Why Expression of Phosphatidylethanolamine N-Methyltransferase Does Not Rescue Chinese Hamster Ovary Cells That Have an Impaired CDP-Choline Pathway*

Kristin A. Waite‡ and Dennis E. Vance§

From the Department of Biochemistry and Canadian Institutes of Health Research Group on Molecular and Cell Biology of Lipids, University of Alberta, Edmonton, Alberta T6G 2S2, Canada

The mutant Chinese hamster ovary cell line (CHO), MT58, has a temperature-sensitive mutation in CTP:phosphocholine cytidylyltransferase (CT), preventing phosphatidylcholine (PC) synthesis at 40 °C which results in apoptosis. Previous studies (Houweling, M., Cui, Z., and Vance, D. E. (1995) J. Biol. Chem. 270, 16277–16282) showed that expression of wild-type CT-α rescued the cells at 40 °C, whereas expression of phosphatidylethanolamine N-methyltransferase-2 (PEMT2) did not, even though PC levels appeared to be maintained at wild-type levels after 24 h at the restrictive temperature. We report that the failure of PEMT2 to rescue the MT58 cell line is due to inadequate long term PC synthesis. We found that changing the medium every 24 h rescued the PEMT2-expressing MT58 cells grown at 40 °C. This was due to the uptake and utilization of lipids in the serum. At 40 °C, PC levels in the wild-type CHO cells and CT-expressing MT58 cells increased over time whereas PC levels did not change in both the MT58 and PEMT2-expressing MT58 cell lines. Further investigation found that both the PEMT2-expressing MT58 and MT58 cell lines accumulated triacylglycerol at 40 °C. Pulse-chase experiments indicated that lyso-PC accumulated to a higher degree at 40 °C in the PEMT2-expressing MT58 cells compared with CT-expressing MT58 cells. Transfection of the PEMT-expressing MT58 cells with additional PEMT2 cDNA partially rescued the growth of these cells at 40 °C. Inhibition of PC degradation, by inhibitors of phospholipases, also stimulated PEMT-expressing MT58 cell growth at 40 °C. Best results were observed using a calcium-independent phospholipase A₂ inhibitor, methyl arachidonyl fluorophosphonate. This inhibitor also increased PC mass in the PEMT2-expressing MT58 cells. When the cells are shifted to 40 °C, PC degradation by enzymes such as phospholipases is greater than PC synthesis in the mutant PEMT2-expressing MT58 cells. Taken together, these results indicate that PEMT2 expression fails to rescue the mutant cell line at 40 °C because it does not maintain PC levels required for cellular replication.

Phosphatidylcholine (PC)¹ is the most abundant phospholipid in mammalian cellular membranes. Besides having a structural role in membranes and lipoproteins, PC plays an important role in signal transduction as it is a major source of lipid second messengers (1). In all nucleated cells, PC is made primarily through the CDP-choline pathway in which the key enzyme is CTP:phosphocholine cytidylyltransferase (CT) (2). However, in hepatic cells, an alternative pathway exists utilizing phosphatidylethanolamine N-methyltransferase (PEMT), which converts phosphatidylethanolamine (PE) to PC via three sequential methylation events (3).

There are several reasons why hepatic cells may maintain two pathways for providing PC. One possibility is that PEMT is necessary for the endogenous production of choline, which may have several fates: including biosynthesis of acetylcholine, a source for betaine (4) and conversion to PC by the CDP-choline pathway. Other studies have suggested that PEMT-derived PC may be preferentially secreted with lipoproteins (5). Recent studies with the PEMT knockout mouse indicate that the PEMT gene may have been conserved during evolution, to provide PC when dietary choline levels are insufficient during such times as pregnancy or starvation (6). Curiously, previous studies indicated that PC generated via the PEMT pathway did not substitute for PC derived from the CDP-choline pathway in cells that are defective in CT (7).

Esko and co-workers (8) first described the mutant Chinese hamster ovary (CHO) cell line, MT58, which has a temperature-sensitive mutation in CT. These cells divide normally at 33 °C, but at 40 °C CT activity is diminished, PC levels are decreased and the cells die via apoptosis (7, 9). Using this cell line, Houweling and co-workers (7) showed that at the restrictive temperature (40 °C), cDNA encoding CT-α rescued the cells whereas cDNA encoding an isofrom of PEMT, PEMT2, did not. This occurred even though PC levels, based upon percentage of total phospholipid, appeared to be restored (7). These cells provide an excellent system in which to analyze the role of both CDP-choline- and PEMT-derived PC in cellular replication and investigate why PEMT-derived PC does not rescue the mutant cell line.

There are several hypotheses as to why PEMT-derived PC does not rescue the mutant cell line, including subcellular location and differences in the molecular species of PC produced by the two pathways. We investigated these and other

---

* This work was supported in part by the Medical Research Council of Canada. 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.

