The pioneering work of Eugene Kennedy in the 1950s established the choline pathway for phosphatidylcholine (PC) biosynthesis. However, the regulation of PC biosynthesis was poorly understood at that time. When I started my lab at the University of British Columbia in the 1970s, this was the focus of my research. This article provides my reflections on these studies that began with enzymology and the use of cultured mammalian cells, and progressed to utilize the techniques of molecular biology and gene-targeted mice. The research in my lab and others demonstrated that the regulated and rate-limiting step in the choline pathway for PC biosynthesis was catalyzed by CTP: phosphocholine cytidylyltransferase. This enzyme is regulated by its movement from a soluble form (largely in the nucleus) to a membrane-associated form where the enzyme becomes activated. Gene targeting in mice subsequently demonstrated that this gene is essential for development of mouse embryos. The other mammalian pathway for PC biosynthesis is catalyzed by phosphatidylethanolamine N-methyltransferase (PEMT) that converts phosphatidylethanolamine to PC. Understanding of the regulation and function of the integral membrane protein PEMT was improved when the enzyme was purified (a masochistic endeavor) in 1987, leading to the cloning of the Pemt cDNA.

Generation of knock-out mice that lacked PEMT showed that they were protected from atherosclerosis, diet-induced obesity, and insulin resistance. The protection from atherosclerosis appears to be due to decreased secretion of lipoproteins from the liver. We continue to investigate the mechanism(s) by which Pemt−/− mice are protected from weight gain and insulin resistance.

I am extremely fortunate to have had such excellent mentors throughout my scientific career. Without these dedicated teachers and colleagues, I am certain that I would not have become a biochemistry professor, nor would I have learned how to become an effective mentor myself.

I became interested in chemistry because of my teacher, Malachi Pancost, in 11th grade at Springfield High School in Springfield, Pennsylvania. He exhibited intense enthusiasm for chemistry and challenged his students to understand and appreciate this subject. He was a caring person, as shown by his sending birthday cards each year to his students. Even after he retired from teaching, he continued his tradition of sending birthday cards until his death in 2010; his obituary estimated that he sent about 1,000 birthday greetings each year.

As an undergraduate, I attended Dickinson College in Carlisle, Pennsylvania and majored in chemistry. A central reason I went to Dickinson was the opportunity to take non-science courses as well. Among those courses that were memorable were Philosophy of Religion, Art History, Abnormal Psychology, and American Literature. In the 3rd year, I took Organic Chemistry with Dr. M. Benton Naff. He explained the subject well and stimulated our critical thinking. Many of the students especially the pre-meds did not appreciate him because he demanded such a high standard. I grew to really enjoy organic chemistry and even did a small research project with Dr. Naff. I do remember that he sometimes became angry when students arrived late to class; consequently, he locked the classroom door at 10 a.m. when the class was due to start. Of course, this occurred in an era before lectures were posted on-line so taking notes in class was important.

One evening Dr. Naff organized a talk on cholesterol biosynthesis by Dr. Charles (Chuck) Sweeley (Fig. 1) from the University of Pittsburgh. At this time (1963), biochemistry was not taught at the undergraduate level in most colleges and universities in the United States (biochemistry was taught in medical schools). Dr. Sweeley’s lecture was truly an eye-opener for me and led to my application to the University of Pittsburgh for PhD studies. Another motivating factor lurking in the background was the increase in American involvement, particularly for young men of my age, in the Vietnam war. As history has now recorded, this involvement was a tragic mistake resulting in millions of unnecessary deaths. In the spring of 1964, a letter arrived offering me a position as a Ph. D. student in Dr. Sweeley’s laboratory. I was astounded to learn that the position came tuition-free and with a stipend of $225 per month! I accepted and moved to Pittsburgh in September.

Why do glycolipids accumulate in Fabry’s disease?

I was Prof. Sweeley’s second graduate student, and he mentored me in great depth from the start of my Ph. D. studies until graduation in 1968. At least once a day, he came into the lab to ask how my research was going and make suggestions. He was a smoker at that time and often leaned over the lab bench where I was using organic solvents such as ether; luckily, the lab did not burst into flames. When the Dean of the School died of lung
cancer, Chuck quit smoking the next day and never, to my knowledge, smoked again.

In 1963, before I arrived, Dr. Sweeley with his colleague Bernard Klionsky had elucidated the structure of the glycosphingolipid, trihexosyl ceramide, that accumulates abnormally in the inherited, lysosomal storage Fabry’s disease and leads to death (from kidney failure or heart disease) in mid-life in males who inherit the abnormal gene (1). Dr. Tamio Yamakawa and his colleague Shizue Suzuki had determined the structure of the related glycosphingolipid, globoside, which accounts for ~10% of lipids in red cells (2). Thus, the hypothesis presented to me for my Ph. D. studies was: because ~1% of red cells are degraded each day in humans, the catabolism of globoside could account for much of the accumulating trihexosyl ceramide observed in Fabry patients. The first step in testing this idea was to develop a method for separation of the various glycosphingolipids from human red cells and plasma. Chuck was a skilled analytical biochemist. Thus, under his supervision, I developed a thin-layer chromatographic procedure to separate the main neutral glycolipids from red cells and plasma. We submitted an abstract to the Federation of American Societies for Experimental Biology (FASEB) meeting in Chicago in 1967. Luckily, the abstract was selected for an oral presentation, as the main neutral glycolipids from red cells and plasma using one of the first machines for this technique, the LKB 9000. With a detection method in hand, we infused 35 g of 6,6-dideutero glucose into a control patient and a Fabry patient over 12 h. Despite the huge amount of labeled glucose administered, we did not detect enough label in globoside to support the hypothesis that red cell globoside was a precursor of plasma trihexosyl ceramide. Nevertheless, we did see ~2% plasma lactosyl ceramide and glucosyl ceramide labeled with 6,6-dideutero glucose, which exchanged onto the red cells (4).

