Feedback Regulation of CTP: Phosphocholine Cytidylyltransferase Translocation between Cytosol and Endoplasmic Reticulum by Phosphatidylcholine*

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The mechanism for the increased association of CTP: phosphocholine cytidylyltransferase (CT) with membranes of hepatocytes derived from choline-deficient, compared with choline-supplemented rats, has been investigated. The cells were maintained in culture for 4 h in a choline- and methionine-deficient medium. (Methionine is required for synthesis of phosphatidylcholine (PC) via methylation of phosphatidylethanolamine.) Afterward, the cells were incubated ± choline for various times up to 4 h. In the presence, but not in the absence of choline, there was a translocation of CT activity from membranes to cytosol. During this time period there was no change in the amounts of unesterified fatty acids or dioleic glycerol recovered from the hepatocytes. In addition, there was no evidence for a difference in the incorporation of 32P into CT or other cytosolic proteins isolated from hepatocytes ± choline. In contrast, there was a highly significant correlation between the concentration of PC in the membranes and the increased activity of CT in the cytosol (R = 0.98) and the decreased activity in the membranes (R = 0.93). The concentration of PC could alternatively be altered by incubation of the choline-deficient hepatocytes with methionine or lyso-PC. With either of these supplements highly significant correlation coefficients were observed between the concentration of PC in membranes and decreased activity of CT in membranes or increased activity in cytosol. The concentration of PC was reduced in the endoplasmic reticulum, but not the Golgi membranes, isolated from choline-deficient compared with choline-supplemented livers. The data suggest that the amount of PC in the endoplasmic reticulum feedback regulates the amount of CT associated with this membrane.

The preceding paper (1) demonstrated that choline deficiency caused a translocation of CT from the cytosol to the ER of rat liver. When the hepatocytes from the choline-deficient liver were cultured in monolayers and maintained in a choline- and methionine-deficient medium, the increased binding of enzyme to microsomes was retained. Supplementation with choline caused release of CT into the cytosol (1). In the present investigation we have tested possible mechanisms which might be responsible for the regulation of the CT movement in the choline-deficient hepatocytes. Evidence is provided that translocation of CT is not caused by changes in the levels of fatty acids or DG nor was a change in the phosphorylation state of CT apparent. The data, however, are consistent with a change in the level of PC in the ER being solely responsible for the increased binding of CT to these membranes.

EXPERIMENTAL PROCEDURES

Materials—32P (carrier free) was purchased from Du Pont-New England Nuclear, and [2-14C]glycerol (1 Ci/mmol) and [1-14C]acetate (56 mCi/mmol) were from Amersham International, United Kingdom. Nitrocellulose membranes were obtained from Bio-Rad. pH 3-10 two-dimensional Pharmalyte and pH 5-8 Pharmalyte were obtained from Pharmacia Fine Chemicals AB, Uppsala, Sweden. The free fatty acid concentration measurement kit (NEFA C) was supplied by Wako Pure Chemical Industries, Ltd., Japan. Source of all the other chemicals is described in the preceding paper (1).

Pulse-Chase Experiments—Hepatocytes were obtained from a single choline-deficient rat by a collagenase perfusion technique (1). In [14C]acetate pulse-chase experiments, hepatocytes were plated in a medium containing 5 μCi/dish of [14C]acetate for 4.5 h and then chased in serum-free medium supplemented with ±100 μM choline or 100 μM lysophosphatidylcholine. At various times, the culture medium was removed, and the cells were immediately harvested in 1 ml of cold methanol. Lipids in hepatocytes were extracted with chloroform/methanol (2:1, v/v).