‡ Postdoctoral fellow of the Alberta Heritage Foundation for Medical Research.

§ Medical scientist of the Alberta Heritage Foundation for Medical Research. To whom correspondence should be addressed. Fax: 780-492-3383; E-mail: dennis.vance@ualberta.ca.

¹ The abbreviations used are: PC, phosphatidylcholine; CT, CTP: phosphocholine cytidylyltransferase; PE, phosphatidylethanolamine; CHO, Chinese hamster ovary; PEMT, phosphatidylethanolamine N-methyltransferase; LPC, lysophosphatidylcholine; MAFP, methyl arachidonyl fluorophosphonate; TG, triacylglycerol; TLC, thin layer chromatography; FBS, fetal bovine serum; PBS, phosphate-buffered saline; DOTAP, dioleoyl-trimethylammonium phosphate.
hypotheses and have determined the rate of PC degradation is greater than the rate of PC synthesis in the PEMT2-expressing MT58 cells grown at 40 °C. Therefore, PEMT2-expressing MT58 cells do not produce enough PC to maintain cellular replication after 24 h at 40 °C.

**EXPERIMENTAL PROCEDURES**

**Materials**—Ham's F-12 medium, fetal bovine serum (FBS), and G418 were from Life Technologies, Inc., and culture dishes and flasks were from Beckton Dickinson. Silica gel G60 plates were obtained from Merck, [methyl-3H]Choline chloride, [methyl-3H]methionine, and [1-3H]homoalanine were obtained from Amersham Pharmacia Biotech. Methyl arachidonyl fluorophosphonate, mannoalide, and D609 were obtained from Calbiochem. t-659989, a platelet-activating factor receptor antagonist, which inhibits phospholipase D, was a generous gift from Merck Frost. Lyso-PC (LPC), alkyl-LPC, and DOTAP were purchased from Avanti Polar Lipids. All other chemicals and reagents were obtained from standard commercial sources.

**Cell Culture and Growth Curves**—Wild-type and MT58 cell lines were cultured in Ham's F-12 medium supplemented with 10% FBS. PEMT2-expressing and CT-expressing MT58 cell lines (7) were cultured in Ham's F-12 medium supplemented with 10% FBS and 0.3 mg/ml G418. All cells were maintained in 75-mm culture flasks at 5% CO2 in a humidified atmosphere at 33 °C. For growth curves, cells were plated at 2.5 × 104 in 60-mm dishes containing 4 ml of medium and incubated at the indicated temperature. At the indicated times, culture medium was removed, the adherent cells were harvested with trypsin and viable cells that excluded trypan blue were counted. Where indicated, cells were also grown in the presence of delipidated FBS or phospholipase inhibitors.

**Transfection of PEMT-expressing MT58 cells**—PEMT-expressing MT58 cells were plated at 2 × 105 cells/60-mm dish and grown overnight. Transfection with the PEMT2 expression vector or the empty vector (7) was performed the next day via a DOTAP transfection method (10). DOTAP was dried under vacuum and resuspended in sterile water to a concentration of 1 mg/ml. 15 µl of the DOTAP solution and 0.5 µg of DNA were incubated at room temperature with serum-free medium for 20 min. During this time, the dishes were washed with serum-free medium and 3 ml of serum-free medium was added to each dish. After this, the dishes were incubated at either 33 °C or 40 °C. At the indicated times, the culture medium was removed, the adherent cells were harvested with trypsin, and viable cells that excluded trypan blue were counted.

**Incorporation of Radiolabeled Precursors into PC**—For in vivo PEMT activity analysis, PEMT2-expressing MT58 cells were grown to 40% confluence and then shifted to 40 °C. At the indicated times, cells were labeled with [3H]methionine (5 µCi/dish) for 2 h and then harvested in phosphate-buffered saline (PBS) as described below. For ethanolamine, oleate, and choline labeling experiments, cells were incubated with the appropriate radiolabel ([3H]Holeate, [3H]ethanolamine or [3H]choline, respectively) at 5 µCi/dish at 33 °C for 24 h. The medium was removed from the cells, fresh medium was added, and the cells were incubated at 40 °C. At the indicated times, cells were washed three times with cold PBS and harvested in 10 ml of PBS. The cells were pelleted by low speed centrifugation, resuspended in PBS, and sonicated. Lipids were extracted as described by Sundler and co-workers (12) and separated by thin layer chromatography (TLC) on Silica gel G60 plates as described below. For analysis of lyso-PC (LPC), phospholipids were separated with chloroform/methanol/acidic acid/formic acid/water (70:30:12:4:1) as a developing solvent. After TLC, LPC was visualized by iodine vapor and the radiolabel incorporated into the bands of interest was measured by liquid scintillation counting after being scraped off the plate.