I completed my Ph. D. in 1968 and stayed in Pittsburgh for postdoctoral studies while my wife, Jean (who changed her name to Jean Vance), completed her Ph. D. training with Ronald Bentley. I was very fortunate to be able to join the laboratory of David Feingold in the Department of Microbiology where I learned enzymology. In his lab, I developed a technique for N-terminal sequencing of proteins and peptides using Edman degradation (5). We used methyl-thiohydantoin to react with the N-terminal amino acid and detected the product by gas–liquid chromatography. The novelty of our procedure was that after the first cycle of the Edman reaction, we removed one aliquot for gas chromatographic analysis. We repeated the Edman reaction in the remaining sample, and then removed another aliquot on which we performed gas chromatography. Thus, after the first round, we generated a phenylalanine derivative, and after the second round, the gas–chromatographic profile showed phenylalanine and aspartate. We, therefore, were able to obtain the N-terminal sequence for up to 4 amino acids of a peptide without difficulty. During this time, I also took the opportunity to broaden my horizons by attending the medical student lectures in microbiology and virology.

An unexpected regulatory mechanism

In 1970, Jean and I moved to Massachusetts where I became a postdoctoral fellow with Konrad Bloch at Harvard University. When I joined his lab, I had thought I would work on purification of one of the membrane-bound enzymes involved in cholesterol biosynthesis. Such endeavors are certainly masochistic. However, his lab had discovered that a polysaccharide stimulated the fatty-acid synthase from Mycobacterium phlei (later identified as Mycobacterium smegmatis) and was trying to identify the major carbohydrate of the polysaccharide. My expertise in gas chromatography of carbohydrates (from Chuck Sweeley’s lab) allowed me to quickly identify the carbohydrate as 3-O-methyl mannose. Thus, 6 weeks after arrival in the lab, I was an author of a paper in Proceedings of the National Academy of Sciences (PNAS) (6).
Dr. Bloch (“KB” as we all called him) then asked me to determine the mechanism by which this polysaccharide stimulated the activity of the fatty-acid synthase in M. phlei. This was a challenging project in which I became totally immersed. Almost 2 years later, in the last 2 weeks that I was in Bloch’s lab, we solved the riddle. It turned out that the polysaccharide was not actually “stimulating” the reaction but rather relieving feedback inhibition by long-chain acyl-CoAs (24-carbon chain) that were made by the fatty-acid synthase. The polysaccharide bound the acyl-CoAs, promoting diffusion of the “sticky” acyl-CoA product away from the active site of the enzyme (7).

Konrad Bloch (Fig. 2) was an extraordinary human being and scientist in so many ways. Before I joined his lab, I naively worried that because he had a Nobel Prize (awarded in 1964), he might not be so interested in his research. I was totally wrong! His intense curiosity and the science drove him, and this did not change after the Nobel Prize. Indeed, his interest and curiosity in science continued for the rest of his life. We kept in touch with him and his very supportive wife, Lore, on many occasions after I left his laboratory. One time I asked him whether there was any part of his career that he regretted. As I recall, he said, “My only regret is that I wasted so much effort and time of many trainees in my lab by asking them to purify membrane proteins/enzymes” — and to think that I went to his lab to learn how to purify integral membrane enzymes!

While I was studying the regulation of fatty-acid synthase in Bloch’s lab, there were many other first-rate labs also working on fatty-acid synthase and its regulation. Thus, it became abundantly clear that, when I started my own lab, it would not be wise to work on this topic. However, I realized that, although the choline pathway for phosphatidylcholine (PC)2 biosynthesis (Fig. 3) had been established in the 1950s through the groundbreaking work of Eugene Kennedy (Fig. 4) and colleagues, in 1972, almost nothing was known about its regulation. This was a fundamental question in biochemistry that was wide open.

Because I had audited a virology course in Pittsburgh, I was aware of certain viruses that were surrounded by a lipid envelope that was obtained from the host cell. Thus, I hypothesized that because the virus was “stealing” lipids from the host cells, PC synthesis in the host cells might be stimulated after viral infection. For that reason, I wanted to test this idea in a virology laboratory. I was fortunate that Derek C. Burke, a British virologist at the University of Warwick in England, was willing to allow me to spend a year in his lab to test this hypothesis. The work went well, and I learned how to work with animal viruses and cultured cells. The unexpected result was that replication of the lipid-enveloped Semliki Forest virus did not stimulate PC biosynthesis (8), whereas replication of the non-enveloped polio virus did stimulate PC biosynthesis. The mechanism for the latter effect appeared to be that the concentration of cytoplasmic CTP increased and accelerated the rate-limiting step in PC biosynthesis catalyzed by CTP:phosphocholine cytidylyltransferase, which converts phosphocholine to CDP-choline (9) (Fig. 3). This result was an important first step in establish-

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2 The abbreviations used are: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PEMT, phosphatidylethanolamine N-methyltransferase CT, cytidylyltransferase.
ing myself in the field, and gave me more confidence in envisioning my own laboratory.

