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Choline-Lipid Release from Normal and Transformed Cells

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Abstract: The effect of albumin on phosphatidylcholine (PC) metabolism in Hep-G2, 3T3-H.ras, and 3T3 cells pre-labelled with [*Me*-^{3}H]choline was studied. The [^{3}H]choline was more efficiently taken up and incorporated into cellular phospholipids in 3T3-H.ras cells than in Hep-G2 and 3T3 cells. In each of the three cell lines, most of the [^{3}H]choline metabolized into the phospholipids was incorporated into PC and only minor was incorporated into lysophosphatidylcholine (LPC). Bovine serum albumin stimulated the release of [^{3}H]LPC and [^{3}H]PC from each of the three cell lines pre-labelled with [^{3}H]choline. [^{3}H]PC was also released in the absence of albumin but [^{3}H]LPC was not. The efficiency of LPC secretion represented as the proportion of medium [^{3}H]LPC to cellular [^{3}H]choline lipid during a chase period is approximately 9 to 14 times greater in 3T3 cells compared with the transformed 3T3-H.ras and Hep-G2 cells. A similar comparison of published data for rat hepatocytes with Hep-G2 shows secretion to be 35~75 times greater from the rat hepatocytes than from Hep-G2. Also, PC secretion from 3T3 cells was 1.6 times more effective than from 3T3-H.ras, whereas rat hepatocytes secrete PC 2.8~3.8 times more effectively than does Hep-G2. The measurement of specific radioactivity of cellular PC in pre-labelled 3T3 cells showed it to be similar to that of the secreted PC. However, the specific radioactivity of secreted LPC was markedly lower than that of the cellular PC, which suggests that LPC is being secreted from a PC pool distinct from that used for PC secretion.

Key words: albumin, lysophosphatidylcholine, phosphatidylcholine.

Phosphatidylcholine (PC) is the major membrane phospholipid class in mammalian cells and has a number of roles in the cell. As an example, the lipid form of PC plays a structural role in membrane. On the contrary, LPC generated by the hydrolysis of a fatty acid from PC also occurs naturally in mammalian tissues at very much lower levels than PC, although LPC is a significant component of blood plasma. PC is synthesized from the taken up choline via the CDP-choline pathway (Kent, 1990). PC biosynthesis is mostly regulated by the CTP-phosphocholine cytidylyltransferase. The enzyme becomes active when it translocates from cytosol to endoplasmic reticulum membrane, which might be stimulated by free fatty acid. PC may also be synthesized by methylation of phosphatidylethanolamine (PE) with S-adenosylmethionine. Vance and Vance showed in rat hepatocytes that the PC pool synthesized from the CDP-choline pathway is preferred for PC secretion based on the observation that the specific radioactivity of PC derived from [*Me*-^{3}H]choline was approximately equal in cells and medium (Vance and Vance, 1986). They also showed that although PC synthesized by the methylation of PE derived from ethanolamine is not preferred for secretion, PC synthesized by the methylation of PE derived from serine is a preferred source of PC for secretion. This indicates that random and homogeneous PC pools are not selected for PC secretion. Instead, a specific pool of PC molecular species is selected for PC turnover into LPC (Mangiapane and Brindley, 1986). It has long been known that certain hormones, growth factors, and phorbol esters stimulate both PC synthesis (Kent, 1990; Warden and Friedkin, 1984, 1985) and PC hydrolysis (Besterman *et al.*, 1986; Kolesnick, 1987) in 3T3 cell lines and other cells. These factors thereby increase the PC turnover rate. Since PC degradation can lead to the release of arachidonic acid and diacylglycerol, the PC turnover cycle is recognized as a potential source of these second messengers during signal transduction by a hormone (Pelech and Vance, 1989; Exton, 1990).