**Determination of Phospholipid Mass, Triacylglycerol (TG) Mass, and Protein**—Cells were cultured and harvested as described above except radiolabeled precursors were omitted. Lipids from 0.5 mg of protein were extracted as described previously. For analysis of PC, phospholipids were separated using TLC with chloroform/methanol/acidic acid/formic acid/water (70:30:12:4:1) as a developing solvent. For TG analysis, TLC using heptane/diisopropyl ether/acidic acid (60:40:4) as a developing solvent separated neutral lipids. After TLC, and visualization with iodine vapor, the bands of interest were scraped and analyzed. PC mass was determined by measuring the phosphorous content (13), and TG mass was determined by the hydroxylamine method described by Snyder and Stephens (14). Protein concentrations were determined using the Coomassie Plus protein protocol from Pierce, which is based on the Bradford method (15). Bovine serum albumin was used as a standard.

**RESULTS**

**Changing the Medium Rescues PEMT2-expressing MT58 Cells at 40 °C**—We investigated, without success, a variety of hypotheses as to why PEMT-derived PC does not rescue the mutant cell line. First, we analyzed the contribution of the PEMT pathway and CDP-choline pathway in generating nuclear PC. Using radiolabeling studies, we found that the percent of PC levels derived from the PEMT pathway or the CDP-choline pathway was similar in PEMT2-expressing MT58 and CT-expressing MT58 cells (3.1% and 3.3% of total choline containing phospholipids, respectively). Next, we determined if there were any alterations in downstream signaling by PC derived second messengers in PEMT-expressing MT58 cells compared with wild-type and CT-expressing MT58 cells. We observed no differences in levels of protein phosphorylation, particularly tyrosine phosphorylation and mitogen-activated protein kinase activation.

Differences in the molecular composition of the PC species from the two pathways were also examined. Using gas chromatography, we found negligible differences in the fatty acid composition of PC derived from the PEMT-expressing MT58 cells compared with PC derived from CT-expressing MT58 cells grown at 40 °C (data not shown). Furthermore, we found that alkyl-LPC could rescue the PEMT-expressing MT58 cells grown at 40 °C (22 ± 5 × 105 cells/dish for PEMT-expressing MT58 cells; 32 ± 6 × 105 for wild-type cells after 96 h, n = 6). These data suggest that potential differences in the molecular composition of PC created by the PEMT pathway are not the reason why PEMT2 does not rescue MT58 cells.

In the process of performing the above experiments, we observed that the PEMT2-expressing MT58 cells could be rescued at 40 °C simply by changing the growth medium every 24 h. Fig. 1 shows that at 40 °C the PEMT2-expressing MT58 cells divide once and then fail to replicate as described earlier (7). When the medium was replaced every 24 h with medium containing...
taining serum, the PEMT2-expressing MT58 cells had a growth rate comparable to those cells grown at 33 °C as well as wild-type cells grown at 40 °C (32 ± 6 × 10^5 wild-type cells/dish after 96 h, n = 6). This was not due to the cellular uptake and utilization of serine, ethanolamine, or methionine, precursors to PE that could then be methylated to PC, since supplementing the medium with these compounds every 24 h did not rescue the cells (data not shown).

**Delipidated Serum Does Not Support the Growth of PEMT2-expressing MT58 Cells at 40 °C**—Subsequently, we incubated PEMT-expressing MT58 cells with medium containing delipidated serum at 40 °C (Fig. 2). Fig. 2A shows that PEMT2-expressing MT58 cells failed to undergo replication when grown at 40 °C with medium containing delipidated serum, even when the medium was changed every 24 h. This was not due to the loss of a growth factor during the delipidation process, as medium containing delipidated serum did not retard the growth of the PEMT2-expressing MT58 cells at 33 °C (Fig. 2B). When the delipidated serum was supplemented with either LPC or lyso-platelet-activating factor (alkyl-LPC), we found that PEMT2-expressing MT58 cells divided at the restrictive temperature yielding 20 × 10^5 and 22 × 10^5 cells/dish at 96 h, respectively (n = 2). Taken together, these data suggested that PC levels might not be sufficient in the PEMT2-expressing MT58 cells for continued growth at 40 °C.

**PC Levels Are Not Maintained in PEMT2-expressing MT58 Cells Grown at 40 °C**—Based on the above data, we next measured PC levels in four CHO cell lines: wild-type, MT58, CT-expressing MT58, and PEMT2-expressing MT58 cells (7). At 33 °C in all four cell lines, PC content varied little over 72 h (~70 nmol of PC/mg of protein). When the cells were shifted to 40 °C, we found that the wild-type and CT-expressing MT58 cells increased their PC content by 2- and 1.7-fold, respectively, over 72 h (Fig. 3A). In contrast, MT58 cells did not have an increase in PC content once shifted to the restrictive temperature, as previously reported (7, 8). Initially, PC levels in the PEMT2-expressing cell lines were maintained at 24 h. However, the PEMT2-expressing MT58 cells also did not have an increase in PC over time (Fig. 3A, triangles) when compared with the wild-type and CT-expressing cell lines.