In Vivo Labeling of Cytosolic Proteins with 32P Phosphate—To measure the effect of choline on the phosphorylation level of cytidylyltransferase, hepatocytes were prepared from 3-day choline-deficient rats and cultured in a choline- and methionine-free medium containing 17% delipidated serum. Four hours after plating the cells were washed with choline-, methionine-, phosphate-, and serum-free medium and incubated in the same medium containing 50 μCi/ml 32P phosphate. After 2 h of labeling fresh medium was added containing 32P phosphate ± choline, and the incubation was continued for another 3 h. Cells were washed with 2 ml of ice-cold phosphate-buffered saline, harvested, and homogenized after combining cells from four dishes (1). The homogenate was centrifuged at 125,000 X g for 60 min to separate cytosol from membranes. CT from cytosolic samples was partially purified (25-fold purification) by octyl glucoside extraction (2) and subjected to two-dimensional electrophoresis. Two-dimensional Electrophoresis—Partially purified CT from cytosolic samples was precipitated with 10% trichloroacetic acid. The precipitated samples were kept on ice for 30 min and subsequently centrifuged at 10,000 rpm for 20 min in a bench-top Eppendorf microcentrifuge. The pellets were washed twice with 1 ml of acetone at -20°C and dissolved in 9.8 M urea, 2% Triton X-100, 100 mM diethiothreitol, 6% pH 5-8 ampholytes and 1.5% pH 3-10 ampholytes. Two μg of pure CT (2) was added to each sample as a carrier. Two-g
dimensional gel electrophoresis was performed according to the method of O'Farrell (3). Isoelectric focusing gels contained 9.2 M urea, 5% acrylamide + 0.8% bisacrylamide, 2% Triton X-100, 6% pH 5-8 ampholytes and 1.2% pH 3-10 ampholytes. Gels were prefocused for 3 min at 200 V, and samples were loaded at the cathodes. Electrophoresis was at 400 V for 12 h followed by 1 h at 300 V. The first dimension gels were stained and soaked in 60 mM Tris-HCl, pH 8.5, 5% 2-mercaptoethanol, 2% sodium dodecyl sulfate, and 10% glycerol for 10 min. Gels were applied to a 1% agarose (dissolved in the same buffer plus 0.01% bromophenol blue) on a 1,2-DG and triacylglycerol gel. Electrophoresis was performed at 15 mA/gel until the bromophenol blue dye reached the bottom of the gel. Proteins were transferred onto a nitrocellulose membrane and im munoblotted with anti-cytidylyltransferase antibody to identify the cytldylyltransferase (1).

Thin Layer Chromatography of Lipids—Silica Gel 60 thin layer plates (20 x 20 cm) were used for all chromatography. Free fatty acids (extracted from about 0.2 g of liver), together with heptadecanoic acid as internal standard, were chromatographed in hexane/diethylether/acetic acid (50:50:1, by volume). 1,2-DG and 1,3-DG, derived from 0.05 g of liver, were chromatographed in diethyl ether/benzene/ethanol/acetic acid (40:50:9:2, by volume), and triacylglycerol (from 0.01 g of liver) was separated in hexane/diethyl ether/acetic acid (30:70:3, by volume). Heptadecanoic acid was applied to the area of the thin layer plate containing 1,2 DG and triacylglycerol, respectively, after chromatography. Neutral lipids were visualized under ultraviolet light with Primulin spray (1 mg in 80 ml of acetone and 20 ml of H2O) (4) and quantified by gas-liquid chromatography (5). Phospholipids were chromatographed in chloroform/methanol/acetic acid/formic acid/H2O (70:30:12:4, v/v) and visualized with iodine vapor.

Gas-Liquid Chromatography of Fatty Acid Methyl Esters—The lipid-containing bands from the thin layer plates were scraped and methyl esters of fatty acids derived from unesterified fatty acids, 1,2-DG, and triacylglycerol samples were prepared by methanolation of lipids (on the silica gel) with 1 ml of BF3-methanol at 100°C as described (5). The fatty acid methyl esters (in hexane) were separated on a 6-foot x ½-inch steel column packed with 10% diethylene glycol succinate on 100/120-mesh Supelcoport (Supelco Inc., Bellefonte, PA) using a Perkin-Elmer 8420 gas chromatograph. Temperature was programmed from 155 to 200°C, and helium was used as a carrier gas. 1,2-DG and 1,3-DG, oleic acid as internal standards, were chromatographed in hexane/2-propanol/methanol/acetic acid (95:5:100, by volume). Lipids from cytosol and microsomes were chromatographed in chloroform/methanol/acetic acid/formic acid/H2O (70:30:12:4, v/v) and visualized with iodine vapor.