At present it is unknown whether LPC, generated in the PC turnover cycle, may play any role in signal

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transduction. However, LPC seems to play important physiological roles either in the form of cellular LPC or as plasma LPC. Although cellular LPC constitutes less than 2% of the total phospholipids in the membranes of most cells, it is present in significant amounts in plasma (Gjone *et al.*, 1959). The circulating plasma LPC exerts several important physiological effects such as platelet aggregation (Besterman and Gillett, 1971), erythrocyte agglutination (Maraikovsky *et al.*, 1976) and cardiac arrhythmia (Man and Choy, 1982). Plasma LPC is mostly produced in the plasma by the action of lecithin-cholesterol acyltransferase (Glomset, 1968). Since LPC is readily taken up by cells, the circulating plasma LPC may be regarded as a source of fatty acid and choline by extravascular tissues (Graham *et al.*, 1988a). However, plasma LPC has also been shown to originate directly from liver in addition to being generated by plasma lecithin-cholesterol acyltransferase (Sekas *et al.*, 1985). It has been reported that albumin stimulates the release of LPC from isolated perfused rat liver and monolayer cultures of rat hepatocyte (Graham *et al.*, 1988b; Baisted *et al.*, 1988; Robinson *et al.*, 1989; Graham *et al.*, 1991). The released LPC from the liver and hepatocyte appear to be formed by the action of phospholipase A1 or A2 on cellular PC. This hepatic LPC is predominantly unsaturated, which is markedly dissimilar from the highly saturated LPC synthesized by plasma lecithin-cholesterol acyltransferase activity in plasma. In previous works, it was proposed that LPC secreted from liver is a major source of plasma lysophosphatidylcholine due to the fact that LPC concentration in the plasma remains relatively high even in patients suffering from a deficiency of lecithin-cholesterol acyltransferase (Norum and Gjone, 1967).

One of the purposes of this research is to test if albumin-stimulated secretion of LPC is unique to hepatocytes, which has been reported already (Baisted *et al.*, 1988), or is a phenomenon which might be displayed by undifferentiated and also transformed cells which have an active lipid metabolism. In addition, since Vance has already shown that the PC secreted by hepatocytes originates from a selected cellular PC pool, the second objective of this research is to compare the specific radioactivities of cellular and secreted PC and LPC pools generated from [*Me*-^{3}H]choline. Such a comparison might enable us to establish whether the secreted LPC has an origin different from that selected for PC secretion. In previous studies, it was proposed that the release of LPC from rat hepatocytes stimulated by albumin results from the ability of albumin to bind to cellular LPC located in the plasma membrane. Cancer cells show different and decreased amounts of glycoprotein and glycolipids in the cell membranes compared with host cells (Fishman and Brady, 1976; Meyer and Burger, 1977). Differences are also observed in membrane-bound proteolytic enzymes and the cell skeleton. However, tests so far show that the phospholipids are unchanged in cancer-transformed cells. Comparisons of the rates of LPC secretion among 3T3, 3T3-H.ras, Hep-G2 and the previously studied rat hepatocytes might reveal differences which correlate with the difference in their plasma membrane structures resulting from the transformations.

Materials and Methods

Materials

Fetal calf serum was purchased from HyClone Laboratories Inc. (Logan, USA). Powdered media and other miscellaneous chemicals were purchased from the Sigma Chemical Co. (St. Louis, USA). [*Me*-^{3}H]choline chloride (specific radioactivity 76 Ci/mmol) was obtained from the Amersham Corporation (Arlington Heights, USA). Coomassie brilliant blue G-250 reagent was from Bio-Rad Laboratories (Richmond, USA). Cell culture plastics used in this study was Falcon brand (Becton Dickinson Co., Franklin Lakes, USA).

Cell lines

All the cell lines used in this study were kindly provided by Dr. David Barnes, Department of Biochemistry and Biophysics, Oregon State University. The human hepatocellular carcinoma cell line Hep-G2 and mouse embryo fibroblast BALB/3T3 clone A31 cell line (3T3) were from the American Type Culture Collection (ATCC) cell bank. The 3T3-H.ras line was prepared from plasmid pUCEJ6.6 which contained the activated H.ras gene under the control of the endogenous promotor.

Incubation of cells and incorporation of [*Me*-^{3}H] choline

Hep-G2 cells were grown in Dulbecco's modified Eagle's medium HAMS : MEM (1 : 1), pH 7.4, containing 1.7 mM insulin, and 10% fetal calf serum. 3T3 and 3T3-H.ras cells were grown in HAMS : MEM (1 : 9), pH 7.4, containing 10% fetal calf serum. To label the cells with [*Me*-^{3}H]choline, equal numbers (1 to 1.5×10^6) of cells were dispersed into plastic culture dishes in 2 ml of the medium to give a subconfluent layer. Before labelling, the cells were maintained overnight in the choline- and methionine-free medium to deplete the cellular choline and phosphocholine pools. Each dish of cultured cells was incubated with 2 ml of choline-free medium that contained 10 μCi of [*Me*-^{3}H] choline chloride after washing with 2 ml of minimal