**PEMT Activity Is Maintained in PEMT2-expressing MT58 Cells at 40 °C**—Possible explanations for the lack of increase in PC levels once the cells were shifted to the restrictive temperature were either that the PEMT2 enzyme was no longer functional or that less PEMT2 was present at the restrictive temperature. We found that there was no change in the amount of immunoreactive PEMT2 after 72 h at 40 °C (data not shown). Therefore, decreased amounts of the protein did not account for
the lack of PC. We next determined if the PEMT2 protein was active at 40 °C over the 72-h time course. Fig. 3B shows that the incorporation of radiolabeled methionine into PC occurs at 40 °C, indicating PEMT activity. In fact, at 40 °C, methionine incorporation is severalfold higher than at 33 °C. Therefore, the PEMT2 protein is still functional after 72 h at 40 °C.

PEMT2-expressing MT58 and MT58 Cells Accumulate TG at 40 °C—When analyzing the PC content in the above experiments, we noticed that there was a prominent band during TLC in the neutral lipids from PEMT2-expressing MT58 cells and MT58 cells at 40 °C. Upon further investigation, we determined that this was due to TG accumulation. Fig. 4 shows that, at 40 °C, TG accumulated in wild-type and CT-expressing MT58 cells, 3.7- and 3.8-fold, respectively. This accumulation, however, was similar to the levels of all four cell lines grown at 33 °C (Fig. 4). In contrast, at 40 °C there was a much greater increase in TG accumulation in both the mutant and PEMT2-expressing MT58 cells, 10- and 16-fold increase, respectively (Fig. 4). At 40 °C, the PEMT2-expressing MT58 cells accumulated approximately 1.6-fold more TG than MT58 cells after 72 h (compare closed triangles to closed diamonds).

Recently, studies have shown that phospholipase A2 plays a role in regulating cellular PC levels (16, 17). Based on these studies, we analyzed the LPC levels in both PEMT2-expressing MT58 cells and CT-expressing MT58 cells. PEMT2-expressing MT58 cells were labeled with [3H]ethanolamine, which would be expected to result in labeled PC by methylation of PE by PEMT. CT-expressing MT58 cells were labeled with [3H]choline. After 24 h at 40 °C, labeled LPC levels were 2-fold higher in PEMT2-expressing MT58 cells compared with the CT-expressing MT58 cells, and they remained 1.5–2-fold higher over 72 h at 40 °C. On the basis of this result, we hypothesized that at 40 °C the rate of PC synthesis was less than the rate of PC degradation in the PEMT2-expressing and MT58 cells when compared with the wild-type and CT-expressing MT58 cells. If this were indeed the case, we hypothesized that PEMT2-expressing MT58 cells may be rescued by stimulating PC synthesis or by inhibiting PC degradation.

Transfection of PEMT2 cDNA Partially Rescues PEMT2-expressing MT58 Cells at 40 °C—We attempted to increase PC synthesis in the PEMT2-expressing MT58 cells, at 40 °C, by transfecting these cells with additional PEMT2 cDNA. Fig. 5 shows that transient transfection of 0.5 μg of PEMT2 containing vector increased PEMT2-expressing MT58 cell growth at 40 °C compared with empty vector-transfected controls. Transfection with additional PEMT2 cDNA increased the number of cells per dish at 72 h after transfection compared with control cells, 11 × 10^5 and 3.4 × 10^5 cell/dish, respectively (Fig. 5). This result suggested that, by shifting the synthesis/degradation equilibrium in favor of PC synthesis, PEMT2-expressing MT58 cells were able to replicate at 40 °C.

Phospholipase Inhibitors Partially Rescue the Growth of PEMT2-expressing MT58 Cells at 40 °C—We next determined if inhibition of PC degradation would rescue the PEMT2-expressing MT58 cells using four phospholipase inhibitors. D609 is a PC phospholipase C inhibitor that has also been shown to inhibit phospholipase D (18, 19). Manoalide and MAFP are phospholipase A2 inhibitors (20–22), and L-659989 (Merck Frost) is a platelet-activating factor receptor antagonist that has been shown to inhibit phospholipase D (23). We achieved the best results with these inhibitors when added once, after the cells had been grown at 40 °C for 24 h. Further additions of the drugs resulted in increased cell death (data not shown). Fig. 6 shows that the addition of these phospholipase inhibitors rescued the PEMT2-expressing MT58 cells to various degrees with the most effective being 300 nM MAFP, the calcium-independent phospholipase A2 inhibitor (22). The addition of MAPF, at 24 h, rescued PEMT2-expressing MT58 cells by about 50% at 48 and 72 h. Fig. 7 shows that MAPF treatment also increased the cellular level of PC at 48 and 72 h. Although PC levels were not restored to the same levels as wild-type cells grown at 40 °C (94 ± 6 and 113 ± 6 nmol/mg protein at 48 and 72 h, respectively), the levels were comparable to PC levels in PEMT2-expressing MT58 cells levels grown at 33 °C (75 ± 6 and 69 ± 7 nmol/mg protein at 48 and 72 h, respectively).

