Fatty Acids Promote Translocation of CTP:Phosphocholine Cytidylyltransferase to the Endoplasmic Reticulum and Stimulate Rat Hepatic Phosphatidylcholine Synthesis*

(Received for publication, November 29, 1982)

Steven L. Pelech†, P. Haydn Pritchard, David N. Brindley§, and Dennis E. Vance

From the Department of Biochemistry, Faculty of Medicine, University of British Columbia, Vancouver, British Columbia, Canada V6T 1W5 and the §Department of Biochemistry, Queen’s Medical Center, Clifton Boulevard, Nottingham NG7 2UH, United Kingdom

The mechanism by which fatty acids stimulate the biosynthesis of phosphatidylcholine has been studied in cultured rat hepatocytes. Long chain fatty acids (1 mM) stimulated approximately 1.9-fold [methyl-3H]choline incorporation from phosphocholine into phosphatidylcholine by isolated hepatocytes. Oleate and palmitate (4 mM) enhanced phosphatidylcholine production by 3- and 2.2-fold, respectively. Stimulation of phosphatidylcholine synthesis by oleate was evident within 30 min after addition of the fatty acid to the hepatocyte medium. The effect could be correlated with a doubling of the microsomal CTP:phosphocholine cytidylyltransferase activity. Additional evidence for a direct effect by fatty acids and their CoA derivatives on the cytidylyltransferase was obtained in vitro. Arachidonate, oleate and palmitate (0.1 mM) stimulated rat liver cytosolic cytidylyltransferase activity 4.1-, 3.5-, and 3.2-fold, respectively. Activation by oleate was accompanied by a 3.6-fold reduction in the apparent Kₘ of the cytidylyltransferase for CTP and aggregation of the enzyme to high molecular weight species. Acceleration of the cytidylyltransferase reaction by fatty acids provides a positive feed-forward mechanism for regulation of phosphatidylcholine anabolism.

Fatty acids complexed with albumin are cleared from the circulation by the liver which has a specific receptor for the uptake of albumin-bound substances (1). Depending on the nutritional state of the rat, internalized fatty acids are either oxidized to CO₂ and ketone bodies, or esterified to form glycerolipids (2). In fed rats, fatty acids are largely channelled into triacylglycerol formation. Studies with liver slices (3, 4) and isolated hepatocytes (5) have shown that 1 mM concentrations of fatty acid in the medium can stimulate [³H]glycerol incorporation into triacylglycerol over 15-fold, whereas incorporation into phosphatidylcholine is enhanced by only 1.4-fold. However, when the cytoplasmic pool of fatty acid is diminished, these fatty acids are preferentially esterified into phospholipid at the expense of triacylglycerol synthesis (2). The mechanism by which the phosphatidylcholine require-

ments of the hepatocyte are satisfied before large amounts of triacylglycerol can be produced is unclear.

In several other model systems, alterations in the rate of phosphatidylcholine synthesis have correlated with changes in the activity of CTP:phosphocholine cytidylyltransferase (6-11). Cytidylyltransferase features a number of properties which are consistent with its proposed regulatory role (12). This enzyme is sensitive to activation by phospholipids (13-15), and substrate inhibition by CTP (16) and phosphocholine (17). Cytidylyltransferase is also controlled by a phosphorylation-dephosphorylation cycle (17). This enzyme is ambigious (18) in that in is recovered in both the cytosolic and microsomal fractions of rat liver (19). Furthermore, there is some evidence that the cytidylyltransferase is activated upon translocation from the cytoplasm to the endoplasmic reticulum (10, 11, 20-22). Some glycerolipids (diacylglycerol, phosphatidylglycerol, and phosphatidylerserine), have been shown to promote aggregation of rat liver cytosolic cytidylyltransferase while other glycerolipids (phosphatidylcholine, phosphatidylethanolamine, lysophosphatidylcholine, and lysophosphatidylethanolamine) do not (14, 23).

Fatty acids and their CoA derivatives exert inhibitory effects on a number of lipogenic enzymes (24), but rat brain phospholipase D is stimulated by fatty acids (25). Recently, Feldman et al. (26) reported that cytidylyltransferase from rat lung can be stimulated by unsaturated fatty acids in vitro. We now provide evidence that cytidylyltransferase activity and phosphatidylcholine synthesis in monolayer cultures of rat hepatocytes are regulated by the supply of fatty acids. However, fatty acids are not activators of the cytidylyltransferase, but rather promote binding of this enzyme to a membrane where it is stimulated by the lipid environment.