RESULTS

Increase in Fatty Acid Concentration Is Not Responsible for CT Translocation in Choline-deficient Hepatocytes—The first mechanism for CT translocation that we considered was that choline deficiency might alter the metabolism of fatty acids in the hepatocytes. The accumulation of triacylglycerol in the choline-deficient liver reflects an abnormal metabolism of fatty acids, and we observed a slight increase in the concentration of the unesterified fatty acids in choline-deficient compared with choline-supplemented livers (1).

Hepatocytes isolated from a 3-day choline-deficient rat were maintained in culture for 5 h. Subsequently, the cells were incubated with choline for up to 2 h, harvested, and the concentrations of the unesterified fatty acids determined by gas chromatography (Fig. 1). Significant translocation of CT occurs in 1 h (1). The results show no significant differences in the fatty acid concentrations between 15 min and 2 h in choline-deficient compared with choline-supplemented hepatocytes. Nonesterified fatty acid concentrations were also measured in subcellular fractions of hepatocytes by an enzymatic colorimetric method as described under “Experimental Procedures.” The values obtained were 5.17 ± 0.9 and 4.8 ± 0.6 nmol/mg protein in cytosol and 39.5 ± 5.2 and 40.3 ± 5.5 nmol/mg protein in microsomes after incubation of hepatocytes in the absence and in the presence of 100 μM choline for 2 h, respectively. Furthermore, in a more sensitive approach the hepatocytes were prelabeled with [14C]acetate for 5 h and incubated in the presence and absence of 100 μM choline. Lipids were extracted from cytosol and microsomes after 1 and 2 h, and the radioactivity in free fatty acids was determined after separation on thin layer chromatography. No difference in the labeling of free fatty acids was observed in the addition of 100 μM choline (results not shown).

We wished to confirm this lack of a difference in fatty acid metabolism by a pulse-chase experiment with [14C]acetate (Fig. 2, left). It is evident that disappearance of radioactivity in the unesterified fatty acid pool is the same ± the addition of choline to the chase medium.

From these studies we have been unable to garner any evidence to suggest that a change in the metabolism or levels of fatty acids might be responsible for the increased association of CT with microsomes.

An Increase in DG Levels Is Not Responsible for CT Translocation in Choline-deficient Hepatocytes—In vitro studies have shown that DG can promote the translocation of CT to membranes or PC vesicles (6). Similarly, treatment of embryonic chick muscle cells with phospholipase C caused a translocation of CT to membranes and an increase in the levels of DG (7). Thus, it was reasonable to postulate that a change in DG concentration in the choline-deficient hepatocytes might be responsible for the increased translocation of CT to membranes. As a result, in the [14C]acetate pulse-chase experiment we also examined the [14C]acetate labeling of DG. Whereas in the cells maintained in the absence of choline we saw a gradual decay of radioactivity, the response was markedly accelerated within minutes after the addition of choline to the medium (Fig. 2, right). Two possible explanations for this result are: 1) the levels of DG are elevated in the choline- deficient hepatocytes. With the addition of choline, there is
FIG. 2. Effect of choline on [14C]acetate-labeled fatty acids and 1,2-diacylglycerol in hepatocytes. Left, hepatocytes obtained from a single choline-deficient liver were plated in a choline- and methionine-free medium containing 17% delipidated serum in the presence of [14C]acetate (5 μCi/dish). Five h after plating, the radioactivity was chased in a serum-free medium with (M) or without (O) 100 μM choline for another 2 h. At the indicated times, the cells were immediately harvested in 1 ml of cold methanol, and lipids were extracted with 2 ml of chloroform and 1 ml of H2O. [14C]Labeled fatty acids were separated by thin layer chromatography using oleic acid as a carrier, and the amount of radioactivity was determined. Right, the experiment was performed as described above, except in the cells incubated with (closed symbols) or without (open symbols) 100 μM choline DG was analyzed. In an experiment in which 100 μM lyso-PC (instead of 100 μM choline) was used, the change in 1,2-[14C] DG was the same as that of no addition. All points are the average of two dishes which do not differ by more than 10%. The experiment was repeated, and similar results were obtained.

increased synthesis of PC and thus a more rapid decrease in DG levels. 2) The DG levels are not changed, but the turnover rate of DG is enhanced in the choline-supplemented cells due to accelerated PC synthesis. The first hypothesis was tested by measurement of DG levels in the hepatocytes as a function of time of addition of choline to the medium (Fig. 3). Evidently, there was no significant alteration in the pool size of DG in the choline-deficient hepatocytes after the addition of choline. Thus, explanation 2 seems to be correct. These experiments suggest that the translocation of CT seen in the choline-deficient cells is not a result of a change in DG levels.

