Chinese Hamster Ovary Cell Lysosomes Rapidly Exchange Contents

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Abstract. We have used cell fusion to address the question of whether macromolecules are rapidly exchanged between lysosomes. Donor cell lysosomes were labeled by the long-term internalization of the fluid-phase pinocytic markers, invertase (sucrase), Lucifer Yellow, FITC-conjugated dextran, or Texas red–conjugated dextran. Recipient cells contained lysosomes swollen by long-term internalization of dilute sucrose or marked by an overnight FITC-dextran uptake. Cells were incubated for 1 or 2 h in marker-free media before cell fusion to clear any marker from an endosomal compartment. Recipient cells were infected with vesicular stomatitis virus as a fusogen. Donor and recipient cells were co-cultured for 1 or 2 h and then fused by a brief exposure to pH 5. In all cases, extensive exchange of content between donor and recipient cell lysosomes was observed at 37°C. Incubation of cell syncytia at 17°C blocked lysosome/lysosome exchange, although a "priming" process(es) appeared to occur at 17°C. The kinetics of lysosome/lysosome exchange in fusions between cells containing invertase-positive lysosomes and sucrose-positive lysosomes indicated that lysosome/lysosome exchange was as rapid, if not more rapid, than endosome/lysosome exchange. These experiments suggest that in vivo the lysosome is a rapidly intermixing organellar compartment.

THE lysosome, a major component of the vacuolar apparatus of eukaryotic cells, is active in many vesicle-mediated transport events (for review, see Steinman et al., 1983). It is the major site of accumulation and degradation of macromolecules internalized by fluid-phase pinocytosis or by receptor-mediated endocytosis. Phagolysosomes degrade bacteria and other phagocytized particles. Lysosomes may also be active in antigen processing and presentation (Ziegler and Unanue, 1981, 1982). These varied tasks are accomplished by a wide spectrum of proteins found principally in the lysosome. To date, all lysosomal proteins, whether luminal or membrane, have proved to be glycoproteins which are synthesized on membrane bound polysomes of the rough endoplasmic reticulum and then transported via vesicular carriers to the lysosome (for review, see Storrie, 1987). Although the exact nature of the carriers and their role in the biogenesis of the lysosome remains controversial, the biosynthesis of this organelle can be thought of as an example of secretion. Hence the lysosome is an intersection point for both endocytic and exocytic pathways.

Recent evidence from cell fusion experiments suggests that in vivo the Golgi apparatus, also a component of the vacuolar apparatus, rapidly exchanges membrane-bound macromolecules between separate stacks of cisternae (Rothman et al., 1984*a*, *b*). In fact, the efficiency of this exchange appears to be as great as that from one cisterna to another within the same Golgi stack. Cell fusion was used to introduce Golgi apparatus of differing biochemical capability into the same cytoplasm. Macromolecule exchange was measured as the successful completion of a series of biochemical steps.

In the present work, we have used a cell fusion approach to address the question of whether macromolecules are rapidly exchanged between lysosomes. In these experiments, differentially labeled lysosome populations were brought together in the same cytoplasm by vesicular stomatitis virus (VSV)-mediated cell fusion. Donor cell lysosomes were labeled by the long-term internalization of the fluid-phase pinocytic markers, invertase (sucrase), Lucifer Yellow (LuY),[1] FITC-dextran, or Texas red–conjugated dextran. Recipient cells contained lysosomes swollen by long-term internalization of dilute sucrose (Cohn and Ehrenreich, 1969) or marked by an overnight FITC-dextran uptake. In all cases, exchange of content between donor and recipient lysosomes was observed. These results suggest that in vivo the lysosome is an intermixing organellar compartment.

Materials and Methods

Cell Culture

For most experiments, 2×10^6 Chinese hamster ovary (CHO-K1) cells

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1. *Abbreviations used in this paper*: F12, Ham's F12 media; FC2, Ham's F12 media supplemented with 2% FCS; LuY, Lucifer Yellow; FC10, Ham's F12 media supplemented with 10% heat-inactivated FCS; P, 50 U/ml of penicillin; S, 50 μg/ml of streptomycin.

were inoculated in 25-cm² plastic T-flasks in Ham's F12 (F12) media supplemented with 10% heat-inactivated FCS (FC10). In some experiments, 2.5 × 10^5 cells were plated on 22-mm², No. 1½ glass coverslips. For viral infection, the serum supplement was lowered to 2% (FC2) and 50 U/ml of penicillin (P) and 50 μg/ml of streptomycin (S) were added to the media. Virus infection was ~18-h post cell plating. In experiments to localize invertase and FITC-dextran by cell fractionation, suspension-cultured CHO-S(C2) cells were used (Pool et al., 1983).

Viral Stocks

VSV stocks were produced by harvesting the growth media from infected cultures in 75-cm² plastic T-flasks. Late log phase CHO-K1 cells were incubated with 1:100 diluted virus stock for 1 h at room temperature. Fresh F12FC2PS was then added to the culture and cells were grown for 18–20 h at 37°C. The media was removed and centrifuged to pellet cell debris. The resulting supernatant was divided into 1 ml portions and stored at −75°C. In most cases cells were cultured with 30 ml media. To produce a concentrated virus stock, cells were cultured with 5 ml media.

Lysosomal-labeling Conditions

All marker uptakes were for 16–20 h at 37°C in complete culture media unless otherwise stated. Cells were incubated with sucrose at 0.03 M. The fluorescent markers LuY (100 μg/ml) and FITC-dextran (M_r = 72 kD, 1 mg/ml in most experiments) were obtained from Sigma Chemical Co., St. Louis, MO. In double-label dextran experiments, cells were incubated with FITC-dextran at 300 μg/ml and Texas red-dextran (M_r = 70 kD) from Molecular Probes, Inc., Eugene, OR, at 200 μg/ml. Yeast invertase (grade VII; Sigma Chemical Co.) concentration was 1 mg/ml. Uptakes were stopped by rinsing the cultures three times with 37°C culture media. To clear marker from an endosomal compartment, cultures were incubated for an additional 1–2 h at 37°C in marker-free media. This incubation should localize marker in the lysosomal compartment (Storrie et al., 1984*a*).

Acid Phosphatase Cytochemistry

CHO-K1 cells were grown overnight in the presence of 0.03 M sucrose to swell lysosomes. After a 1-h chase in sucrose-free media, the cells were fixed with 1.25% glutaraldehyde in 0.1 M sodium phosphate buffer, pH 7.4, for 10 min at 4°C (Storrie et al., 1984*b*). Cells were then stained for acid phosphatase by incubating in Novikoff's cytidine monophosphate medium (Novikoff, 1963) for 1 h at 37°C, postfixed in 1% osmium tetroxide, and embedded in Epon. Sections were observed with a Zeiss EM 10CA electron microscope at an accelerating voltage of 60 or 80 kV.

Cell Fractionation

To determine the subcellular location of invertase or FITC-dextran after an overnight uptake, CHO-S cells were homogenized and fractionated in 10% Percoll gradients as previously described (Storrie et al., 1984*b*).

