Experimentally caused proliferation of lysosomes in cultured BHK cells involving an increase of biphosphatidic acids and triglycerides

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Abstract When cultured hamster fibroblasts (BHK 21 cells) were incubated in a synthetic serum-free medium up to 4 days, they developed signs of a progressive proliferation of lysosomes. The cells became filled with vacuoles that contained polymorphic debris and showed acid phosphatase activity. The specific activities of acid protease and acid phosphatase in the cell cultures increased three- to fourfold. The process was accompanied by a marked decrease in the contents of protein, deoxyribonucleic acid, and total phospholipids of the cultures. The concentration of lysobisphosphatidic acid increased during the incubation from about 1.5% to 3–6% of the cellular phospholipids. The concentrations of two related lipids, bisphosphatidic acid and semilysobisphosphatidic acid also increased substantially. The triglyceride content of the cells increased several fold, whereas the concentration of phosphatidylcholine decreased markedly. Lysobisphosphatidic acid did not increase upon induction of vacuolization by exogenous sucrose, nor when there was an accumulation of triglyceride due to addition of oleic acid to the growth medium. These findings suggest that the formation of the bisphosphatidic acids may be specifically linked to the autolysis of the phospholipids of the cellular membranes and the formation of triglycerides associated with this process.

Supplementary key words lysobisphosphatidic acid · semilysobisphosphatidic acid

Lysobisphosphatidic acid (LBPA) is found in a variety of cells and tissues in relatively small concentrations (1, 2). This lipid has a unique stereoconfiguration; it is a derivative of bis(*sn*-glyceryl-1-)-phosphate (3, 4). LBPA is enriched in the lysosomes of cultured hamster fibroblasts (BHK 21 cells) (5), rat liver (6, 7), human liver (8) and rabbit alveolar macrophages (9). It is not present in appreciable concentrations in the other organelles (6, 10, 11).

Other bis(glyceryl)phosphate derivatives are also present in the BHK cells. They include semilysobisphosphatidic acid (SLBPA) and bisphosphatidic acid, different from LBPA in that they contain three and four fatty chains, respectively, instead of two (12). Their detailed structures and cellular locations are not known.

The curious structure and localization of LBPA led us to postulate that its biosynthesis may be associated with the lysosomal degradation of other glycerolipids (3). If this is true, the concentration of LBPA should increase under conditions where the cells are autolyzing their membrane lipids. The present report provides evidence to support this hypothesis. The simultaneous increase of SLBPA and bisphosphatidic acid suggests that they are closely related to LBPA. A preliminary report of some of the findings has been presented previously (11).

MATERIALS AND METHODS

Cell cultivation

BHK 21 cells (clone Wi-2) were grown at 37°C in air mixed with 5% CO_2 on plastic dishes (6 cm in diameter, Falcon Plastics, Oxnard, CA) in a medium consisting of 80% BHK medium (Gibco, Grand Island, NY), 10% tryptose phosphate broth (Difco, Detroit, MI) and 10% calf serum (Orion, Helsinki,Finland). The medium contained 200 units/ml of penicillin G and 100 μg/ml of streptomycin. The confluent cultures were then incubated in Eagle's minimum essential medium (13) without serum but supplemented with 0.2% bovine serum albumin (Fraction V, Sigma, St. Louis, MO). Control cultures were

Abbreviations: LBPA, lysobisphosphatidic acid; SLBPA, semilysobisphosphatidic acid.

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maintained in fresh growth medium. In some experiments albumin was omitted from the medium or replaced by fatty acid-free bovine serum albumin (Sigma). Two experiments were carried out with cells grown in glass bottles (5).

In two experiments the full-grown monolayers of cells were incubated in a fresh growth medium that contained sucrose (15 mg/ml or 34 mg/ml). One experiment was performed growing the cells in the presence of 0.07 mM sodium oleate (Nu Chek Prep, Austin, MN).

In some experiments the cells were first labeled with [^{32}P]orthophosphate (Institutt for Atomenergi, Kjeller, Norway) to isotopic equilibrium (14) and then incubated in the minimum essential medium, which contained [^{32}P]orthophosphate at the same specific activity as the growth medium.

Electron microscopy

The electron microscopic examination of the cells and the cytochemical staining of acid phosphatase were carried out as previously described (5).

Extraction and analysis of lipids

The cells were dissolved in 2 mM sodium dodecyl sulfate and the lipids were extracted with chloroform–methanol 2:1 (v/v). The extract was partitioned with water according to Folch, Lees, and Sloane Stanley (15); the detergent remained in the aqueous phase. Separation and quantitation of the individual phospholipids and neutral lipid classes were carried out as described previously (5, 12, 16). The results for the individual glycerolipid classes included the corresponding alkylacyl and alkenylacyl forms, if present (16). ^{32}P-Labeled phospholipids were detected by radioautography on Medichrome S.M1 X-ray film (Agfa-Gevaert, Antwerpen, Belgium) and the spots were scraped directly into scintillation vials. Radioactivity was measured either by counting the Cerenkov radiation in chloroform–methanol 2:1 (v/v) or by conventional liquid scintillation counting in a toluene-based scintillation mixture (14). Both methods gave identical results.

