# Involvement of the lysosome in the catabolism of intracellular lysophosphatidylcholine and evidence for distinct pools of lysophosphatidylcholine

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**Abstract** The role of the lysosome in the metabolism of lysophosphatidylcholine was investigated in isolated rat hepatocytes. Chloroquine, primaquine, and ammonium chloride caused a 2.5-fold increase in radioactive lysophosphatidylcholine in [*methyl*-$^3$H]choline-labeled cells. This effect was confirmed by a 1.7-fold increase in lysophosphatidylcholine mass in chloroquine-treated hepatocytes. Chloroquine caused a 2.7-fold increase in radioactive lysophosphatidylethanolamine in [1-$^3$H]ethanolamine-labeled cells and a 2.3-fold increase in radioactive lysophosphatidylcholine in [*methyl*-$^3$H]methionine-labeled cells. Chloroquine did not affect formation of choline-containing aqueous metabolites or the level of radioactivity in phosphatidylcholine (PC). The effect of chloroquine on radioactive lysophosphatidylcholine accumulation was concentration-dependent and occurred within 10 min, consistent with rapid inhibition of lysosomal function. As there was no observed decrease in the $^3$H in PC, the accumulation of lysophosphatidylcholine was likely due to the inhibition of acid lysophospholipase activity in chloroquine-treated cells. The accumulation of lysophosphatidylcholine in the presence of chloroquine was observed in both short-term- (30 min) and equilibrium-(24 h) [*methyl*-$^3$H]choline-labeled cells. Simultaneous incubation of hepatocytes with both albumin and chloroquine increased the radioactivity in lysophosphatidylcholine in the medium independently of the accumulation of radioactive lysophosphatidylcholine in the cells. The results suggest that there are separate pools of lysophosphatidylcholine in the hepatocyte and that the pool donated to an extracellular acceptor is different from the lysosomal pool. This study is the first to suggest that lysosomes are involved in the catabolism of intracellular lysophosphatidylcholine in isolated rat hepatocytes.—**Hatch, G. M., A. Oskin, and D. E. Vance.** Involvement of the lysosome in the catabolism of intracellular lysophosphatidylcholine and evidence for distinct pools of lysophosphatidylcholine. *J. Lipid Res.* 1993. **34:** 1873–1881.

**Supplementary key words** chloroquine • lysophosphatidylethanolamine • phosphatidylcholine • lysophospholipase • brefeldin A • glycerophosphocholine • hepatocytes

Phosphatidylcholine (PC) is the most abundant phospholipid in mammalian tissues (1). Although the regulation of the biosynthesis of PC has been studied intensely, information on the mechanism of PC catabolism in intact cells has only recently begun to emerge (2). PC is catabolized to lysophosphatidylcholine (LPC) via the action of phospholipase(s) A (3). LPC can be further catabolized to glycerophosphocholine (GPC) via the action of lysophospholipase(s). It is generally accepted that GPC is the major catabolite of PC (4). These enzymatic reactions have been well characterized in isolated mitochondrial and microsomal fractions (3).

The involvement of isolated rat liver lysosomal fractions in the catabolism of exogenously added phospholipid is well documented. When rat liver microsomal membranes, containing labeled PC and phosphatidylethanolamine (PE) were incubated with lysosomal extracts, hydrolysis of the PC and PE proceeded mainly by a phospholipase $A_1$ deacylation pathway with accumulation of LPC and lysophosphatidylethanolamine (LPE), respectively (5). These lysophospholipids were further degraded by lysosomal lysophospholipases to fatty acids and GPC and glycerophosphoethanolamine (GPE) in the lysosome (6). The resultant phosphodiesters were not hydrolyzed further in the lysosomes (7). An alternative pathway exists in which lysophospholipids are hydrolyzed at the glycerophosphate ester bond followed by a monoacylglycerol lipase (6). An additional pathway involves the hydrolysis of the parent phospholipids by a phospholipase A (8). Furthermore, in rat kidney cortex, a lysosomal phospholipase C activity was described (9). However, the quantitative contribution of these latter two pathways to lysosomal degradation of phospholipids is unknown. Lysophospholipases purified from rat liver were shown to be active on lysophospholipid embedded in model membranes (10) and microsomal membranes (11).

Abbreviations: LPC, lysophosphatidylcholine; LPE, lysophosphatidylethanolamine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; GPC, glycerophosphocholine; GPE, glycerophosphoethanolamine.

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Chloroquine, which causes an increase in the pH of lysosomes, was demonstrated to induce intracellular phospholipidosis (12). Administration of chloroquine to rats resulted in an accumulation of millimolar concentrations of the drug in isolated rat liver lysosomes (13). This chloroquine-mediated accumulation of phospholipids in the lysosome was due to competitive inhibition of the rat liver lysosomal phospholipase $A_1$ (14). Although isolated lysosomal fractions hydrolyze exogenous PC, PE, and their respective lysophospholipids, the role of the lysosome in degradation of phospholipids generated within intact cells is not well understood.