The Concentration of PC in Hepatocytes Correlates with CT Translocation—A third explanation for increased translocation of CT to membranes of choline-deficient hepatocytes might be that a change in the amount of PC in the cells somehow influences the binding of CT. Such a proposal has previously been made to explain the translocation of CT in choline-deficient LM (a line of mouse fibroblasts) or Chinese hamster ovary cells, but evidence in support of this hypothesis was not presented (8). Our initial approach to this hypothesis was to study the effect of choline supplementation on CT levels in the cytosol and microsomes and correlate the result with the concentration of PC in the hepatocytes. Thus, supplementation of the cells with choline showed the expected decrease in membrane-associated CT activity (Fig. 4B) and release of enzyme into the cytosol (Fig. 4A). At the same time there was a 23% increase in the amount of PC in the cells (Fig. 4C). The concentrations of other phospholipids did not change during the course of the experiment (PE = 72, phosphatidylserine = 4.3, and phosphatidylinositol = 3.7 nmol/mg membrane protein). When the CT activity in the cytosol (Fig. 5A) and membranes (Fig. 5B) was plotted as a function of PC concentration, there was a nearly linear correlation. This provided the first evidence that PC levels might alter the distribution of CT between microsomes and cytosol.

However, other explanations were plausible. For example, the concentrations of phosphocholine or CDP-choline might be the factors that modulate the binding of CT to membranes. The concentration of both of these metabolites would be expected to be increased in the choline-supplemented hepatocytes. An advantage of working with hepatocytes compared...
FIG. 4. Effect of choline on the distribution of cytidylyltransferase and change in PC concentration in cultured hepatocytes. Hepatocytes were prepared from a single liver of a rat fed with a choline-deficient diet for 3 days and cultured in a choline- and methionine-free medium + 17% delipidated serum for 4 h. To start the incubation the medium was replaced with a serum-free medium in the absence (open symbols) or the presence (closed symbols) of 100 µM choline. At various times the cells were treated with digitonin to measure release of cytosolic CT (A). After the digitonin treatment the ghosts of the cells were collected and sonicated in buffer R (10 mM Tris-HCl, pH 7.4, 0.25 M sucrose, and 0.1 mM phenylmethylsulfonyl fluoride) 7 X 1 s. CT activity (B) and PC concentration (C) were measured in the cell ghosts. A milliunit of cytosolic enzyme activity is 1 nmol of CDP-choline formed per mg of membrane protein. Membrane CT activity was measured as nanomoles of CDP-choline formed per mg of membrane protein. PC concentration was also measured per mg of protein in the membrane samples. Each point is the average of four dishes from two different experiments. The experiment has been repeated 20 times, and similar results were obtained.

FIG. 5. Correlation between increased PC concentration and an increase in cytosolic CT activity (A) and a decrease in microsomal CT activity (B). Data from Fig. 4 have been replotted and correlation coefficients calculated.
with other cultured cells is the presence of significant PE methyltransferase activity (9). Thus, we had the ability to change the levels of PC in the choline-deficient hepatocytes without altering the pool sizes of the choline-containing precursors. The results in Fig. 6 show that addition of methionine to the medium of choline-deficient hepatocytes caused an increase in the activity of CT in the cytosol and a decreased association of activity with membranes. The methionine time course was slower than that for choline, requiring up to 12 h for a new steady state level. During the course of these experiments the level of PC increased from 90 to 114 nmol/mg cellular protein after supplementation with choline or methionine. Correlation coefficients were calculated for the concentration of PC and release of CT activity into cytosol (R = 0.86) and decrease on membranes (R = 1.0). The PE levels dropped from 79 to 50 nmol/mg membrane protein after the addition of methionine, reflecting the ability of methionine-supplemented cells to convert PE to PC. In cells supplemented with choline or no addition, the PE levels decreased slightly from 79 to 69 nmol/mg cellular protein. In related studies supplementation of the choline-deficient hepatocytes with 200 μM ethanolamine for 4 h had no effect on CT translocation (data not shown). Addition of 200 μM ethanolamine with 200 μM methionine gave the same translocation of CT as supplementation with 200 μM methionine alone (data not shown). Moreover, blockage of the synthesis of PC from PE by the addition of 3-deazaadenosine to the medium (10) prevented the methionine-induced translocation of CT (data not shown). These results provided important new evidence for the regulation of CT translocation by changes in the amount of PC in the cells.