EXPERIMENTAL PROCEDURES

Materials and Cells—Fatty acids, oleoyl-CoA, trypsin, and soybean trypsin inhibitor used in this study were purchased from Sigma. Fatty acid-poor bovine serum albumin was obtained from Calbiochem-Behring Corp. [Methyl-³H]choline was bought from American International. Cultured monolayers of rat hepatocytes were isolated by a collagenase-perfusion technique (27) and resuspended in medium (1 × 10⁶ cells/ml) as previously described (28). These cells were dispersed into collagen-coated plastic culture dishes (Lux Contur, 60 mm) (3 ml/dish) and incubated at 37°C under an atmosphere of 95% air/5% CO₂.

Pulse-Chase Studies—Twenty-four h after plating, hepatocytes were incubated for 2 h in serum-free medium (arginine-free Dulbecco’s modified Eagle’s medium with 28 µM choline chloride, 100 nM insulin, 0.4 mM ornithine, 100 µg/ml of streptomycin sulfate, 100 units/ml of penicillin G and 10 mM Hepes, pH 7.4). Cells were pulsed

* This work was supported by grants from the Medical Research Council of Canada and the North Atlantic Treaty Organization. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† Supported by a Studentship Award from the Medical Research Council.

§ The abbreviations used are: Hepes, 4-(2-hydroxyethyl)-1-piper-
for 30 min with 15 μCi of [methyl-3H]choline (0.18 Ci/mmol), dissolved in medium that contained 10 mg/ml bovine serum albumin and subsequently incubated for up to 2 h in an equivalent but unlabeled medium in the presence of 0.4 mM fatty acid. At the end of the chase period, the cells were harvested and the radioactivity quantitated in the various choline metabolites after thin layer chromatography (28). Diacylglycerol was measured by the method of Schneider (29).

Measurement of Enzyme Activities—Hepatocytes were incubated for 3 h in serum-free medium (as described above) and subsequently incubated for an additional h in medium which contained 2 mM oleate and 10 mg/ml bovine serum albumin. The cells from 4 dishes were scraped into 2.5 ml of ice-cold 0.145 M NaCl; 10 mM Tris-HCl, pH 7.4; 1 mM EDTA, and 10 mM NaF. Cytosolic CTP:phosphocholine cytidylyltransferase activity was measured (8) within 30 min of completion of ultracentrifugation. Choline kinase (30) and CDP-cholineacylcholine phosphotransferase (31) were assayed as described. Protein was estimated by the Bio-Rad protein assay based on the method of Bradford (32). Microsomal recovery (~15%) was determined by measurement of esterase activity in the cell homogenate and microsomal fractions (33).

Activation of Cytidylyltransferase by Exogenous Fatty Acids—Stock solutions of 100 mM fatty acid were prepared by dissolving the fatty acid in 0.12 M KOH in 95% ethanol. Ten μl of the stock fatty acid solution was dried under nitrogen and resuspended in 5 ml of distilled H2O by sonication twice for 1 min at the 60 setting with a Sonic Dismembrator (Quigley-Rochester). Cytidylyltransferase was assayed in the cytosol (170,000 g x 1 h supernatant prepared from a rat liver homogenate in 4 volumes of isotonic saline with 7 strokes of a Potter-Evrevhjem homogenizer) as described (8) in the presence of 0.12 mM fatty acid. For some experiments, only the clear intranatant portion of the 150,000 g x 1 h supernatant was sampled as a source of cytidylyltransferase. The L-form of the cytidylyltransferase was prepared as previously described (17) and also tested for fatty acid activation.

Preparation of Trypsinized Membrane Fragments—The 100,000 g x 1 h supernatant of a 25% (v/w) rat liver homogenate was incubated at 20°C for 5 h. The aged cytosol was adjusted to 15% ammonium sulfate and 10 ml were centrifuged at 10,000 g x 20 min. The supernatant was adjusted to 30% ammonium sulfate and incubated at 20°C for 1 h. The precipitate was pelleted at 10,000 g x 20 min and resuspended in 1.5 ml of Buffer A (20 mM Tris-HCl, pH 7.0; 100 mM NaCl). The sample was incubated at 37°C for 15 min with 30 μl of 10 mg/ml trypsin, and incubated with 75 μl of 10 mg/ml soybean trypsin inhibitor for an additional 5 min. The trypsinated sample was applied to a Sephacry 6B column (180 ml of bed volume) and eluted with Buffer A. The void volume was collected (9 ml) and adjusted to 30% ammonium sulfate concentration. The 30% ammonium sulfate pellet was resuspended in 1 ml of Buffer A and stored at –20°C in 100-μl aliquots.

