Diacylglycerol cholinephosphotransferase (EC 2.7.8.2) and diacylglycerol ethanolaminophosphotransferase (EC 2.7.8.1) activities were investigated in microsomes from isolated rat fat cells. Assays based on the conversion of CDP-[3H]choline or CDP-[3H]ethanolamine to phosphatidylcholine or phosphatidylethanolamine utilized ethanol-dispersed diacylglycerols and 1 to 5 μg of protein. Cholinephosphotransferase and ethanolaminophosphotransferase activities had similar dependences on MgCl₂ and pH, and were inhibited similarly by CaCl₂, organic solvents, Triton X-100, Tween 20, and dithiothreitol. Ethylene glycol bis-(β-aminoethyl ether)-N,N,N',N'-tetraacetic acid stimulated both activities similarly. With 1,2-dioleoyl-sn-glycerol, the cholinephosphotransferase activity had an apparent Kₘ of 23.9 μM and a Vₘₐₓ of 8.54 nmol/min/mg. CDP-ethanolamine and CDP were competitive inhibitors of the cholinephosphotransferase activity (apparent Kᵢ values of 227 μM and 360 μM, respectively). With 1,2-dioleoyl-sn-glycerol, the ethanolaminophosphotransferase activity had an apparent Kᵢ of 18.3 μM for CDP-ethanolamine and a Vₘₐₓ of 1.14 nmol/min/mg. CDP-choline appeared to be a noncompetitive inhibitor of the ethanolaminophosphotransferase activity (apparent Kᵢ of 1620 μM). Inhibition of the ethanolaminophosphotransferase activity by CDP appeared to be of a mixed type. The dependences on diacylglycerols containing fatty acids 6 to 18 carbons in length were investigated. Highest activities for both ethanolaminophosphotransferase and cholinephosphotransferase activities were observed with 1,2-dioleoyl-sn-glycerol. The apparent Kᵢ for 1,2-dioleoylglycerol was 50.9 μM for the cholinephosphotransferase and 11.8 μM for the ethanolaminophosphotransferase activity. The ethanolaminophosphotransferase and cholinephosphotransferase activities had different dependences on bacterial, 1-stearoyl-2-oleoylglycerol, 1,2-didecanolylglycerol, and 1,2-dipalmitoylglycerol diacylglycerol substrates. 1,2-Diacylglycerols containing fatty acids 6, 12, and 14 carbons in length were substrates for the cholinephosphotransferase but were not substrates for the ethanolaminophosphotransferase under the conditions employed. The ethanolaminophosphotransferase and cholinephosphotransferase activities were differentially inactivated by heating, by trypsin digestion, and by addition of manganese chloride or palmitoyl-CoA to the reaction mixture. Bovine serum albumin increased ethanolaminophosphotransferase activity over 2-fold, but had no effect on the cholinephosphotransferase. A tissue survey in the rat indicated that the two activities varied independently.

These data provide the first characterization of the cholinephosphotransferase and ethanolaminophosphotransferase activities from adipose tissue or fat cells and the first systematic investigation of the diacylglycerol dependences of these activities from any tissue. Taken as a whole, the data strongly suggest that the cholinephosphotransferase and ethanolaminophosphotransferase are separate microsomal enzymes.

The principal function of the mammalian fat cell is the synthesis, storage, and degradation of triacylglycerol in response to physiologic requirements. The adipocyte is unique among mammalian cell types in that one material, triacylglycerol, comprises up to 90% of its weight (including water). Triacylglycerol synthesis is known to be regulated at the level of pyruvate dehydrogenase (1, 2), acetyl-CoA carboxylase (3, 4), fatty acid synthetase (4), and fatty acid CoA ligase (5). Triacylglycerol degradation in fat cells is controlled by hormonal regulation of hormone-sensitive lipases (6). While the synthesis of triacylglycerol in adipocytes varies markedly with nutritional intake and energy expenditure, the synthesis of membrane phospholipid must occur continuously in the adipocyte as in other types of mammalian cells.

