Phosphatidylcholine metabolism in rat liver after partial hepatectomy

Evidence for increased activity and amount of CTP:phosphocholine cytidyltransferase

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The effect of partial (70%) hepatectomy on phosphatidylcholine (PC) synthesis in rat liver was investigated during the first 4 post-operative days. Between 4 and 96 h after partial hepatectomy, the mass of PC increased from 30% to 80% of sham-operation values, being comparable with the restoration of total liver mass after partial hepatectomy. Relative to control (sham-operation), the incorporation *in vivo* of [³H]choline into PC was stimulated 2.6-fold at 22 h after partial hepatectomy. Moreover, CTP:phosphocholine cytidyltransferase (EC 2.7.7.15) activity was significantly enhanced, and the pool size of phosphocholine decreased at 22 and 48 h after partial hepatectomy, whereas the activity of choline kinase (EC 2.7.1.32) was augmented at a later stage of liver regeneration (48 and 96 h). Stimulation of CTP:phosphocholine cytidyltransferase activity by partial hepatectomy occurred in both the microsomal and cytosolic fractions. The stimulatory effect in the cytosolic fraction was mainly due to an increase in the number of enzyme molecules, as demonstrated by immunotitration of the amount of cytosolic cytidyltransferase protein.

INTRODUCTION

Adult rat liver is normally in a quiescent state. However, after partial (70%) hepatectomy a synchronous wave of DNA synthesis and subsequent mitosis develop in the remaining hepatocytes within 20–30 h [1,2]. Although mitogenic factors such as epidermal growth factor and phorbol esters are known to stimulate phosphatidylcholine (PC) synthesis in various cell types [3–5], little information is yet available on the synthesis of PC and other phospholipids in the regenerating liver system.

It is widely held that the main mechanism for stimulation of PC biosynthesis is located at the level of CTP:phosphocholine cytidyltransferase [6] and that the activity of this enzyme is predominantly regulated by a translocation mechanism. The cytidyltransferase is reversibly distributed between the cytosol, where it is inactive, and the endoplasmic reticulum, where it becomes activated [7,8]. Nevertheless, in view of the co-variance between choline kinase activity and rate of PC synthesis in certain cell systems [3,9,10], and of the importance of diacylglycerol supply [11,12], additional regulatory mechanisms have to be considered as well.

In this study we provide evidence that during the first 4 post-surgical days partial hepatectomy leads to: (i) increased incorporation of labelled choline into PC *in vivo*; (ii) asynchronous stimulation of the activities of choline kinase (EC 2.7.1.32) and CTP:phosphocholine cytidyltransferase (EC 2.7.7.15), with cholinephosphotransferase (EC 2.7.8.2) activity not being affected; and (iii) a definite increase in the amount of cytosolic CTP:phosphocholine cytidyltransferase protein. To our knowledge, the last result shows for the first time that not only the activity, but also the number of cytidyltransferase molecules, are directly involved in the regulation of PC biosynthesis in rat liver.

EXPERIMENTAL

Materials

All radioactive compounds were from Amersham International, Amersham, Bucks., U.K. Choline kinase from yeast, glycerokinase and glycerol-3-phosphate dehydrogenase were purchased from Boehringer, Mannheim, Germany, and Protein A–Sepharose CL 4B was from Pharmacia LKB, Uppsala, Sweden. All other chemicals were of analytical grade, obtained from Baker Chemical Co., Deventer, The Netherlands.

Animals and surgery

Partial (70%) hepatectomies [13] were routinely performed between 09:00 and 10:00 h on male Wistar rats (150–200 g) under light diethyl ether anaesthesia. The animals, which had free access to water and pelleted standard diet [14] in both the pre- and post-surgical periods, had been maintained on an inverted light/dark cycle (dark between 06:00 and 18:00 h) for at least 2 weeks. In accordance with Gove & Hems [15], feed intake of partially hepatectomized and sham-operated animals did not differ.