medium to remove detached cells. After 1 h the cells were washed twice with the chase medium, which was composed of MEM : HAMS (1 : 1) containing unlabelled 28 μM choline. The labelled cells were subsequently incubated for up to 4 h with 2 ml of the chase medium. The chase medium was either albumin-free as a control or contained 5 mg/ml of fatty acid-free bovine serum albumin for the experimental runs. At the end of the incubation period, the medium was removed and the monolayer of cells was washed with 1 ml of PBS. The medium and cell washings were combined, and centrifuged at 10000×*g* for 20 min to remove detached cells and cell debris. The supernatant was used for extraction and analysis of separated lipids. The cells were scraped from the dishes in 2 ml of distilled water with a cell scraper. The cell suspension was sonicated for 30 sec and, after removal of 50 μl for protein analysis, was used for extraction and analysis of lipids.

Extraction and analysis of cell and medium lipid

Lipid was extracted from sonicated cell preparations and centrifuged media by adding 3 volumes of chloroform/methanol (2 : 1, v/v) containing the antioxidant 2,6-di-*t*-butyl-4-methylphenol (50 mg/l). After centrifugation of the extract at 300×*g* for 1 h, the upper aqueous phase was removed and the lower chloroform phase was washed once with 4 ml of methanol/water (1 : 1, v/v). A portion of the lower chloroform phase (0.1 ml) containing lipids was evaporated to dryness under N_2 in a scintillation vial. The residue was dissolved in 4 ml of scintillation fluid (0.4%, w/v, of PPO in toluene : Triton X-100 : H_2O, 6 : 3 : 1, v/v/v) and radioactivity was measured with a Beckman liquid scintillation counter. The remainder of the lower chloroform phase containing phospholipids was evaporated to small volume and applied to a silica-gel 60 TLC plate, which was developed in the solvent system chloroform/methanol/acetic acid/water (50 : 30 : 8 : 3 by volume) for the separation of phospholipids. After the plate was air-dried, the radioactivity spots of PC and LPC were observed with I_2 vapor and by radioactivity scanning of the plate using a Bioscanner. The labeled PC and LPC zones were scraped from the TLC plate and transferred to pasteur pipetes that were plugged with a small cotton plug to retain the scrapings. The lipids were eluted from the SiO_2 scrapings with 15 ml of chloroform/methanol (2 : 1). The eluate was evaporated to a volume of 2 ml and an aliquot (0.1 ml) was used for radioactivity determination. The remaining 1.9 ml was used for phosphate assay.

Phosphate assay

The phosphate content of phospholipids was measured by a modified method (Chen *et al.*, 1956). The phospholipid samples isolated in chloroform and methanol mixture were dried by using N_2 gas. Concentrated perchloric acid (450 μl) was added to the dried sample and heated in a sand bath at 180℃. After 1 h, 2.5 ml distilled water, 0.5 ml 10% ascorbic acid, and 0.5 ml 2.5% ammonium molybdate were added and vortexed in order. The mixture was heated at 100℃ for 15 min. After cooling to room temperature, the absorbance at 820 nm was measured. Inorganic phosphate was used as the standard.

Protein determination

The amount of cellular protein was determined using Coomassie brilliant blue G-250. The sonicated cell suspension, 50 μl, was dissolved in 0.1 M NaOH, 0.1 ml, and then incubated at 55℃ for 10 min. After addition of 0.2 ml of the Coomassie reagent, the optical density was measured at 595 nm. Typically, the protein content of the cells in a culture dish was 280 to 380 μg/ml.

