4 and 6 hr. Appreciable increases occurred in 18:2 and 20:4, accompanied by decreases in 16:0, 16:1, and 18:1. Since PGE₂ production by the linoleate-enriched cultures was similar to that of the un-supplemented controls, it appears that the increase in the phospholipids 20:4 content is not effective in enhancing the capacity of the cells to produce PGE₂ during short-term exposures.

Prostaglandin production in growing cultures

Prostaglandin release from 3T3 cells is reported to be highest early in the culture period when the cells are growing most rapidly (21). Because of the apparent influence of growth rate or cell density on prostaglandin production, we investigated whether supplementation with linoleic acid would have the same effect in rapidly growing cultures as was noted in the above experiments where the fatty acid supplement was added after the cultures became confluent. Table 6 contains the results of an experiment in which linoleic acid was added 24 hr after the cells were seeded. In both the linoleate-supplemented and control cultures, the highest PGE₂ production per mg cell protein occurred 24 hr later, the first time that enough cells were present to make accurate measurements. At this time, the cultures supplemented with linoleic acid produced less PGE₂ than the corresponding controls, a different result than that obtained

Compositions ^a					
Inositol Glycerophospholipids			Serine Glycerophospholipids		
Control	Oleic	Linoleic	Control	Oleic	Linoleic
%					
48.2	47.2	52.1	48.3	48.8	51.7
28.0	32.2	22.5	28.2	27.0	24.0
23.8	20.6	25.4	23.5	24.2	24.3
11.6 ± 0.9	13.3 ± 1.9	15.1 ± 2.4	16.4 ± 1.4	18.5 ± 0.3	21.3 ± 0.5 ^d
1.8 ± 0.3	2.4 ± 0.2	2.8 ± 0.6	3.6 ± 0.3	4.2 ± 0.3	4.1 ± 0.2
35.5 ± 4.4	33.0 ± 0.7	35.9 ± 1.6	30.8 ± 0.5	28.6 ± 1.0	29.3 ± 0.8
25.5 ± 1.8	29.6 ± 1.6	19.4 ± 0.3 ^d	24.1 ± 1.2	22.4 ± 1.4	19.9 ± 0.6 ^d
4.4 ± 0.1	5.2 ± 0.1 ^e	5.3 ± 0.2 ^d	4.9 ± 0.4	6.0 ± 0.7	5.7 ± 0.1
3.0 ± 1.0	5.3 ± 0.7	1.1 ± 0.2	4.6 ± 0.3	2.5 ± 0.1 ^e	0.2 ± 0.1 ^f
4.7 ± 0.8	4.4 ± 1.8	9.6 ± 1.4 ^d	3.6 ± 0.3	1.7 ± 0.1 ^e	4.4 ± 0.4
4.1 ± 0.8	3.3 ± 0.7	4.3 ± 0.2	2.3 ± 1.1	N.D.	N.D.
N.D.	N.D.	N.D.	0.7 ± 0.4	0.3 ± 0.2	N.D.
N.D.	N.D.	N.D.	0.6 ± 0.3	0.2 ± 0.1	N.D.

when confluent cultures were supplemented with linoleic acid (Figs. 1-3). Subsequently, when the cell density increased and the amount of PGE₂ produced per mg cell protein was reduced, supplementation with linoleate en-

hanced PGE₂ production, as was noted above with the confluent cultures. The reason for this qualitative difference in the effect of linoleate during the early as compared with latter phases of the culture period is not