In the fall of 1972, I sent job applications to many universities. In particular, the University of British Columbia (UBC) had advertised a tenure track job for someone to teach a graduate course on lipids. I interviewed and was delighted when they offered me the position. As a typical American, I knew very little about Canada. The Department of Biochemistry at UBC provided a very good opportunity for me to start my lab, and Vancouver was a very attractive place to live. Moreover, because I was married to a British citizen, Canada seemed appropriate. I always expected my first job would be on the East Coast of the United States; that one advertisement changed the direction of my life.

The UBC years

The Ph. D. project of my first graduate student, Chris Richardson, naturally built on my studies in Warwick. He was to study the mechanism of assembly of the lipid envelope of Semliki Forest virus. He demonstrated that the virus obtained its envelope by budding from the plasma membrane of infected cells (10). Because none of the three enzymes in the choline pathway for PC biosynthesis had been purified, we started to purify the soluble enzymes choline kinase and CTP:phosphocholine cytidylyltransferase (CT). We wisely left the integral membrane enzyme, cholinephosphotransferase, in the “to do bucket.” We made good progress on purification of choline kinase (11) and CT (12). CT was eventually purified to a single band on an SDS gel by Paul Weinhold’s lab (13), and choline kinase was purified by Kozo Ishidate’s lab in Japan (14). To my knowledge, no one has ever purified cholinephosphotransferase.

Regulation of CTP:phosphocholine cytidylyltransferase

In 1978, Pat Choy, a postdoctoral fellow in my lab, discovered that CT was activated by lipids (15). Research from Claudia Kent’s lab (16) and our own lab (17) showed that the lipid activation in vitro reflected the observation that CT was inactive in cytosol and activated only upon translocation to cellular membranes (reviewed in Ref. 18). In the last 30 years, the mechanism underlying CT translocation has been exquisitely elucidated by research in Rosemary Cornell’s lab (19).

The primary focus of our lab subsequently became an understanding of the mechanism(s) of regulation of the choline pathway for PC biosynthesis in mammalian cells. Initial results demonstrated that the rate-limiting step in the pathway was catalyzed by CT (Fig. 3). When choline is taken up by cells, it is rapidly phosphorylated by choline kinase. The subsequent conversion of phosphorylcholine (P-choline) to CDP-choline then determines the rate of PC biosynthesis because the levels of CDP-choline are very low and it rapidly reacts with diacylglycerol to form PC (20). It is a somewhat unique situation that the first step in a biosynthetic pathway (choline kinase in this case) is apparently not regulated or rate-limiting for the pathway. Subsequent research has demonstrated that the rate of the CT reaction increases when levels of PC in the membranes decrease, when the cells are supplemented with fatty acids, or when the enzyme becomes dephosphorylated (17, 21, 23, 24), in addition to when the concentration of CTP increases.

Under normal metabolic conditions in mammalian cells and tissues, there is an excess pool of CT that is not associated with membranes; translocation of CT to membranes activates the enzyme. Thus, the store of inactive CT allows cells to activate CT rapidly without increasing gene transcription or protein synthesis. Although transcriptional regulation of CT might not be important in short-term regulation of CT activity, we investigated mechanisms by which transcription of CT is regulated over longer periods (reviewed in Ref. 25). The results from over a decade of research in my lab and the lab of Hiroyuki Sugimoto demonstrated that the transcription factors Sp1, Rb, TEF4, Ets-1, and E2F enhance the expression of CT, whereas Net represses CT expression. In contrast, transcription factors (sterol regulatory element–binding proteins (SREBPs), liver X receptors (LXRs), and peroxisome proliferator–activated receptors (PPARs)) involved in the metabolism of cholesterol and fatty acids are not major players in the regulation of CT. Perhaps the explanation for this difference between the regulation of cholesterol/fatty acids and phospholipid synthesis is that instead of being linked to energy metabolism, transcriptional regulation of CT expression is linked to the cell cycle, as well as cell growth and differentiation.

A surprising finding from Claudia Kent’s lab was that CT is primarily located in the nucleus of cells (26). Much more research on this topic has been performed, and it is now clear that CTo, which contains a nuclear localization signal, is primarily nuclear, whereas CTβ (encoded by a distinct gene) lacks a nuclear localization signal and is cytoplasmic (reviewed in Ref. 27). The reason why CTo is primarily localized to the nucleus has not yet been established and is a challenging area of research.