Results

Incorporation of [*Me*-^{3}H]choline into cellular phospholipid

After preincubation of cells with [*Me*-^{3}H]choline, continued incubation in the presence of cold choline (the chase period) allowed the cells to metabolize and secrete the newly generated lipids. The extracted lipids from the cells and the incubation media were separated by TLC. After the initial 1 h pulse with the labelled substrate, the percent radioactivity absorbed by 3T3, 3T3-H.ras and Hep-G2 cell line was 32.8± 0.6, 65.3± 0.4 and 51.3± 1.3, respectively (data not shown). In order to monitor the effect of albumin on the incorporation of radioactive choline, the radioactivities incorporated in the total cellular lipid, LPC and PC were measured (Fig. 1 and 2). The presence of albumin in the incubation medium during the chase period has no marked effect on the radioactivity in the total cellular lipid or in either cellular PC or LPC in any of the cell lines. Whereas Hep-G2 and 3T3 cells continue to accumulate [^{3}H]choline in their lipids during the 4 h chase, PC labelling in 3T3-H.ras cells is close to a plateau level during the chase (Fig. 2). In addition, most of the radioactivity was incorporated into PC in each of the three cell lines. So, it is not surprising then that the radioactivity incorporation patterns of total cellular [^{3}H]lipid from [^{3}H]choline in Fig. 1 reflects the radioactivity profiles of cellular [^{3}H]PC in Fig. 2. Cellular [^{3}H]LPC during the chase period shows some small accumulation but the presence of albumin has no significant effect in any of the three cell lines.

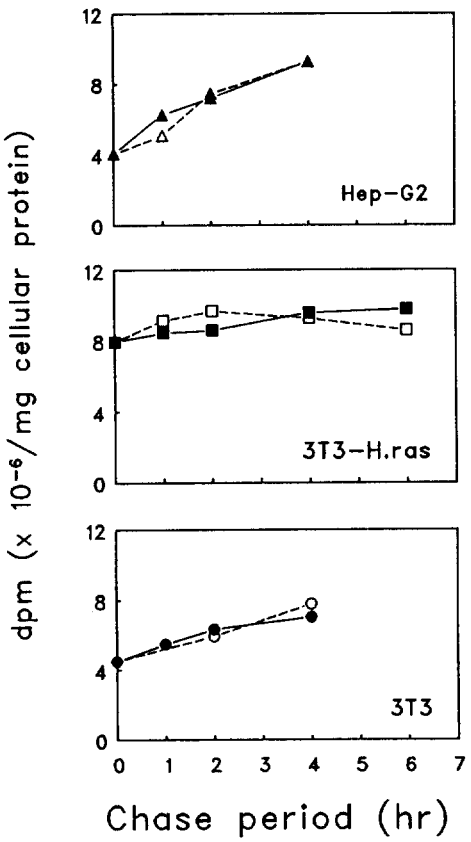

Fig. 1. Effect of albumin on the metabolism of cellular [^{3}H]lipids in three cell lines prelabelled with [*Me*-^{3}H]choline. Each point represents the mean of two or three dishes, and the ranges were less than 10% of the mean. Closed and open symbols represent the values from the incubation with and without albumin in the media, respectively.

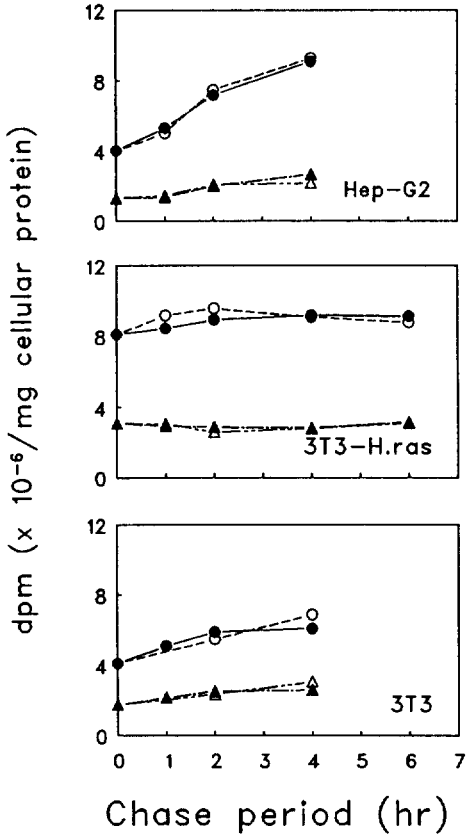

Fig. 2. Effect of albumin on the cellular [^{3}H]PC and [^{3}H]LPC radioactivity changes in three cell lines prelabelled with [*Me*-^{3}H] choline. Each point represents the mean of two or three dishes, and the ranges were less than 10% of the mean. Circles and triangles represents the values of PC and LPC, respectively. Closed and open symbols represent the values from the incubation with and without albumin in the media, respectively.