The choline-deficient rat has recently been used to study several aspects of PC and sphingomyelin metabolism in isolated rat hepatocytes (15–19). Choline-deficient rats have a reduced pool of phosphocholine in their livers. Thus, incubation of hepatocytes isolated from these animals with radioactive PC precursors (e.g., [*methyl*-$^3$H]choline) produces a highly labeled pool of PC. The radioactivity in the PC pool may be subsequently chased into its metabolites thus facilitating studies on the catabolism of PC and its products under various metabolic conditions. In this study we have used choline-deficient rat hepatocytes incubated in the absence or presence of chloroquine to investigate the role of the lysosome in the degradation of intracellularly synthesized PC. We show for the first time that the lysosome does indeed function in the catabolism of intracellular LPC in isolated hepatocytes.

## MATERIALS AND METHODS

[*Methyl*-$^3$H]choline (75 Ci/mmol), [1-$^3$H]ethanolamine (20 Ci/mmol), L-[*methyl*-$^3$H]methionine (70 Ci/mmol), and aqueous counting scintillant were obtained from Amersham, International. Silica gel thin-layer plates were obtained from Merck. Fetal bovine serum and Dulbecco's modified Eagle's medium (without choline, methionine, and arginine) were obtained from Gibco Laboratories, Grand Island, NY. Choline-deficient diet was obtained from ICN Biochemicals, Canada. The composition of the diet has been described previously (15). Albumin was obtained from Sigma (A-7030). Chloroquine and all other biochemicals were of analytical grade and were obtained from either Sigma Chemical Company, St. Louis, MO, or Fisher Scientific, Edmonton, Alberta. Male Sprague-Dawley rats (40–50 g) were used throughout the study. Rats were fed the choline-deficient diet and tap water, ad libitium, in a temperature- and light-controlled room.

### Isolation, incubation, and harvest of hepatocytes

Rats were fed a choline-deficient diet for 3 days. Subsequently, hepatocytes were isolated by collagenase perfusion (20) except that choline-deficient medium containing 10% delipidated fetal bovine serum was used. Trypan blue was excluded by greater than 90% of the isolated cells. Hepatocytes (4–6 × $10^6$ cells/2 ml medium) were incubated, 90 rpm on a Georotatory shaker, with [*methyl*-$^3$H]choline (1 μCi/flask), [1-$^3$H]ethanolamine (10 μCi/flask), or [*methyl*-$^3$H]methionine (10 μCi/flask) in 25-ml Ehrlenmeyer flasks for 30 min. Cells were pelleted by centrifugation at 50 *g* for 2 min in a bench-top centrifuge and the cells were resuspended in nonlabeled (chase, without unlabeled substrate) medium for up to 60 min. At the specified times the cells were centrifuged as before and the medium was removed and placed on ice. Cells were washed with phosphate-buffered saline, and radioactivity incorporated into choline-containing metabolites was determined in both the cells and medium (21), except that the initial extraction contained chloroform–methanol–0.05 M potassium chloride. Radioactive LPE and PE were separated in a solvent system containing chloroform–methanol–ammonium hydroxide–water 70:35:5:2 (v:v:v:v). In some experiments chloroquine (100 μM), albumin (2%), both, or other compounds were added to the chase incubation medium. In endogenous LPC labeling experiments, 0.2 mCi [*methyl*-$^3$H]choline was injected into the tail vein of rats and hepatocytes from these rats were prepared 24 h post-injection. These hepatocytes (4–6 × $10^6$/flask) were incubated in 2 ml medium per flask of cells in the absence or presence of 100 μM chloroquine, 2% albumin, or both. In some experiments, the labeled LPC from these livers was isolated and used as the source of radioactive LPC in the acid lysophospholipase assay (16).

### Assay of acid lysophospholipase activity

Choline-deficient rat livers were perfused with saline for 2 min to remove blood. The livers were placed in ice-cold saline, weighed, and a 10% homogenate was prepared in buffer A (50 mM Tris-HCl, pH 7.4, 0.15 M NaCl, 0.5 mM phenylmethylsulfonyl chloride, 1.0 mM EDTA, 2 mM dithiothreitol, 0.025% sodium azide) using a Potter-Elvejhem homogenizer. The homogenate was immediately centrifuged at 2000 *g* for 5 min to sediment cellular debris and unbroken cells. The resulting supernatant was centrifuged at 12,000 *g* for 10 min and the supernatant resulting from this centrifugation was centrifuged at 105,000 *g* for 1 h. All procedures were performed at 4°C. The 12,000-*g* and 105,000-*g* pellets were resuspended in 5 ml of buffer containing 0.25 M sucrose, 10 mM Tris-HCl, pH 7.4, 0.145 M NaCl. Acid lysophospholipase activity was assayed in the 12,000-*g* and 105,000-*g* pellets. The incubation mixture contained 20 mM acetate buffer, pH 4.4, 0.3 mM [*methyl*-$^3$H]LPC (sp act 1 μCi/μmol), 100 μg protein, and water in a final volume of 100 μl. Incubations were performed at 37°C for 30 min in a shaking water bath. The incubation was terminated by the addition of 2 ml of chloroform–methanol

2:1 (v/v); 1.5 ml 0.05 M KCl was added to facilitate phase separation. The upper aqueous phase was removed and an aliquot was taken for the determination of radioactivity. The aqueous phase was also analyzed for radioactivity in choline-containing metabolites and the majority of radioactivity was in GPC. The lower phase was analyzed for radioactivity incorporated into PC, which was negligible. In some experiments, 10 mM chloroquine was added to the incubation mixture prior to assay.

