# ENDOCYTOSIS AND CHLOROQUINE ACCUMULATION DURING THE CELL CYCLE OF HEPATOMA CELLS IN CULTURE

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ABSTRACT

Variations of endocytic and of lysosomal functions during the cell cycle have been investigated in synchronized hepatoma cells (derived from Morris hepatoma 7288c) by following the cellular uptake of horseradish peroxidase, dextran (mol wt, 70,000), and chloroquine.

Cell fractionation and cytochemistry show that in asynchronously growing cells exposed for 1 h to 5 mg/ml peroxidase, the bulk of the enzyme taken up by the cells is found in phagosomes. By using the same experimental system with synchronized HTC cells, large variations of endocytosis are observed during the cell cycle. Peroxidase uptake is lowest during mitosis, increases 5–10 times during G1 phase, reaches a plateau, and finally decreases at the end of S phase and during G2 phase. A similar evolution is observed for the uptake of dextran (0.5 or 1 mg/ml), but it is likely that a significant part of the polysaccharide is still associated with the pericellular surface after 1 h. Moreover, dextran is transferred more slowly than peroxidase to lysosomes.

Cellular accumulation of chloroquine is related to intralysosomal pH or to the buffering capacity of lysosomes. Our results show that this drug is taken up more rapidly during G1 and S phases while the rate of accumulation is lowest in mitotic cells.

The results are discussed in relation to the modifications of the physical properties of lysosomes during the cell cycle observed previously by cell fractionation and electron microscopy, and to the possible role of lysosomes in the initiation of mitosis. Cyclic changes of endocytosis in actively dividing cells are demonstrated by our observations and may induce large differences in the uptake rate of extracellular substances.

KEY WORDS    cell cycle · hepatoma cells · lysosomes · endocytosis · peroxidase · dextran · chloroquine

Variations in the endocytic capacity and in the functional properties of lysosomes in an actively dividing cell population have obvious physiological and pathological implications. Moreover, they bear directly upon the applications of the "lysosomotropic" concept of cancer chemotherapy (11, 38) based on the use of endocytozable drug-carrier complexes and upon cell pharmacokinetics of compounds (weak bases) which are concentrated into lysosomes.

We report here a study of endocytosis in synchronized hepatoma tissue culture (HTC)[1] cells in which the accumulation of horseradish peroxidase (HRP) and radiolabeled dextran was followed. Chloroquine uptake was investigated as a model of the accumulation of a weak base. Results are discussed in relation to modifications in the physical properties of lysosomes described elsewhere (28), together with some implications with respect to the role of endocytosis in the nutrition of cultured cells, to the rate of membrane interiorization, and to the eventual function of lysosomes in mitosis initiation (1). Preliminary reports of this work have previously been published in abstract form (26, 27, 29).

## MATERIALS AND METHODS

### Biochemical Methods and Cell Synchronization

Methods for culture, determination of mitotic indices, thymidine incorporation, and fractionation of HTC cells have been previously described (4). A method derived from that of Sellers and Granner (33) was used for synchronizing the cells. It rests on selective detachment at mitosis combined with a metaphase block with Colcemid. After 12- to 24-h culture in 5-liter flasks (Arthur H. Thomas Co., Philadelphia, Pa.), the cells were cultured for 5 h in a medium containing $10^{-7}$ M Colcemid and 2.4 mM $CaCl_2$. Mitotic cells were then harvested by gentle shaking and decantation, washed two times with culture medium without Colcemid and $CaCl_2$, and further cultivated in suspension. Time 0 refers to the resuspension in fresh medium.

HRP, dextran, and chloroquine were added to the culture medium in solution in phosphate-buffered saline (PBS), the dilution of the medium being <20%. After incubation, cells were washed by centrifugation at 1,000 rpm for 5 min (Damon/IEC Div., Damon Corp., Needham Heights, Mass.), twice with culture medium including 10% serum and twice with PBS. Cells were then either sonicated at 0°C (30 s at 75 W; ultra-Sonicator from Branson Sonic Power Co., Danbury, Conn.) for the determination of HRP activity by the method of Strauss (37) or digested in 1 N NaOH, followed by neutralization with acetic acid for measurement of [$^3$H]dextran uptake. Large variations in dextran uptake were observed between batches; filtration on Sephadex G-25 (PD-10 columns, Pharmacia Fine Chemicals, A.B., Uppsala, Sweden) was found to reduce this variability and was performed in most of the experiments reported here. Chloroquine was measured by fluorimetry in 1% deoxycho-

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late, at pH 11.3 (excitation, 340 nm; emission, 370 nm) using a Perkin-Elmer fluorimeter model 1,000 (Beaconsfield, England).