It is apparent in Fig. 6 that the total CT activity and CT activity in cytosol are decreasing between 4 and 12 h after the addition of choline without a change in the amount of membrane-associated CT activity. The reason for this is not clear. When a high titer antibody to CT becomes available, we shall be able to determine if the decreased activity is due to a decrease in CT protein in the cytosol. A third option that was available for alteration of PC concentrations in choline-deficient hepatocytes was supplementation of the cells with lysosphatidylcholine (lyso-PC). This lipid is taken up by hepatocytes and converted largely to PC (11). Initial studies with 100 μM lyso-PC showed no effect on the release of cytosolic CT after the treatment of cells with digoxin. Yet in the same studies there was a 15-20% increase in the concentration of PC in the cells. This was taken as evidence that the levels of PC did not correlate with CT translocation. This mistaken impression was corrected when we realized that incubation of hepatocytes with 100 μM lyso-PC caused cytosolic CT to leak from the hepatocytes into the medium. When the study was redesigned and the CT activity in the medium and digoxin-released cytosol were both measured (inset of Fig. 7A), we found that lyso-PC supplementation did mediate release of CT from membranes into the cytosol (Fig. 7). Correlation coefficients were calculated between the concentration of PC in the cells and release of CT activity into the cytosol (R = 0.97) and decrease on membranes (R = 0.99). Thus, three different approaches which caused an increase in PC concentration in the choline-deficient hepatocytes also resulted in translocation of CT from membranes to cytosol.

PC Levels Are Reduced in the ER, but Not the Golgi, of Choline-Deficient Rat Livers—In the previous study (1), we found that the translocation of CT in choline-deficient livers from cytosol was confined to ER, but not Golgi. If the PC levels were important in modulating CT movement in the cells, then we would expect that the PC levels would be decreased in the ER, but not the Golgi, from choline-deficient livers. Golgi and ER were isolated from the livers of choline-deficient and choline-supplemented rats, and the amount of PC in these membranes was measured (Table I). A small but significant decrease in PC of the ER, but not the Golgi, was found. The DG levels were also measured in these subcellular fractions, and no significant differences were observed between choline-deficient and choline-supplemented livers (Table II). The recovery of ER and Golgi from the livers was the same (1). These data are consistent with the model that PC levels can regulate the translocation of CT to membranes in livers and cultured hepatocytes.

**Evidence That the State of Phosphorylation of CT and Other Cytosolic Proteins Is Not Affected by Choline Deficiency**—Although the above experiments on PC levels and CT activation in choline-deficient cells were convincing, an additional mechanism for causing CT translocation should be considered. CT is a substrate for cAMP protein kinase which causes release of the enzyme from membranes in vitro (13). Consistent with this result, treatment of hepatocytes with cAMP analogues resulted in a decrease in the microsomal CT activity (14). It was conceivable that the choline-induced release of CT might be due to an induced phosphorylation of CT:Phosphocholine Cytidyltransferase Translocation.