### Other procedures

Lactate dehydrogenase activity was assayed as previously described (22). Phosphorus content was estimated as described (23). Protein was determined by the method of Lowry et al. (24). Student's unpaired *t*-test was used for statistical analysis. The level of significance was defined as $P < 0.05$.

## RESULTS

### Chloroquine causes an accumulation of LPC in hepatocytes

To determine whether the lysosome is involved in intracellular phospholipid catabolism in intact cells, choline-deficient hepatocytes were isolated and pulse-labeled with [*methyl*-$^3$H]choline or [1-$^3$H]ethanolamine for 30 min. The radioactivity was chased in the absence or presence of chloroquine (100 $\mu$M) for 60 min. The radioactivity incorporated into PC and PE at 60 min was $4.07 \pm 0.10 \times 10^5$ dpm/$10^6$ cells and $6.19 \pm 0.20 \times 10^5$ dpm/$10^6$ cells, respectively. The presence of chloroquine in the incubation medium did not affect the radioactivity incorporated into PC or PE but caused a 2.5-fold increase in the radioactivity incorporated into LPC and a 2.7-fold increase in radioactivity incorporated into LPE compared to untreated cells (**Table 1**). Greater than 90% of the cells excluded Trypan blue and assay of medium lactate dehydrogenase revealed that the cells were not leaky in the presence of chloroquine.

The time- and concentration-dependence of the chloroquine effects on radioactive LPC formation was investigated. Choline-deficient hepatocytes were incubated with [*methyl*-$^3$H]choline for 30 min and the radioactivity was chased into LPC for various times. The increased formation of LPC in chloroquine-treated hepatocytes was evident within 10 min and highly significant by 30 min of chase (**Fig. 1A**). In addition, the effect of chloroquine on increasing LPC formation in hepatocytes was concentration-dependent (Fig. 1B). Thus, the effect of chloroquine on LPC formation in choline-deficient hepatocytes was concurrent with the rapid inhibition of lysosomal function caused by this agent (25). The radioactivity in choline, phosphocholine (**Fig. 2A**), GPC, and betaine (Fig. 2B) was unaltered by the presence of chloroquine. Thus, chloroquine caused a specific and rapid accumulation of radioactive LPC in hepatocytes.

PC may also be synthesized by the progressive methylation of PE (26). We investigated whether or not the formation of LPC derived from PC synthesized by the methylation of PE was altered in chloroquine-treated cells. Choline-deficient hepatocytes were incubated with [*methyl*-$^3$H]methionine and the radioactivity was chased in the absence or presence of chloroquine for 30 min. We chose 30 min because at this time the increase in LPC formation in choline-labeled cells was highly significant. Radioactivity incorporated into PC ($2.53 \pm 0.20 \times 10^5$ dpm/$10^6$ cells) was not altered but radioactivity incorporated into LPC was increased 2.3-fold in chloroquine-treated hepatocytes compared to untreated cells (Table 1). Thus, inhibition of lysosomal function caused a rapid accumulation of radiolabeled LPC derived from PC synthesized from both the CDP-choline and methylation pathways.

The effect of other lysosomotropic agents on the formation of LPC was investigated. Primaquine and $NH_4Cl$ were added to the chase medium of hepatocytes pulse-

TABLE 1. The effect of chloroquine on LPC and LPE production in choline-deficient hepatocytes

| | | | Chloroquine-Treated | |
|---|---|---|---|---|
| Label | Control LPC | LPE | LPC | LPE |
| | *dpm* × *$10^{-3}$/$10^6$ cells* | | | |
| [*Methyl*-$^3$H]choline | 2.7 ± 0.5 | N.D. | 6.8 ± 0.7[a] | N.D. |
| [1-$^3$H]ethanolamine | N.D. | 5.4 ± 0.6 | N.D. | 14.7 ± 1.0[a] |
| [*Methyl*-$^3$H]methionine | 0.24 ± 0.013 | N.D. | 0.57 ± 0.035[a] | N.D. |

Isolated choline-deficient hepatocytes were incubated with [*methyl*-$^3$H]choline, [1-$^3$H]ethanolamine or [*methyl*-$^3$H]methionine for 30 min. For the choline- and ethanolamine-labeled cells, the radioactivity was chased into LPC and LPE in the absence or presence of chloroquine for 60 min. In methionine-labeled cells, the radioactivity was chased in the absence or presence of chloroquine for 30 min. The radioactivity in LPC and LPE was subsequently determined. Data represent the mean ± SD of three flasks. The experiment was repeated twice with similar results. N.D.; not determined.

[a] $P < 0.05$.