Enzyme analyses

Cells were scraped off in 0.3 M sucrose, 5 mM Tris, 0.5 mM EDTA, pH 7.4, and then centrifuged. The pellet of cells was suspended in 0.1 M sucrose, buffered as above, and homogenized in an all-glass Dounce homogenizer (Thomas, Philadelphia, PA) with 10 strokes of the tight-fitting B-pestle. The homogenates were stored frozen at −20°C up to one week. Before the measurement of the lysosomal enzymes the samples were quickly thawed and refrozen three times.

Acid protease (E.C. 3.4.4.23) was assayed using the method of Anson (17). Acid ribonuclease (E.C. 2.7.7.16) and acid phosphatase (E.C. 3.1.3.2) were measured by the methods of Allison and Sandelin (18). Succinate dehydrogenase (E.C. 1.3.99.1) was assayed using a method described earlier (5).

Other determinations

Protein was measured (16) using bovine serum albumin as a standard. Deoxyribonucleic acid was measured (19) using calf thymus DNA as a standard.

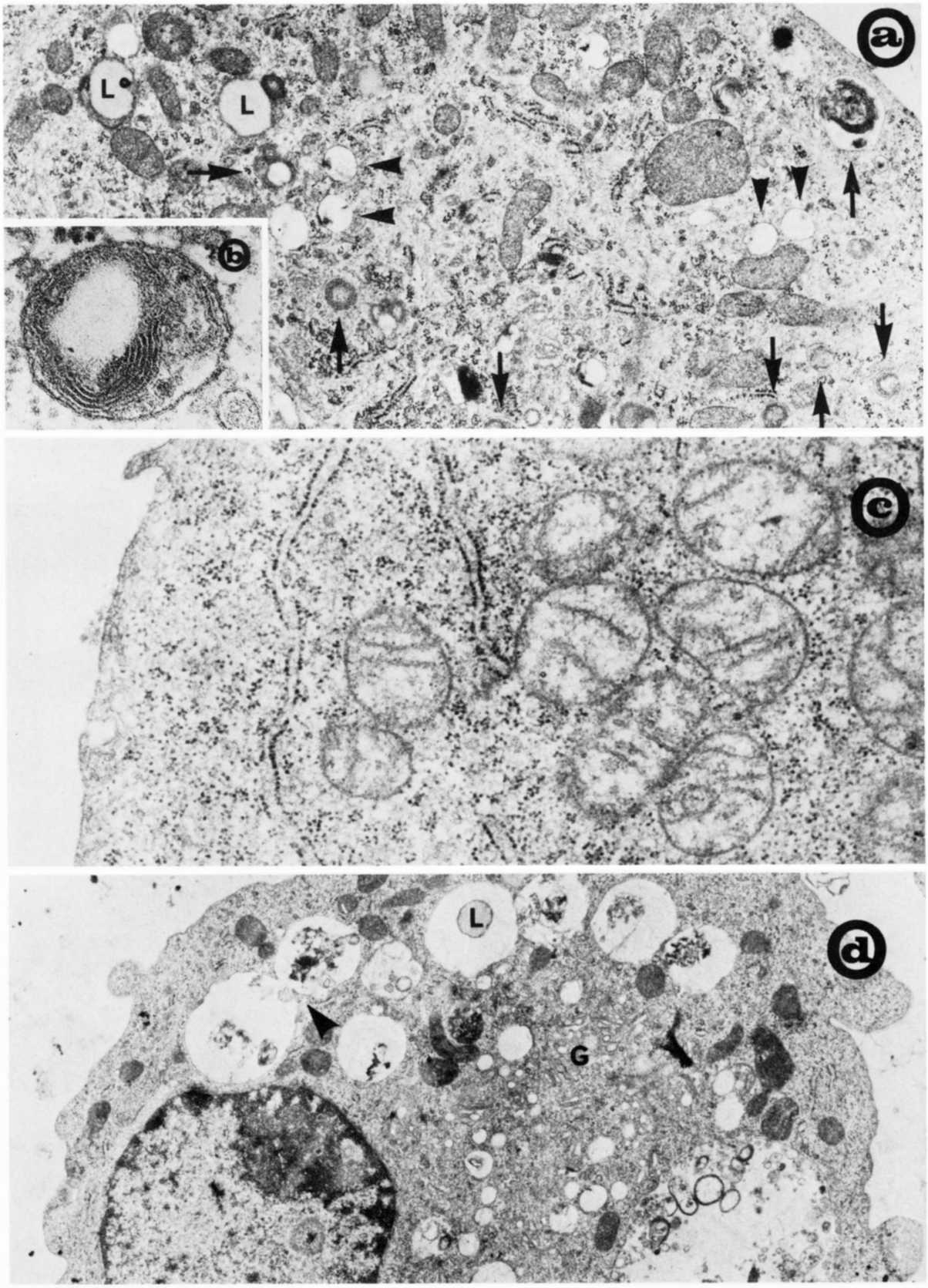
RESULTS

The present report describes changes induced in BHK 21 cells by prolonged incubation in Eagle's minimum essential medium. In most experiments the basal medium was supplemented with 0.2% bovine serum albumin; the resulting mixture is called "maintenance medium" for brevity. The control cells were incubated in the full growth medium.