Studies *in vivo*

To estimate the rate of PC synthesis *in vivo*, rats were intraperitoneally injected with 0.5 ml of physiological saline containing 10 μ Ci of [³H]choline (15 Ci/mmol) at different times after surgery. At 1 h after label injection, livers were homogenized with 4 vol. of ice-cold buffer containing 145 mM-NaCl, 10 mM-Tris/HCl (pH 7.4), 1 mM-EDTA and 10 mM-NaF. Lipids were extracted from homogenates as described by Sundler *et al.* [16], and PC was separated from other phospholipids by t.l.c. on silica gel G with chloroform/methanol/conc. NH₃ (sp.gr. 0.880) (13:7:1, by vol.) as developing agent. Label incorporation into

Abbreviation used: PC, phosphatidylcholine.

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Fig. 1. Effect of partial hepatectomy on level (a) and rate of synthesis (b) of hepatic PC at different times after surgery

(a) PC was isolated from livers of sham-operated (○) or partially hepatectomized (●) rats. Data are expressed as a function of total liver weight after surgery. Each time point represents the mean $\pm$ S.D. of four different rats. (b) The incorporation of [^{3} H]choline into PC *in vivo* was determined in livers of sham-operated (□) and partially hepatectomized (▨) rats, 1 h after intraperitoneal injection of [^{3} H]choline. Each time point represents the mean $\pm$ S.D. of three different rats. Significantly different from sham-operated rats: * $P < 0.02$; ** $P < 0.05$.

PC was quantified by liquid-scintillation counting, whereas the mass of hepatic PC was determined by phosphorus analysis as described by Bartlett [17].

Enzyme assays

At different times after surgery, rats were killed and livers were removed and homogenized as described above. Homogenates were centrifuged for 15 min at 12000 *g*, followed by centrifugation of the resulting supernatant for 60 min at 105000 *g*. The final supernatant was employed as cytosolic fraction, whereas the pellet (microsomal fraction) was resuspended in buffer [containing 250 mM-sucrose, 10 mM-Tris/HCl (pH 7.4), 1 mM-EDTA and 10 mM-NaF] at a final concentration of 5 mg of protein/ml.

Choline kinase activity was determined in the cytosolic fraction essentially as described by Weinhold & Rethy [18]. Labelled phosphocholine was separated from choline by paper chromatography (Whatman 3 MM), with ethanol/propanol/conc. NH_3 (13:4:7, by vol.) as developing solvent.

CTP:phosphocholine cytidylyltransferase activity was measured in cytosolic and microsomal fractions as described in [19], the only difference being the assay time (10 or 20 min). The cytosolic activity of CTP:phosphocholine cytidylyltransferase was determined in the absence as well as in the presence of lipid (oleate/rat liver phospholipid) vesicles [20].

The activity of cholinephosphotransferase was measured in the microsomal fraction with CDP-[methyl- ^{14} C]choline and endogenous diacylglycerol as substrates [21]. Incubations were carried out for 8 min, and formation of radioactive PC was quantified by the filter-disc method as described by Goldfine [22].

Quantification of the amount of CTP:phosphocholine cytidylyltransferase

Antiserum against a synthetic peptide fragment (sequence between amino acids 164 and 176, according to ref. [23]) of rat liver CTP:phosphocholine cytidylyltransferase was raised in rabbits and subjected to affinity purification with Protein A-Sepharose CL 4B and BSA-peptide conjugated to CNBr-activated Sepharose 4B (H. Jamil & D. E. Vance, unpublished work). To quantify the amount of cytosolic cytidylyltransferase in rat liver, a 105000 *g* supernatant was prepared as described above, and antibody-antigen titrations were carried out using a fixed amount of the polyclonal antibody with variable amounts of 105000 *g* supernatant. The mixture of antibody and antigen

was incubated at 4 °C for 18 h (final volume 200 μ l), followed by addition of 25 μ l of Protein A-Sepharose suspension, prolonged incubation at 4 °C for 1 h and removal of the antibody-antigen complex by centrifugation, as published by Rooney *et al.* [24]. Cytidylyltransferase activity was then assayed by using 50 μ l of the supernatant. Units of enzyme activity (pmol/min) remaining after immunoprecipitation were plotted against the units originally added, and the equivalence point (amount of enzyme removed by the antibody) was determined [24].