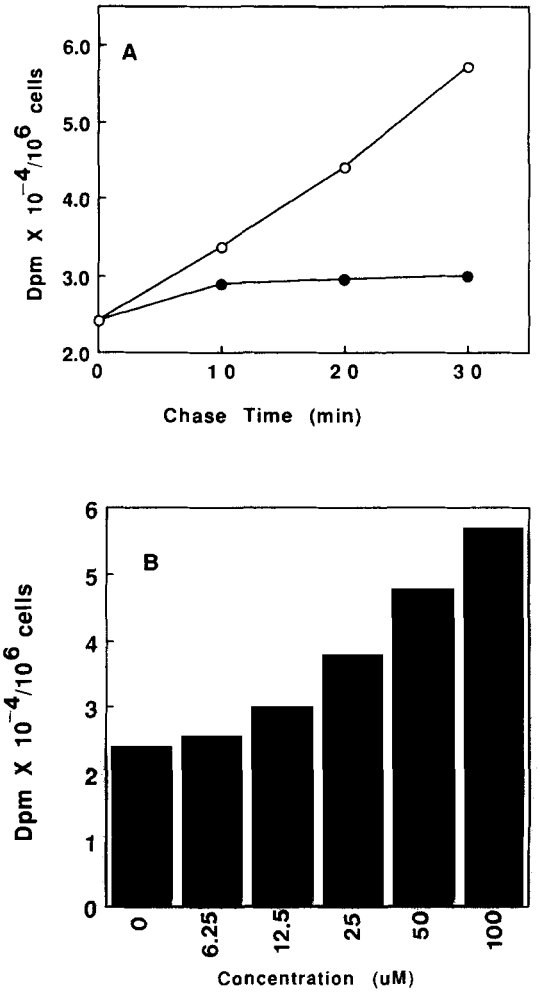


**Fig. 1.** Time course and concentration dependence of chloroquine-induced LPC formation in choline-deficient hepatocytes. A: Choline-deficient hepatocytes were incubated for 30 min with [*methyl*-$^{3}$H]choline (2 $\mu$Ci/flask) and the radioactivity was chased for up to 30 min in the absence or presence of 0.1 mM chloroquine in the medium. Subsequently, the radioactivity in LPC was determined; closed symbols, control; open symbols, chloroquine-treated. B: Choline-deficient hepatocytes were pulse-labeled as described above and subsequently incubated for 30 min in the absence or presence of various concentrations of chloroquine in the medium. The radioactivity in LPC was then determined. Each point represents the average of two separate experiments performed in duplicate.

labeled with radioactive choline. Both primaquine and $NH_4Cl$ increased the formation of LPC compared to controls (**Fig. 3A**). Thus, the enhanced formation of LPC in chloroquine-treated cells appears to be the result of inhibition of lysosomal function.

The above studies investigated the effect of chloroquine on the production of LPC from newly synthesized PC. Therefore, we also determined whether or not LPC derived from pre-existing PC accumulated in chloroquine-treated hepatocytes. Rats were injected with radioactive choline and the cells were isolated after 24 h. This should allow for radioequilibration of all choline-containing metabolites (16). Incubation of these cells with chloroquine caused a rapid, time-dependent increase in radioactivity in cellular LPC compared to that in untreated cells (**Fig. 4**). In addition, the presence of chloroquine had no effect on the level of radioactive LPC in the medium. The labeling of the aqueous choline-containing metabolites was unchanged upon treatment of the cells with chloroquine. The major catabolite was GPC. These results suggest that both newly synthesized and pre-existing PC can be readily catabolized to LPC and that both pools of LPC contribute to the lysosomal LPC pool.

Because the radiolabeling experiments might be misinterpreted, we investigated whether the mass of LPC was increased in the presence of chloroquine. Choline-deficient hepatocytes were incubated for 30 min in the absence or presence of 0.1 mM chloroquine. Subsequently,