Taken together, the data suggest that the reason PEMT2
individual experiments. and viable cells were counted. Shown are the means of five dishes. At the indicated times, the cells were harvested by trypsin; No Treatment

grown on 100-mm dishes at 40 °C. After 24 h, 300 nM MAFP was added to some dishes. At the indicated times, the cells were harvested and subjected to lipid extraction followed by TLC to separate PC. Phospholipase inhibitors partially rescue growth of PEMT2-expressing MT58 cells at 40 °C. When the cells are shifted to 40 °C, PC degradation by phospholipases is greater than PC synthesis due to the change in temperature from 33 °C to 40 °C, we did not anticipate such an increase in PC mass. Several groups have reported that PC mass does not increase even when CT activity increases (16, 27–29). Excess PC appears to be degraded by a lipid remodeling pathway. However, we found no major differences in the fatty acid composition of PC, by gas chromatography analysis, between CT-expressing and PEMT2-expressing MT58 cells grown at 40 °C after 48 or 72 h. We selected these time points for our study as these are the times when the PEMT2-expressing MT58 cells failed to proliferate. These apparently conflicting results may be due to lipid remodeling occurring in the McA-RH7777 cells.

We now provide evidence that expression of PEMT2 does not rescue the MT58 cells simply because not enough PC is produced after 24 h. Four lines of evidence support this conclusion. 1) Medium containing delipidated serum failed to rescue PEMT2-expressing MT58 cells at 40 °C, whereas medium containing lipids did. 2) PC levels did not increase in PEMT2-expressing MT58 cells at 40 °C, whereas PC levels increased in wild-type and CT-expressing MT58 cells. 3) Increased expression of PEMT2 by transient transfection enhanced the growth of PEMT2-expressing MT58 cells at 40 °C. 4) Inhibition of phospholipases allowed these cells to replicate at 40 °C.

We found that, at 40 °C, the PC content of wild-type and CT-expressing MT58 cells increased (Fig. 3A). Although this was not totally unexpected, as the laws of thermodynamics would dictate that enzymatic rates increase due to the change in temperature from 33 °C to 40 °C, we did not anticipate such an increase in PC mass. Several groups have reported that PC mass does not increase even when CT activity increases (16, 27–29). Excess PC appears to be degraded by a lipid remodeling pathway. However, we found no major differences in the fatty acid composition of PC, by gas chromatography analysis, between CT-expressing and PEMT2-expressing MT58 cells grown at 40 °C after 48 or 72 h. We selected these time points for our study as these are the times when the PEMT2-expressing MT58 cells failed to proliferate. These apparently conflicting results may be due to lipid remodeling occurring in the McA-RH7777 cells.

We now provide evidence that expression of PEMT2 does not rescue the MT58 cells simply because not enough PC is produced after 24 h. Four lines of evidence support this conclusion. 1) Medium containing delipidated serum failed to rescue PEMT2-expressing MT58 cells at 40 °C, whereas medium containing lipids did. 2) PC levels did not increase in PEMT2-expressing MT58 cells at 40 °C, whereas PC levels increased in wild-type and CT-expressing MT58 cells. 3) Increased expression of PEMT2 by transient transfection enhanced the growth of PEMT2-expressing MT58 cells at 40 °C. 4) Inhibition of phospholipases allowed these cells to replicate at 40 °C.

We found that, at 40 °C, the PC content of wild-type and CT-expressing MT58 cells increased (Fig. 3A). Although this was not totally unexpected, as the laws of thermodynamics would dictate that enzymatic rates increase due to the change in temperature from 33 °C to 40 °C, we did not anticipate such an increase in PC mass. Several groups have reported that PC mass does not increase even when CT activity increases (16, 27–29). Excess PC appears to be degraded by a lipid remodeling pathway. However, we found no major differences in the fatty acid composition of PC, by gas chromatography analysis, between CT-expressing and PEMT2-expressing MT58 cells grown at 40 °C after 48 or 72 h. We selected these time points for our study as these are the times when the PEMT2-expressing MT58 cells failed to proliferate. These apparently conflicting results may be due to lipid remodeling occurring in the McA-RH7777 cells.