### Morphology

Cells were fixed for at least 20 min with 2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.4, containing 1% sucrose. Cytochemical staining of peroxidase was performed by the method of Graham and Karnovsky (17), except that cells in suspension were incubated for 30 min in the presence of 0.05% diaminobenzidine. After washings in 0.05 M Tris-HCl buffer, pH 7.6, the cells were concentrated by filtration on Millipore filters (Millipore Corp., Bedford, Mass.) (5). They were postfixed with 1% $OsO_4$ in 0.05 M Veronal-acetate HCl, pH 7.4, containing 5% sucrose and were eventually stained with 0.5% uranyl acetate in 0.06 M veronal-acetate HCl buffer, pH 6, containing 4% sucrose (21). Pellicles were dehydrated in alcohol and embedded in Epon 812 as described by Luft (23). Sections were stained with lead citrate (31).

### Materials

HRP and 3,3'-diaminobenzidine tetrahydrochloride were purchased from Sigma Chemical Co. (St. Louis, Mo.); [$^3$H]dextran (mol wt, 70,000) from Radiochemical Center (Amersham, England); Colcemid and fetal calf serum from GIBCO (Paisley, England).

## RESULTS

### Peroxidase Uptake

In asynchronously growing cells exposed for 1 h to 5 mg/ml HRP, the uptake of peroxidase at 37°C was 1.13 ± 0.39 µg/mg cellular protein (mean of 12 experiments ± SD). The corresponding value at 0°C was 0.15 ± 0.08 µg HRP/mg cellular protein. Fixation of the protein at 0°C is most likely reflecting adsorption to the pericellular membrane. Hence this value was subtracted from the value obtained at 37°C to evaluate endocytic uptake. As shown in Fig. 1, the uptake is linear for at least 3 h under our conditions.

In all five experiments where HRP uptake was measured in synchronized cells (Fig. 2), the accumulation over a 1-h period was lowest at mitosis; thereafter, an increase was observed during the first hours of the G1 phase. A plateau corresponding an average of nearly eight times the amount accumulated at mitosis was reached after 8 h. Between 16 and 20 h after mitosis, accumulation decreased concomitant with thymidine incorporation.

An effect of Colcemid on endocytosis appears unlikely for two reasons. First, when asynchro-
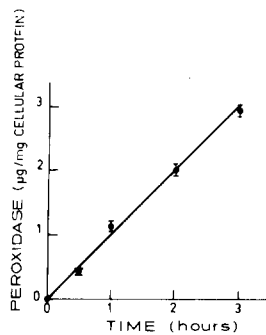
FIGURE 1 Kinetics of HRP uptake in asynchronous culture. Values are differences between the 37° and 0°C uptakes observed for an external concentration of 5 mg/ml HRP. Means of three experiments ± SD. Conditions for incubation, washing, and determination of activity are described in Materials and Methods.
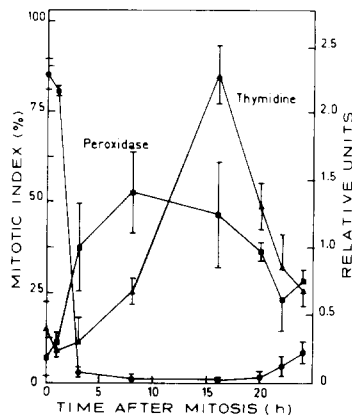


FIGURE 2 Peroxidase uptake by cells synchronized in the presence of $10^{-7}$ M Colcemid. At various times after mitosis, synchronized cells were incubated for 1 h in the presence of HRP (5 mg/ml). HRP uptake (■), thymidine incorporation (▲), and mitotic index (●) were observed. Values are means of five experiments, except for the sample at 1 h (two experiments). The vertical bars represent SD. The HRP uptake is the difference between cell-associated HRP at 37°C and that at 0°C, expressed with respect to the average value over the cycle (1.21 μg HRP/mg cellular protein). For thymidine incorporation measured over ½ h incubation, the average value over the cycle, taken as unity, was 8.53 pmol/h/mg cellular protein.