Gel Filtration Studies—Cytosol (1 ml) was incubated at 37°C for 15 min in the presence of 100 μM oleate or oleoyl-CoA, and 400 μl was then immediately applied to a Sepharose 6B column (20 ml bed volume). Cytidylyltransferase was eluted from the column with Buffer A in 50-drop fractions at a flow rate of 1 drop every 3 s. Aliquots (60 μl) of the column fractions were assayed for cytidylyltransferase activity in the presence of total rat liver phospholipid (34).

L-form (17) (300 μl of 13 nmol min⁻¹ ml⁻¹ of preparation) was incubated with 50 μl of trypsin-treated membrane fragments and 50 μl of 0.5 mM oleate or oleoyl-CoA in a final volume of 500 μl for 10 min at 37°C. The membrane vesicles were immediately subjected to Sephacry 6B chromatography as described above.

RESULTS

Pulse and Chase Studies—Oleate (1.0 mM) has previously been shown to stimulate the incorporation of [3H]glycerol and [32P]phosphate into hepatic phosphatidylcholine by 2.3- and 3.5-fold, respectively (35). Similarly, we have found palmitate to increase incorporation of [3H]glycerol into phosphatidylcholine by 2.3-fold (data not shown). A major criticism of pulse-labeling studies is that apparent changes in the labeling of metabolites can sometimes be explained by isotope dilution due to differences in pool sizes of intermediate metabolites or alterations in isotope uptake (16). To circumvent these problems, hepatocytes were preincubated for 30 min with 15 μCi of [methyl-3H]choline. At the end of the pulse period, 90% of the label which was not oxidized to betaine was associated with the phosphocholine pool of the cells (Fig. 1A). The subsequent addition of oleate accelerated the rate of disappearance of label from phosphocholine and its appearance into phosphatidylcholine. Since hepatocytes were exposed to only oleate only in the chase medium, at the conclusion of the pulse, the specific radioactivity of [3H]phosphocholine was the same as in control cells. Hence, the stimulated transfer of radioactivity from phosphocholine to phosphatidylcholine reflected a net increase in the rate of the reaction catalyzed by CTP:phosphocholine cytidylyltransferase.

The influence of various fatty acids (1 mM) on [methyl-3H]choline incorporation into phosphatidylcholine was evaluated with a pulse-chase study (Fig. 2A). The short chain fatty acids, butyrate and octanoate, failed to elevate phosphatidylcholine synthesis, but all of the long chain fatty acids produced stimulation. Activation by saturated fatty acids was slightly reduced compared to unsaturated fatty acids. However, the saturated species were difficult to maintain in solution even in the presence of 10 mg/ml bovine serum albumin.

The concentration dependence for 3 fatty acids was further examined (Fig. 2B). Oleate, palmitate, and arachidonate (1 mM) stimulated [methyl-3H]choline incorporation into phosphatidylcholine approximately 1.9-fold. At higher concentrations, these fatty acids differed in their degree of activation. Oleate (4 mM) increased incorporation 3-fold, while palmitate (4 mM) stimulated incorporation only 2.2-fold. By contrast, arachidonate activation at 4 mM was only 1.2-fold. This was

![Figure 1](http://www.jbc.org/)

**Fig. 1.** The effect of 2 mM oleate on the incorporation of [methyl-3H]choline into phosphatidylcholine. Monolayer cultures of rat hepatocytes were preincubated for 2 h in serum-free medium at 37°C. The cells were pulsed with 15 μCi of [methyl-3H]choline for 30 min, and subsequently chased with 28 μM choline in the presence of 2 mM oleate and 10 mg/ml bovine serum albumin. At various times up to 2 h, the cells were harvested and the radioactivity incorporated into choline metabolites determined. A, phosphocholine (Ο, ●); phosphatidylcholine (Ο, ▲). B, cellular betaine (Ο, ▲), medium betaine (Ο, ○); and total medium radioactivity (Ο, ▼). Open symbols, solid lines, controls; closed symbols, dashed lines, 2 mM oleate-treated. Each point represents the mean of three dishes, and S.E. is indicated by bars.
likely due to disruption of the hepatocytes since they released most of their [methyl-3H]phosphocholine into the medium.