We now provide evidence that expression of PEMT2 does not rescue the MT58 cells simply because not enough PC is produced after 24 h. Four lines of evidence support this conclusion. 1) Medium containing delipidated serum failed to rescue PEMT2-expressing MT58 cells at 40 °C, whereas medium containing lipids did. 2) PC levels did not increase in PEMT2-expressing MT58 cells at 40 °C, whereas PC levels increased in wild-type and CT-expressing MT58 cells. 3) Increased expression of PEMT2 by transient transfection enhanced the growth of PEMT2-expressing MT58 cells at 40 °C. 4) Inhibition of phospholipases allowed these cells to replicate at 40 °C.

We found that, at 40 °C, the PC content of wild-type and CT-expressing MT58 cells increased (Fig. 3A). Although this was not totally unexpected, as the laws of thermodynamics would dictate that enzymatic rates increase due to the change in temperature from 33 °C to 40 °C, we did not anticipate such an increase in PC mass. Several groups have reported that PC mass does not increase even when CT activity increases (16, 27–29). Excess PC appears to be degraded by a lipid remodeling pathway. However, we found no major differences in the fatty acid composition of PC, by gas chromatography analysis, between CT-expressing and PEMT2-expressing MT58 cells grown at 40 °C after 48 or 72 h. We selected these time points for our study as these are the times when the PEMT2-expressing MT58 cells failed to proliferate. These apparently conflicting results may be due to lipid remodeling occurring in the McA-RH7777 cells.

We now provide evidence that expression of PEMT2 does not rescue the MT58 cells simply because not enough PC is produced after 24 h. Four lines of evidence support this conclusion. 1) Medium containing delipidated serum failed to rescue PEMT2-expressing MT58 cells at 40 °C, whereas medium containing lipids did. 2) PC levels did not increase in PEMT2-expressing MT58 cells at 40 °C, whereas PC levels increased in wild-type and CT-expressing MT58 cells. 3) Increased expression of PEMT2 by transient transfection enhanced the growth of PEMT2-expressing MT58 cells at 40 °C. 4) Inhibition of phospholipases allowed these cells to replicate at 40 °C.

We found that, at 40 °C, the PC content of wild-type and CT-expressing MT58 cells increased (Fig. 3A). Although this was not totally unexpected, as the laws of thermodynamics would dictate that enzymatic rates increase due to the change in temperature from 33 °C to 40 °C, we did not anticipate such an increase in PC mass. Several groups have reported that PC mass does not increase even when CT activity increases (16, 27–29). Excess PC appears to be degraded by a lipid remodeling pathway. However, we found no major differences in the fatty acid composition of PC, by gas chromatography analysis, between CT-expressing and PEMT2-expressing MT58 cells grown at 40 °C after 48 or 72 h. We selected these time points for our study as these are the times when the PEMT2-expressing MT58 cells failed to proliferate. These apparently conflicting results may be due to lipid remodeling occurring in the McA-RH7777 cells.

We now provide evidence that expression of PEMT2 does not rescue the MT58 cells simply because not enough PC is produced after 24 h. Four lines of evidence support this conclusion. 1) Medium containing delipidated serum failed to rescue PEMT2-expressing MT58 cells at 40 °C, whereas medium containing lipids did. 2) PC levels did not increase in PEMT2-expressing MT58 cells at 40 °C, whereas PC levels increased in wild-type and CT-expressing MT58 cells. 3) Increased expression of PEMT2 by transient transfection enhanced the growth of PEMT2-expressing MT58 cells at 40 °C. 4) Inhibition of phospholipases allowed these cells to replicate at 40 °C.

We found that, at 40 °C, the PC content of wild-type and CT-expressing MT58 cells increased (Fig. 3A). Although this was not totally unexpected, as the laws of thermodynamics would dictate that enzymatic rates increase due to the change in temperature from 33 °C to 40 °C, we did not anticipate such an increase in PC mass. Several groups have reported that PC mass does not increase even when CT activity increases (16, 27–29). Excess PC appears to be degraded by a lipid remodeling pathway. However, we found no major differences in the fatty acid composition of PC, by gas chromatography analysis, between CT-expressing and PEMT2-expressing MT58 cells grown at 40 °C after 48 or 72 h. We selected these time points for our study as these are the times when the PEMT2-expressing MT58 cells failed to proliferate. These apparently conflicting results may be due to lipid remodeling occurring in the McA-RH7777 cells.

We now provide evidence that expression of PEMT2 does not rescue the MT58 cells simply because not enough PC is produced after 24 h. Four lines of evidence support this conclusion. 1) Medium containing delipidated serum failed to rescue PEMT2-expressing MT58 cells at 40 °C, whereas medium containing lipids did. 2) PC levels did not increase in PEMT2-expressing MT58 cells at 40 °C, whereas PC levels increased in wild-type and CT-expressing MT58 cells. 3) Increased expression of PEMT2 by transient transfection enhanced the growth of PEMT2-expressing MT58 cells at 40 °C. 4) Inhibition of phospholipases allowed these cells to replicate at 40 °C.