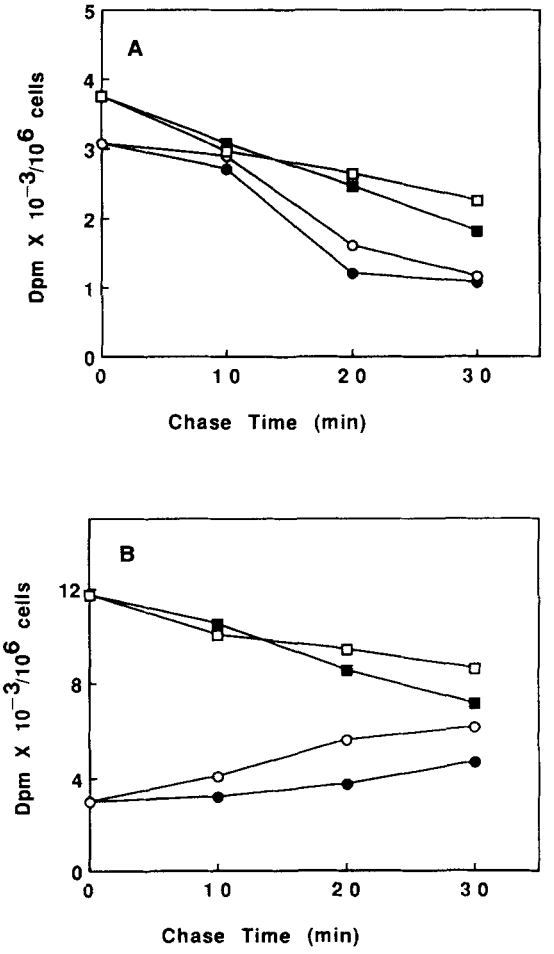


**Fig. 2.** Effect of chloroquine on labeling of aqueous choline-containing metabolites. Choline-deficient hepatocytes were incubated as in Fig. 1A and the radioactivity in the aqueous choline-containing metabolites was determined. A: squares, phosphocholine; circles, choline. B: squares, betaine; circles, glycerophosphocholine. Closed symbols, control; open symbols, chloroquine-treated. Each point represents the average of two separate experiments performed in duplicate.

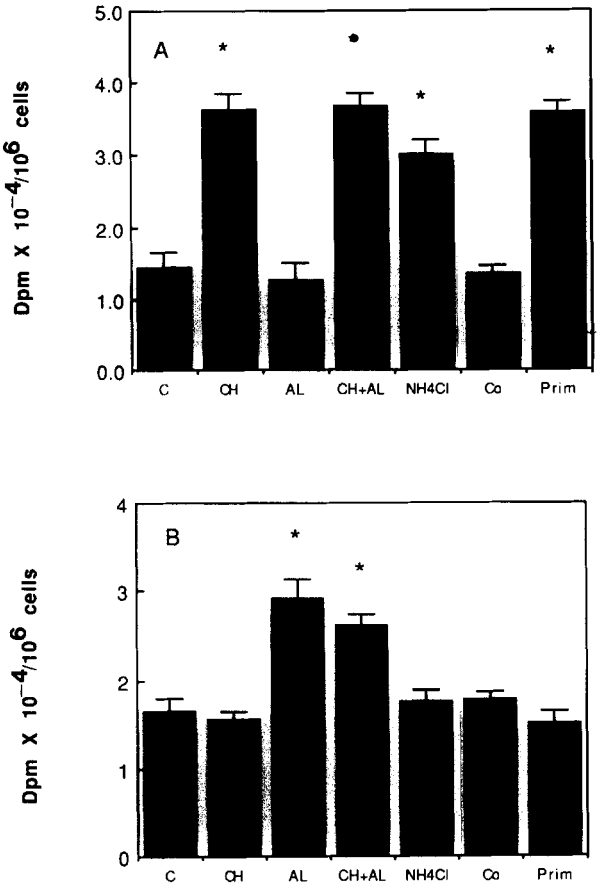


**Fig. 3.** Effect of various agents on LPC formation in choline-deficient hepatocytes. Choline-deficient hepatocytes were incubated for 30 min with [*methyl*-$^3$H]choline (1 $\mu$Ci/flask) and the radioactivity was chased for 30 min in the absence or presence of various lysosomotropic agents. Subsequently, the radioactivity in LPC was determined. A: cells; B: medium. Control, C; chloroquine, CH; albumin, AL; colchicine, Co; primaquine, Prim; ammonium chloride, $NH_4Cl$. Each point represents the mean ± standard deviation of three flasks. The experiment was repeated twice with similar results. $^*P < 0.05$.

the cells from three flasks (12–18 × $10^6$ cells) were pooled. The lipids were extracted and LPC was isolated by thin-layer chromatography. The mass of LPC was determined. Chloroquine caused a 1.7-fold ($P < 0.05$) increase in cellular LPC mass from 1.03 ± 0.20 to 1.73 ± 0.39 nmol phosphorus/mg protein (average ± SD of three separate experiments). The mass of PC in these hepatocytes was 100.6 ± 6.4 nmol/mg protein and was unchanged in the presence of chloroquine.

Lysosomotropic agents inhibit lysosomal phospholipase A activity (14) and addition of micromolar levels of cholorquine to cells causes the intralysosomal concentration of chloroquine to increase rapidly to millimolar concentrations (25). We investigated whether or not the inhibition of acid lysophospholipase activity by chloroquine contributed to the accumulation of LPC in intact cells by assay of acid lysophospholipase activity. Subcellular fractions were incubated for 30 min in the absence or presence of 10 mM chloroquine. The presence of chloroquine in the incubation caused a 65% reduction in acid lysophospholipase activity in the 12,000-*g* pellet from 0.20 ± 0.04 to 0.07 ± 0.02 nmol/min per mg protein. There was negligible acid lysophospholipase activity in the 105,000-*g* pellet and the presence of chloroquine did not affect this activity. In addition, the formation of radioactive PC in these incubations was negligible. Thus, the inhibition of acid lysophospholipase activity by chloroquine appears to be correlated with the accumulation of LPC in intact cells. As a control, the effect of chloroquine on the activity of a nonlysosomal enzyme, CTP:phosphocholine cytidylyltransferase, was determined. Hepatocytes were incubated in the absence or presence of 0.1 mM chloroquine and subsequently permeabilized with digitonin (21). Cytidylyltransferase activity was assayed in both the digitonin-released cytosol and the membrane fraction. Cytidylyltransferase activity was 2.24 ± 0.25 and 2.72 ± 0.14 nmol/min per mg protein in membrane and released cytosolic fractions, respectively, and was unaltered in the presence of chloroquine.