PEMT brings us to masochistic enzymology

Although the choline pathway for PC biosynthesis is present in all nucleated mammalian cells, an alternative pathway in which phosphatidylethanolamine (PE) is converted to PC by PEMT is present in the liver, but only at very low levels (<1% of the activity in liver) in a few other tissues. PEMT catalyzes three
Figure 5. Pathway for the conversion of PE to PC, showing the methylation status of the ethanolamine headgroup (above) in each case. PMME, phosphatidylmonomethyl ethanolamine; PDME, phosphatidyl dimethyl ethanolamine; AdoMet, 5'-adenosyl methionine; AdoHcy, 5'-adenosyl homocysteine.

reactions in which PE is sequentially methylated to PC with 5'-adenosyl methionine as the methyl donor (Fig. 5). Although PEMT is an integral membrane protein of microsomes, Wolfgang Schneider, a postdoc in my lab, made a heroic effort to purify PEMT from rat liver (28). He obtained a partial purification and solubilization of the protein. Subsequently, in the mid-1980s, Neale Ridgway joined my lab as a Ph. D. student and, after tremendous efforts, was successful in purifying PEMT to homogeneity (29). A major problem was that the detergent-solubilized enzyme was quite unstable in the Tris buffer that we were using. One day Neale had the idea of trying a phosphate buffer instead. The solubilized enzyme was perfectly stable in phosphate buffer, allowing Neale to complete the purification. The term “masochistic enzymology” refers to the daunting, almost impossible, job of purifying membrane proteins. Of course, in 2017, almost no one purifies proteins from tissues due to the ease of cloning the cDNAs, allowing expression of large amounts of defined proteins.

PC and lipoproteins

In 1982, I thought it was time to learn about lipoproteins because PC is a major component of these cholesterol-carrying particles that are involved in atherosclerosis. The PC component of lipoproteins had, until this time, been largely ignored. Thus, I took a sabbatical year in Dan Steinberg’s lab at the University of California at San Diego. I worked with David Weinstein, a young faculty member, to develop protocols for culture of primary rat hepatocytes and isolation of the lipoproteins secreted from these cells (30). Using this method, we initiated studies on the role of PC biosynthesis in the secretion of very low-density lipoproteins (VLDLs). At this time, Zemin Yao joined my lab as a Ph. D. student, one of the first students to come out of China after the “Cultural Revolution” ended. He demonstrated that PC biosynthesis was required for normal secretion of VLDLs from rat hepatocytes (31). In contrast, the formation of high-density lipoproteins was not dependent on PC biosynthesis. In more recent years, with the advent of gene-targeted mice, we have been able to demonstrate that both the choline pathway and the PEMT pathway for PC biosynthesis are required for normal secretion of VLDLs from mouse hepatocytes (32) (33). Why one PC biosynthetic pathway does not substitute for the other in VLDL secretion is not clear at this time.

Important diversions

In 1978, I was appointed Associate Dean for Research in the Faculty of Medicine at UBC. It was a half-time appointment, and I wanted to try administration. Bill Webber was the Dean of Medicine at that time, and he was committed to ensuring that my research could continue during my time in the Dean’s Office. Consequently, he made certain that the 3-year appointment really did constitute a maximum of 50% time commitment. Although it was interesting to learn the challenges of faculty administration, I decided that I did not wish to pursue administration at that level. Instead, I thought that being Chair of the Biochemistry Department at UBC would better fit my interests and provide more time for research. I was given that opportunity in 1983. Although I enjoyed some aspects of being Chair, such as recruiting and helping new faculty members become established, I concluded without any doubt that I preferred to put my energies into full-time research and teaching.

Other diversions were more closely related to research. I was appalled at the level of lipid biochemistry presented in biochemistry textbooks. Even the wonderful textbooks by Lehninger and Stryer gave at best a cursory treatment of lipids with no information on lipoproteins. I knew there was much more that students should learn about lipids at the introductory level. Thus, when Geoff Zubay asked me to contribute the lipid chapters to his textbooks Biochemistry (four editions) and Principles of Biochemistry, I agreed with enthusiasm. I’m proud to say that lipid biochemistry is now much better represented at the introductory level. Moreover, the treatment of lipids in textbooks at the advanced level was poor to nonexistent. Thus, Jean Vance and I coedited an advanced textbook called Biochemistry of Lipids, Lipoproteins and Membranes (46). The first edition was published in 1985, and we continued to serve as editors through the 5th edition that was published in 2008. It was gratifying to see that this book appeared to have an impact on graduate students and postdoctoral training throughout the world. When we traveled to other countries, we always saw it being widely used. The sixth edition of Biochemistry of Lipids, Lipoproteins and Membranes, now with Neale Ridgway and Roger McLeod as editors, was published in 2016.

Finally, as a research-active professor, one is obliged to assist in the review of research manuscripts and grants submitted by other scientists. The system depends on these external reviews, and virtually all scientists accept that duty and perform it at a high level. Perhaps because I consider this to be such an important responsibility, I dedicated more time to review than I could have, serving on many journal editorial boards (including JBC) and as Executive Editor of the lipid section and Editor-in-Chief of Biochimica Biophysica Acta (BBA) for 14 years total.