Triacylglycerol, phosphatidylcholine, and phosphatidylethanolamine are derived from a common 1,2-diacylglycerol intermediate. Three microsomal (7) enzyme activities exist at this 1,2-diacylglycerol branch point between phospholipid and triacylglycerol synthesis. The 1,2-diacylglycerol acyltransferase (EC 2.3.1.20) activity functions to convert the common diacylglycerol substrate to triacylglycerol while the 1,2-diacylglycerol cholinephosphotransferase (EC 2.7.8.2) and the 1,2-diacylglycerol ethanolaminophosphotransferase (EC 2.7.8.1) activities function to synthesize phosphatidylcholine and phos-
phatidylethanolamine, respectively. Since the amounts of triacylglycerol and phospholipid synthesized appear to be regulated, we have initiated studies on these three microsomal activities from isolated fat cells. We have recently reported studies on the 1,2-diacylglycerol and acyl-CoA dependences of the diacylglycerol acyltransferase activity from isolated rat fat cells using ethanol-dispersed diacylglycerols (8). The cholinephosphotransferase and ethanolaminephosphotransferase activities from rat and chick liver (g-131, rat brain (14, 151, cat intestinal mucosa (16), and cultured hamster kidney cells (17, 18) have been investigated. No reports on the activities from adipose tissue or isolated fat cells have appeared.

In this paper, we present the first characterization of the cholinephosphotransferase and the ethanolaminephosphotransferase activities from isolated rat fat cells, and the first systematic study of their diacylglycerol dependences using ethanol-dispersed diacylglycerols. In addition, the hypothesis that these microsomal enzymes are dual activities of a single microsomal enzyme was tested. Several lines of evidence indicate that the cholinephosphotransferase and ethanolaminephosphotransferase are separate microsomal enzymes.

**EXPERIMENTAL PROCEDURES**

**Materials**

Bovine serum albumin (essentially fatty acid-free), CTP, CDPA, CMP, CDP-ethanolamine, and CDP-choline were purchased from Sigma. 3-Phosphoprop-1-lyalamido-2-phenyl ethyl chloromethyl ketone were from Worthington Biochemical Corp. Silica Gel G plates, 250 +, were from Analtech, Inc. 1,2-DiC,,:-sn-glycerol ether, 1,2-diacyl-sn-glycerols containing saturated fatty acids 10 to 18 carbons in chain length, 1,2-diC,,:-sn-glycerol, diC,,:-phosphatidylethanolamine, and egg lecithin were from Scharlau Reagents. CDP-[3H]ethanolamine (1,2,3H) ethanolamine was from ICN Pharmaceuticals Inc. Bacterial 1,2-diacyl-sn-glycerol was prepared as described by Jarrett (22). Protein was determined by the method of Lowry et al. (23) using bovine serum albumin as the standard. Microsomal preparations at 1.0 mg/ml in Medium I were in stored in small tubes at -15°C. Once thawed, the microsomes were discarded. Under these conditions the cholinephosphotransferase and ethanolaminephosphotransferase activities were stable for several months.

**Isolation of Fat Cells** - Fat cells were isolated from parametrial adipose tissue of Charles River CD strain female rats (200 to 250 g) according to the method of Kodbell (21). Cells were broken by 10 rapid up and down strokes using a motor-driven Teflon-glass homogenizer in Medium I (0.25 M sucrose, 1 mm EDTA, and 10 mm Tris/Cl, pH 7.4) (22). Subcellular fractionation of the isolated fat cells was performed as described by Jarrett (22). Protein was determined by a method analogous to that described above for the cholinephosphotransferase activity except that 100 mM CDP-[3H]ethanolamine (10 µCi/µmol) replaced the CDP-[3H]choline. Greater than 95% of the extracted labeled material migrated like authentic phosphatidylethanolamine on thin layer chromatography in Solvent I. Variations in these conditions are noted in the appropriate figure and table legends.