Invertase and FITC-Dextran Assays

Invertase activity was assayed by a two step reaction protocol based on that of Goldstein and Lampen (1975). In the first step, 0.1 ml of cell fraction or diluted invertase (12.5–50 ng/ml) was combined with 0.2 ml of a reaction mix containing 0.15% Triton X-100 and 0.47 M sucrose in 0.09 M sodium acetate buffer, pH 4.9, and incubated at 37° or 17°C. The mixture was then combined with 0.8 ml of a second reaction mix containing 6.9 μg/ml horseradish peroxidase, 12.7 μg/ml glucose oxidase, and 85.8 μg/ml o-dianisidine in 0.17 M sodium imidazole buffer, pH 7.0. The resulting solution was quickly mixed and the development of colorimetric product monitored at room temperature for 2 min at 460 nm with a spectrophotometer. Color development was linear for the full 2-min reaction period. Invertase activity at 17°C was 50% of that at 37°C. For the assay of gradient fractions, the first step incubation was typically for 8–16 h.

FITC-dextran activity was assayed by combining 0.3 ml of each gradient fraction with 0.9 ml of 0.5 M glycine, 0.5 M carbonate buffer, pH 9.5. Fluorescence was measured with a fluorescence spectrophotometer (model 650-10S; Perkin-Elmer Corp., Norwalk, CT) set at an excitation wavelength of 490 nm and an emission wavelength of 525 nm with slit widths of 10 nm. Fluorescence values from a blank, parallel Percoll gradient were subtracted from the experimental values.

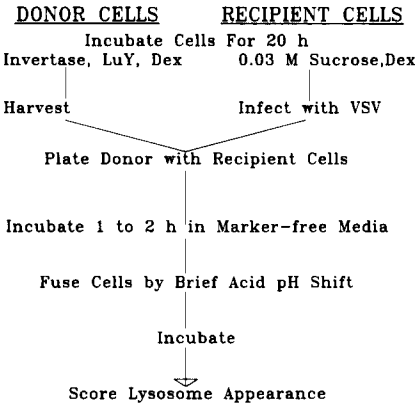


Figure 1. Flow chart for experimental protocol. Donor cell lysosomes were labeled by the long-term internalization of invertase (1 mg/ml) or one of the fluorescent tracers, LuY (0.1 mg/ml), FITC-dextran (72 kD, 300 μg/ml or 1 mg/ml), or Texas red–conjugated dextran (70 kD, 200 μg/ml). Recipient cell lysosomes were swollen with sucrose (0.03 M) or incubated overnight with FITC-dextran (72 kD, 200 μg/ml).

Cell Fusion

For cell fusion in T-flasks CHO-K1 cell monolayers, ~80% confluent, were infected with viral stock (diluted 1:1 with F12FC2PS ± 0.03 M sucrose as appropriate) for 1 h at room temperature. For cell fusion on coverslips, CHO-K1 monolayers were 95% confluent and infected with concentrated viral stock diluted 1:5–1:8 with F12FC2PS. Flasks or coverslips were rocked gently every 10 min to ensure uniform viral distribution. After a 1-h incubation, free virus was removed by rinsing the culture three times with 37°C F12FC2PS ± 0.03 M sucrose. Fresh media was added (± sucrose) and the virally infected cells were cultured at 37°C for 4 h to permit expression of the VSV G protein at the cell surface. The virally infected cells will be referred to henceforth as recipient cells. For coverslip cultures, 3 × 10^5 cells of a second cell population (donor cells) that had accumulated either LuY, FITC-dextran, or Texas red-dextran were added to the recipient culture 2-h postinfection and co-cultured with the recipient cells for 2 h in F12FC2PS. For recipient cultures in plastic T-flasks, 2 × 10^6 donor cells that had accumulated either invertase, LuY, FITC-dextran or Texas red–dextran were added to the recipient culture 2 to 3 h post-infection. At 4-h postinfection, the growth media was removed and cells were rinsed once with 2–5 ml of warm pH 5.0 fusion medium (Rothman et al., 1984*a*). T-flask cultures were then incubated in fusion medium for 5 min at 37°C. Coverslip cultures were incubated in fusion medium for 10 min at 37°C. After fusion, cells were rapidly rinsed with growth media and incubated at either 17° or 37°C. This experimental protocol is outlined schematically in Fig. 1.

Light Microscopy

LuY fluorescence was observed with a Zeiss lucifer yellow filter set (excitation, 400–440 nm; emission, LP470 nm, reflector, 460 nm) FITC-dextran fluorescence with a selective Zeiss fluorescein filter set (excitation, 450–490 nm; emission, 520–560 nm; reflector, 510 nm) and Texas red fluorescence with a Zeiss rhodamine filter set (excitation, interference filter BP546 nm; emission, LP590 nm; reflector, 580 nm). In control experiments, no FITC fluorescence was detectable with the rhodamine filter set, and likewise, no Texas red fluorescence was detectable with the fluorescein filter set.

Results

The strategy for these experiments was to bring together in the same cytoplasm differentially labeled lysosome populations and then score the status of the populations. To accomplish this, lysosomes were first differentially labeled by long-term incubation of separate CHO-K1 cell populations with