Chemical analyses

Lipids were extracted and phospholipids separated as described above (see 'Studies *in vivo*'). Diacylglycerol was further purified by t.l.c. on silica gel G with light petroleum (b.p. 40–60 °C)/diethyl ether/acetic acid (35:15:1, by vol.) as eluent. Next it was extracted from the silica, hydrolysed and determined by a modification of the procedure of Wieland [25] as published [26].

The water-soluble PC precursors choline, phosphocholine and CDP-choline were extracted and subsequently separated by h.p.l.c., followed by a sensitive chemical analysis, exactly as described by Tijburg *et al.* [27].

Protein was determined as described by Lowry *et al.* [28], with BSA as standard.

Expression of results

Results are presented as means $\pm$ S.D. of the numbers of experiments indicated. Significance was determined by Student's *t* test.

RESULTS

PC synthesis *in vivo* after partial hepatectomy

In response to 70 % partial hepatectomy, most of the hepatocytes in the liver remnant start to divide within 30 h after surgery [1,2]. Cell proliferation involves biosynthesis of new membranes, and thus of new membrane components. Fig. 1(a) shows the levels of PC in rat liver as a function of time after partial hepatectomy or sham operation. The increase in PC mass after partial hepatectomy correlated well with the overall restoration of liver mass (results not shown), whereas sham operation caused no significant change in phospholipid levels. As clearly indicated by Fig. 1(b), this increase in PC mass was also reflected by enhanced incorporation *in vivo* of [^{3} H]choline into

Table 1. Effect of partial hepatectomy on the enzyme activities in the CDP-choline pathway at different times after surgery

At the indicated times after partial hepatectomy (PH) or sham operation (SO), subcellular fractionation of rat liver and subsequent determination of the activities of choline kinase, CTP:phosphocholine cytidyltransferase (CPCT) and cholinephosphotransferase were carried out. CPCT values represent the summation of cytosolic and microsomal fractions. Choline kinase and cholinephosphotransferase activity were only measured in the cytosolic and microsomal fraction, respectively. CTP:phosphocholine cytidyltransferase and cholinephosphotransferase activities have been adjusted for recovery of glucose-6-phosphatase activity [29] in the microsomes, which was approx. 30 % in preparations from either partially hepatectomized or sham-operated animals. Control ($t = 0$) values of the three enzyme activities were 79.3 ± 4.1 , 55.2 ± 2.5 and 32.4 ± 1.5 nmol/min per g of liver respectively. Values are expressed as means $\pm$ S.D. for n rats. Significantly different from sham-operated rats: * $P < 0.001$; † $P < 0.01$; ‡ $P < 0.05$.

Treatment	Time after surgery (h)...	Activity (nmol/min per g of liver)			
		4	22	48	96
Choline kinase ($n = 3$)	SO	75.7 ± 1.6	89.5 ± 6.5	82.1 ± 6.1	84.3 ± 3.9
	PH	80.1 ± 12.6	91.2 ± 2.0	$107.9 \pm 5.4^\dagger$	$95.6 \pm 2.8^\dagger$
CPCT ($n = 5$)	SO	62.6 ± 5.6	63.8 ± 7.1	52.6 ± 5.6	56.4 ± 6.1
	PH	70.0 ± 3.9	$95.8 \pm 9.1^*$	$66.6 \pm 3.2^\dagger$	62.3 ± 3.3
Cholinephosphotransferase ($n = 3$)	SO	33.9 ± 0.3	30.4 ± 0.2	32.4 ± 1.0	32.2 ± 4.9
	PH	30.1 ± 3.7	28.4 ± 2.2	29.4 ± 1.6	30.6 ± 1.9

PC at 4, 22 and 48 h after partial hepatectomy, as compared with incorporation after sham operations. A pronounced stimulation (2.6-fold increase) of [^3H]choline incorporation occurred at 22 h after partial hepatectomy (Fig. 1b), i.e. just before the first wave of DNA synthesis reached its optimum [1,2]. A similar 2-fold increase in incorporation of [^3H]choline into PC was observed *in vitro* with hepatocytes isolated at 22 h after partial hepatectomy, relative to hepatocytes isolated from sham-operated animals (results not shown). It may be noted (see Fig. 1b) that, apparently caused by the surgical stress, the rate of [^3H]choline incorporation was also elevated at the 4 h time point of sham-operated animals as compared with non-operated animals ($t = 0$ h).