We found that, at 40 °C, the PC content of wild-type and CT-expressing MT58 cells increased (Fig. 3A). Although this was not totally unexpected, as the laws of thermodynamics would dictate that enzymatic rates increase due to the change in temperature from 33 °C to 40 °C, we did not anticipate such an increase in PC mass. Several groups have reported that PC mass does not increase even when CT activity increases (16, 27–29). Excess PC appears to be degraded by a lipid remodeling pathway. However, we found no major differences in the fatty acid composition of PC, by gas chromatography analysis, between CT-expressing and PEMT2-expressing MT58 cells grown at 40 °C after 48 or 72 h. We selected these time points for our study as these are the times when the PEMT2-expressing MT58 cells failed to proliferate. These apparently conflicting results may be due to lipid remodeling occurring in the McA-RH7777 cells.
MT58 cells and wild-type cells was more pronounced. We found that the difference in PC levels between the PEMT2-expressing MT58 cells and wild-type or CT-expressing MT58 cells is also more pronounced after growth at 40 °C for 48 and 72 h (Fig. 3A). We considered the possibility that PEMT2 may not be produced or active in PEMT2-expressing MT58 cells grown at 40 °C for over 24 h. However, active PEMT2 was present in cells grown at 40 °C for at least 72 h (Fig. 3B). In fact, the level of PEMT activity at 40 °C was higher than the activity at 33 °C (Fig. 3B), consistent with thermodynamic predictions.

We found that products of further PC metabolism, such as TG and LPC, accumulated in PEMT2-expressing MT58 cells compared with wild-type cells grown at 40 °C (Fig. 4, and this report). Recently, Jackowski and co-workers have shown that TG levels increase in the MT58 cells due to the diversion of newly synthesized diacylglycerol to the TG pool (17). As shown in Fig. 4, we too see these results. However, some of the TG accumulated at 40 °C may also result from PC degradation. We found that the accumulation of TG was greater in the PEMT2-expressing MT58 cells compared with MT58 cells. This increase in the accumulation of TG in PEMT2-expressing MT58 cells is probably due to increased synthesis of PC and its subsequent catabolism, compared with the MT58 cells. Enhanced catabolism of PC is indicated, since LPC levels were higher in PEMT2-expressing MT58 cells compared with CT-expressing MT58 cells.

Based on the above results, we postulate that at 40 °C, the rate of PC synthesis is greater than or equal to the rate of PC degradation in wild-type and CT-expressing MT58 cells and that in MT58 and PEMT2-expressing MT58 cells grown at 40 °C the rate of PC synthesis is less than or equal to the rate of PC degradation. If this hypothesis were true, we should therefore be able to rescue, at least partially, the PEMT2-expressing MT58 cells if the equilibrium between synthesis and degradation was shifted in the direction of synthesis. By transfecting the PEMT2-expressing MT58 cells with additional amounts of PEMT2 cDNA, the number of viable cells increased at 40 °C, presumably due to increased synthesis of PC (Fig. 5). In our attempts to modulate the rate of PC degradation, we showed that several phospholipase inhibitors increased the growth of PEMT-expressing MT58 cells to varying degrees (Fig. 6). As expected, based upon the reports of others (16, 28–30), which implicate a role for the calcium-independent phospholipase A2 in lipid remodeling, the most effective restoration of growth occurred with inhibitors of calcium-independent phospholipase A2: MAFP (22) and manoolide (20, 21). Cell growth was not completely rescued at 40 °C, as high levels of MAFP were cytotoxic, even at 33 °C. Treatment with MAFP also increased PC levels in the PEMT-expressing MT58 cells grown at 40 °C (Fig. 7). These inhibitors did not partially rescue MT58 cells, as we would expect since these cells contain no active PC-producing pathway at the restrictive temperature.

Taken together, we have shown that the reason why PEMT2 does not rescue growth of the MT58 cells at 40 °C is that insufficient PC is produced after 24 h. At 40 °C, the rate of PC degradation is greater than the rate of PC synthesis in the MT58 and PEMT2-expressing MT58 cells. It remains to be seen if PEMT-derived PC has a specialized role in the whole animal, particularly the liver. The generation of the PEMT null mouse (31) will help to elucidate further why the liver has maintained two pathways for PC production. Studies to address this question are currently underway and should prove to increase our knowledge on the roles of PC. Nonetheless, the data shown here suggest that in cell culture, PEMT-derived PC can substitute for PC made by the CDP-choline pathway in maintaining cellular homeostasis and further indicates the importance of PC for cellular replication.

Acknowledgments—We thank Susanne Lingrell for excellent technical assistance on this project and Dr. Jean Vance for helpful comments.