The University of Alberta years

At the 1985 Gordon Conference on Lipid Metabolism at the Kimball Union Academy in Meriden, New Hampshire, I met Mark Poznansky, a lipid biochemist and Associate Dean for Research in the Faculty of Medicine at the University of Alberta, Canada. A new medical research foundation (the Alberta Heritage Foundation for Medical Research) had just been established in the province of Alberta. I applied for one of the faculty positions supported by this foundation with the idea of establishing a group of researchers with a common research interest in lipid metabolism. I was appointed as a Professor of Biochemistry at the University of Alberta with a generous establishment grant. In addition, a new building would house the “Lipid Group” on one floor, and I was given permission to appoint five new professors in cooperation with the established...
Molecular biology meets lipids

The move to the University of Alberta in 1986 also came at the time when molecular biological techniques were starting to have a major impact on lipid research. After the purification of PEMT in 1987, we were able to obtain some protein sequence. At that time, a molecular biologist, Zheng Cui, joined the lab and cloned and expressed the cDNA encoding PEMT (34). Surprisingly, using an excellent antibody raised against a PEMT dodecapeptide, we discovered that the enzyme was highly enriched in mitochondria-associated membranes (34), a fraction of endoplasmic reticulum that transiently and reversibly contacts mitochondrial outer membranes, as described by Jean Vance in 1990 (35).

We were curious about the function of PEMT because PC is also made by the choline/CT pathway. From the cDNA sequence of PEMT, we were in a position to undertake the arduous task of generating a knock-out mouse in which the Pemt gene was no longer expressed. Fortunately, a new graduate student, Chris Walkey, accepted the challenge. In close collaboration with our colleague, Lou Agellon, Chris characterized the mouse Pemt gene (36) and disrupted it in mice (37). In 2017, gene targeting is almost routine; in contrast, in the mid-1990s, this was not a trivial task.

The Pemt<sup>−/−</sup> mice grew normally and did not exhibit obvious abnormalities. In the knock-out mice, the amount of the active form of hepatic CT was 60% higher than in Pemt<sup>+/+</sup> mice, which probably explained why the levels of PC were not lower in the livers of Pemt<sup>−/−</sup> mice. This result was, at first, disappointing but, upon reflection, not unexpected because the livers still contained abundant CT activity, which apparently compensated for the lack of PEMT. Chris then fed the Pemt<sup>−/−</sup> mice a choline-deficient diet so that availability of the substrate for the choline pathway for PC synthesis was reduced. After 3 days, the Pemt<sup>−/−</sup> mice that lacked choline in their diet developed end-stage liver failure (38), and the experiment had to be stopped (Fig. 6). The conclusion was that PEMT expression in the liver had been maintained during evolution to provide a source of PC when dietary choline was insufficient. The requirement for choline is very specific because dimethylthanolamine, which is converted into phosphatidyl(dimethylethanolamine, a phospholipid with physical properties similar to those of PC, did not prevent liver failure in Pemt<sup>−/−</sup> mice fed a choline-deficient diet (39).

Transition to mechanistic physiology and disease

The development of gene-targeting technology has profoundly altered our understanding of the function of enzymes and proteins in biology. Previously, one could possibly have developed an inhibitor of PEMT that might have revealed the role of PEMT in evolutionary biology. However, there is always concern about incomplete inhibition and off-target effects of inhibitors, whereas gene-targeting technology is very specific and unambiguous. Thus, in 1997, we were in the enviable position of exploring and understanding the role of PEMT in mouse physiology and disease.

As our first project, we wanted to test a hypothesis that Pemt<sup>−/−</sup> mice might be protected from atherosclerosis because hepatic VLDL secretion is attenuated by elimination of PEMT. We used mice that lacked the LDL receptor because when these mice are fed a high-cholesterol diet, they develop severe atherosclerosis. When PEMT was eliminated from mice lacking the LDL receptor (and the mice were fed a high-cholesterol diet), atherosclerosis in the artery (aortic root) was strikingly decreased by 80% (40) (Fig. 7). This result was very surprising because no one had predicted that PEMT and PC biosynthesis might be directly involved in atherosclerosis. Thus, these observations indicated that inhibition of PEMT might be a novel approach to reducing cardiovascular disease.

As we were feeding high-cholesterol diets to Pemt<sup>−/−</sup> mice, we designed a curiosity-driven experiment testing what seemed like an even more far-fetched hypothesis: that there might be a link between PEMT and obesity/diabetes. A postdoctoral fellow in the lab, René Jacobs, fed Pemt<sup>−/−</sup> and Pemt<sup>+/+</sup> mice a high-fat diet (60% fat as lard). As predicted, the Pemt<sup>+/+</sup> mice gained weight and developed insulin resistance (which can lead to type 2 diabetes). However, the Pemt<sup>−/−</sup> mice were protected against weight gain and insulin resistance (41) (Fig. 8). The diet we used contained normal concentrations of choline (1.3 g of choline/kg of diet), so the mice were not technically choline-deficient. We hypothesized, however, that because the PEMT reaction generates PC that can be degraded to choline, perhaps the Pemt<sup>−/−</sup> mice were choline-insufficient. Supplementation of the diet with choline eliminated the protective phenotype of
Figure 7. Sections from the aortic roots of mice fed a cholesterol-enriched diet. Ldlr−/− mice lack the low-density lipoprotein receptor and so are susceptible to atherosclerosis; Ldlr−/−/Pemt−/− mice lack both the low-density lipoprotein receptor and phosphatidylethanolamine N-methyltransferase. Samples were stained with oil red O (red) and hematoxylin (light blue). Reprinted from Vance, D. E. (2013) Biochim. Biophys. Acta - Molecular and Cell Biology of Lipids, Physiological roles of phosphatidylethanolamine N-methyltransferase, Volume 1831, Issue 3, 626–632 (47). Copyright (2013), with permission from Elsevier.