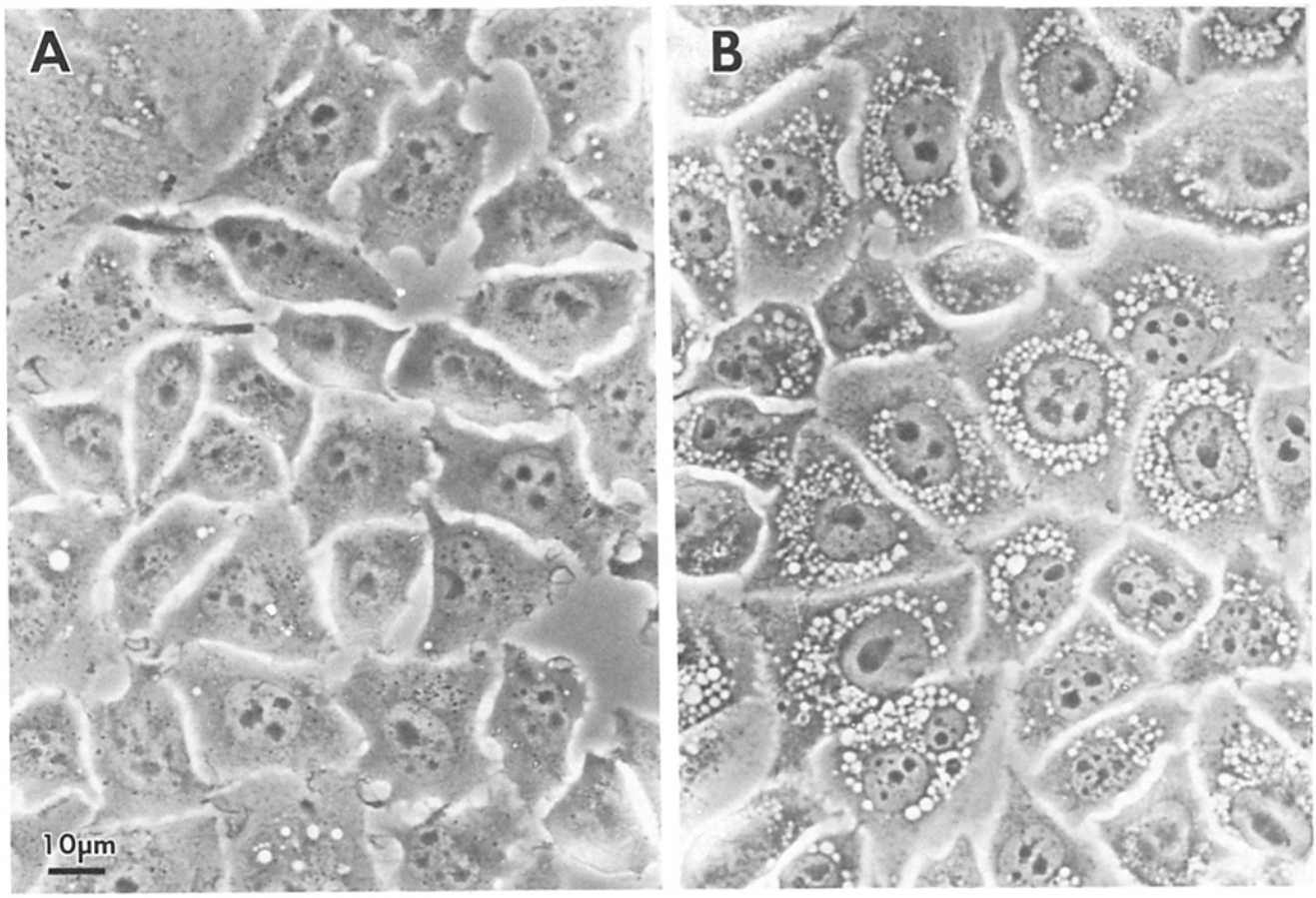


Figure 2. Morphological appearance of control cells (*A*) and cells grown in the presence of sucrose (*B*). CHO-K1 cells were cultured for 20 h on 22-mm² No. 1½ glass coverslips in complete culture media or in media containing 0.03 M sucrose. At the end of this time, the coverslips were transferred into fresh, sucrose-free, complete media, and the cells cultured for an additional 1 h. Occasional phase bright inclusions are seen in control cells (*A*). These are probably lipid droplets. Sucrose-treated cells (*B*) contain numerous perinuclear, phase bright inclusions. These are sucrosomes. For photography, the coverslips were mounted in complete culture media and a short working distance, 1.4 NA Olympus phase contrast condenser was used. The objective lens was an Olympus 40/0.65 SPlan achromat phase contrast objective.

fluid-phase pinocytic tracers, followed by a 1–2-h incubation in marker-free media to chase tracer from an endosomal compartment. The differentially labeled lysosomes present in recipient and donor cells were then brought together in the same cytoplasm by a VSV-mediated cell fusion step. The experimental protocol is outlined in Fig. 1.

For most experiments, the recipient cell population contained lysosomes labeled by sucrose induced osmotic swelling. Preliminary experiments based on the work of Cohn and Ehrenreich (1969) demonstrated that CHO cells became highly vacuolated during an 18–20-h exposure to 0.03 M sucrose (compare Fig. 2 *A* and *B*). Untreated CHO cells contained a small number of large phase-bright inclusions (Fig. 2 *A*). These inclusions were probably lipid droplets. The numerous phase-bright vacuoles in sucrose-treated cells were easily seen with the light microscope and remained stable for several hours (>5 h) after sucrose was removed from the extracellular media. To test whether the vacuoles were lysosomes that had swollen in response to sucrose accumulation, the subcellular localization of the standard cytochemical lysosome marker, acid phosphatase, was determined. By electron microscopy, the large vacuoles found in sucrose-treated cells were positive for luminal lead deposits characteristic of acid phosphatase reaction product (Fig. 3). Almost all the acid phosphatase activity in these cells was present in the large vacuoles, suggesting that the vacuoles are representative of the entire lysosome population. By definition, then, these vacuoles, which we refer to as sucrosomes, are lysosomes.

In many experiments, donor cell lysosomes contained invertase. Invertase, an enzyme that cleaves sucrose into its monosaccharide components, is internalized by CHO cells. To establish the subcellular localization of invertase in CHO cells after an overnight uptake CHO-S cells were fractionated in a 10% Percoll gradient (Storrie et al., 1984*a*). Invertase activity after a 10-min uptake was present in endosomes and after a 18-h uptake distributed similarly to the lysosomal marker, β-hexosaminidase (Fig. 4). In other experiments, donor cell lysosomes contained either LuY, FITC-dextran, or Texas red–dextran. LuY has previously been shown to ac-