**RESULTS**

**Dependences of Diacylglycerol Cholinephosphotransferase Activity** - Microsomal cholinephosphotransferase activity from isolated rat fat cells was proportional to the amount of protein added to 15 µg and with time to 14 min when 5 µg of protein were employed (see Fig. 1). The activity had a broad pH optimum from 8.5 to 9.3. Dependences of the microsomal cholinephosphotransferase are shown in Table I. The reaction was completely dependent upon the addition of microsomes, MgCl, and diacylglycerol. CDP-[3H]choline (0.5 mm) stimulated activity 40% and was routinely included in the incubation mixture. DiC,,:-glycerol added in 10 µl of ethanol (final volume of 200 µl) proved optional. When the diC,,:-glycerol was added in 20 µl of ethanol, the reaction was inhibited 46%. Higher activity was obtained when the diacylglycerol was added immediately after addition of microsomes; if the diacylglycerol was added prior to the microsomes, the activity was 48% lower.

No change in activity was seen with the addition of up to 125 mm NaCl, KCl, or KBr. CaCl, was strongly inhibitory at concentrations above 0.5 mm. At 10 mm CaCl, no activity was detected. Several detergents and organic solvents severely inhibited the reaction. No activity was obtained with the addition of Triton X-100 (0.5 mg/ml), Triton WR 1339 (0.25 mg/ml), Tween 20 (0.5 mg/ml), or 1-butanol (10 µl) to the incubation mixture. The addition of 10 µl of methanol, acetone, or dioxane inhibited activity 23, 30, or 38%, respectively. Preincubation of microsomes (0.8 mg/ml) with phospholipase C (1 unit/ml) for 10 min at 23°C stimulated the activity 14%. Dithiothreitol (0.5 mm) inhibited the activity 50%. Freezing and thawing the microsomal preparation five times inhibited the activity 50%.

**Dependences of Diacylglycerol Ethanolaminephosphotransferase Activity** - The microsomal ethanolaminephosphotransferase activity was proportional with the amount of protein added to 15 µg and with time to 15 min when 4 µg of protein were employed. A steady increase in activity was detected in the reaction mixture contained 175 mm Tris/Cl, pH 8.5, 8 mm MgCl, 1 µg of bovine serum albumin, 100 µM CDP-[3H]choline (10 µCi/µmol), 500 µg EDTA, and 10 µl of 2.00 mm diC,,:-glycerol dissolved in absolute ethanol (final concentration 100 µM). The ethanol-diacylglycerol solution was clear. The reaction was started by adding 2 to 5 µl of protein followed immediately by the diC,,:-glycerol. The reaction was terminated after 10 min by adding 0.6 ml of 1% HClO,. Phospholipids were extracted by the method of Bligh and Dyer (24) using 3 ml of CHCl, : methanol, 1:2 (v/v), followed by 1 ml of CHCl, and 1 ml of 1% HClO,. The resulting CHCl, phase was extracted 3 times with 2 ml of 1% HClO,. Greater than 95% of the extracted labeled material migrated like authentic phosphatidylethanolamine on thin layer chromatography in Solvent I. Variations in these conditions are noted in the appropriate figure and table legends.

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were employed (see Fig. 1). There was a broad pH optimum from 8.5 to 9.3. The dependences of the ethanolaminephosphotransferase activity are shown in Table I. The activity was completely dependent on the addition of microsomes and MgCl$_2$. The 18.3% activity observed independent of added diacylglycerol was proportional to the amount of microsomal protein added to 7.5 µg. This residual activity probably arises from the utilization of diacylglycerols endogenous to the microsomal fraction. EGTA stimulated activity 60% and was routinely included in the incubation mixture. Omission of bovine serum albumin resulted in only 47% activity. Addition of the diacylglycerol in 10 µl of ethanol proved optimal. If the diacylglycerol was added to the incubation mixture prior to the addition of microsomes, the activity was 46% lower than observed when the diacylglycerol was added immediately following the addition of microsomes.