Enzyme activities in the CDP-choline pathway of PC biosynthesis

The results presented in Table 1 show that the activities of both choline kinase (at 48 and 96 h after surgery) and CTP:phosphocholine cytidyltransferase (at 22 and 48 h after surgery) were enhanced in liver remnants of partially hepatectomized rats, relative to livers from sham-operated rats. Choline kinase activity was clearly elevated at 48 h after partial hepatectomy (31 % increase) and cytidyltransferase activity at 22 h after partial hepatectomy (50 % increase). It should be noted that the data in Table 1 represent total (cytosolic+microsomal) activity of the cytidyltransferase per g of liver. Cholinephosphotransferase, the terminal enzyme in the CDP-choline pathway, showed no significant changes during the first 4 post-operative days.

There is substantial evidence from studies with a number of cell types that activation of CTP:phosphocholine cytidyltransferase is commonly caused by its translocation from cytosol to endoplasmic reticulum [7,8]. Therefore, we measured CTP:phosphocholine cytidyltransferase activity in both microsomal and cytosolic fractions. Fig. 2 demonstrates that the enhanced activity of CTP:phosphocholine cytidyltransferase, as observed at 22 h after partial hepatectomy, was caused by an increased activity in the microsomal (Fig. 2a) as well as in the cytosolic (Fig. 2b) fraction. Rather than exclusively pointing to an intracellular translocation of the enzyme, the data of Fig. 2 suggest that the augmented cytidyltransferase activity was, at least in part, due to an increase in the amount of enzyme molecules. This led us to quantify the cytosolic amount of cytidyltransferase protein, using antibodies directed against a distinct amino acid sequence in the rat liver enzyme.

Fig. 2. Effect of partial hepatectomy on the activity of CTP:phosphocholine cytidyltransferase during the first 4 post-operative days

Enzyme activities were measured in livers of sham-operated (○) or partially hepatectomized (●) rats. CTP:phosphocholine cytidyltransferase activity was determined in the microsomal fraction (a) and in the cytosolic fraction (b), in either the absence (---) or the presence (—) of lipid (oleate/rat liver phospholipid) vesicles. Each time point represents the mean $\pm$ S.D. of five different rats. Significantly different from sham-operated rats: * $P < 0.001$; ** $P < 0.01$.

Immunotitration of cytosolic CTP:phosphocholine cytidyltransferase

The results of such immunotitrations, using a fixed amount of antibody and variable amounts of liver cytosol, are shown in Fig. 3; both the titration values obtained with liver cytosol from partially hepatectomized rats (30 h after surgery) and the corresponding values obtained with cytosol from sham-operated rats can be considered as located on virtually the same straight line. In other words, the amount of cytidyltransferase activity removed by the antibody (= equivalence point) was nearly the same: 353 ± 23 and 371 ± 18 pmol/min precipitated by $4 \mu\text{l}$ of antibody for liver cytosol from sham-operated and partially

Fig. 3. Equivalence-point determination by immunotitration

A fixed amount of antibody (4 μ l, corresponding to 1.05 μ g of protein) was incubated with increasing amounts of cytosol from sham-operated (○) or partially hepatectomized (●) rats, after which CTP:phosphocholine cytidyltransferase activity was assayed in the presence of lipid vesicles, as described in the Experimental section. Each data point represents the mean $\pm$ S.D. of four separate experiments with different animals. The specific activity (pmol/min per μ g of cytosolic protein) of cytidyltransferase was normalized to allow comparison of the data sets. The equivalence point, units of cytidyltransferase (pmol/min) immunoprecipitated, is the intercept on the abscissa when the initial (added) versus the final (recovered) units of enzyme activity are plotted by using linear regression. Instead of drawing the two plots, their equations are shown.

hepatectomized rats respectively. In these experiments partial hepatectomy increased the specific activity of cytosolic cytidyltransferase, assayed in the presence of lipid vesicles, by 52% relative to sham operations (results not shown) and increased the amount of activity immunoprecipitated by about 5% (see the above-mentioned equivalence points). Because a fixed amount of antibody should react with the same amount of antigen (regardless of its origin from livers of either hepatectomized or sham-operated animals), these data show that the increase in cytosolic cytidyltransferase activity induced by partial hepatectomy was mainly due to an increase in enzyme mass rather than to increased activation of pre-existing enzyme molecules. Whether a similar increase in enzyme mass accounts for the stimulation of microsomal cytidyltransferase activity (Fig. 2a) requires further investigation. It may be noted that

phosphorylation of the cytidyltransferase had no effect on its immunoprecipitation (H. Jamil & D. E. Vance, unpublished work), and that the increase in cytosolic cytidyltransferase activity was also observed when the assays were performed in the absence of lipid vesicles (results not shown).