nously growing cells are pretreated for 5 h with $10^{-7}$ M Colcemid, then washed and exposed to a medium containing 5 mg/ml HRP for 1 h, no significant effect of Colcemid on HRP uptake was found. At most, a 20–30% decrease in peroxidase uptake was observed after a 5-h pretreatment with

$10^{-7}$ M Colcemid, provided the drug remained present during the uptake period. Secondly, an HTC cell population can be partially synchronized in absence of Colcemid by relying solely on selective cell detachment at mitosis. After collection and washings as described for synchronization in the presence of Colcemid, a mitotic index of 20.5% (Fig. 3) was obtained and increased again to nearly 10% after 17 h. Peroxidase uptake presented clearly a peak 3 h after the beginning of the experiment. This is somewhat earlier than in the presence of Colcemid, but the shift is also occurring for the peak of thymidine incorporation.

## Dextran Uptake

For an extracellular concentration of 0.5 mg/ml dextran, the amount of dextran fixed in 1 h at 0°C (0.073 μg/mg cellular protein) represented ~30% of that obtained at 37°C (0.255 μg/mg). While the accumulation was linear as a function of concentration up to 2 mg/ml dextran, the time curve showed somewhat more rapid fixation of dextran during the first 15 min (Fig. 4).

In synchronized cell populations, the uptake of dextran doubled roughly between mitosis and the middle of G1 phase (Fig. 5). It then decreased during S phase and was minimal during G2 phase.

## Intracellular Fate of Peroxidase and Dextran

For the interpretation of our observations on uptake of peroxidase and dextran, it is crucial to ascertain to what extent our accumulation measurements reflect interiorization by endocytosis or
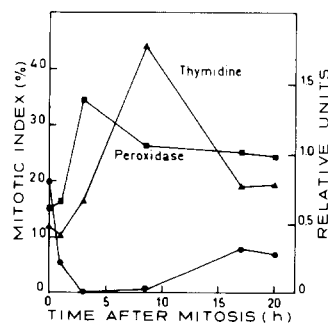


FIGURE 3 Peroxidase uptake by cells synchronized in the absence of $10^{-7}$ M Colcemid. Experimental conditions and presentation as in Fig. 2, except that the average values taken as unity for peroxidase uptake and thymidine incorporation here were 0.86 μg HRP/mg cellular protein and 11.96 pmol/h/mg cellular protein, respectively.
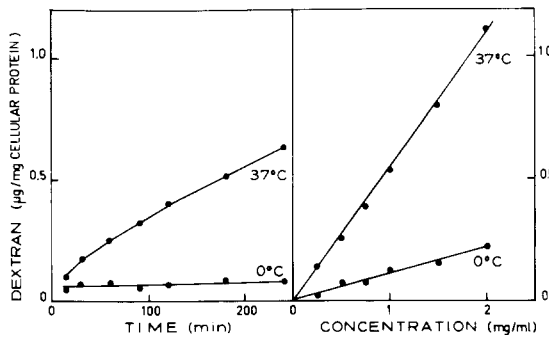
FIGURE 4 Time- and concentration-dependent dextran uptake in asynchronously growing cells. Kinetics was measured with an external concentration of 0.5 mg/ml dextran. The uptake as a function of dextran concentration refers to a 1-h incubation. Values are means of two experiments.
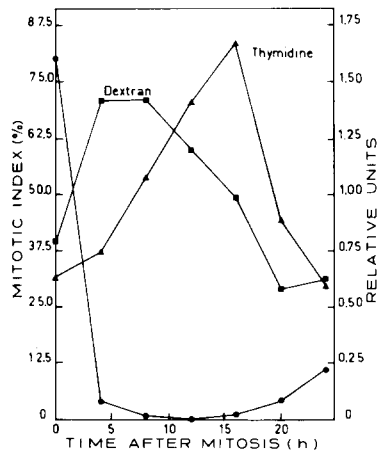


FIGURE 5 Dextran uptake by synchronized cells. Mean of two experiments performed at two different dextran concentrations (0.5 and 1.0 mg/ml). Dextran uptake (■) is expressed as the difference between cell-associated activity measured at 37°C and that at 0°C after 1-h incubation. Average values taken as unity for dextran uptake and for thymidine incorporation are 0.593 μg dextran/mg cellular protein and 10.85 pmol/h/mg cellular protein, respectively. Other experimental conditions as in Fig. 2.