Ultrastructural changes in BHK cells incubated in maintenance medium

The cells were grown to full monolayers (usually for 3 days) and then incubated in the maintenance medium for 4 more days. After 1 day in the maintenance medium, numerous small vacuoles appeared in the cells (**Fig. 1A**). Some of the vacuoles had clear contents whereas others contained more dense materials which frequently revealed stacked

Fig. 1. *A*. BHK cell incubated in the maintenance medium for 24 hr. The cell contains numerous vacuoles, about the size of the mitochondria or smaller. Some vacuoles are clear (arrowheads), others contain dense amorphous or multilamellar material and occasionally small lipid droplets (arrows). The large lipid droplets (L) often are partially surrounded by darkly staining material, which at a high magnification reveals a laminated fine structure. Magnification ×19,300. *B*. A membrane-bound vacuole that contains a small lipid droplet surrounded by prominent multilamellar substance in a BHK cell incubated 48 hr in the maintenance medium. Magnification ×110,000. *C*. Control cell incubated for 24 hr in the growth medium. The endoplasmic reticulum is well preserved but the mitochondria are slightly swollen. Magnification ×36,000. *D*. Cell after 72 hr incubation in the maintenance medium. The cell contains several large vacuoles inside which lamellar whorls, small vesicles, and amorphous flocculent material are visible. Lipid droplet (L) is in one vacuole. G = Golgi zone. Magnification ×12,000.



multilamellar figures at a high magnification. Lipid droplets, more osmiophilic than the clear vacuoles, also increased. Many of them were surrounded by a membrane, the inner side of which was lined with more electron-dense material. On a high magnification, this material resolved into myelin-like multiple layers (Fig. 1B). This kind of structure has been tentatively identified as a lipid droplet within a lysosome (5).

No accumulation of cytoplasmic vacuoles was observed in the control cells (Fig. 1C).

The size of the intracellular vacuoles increased at later stages of the incubation in the maintenance medium. After 3 days most cells were almost filled with large vacuoles (Fig. 1D), which contained small vesicles, membrane whorls, and amorphous flocculent material. Occasionally, recognizable lipid droplets, mitochondria, and ribosome-rich cytoplasmic material were encountered, which indicates an autophagic or heterophagic origin for the intravacuolar material. The vacuolization seemed to proceed mainly at the expense of rough endoplasmic reticulum, the profiles of which appeared to decrease in length and number (compare Figs. 1A, 1C, and 1D).

Acid phosphatase staining identified many of the vacuoles as lysosomes. Deposits of lead phosphate were observed in association with the clear vacuoles (**Fig. 2A**), vacuoles containing membraneous whorls (Fig. 2A) and with some lipid droplets (Fig. 2B).

Assuming that our selection of morphological data is correct in the quantitative sense, it thus appeared that rough endoplasmic reticulum decreased, whereas lysosomes increased during the incubation of the BHK cells in the maintenance medium. The abundance of fat droplets, both the naked cytoplasmic droplets and the lysosome-associated droplets, appeared to increase in these cells, too.

Changes in enzyme activities in BHK cells incubated in maintenance medium

The specific activities of acid protease and acid phosphatase increased evenly with respect to the time the cells were kept in the maintenance medium, being 300–400% of the zero-time value after 72 hr (**Fig. 3**). In the control cells the specific activities of the two enzymes increased slowly, up to 130–140% of the zero-time value after 72 hr in fresh growth medium (data not shown). These findings are in agreement with the morphological evidence of lysosomal proliferation caused by the maintenance medium. However, the specific activity of acid ribonuclease did not increase markedly (Fig. 3). The specific activity of succinate dehydrogenase, a mitochondrial enzyme, remained constant during the incubation in the maintenance medium (Fig. 3).