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**Table I**

<table>
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<th>Diet</th>
<th>ER I</th>
<th>ER II</th>
<th>Golgi</th>
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<tr>
<td>Choline-deficient</td>
<td>216.5 ± 9.1</td>
<td>291.3 ± 11.0</td>
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<tr>
<td>Choline-supplemented</td>
<td>265.1 ± 18.6</td>
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<td></td>
<td>p &lt; 0.025</td>
<td>p &lt; 0.005</td>
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**Fig. 6.** Effect of methionine on the distribution of cytidylyltransferase activity in cultured choline-deficient hepatocytes. Experimental conditions were the same as in Fig. 4 except that the hepatocytes were incubated in the absence (□) or in the presence of 100 μM choline (▲) or 200 μM methionine (■) for 12 h. Cytidylyltransferase activity was measured in the digoxin-released samples (A) to measure cytosolic activity and in the cellular membranes to measure membrane enzyme activity (B). 1 millimicron of CT activity is 1 pmol of CDP-choline formed per min. Cytosolic enzyme activity in digitonin-released samples is plotted as millimicrons of enzyme activity released per mg of membrane protein. Membrane enzyme activity was measured per mg of membrane protein. Each point is the average of four assays from two independent experiments.
to incubate solubilized 32P-labeled cellular extracts with a choline. A more definitive experiment would have been with (Fig. 8A) or without (Fig. 8B) choline. The location of partially identical profiles were obtained from cells supplemented from other proteins, and perform two-dimensional gel analysis. Nevertheless, there was no evidence for a change in the state of phosphorylation of CT whether or not the cells were supplemented with choline. A more definitive experiment would have been to incubate solubilized 32P-labeled cellular extracts with a high titer antibody to CT, separate the CT-antibody complex from other proteins, and perform two-dimensional gel analysis. However, such an antibody is not available. Nevertheless, the results in Fig. 8 provide no support for a change in enzyme phosphorylation causing the choline-mediated redistribution of CT.

Fig. 7. Effect of lyso-PC on the distribution of cytidylyltransferase activity and PC concentration in cultured choline-deficient hepatocytes. Hepatocytes were prepared from a single liver of a rat fed with a choline-deficient diet for 3 days and cultured in a choline- and methionine-free medium + 17% delipidated serum. After 4 h the cells were incubated in a serum-free medium in the absence (open symbols) or in the presence (closed symbols) of 100 µM lyso-PC. At various times the medium was collected and cells were treated with digitonin. Cytidylyltransferase activity was measured both in the cytosol (●) and the medium (○) (see inset) which is a measure of total cytosolic activity (A) and plotted as CT activity released per mg of membrane protein. Cell membranes were scraped in buffer R and sonicated 7 times for 1 s. Membrane CT activity and PC concentrations were measured in cell membrane samples and plotted as milliunits of CDP-choline formed per mg of membrane protein (B) and nanomoles of PC per mg of membrane protein (C), respectively. All points are the average of two dishes, the individual values of which do not differ by more than 5%. The experiment was repeated three times, and similar results were obtained.

### Table II

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<td>Choline-deficient</td>
<td>5.6 ± 0.4</td>
<td>7.0 ± 1.9</td>
<td>17.4 ± 1.6</td>
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<td>Choline-supplemented</td>
<td>5.6 ± 1.9</td>
<td>8.8 ± 4.3</td>
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**CTP-Phosphocholine Cytidylyltransferase Translocation**

**Discussion**

**Feedback Regulation of PC Biosynthesis**—A fundamental principle of metabolic regulation is feedback regulation of biosynthesis by the end product. Until the present investigations, such a mechanism for regulation of PC biosynthesis (or any phospholipid) had not been demonstrated, although the concept was introduced by Sleight and Kent in 1983 (8). The results presented herein are entirely consistent with a very sensitive mechanism for feedback regulation by PC of CT translocation in rat liver and cultured hepatocytes. Since CT is usually rate-limiting in the biosynthesis of PC (15), it appears that the concentration of PC in the ER membrane has the capacity to regulate its biosynthesis.

Several lines of evidence support this hypothesis. In choline-deficient hepatocytes there is increased binding of CT to membranes when the PC level in the cells decreases. Restoration of PC biosynthesis by the addition of CT results in a rise in the PC concentration of cellular membranes and a release of CT from membranes into cytosol. The translocation of CT could alternately be due to an increase in concentration of one of the choline-containing precursors (e.g., phosphocholine, CDP-choline, or choline itself) which might alter the binding of CT to membranes. Thus, it was important to test other mechanisms for altering the pool size of PC without affecting the choline-containing precursors. The PC levels were increased by the addition of lyso-PC or methionine to the medium. In both instances there was a high correlation between the concentration of PC in the membranes and release of CT into the cytosol. In contrast to the above results, we tested other mechanisms that are known to alter CT translocation in vitro or in cultured cells (1, 15). We found no evidence for a change in the levels of fatty acids, diacylglycerol content of microsomal subfractions of choline-deficient and choline-supplemented rat livers.