Release of lysophosphatidylcholine and phosphatidylcholine by albumin as a function of time

The most conspicuous result in this study was the difference in release of LPC into the albumin-containing medium from cultured 3T3, 3T3-H.ras, and Hep-G2 cells during the time course. In this experiment, bovine serum albumin, free of lipid and other small metabolites, was used to promote LPC secretion. Fig. 3 shows the influence of albumin on the release of total [^{3}H]choline lipids into the media of each of the three cell lines during the 4 h chase. It is clear that albumin has a stimulating effect upon this release of lipid with the effect being most marked for 3T3 cells. A time course of the albumin-mediated release of [^{3}H] LPC from each of the three cell lines is presented in Fig. 4. The most striking observation is that no [^{3}H] LPC is released into the medium from any of the three cell lines in the absence of albumin and that release of [^{3}H]LPC from the 3T3 cells is much greater than that from the transformed cell lines. Clearly, with albumin present, all three cell lines release [^{3}H]LPC but they do so with markedly different efficiencies. Table 1 compares these efficiencies by expressing them as ratios of the [^{3}H]LPC released during a 1 h interval divided by the [^{3}H]choline lipid present in the cell at the beginning of the interval. The results show that 3T3 secretes [^{3}H]LPC 9~14 times more effectively than that from 3T3-H.ras. In addition, the calculation shows that rat hepatocytes secrete LPC 35~75 times more effectively than do Hep-G2 cells. It is obvious that Hep-G2 is the least efficient in [^{3}H]LPC secretion among these cells while rat hepatocytes are the most efficient. Albumin also stimulates PC secretion into the chase medium (Fig. 5). It is clear that PC is secreted into the medium in each of the three cell lines in the absence of albumin. However, Hep-G2 secretes PC most effectively among the three cell lines tested. It is noteworthy that albumin is more stimulatory on the 3T3 and 3T3-H.ras cells than Hep-G2 cells. At 4 h of chase, PC secretion by albumin from Hep-G2 is increased about 25% whereas the increase is about 60% and 40% for 3T3-H.ras and 3T3 cells, respectively. Even so, the liver cell line is much more efficient

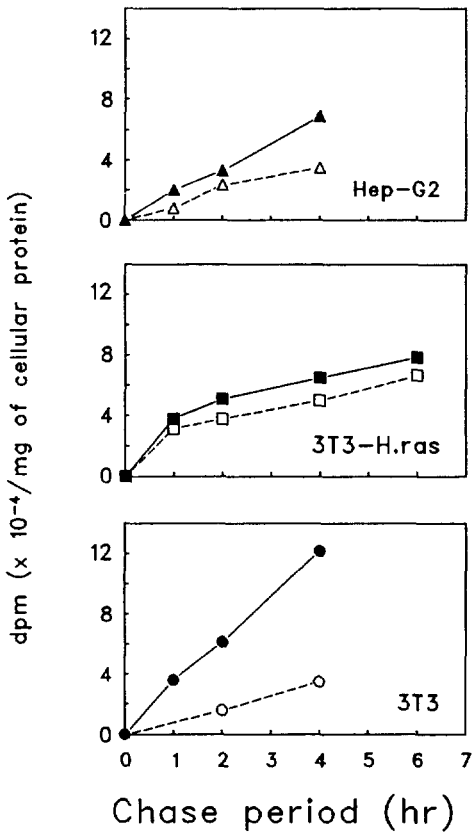

Fig. 3. Effect of albumin on the release of [^{3}H]lipids from three cell lines prelabelled with [*Me*-^{3}H]choline. Each point represents the mean of two or three dishes, and the ranges were less than 10% of the mean. Closed and open symbols represent the values from the incubation with and without albumin in the media, respectively.

at [^{3}H]PC secretion than the two fibroblast lines with or without albumin present. Table 2 shows that the transformed cells release [^{3}H]PC less effectively than nontransformed cells in the presence of albumin. Rat hepatocytes secrete PC 2.8~3.8 times more effectively than Hep-G2, whereas 3T3 cells secrete PC 1.6 times more effectively than 3T3-H.ras.