Compositional changes in BHK cells incubated in maintenance medium

Incubation of full monolayers of cells in the maintenance medium led to a gradual decrease of phospholipids and cellular macromolecules (**Fig. 4**). About half of the phospholipids disappeared during the third and fourth days of the incubation (**Table 1**). The control cells accumulated about 50%

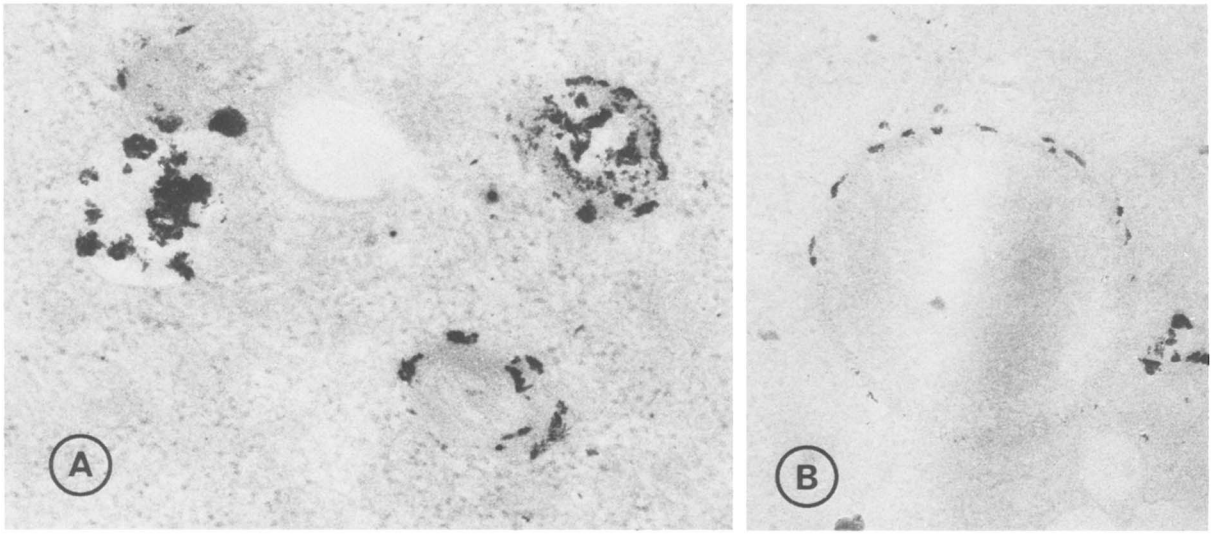


Fig. 2. Cytochemical staining of acid phosphatase of BHK cells incubated for 48 hr in the maintenance medium. *A*. The reaction product appears in association with a clear vacuole (left) and with two vacuoles containing electron-dense materials (right). Magnification ×83,000. *B*. A round body, probably a lipid droplet, surrounded by a more dense rim where small deposits of the reaction product are seen. The wave-like sectioning artifact is characteristic for lipid droplets. Magnification ×60,000.

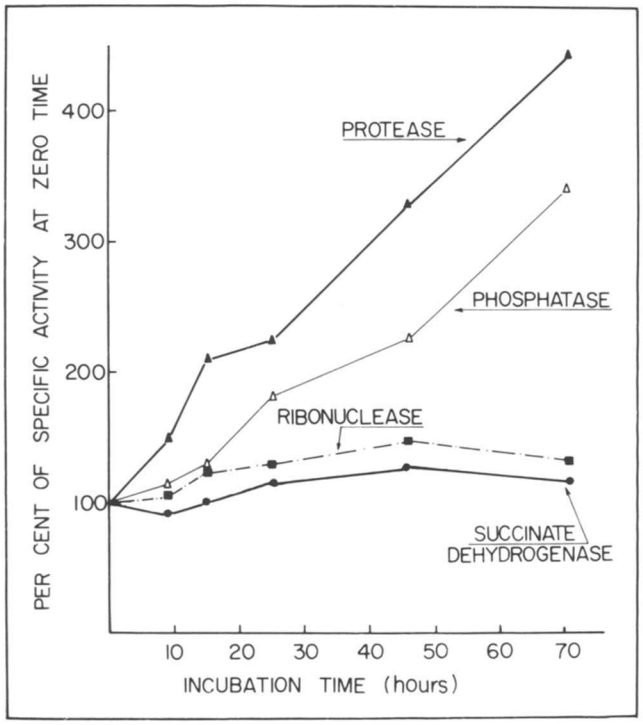


Fig. 3. Rise in the specific activity of acid protease and acid phosphatase during the incubation of BHK cells in the maintenance medium. The data are from one experiment with cells from five glass bottles per time point.

additional phospholipids during the first and second days, and maintained this level during the next two days (Table 1).

The phospholipid composition of the cells was

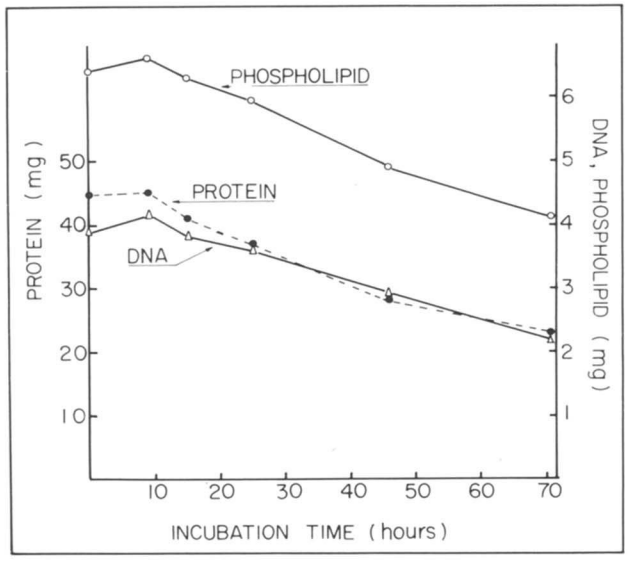


Fig. 4. Decline of the content of phospholipid, protein, and DNA of BHK cell monolayers incubated in the maintenance medium. The ordinate scales indicate the total amounts of the substances in five glass bottles in the same experiment as in Fig. 3.