Essentially no change in activity was seen with the addition of NaCl, KCl, or KBr up to 125 mM to the incubation mixture. CaCl$_2$ (0.5 mM) inhibited activity 20% and 1.0 mM CaCl$_2$ inhibited activity 80%. No activity was observed with the addition of Triton X-100 (0.5 mg/ml) or 1-butanol (10 µl) to the incubation mixture. Triton WR 1339 (0.25 mg/ml) or Tween 20 (0.5 mg/ml) inhibited the activity 83 or 92%, respectively. The addition of 10 µl of acetone, methanol, or dioxane inhibited activity 40, 44, or 55%, respectively. Preincubation of microsomes (0.8 mg/ml) with phospholipase C (1 unit/ml) for 10 min at 23°C stimulated the activity 26%. Dithiothreitol (0.5 mM) inhibited the activity 48%. Freezing and thawing the microsomal preparation five times inhibited the activity 54%.

**Diacylglycerol Dependences**—The cholinephosphotransferase and ethanolaminephosphotransferase activities showed different dependences on added diC$_{1,2}$glycerol (see Fig. 2). When the data were plotted according to the method of Lineeweaver and Burk (25), the apparent $K_m$ for diC$_{1,2}$glycerol was 50.9 µM for the cholinephosphotransferase activity and 11.8 µM for the ethanolaminephosphotransferase activity (Table I). The complete system is described under "Experimental Procedures." Specific activities for the complete system were 7.61 ± 1.62 for the cholinephosphotransferase and 0.88 ± 0.31 for the ethanolaminephosphotransferase. These activities are the mean ± the standard deviation for four independent microsomal preparations.

**III.** Diacylglycerol cholinephosphotransferase activity was almost entirely dependent on added diC$_{1,2}$glycerol (see Table I). Measurable diacylglycerol ethanolaminephosphotransferase activity, on the other hand, was present when no diC$_{1,2}$glycerol was added (see Table I).

The two microsomal activities exhibited different dependences on added diacylglycerols (see Fig. 2 and Table II). Highest ethanolaminephosphotransferase activity was observed with diC$_{1,2}$glycerol. The activities observed using 150 µM bacterial diacylglycerol or 1-C$_{1,2}$-2-C$_{1,2}$glycerol were 49% or 57%, respectively, of that seen with diC$_{1,2}$glycerol for the cholinephosphotransferase activity. With the ethanolaminephosphotransferase activity, however, 150 µM bacterial diacylglycerol or 1-C$_{1,2}$-2-C$_{1,2}$glycerol were 18% or 51% active, respectively, as was diC$_{1,2}$glycerol. DiC$_{1,2}$glycerol was 31% as active as diC$_{1,2}$glycerol for the cholinephosphotransferase but only 14% as active for the ethanolaminephosphotransferase activity. Apart from diC$_{1,2}$glycerol which was 2.6% as active as diC$_{1,2}$glycerol, the other diacylglycerols tested were not substrates for the ethanolaminephosphotransferase activity under the conditions employed. DiC$_{1,2}$, diC$_{2,3}$, and diC$_{3,4}$glycerol were, however, utilized as substrates by the cholinephosphotransferase activity (see Fig. 2A, Table II).

Neither the cholinephosphotransferase nor the ethanolaminephosphotransferase activity was able to utilize 1,3-diC$_{1,2}$glycerol or 1,2-diC$_{1,2}$glycerol ether as substrates.

**CDP-choline and CDP-ethanolamine Dependencies**—The cholinephosphotransferase activity had an apparent $K_m$ of 23.9 µM (see Fig. 3). When 250 µM CDP-ethanolamine was added to the incubation mixtures, it proved to be a competitive inhibitor of the reaction with an apparent $K_i$ of 227 µM. CDP was also a competitive inhibitor with an apparent $K_i$ of 360 µM. At 100 µM CDP-choline, 1 mM CMP, or 1 mM CTP inhibited the activity 53 or 25%, respectively. A double reciprocal plot of the data (not shown) revealed a mixed type of inhibition.

The ethanolaminephosphotransferase activity had an ap-
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Fig. 2. Diacylglycerol dependences. Diacylglycerol solutions were prepared such that addition of 10 μl resulted in the desired concentration. See "Experimental Procedures." Activity independent of added diacylglycerol was subtracted. A, diacylglycerol cholinephosphotransferase; B, diacylglycerol ethanolaminophosphotransferase.

**TABLE II**

Diacylglycerol dependences of cholinephosphotransferase and ethanolaminophosphotransferase

The table entries are derived from data shown in Fig. 2.