Figure 8. The connections between PEMT and a high-fat diet. Shown are mice that either express PEMT (WT) or lack PEMT (KO) that were fed a high-fat diet for 10 weeks. Reprinted from Vance, D. E. (2013) Biochim. Biophys. Acta - Molecular and Cell Biology of Lipids, Physiological roles of phosphatidylethanolamine N-methyltransferase, Volume 1831, Issue 3, 626–632 (47). Copyright (2013), with permission from Elsevier.

the Pemt−/− mice; thus, the choline-supplemented Pemt−/− mice gained weight and became insulin-resistant. These observations indicated that Pemt−/− mice are choline-insufficient when fed a high-fat diet. Despite these exciting findings, at this time in 2017, we still have established neither the mechanism by which Pemt−/− mice are protected from obesity and insulin resistance, nor the mechanism by which excess choline reverses this phenotype. We hope to have the answer(s) in the next year or two — stay tuned.

Recently, we’ve returned to one of our previous results, that Pemt−/− mice fed a choline-deficient diet experience liver failure. An enterprising graduate student, Zhaoyu Li, took on the challenge of understanding the mechanism leading to this liver failure. In a variety of experimental approaches, we demonstrated that liver failure in choline-deficient Pemt−/− mice is due to loss of plasma membrane integrity caused by a decrease in the molar ratio of PC to PE (42). In livers of mice in which PEMT was eliminated, the amount of hepatic PC was decreased, whereas the amount of PE, the substrate for the PEMT reaction, was increased. PC is a bilayer-forming lipid, whereas PE is an non-bilayer-forming lipid. A decrease of PC in the plasma membrane of hepatocytes, as well as an increase in PE, promotes loose packing of the membrane and, consequently, impairs membrane integrity, causing leakage. Thus, these and other studies suggest that the PC/PE ratio is a key regulator of cell membrane integrity and that a low PC/PE ratio plays a role in the progression of steatosis (fatty liver) to steatohepatitis (fatty liver disease) (reviewed in Ref. 43). This conclusion is supported by studies showing that the molar ratio of PC to PE is a predictor of non-alcoholic fatty liver disease (NAFLD) and survival of mice after partial hepatectomy (44), suggesting that the lack of PEMT leads to non-alcoholic fatty liver disease. At this point, there are no examples of humans that lack PEMT.

Similarly, mitochondrial function can be impaired by alterations in the PC/PE ratio. Using Pemt−/− mice, Jelske van der Veen, a postdoctoral fellow in the lab, revealed a strong positive correlation between the mitochondrial PC/PE molar ratio and cellular ATP levels (45). In addition, Jean Vance demonstrated that in cells with diminished activity of the mitochondrial enzyme phosphatidylserine decarboxylase, mitochondrial PE levels are reduced, the PC/PE ratio is increased, and ATP synthesis is inhibited (22). On the other hand, in PEMT-deficient mice, as the level of mitochondrial PE increases, pyruvate is diverted from decarboxylation and into the tricarboxylic acid cycle for ATP production. As a result, glucose production from pyruvate is diminished, possibly contributing to the protection of Pemt−/− mice from insulin resistance. Moreover, the sensitivity of the response of cellular functions to the PC/PE ratio might explain why the biosynthesis of PC and PE is so tightly regulated and why the rate of phospholipid synthesis is programmed to change within seconds.

When I started investigating regulation of PC biosynthesis, I certainly did not predict where we would be in 2017. It has been gratifying to be part of the process of elucidating control mechanisms in PC biosynthesis. It was challenging and very stimulating to see the field evolve into an understanding of the functions of PC biosynthesis in animal physiology and disease. There is still much to do. How our understanding of PC biosynthesis and function will evolve during the next 45 years is not predictable, but certainly an exciting prospect.

**Appreciation**

I have indicated the crucial roles that my mentors played in my career. A successful career is also dependent on many other factors. I have had a stable and supportive family life in spades. In particular, Jean Vance has been immensely important in so many ways, both personally and scientifically. Good fortune in recruiting outstanding postdoctoral fellows and graduate students from the very start of my appointment at UBC has provided an exciting scientific environment and many long-term friendships. Stable, continuous grant funding from 1973 through 2019 from the Canadian government has been a crucial factor in my research productivity. Important funding has also been awarded by the Alberta Heritage Foundation for Medical
References

1. Sweetley, C. C., and Klionsky, B. (1963) Fabry’s disease: classification as a sphingolipidosis and partial characterization of a novel glycolipid. J. Biol. Chem. 238, 3148–3150

2. Yamakawa, T., and Suzuki, S. (1952) The chemistry of the lipids of post-hemolytic residue or stroma of erythrocytes. III. Globoside, the sugar-containing lipid of human blood stroma. J. Biochem. 39, 393–402

3. Vance, D. E., and Sweetley, C. C. (1967) Quantitative determination of the neutral glycolipids in plasma of a normal human and a patient with Fabry’s disease. J. Biol. Chem. 250, 8119–8125

4. Vance, D. E., Krivit, W., and Sweetley, C. C. (1975) Metabolism of neutral glycosphingolipids in plasma of a normal human and a patient with Fabry’s disease. J. Biol. Chem. 250, 39, 621–630