### Chloroquine-mediated increase in intracellular LPC is not altered by LPC entrapment in the medium

As albumin rapidly removes LPC from hepatocytes (27), we investigated whether or not the lysosome contributed to the pool of LPC removed by albumin. Choline-deficient hepatocytes were incubated with radioactive choline for 30 min and subsequently the radioactivity was chased for 30 min in the absence or presence of 2% albumin or chloroquine in the medium. A 2.5-fold increase in radioactive LPC formation by 30 min was observed in chloroquine-treated cells compared to control cells (Fig. 3A). The increased formation of radioactive

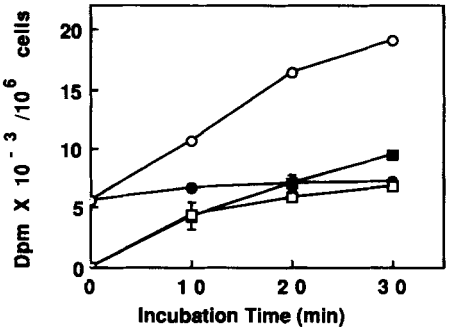


**Fig. 4.** Effect of chloroquine and albumin on the accumulation of LPC and aqueous-metabolites in cells labeled to equilibrium with [*methyl*-$^3$H]choline. Choline-deficient rats were injected with [*methyl*-$^3$H]choline (0.2 mCi/rat). Hepatocytes were isolated 24 h later and incubated for up to 30 min in the absence (closed symbols) or presence of 0.1 mM chloroquine (open symbols) and the radioactivity in LPC in the cells (circles) or medium (squares) was determined. Each point represents the mean ± SD of three flasks. The experiment was repeated once with similar results.

LPC in the cell in the presence of chloroquine was unaltered by the presence of albumin. In addition, in the presence of albumin or albumin plus chloroquine, a 2-fold increase in radioactive LPC was observed in the medium (Fig. 3B). However, chloroquine alone did not affect the level of radioactive LPC in the medium.

In another set of experiments, hepatocytes were prepared from rats injected with [*methyl*-$^3$H]choline 24 h earlier and subsequently the cells were incubated in the absence or presence of chloroquine, plus or minus albumin. As seen in **Fig. 5,** the chloroquine-induced increase in radioactive LPC formation in the cells was independent of albumin in the medium as was also observed in the short-term pulse-chase experiments. Thus, the pool of LPC available to albumin (i.e., likely plasma membrane) appeared to be separate from the intracellular pool.

### Does brefeldin A or colchicine affect the chloroquine-mediated increased labeling of LPC?

If PC transport in cells occurred by a mechanism that involved cytoskeletal elements, colchicine, a cytoskeletal disruption agent, would be expected to interfere with the transport process. Choline-deficient hepatocytes were incubated with radioactive choline and the radioactivity was chased in the absence or presence of colchicine or colchicine plus chloroquine. Colchicine did not affect the labeling of LPC (Fig. 3A) or the chloroquine-mediated increase in LPC labeling (data not shown). In addition, colchicine did not affect the radioactivity in medium LPC (Fig. 3B), nor were there any effects of colchicine on the aqueous choline-containing metabolites. Apparently the formation of LPC from PC and its catabolism are not affected by cytoskeleton disruption.

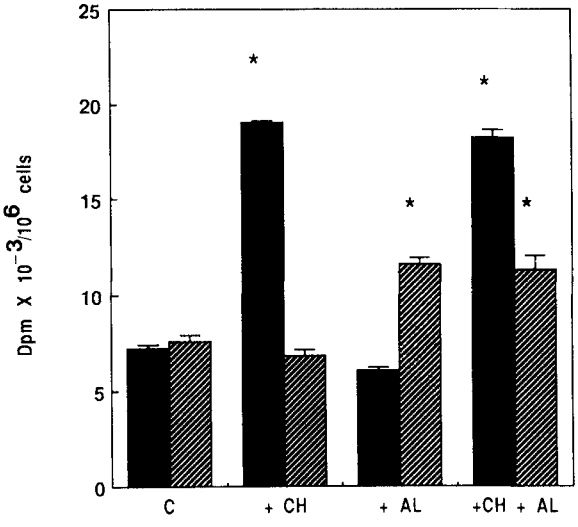


**Fig. 5.** Effect of chloroquine and albumin on cellular and medium LPC content in cells labeled to equilibrium with [*methyl*-$^3$H]choline. Choline-deficient hepatocytes were incubated as described in Fig. 4 and the radioactivity was determined in LPC in cells (solid bars) or medium (hatched bars) after 30 min of incubation. Control (C), albumin (+ AL), chloroquine (+ CH), chloroquine and albumin (+ CH + AL). Each point represents the mean ± SD of three flasks. The experiment was repeated once with similar results. *$P < 0.05$.