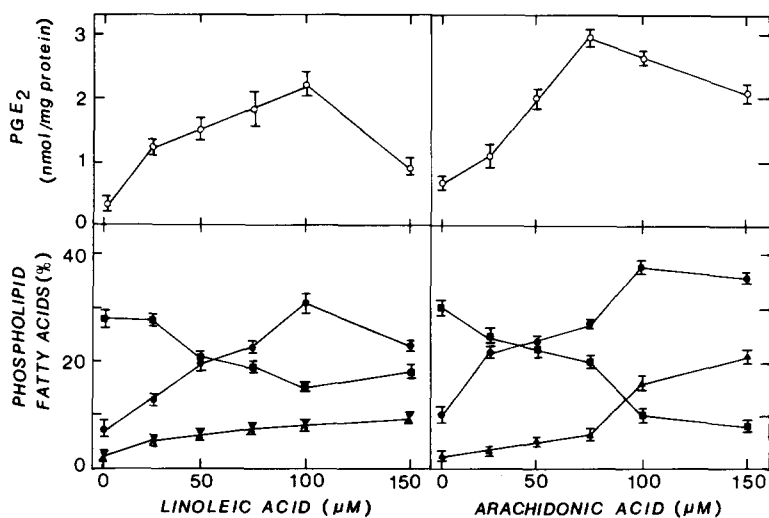


Fig. 3. Effect of linoleic acid or arachidonic acid supplementation on PGE₂ release and phospholipid fatty acyl composition. All cultures were grown to confluence and then exposed to medium supplemented with the indicated concentrations of the fatty acids (0-150 μM). After 48 hr, the cells were washed and incubated with 10 μM calcium ionophore A23187 for 5 min. PGE₂ release was measured by radioimmunoassay. Each point represents the mean ± SE of values obtained from four separate cultures. In additional sets of cultures, the fatty acid composition of the cell phospholipids was determined by gas-liquid chromatography. Only fatty acyl groups that changed in composition appreciably are shown: 18:1, ■; 18:2, ×; 20:4, ●; 22:4, ▲. The fatty acid values are the mean ± SE of three separate cultures.

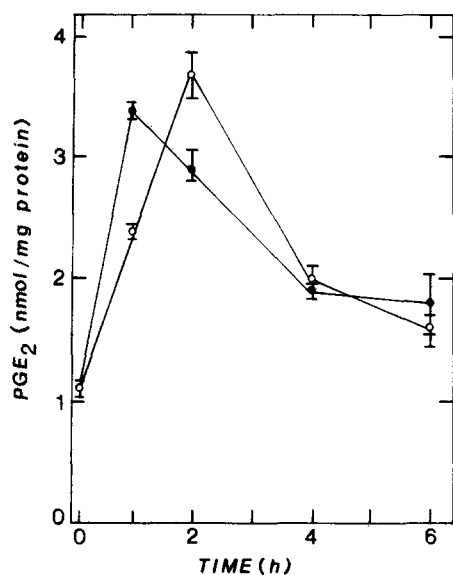


Fig. 4. Effect of short-term exposure to supplemental fatty acids on PGE₂ production. Cells were grown to confluence and then exposed to either a medium containing no supplemental fatty acid (○) or one supplemented with 100 μM linoleic acid (●) for up to 6 hr. After the cultures were washed, they were exposed to 10 μM calcium ionophore A23187 for 5 min. PGE₂ production was measured by radioimmunoassay. Each point is the mean ± SE of three separate cultures.

known at present. Likewise, it is not clear why PGE₂ production per mg of cell protein was greater on Day 6, after the cultures became confluent, as compared with Days 2 and 3 when they were still growing fairly rapidly.

Reversion of prostaglandin and lipid changes

Additional studies revealed that the changes produced by supplementation of confluent cultures with linoleic

acid are to a large extent reversible. As seen in Fig. 5, the capacity of linoleate-supplemented cultures to produce PGE₂ in response to stimulation with the calcium ionophore approached that of the unsupplemented controls within 3 to 5 days after they were transferred to an unsupplemented medium. After 48 hr of supplementation, the linoleate-enriched cells produced 11 times more PGE₂ in response to the calcium ionophore than the unsupplemented controls. At this time, the medium was removed from the remaining cultures and, after washing, unsupplemented medium was added. The remaining unsupplemented control cultures were treated similarly. Cultures were taken at various times following transfer for measurement of PGE₂ release and lipid composition. Two days after transfer to the unsupplemented medium, the cultures that were exposed originally to linoleic acid produced only 3 times more PGE₂ as the corresponding controls, and after 5 days they produced only 1.3 times more. This reduction was associated with changes in the phospholipid fatty acid composition of the cells originally enriched with linoleic acid. As seen in the bottom half of Fig. 5, the percentage of 16:0 increased and 18:2 decreased during the 5-day reversion period, and the final 16:0 and 18:2 percentages were similar to those found in the corresponding unsupplemented control cultures. Although the phospholipid 18:1 and 20:4 percentages also reverted towards those found in the unsupplemented controls, these reversions were incomplete. In particular, 20:4 still accounted for 12% of the phospholipid fatty acids 5 days after transfer, whereas it accounted for only 4% in the unsupplemented controls.