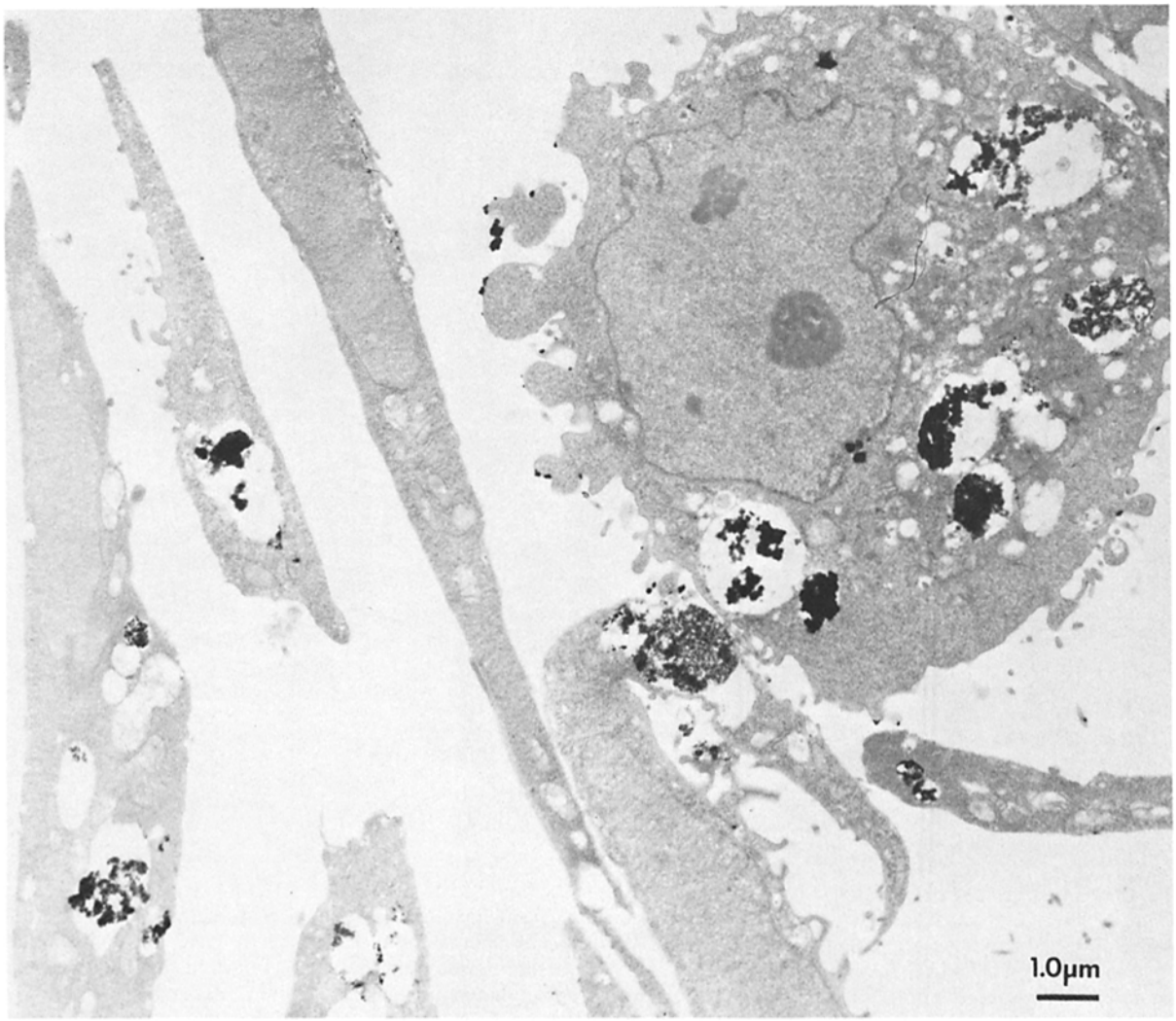
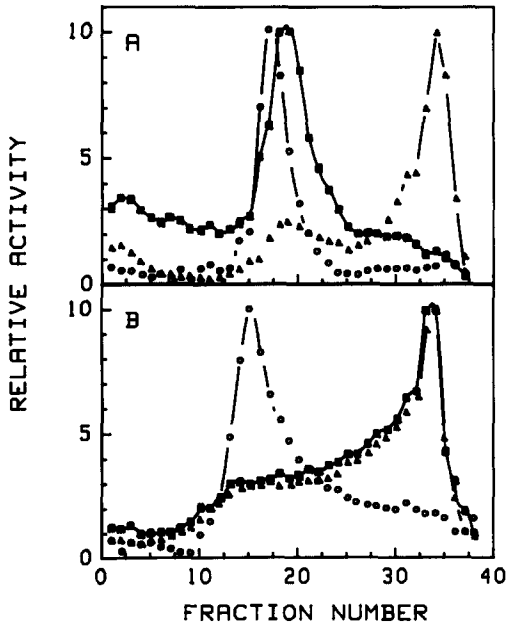


Figure 3. Acid phosphatase-specific staining of sucrosome-containing cells. CHO-K1 cells were grown for 20 h in media containing 0.03 M sucrose, the media was removed, and the cells were cultured for an additional 1 h in sucrose-free, complete media. Cells were then briefly fixed with 1.25% glutaraldehyde at 4°C and processed for acid phosphatase cytochemistry. The acid phosphatase substrate was cytidine 5'-monophosphate. The acid phosphatase reaction product appears as dense black deposits within large intracellular vacuoles, identifying these vacuoles as swollen lysosomes (sucrosomes).



cumulate in lysosomes under our uptake and chase conditions (Buckmaster et al., 1987). To establish the lysosomal localization of FITC dextran, CHO-S cells were fractionated in Percoll gradients. The distribution of long-term internalized FITC-dextran coincided with that of β-hexosaminidase in these gradients (data not shown). Texas red–conjugated dextran of the same molecular mass as the FITC-dextran (70 vs. 72 kD) was assumed to localize in lysosomes.

In initial experiments, the recipient/donor lysosome pairs were sucrosomes and invertase-positive lysosomes. This combination was chosen for ease of scoring lysosome/lysosome exchange. Cohn and Ehrenreich (1969) have previously reported that invertase delivered to sucrosomes cleaves sucrose to glucose and fructose; the monosaccharides diffuse

Figure 4. Invertase is internalized into endosomes and accumulates in lysosomes. Cells were incubated at 37°C for either 10 min (*A*) or 18 h (*B*) with 1 mg/ml invertase. Postnuclear supernatants were prepared and centrifuged in 10% Percoll gradients. Fractions were collected and analyzed for invertase (■), β-hexosaminidase (△, lysosomal marker), and alkaline phosphodiesterase I (○, plasma membrane marker) activity.