5. Vance, D. E., and Feingold, D. S. (1971) Additive Edman degradation to sequence small peptides. Nature 229, 121–123

6. Ilton, M., Jevas, A. W., McCarthy, E. D., Vance, D., White, H. B., 3rd, and Bloch, K. (1971) Fatty acid synthetase activity in Mycobacterium phlei: regulation by polysaccharides. Proc. Natl. Acad. Sci. U.S.A. 68, 87–91

7. Vance, D. E., Mitsushashi, O., and Bloch, K. (1973) Purification and properties of the fatty acid synthetase from Mycobacterium phlei. J. Biol. Chem. 248, 2303–2309

8. Vance, D. E., and Burke, D. C. (1974) Inhibition of 3-sulfaphosphatidylcholine biosynthesis in baby-hamster kidney-21 cells infected with Semliki Forest virus. Eur. J. Biochem. 43, 327–336

9. Choy, P. C., Paddon, H. B., and Vance, D. E. (1980) An increase in cytoplasmic CTP accelerates the reaction catalyzed by CTP:phosphocholine cytidylyltransferase in poliovirus-infected HeLa cells. J. Biol. Chem. 255, 1070–1073

10. Richardson, C. D., and Vance, D. E. (1976) Biochemical evidence that Semliki Forest virus obtains its envelope from the plasma membrane of the host cell. J. Biol. Chem. 251, 5544–5550

11. Brophy, P. J., and Vance, D. E. (1976) Copurification of choline kinase and ethanolamine kinase from rat liver by affinity chromatography. FEBS Lett. 62, 123–125

12. Choy, P. C., Lim, P. H., and Vance, D. E. (1977) Purification and characterization of CTP: cholinephosphate cytidylyltransferase from rat liver cytosol. J. Biol. Chem. 252, 7673–7677

13. Feldman, D. A., and Weinhold, P. A. (1987) CTP:phosphocholine cytidylyltransferase from rat liver: isolation and characterization of the catalytic subunit. J. Biol. Chem. 262, 9075–9081

14. Ishidate, K., Nakagomi, K., and Nakazawa, Y. (1984) Complete purification of choline kinase from rat kidney and preparation of rabbit antibody against rat kidney choline kinase. J. Biol. Chem. 259, 14706–14710

15. Choy, P. C., and Vance, D. E. (1978) Lipid requirements for activation of CTP:phosphocholine cytidylyltransferase from rat liver. J. Biol. Chem. 253, 5163–5167

16. Kent, C. (1979) Stimulation of phospholipid metabolism in embryonic muscle cells treated with phospholipase C. Proc. Natl. Acad. Sci. U.S.A. 76, 4474–4478

17. Pelech, S. L., Pritchard, P. H., Brindley, D. N., and Vance, D. E. (1983) Fatty acids promote translocation of CTP:phosphocholine cytidylyltransferase to the endoplasmic reticulum and stimulate rat hepatic phosphatidylcholine synthesis. J. Biol. Chem. 258, 6782–6788

18. Vance, D. E., and Pelech, S. L. (1984) Enzyme translocation in the regulation of phosphatidylcholine biosynthesis. Trends Biochem. Sci. 9, 17–20

19. Cornell, R. B., and Ridgway, N. D. (2015) CTP:phosphocholine cytidylyltransferase: function, regulation, and structure of an amphibiotic enzyme required for membrane biogenesis. Prog. Lipid Res. 59, 147–171

20. Vance, D. E., Trip, E. M., and Paddon, H. B. (1980) Poliovirus increases phosphatidylcholine biosynthesis in HeLa cells by stimulation of the rate-limiting reaction catalyzed by CTP:phosphocholine cytidylyltransferase. J. Biol. Chem. 255, 1064–1069

21. Pelech, S. L., and Vance, D. E. (1982) Regulation of rat liver cytosolic CTP:phosphocholine cytidylyltransferase by phosphorylation and dephosphorylation. J. Biol. Chem. 257, 14198–14202

22. Tasseva, G., Bai, H. D., Davidescu, M., Haromy, A., Michelakis, E., and Vance, J. E. (2013) Phosphatidylethanolamine deficiency in mammalian mitochondria impairs oxidative phosphorylation and alters mitochondrial morphology. J. Biol. Chem. 288, 4158–4173

23. Cornell, R., and Vance, D. E. (1987) Translocation of CTP:phosphocholine cytidylyltransferase from cytosol to membranes in HeLa cells: stimulation by fatty acid, fatty alcohol, mono- and diacylglycerol. Biochim. Biophys. Acta 919, 26–36

24. Yao, Z. M., Jami, H., and Vance, D. E. (1990) Choline deficiency causes translocation of CTP:phosphocholine cytidylyltransferase from cytosol to endoplasmic reticulum in rat liver. J. Biol. Chem. 265, 4326–4331

25. Sugimoto, K., Bancho, K., and Vance, D. E. (2008) Transcriptional regulation of phosphatidylcholine biosynthesis. Prog. Lipid Res. 47, 204–220

26. Wang, Y., SweiZer, T. D., Weinhold, P. A., and Kent, C. (1993) Nuclear localization of soluble CTP:phosphocholine cytidylyltransferase. J. Biol. Chem. 268, 5899–5904