Effect of partial hepatectomy on the amount of precursors of PC at different times after surgery

If partial hepatectomy stimulates the CDP-choline pathway via a stimulation of the cytidyltransferase-catalysed reaction, one would expect to find lower phosphocholine levels in livers from partially hepatectomized rats than in livers from sham-operated rats. Table 2 indeed shows that the pool size of phosphocholine decreased 1.4- and 1.9-fold at 22 and 48 h after partial hepatectomy respectively. On the other hand, there was no significant change in the amount of choline, and the pool size of CDP-choline was only slightly enhanced ($P < 0.02$, 48 h after partial hepatectomy). Furthermore, Table 2 shows that, in liver homogenates from partially hepatectomized rats, diacylglycerol levels were not significantly increased at the indicated time points, relative to homogenates from sham-operated animals.

DISCUSSION

In the present study we investigated the effect of partial hepatectomy on the biosynthesis of PC, one of the major membrane phospholipids in liver. PC synthesis *in vivo*, as measured by [^{3} H]choline incorporation, was found to be enhanced (Fig. 1b), in line with the restoration of PC mass after partial hepatectomy (Fig. 1a). The increase of [^{3} H]choline incorporation at 4 h after surgery in both the partially hepatectomized and sham-operated rats was probably due to stress factors, such as an increased influx of fatty acids, which are known to stimulate PC synthesis [20,30]. A similar phenomenon was reported previously for phosphatidate phosphohydrolase in regenerating liver at 6 h after surgery [31].

As to the effect of partial hepatectomy on the CDP-choline pathway, several parameters were studied. CTP:phosphocholine cytidyltransferase is generally acknowledged as the rate-limiting enzyme in this anabolic pathway [6,7], although some studies have been published demonstrating a correlation between the activity of choline kinase and the rate of PC synthesis [3,9,10]. The activity of the latter enzyme was increased at 48 and 96 h after partial hepatectomy (Table 1). This increase in activity may explain the quick replenishment of the phosphocholine pool size

Table 2. Effect of partial hepatectomy on the amount of precursors of PC at different times after surgery

Choline and choline metabolites were separated and quantified as described in the Experimental section. Each value represents the mean $\pm$ S.D. for n rats. Abbreviations: n.d., not determined; PH, partial hepatectomy; SO, sham operation. The amounts of precursors in control rats were 0.18 ± 0.01 (choline), 1.72 ± 0.17 (phosphocholine), 0.13 ± 0.01 (CDPcholine) and 0.25 ± 0.05 (diacylglycerol) μ mol/g of liver. Significantly different from sham-operated rats: * $P < 0.01$; † $P < 0.02$.

Treatment	Time after surgery (h)...	Amount (μ mol/g of liver)			
		4	22	48	96
Choline ($n = 3$)	SO	0.23 ± 0.02	0.25 ± 0.01	0.27 ± 0.03	0.22 ± 0.02
	PH	0.27 ± 0.01	0.34 ± 0.08	0.24 ± 0.05	0.30 ± 0.04
Phosphocholine ($n = 3$)	SO	1.54 ± 0.21	1.62 ± 0.13	1.70 ± 0.15	1.88 ± 0.08
	PH	1.38 ± 0.02	$1.14 \pm 0.05^*$	$0.90 \pm 0.05^*$	1.62 ± 0.16
CDP-choline ($n = 3$)	SO	0.13 ± 0.01	0.11 ± 0.01	0.11 ± 0.01	0.12 ± 0.01
	PH	0.15 ± 0.01	0.15 ± 0.01	$0.16 \pm 0.02^\dagger$	0.13 ± 0.01
Diacylglycerol ($n = 4$)	SO	0.25 ± 0.05	0.22 ± 0.03	0.20 ± 0.04	n.d.
	PH	0.30 ± 0.06	0.25 ± 0.03	0.25 ± 0.06	n.d.