REFERENCES
1. Exton, J. H. (1994) Biochim. Biophys. Acta 1212, 26–42
2. Kennedy, E. P. (1986) in Lipids and Membranes: Past Present and Future (Op-den-Kamp, J. A. F., Roelofsen, B., and Wirtz, K. W. A., eds) pp. 171–206, Elsevier Sciences Publishers B. V., Amsterdam
3. Vance, D. E., and Ridgway, N. D. (1988) Prog. Lipid Res. 27, 61–79
4. Zeisel, S. H., and Blustejn, J. K. (1984) Annu. Rev. Nutr. 14, 269–296
5. Vance, J. E., and Vance, D. E. (1986) J. Biol. Chem. 261, 4486–4491
6. Walskey, C. J., Yu, L., Agellon, L. B., and Vance, D. E. (1998) J. Biol. Chem. 273, 37043–37046
7. Houweling, M., Cui, Z., and Vance, D. E. (1995) J. Biol. Chem. 270, 16277–16282
8. Eske, J. D., Wermuth, M. M., and Raetz, C. H. R. (1981) J. Biol. Chem. 256, 7388–7395
9. Cui, Z., Houweling, M., Chen, M. H., Record, M., Chap, H., Vance, D. E., and Terre, F. (1996) J. Biol. Chem. 271, 14668–14671
10. Holmen, S. I., Vanbroekhin, M. W., Eversole, R. R., Stapleton, S. R., and Ginsberg, L. C. (1995) In Vitro Cell. Dev. Biol. 31, 347–351
11. Bakovic, M., Waite, K. A., Tang, W., Tabas, I., and Vance, D. E. (1999) Biochim. Biophys. Acta 1438, 147–165
12. Sundler, R., Axesson, B., and Nilsson, A. (1974) J. Biol. Chem. 249, 5102–5107
13. Rouser, G., Giakatos, A. N., and Fleischer, S. (1966) Lipids 13, 85–86
14. Synder, F., and Stephens, N. (1959) Biochim. Biophys. Acta 34, 244–245
15. Bradford, M. M. (1976) Anal. Biochem. 72, 248–254
16. Barbour, S. E., Kapur, A., and Deal, C. (1999) Biochim. Biophys. Acta 1439, 77–88
17. Jackowski, S., Wang, J., and Baburina, I. (2000) Biochim. Biophys. Acta 1483, 310–315
18. Gratas, C., and Powis, G. (1993) Anticancer Res. 13, 1239–1244
19. Cobb, R. R., Felts, K. A., Parry, G. C., and Mackman, N. (1996) Mol. Pharma. 49, 988–1004
20. Soriente, A., DeRosa, M. M. C., Scettri, A., Sodano, G., Terencio, M. C., Paya, M., and Aleanz, M. J. (1999) Curr. Med. Chem. 6, 415–431
21. Grange, E., Rabin, O., Bell, J., and Chang, M. C. (1998) Neurochem. Res. 23, 1251–1257
22. Lio, Y. C., Reynolds, L. J., Balsinde, J., and Dennis, E. A. (1996) Biochim. Biophys. Acta 1302, 55–60
23. Gomez-Munoz, A., O’Brien, L., and Steinbrecher, U. P. (1999) Biochim. Biophys. Acta 1438, 247–252
24. Lykidiadis, A., Murti, K. G., and Jackowski, S. (1998) J. Biol. Chem. 273, 14222–14209
25. Lykidiadis, A., Baburina, I., and Jackowski, S. (1999) J. Biol. Chem. 274, 26992–27001
26. DeLong, C. J., Shen, Y.-J., Thomas, M. J., and Cui, Z. (1999) J. Biol. Chem. 274, 29683–29688
27. Walskey, C. J., Kahlmar, G. B., and Cornell, R. B. (1994) J. Biol. Chem. 269, 5742–5749
28. Balsinde, J., and Dennis, E. A. (1996) J. Biol. Chem. 271, 6758–6765
29. Baburina, I., and Jackowski, S. (1999) J. Biol. Chem. 274, 9400–9408
30. Balsinde, J., Bianco, I. D., Ackerman, E. J., Conde-Frieves, K., and Dennis, E. A. (1995) Proc. Natl. Acad. Sci. U. S. A. 92, 8527–8531
31. Walskey, C. J., Donohue, L. B., Bronson, R., Agellon, L. B., and Vance, D. E. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 12880–12885
Why Expression of Phosphatidylethanolamine-N-Methyltransferase Does Not Rescue Chinese Hamster Ovary Cells That Have an Impaired CDP-Choline Pathway
Kristin A. Waite and Dennis E. Vance

J. Biol. Chem. 2000, 275:21197-21202. doi: 10.1074/jbc.M003539200 originally published online May 4, 2000

Access the most updated version of this article at doi: 10.1074/jbc.M003539200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 30 references, 15 of which can be accessed free at http://www.jbc.org/content/275/28/21197.full.html#ref-list-1