TABLE 1. Changes in the phospholipid contents of monolayer cultures of BHK cells incubated in different media[a]

Duration of Incubation	Maintenance Medium	Fresh Growth Medium
	μg of lipid P	
Fresh monolayers	12.9 ± 1.6 (7)	14.1 ± 1.1 (2)
24 hr	12.5 ± 0.7 (3)	20.4 ± 1.5 (2)
48 hr	12.3 ± 1.9 (8)	23.0 ± 0.7 (2)
72 hr	8.9 ± 1.4 (5)	19.8 (1)
96 hr	6.4 ± 1.0 (8)	20.1 ± 1.8 (2)

[a] Values are mean ± standard deviation or half range (in the case of two analyses) of the phospholipid contents of single culture dishes. The number of independent analyses is given in parentheses.

analyzed by two-dimensional thin-layer chromatography (**Fig. 5**). The relative concentrations of lysobisphosphatidic acid (LBPA) and semilysobisphosphatidic acid (SLBPA) increased over the whole period of incubation in the maintenance medium (**Table 2**). After 96 hr they amounted to about 5% of the total cellular phospholipid, which represented a nearly fourfold increase over the initial value. Even the absolute amounts of LBPA and SLBPA per culture dish increased in spite of the 50% reduction of phospholipids, which indicates that a net synthesis of these lipids was taking place during the

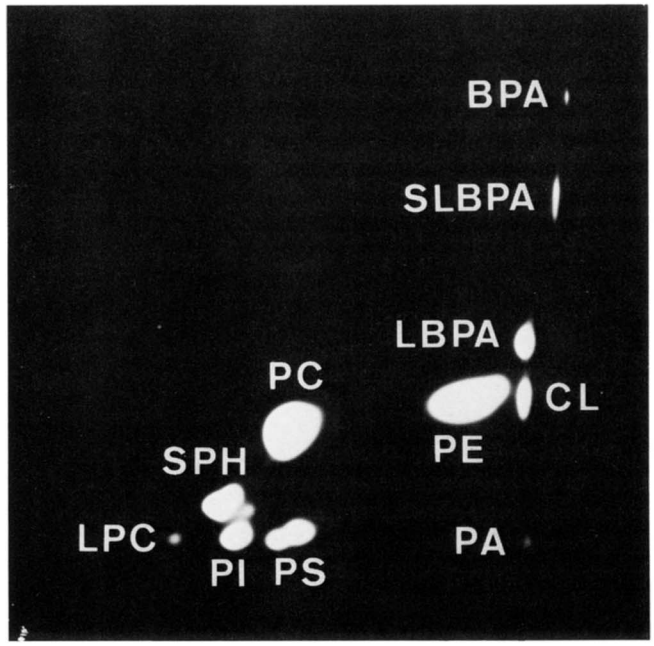


Fig. 5. Radioautogram of a two-dimensional thin-layer chromatogram of lipids extracted from BHK cells that were labeled to isotopic equilibrium with [^{32}P]phosphate and incubated in the maintenance medium for 4 days. Symbols: BPA, bisphosphatidic acid; SLBPA, semilysobisphosphatidic acid; LBPA, lysobisphosphatidic acid; CL, cardiolipin; PA, phosphatidic acid; PE, phosphatidylethanolamine; PC, phosphatidylcholine; SPH, sphingomyelin; LPC, lysophosphatidylcholine; PI, phosphatidylinositol, PS, phosphatidylserine.

TABLE 2. Changes in some phospholipids of BHK cells during incubation in maintenance medium[a]

Lipid	Incubation in Maintenance Medium			Incubation in Fresh Growth Medium	
	0 hr	48 hr	96 hr	48 hr	96 hr
Lysobisphosphatidic acid	1.4 ± 0.5 (11)	2.9 ± 0.7 (9)	3.6 ± 1.3 (9)	1.1 ± 0.1 (2)	1.1 ± 0.4 (3)
Semilysobisphosphatidic acid	0.03 ± 0.06 (8)	0.3 ± 0.3 (8)	1.7 ± 1.0 (9)	ND[b] (2)	0.3 ± 0.4 (3)
Phosphatidylcholine	55 ± 3 (11)	45 ± 5 (10)	40 ± 2 (9)	50 ± 3 (2)	48 ± 4 (3)
Sphingomyelin	6.5 ± 0.8 (11)	7.8 ± 2.1 (10)	12.8 ± 2.6 (9)	6.7 ± 0.9 (2)	6.7 ± 0.3 (3)

[a] Values are mol percent of the lipid phosphorus ± standard deviation, or half range (number of analyses in parentheses).
[b] ND, not detected.

incubation. The control cells showed no relative increase in the two lipids (Table 2).