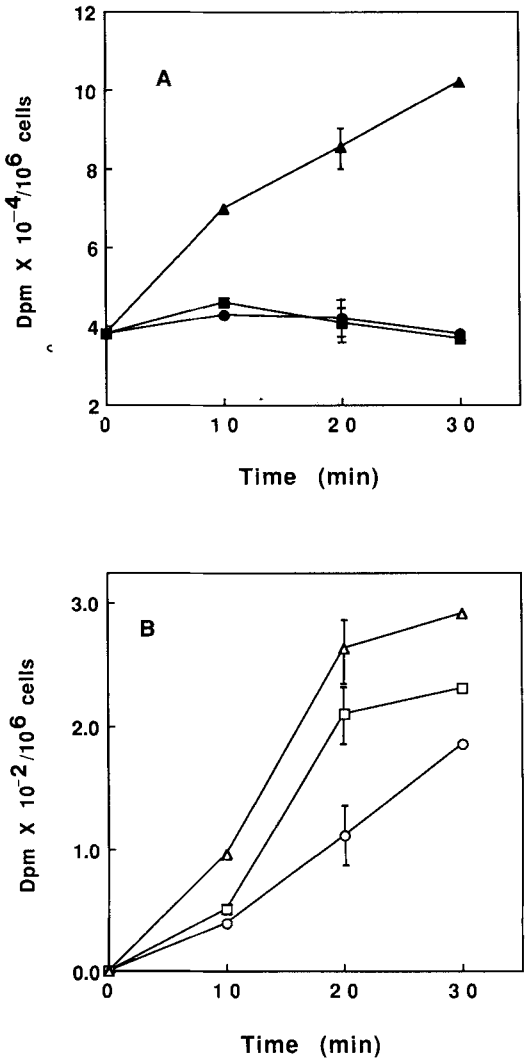


**Fig. 6.** Effect of brefeldin A and chloroquine on LPC and GPC formation in choline-deficient hepatocytes. Choline-deficient hepatocytes were incubated for 30 min with [*methyl*-$^3$H]choline (2.5 μCi/flask) and the radioactivity was chased for up to 30 min in the absence or presence of 5 μg/ml brefeldin A alone or brefeldin A with 0.1 mM chloroquine in the medium. Subsequently, the radioactivity in LPC (panel A) and GPC (panel B) was determined. Control, circles; brefeldin A, squares; brefeldin A and chloroquine, triangles. Each point represents the average of two separate experiments performed in duplicate. The 20-min time points were from three separate experiments ± SD.

To determine whether LPC labeling was dependent upon vesicular transport, choline-deficient hepatocytes were incubated with radioactive choline and the radioactivity was chased in the absence or presence of brefeldin A or brefeldin A plus chloroquine. The radioactivity in PC was $2.91 \times 10^{-5}$ dpm/$10^6$ cells and was unaltered under all incubation conditions. The presence of brefeldin A did not affect the formation of labeled LPC or the chloroquine-mediated increase in LPC (Fig. 1 and **Fig. 6A**). The formation of GPC increased with time of incu-

bation (Fig. 6B). Surprisingly, the presence of brefeldin A caused a 1.9-fold increase in radioactivity in GPC by 20 min of incubation compared to controls (Fig. 6B). Furthermore, the presence of both brefeldin A and chloroquine caused a 2.4-fold increase in GPC formation. Recall that chloroquine by itself had no effect on the labeling of GPC. Thus, brefeldin A had no effect on the labeling of LPC. The apparent stimulation of GPC formation will need to be investigated in a separate study. Another possibility is that brefeldin A inhibited GPC catabolism.

## DISCUSSION

The major findings of this paper are as follows. *1)* An intracellular pool of LPC is derived from PC synthesized from both the CDP-choline and methylation pathways and rapidly accumulates when lysosomal function is compromised. *2)* Both newly synthesized and pre-existing PC contribute to this intracellular LPC pool. *3)* There is a pool of LPC, distinct from the lysosomal LPC pool, which is extracted from cells by addition of a suitable acceptor to the medium. *4)* The results suggest that lysosomes play an important role in the catabolism of intracellularly generated lysophospholipids in intact cells.

### Intracellular LPC is derived from PC synthesized from both the CDP-choline and methylation pathways, and LPC accumulates when lysosomal function is impaired

It had previously been demonstrated by Matsuzawa and Hostetler (28) that oral administration of chloroquine to rats for 7 days resulted in an accumulation of LPC and other phospholipids in their livers. However, these studies did not determine whether this LPC was derived from an endogenous or exogenous source. In the present study, radioactive LPC was formed in hepatocytes labeled with either choline or methionine, indicating that the intracellular LPC pool could be derived from PC synthesized from both the CDP-choline and methylation pathways. When lysosomal function was compromised by the addition of chloroquine to these cells, radioactive LPC, and LPC mass (but not detectable PC mass), rapidly accumulated. These results indicate that the lysosome of hepatocytes may be an important organelle for the degradation of intracellularly generated LPC. The ratio of radioactivity in LPC:PC was similar in choline- and methionine-labeled cells which might indicate that the contribution of each of these pathways to intracellular LPC is similar. Moreover, we observed a similar effect of chloroquine on radioactive LPE formation in these hepatocytes. Thus, lysosomal catabolism may occur for all intracellular lysophospholipids. In contrast to earlier studies (13, 28) we did not observe a change in PC mass in our chloroquine-treated cells. Based upon one of these studies (13) we should have observed an increase in PC mass by 30 min of incubation with chloroquine. However, the calculated increase in PC mass would only be 4% and would not be detected with our existing methodology. In addition, it is quite possible that some of the observed increase in lysosomal PC mass (13, 28) may have been due to acylation of accumulated LPC.