Lysophosphatidylcholine and phosphatidylcholine secretion pools

Comparison of the specific radioactivity of the cellular and secreted PC and LPC were measured by separation of the two lipids and quantitation of their [^{3}H] radioactivities and phosphate contents. Table 3 shows the specific radioactivities of cellular and medium PC and LPC, respectively. As we can see from the table, the specific radioactivity of cellular PC was approximately the same as that of secreted PC, although the medium LPC was markedly lower than that of cellular LPC or of cellular PC.

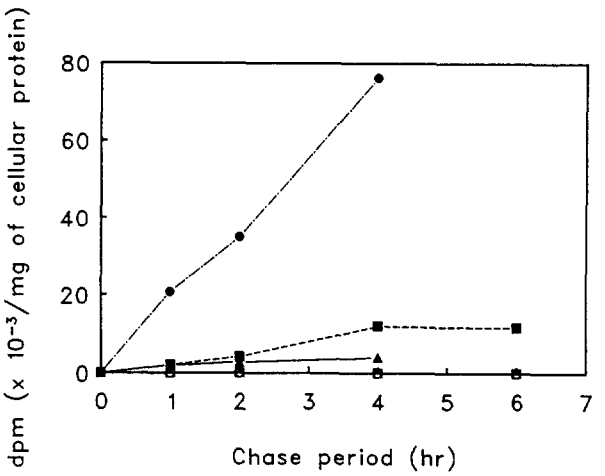

Fig. 4. Effect of albumin on the release of [^{3}H]LPC from three cell lines prelabelled with [*Me*-^{3}H]choline. Each point represents the mean of two or three dishes, and the ranges were less than 10% of the mean. Closed and open symbols represent the values from the incubation with and without albumin in the media, respectively. Triangles, rectangles and circles represent the values from Hep-G2, 3T3-H.ras and 3T3 cells, respectively.

Discussion

In this study, we compared LPC release from 3T3 cells with that from 3T3-H.ras cells. In addition, LPC release from Hep-G2 is compared with previously published results on the rat hepatocytes (Quinn *et al.*, 1988; Naderi *et al.*, 1989; Low *et al.*, 1983; Smith *et al.*, 1982; Love *et al.*, 1986; Triarhou and Herndon, 1986). Generally, choline uptaken was much more efficient in the transformed cell lines. In addition, it is clear that the 3T3-H.ras cells also incorporate the absorbed

Table 1. Comparison of the efficiencies of release of [^{3}H]LPC from 3T3, 3T3-H.ras, Hep-G2 and rat hepatocytes[a]

Incubation time	Medium LPC/CC in 3T3-H.ras (%)	Medium LPC/CC in 3T3 (%)	Medium LPC/CC in Hep-G2 (%)	Medium LPC/CC in rat hepatocytes[b] (%)
1	0.033± 0.001	0.462± 0.019	0.049± 0.000	1.71± 0.17
2	0.031± 0.007	0.273± 0.007	0.019± 0.009	1.43± 0.14

[a]The radioactivity ratios represent the released labelled LPC during a 1 h chase interval divided by the amount of labelled cellular choline-lipids present at the beginning of the interval. Each value of the ratios is the mean± S.D. of two or three analyses. CC means total cellular choline lipid.

[b]Calculated values from previously published data obtained under the same experimental conditions (Baisted *et al.*, 1988).

substrate into lipid more efficiently probably due to their high growth rate (Fig. 1). During the chase period, the [^{3}H] content of cellular PC and LPC increases in Hep-G2 and 3T3 cells but is relatively constant in 3T3-H.ras (Fig. 2). Since the cytidylyltransferase is the rate-limiting enzyme in mammalian PC synthesis, phosphocholine is the major metabolic pool in the pathway. Such differences in the size of the endogenous phosphocholine pools among the three cell lines would explain the higher incorporation of label into PC by 3T3-H.ras and also the steadily increasing label in PC during the chase period in Hep-G2 and 3T3.

After Sekas *et al.* reported that an isolated rat liver directly secreted LPC in the presence of albumin, LPC secretion from isolated rat hepatocytes by albumin was studied (Sekas *et al.*, 1985). All of the research showed that rat hepatocytes strongly secreted LPC into albumin-containing medium while there was no secretion of LPC in the absence of albumin. Curiously, Mangiapane and Brindley reported that bovine hepatocytes do not display the capacity to release LPC (Mangiapane and Brindley, 1986). Our result (Fig. 4) shows that not only Hep-G2 but also the two cell lines derived from fibroblasts, 3T3 and 3T3-H.ras, release LPC into the medium only in the presence of albumin. A comparison of the efficiency with which [^{3}H]LPC is released from the three cell lines shows that the amount of radioactive LPC appearing in the medium during a 1 h time period is a measure of the efficiency of release of this newly synthesized lipid. Clearly, the normal cell line, 3T3, is much more efficient compared with the transformed cells. Comparison of the Hep-G2 data with that calculated from a previously published work on rat hepatocytes also shows that the normal cell line releases [^{3}H]LPC more readily than does the transformed cell.