27. Ridgway, N. D. (2013) The role of phosphatidylcholine and choline metabolites to cell proliferation and survival. Crit. Rev. Biochem. Mol. Biol. 48, 20–38

28. Schneider, W. J., and Vance, D. E. (1979) Conversion of phosphatidylethanolamine to phosphatidylcholine in rat liver: partial purification and characterization of the enzymatic activities. J. Biol. Chem. 254, 3886–3891

29. Ridgway, N. D., and Vance, D. E. (1987) Purification of phosphatidylethanolamine N-methyltransferase from rat liver. J. Biol. Chem. 252, 17231–17239

30. Vance, D. E., Weinstein, D. B., and Steinberg, D. (1984) Isolation and analysis of lipoproteins secreted by rat liver hepatocytes. Biochim. Biophys. Acta 792, 39–47

31. Yao, Z. M., and Vance, D. E. (1988) The active synthesis of phosphatidylcholine is required for very low density lipoprotein secretion from rat hepatocytes. J. Biol. Chem. 263, 2998–3004

32. Noga, A. A., Zhao, Y., and Vance, D. E. (2002) An unexpected requirement for phosphatidylethanolamine N-methyltransferase in the secretion of very low density lipoproteins. J. Biol. Chem. 277, 42358–42365

33. Jacobs, R. L., Devlin, C., Tabas, I., and Vance, D. E. (2004) Targeted deletion of the host cell. J. Biol. Chem. 279, 47402–47410

34. Cui, Z., Vance, J. E., Chen, M. H., Voelker, D. R., and Vance, D. E. (1993) Cloning and expression of a novel phosphatidylethanolamine N-methyltransferase: a specific biochemical and cytological marker for a unique membrane fraction in rat liver. J. Biol. Chem. 268, 16655–16663

35. Vance, J. E. (1990) Phospholipid synthase in a membrane fraction associated with mitochondria. J. Biol. Chem. 265, 7248–7256
36. Walkey, C. J., Cui, Z., Agellon, L. B., and Vance, D. E. (1996) Characterization of the murine phosphatidylethanolamine N-methyltransferase-2 gene. *J. Lipid Res.* 37, 2341–2350
37. Walkey, C. J., Donohue, L. R., Bronson, R., Agellon, L. B., and Vance, D. E. (1997) Disruption of the murine gene encoding phosphatidylethanolamine N-methyltransferase. *Proc. Natl. Acad. Sci. U.S.A.* 94, 12880–12885
38. Walkey, C. J., Yu, L., Agellon, L. B., and Vance, D. E. (1998) Biochemical and evolutionary significance of phospholipid methylation. *J. Biol. Chem.* 273, 27043–27046
39. Waite, K. A., and Vance, D. E. (2004) Dimethyl ethanolamine does not prevent liver failure in phosphatidylethanolamine N-methyltransferase-deficient mice fed a choline-deficient diet. *Biochim. Biophys. Acta* 1636, 175–182
40. Zhao, Y., Su, B., Jacobs, R. L., Kennedy, B., Francis, G. A., Waddington, E., Brosnan, J. T., Vance, J. E., and Vance, D. E. (2009) Lack of phosphatidylethanolamine N-methyltransferase alters plasma VLDL phospholipids and attenuates atherosclerosis in mice. *Arterioscler. Thromb. Vasc. Biol.* 29, 1349–1355
41. Jacobs, R. L., Zhao, Y., Koonen, D. P., Sletten, T., Su, B., Lingrell, S., Cao, G., Peake, D. A., Kuo, M. S., Proctor, S. D., Kennedy, B. P., Dyck, J. R., and Vance, D. E. (2010) Impaired de novo choline synthesis explains why phosphatidylethanolamine-methyltransferase-deficient mice are protected from diet-induced obesity. *J. Biol. Chem.* 285, 22403–22413
42. Li, Z., Agellon, L. B., Allen, T. M., Umeda, M., Jewell, L., Mason, A., and Vance, D. E. (2006) The ratio of phosphatidylcholine to phosphatidylethanolamine influences membrane integrity and steatohepatitis. *Cell Metab.* 3, 321–331
43. van der Veen, J. N., Kennelly, J. P., Wan, S., Vance, J. E., Vance, D. E., and Jacobs, R. L. (2017) The critical role of phosphatidylcholine and phosphatidylethanolamine metabolism in health and disease. *Biochim. Biophys. Acta* 1859, 1558–1572
44. Ling, J., Chaba, T., Zhu, L. F., Jacobs, R. L., and Vance, D. E. (2012) Hepatic ratio of phosphatidylcholine to phosphatidylethanolamine predicts survival after partial hepatectomy in mice. *Hepatology* 55, 1094–1102
45. van der Veen, J. N., Lingrell, S., da Silva, R. P., Jacobs, R. L., and Vance, D. E. (2014) The concentration of phosphatidylethanolamine in mitochondria can modulate ATP production and glucose metabolism in mice. *Diabetes* 63, 2620–2630
46. Vance, D. E., and Vance, J. E. (1985) *Biochemistry of Lipids, Lipoproteins and Membranes*, Benjamin/Cummings Publishing Company, Menlo Park, CA
47. Vance, D. E. (2013) Physiological roles of phosphatidylethanolamine N-methyltransferase. *Biochim. Biophys. Acta* 1831, 626–632