<table>
<thead>
<tr>
<th>Diacylglycerol</th>
<th>Cholinephosphotransferase</th>
<th>Ethanolaminophosphotransferase</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Activity dependent on added diacylglycerol at 150 μM</td>
<td>Apparent $K_m$</td>
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<tr>
<td></td>
<td>nmol/min/mg</td>
<td>μM</td>
</tr>
<tr>
<td>DiC₁₂:0:glycerol</td>
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<tr>
<td>DiC₁₀:0:glycerol</td>
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<td>110</td>
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<tr>
<td>DiC₁₀:2:glycerol</td>
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<td>5.7</td>
</tr>
<tr>
<td>DiC₀:0:glycerol</td>
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<td>14.1</td>
</tr>
<tr>
<td>DiC₀:2:glycerol</td>
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<tr>
<td>Bacterial diacylglycerol</td>
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<td>40.5</td>
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<tr>
<td>1-C₁₀:0-2-C₁₀:0:glycerol</td>
<td>3.2</td>
<td>126</td>
</tr>
</tbody>
</table>

* The activity independent of added diacylglycerol was subtracted (see Table I).
* Derived from a computer-assisted least squares analysis of the data according to the method of Lineweaver and Burk (25). Regression coefficients were greater than 0.975. Also see Footnote 3.
* From *Escherichia coli* phospholipids.

Parent $K_m$ for CDP-ethanolamine of 18.3 μM (see Fig. 4). CDP-choline appeared to be a noncompetitive inhibitor with an apparent $K_i$ of 1620 μM. Inhibition of a mixed type was apparent with CDP. At 100 μM CDP-ethanolamine, 1 mM CMP or 1 mM CTP inhibited the activity 54 or 14%, respectively.

**Thermal Inactivation** — Microsomes were heated and samples were taken at various times for assay of cholinephosphotransferase and ethanolaminophosphotransferase activities at 23°. Both activities were stable with preincubation at 37° for 19 min. At 49°, 50% of the cholinephosphotransferase activity was lost after 15 min of heating (see Fig. 5). Ethanolaminophosphotransferase activity was more thermostable with 50% of its activity lost during 4 min of heating at 49° (see Fig. 5). Preincubation of microsomes at 51° confirmed the greater temperature stability of the cholinephosphotransferase activity (data not shown).

**Inhibition by Trypsin** — Cholinephosphotransferase and ethanolaminophosphotransferase activities were differentially inactivated by trypsin (see Fig. 6). Both activities were inhibited 20% by 4 min of exposure to 10 μg/ml of trypsin. The ethanolaminophosphotransferase was stable with exposure to increasing concentrations of trypsin while cholinephosphotransferase activity continued to decline (see Fig. 6A). A time course of inactivation with 80 μg/ml of trypsin showed an initial rapid loss for both microsomal activities (see Fig. 6B). The ethanolaminophosphotransferase activity remaining after 0.8 min of incubation with trypsin (approximately 10%) was stable. Cholinephosphotransferase activity, however, was inhibited 55% after 0.6 min of preincubation with trypsin. Only 16% of the activity remained after 9 min.

**Effect of Bovine Serum Albumin** — The addition of up to 2.0 mg/ml of bovine serum albumin to the incubation mixture had essentially no effect on the cholinephosphotransferase activity (see Fig. 7). Ethanolaminophosphotransferase activity, however, was increased 2-fold in the presence of 1 mg/ml of bovine serum albumin.

**Inhibition by Palmitoyl-CoA and Manganese Chloride** — Palmitoyl-CoA inhibition of liver cholinephosphotransferase has been reported (11). Therefore, the effects of palmitoyl-CoA on the cholinephosphotransferase and ethanolaminophosphotransferase activities from fat cells were investigated (see Fig. 8). At concentrations greater than 50 μM both activities were inhibited. Ethanolaminophosphotransferase activity was reduced only 22% at palmitoyl-CoA concentrations from 100 to...
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Fig. 5. Thermolability of microsomal cholinephosphotransferase and ethanolaminephosphotransferase activities. Microsomes (0.2 mg/ml) were heated at 49° in Medium I and samples containing 4 μg were taken at the times indicated for immediate assay at 23°. Specific activities for the unheated microsomes were 9.09 and 1.74 nmol/min/mg of protein for the choline- and ethanolaminephosphotransferase activities, respectively.