One experiment was carried out with cells labeled to isotopic equilibrium with [^{32}P]phosphate to study the concentration of bisphosphatidic acid, which was undetectable by chemical means. At the start of the incubation in the maintenance medium this lipid amounted to 0.02% of the total phospholipid radioactivity; it increased to 0.04% in 48 hr and to 0.14% in 72 hr. Thus the behavior of bisphosphatidic acid paralleled that of the more abundant members of this family of phospholipids.

LBPA and SLBPA increased in the maintenance medium in the same way in BHK cells of both low and high passage number (from 10 passages to 43 passages, data not shown). The effect was also independent of the culture vessels (plastic dishes or glass bottles, data not shown). Furthermore, the increase of LBPA was independent of the cell density. When sparse monolayers of cells, containing only 5 μg of lipid-P per dish were incubated in the maintenance medium, the LBPA content of the cells increased in 3 days from 1.5 to 5.3% of lipid-P. The total amount of phospholipids did not change.

Besides the increase of the three bisphosphatidic acids, there were other changes in the phospholipids of the cells in the maintenance medium. The relative concentration of phosphatidylcholine decreased by about 30% and that of sphingomyelin increased twofold after 4 days of incubation (Table 2). These changes were not observed in control cells incubated in fresh growth medium. Cardiolipin, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylserine remained fairly constant in relation to the total phospholipids (data not shown).

Neutral lipids exhibited marked changes during incubation of the cells in the maintenance medium. The concentration of triglycerides, relative to phospholipid, increased fivefold in 4 days (**Table 3**). The absolute increase of the triglyceride fatty acids (0.16 μmol/dish) amounted to a sizable fraction of the decrease of the phospholipid fatty acids (0.42 μmol/dish). Free cholesterol also increased markedly in relation to the phospholipids (Table 3).

TABLE 3. Changes in neutral lipids of the BHK cells during incubation in maintenance medium[a]

Lipid	Duration of Incubation		
	0 hr	48 hr	96 hr
Triglyceride	11.3 ± 2.8	19.1 ± 4.1	49.1 ± 5.6
Cholesterol	27.4 ± 1.3	32.0 ± 1.6	70.3 ± 3.4
Cholesteryl ester	14.9 ± 4.0	12.6 ± 3.2	22.4 ± 4.1
Free fatty acid	11.3 ± 3.2	12.4 ± 3.9	12.8 ± 2.3

[a] Values are mol per 100 mol of phospholipid, mean ± half range of two independent experiments.

Changes in BHK cells incubated in fatty acid-free media

To check the possible role of the albumin-bound fatty acids in the proliferation of lysosomes (20) and in the increase of triglycerides (21–24), we repeated the experiments using fatty acid-free bovine serum albumin in the medium.

Electron microscopy revealed a smaller number of the electron-lucent vacuoles, probably of pinocytic origin (20), than observed in the presence of normal albumin (**Fig. 6**). Otherwise, the proliferation of the lysosomes appeared to progress similarly as in the previous experiments (Fig. 6).

The compositional changes were almost identical to those observed in the presence of normal albumin (**Table 4**). The phospholipids remained constant for about 2 days and then dropped by about 50% in another 2 days. The concentrations of LBPA and SLBPA increased several-fold. The increase of the triglycerides was similar to that noted when normal albumin was present, indicating that the majority of the triglyceride fatty acids was endogenous.

Fairly similar changes were also seen when albumin was totally omitted from the medium (Table 4).

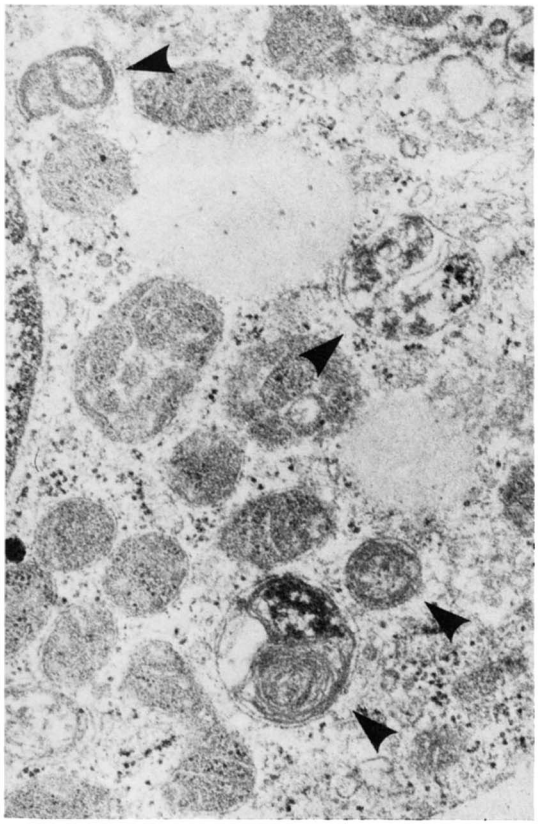


Fig. 6. BHK cell incubated for 48 hr in the maintenance medium that contained fatty acid-free albumin. There are numerous vacuoles with membranous or amorphous contents (arrows) but few clear vacuoles. Magnification ×36,500.