At the end of the 30-min pulse with 15 μCi of [methyl-3H]choline, 75% of the choline transported into the cells had been oxidized to betaine (Fig. 1B). During the 2-h chase, the loss of radioactive betaine correlated with the accumulation of label in the medium (Fig. 1B). Oleate did not affect the rate of disappearance of betaine from the cells. None of the fatty acids examined altered the appearance of labeled betaine into the medium (data not shown).

A rather surprising discovery was made when a portion of the hepatocyte medium was analyzed after thin layer chromatography. Over 90% of the radioactivity recovered, co-migrated with betaine, but the total recovery of the radioactivity applied to the plate was less than 50% (Fig. 1B). Evaporation of the medium at 20 or 100°C also produced a loss of approximately 58% of the radioactivity in the medium. The identity of the volatile compound(s), probably a metabolite of betaine, is unknown.

Enzyme Studies—The activities of the enzymes of de novo phosphatidylcholine biosynthesis from control and 2 mM oleate-treated hepatocytes were measured in an attempt to correlate the elevation of phosphatidylcholine synthesis with an activation of one of the enzymes. Choline kinase and CDP-choline:diacylglycerol cholinephosphotransferase activities were unchanged after exposure of the hepatocytes to oleate (Table I). The cytosolic and microsomal cytidylyltransferase activities were increased 2.2- and 1.7-fold, respectively. However, when the cytosolic cytidylyltransferase was assayed in the presence of rat liver phospholipid (which stimulates the cytosolic enzyme activity) there was 26% less activity (p < 0.001) in the cytosols from oleate-treated cells. Hence, the concomitant increase in the microsomal cytidylyltransferase activity could have resulted from translocation of the enzyme.

The subcellular distribution of the cytidylyltransferase was further examined in isolated hepatocytes. In control hepatocytes, approximately 76% of the total cytidylyltransferase activity was detected in the 150,000 g supernatant of total rat liver phospholipid were included in the cytidylyltransferase assay. Phospholipids do not stimulate the microsomal-associated cytidylyltransferase activity.

Exogenous diacylglycerol was included in the cholinephosphotransferase assay.

**Fig. 2.** The effect of various fatty acids on the incorporation of [methyl-3H]phosphocholine into phosphatidylcholine. Cells were pulse-labeled as described in Fig. 1 and subsequently chased with 28 μM choline in the presence of 0-4 mM fatty acid and 10 mg/ml bovine serum albumin. A, effect of 1 mM fatty acids on [methyl-3H]phosphocholine incorporation into phosphatidylcholine during a 2-h chase. B, concentration dependence of arachidonate (○), oleate (□), and palmitate (△) on [methyl-3H]phosphocholine incorporation into phosphatidylcholine during a 2-h chase. Each point represents the mean of three dishes and S.E. is indicated by bars.

**Fig. 3.** The subcellular distribution of cytidylyltransferase in oleate-treated hepatocytes. Monolayer cultures of rat hepatocytes were incubated for 2 h in serum-free medium and for an additional h in medium that also contained 2 mM oleate and 10 mg/ml bovine serum albumin. Cytosols and microsomes were prepared as described under “Experimental Procedures.” Cytosolic cytidylyltransferase activity was determined from the volume, protein concentration, microsomal recovery, and specific cytidylyltransferase activity in the cytosolic and microsomal fractions of the hepatocytes. Height of each bar represents the mean of 5 sets of pooled hepatocytes (5 dishes/set) and the S.D. is shown. This experiment was performed 3 times with different batches of hepatocytes, but similar results.
Fatty Acids Stimulate Hepatic Phosphatidylcholine Synthesis

Activation of Cytosolic Cytidylyltransferase by Fatty Acids—
Since fatty acids rapidly promoted phosphatidylcholine metabolism in the intact hepatocytes (Fig. 1A), could fatty acids directly stimulate the cytosolic cytidylyltransferase of rat liver? Oleate, palmitate, and arachidonate activated the cytidylyltransferase and maximal stimulation was achieved with 80 \( \mu M \) fatty acid (Fig. 4). Maximal stimulation with oleate was still about 40% lower than the maximal stimulation of cytosolic cytidylyltransferase obtained with total rat liver phospholipid. Higher concentrations of these fatty acids reversed the activation of the enzyme so that stimulation was abolished by 0.6 mM fatty acid.