TABLE 5. Short-term alterations in the phospholipid fatty acid composition of 3T3 cells

Fatty Acid	Phospholipid Composition ^a						
	2 Hours ^c		4 Hours		6 Hours		
	Control ^b	Control ^d	Linoleic ^e	Control	Linoleic	Control	Linoleic
	%						
16:0	20.3 ± 2.7	16.4 ± 2.1	12.9 ± 0.5	19.7 ± 1.8	15.7 ± 2.0	18.2 ± 2.5	14.9 ± 1.3
16:1	8.7 ± 1.5	7.6 ± 1.5	4.4 ± 1.7	8.6 ± 1.7	5.8 ± 1.5	6.9 ± 0.8	4.9 ± 0.9
18:0	12.3 ± 0.3	12.2 ± 1.2	13.5 ± 1.0	12.6 ± 0.7	12.0 ± 0.6	13.2 ± 0.2	13.6 ± 0.6
18:1	41.3 ± 1.3	41.0 ± 0.7	40.0 ± 1.8	39.4 ± 1.5	35.3 ± 0.1 ^g	41.9 ± 2.0	35.0 ± 0.6 ^g
18:2	2.3 ± 0.7	1.7 ± 0.1	6.7 ± 0.1 ^f	1.5 ± 0.1	8.5 ± 0.2 ^f	1.7 ± 0.2	9.6 ± 0.3 ^f
20:4	4.2 ± 0.3	5.3 ± 0.3	6.8 ± 0.6 ^g	4.9 ± 0.6	8.1 ± 0.5 ^f	5.2 ± 0.5	9.8 ± 0.3 ^f
22:4	1.2 ± 0.2	1.2 ± 0.1	1.4 ± 0.1	1.2 ± 0.4	1.6 ± 0.3	1.6 ± 0.5	2.2 ± 0.2
22:5	1.4 ± 0.4	1.7 ± 0.2	2.3 ± 0.2	1.5 ± 0.4	2.1 ± 0.3	1.8 ± 0.8	1.5 ± 0.1
22:6	1.5 ± 0.3	1.9 ± 0.1	2.1 ± 0.1	1.7 ± 0.5	1.8 ± 0.1	2.1 ± 0.9	2.2 ± 0.2

^a Mean ± SE of values obtained from four separate cultures. Only the most abundant fatty acids are listed and, therefore, the percentage values do not add up to 100%.

^b Values for the unsupplemented cultures before washing and transfer to the second medium.

^c Time of exposure to the second medium.

^d No supplemental fatty acid was added.

^e Medium supplemented with 100 μM linoleic acid.

^f Significantly different from the control value, $P < 0.001$.

^g Significantly different from the control value, $P < 0.01$.

Prostaglandin production from extracellular arachidonic acid

In an attempt to distinguish between effects on the prostaglandin-forming enzymes as opposed to availability of substrate in cellular phospholipids, we investigated the capacity of the cells to produce prostaglandins from externally added arachidonic acid. The arachidonic acid concentration was varied from 0.2 to 8 μM , and the assay medium, which was buffered at pH 7.4, contained no albumin or serum. Likewise, neither the calcium ionophore nor any other external stimulus was added to the medium so that the contribution of internal substrate to prostaglandin production would be negligible. Preliminary studies indicated that most of the prostaglandin production under these conditions occurred during the first 5 min, and this time was selected for all subsequent measurements. Fig. 6 shows the quantity of PGE₂ formed relative to the arachidonic acid concentration of the assay medium. At each concentration tested, more PGE₂ was produced by the cells enriched with linoleic acid as compared with either the unsupplemented control cultures or those enriched with oleic acid. Although the cells enriched with oleic acid also produced somewhat more PGE₂ than the unsupplemented controls at several of the intermediate arachidonic acid concentrations, this difference was overcome when the concentration was raised to 8 μM . Saturation was not achieved in these experiments, but it was not possible to further increase the arachidonic acid concentration of the assay medium because of aggregate formation. This experiment was repeated three times, and a similar result was obtained in each case; the cultures supplemented with linoleate produced more PGE₂ from exogenous arachidonate than either the control or oleic acid-supplemented cultures.

TABLE 6. PGE₂ production by cells modified during growth

Time in Culture ^a	PGE ₂ Produced ^b	
	Control ^c	Linoleic ^d
days	nmol/mg protein	
1	3.90 ± 0.19	2.60 ± 0.16
2	0.37 ± 0.06	0.80 ± 0.02
3	0.33 ± 0.04	0.67 ± 0.05
6	0.73 ± 0.05	1.70 ± 0.17

^a This does not include the 24 hr after seeding, during which time cell attachment occurred.

^b PGE₂ production was measured after the cells were exposed to the calcium ionophore A23187 for 5 min. The culture medium was removed and the cells were washed prior to exposure to the ionophore. Each value is the mean ± SE of three separate cultures.

^c No supplemental fatty acid was present in the media during culture.

^d Media supplemented with 100 μM linoleic acid during culture. All of these values are significantly different from the corresponding control values, $P < 0.01$.