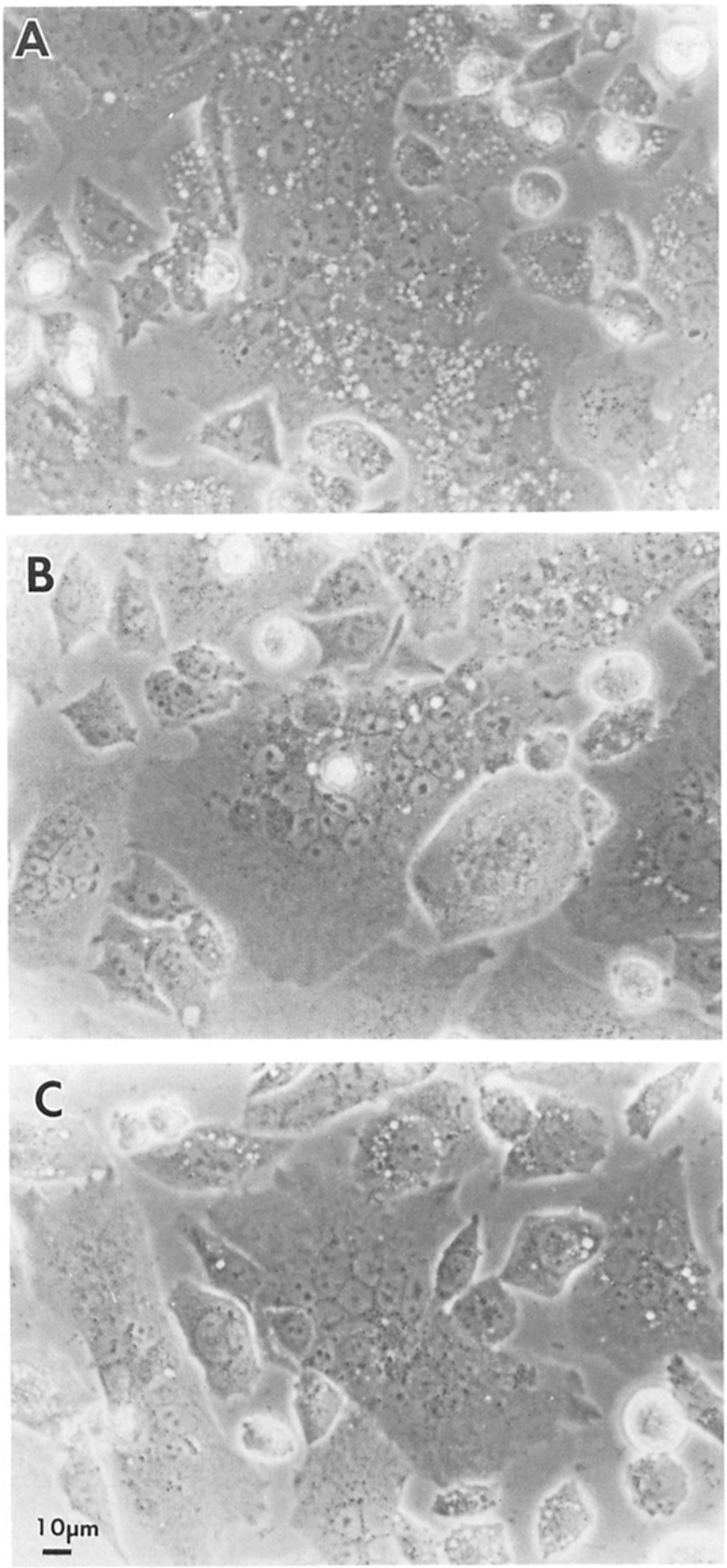


Figure 5. Disappearance of sucrosomes after fusion of cells containing sucrosomes with cells containing invertase-positive lysosomes. T-flask cultures (25 cm^2) of cells containing sucrosomes were fused with cells containing invertase-positive lysosomes. The cultures were photographed after 10 min (*A*), 1 (*B*) and 3 (*C*) h of incubation at 37°C. Micrographs were taken with a long working-distance (70 mm), 0.3 NA Zeiss condenser and a Zeiss 40/0.75 water immersion achromat phase contrast objective lens.

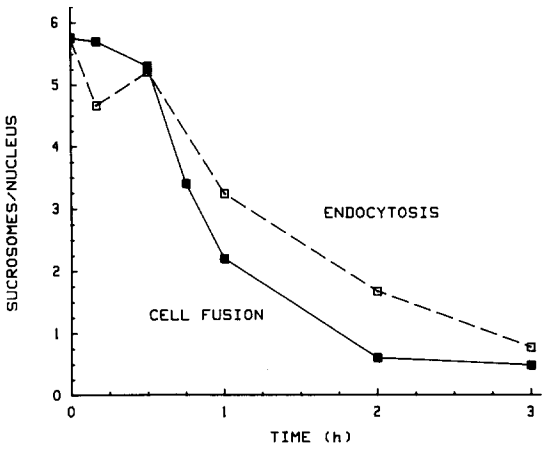


Figure 6. Comparative kinetics of sucrosome disappearance. T-flask cultures (25 cm^2) of cells containing sucrosomes were established. To one flask, invertase at a final concentration of 1 mg/ml was added and the flask photographed after various times at 37°C. To the second flask, cells containing invertase-positive lysosomes were added and after co-culturing for 1 h in marker-free media the cells were fused. Flasks were then photographed after incubation for various times at 37°C. Micrographs were taken as described in Fig. 5. For scoring, micrographs were printed at a magnification of 770×. Large phase-bright inclusions were scored as sucrosomes. Between 130 and 150 nuclei together with associated sucrosomes were scored for each time point.

across the sucrosome membrane and the sucrosomes shrink to normal size lysosomes. If lysosome/lysosome exchange occurs between sucrosomes and invertase-positive lysosomes in the mixed cytoplasm, then the large, phase-bright sucrosomes should disappear. In our experiments, rapid disappearance of sucrosomes was observed after fusion of recipient and donor cells. As shown in Fig. 5 *A*, initially the large cell syncytia had many sucrosomes. Within 1 h after fusion, many fewer sucrosomes were present (Fig. 5 *B*) and after 3 h a further decrease in large, phase-bright inclusions was apparent (Fig. 5 *C*). In control experiments in which sucrose-positive cells were fused to invertase-negative cells, sucrosomes were stable for at least 3 h after fusion. In experiments in which sucrose-positive and invertase-positive cells were co-cultured in the same cell ratio as in cell fusion experiments but were not fused, the sucrosomes were also stable. These experiments indicate that lysosome/lysosome exchange is occurring.

To investigate the rapidity of lysosome/lysosome exchange and to compare the kinetics of interlysosomal transfer of invertase with that of endocytic delivery of invertase to lysosomes, we scored the disappearance of sucrosomes in fused cells and in cells incubated with 1 mg/ml exogenous invertase. As shown in Fig. 6, after either cell fusion or endocytic delivery a time lag of ~30 min was observed. Little decrease in sucrosome number was apparent during this period. After the initial lag, a rapid decrease in sucrosome number was observed with cell fusion and a slower decrease with endocytic delivery. For each condition, the best curve fit for the rapid disappearance phase was found to be an exponential decay process with a $t_{1/2}$ of 22 min for the cell fusion conditions[2] and 43 min for the endocytic delivery conditions. It should be noted that Cohn and Ehrenreich (1969) have previously reported a similar time lag for endocytic disappearance of sucrosomes in mouse macrophages.

The disappearance of sucrosomes after cell fusion may require the transfer of only catalytic amounts of the enzyme invertase from one lysosome population to another. To test for bulk exchange of material among lysosomes fusion experiments were done substituting the fluorescent compound, FITC-dextran, for invertase in the donor population. FITC-dextran accumulates in lysosomes after fluid phase endocytosis. Cell syncytia were observed 2 h after fusion of sucrose- and FITC-dextran–positive cells. Based on the kinetics of sucrosome disappearance in fusions between sucrose- and invertase-positive cells, bulk exchange of marker would be expected 2 h post cell fusion. As shown in Fig. 7, many, but not all, sucrosomes were visibly positive for FITC-dextran at 2-h post cell fusion. In general, the fluorescence associated with the sucrosomes was faint in comparison with that in other portions of the cell syncytia. Based upon the large volume dilution of the fluor that should occur upon the transfer of FITC-dextran into sucrosomes, this faintness is to be expected. Considerable variation in fluorescence intensity within the sucrosome population was observed. As lysosome/lysosome exchange is likely to be a random process, this type of variation is to be expected. All sucrosomes may be positive for at least trace amounts of FITC-dextran. Similar results were observed using LuY as the fluorescent marker (data not shown).