The effect of oleate (100 \( \mu M \)) on the kinetics of the cytosolic cytidylyltransferase reaction was examined (Fig. 5). Although the affinity of the cytidylyltransferase for phosphocholine (apparent \( K_m \) = 0.2 mM) was not influenced by oleate, the apparent \( K_m \) for CTP was reduced 3.6-fold from 2 to 0.6 mM. Oleate also increased the \( V_{max} \) of the cytidylyltransferase-catalyzed reaction.

When fresh cytosol is applied to a Sepharose 6B column, cytidylyltransferase elutes as the L-form (19). This L-form requires exogenous phospholipid for appreciable activity. If oleate is an activator of the cytidylyltransferase then it should also activate L-form. Although L-form was activated 3-fold by 20 \( \mu M \) oleate, liposomes prepared from total rat liver phospholipid stimulated L-form activity by 43-fold (Fig. 6). Apparently a factor was resolved from the cytidylyltransferase by gel filtration which augmented the activation of this enzyme by fatty acids. This factor did not seem to be a cytosolic protein since the cytidylyltransferase in the clear intranatant portion of 150,000 \( \times g \times 1 \) h supernatant was also extremely sensitive to activation by phospholipid but not by oleate. Maximal stimulation by oleate was noted if the milky lipid-rich layer (which floated at the air-solution interface after ultracentrifugation) was dispersed into the cytosol.

Aggregation of Cytosolic Cytidylyltransferase by Oleate—
Activation of cytidylyltransferase upon incubation of rat liver...
cytosol at 4°C for 5 days (19) or 20°C for 8 h (54) is accompanied by an aggregation of the enzyme to H-form. We were interested if oleate activation coincided with increased aggregation of the cytidylyltransferase in cytosol. When complete 150,000 x g x 1 h supernatant was incubated for 15 min at 37°C, approximately 25% of the total activity eluted from a Sepharose 6B column as H-form (Fig. 7). When 100 μM oleate was also included in the preincubation, over 70% of the total activity was present as H-form. On the other hand, only 20% of the applied cytidylyltransferase activity was recovered as H-form when 100 μM oleate was incubated with the clear intranatant portion of the 150,000 x g x 1 h supernatant for 15 min at 37°C prior to gel filtration (data not shown).

Possibly, the membrane fragments which contaminate the cytosolic fraction, were required for aggregation of the cytidylyltransferase and subsequent activation. This was tested with L-form and H-form which was pretreated with trypsin to abolish endogenous cytidylyltransferase activity. Soybean trypsin inhibitor was included with the subsequent incubations to activate the trypsin. When L-form was subjected to Sepharose 6B chromatography with the trypsin-treated H-form, only about 13% of the applied enzyme activity eluted as H-form, while the total recovery of enzyme activity was 33% (Fig. 8). Total recovery from the column was only 18% when L-form was incubated with 50 μM oleate and applied over Sepharose 6B, and H-form recovery was less than 7% (data not shown). However, if oleate (50 μM), L-form, and trypsin-treated H-form were all included in the 10-min preincubation at 37°C prior gel filtration, total recovery of enzyme activity increased to 60% and recovery as H-form was 47% (Fig. 8).

Effect of Oleoyl-CoA on Cytidylyltransferase—Fatty acids in the cytoplasm are usually found in their activated form as Coenzyme A derivatives. Oleoyl-CoA (50 μM) activated cytosolic cytidylyltransferase 1.9-fold, whereas oleate (50 μM) activated the enzyme 2.7-fold. Stimulation of cytosolic cytidylyltransferase by oleoyl-CoA was also accompanied by increased aggregation of the enzyme (Fig. 7), but again oleate proved to be a better aggregator. Oleoyl-CoA (50 μM) also increased L-form binding to trypsin-treated H-form by 2.7-fold after 10 min at 37°C (Fig. 8).