The mechanism for LPC secretion is not well understood yet. It is generated by phospholipase A activities which have been found in almost every mammalian cell (Bosch, 1980). However, many researchers have suggested that LPC arises mainly by the action of phospholipase A1 on PC because of the unsaturated nature of the secreted LPC (Sekas *et al.*, 1985; Graham *et al.*, 1988a; Graham *et al.*, 1988b; Baisted *et al.*, 1988; Robinson *et al.*, 1989; Graham *et al.*, 1991). A major site for the origin of the LPC-deacylating activity does not occur in the plasma membrane (Bosch, 1980). LPC so formed may then be sequestered into the medium by attachment to albumin or be reacylated to PC. The basis for the more efficient release of newly formed LPC from normal cells compared with transformed cells is not clear. As Mangiapane and Brindley pointed out, this LPC, which contains mostly unsaturated fatty acids, can supply other organs with choline and unsaturated fatty acids (Mangiapane and Brindley, 1986). We may assume that the tumor cell, in order to maintain rapid growth, might restrict the loss of materials for new membrane synthesis, such as choline and unsaturated fatty acids. However, it is not clear why 3T3, and particularly 3T3-H.ras cells, release PC into the medium even in the absence of albumin (Fig. 5), unless this PC is a component of a lipoprotein and the fibroblast may possess an active lipoprotein secretion pathway.

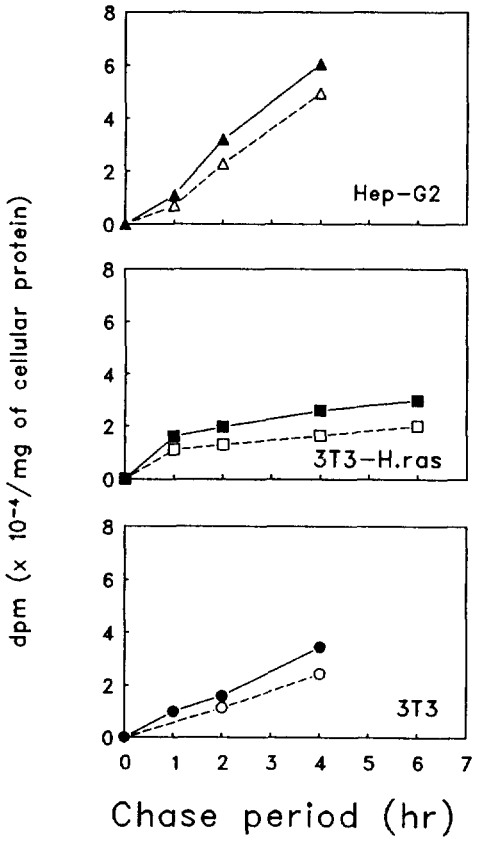

Fig. 5. Effect of albumin on the release of [^{3}H]PC from three cell lines prelabelled with [*Me*-^{3}H]choline. Each point represents the mean of two or three dishes, and the ranges were less than 10% of the mean. Closed and open symbols represent the values from the incubation with and without albumin in the media, respectively.

With regard to the cell surfaces of the three cell lines, it is likely that the topography of the cell surfaces plays a role in the sequestering of LPC by albumin. When 3T3 cells are transformed by oncogenic viruses or chemical carcinogens, the transformed cells have an altered and simpler oligosaccharide moiety of their complex ganglioside, an acidic glycolipid (Fishman and Brady, 1976). It has also been observed that the oligosaccharide moieties of tumor cell glycoproteins are different and simpler from those found in normal cell plasma

Table 2. Comparison of the efficiencies of release of [^{3}H]PC from 3T3, 3T3-H.ras, Hep-G2 and rat hepatocytes[a]