merely adsorption to the pericellular membrane. Preliminary experiments, in which 32 strokes of a tight Dounce pestle (Kontes Co., Vineland, N. J.) were used to homogenize the cells as described elsewhere (4), indicated that after 1-h uptake of HRP or dextran, a significant fraction of the material taken up by the cells occurred in soluble form. The amount of HRP, dextran, phosphoglucomutase, and N-acetyl-β-glucosaminidase solu-

bilized during the homogenization process was thus determined as a function of the number of pestle strokes to find optimal conditions. After a 1-h uptake period at 37°C, HRP and especially dextran were more readily solubilized than lysosomal N-acetyl-β-glucosaminidase (Fig. 6). Homogenization was thus restricted to 10 strokes for fractionation experiments after short uptake periods. Under these conditions, cytosol phosphoglucomutase was nearly completely solubilized, while ~70% of the peroxidase remained sedimentable. The situation was much less favorable for dextran because it was largely (70%) soluble under these conditions.

Fractionation of asynchronous cell populations by density equilibration (Fig. 7) shows that, after 1-h incubation, peroxidase is found partly in soluble form and partly in the density region where alkaline phosphodiesterase 1 is concentrated. When the cells are further cultured for 7 h in fresh medium after the peroxidase uptake, the distribution of HRP becomes very similar to that of acid hydrolases. Experiments (results not shown) suggest that a period of 5 h is already sufficient for obtaining a large coincidence between peroxidase and acid hydrolases distributions. Independent determinations of soluble activity from the homogenates (centrifugation 30 min at 40,000 rpm; W = 3·10^{10} rad^2·s^{-1}) show also a significant decrease (from 25 to 8%) of soluble peroxidase 7 h after the uptake.

The general appearance of HTC cells in the electron microscope is presented in Fig. 8A. In cells exposed for 1 h to 5 mg/ml peroxidase, the
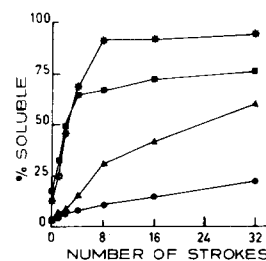


FIGURE 6 Effect of the homogenization procedure upon solubilization of HRP and [³H]dextran. Asynchronously growing cells incubated for 1 h at 37°C in the presence of HRP (5 mg/ml) or dextran (0.5 mg/ml) were homogenized with the tight pestle of a Dounce homogenizer. Soluble activities of phosphoglucomutase (*), [³H]dextran (■), HRP (▲), and N-acetyl-β-glucosaminidase (●) were determined in the supernate of the homogenates after 30-min centrifugation at 40,000 rpm (W = 3·10^{10} rad^2·s^{-1}).
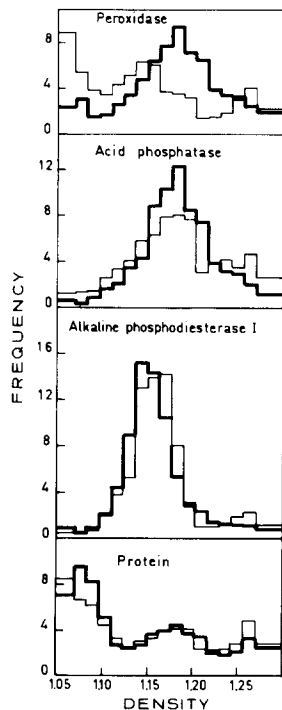
FIGURE 7 Density equilibration of homogenates from asynchronous cells after HRP uptake. Cells were cultivated at 37°C either for 1 h in the presence of HRP (thin line) or for 1 h in the presence of HRP and subsequently for 7 h in fresh medium (thick line). HRP concentration in the culture medium was 5 mg/ml. They were then homogenized and submitted to density equilibration in a sucrose gradient. Results are represented in the form of standardized histograms (7). Recoveries (sum of the activity of all fractions with respect to the activity of the homogenate) ranged between 81.9 and 108.8%.

diaminobenzidine reaction showed numerous positive intracellular structures, while the plasma membrane was unstained (Fig. 8 B).

Fractionation experiments of asynchronous cells incubated with dextran at 37°C confirmed that the polysaccharide was mostly soluble after 1-h uptake (Fig. 9 A). When cells were further incubated in fresh medium at 37°C, a transfer of dextran towards the fractions rich in N-acetyl-β-glucosaminidase occurred progressively (Fig. 9 B). Larger overlapping between the distributions of dextran and lysosomal hydrolases was observed after further culture in fresh medium for 19 h (Fig. 9 C).