**DISCUSSION**

Although it has been known for a number of years that fatty acids probably stimulate phosphatidylcholine synthesis in the liver (2-5), the mechanism of this effect has not been delineated. Our results provide evidence that this effect is mediated by an activation of the synthesis of CDP-choline catalyzed by CTP:phosphocholine cytidylyltransferase. The acceleration of this reaction was clearly demonstrated by pulse-chase studies with cultured hepatocytes and correlated with increased biosynthesis of phosphatidylcholine. Moreover, a 2-fold stimulation of the cytidylyltransferase could be shown in the cytosol and microsomes from hepatocytes treated with oleate. In addition, the cytosolic activity of cytidylyltransferase from rat liver could be stimulated *in vitro* by free fatty acids. Thus, this study supports many other findings (6-12) which strongly suggest that the rate of phosphatidylcholine synthesis is governed by the activity of cytidylyltransferase.

Although less clear-cut, the results also support the hypothesis that activation of the cytidylyltransferase results from a translocation of the enzyme from the cytosol to microsomes (10, 11, 20-22). The strongest data in support of this concept is that the total cytidylyltransferase activity associated with the microsomes was doubled in the oleate-supplemented hepatocytes, with a corresponding decrease of total cytidylyl-
transferase activity recovered from cytosol. In concordance are the in vitro studies which show that the cytosolic enzyme from rat liver can be converted to a high molecular weight form by exogenously supplied fatty acid or acyl-CoA. Moreover, partially purified cytidylyltransferase (L-form) will bind much more readily to membranes in the presence of fatty acid. The activation of cytidylyltransferase by translocation to microsomes is theoretically appealing since membrane phospholipids have been shown to stimulate this enzyme (13-15, 21). Furthermore, the product of the cytidylyltransferase-catalyzed reaction, CD-P-choline, is in close proximity to the next and final enzyme of the pathway, cholinephosphotransferase, which is membrane-bound. The rapid utilization of CD-P-choline could explain the very low levels of this metabolite reported for rat liver (36, 37).

Rat liver cytosol can account for 90% of the total cytidylyltransferase and the cytosolic enzyme activity might be expected to catalyze a significant portion of total CD-P-choline synthesis. The substrates of the cytidylyltransferase-catalyzed reaction are water-soluble, and presumably found in the cytoplasm. However, it seems more likely that the cytoplasmic pool of cytidylyltransferase represents a reservoir of inactive enzyme. The apparent K m of the cytosolic cytidylyltransferase for CTP (as high as 5 mM (17)) is an order of magnitude higher than the apparent K m of the microsomal enzyme for CTP (0.5 mM), and almost two orders of magnitude higher than the intracellular concentration of CTP in rat liver (0.07 mM (20, 38)). Consequently, the cytoplasmic synthesis of CD-P-choline is probably minor. On the other hand, although the supply of CTP may still limit phosphatidylcholine synthesis, the microsomal cytidylyltransferase has a 10-fold greater affinity for CTP. In this respect, it is relevant that the oleate-induced aggregation of cytosolic cytidylyltransferase was accompanied by a reduction in the apparent K m of the enzyme for CTP to micromolar values.

When the cytidylyltransferase in cytosols from oleate-treated hepatocytes was assayed in the absence of exogenous phospholipid, a doubling of enzyme activity was noted. This stimulation probably arose from increased amounts of fatty acid or acyl-CoA and enhanced generation of active H-form in the cytosols of the oleate-treated hepatocytes. The partially purified cytidylyltransferase from cytosol was shown to be relatively insensitive to activation by oleate. However, oleate promoted aggregation and subsequent activation of the enzyme if membrane was present.

We considered possible alternative mechanisms for the fatty acid effect on hepatocyte phosphatidylcholine synthesis, one of which was that fatty acid increased the supply of diacylglycerol which was previously limiting the formation of phosphatidylcholine. We knew the flux of substrate through diacylglycerol was greatly enhanced due to the pronounced stimulation of triacylglycerol synthesis by fatty acid (2). In addition, we measured the amount of diacylglycerol in our hepatocytes and detected a 1.6-fold increase (27.2 ± 1.5 (S.D.) and 44.4 ± 2.5 (S.D.) nmol/6·10^6 cells for control and 2 mM oleate-treated cells, respectively, after 1 h (n = 4)). An increased supply of diacylglycerol seems to us to be an unlikely explanation for the stimulation of phosphatidylcholine synthesis. Free fatty acids have been reported to stimulate cholinephosphotransferase activity in vitro, provided that exogenous diacylglycerol was also included in the enzyme assay (39, 40). However, we were unable to detect an increase in cholinephosphotransferase activity (+ exogenous diacylglycerol) in microsomes from oleate-treated hepatocytes. This contrasts with the very marked effect on cytidylyltransferase activity in our experiments. The finding that free fatty acids accelerate the cytidylyltransferase-catalyzed reaction is a more satisfying explanation for the stimulation of phosphatidylcholine synthesis since the amount of CD-P-choline seems to be rate-limiting in rat liver (12, 28). Perhaps the elevated supply of diacylglycerol in fatty acid-supplemented hepatocytes ensures that the cytidylyltransferase reaction continues to be rate-limiting despite the increased production of CD-P-choline.