Chloroquine inhibits lysosomal enzymes such as cathepsin B (29) and inhibition of the rat liver lysosomal phospholipase A by antimalarial agents has been documented (14, 30). Other lysosomotropic agents (primaquine and $NH_4Cl$) had an effect similar to that of chloroquine on LPC formation. Chloroquine did not affect the activity of the nonlysosomal enzyme CTP:phosphocholine cytidylyltransferase.

The accumulation of LPC mass and radioactivity in chloroquine-treated cells could have been the result of an increase in the rate of PC catabolism to LPC and/or a blockage in the degradation of LPC to GPC. However, chloroquine did not decrease the radioactivity or mass of PC. Possibly, an effect on PC levels might not have been detected as both the mass and radioactivity in PC greatly exceed that of LPC ($>$ 100-fold) and the turnover of PC is slow, with a half time of approximately 15 h (31). Any increase in PC catabolism was therefore modest. A more likely reason for the accumulation of LPC upon chloroquine treatment is a reduction in LPC catabolism by a lysosomal lysophospholipase.

### Both newly synthesized and pre-existing PC contribute to LPC formation

Similar kinetics of LPC accumulation in the presence of chloroquine was observed in both short-term- and equilibrium-labeled cells. This indicates that both newly synthesized and pre-existing intracellular LPC are degraded in the lysosome. In addition, the data suggest that the accumulation of intracellularly derived LPC by the lysosome is rapid. However, the precise quantitative contribution of the lysosome to intracellular LPC catabolism is unknown and estimation is difficult, if not impossible.

### Effect of other agents on LPC accumulation in hepatocytes—evidence for distinct cellular pools of LPC

The primary effect of cholchicine is to disrupt the cytoskeleton, whereas brefeldin A disrupts vesicular traffic (32). Neither agent altered the increased formation of LPC in chloroquine-treated hepatocytes, suggesting that the PC converted to LPC moves within the cell by mechanisms independent of the cytoskeleton and vesicular traffic. On the other hand, if the LPC was generated exclusively from PC in the lysosome, there would be no reason to expect that cholchicine or brefeldin A would affect LPC accumulation. In subcellular fractionation studies we found a similar accumulation of LPC in the

12,000- and 105,000-*g* pellets (data not shown). Thus, perhaps LPC accumulated in areas of the cell other than the lysosomes (which would be in the 12,000-*g* pellet). If true, it seems that the cytoskeleton and vesicular traffic had no role in LPC transport. Alternatively, the LPC could have been redistributed during the homogenization of the cell.

More revealing and surprising were the studies with albumin. Albumin is known to extract LPC from hepatocytes (27). Thus, we were surprised that when albumin was added to the medium there was no effect on the accumulation of LPC in the chloroquine-treated cells from either short-term or equilibrium labeling experiments. If the lysosomal pool of LPC were rapidly exchanged with LPC of the plasma membrane, we would have expected an increase in radioactive LPC in the medium when albumin was present. In the absence of added albumin some LPC accumulated in the medium of control hepatocytes because serum (10%) which contains albumin was present in our incubations. It thus appears that there are at least two metabolically distinct pools of LPC in hepatocytes. The compartmentation of PC and sphingomyelin, two other choline-containing lipids, is well documented (33).

The total amount of radiolabeled LPC in albumin chloroquine-treated cells (medium plus cells) was greater than in cells treated with albumin or chloroquine alone. Because the increase of LPC was in the medium and there was not a concomitant decrease in the cells, there appears to be a need for a minimum level of LPC in the cell membranes. The function of maintaining such a pool of LPC is unknown. Possibly this pool may be a reserve for reacylation to PC if a particular species of PC (e.g., arachidonoyl-PC) were required.

Other studies presented also provide evidence for the compartmentation of LPC and GPC in hepatocytes. If the sole, or major, source of GPC in the cell were LPC formed in the lysosome, we would have expected a decrease in the labeling of GPC in the chloroquine-treated cells. As no effect was observed, another source of LPC may be contributing to the formation of GPC outside of the lysosome. Furthermore, the generation of the alternate source of GPC may be increased as compensation for the decreased formation of GPC in the lysosome. Apparently the cell may need to maintain the level of GPC and sensitive and rapid regulation of this process might occur. If GPC has an important function in osmotic regulation in the cell as recently suggested (34), careful control of GPC concentration would certainly be warranted.

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