In the albumin-free maintenance medium the final phospholipid content of the cultures was lower than previously, suggesting a more severe degeneration of the cells. The concentrations of SLBPA and sphingomyelin (Table 4) as well as free fatty acids (data not shown) increased to a much greater extent than in the albumin-containing media. In absolute terms, however, the amount of sphingomyelin remained rather constant in this medium as well as in the other maintenance media.

Changes in BHK cells induced by sucrose in the growth medium

When the BHK cells were incubated in the usual growth medium supplemented with 0.1 M sucrose, they developed, quite expectedly (25–27), large numbers of clear vacuoles (**Fig. 7**). The vacuolization was accompanied by a moderate increase of the specific activity of the cellular acid protease (about 1.3-fold that of the control cultures at 48 hr) but the cellular acid phosphatase did not increase. In contrast to the lysosomal proliferation caused by the maintenance medium, the sucrose-induced vacuolization was not accompanied by an increase of the bisphosphatidic acids (**Table 5**); on the contrary, the concentration of LBPA appeared to decrease.

Lipid changes induced by oleic acid in the growth medium

When the BHK cells were grown in the usual growth medium supplemented by 0.07 mM sodium oleate, they accumulated triglycerides as expected (21–24) (**Table 6**). The increase was 3–4-fold in comparison to the control cells and was nearly equivalent to the total added oleate. However, lysobisphosphatidic acid did not increase.

DISCUSSION

The present data suggest that the incubation of BHK cells in the maintenance medium leads in a few days to a marked proliferation of lysosomes. The conclusion is based primarily on electron microscopy, but it is supported by the increase of two lysosomal enzymes, acid protease and acid phosphatase, and also by the increase of sphingomyelin, free cholesterol, and lysobisphosphatidic acid which are known to be lysosomal lipids (5–9, 28).

TABLE 4. Lipid changes in BHK cells during incubation in modified maintenance media

Lipid	Duration of Incubation 0 hr	48 hr	96 hr
Medium containing fatty acid-free albumin:			
Phospholipid (μg P/dish)	12.7 ± 2.2 (3)	13.4 ± 1.5 (3)	6.5 ± 0.9 (3)
Lysobisphosphatidic acid[a]	1.3 ± 0.4 (3)	2.8 ± 0.5 (3)	4.4 ± 0.5 (3)
Semilysobisphosphatidic acid[a]	0.05 ± 0.07 (3)	0.7 ± 0.3 (3)	2.2 ± 0.9 (3)
Phosphatidylcholine[a]	56.3 ± 0.3 (3)	48 ± 2 (3)	40 ± 2 (3)
Sphingomyelin[a]	6.7 ± 0.2 (3)	6.2 ± 0.3 (3)	13.0 ± 0.3 (3)
Triglyceride[a]	11 ± 3 (2)	16 ± 3 (2)	57 ± 9 (2)
Cholesterol[a]	27 ± 1 (2)	29 (1)	62 ± 8 (2)
Medium without albumin:			
Phospholipid (μg P/dish)	13.1 ± 1.9 (4)	14.7 ± 1.1 (4)	4.8 ± 1.2 (4)
Lysobisphosphatidic acid[a]	1.3 ± 0.3 (4)	3.1 ± 0.6 (3)	4.5 ± 1.4 (4)
Semilysobisphosphatidic acid[a]	0.04 ± 0.07 (4)	0.5 ± 0.3 (3)	6.5 ± 2.5 (4)
Phosphatidylcholine[a]	57 ± 1 (4)	50 ± 3 (3)	39 ± 11 (4)
Sphingomyelin[a]	6.5 ± 0.5 (4)	5.9 ± 0.2 (3)	17.0 ± 4.0 (4)
Triglyceride[a]	11 ± 3 (2)	12 ± 2 (2)	42 ± 7 (2)
Cholesterol[a]	27 ± 1 (2)	30 ± 2 (2)	67 ± 14 (2)

[a] Values are mol per 100 mol of phospholipid ± standard deviation, or half range (number of analyses in parentheses).