### Chloroquine Accumulation

Fractionation by density equilibration of cells incubated for 1.5 h in the presence of 100 μM chloroquine showed that the drug had a very disperse distribution (Fig. 10) with a peak in the same region as that of N-acetyl-β-glucosaminidase. The equilibrium density of lysosomal hydrolases was appreciably (~0.035 g/cm³) displaced towards lower densities. A small shift was also observed for alkaline phosphodiesterase I. Under these conditions, intense vacuolization of the cells could be observed in the light microscope.

As is the case for other cell types (11, 15, 40), the kinetics of accumulation are not linear. In HTC cells growing asynchronously (Fig. 11), accumulation levels off after 1 or 2 h, depending on the concentration of the drug. In synchronized cell populations (Fig. 12), the progressive appearance of a peak was observed 8 h after mitosis when accumulation lasted for 2 h. Detailed kinetics (results not shown) indicated that this was essentially a result of changes in the maximal accumulation level of the drug, which did not even reach a plateau for the samples 8 and 16 h after mitosis.

## DISCUSSION

### Peroxidase Uptake

No cytochemical staining of peroxidase can be observed by electron microscopy in the pericellular membrane, and under our experimental conditions the enzyme is found only in cytoplasmic vacuoles. On the other hand, cell fractionation experiments indicate that after 1-h uptake, peroxidase equilibrates at a lower density than lysosomes. The overlapping with the distribution of alkaline phosphodiesterase I, classically considered to be a marker of the plasma membrane, suggests that this enzyme may also be associated with the phagosomal membrane, derived from the plasmalemma. However, it should be noted that other components such as Golgi membranes are also concentrated in these fractions (4). Thus, morphological and cell fractionation data together indicate that after 1-h uptake, HRP is mainly localized in phagosomes. The soluble activity found at that time stems probably from the disruption of some phagosomes upon homogenization.