seen between 48 and 96 h after partial hepatectomy (Table 2). However, it seems plausible that the increase in choline kinase activity was not a major factor for the activation of PC synthesis, because incorporation of [^3H]choline into PC was already decreasing to the levels found in sham-operated rats at these time points after surgery (Fig. 1*b*). On the other hand, the cytidyltransferase activity was clearly stimulated (50%) at 22 h after partial hepatectomy (Table 1). Evaluation of the water-soluble precursors of PC showed that, in line with the elevated activity of the cytidyltransferase, the pool size of phosphocholine was decreased (Table 2). It may be argued that this decrease in pool size of phosphocholine could also account for the enhanced incorporation of [^3H]choline into PC *in vivo*, as shown in Fig. 1*b*). However, if assuming a hepatectomy-induced increase in specific radioactivity of phosphocholine in the liver (22 h after surgery) by a factor 1.62/1.14 (Table 2), this could still explain only partially the 2.6-fold increase in [^3H]choline incorporation observed at this time point (Fig. 1*b*).

A number of mechanisms for activation of CTP:phosphocholine cytidyltransferase have been proposed, such as activation by dephosphorylation [32–36], by non-esterified fatty acids [20,30], by diacylglycerol [20] and by phospholipids [20,37,38]. Yet the activity of this transferase is thought to be mainly regulated by redistribution of the enzyme between the cytosolic and the membranous fractions [7,8], possibly being modulated by one or more of the above activation mechanisms or, as recently suggested [39], by a feedback effect of the microsomal PC concentration. However, after partial hepatectomy (22 and 48 h after surgery) CTP:phosphocholine cytidyltransferase activity in both the cytosolic and microsomal fractions was increased (Fig. 2). By inference, this increased activity of CTP:phosphocholine cytidyltransferase might be considered to be at least partially due to an increase in the number of enzyme molecules. This line of thinking is strongly supported by the immunotitration data (Fig. 3) obtained with a specific antibody against CTP:phosphocholine cytidyltransferase. In fact, most of the hepatectomy-induced stimulation of cytidyltransferase activity in the cytosolic fraction can be explained in terms of enzyme induction. In preliminary experiments using hepatocytes isolated from partially hepatectomized rats, the incorporation of [^{35}S]methionine into cytosolic cytidyltransferase was increased as compared with cells from sham-operated rats (results not shown). This indicates that the increased level of cytidyltransferase is indeed due to enhanced synthesis. To the best of our knowledge, enhanced synthesis of CTP:phosphocholine cytidyltransferase in liver is a novel finding and has not been reported previously (cf. [7,8,24,40]).

In conclusion, the increase in PC mass in regenerating liver is in line with an increased incorporation of choline into PC. The activities of two enzymes involved in PC biosynthesis (choline kinase and CTP:phosphocholine cytidyltransferase) were indeed enhanced after partial hepatectomy. Interestingly, our results also indicate that stimulation of PC synthesis at the level of cytosolic CTP:phosphocholine cytidyltransferase activity in regenerating liver is mainly due to the synthesis of new enzyme molecules.