It is possible that cholinephosphotransferase has a higher specific activity and/or lower apparent K m for diacylglycerol than does diacylglycerol acyltransferase. This could account for why diacylglycerol is preferentially channeled into phosphatidylcholine rather than triacylglycerol. Once the utilization of diacylglycerol for phosphatidylcholine synthesis is limited by the availability of CD-P-choline, then diacylglycerol entry into triacylglycerol becomes more significant. With fatty acid supplementation, the cytidylyltransferase reaction and phosphatidylcholine synthesis can be stimulated 3-fold. However, with the increased availability of diacylglycerol and fatty acyl-CoA, triacylglycerol synthesis can be enhanced 10-fold (2).

Feldman et al. (38) have reported that various unsaturated fatty acids activated lung cytosolic cytidylyltransferase almost 3-fold. Furthermore, endogenous free fatty acids were determined to be the predominant species of cytidylyltransferase activators in lung cytosol. The present study has shown that saturated fatty acids also affect the cytidylyltransferase. However, fatty acids and the CoA derivatives apparently are not true activators of cytidylyltransferase, but instead are aggregators which promote the binding of this enzyme to membranes where it may be stimulated by phospholipid activators.

The concentrations of oleate or oleoyl-CoA required to support aggregation of rat liver cytosolic cytidylyltransferase were between 10 and 80 µM. The level of fatty acyl-CoA in rat liver is in this range at approximately 52 µM (41). Fasting and refeeding after a fast might be expected to influence phosphatidylcholine synthesis since more than a doubling of the hepatic fatty acyl-CoA levels occurs in 48-h fasted, or 48-h fasted and 48-h refed rats (41).

Fatty acid binding proteins in the cytosol probably reduced the free concentration of oleate in our in vitro studies (42). Fatty acid binding proteins do not seem to be required for fatty acid-promoted aggregation of cytidylyltransferase since in the L-form preparations fatty acid binding proteins have been removed by gel filtration. This L-form still bound to trypsinized membrane fragments in the presence of oleate or oleoyl-CoA. Nevertheless, fatty acid binding proteins have been shown to stimulate other lipogenic enzyme (43-45), and the influence of these proteins on cytidylyltransferase should be investigated in future studies.

Acknowledgments—We are most grateful to Harry Paddon and Fateem Jetha for technical assistance.

REFERENCES
1. Weisiger, R., Gollan, J., and Ockner, R. (1981) Science (Wash. D.C.) 211, 1048-1051
2. Ontko, J. A. (1972) J. Biol. Chem. 247, 1788-1800
3. Rose, H., Vaughan, M., and Steinberg, D. (1963) Am. J. Physiol. 206, 345-350
4. Nilsson, T., and Schersten, T. (1969) Stand. J. Clin. Lab. Invest. 24, 237-249
5. Sundler, R., Akesson, B., and Nilsson, A. (1974) J. Biol. Chem. 249, 5102-5107
6. Schneider, W. J., and Vance, D. E. (1978) Eur. J. Biochem. 85, 181-187
Fatty Acids Stimulate Hepatic Phosphatidylcholine Synthesis