Incubation time	Medium PC/CC 3T3-H.ras (%)	in Medium PC/CC 3T3 (%)	in Medium PC/CC Hep-G2 (%)	in Medium PC/CC in rat hepatocytes[b] (%)
1	0.19± 0.01	0.30± 0.05	0.35± 0.05	1.33± 0.13
2	0.19± 0.02	0.32± 0.04	0.38± 0.01	1.07± 0.11

[a]The radioactivity ratios represent the released labelled PC during a 1 h chase interval divided by the amount of labelled cellular choline-lipids present at the beginning of the interval. Each value of the ratios is the mean± S.D. of two or three analyses. CC means total cellular choline lipid.
[b]Calculated values from previously published data obtained under the same experimental conditions (Baisted *et al.*, 1988).

Table 3. Specific radioactivity of medium and cellular [^{3}H]PC and [^{3}H]LPC of 3T3 cells[a]

Time (h)	Cellular PC (dpm/nmol)	Medium PC (dpm/nmol)	Cellular LPC (dpm/nmol)	Medium LPC (dpm/nmol)
0	$1.01(\pm 0.13)\times10^4$		$9.22(\pm 0.17)\times10^3$	
1	$1.39(\pm 0.09)\times10^4$	$1.06(\pm 0.63)\times10^4$	$1.20(\pm 0.14)\times10^4$	$5.28(\pm 0.41)\times10^3$
2	$1.52(\pm 0.04)\times10^4$	$1.13(\pm 0.26)\times10^4$	$1.36(\pm 0.15)\times10^4$	$7.58(\pm 0.09)\times10^3$
4	$1.44(\pm 0.06)\times10^4$	$1.34(\pm 0.15)\times10^4$	$1.31(\pm 0.13)\times10^4$	$8.41(\pm 1.28)\times10^3$

[a]Each value of specific radioactivity is the mean± S.D. of two or three analyses.

membranes (Meyer and Burger, 1977). Thus, the association of albumin with the cell surface may be affected in such a way that there is less effective binding of LPC from the transformed cell.

PC is secreted by the three cell lines regardless of the albumin presence (Fig. 5). In Hep-G2 and rat hepatocyte, the PC secretion was reported as a normal function of the cell in which PC is secreted as a component of lipoprotein. The PC secretion from 3T3 cells may result from the binding of albumin to PC in the plasma membrane. For this to occur, a tight association of albumin with the membrane is needed. Differences in the effective association of albumin with the cell surface between transformed and normal cells may also account for a less efficient release of newly-formed PC from the transformed cells (Table 2). Thus, both LPC and PC release may be governed by the same mechanism. If the PC that appears in the medium has a lipoprotein source, as is the case for Hep-G2, it is conceivable that there may be an enhanced lipoprotein secretion in response to albumin.

The specific radioactivity of cellular LPC in 3T3 cells was lower than that of cellular PC (Table 3). Since the pool size of LPC is always very small, it is clear that cellular LPC is generated from newly-synthesized and endogenous PC. More surprising is the observation that the specific radioactivity of the medium LPC was about 40% of that of the cellular LPC at 1 h of incubation and only increased to 66% after 4 h. Clearly, the pools of newly-synthesized PC and LPC are not the major sources for the LPC that is secreted, and this secretory pool only slowly equilibrates with the LPC generated from cellular PC. Mangiapane and Brindley reached the same conclusion concerning separate pools in LPC production. The specific radioactivity of PC made from [^{3}H]choline was approximately equal in 3T3 cells and the medium as shown in Table 1. This is not surprising considering the data reported from several studies on the rapid rate of equilibration of PC among microsomal, Golgi and plasma membranes of rat liver (Chang *et al.*, 1977). Other studies have shown a $t_{1/2}=2$ min at 25℃ for transfer of PC from the endoplasmic reticulum (the site of synthesis of PC) to the plasma membrane in Chinese hamster ovary cells (Kaplan and Simoni, 1985). It is clear that the pathways by which PC and LPC appear in the medium originate from distinctly different pools of cellular PC. In support of the existence of different pools of cellular PC, Vance and Vance have found that, in the case of lipoprotein secretion by cultured rat hepatocytes, there is a preference for PC made from choline rather than ethanolamine (Vance and Vance, 1986). In addition, from PC and phospholipid secretion using [^{3}H]serine, they concluded that there is no random or homogeneous labelling of phospholipid pools from radioactive precursors.

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