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REFERENCES

- Grisham, J. W. (1962) *Cancer Res.* **22**, 842–849
- McGowan, J. A. & Fausto, N. (1978) *Biochem. J.* **170**, 123–127
- Warden, C. H. & Friedkin, M. (1984) *Biochim. Biophys. Acta* **792**, 270–280
- Pelech, S. L., Paddon, H. B. & Vance, D. E. (1984) *Biochim. Biophys. Acta* **795**, 447–451
- Kolesnick, R. N. (1987) *J. Biol. Chem.* **262**, 14525–14530
- Vance, D. E. & Pelech, S. L. (1984) *Trends Biochem. Sci.* **9**, 17–20
- Pelech, S. L. & Vance, D. E. (1984) *Biochim. Biophys. Acta* **779**, 217–251
- Sleight, R. & Kent, C. (1983) *J. Biol. Chem.* **258**, 831–835
- Warden, C. H. & Friedkin, M. (1985) *J. Biol. Chem.* **260**, 6006–6011
- Nishijima, M., Kuge, O., Maeda, M., Nakano, A. & Akamatsu, Y. (1984) *J. Biol. Chem.* **259**, 7101–7108
- Ide, H. & Weinhold, P. A. (1982) *J. Biol. Chem.* **257**, 14926–14931
- Lim, P., Cornell, R. & Vance, D. E. (1986) *Biochem. Cell Biol.* **64**, 692–698
- Higgins, G. M. & Anderson, R. M. (1931) *Arch. Pathol.* **12**, 186–202
- Tijburg, L. B. M., Houweling, M., Geelen, M. J. H. & van Golde, L. M. G. (1989) *Biochem. J.* **257**, 645–650
- Gove, C. D. & Hems, D. A. (1978) *Biochem. J.* **170**, 1–8
- Sundler, R., Åkesson, B. & Nilsson, Å. (1974) *J. Biol. Chem.* **249**, 5102–5107
- Bartlett, G. R. (1959) *J. Biol. Chem.* **234**, 466–468
- Weinhold, P. A. & Rethy, V. B. (1974) *Biochemistry* **13**, 5135–5141
- Vance, D. E., Pelech, S. L. & Choy, P. C. (1981) *Methods Enzymol.* **71**, 576–581
- Cornell, R. & Vance, D. E. (1987) *Biochim. Biophys. Acta* **919**, 26–36
- Groener, J. E. M., Klein, W. & van Golde, L. M. G. (1979) *Arch. Biochem. Biophys.* **198**, 287–295
- Goldfine, H. (1966) *J. Lipid Res.* **7**, 146–149
- Kalmar, G. B., Kay, R. J., Lachance, A., Aebbersold, R. & Cornell, R. B. (1990) *Proc. Natl. Acad. Sci. U.S.A.* **87**, 6029–6033
- Rooney, S. A., Smart, D. A., Weinhold, P. A. & Feldman, D. A. (1990) *Biochim. Biophys. Acta* **1044**, 385–389
- Wieland, O. (1984) in *Methods of Enzymatic Analysis* (Bergmeyer, H. U., ed.), 3rd edn., vol. 6, pp. 504–510, Verlag Chemie, Weinheim
- Tijburg, L. B. M., Schuurmans, E. A. J. M., Geelen, M. J. H. & van Golde, L. M. G. (1987) *Biochim. Biophys. Acta* **919**, 49–57
- Tijburg, L. B. M., Houweling, M., Geelen, M. J. H. & van Golde, L. M. G. (1988) *Biochim. Biophys. Acta* **959**, 1–8
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Morré, D. J. (1971) *Methods Enzymol.* **22**, 130–148
- Pelech, S. L., Pritchard, P. H., Brindley, D. N. & Vance, D. E. (1983) *J. Biol. Chem.* **258**, 6782–6788
- Mangiapan, E. H., Lloyd-Davies, K. A. & Brindley, D. N. (1973) *Biochem. J.* **134**, 103–112
- Pelech, S. L., Pritchard, P. H. & Vance, D. E. (1981) *J. Biol. Chem.* **256**, 8283–8286
- Pelech, S. L. & Vance, D. E. (1982) *J. Biol. Chem.* **257**, 14198–14202
- Radika, K. & Possmayer, F. (1985) *Biochem. J.* **232**, 833–840
- Sanghera, J. S. & Vance, D. E. (1989) *J. Biol. Chem.* **264**, 1215–1223
- Watkins, J. D. & Kent, C. (1990) *J. Biol. Chem.* **265**, 2190–2197
- Chu, A. J. & Rooney, S. A. (1985) *Biochim. Biophys. Acta* **834**, 346–356
- Feldman, D. A., Rounsifer, M. E. & Weinhold, P. A. (1985) *Biochim. Biophys. Acta* **429**, 429–437
- Jamil, H., Yao, Z. & Vance, D. E. (1990) *J. Biol. Chem.* **265**, 4332–4339
- Tijburg, L. B. M., Geelen, M. J. H. & van Golde, L. M. G. (1989) *Biochim. Biophys. Acta* **1004**, 1–19