The sucrosome is a convenient vehicle for visualizing the results of interlysosome exchange. To test for lysosome/lysosome exchange under conditions where both the donor and recipient lysosomes were as normal as possible, donor cells were labeled with Texas red–dextran (70 kD, 200 g/ml) and recipient cells with FITC-dextran (72 kD, 300 μg/ml). Cells were then fused and incubated for 1.5 h at 37°C and photographed sequentially using different filters. As shown in Fig. 8, extensive overlap in the distribution of FITC and Texas red fluorescence was apparent. Most interestingly, these overlaps included tubular lysosomal elements. Tubular lysosomes have been described previously by Silverstein and colleagues (Swanson et al., 1985, 1987). Cells were photographed unfixed in order to maximize the possibilities of observing tubular lysosomes. Because lysosomes are dynamic structures in living cells, the actual overlap between FITC- and Texas red–dextran distribution at any time point may be greater than in the micrograph series shown here. Included within the micrograph field are two Texas red–dextran–positive cells that have not fused into the cell syncytia. Note that the Texas red fluorescence (Fig. 8 *A*) from these cells did not appear in the fluorescein channel (Fig. 8 *C*).

A membrane delimited transfer of molecules between lysosome populations should be sensitive to lowered temperature (Dunn et al., 1980; see also Sullivan et al., 1987). To test whether low temperature affects the lysosome/lysosome exchange described, cells containing sucrosomes or fluorescent lysosomes (LuY or FITC-dextran) were fused at 37°C as usual, chilled to 17°C, and incubated at 17°C for 3 h. After this time period, no LuY fluorescence was observed to correspond with sucrosomes (Fig. 9). Similar results were observed with FITC-dextran (not shown). These results indicate that lysosome/lysosome exchange is temperature

2. In a separate cell fusion experiment (2- vs. 1-h clearance period for invertase from endosomes) scored by a different individual, the $t_{1/2}$ for sucrosome disappearance was 26 min.

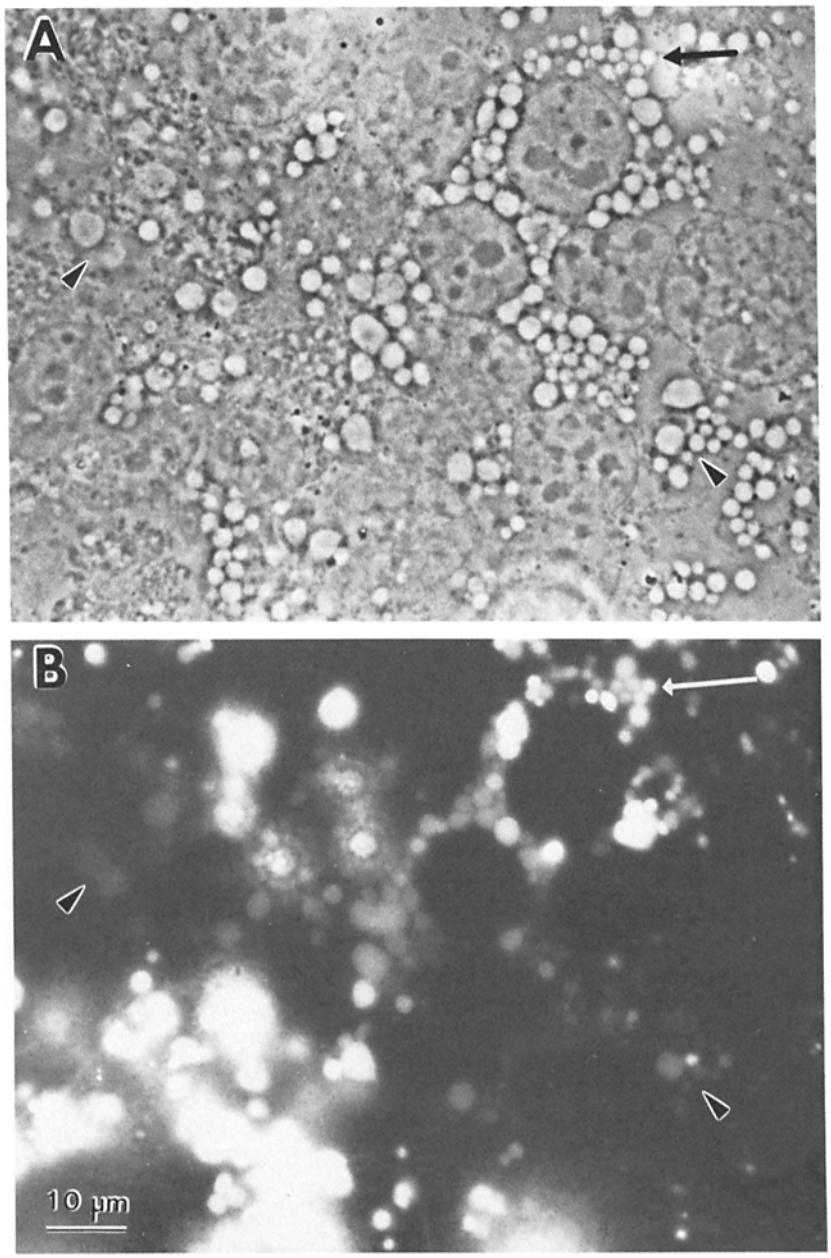


Figure 7. Bulk transfer of FITC-dextran between lysosomes. Cells containing sucrosomes were fused on glass coverslips with cells containing FITC-dextran-positive lysosomes. The FITC-dextran-positive cells were preincubated in FITC-dextran-free media for 2 h to clear FITC-dextran from endosomes. Fused cells were incubated at 37°C in marker-free media for 2 h. A high NA condenser was used for the phase micrograph (*A*). The fluorescence micrograph (*B*) was taken at the same focus as the phase micrograph. The microscope objective was a Zeiss Plan Neofluar 63/1.25 lens. In *B*, arrows point to examples of "bright" sucrosomes and arrowheads to fainter sucrosomes.

sensitive as would be expected for any membrane-mediated process.

Reduced temperature should be a freely reversible block of lysosome/lysosome exchange. To test this under easily quantified conditions, cells pulsed with either sucrose or invertase were fused at 37°C as usual, chilled to 17°C, and incubated for 2.5 h. As expected, the number of sucrosomes in the syncytia did not decrease at 17°C (Fig. 10). Warm media was then added to the culture and the syncytia were incubated at 37°C. Under these conditions, sucrosomes disappeared rapidly. Within 30 min after warming, the number of sucrosomes had decreased by 50% and this was followed by an additional 50% decrease during the subsequent 30 min (Fig. 10). Unexpectedly, in fused cells that were "preincubated" at 17°C, the sucrosomes disappeared without any apparent time lag. These results suggest that "priming" event(s) distinct from organelle exchange occur at 17°C.

Discussion

These experiments were performed to test whether, within the mammalian cell, lysosomes behave as a series of isolated, discrete organelles or as a rapidly intermixing organelle population. The data support the conclusion that lysosomes are a rapidly intermixing organellar population. The lysosomal markers used in these experiments were all content markers and our conclusion applies in the strictest sense to the contents of the organelle rather than to the membrane. Experiments are in progress to test whether lysosomal membrane proteins are rapidly exchanged within the organelle population.