Fig. 6. Inactivation of cholinephosphotransferase and ethanolaminephosphotransferase activities by trypsin. A, dose dependence. Microsomes (0.8 mg/ml) were incubated with various levels of trypsin in Medium I at 23° for 4 min prior to assay. B, time dependence. Microsomes (0.8 mg/ml) were incubated with trypsin (80 μg/ml) in Medium I at 23° for various times prior to assay. Assays utilizing 4 μg of protein were performed as described under "Experimental Procedures" except that diC₂₀:₀ glycerol was present in the incubation mixture prior to the addition of microsomal protein. Specific activities of the untreated microsomes were 3.66 and 0.50 nmol/min/mg of protein, respectively, for the choline- and the ethanolaminephosphotransferase activities.

200 μM. The cholinephosphotransferase activity was inhibited 58 and 69% at palmitoyl-CoA levels of 100 and 200 μM, respectively.

Manganese has been reported to partially substitute for the magnesium requirement of the brain cholinephosphotransferase activity (14) and to substitute totally for the magnesium requirement of brain ethanolaminephosphotransferase activity (15). Therefore, the effect of manganese chloride on fat cell cholinephosphotransferase and ethanolaminephosphotransferase activities was investigated. When 4 mM MnCl₂ replaced the 8 mM MgCl₂ of the standard assays, ethanolaminephosphotransferase activity increased 13% but cholinephosphotransferase activity decreased 75%. When manganese chloride was added to the standard assays containing magnesium, ethanolaminephosphotransferase activity was not affected at concentrations up to 8 mM (see Fig. 8) while the cholinephosphotransferase activity was inhibited 76% by the addition of 1 mM manganese chloride.

Tissue Distribution — A tissue survey in the rat (see Table III) revealed highest microsomal cholinephosphotransferase activities from fat cells, liver, and intestinal mucosa while microsomal ethanolaminephosphotransferase activities were highest in microsomes from liver, brain, and intestinal mucosa. It is apparent that the ratio of cholinephosphotransferase activity to ethanolaminephosphotransferase activity varied independently in the tissues examined. This ratio ranged from over 8 in microsomes from isolated fat cells to about 1 in heart muscle.

DISCUSSION

We have initiated studies on the microsomal diacylglycerol ethanolaminephosphotransferase and diacylglycerol cholinephosphotransferase activities as an initial step in elucidating the regulatory processes controlling the synthesis of triacylglycerols and phospholipids in adipocytes. Our investigations employing ethanol-dispersed diacylglycerols represent the first characterization of these activities from adipose tissue or isolated fat cells. The assays employed 100 to 1000 times less protein than previous methods (9–18) which is essential for studies in adipocytes since they contain little protein (22). The microsomal choline- and ethanolaminephosphotransferase activities resembled the activities from other tissues in their...
of these activities are salient. These investigations represent choline- and ethanolaminephosphotransferase activities are the ethanolaminephosphotransferase activity (13). In view of these findings, our results on the diacylglycerol dependences of these activities are salient. These investigations represent the first systematic investigation of the diacylglycerol dependences of the cholinephosphotransferase and ethanolaminephosphotransferase activities from any tissue. Our data indicate that the microsomal cholinephosphotransferase and ethanolaminephosphotransferase activities differ in their dependences on diacylglycerols (Fig. 2 and Table II).

Interpretation of the diacylglycerol dependences of the microsomal cholinephosphotransferase and ethanolaminephosphotransferase activities in terms of specificity of the enzymes must necessarily be guarded because of the complexity of the system employed. Data demonstrating micelle formation from either long or short chain diacylglycerols are lacking, but if diacylglycerols form micelles, the critical micellar concentration would be expected to decrease with increasing fatty acid chain length. The diacylglycerol dependences are not merely a function of the expected critical micellar concentrations (see Table II). The physical properties of diacylglycerols under the conditions employed remain to be defined, but the data suggest that the cholinephosphotransferase and ethanolaminephosphotransferase do have different specificities for 1,2-diacylglycerols. Differences in diacylglycerol specificities may account, in part, for the differences in fatty acid compositions between phosphatidylcholine and phosphatidylethanolamine (26, 27).