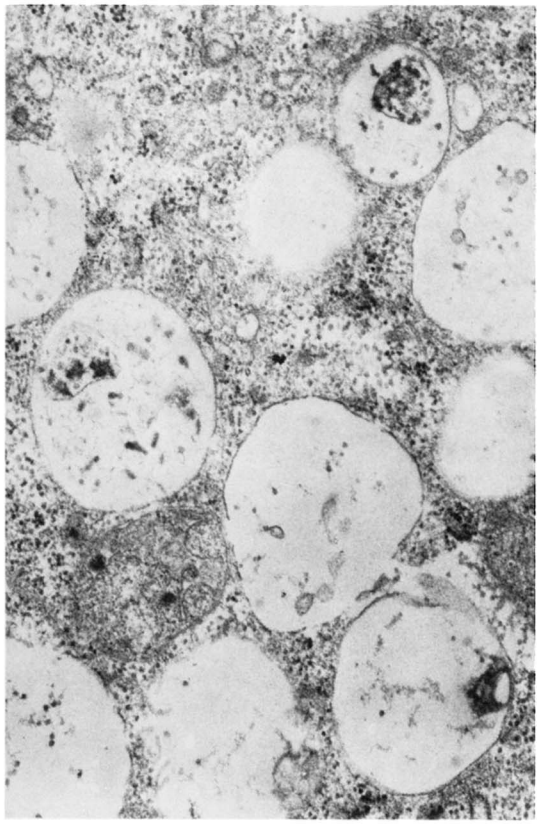


Fig. 7. BHK cell after 48 hr in the presence of 34 mg of sucrose per ml of growth medium. Lysosomes are swollen by the slowly digestible sucrose. Magnification ×29,000.

The increase of the lysosomal apparatus has been observed in stationary cultures previously. Mouse and human fibroblasts kept stationary in fresh growth medium exhibit vacuolization and an increase of the activities of lysosomal enzymes (29–32). In our experiments, involving the use of the maintenance medium, the proliferation of lysosomes develops, however, faster than in experiments where complete media were used. The underlying mechanisms may also be different.

We do not know what causes the proliferation of lysosomes in the maintenance medium, but an attractive view is that it is caused by nutrient deprivation and subsequent autolysis of dispensable material (13, 33). Another possibility is that it is caused by a partial inactivation of the lysosomal functions, for instance, by the high pH of the medium (34).

The proliferation of lysosomes was accompanied by a loss of cellular phospholipids and proteins and a simultaneous increase of triglycerides. Since triglycerides also increased in the absence of all exogenous fatty acids, it seems likely that they were derived mainly from the disappearing phospholipids. We visualize this process as progressive breakdown of the cellular membranes in the proliferating lysosomes followed by the formation of triglycerides from the fatty acids liberated from the phospholipids. The phenomenon may be related to the "fatty degeneration" sometimes seen in crowded and aging cell cultures (29, 31).

TABLE 5. Concentration of bisphosphatidic acids in BHK cells incubated in presence of sucrose[a]

Lipid	Duration of Incubation		
	0 hr	48 hr	96 hr
Lysobisphosphatidic acid	1.8 ± 0.2	1.3 ± 0.2	1.0 ± 0.3
Semilysobisphosphatidic acid	ND	0.03 ± 0.03	0.3 ± 0.3

[a] Values are mol per 100 mol of phospholipid ± half range of two experiments, one with 15 mg/ml, the other with 34 mg/ml of sucrose in the growth medium.

There was a marked increase of lysobisphosphatidic acid (LBPA) in the course of the incubation in the maintenance medium. This increase is likely to be associated to the lysosomal proliferation. LBPA is known to be enriched in the lysosomes of the BHK cell together with neutral lipids (5). The possibility that LBPA is directly connected with the formation of triglycerides in the endoplasmic reticulum seems improbable since the cells synthesized large amounts of triglycerides from exogenous oleic acid without concomitant increase of LBPA.

Sucrose provoked a heavy vacuolization in the BHK cells as expected (25, 27). The concentration of LBPA did not increase in the vacuolated cells although the sucrose-induced vacuoles are believed to represent lysosomal structures (27). An analogous observation is that LBPA is apparently missing from lysosomes in several storage diseases (35).

Our findings are consistent with the hypothesis that LBPA accumulates only in lysosomes that are involved in lipid degradation (35). We think that

TABLE 6. Concentration of triglyceride and lysobisphosphatidic acid in BHK cells grown in the presence of 0.07 mM sodium oleate[a]

Lipid	Oleate in Medium		Normal Growth Medium	
	24 hr after seeding	48 hr after seeding	24 hr after seeding	48 hr after seeding
Phospholipid (μg/dish)	80	177	83	183
Triglyceride (μg/dish)	89	110	28	23
Lysobisphosphatidic acid (mol/100 mol phospholipid)	1.9	1.4	1.1	0.9

[a] Results of one experiment with two culture dishes per each value.

random recombinations of partially digested lipids may be formed in transesterification reactions catalyzed by the lysosomal hydrolases (3). Among the "recombinants" so formed, the derivatives of *sn*-1-glycerophosphate may be the most slowly degraded ones, which would explain their accumulation.

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