Lysosomes from cells differentially labeled by the accumulation of content markers were introduced into the same cytoplasm by virus-mediated cell fusion at pH 5.0. Only the recipient cell population was infected with VSV, hence donor

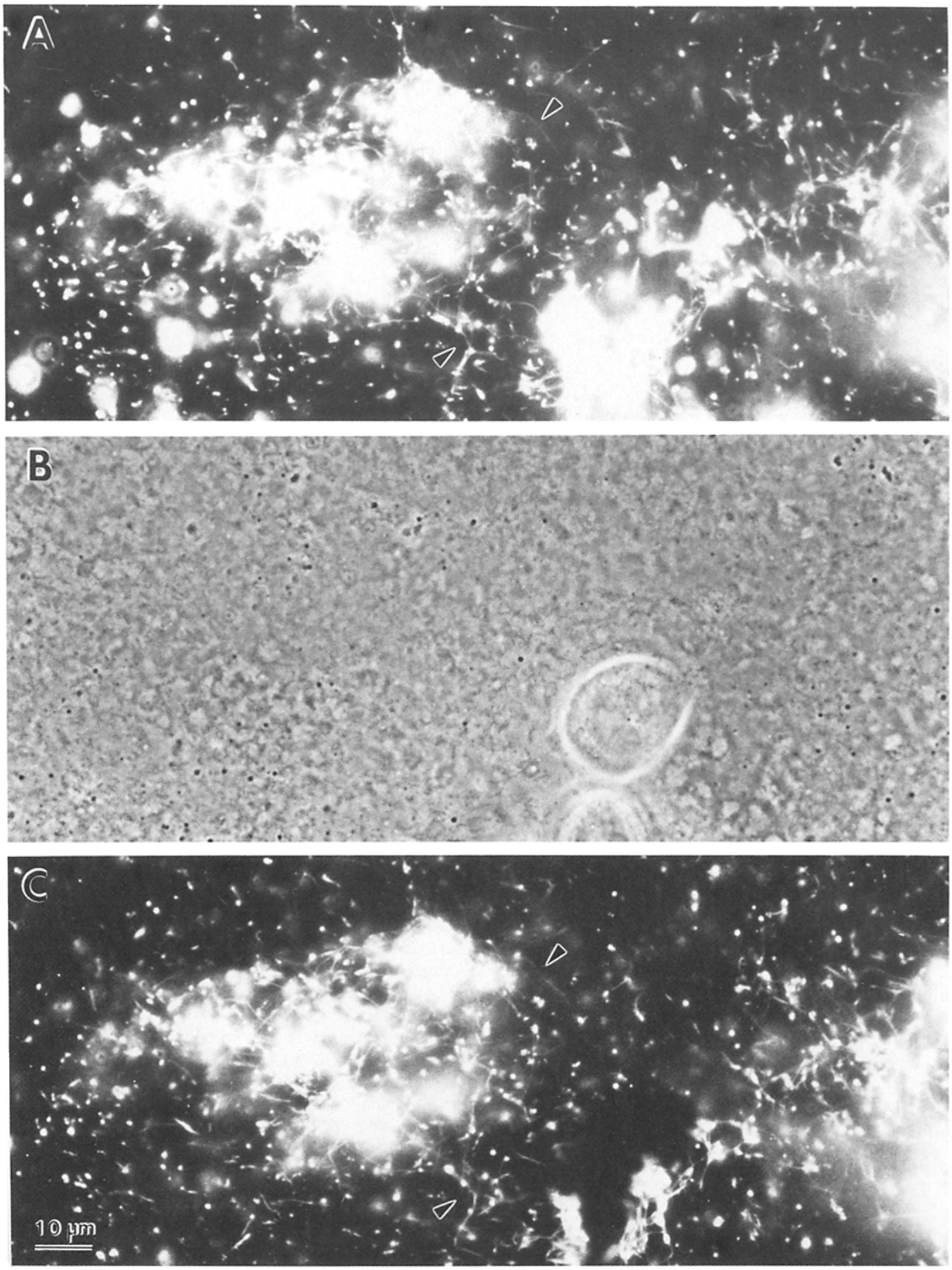


Figure 8. Content exchange between dextran-labeled lysosomes. Donor cells internalized Texas red–conjugated dextran (70 kD, 200 μg/ml) and recipient cells internalized FITC-dextran (72 kD, 300 μg/ml) overnight. Cells were preincubated in marker-free media for 2 h to clear dextran from endosomes. Cells on glass coverslips were fused and incubated at 37°C in marker-free media for 1.5 h. The focus was on tubular lysosomes with the Texas red filter set in place. Cells were unfixed and all micrographs are in the same focal plane. Texas red image (*A*), phase contrast image (*B*), FITC image (*C*). The objective was a Zeiss Plan Neofluar 63/1.25 lens. Arrowheads point to examples of tubular lysosomes labeled by both colored dextrans.