In view of the similarities of the reactants, products, and reactions catalyzed by the microsomal cholinephosphotransferase and ethanolaminephosphotransferase activities, it is not surprising that the two activities were similar in their dependences on pH and magnesium. Both activities were inhibited similarly by calcium, dithiothreitol, organic solvents, detergents, and by freezing and thawing. Both activities were stimulated by EGTA and phospholipase C and were unaffected by several salts.

However, several lines of evidence strongly suggest that the choline- and ethanolaminephosphotransferase activities are separate microsomal enzymes. Although CDP-ethanolamine was a competitive inhibitor of the cholinephosphotransferase activity, its apparent \( K_i \) was 9.5 times greater than the \( K_m \), for CDP-choline. Since CDP was also a competitive inhibitor of the cholinephosphotransferase and ethanolaminephosphotransferase activities on bovine serum albumin. Assays were performed with 5 \( \mu \)g of protein as described under "Experimental Procedures" except that the amount of bovine serum albumin was varied as indicated. Specific activities with 1 mg/ml of bovine serum albumin were 5.63 and 0.57 nmol/min/mg of protein for the choline- and ethanolaminephosphotransferase activities, respectively.

**Fig. 8 (right).** Palmitoyl-CoA and MnCl\(_2\) inhibition of cholinephosphotransferase and ethanolaminephosphotransferase activities. Assays were performed as described under "Experimental Procedures" with 4 \( \mu \)g of protein except that palmitoyl-CoA or MnCl\(_2\) was added as indicated. Specific activities were 6.95 and 0.77 nmol/min/mg of protein for the untreated choline- and ethanolaminephosphotransferase activities, respectively.

**Table III**

<table>
<thead>
<tr>
<th>Tissue distribution of microsomal cholinephosphotransferase and ethanolaminephosphotransferase activities</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Tissue</strong></td>
</tr>
<tr>
<td>Fat cells</td>
</tr>
<tr>
<td>Liver</td>
</tr>
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<td>Intestinal mucosa</td>
</tr>
<tr>
<td>Brain</td>
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<td>Lung</td>
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<td>Kidney</td>
</tr>
<tr>
<td>Heart</td>
</tr>
<tr>
<td>Skeletal muscle</td>
</tr>
</tbody>
</table>

The microsomal fractions were isolated from the various tissues as described under "Experimental Procedures" for fat cells. Specific activities are the average of two independent tissue surveys. Choline- and ethanolaminephosphotransferase activities were determined on the same day using the same microsomal preparations.

The cholinephosphotransferase activity \( K_i \) was 15 times greater than the \( K_m \) for CDP-choline, competitive inhibition based on the structural similarity between CDP, CDP-ethanolamine, and the CDP-choline substrate appears probable. Moreover, CDP-choline was a noncompetitive inhibitor of the ethanolaminephosphotransferase \( K_i \) was 85 times greater than the \( K_m \) for CDP-ethanolamine. These patterns of inhibition are not consistent with the ethanolaminephosphotransferase and cholinephosphotransferase reactions occurring at a single active site. Further, the cholinephosphotransferase and ethanolaminephosphotransferase activities had different diacylglycerol dependences and different tissue distributions, were affected differently by addition of albumin, and were differentially inactivated by heating, trypsin, palmitoyl-CoA, and manganese. Taken as a whole, the data strongly suggest
that the microsomal ethanolaminephosphotransferase and cholinephosphotransferase activities are catalyzed by separate microsomal enzymes. The solubilization and purification of the activities to homogeneity could provide conclusive evidence.

REFERENCES
Phospholipid synthesis in isolated fat cells. Studies of microsomal diacylglycerol cholinephosphotransferase and diacylglycerol ethanolaminephosphotransferase activities.
R Coleman and R M Bell