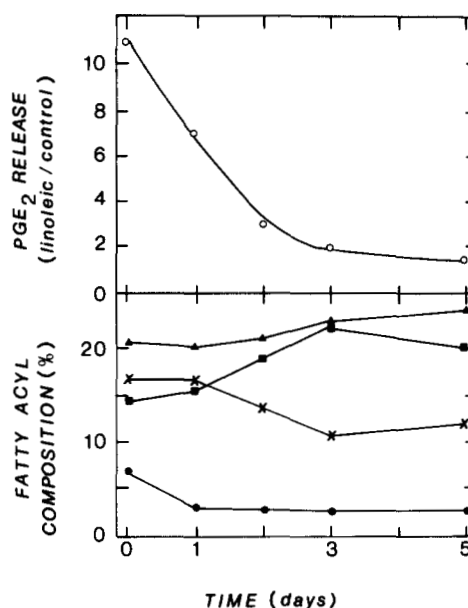


Fig. 5. Changes in PGE₂ release and cellular fatty acid composition during reversion of cells supplemented with linoleic acid. Confluent cells were exposed for an initial 48-hr period to medium supplemented with 100 μM linoleic acid. This medium was then removed and fresh medium containing no supplemental fatty acid was added. The 0 hr controls were immediately exposed to 10 μM calcium ionophore A23187 for 5 min, and PGE₂ release was assayed. Additional cultures were assayed for PGE₂ release at 1, 2, 3, and 5 days after transfer to the unsupplemented medium. At each time, control cultures treated in the same way, except that they were exposed to unsupplemented medium for the initial 48-hr period, were also assayed for PGE₂ release. All values represent the mean ± SE of three separate cultures. Additional cultures were analyzed at each time point for phospholipid fatty acid composition by gas-liquid chromatography. The fatty acid percentages also represent the mean ± SE of three separate determinations. Data from only those fatty acids that changed appreciably during the experiment are shown on the figure: 16:0, \blacktriangle ; 18:1, \blacksquare ; 18:2, \bullet ; 20:4, \times .

Increased prostaglandin production by the cultures enriched with linoleate, also was observed when the experiments were done with [1-¹⁴C]arachidonic acid and assayed by measuring isotope incorporation into prostaglandin products separated by thin-layer chromatography (11, 22). In both the linoleate-enriched and unsupplemented cultures, the largest amount of radioactivity was recovered in PGE₂. As compared with unsupplemented control cultures, those enriched with linoleate incorporated 1.5 to 2.1 times more [1-¹⁴C]arachidonic acid into PGE₂. Likewise, they incorporated 1.2 to 1.8 times more radioactivity into PGF_{2 α} and 1.3 to 1.4 times more radioactivity into an unidentified metabolite that migrated in the region of the gel occupied by the PGD₂ standard. Taken together with the radioimmunoassay results, these data indicate that the greater prostaglandin production by the linoleate-enriched cells cannot be explained entirely on the basis of differences in the availability of intracellular arachidonic acid substrate.

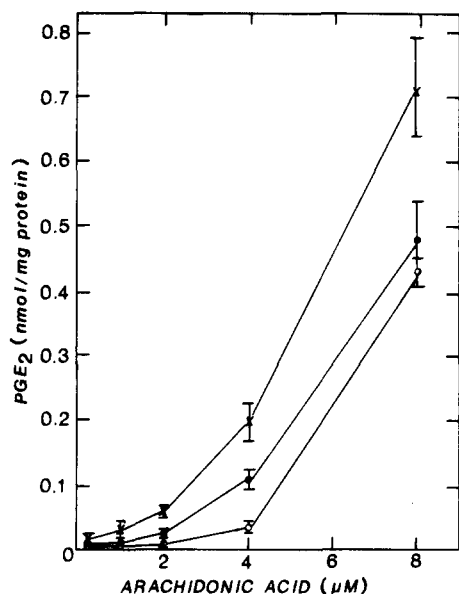


Fig. 6. PGE₂ production from arachidonic acid added to the extracellular fluid. Confluent cultures were exposed 48 hr to medium without added fatty acid (O) or medium supplemented with either 100 μM oleic acid (\bullet) or 100 μM linoleic acid (\times). After the cultures were washed, they were incubated for 5 min with the indicated concentration of arachidonic acid. The media were analyzed for PGE₂ formation by radioimmunoassay. Control flasks containing no cells were assayed at each concentration, and any PGE₂ formed was subtracted from the experimental values. Each data point represents the mean \pm SE of three separate cultures.