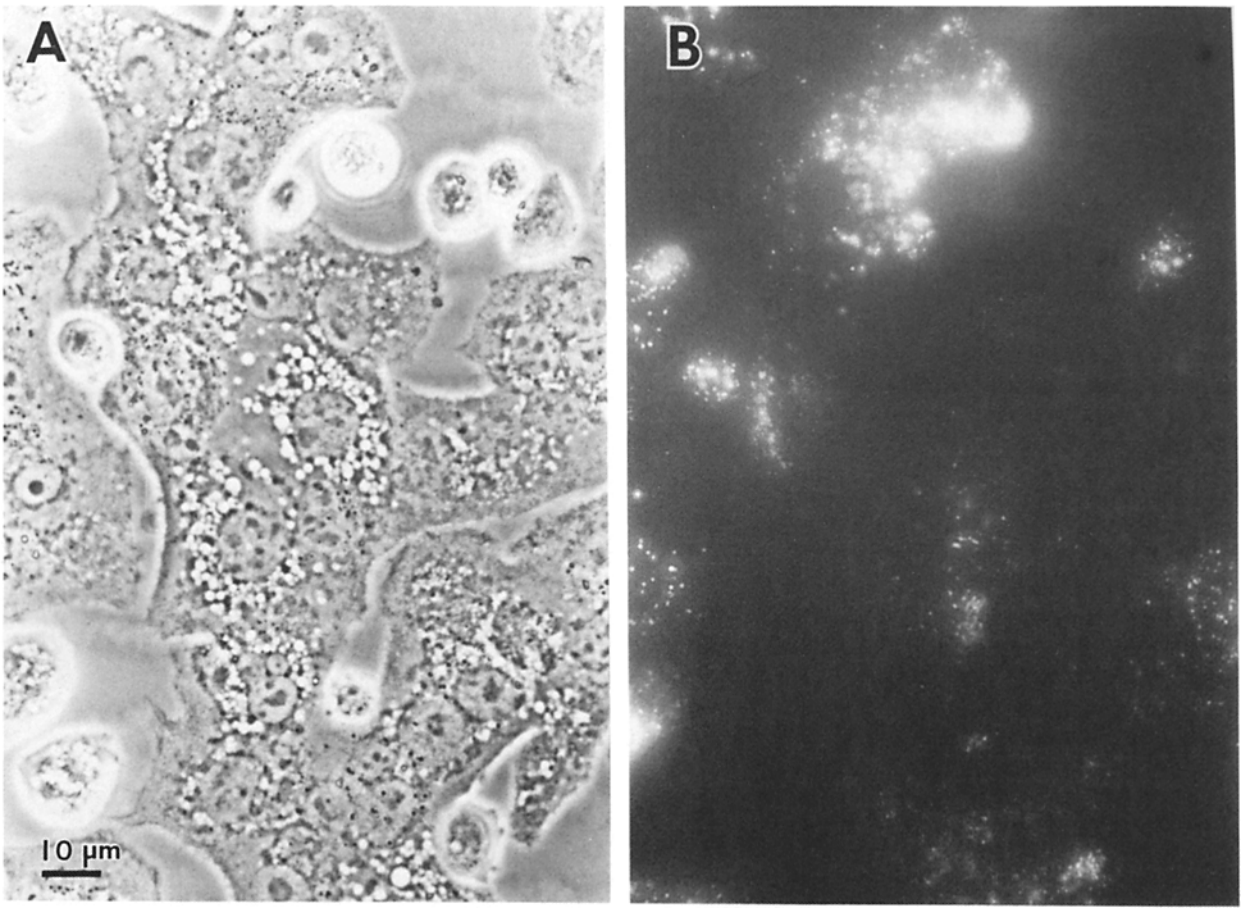


Figure 9. LuY is not transported between lysosomes at 17°C. Sucrosome-positive cells were fused with cells containing LuY-positive lysosomes at 37°C, then chilled to 17°C, and held at 17°C for 3 h. Micrographs were taken as described in Fig. 7. (*A*) Phase contrast; (*B*) LuY fluorescence. No correspondence between sucrosomes and LuY fluorescence was observed.

cells were incapable of fusing directly with each other. Live virus was used rather than UV-inactivated virus or a stable viral transfectant. This was the most convenient way to produce a concentration of the fusogen, VSV G protein, at the cell surface sufficient for the high efficiency cell fusion required in our experiments. For this work, the synchronous formation of large heterokaryons was essential. In cell syncytia containing only a few nuclei, the ratio of cytoplasm from recipient and donor cells is likely to vary greatly. In the large heterokaryons produced here the ratio is likely to be more uniform. The cell syncytia are a good model for normal cell organization. Within 1–2 min of cell fusion, the syncytia resembled normal cells in their organization with the nuclei centrally located and lysosomes perinuclear.

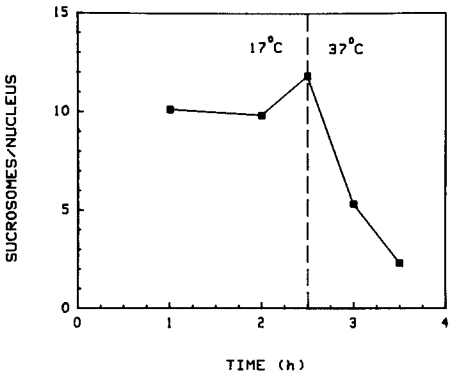


Figure 10. Preincubation at 17°C of a cell fusion between sucrosome-positive cells and cells containing invertase-positive lysosomes leads to a rapid disappearance of sucrosomes when the culture is warmed to 37°C. Sucrosome-positive cells were fused with cells containing invertase-positive lysosomes at 37°C, then incubated at 17°C for 2.5 h. Warm media was added 2.5 h postfusion and the culture was incubated at 37°C. The culture was photographed at various times during the experiment and the incidence of sucrosomes scored. Between 130 and 150 nuclei together with associated sucrosomes were scored for each time point.

Interlysosome content transfer in most experiments was scored between sucrosomes (sucrose swollen lysosomes) and donor lysosomes containing either invertase (sucrase) or fluorescent markers. The choice of sucrosomes for the recipient lysosomal population was based on ease of identification and future prospects for continuously monitoring the transfer process by video microscopy. Sucrosomes, formed readily in CHO-K1 cells incubated with dilute sucrose, were clearly identified as lysosomes by acid phosphatase cytochemistry and in the absence of invertase were stable over the time course of the experiments. Donor or recipient lysosomes containing either long-term internalized invertase, LuY, FITC-dextran or Texas red–dextran should be a normal organelle population. By cell fractionation, these lysosomes behaved identically to native lysosomes.

Content transfer between lysosomes was both rapid and extensive. In experiments where the recipient/donor lysosome pair was sucrosomes and invertase-positive lysosomes, after an initial lag period of ~30 min, sucrosomes disappeared with a $t_{1/2}$ of 22–26 min, and by 2–3-h post cell fusion essentially all sucrosomes had disappeared. Similarly, extensive organellar exchange was indicated by experiments in which the donor lysosomes contained fluorescent tracers. 2-h post cell fusion, many sucrosomes were positive for FITC-dextran or LuY. Our data strongly suggest that content transfer between lysosomes is at least, if not more, rapid and extensive than endocytic delivery to lysosomes. In experiments comparing sucrosome disappearance due to the endocytic delivery of invertase with interlysosomal transfer of invertase, a similar initial time lag of ~30 min was observed. In the exponential decay phase of the kinetic curves, the $t_{1/2}$ for sucrosome disappearance was twice as rapid for interlysosomal exchange as endocytic delivery. However, although the exogenous concentration of invertase was identical during the uptake phase, no effort was made to match the intraorganellar concentration of enzyme.

Intercommunication between lysosomes was reversibly inhibited at 17°C. Many events in membrane trafficking are inhibited at this temperature including endocytic transport to lysosomes (Dunn et al., 1980; see also, Sullivan et al., 1987) and secretory transport from *trans* elements of Golgi apparatus to the plasma membrane (Matlin and Simons, 1983). It was surprising that when the syncytia containing sucrosomes and invertase-positive lysosomes was warmed to 37°C, sucrosomes were lost with no apparent lag phase. These data suggest that a rate limiting step(s) of unknown nature in lysosome/lysosome exchanges can occur at 17°C. This step may be analogous with the priming event of intra-Golgi apparatus transport (for review, see Dunphy and Rotman, 1985). Different steps in protein transport within the Golgi apparatus have been reported to be differentially sensitive to temperatures between 15 and 20°C (Saraste and Kuismanen, 1984). The mechanism of lysosome/lysosome exchange is not known. Transfer may be by direct exchange between lysosomes or budding of vesicular carriers that shuttle from one lysosome to another.

Rapid interlysosome exchange may have several physiologic functions. For example, exchange provides a means to spread digestive load over the entire organellar population and to distribute newly synthesized luminal and conceivably membrane poteins to all preexisting lysosomes. Rapid interorganelle exchanges for similar biological reasons are apt to be a general trait of other subcellular organelle populations. Certainly they are a characteristic of the Golgi apparatus (Rothman et al., 1984*a*, *b*).

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