ER and Golgi apparatus were isolated from the livers of choline-deficient and choline-supplemented rats according to Craze and More (1, 12, 16). Lipids were extracted and DG was quantitated by gas-liquid chromatography. The data of three experiments are presented as means ± S.D. ER I and ER II are two ER fractions separated on the basis of density (12, 16). The significance of difference was determined by the t test. NS, not significant.

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**Feedback Regulation**

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FIG. 8. Two-dimensional gel electrophoresis analysis of 
32P-labeled cytosolic cytidylyltransferase. Panels A and B, 
hepatocytes were prepared from choline-deficient rats and incubated 
with [32P]phosphate for 2 h as described under "Experimental 
Procedures." Subsequently, 10 μl of 20 mM choline (final concentration, 
100 μM, A) or 10 μl of water (B) was added, and cells were incubated 
for a further 3 h. The cells were homogenized and cytosol was prepared 
(1). Cytidylyltransferase activity was partially purified through the 
ocetyl glucoside extraction step (2). Two pg of purified CT (2) was 
added to the samples as a carrier. Samples (100 μl) of cellular extracts 
were analyzed on isoelectric focusing gels (pH 3–10) in one direction 
and on 5–15% sodium dodecyl sulfate electrophoresis in the second 
dimension (3). The nitrocellulose membranes were exposed to Kodak 
XAR-5 film for 12 h. The arrow in A and B autoradiograms indicates 
the position of CT. Panel C, proteins were transferred to nitrocellulose 
membranes and immunoblotted with chicken IgG raised against 
purified CT to identify CT on the gels. The second probe was rabbit 
anti-chicken IgG conjugated with peroxidase.

FIG. 9. Proposed model for feedback regulation of cytidy-
lyltransferase translocation by phosphatidylcholine. It is hy-
pothesized that a decrease in the amount of PC in the endoplasmic 
reticulum causes an enhanced binding of CT to the membrane. 
Restoration of PC to normal levels in the membrane promotes release 
of the enzyme to the cytosol.
change in the distribution of CT between cytosol and microsomes during development of rat liver was observed (19). In addition, young rats fed a diet enriched in cholesterol (5%) and cholate (2%) displayed an apparent 2-fold translocation of CT from cytosol to microsomes compared with control animals (20). In neither of these model systems was the mechanism for translocation elucidated. The possibility that these changes were due to a decrease in the level of PC in the ER should be tested. In addition, other potential mechanisms for CT translocation such as changes in the levels of fatty acids must also be considered. In the lungs from rats 3 h after premature birth on day 21 of gestation, there was a very striking correlation between the increased activity of CT in microsomes and the increased content of unesterified fatty acids (21). In support of these results, Weinhold et al. (21) obtained similar translocation results in vitro by incubation of postmitochondrial supernatants from lung with various concentrations of oleic acid.

**How Does the Level of PC Alter CT Binding to Membranes—**

A striking feature of the results with the choline-deficient animals is the apparent sensitivity of CT to the level of PC in the membranes. Thus, an increase of approximately 10–15% (from 95 to 110 nmol of PC/mg of membrane protein) resulted in a release of 1–2 units of CT from 1 mg of membrane protein to cytosol. Consistent with these results are in vitro studies which showed that PC vesicles bind pure or cytosolic CT to a limited extent (2, 22). However, when oleic acid was incubated with the PC vesicles the amount of CT bound is markedly increased (2, 22). The increased binding of CT may have been due to a positive effect of the oleic acid on binding of CT to the vesicles or may have been due to a dilution of the mole percent of PC in the vesicles. Since oleoyl alcohol, monolein, diolein, and 1-oleoyl-2-acetylglycerol also promoted binding of CT to PC vesicles (22), a dilution of the PC concentration might be more important than previously suspected. Experiments to study this phenomenon in more depth will be important.

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