7. Vance, D. E., Trip, E. M., and Paddon, H. B. (1980) J. Biol. Chem. 255, 1064-1069
8. Pelech, S. L., Pritchard, P. H., and Vance, D. E. (1981) J. Biol. Chem. 256, 8283-8286
9. Possmayer, F., Casola, P. G., Chan, F., MacDonald, P., Ormseth, M. A., Wong, T., Harding, P. G. R., and Tokmakjian, S. (1981) Biochim. Biophys. Acta 664, 10-21
10. Weinhold, P. A., Feldman, D. A., Qasde, M. M., Miller, J. C., and Brooks, R. L. (1981) J. Biol. Chem. 256, 8283-8286
11. Possmayer, F., Casola, P. G., Chan, F., MacDonald, P., Ormseth, M. A., Wong, T., Harding, P. G. R., and Tokmakjian, S. (1981) Biochim. Biophys. Acta 664, 10-21
12. Weinhold, P. A., and Rethy, V. B. (1979) J. Biol. Chem. 254, 2010-2016
13. Schneider, P. B. (1977) J. Lipid Res. 18, 396-399
14. Weinhold, P. A., and Rethy, V. B. (1974) Biochemistry 13, 5135-5141
15. Choy, P. C., and Vance, D. E. (1978) J. Biol. Chem. 253, 5163-5167
16. Feldman, D. A., Dietrich, J. W., and Weinhold, P. A. (1980) Biochim. Biophys. Acta 620, 603-611
17. Whitehead, F. W., Trip, E., and Vance, D. E. (1981) Can. J. Biochem. 59, 38-47
18. Pelech, S. L., and Vance, D. E. (1982) J. Biol. Chem. 257, 14198-14202
19. Wilson, J. E. (1978) Trends Biochem. Sci. 3, 124-125
20. Choy, P. C., Lim, P. H., and Vance, D. E. (1977) J. Biol. Chem. 252, 7673-7677
21. Pritchard, P. H., Chiang, P. K., Cantoni, G. L., and Vance, D. E. (1982) J. Biol. Chem. 257, 6362-6367
22. Slieght, R., and Kent, C. (1983) J. Biol. Chem. 258, 831-835
23. Slieght, R., and Kent, C. (1983) J. Biol. Chem. 258, 836-839
24. Choy, P. C., Farren, S. B., and Vance, D. E. (1979) Can. J. Biochem. 57, 605-612
25. Bloch, K., and Vance, D. E. (1977) Annu. Rev. Biochem. 46, 283-298
26. Chalifour, R., and Kanfer, J. N. (1982) J. Neurochem. 39, 299-305
27. Feldman, D. A., Brubaker, P. G., and Weinhold, P. A. (1981) Biochim. Biophys. Acta 665, 53-59
28. Davis, R. A., Engelhorn, S. C., Pangburn, S. H., Weinstein, D. B., and Steinberg, D. (1979) J. Biol. Chem. 254, 2010-2016
29. Pritchard, P. H., and Vance, D. E. (1981) Bioch. J. 196, 261-267
30. Schneider, P. B. (1977) J. Lipid Res. 18, 396-399
31. Weinhold, P. A., and Rethy, V. B. (1974) Biochemistry 13, 5135-5141
32. Snyder, P. H., and Hubscher, G. (1969) Biochem. J. 113, 429-440
33. Vance, D. E., Pelech, S. L., and Choy, P. C. (1981) Methods Enzymol. 71, 576-578
34. Deleted in proof
35. Wilgram, G. F., Holoway, C. F., and Kennedy, E. P. (1960) J. Biol. Chem. 235, 37-39
36. Schneider, W. C. (1971) J. Natl. Cancer Inst. 46, 435-441
37. Infante, J. P. (1977) Biochem. J. 167, 847-849
38. Sribney, M., and Lyman, E. M. (1973) Can. J. Biochem. 51, 1479-1486
39. Radominska-Pyrek, A., Strosznajder, J., Dabrowiecki, Z., Chojnacki, T., and Horrocks, L. A. (1976) J. Lipid Res. 17, 657-662
40. Tubbs, P. K., and Garland, P. B. (1964) Biochem. J. 93, 550-557
41. Ockner, R. K., Manning, J. A., and Kane, J. P. (1982) J. Biol. Chem. 257, 7872-7878
42. Burnet, D. A., Lynenko, N., Manning, J. A., and Ockner, R. K. (1979) Gastroenterology 77, 241-249
43. Mishkin, S., and Turcotte, R. (1974) Biochem. Biophys. Res. Commun. 60, 376-381
44. O'Doherty, F. J. A., and Kuksis, A. (1975) FEBS Lett. 60, 256-258
Fatty acids promote translocation of CTP:phosphocholine cytidylyltransferase to the endoplasmic reticulum and stimulate rat hepatic phosphatidylcholine synthesis.

S L Pelech, P H Pritchard, D N Brindley and D E Vance

J. Biol. Chem. 1983, 258:6782-6788.