DISCUSSION

Prostaglandin production by Swiss mouse 3T3 cells, like human endothelial cells (2), is affected by enrichment of the cultures with linoleic acid. The nature of the response in the two cell types, however, is quite different. In 3T3 cells, supplementation with linoleic acid enhances prostaglandin production under all conditions except during the first 24 hr after attachment, at which time the amount of PGE₂ produced per mg cell protein is very high. By contrast supplementation with linoleic acid reduces the capacity of the endothelial cells to produce prostaglandins (2). This occurs at linoleic acid concentrations similar to those employed in the present study, 25 to 150 μM (23).

Although other possibilities cannot be excluded, the findings can be explained entirely on the basis of the different ways in which the 3T3 and endothelial cells utilize newly incorporated linoleic acid. The endothelial cells convert little of the incoming linoleic to arachidonic acid (2). Much of the linoleate is incorporated without structural modification and the small quantity that is modified is converted to eicosadienoic acid (20:2 n-6), the direct elongation product (2). Linoleic and eicosadienoic acids accumulate in the cells and are incorporated into cellular phospholipids at the expense of arachidonic acid.

Because of this, the quantity of arachidonic acid in cellular phospholipids decreases, including that in the choline, ethanolamine, and inositol phosphoglyceride fractions, (2), and less substrate is available for prostaglandin synthesis. In addition, the observations made with isolated microsomal preparations from vesicular gland, stomach, and skin (5, 6) suggest that the excessive quantity of linoleic and eicosadienoic acids may competitively inhibit prostaglandin production. A completely different fatty acid compositional change occurs when the 3T3 cultures are enriched with linoleic acid. In this case, the cellular phospholipids become enriched with arachidonic acid so that they contain about 4 times more than the phospholipids of either the unsupplemented control cultures or those supplemented with oleic acid. The increase in arachidonic acid is manifested in the choline, inositol, and ethanolamine glycerophospholipids, the cellular phospholipid fractions that have been reported to provide the substrate for prostaglandin synthesis in the most extensively studied system, the human platelet (24–28). Furthermore, there is only a small increase in the linoleic acid content of the cell phospholipids relative to the increase that occurs in the endothelial cells following linoleate supplementation (2), and no accumulation of eicosadienoic acid was detected. These findings indicate that, unlike cultured endothelial cells, 3T3 cells have the enzymatic capability to desaturate as well as elongate linoleic acid. Therefore, the enhanced ability of the 3T3 cells enriched with linoleic acid to produce prostaglandins can be explained on the basis of two factors; the greater availability of arachidonic acid in substrate phospholipids and the fact that potentially inhibitory polyunsaturates, such as linoleic and eicosadienoic acid, do not accumulate.

Different lipid compositional responses to linoleic acid enrichment have been reported for a number of other cultured cell lines. For example, the human skin fibroblast responds in the same manner as the endothelial cells, with a large decrease occurring in the arachidonic acid content of the cellular phospholipids (3, 19). Rat and mouse lymphocytes (29), hamster NN astroglia (30), and rat hepatoma cells (31) show no appreciable change in arachidonic acid content in spite of the fact that large amounts of linoleic acid accumulate. Finally, like the 3T3 cells, rat C₆ astroblasts (30) and the C1300 mouse neuroblastoma cells (32) show large increases in their phospholipid arachidonic acid contents when they are enriched with linoleic acid.

The results of the reversion experiment (Fig. 5) are consistent with the explanation that the enhanced prostaglandin production is due to the increase in intracellular arachidonic acid content, for PGE₂ production in the cultures initially supplemented with linoleic acid decreased as the intracellular arachidonic acid content decreased. On the other hand, the greater capacity of the

cultures supplemented with linoleic acid to convert extracellular arachidonate into PGE₂ (Fig. 6) probably cannot be explained on the basis of changes in the availability of arachidonic acid in the intracellular substrate pools. It is possible that the 3T3 cells contain more of the prostaglandin-forming enzymes when the cultures are supplemented with linoleic acid. Alternatively, the increase in phospholipid fatty acyl unsaturation may cause a change in membrane properties that enables the prostaglandin-forming enzymes, which are membrane bound (5, 6, 33), to operate more effectively. In this regard, several other membrane-bound enzymes exhibit activity changes in response to modification of the phospholipid fatty acyl composition, including adenylate cyclase (34, 35), (Na⁺ + K⁺)-ATPase (36, 37), calcium-dependent ATPase (38), and acyl coenzyme A:cholesterol acyltransferase (39). Such an explanation is attractive because all of the effects of linoleic acid enrichment on prostaglandin production can be attributed to a single factor, modification of the intracellular phospholipid fatty acyl composition. If this type of membrane lipid structural effect is operative, it may also contribute to the enhanced PGE₂ production that occurs when the 3T3 cells enriched with linoleic acid are stimulated with the calcium ionophore. **■**

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