Density equilibration shows that the transfer of HRP into the lysosomes occurs during further culture of the cells in fresh medium. It should be mentioned that because the half-life of HRP in lysosomes is ~5 h (4), the degradation of HRP can be neglected in our experiments where uptake is restricted to 1 h.
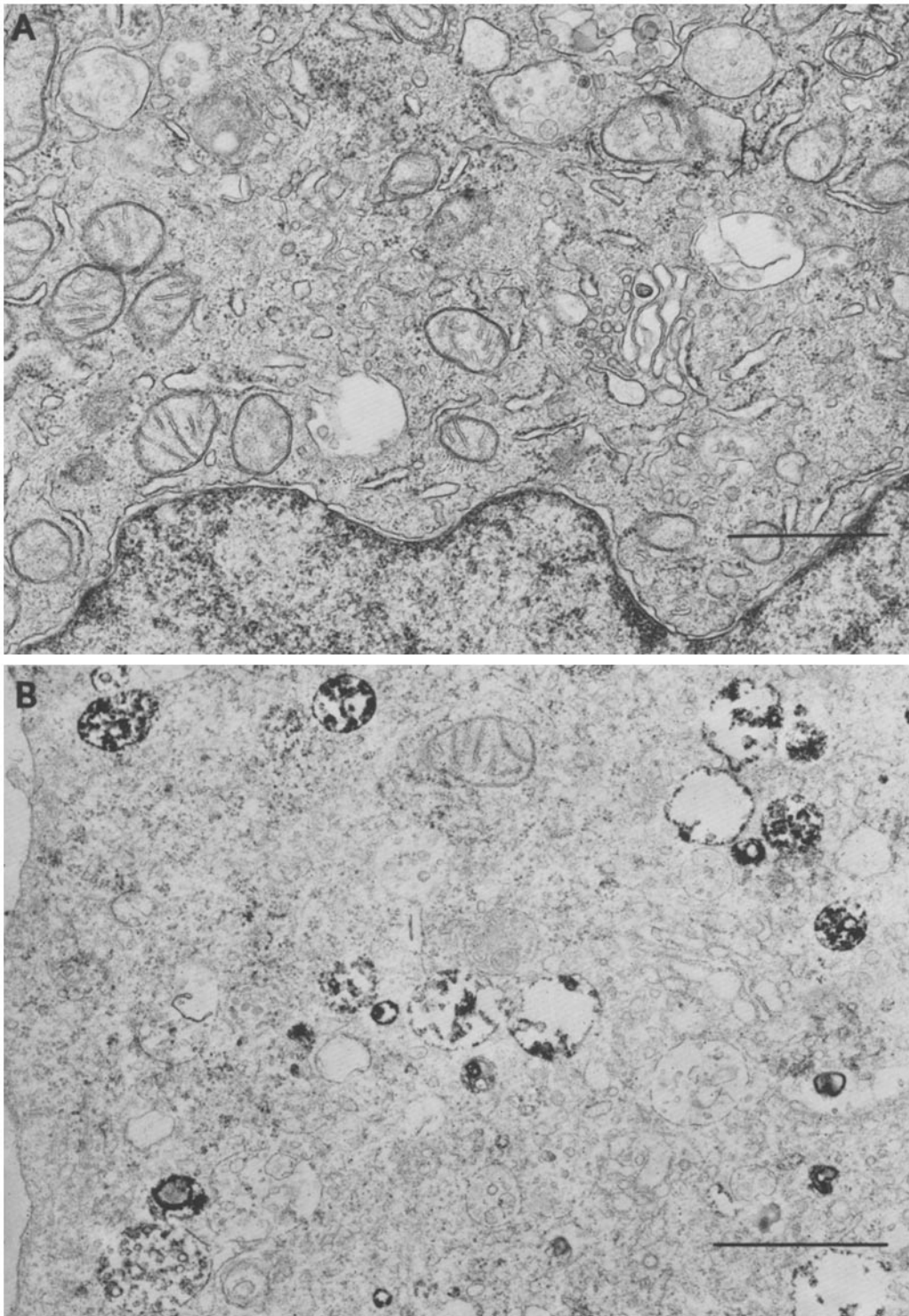
FIGURE 8 Cytochemical localization of peroxidase. (A) General appearance of HTC cells stained in block with uranyl acetate. × 23,500; bar, 1 μm. (B) Staining with diaminobenzidine after 1-h culture at 37°C in the presence of 5 mg/ml HRP. To facilitate identification of HRP-positive structures, no staining with uranyl acetate was performed × 25,000; bar, 1 μm.
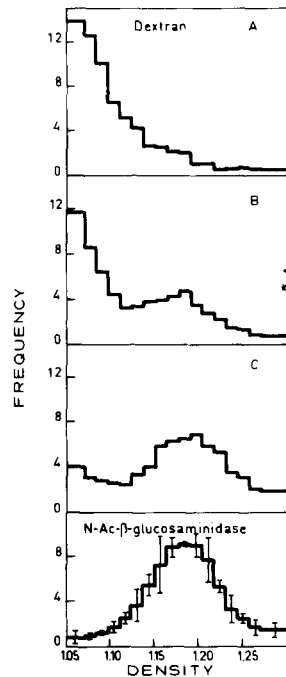
FIGURE 9 Density equilibration of homogenates of asynchronous cells cultivated in the presence of dextran. Cells were incubated at 37°C with [³H]dextran before homogenization. (A) 1-h culture in the presence of dextran (0.5 mg/ml); (B) 16-h culture in the presence of dextran followed by 5 h in normal medium; (C) 19-h culture in the presence of dextran followed by 19 h in normal medium. The average distribution of N-acetyl-β-glucosaminidase in the three experiments is represented in the lower graph, where the vertical bars correspond to the SD. Recoveries ranged between 76.7 and 107.6%.

Maximal endocytosis is occurring during the second half of G1 and the first half of S phase. Endocytosis is lowest at mitosis; it may even be overestimated in our experiments, owing to the ±10% contamination by nonsynchronized cells. Notwithstanding the progressive desynchronization of the cell population, the decrease in HRP uptake at the end of the cycle is, in our opinion, significant. Because it is concomitant with or even slightly precedes the decrease in thymidine incorporation, it may thus be concluded that endocytic uptake is largely decreased at the end of S phase and during G2 phase. In synchronization experiments without Colcemid, a twofold increase in HRP uptake occurs within 3 h. Because this population contained only 20% of mitotic cells, this is compatible with the eightfold increase observed for a cell population synchronized at 80%. The

low initial mitotic index observed for the cell preparation obtained in the absence of Colcemid could be explained by a contamination by cells entering early G1 and not yet adherent. The earlier occurrence of the thymidine peak (8 instead of 16 h) and of the second mitotic burst (16 instead of 24 h) is in agreement with this explanation.
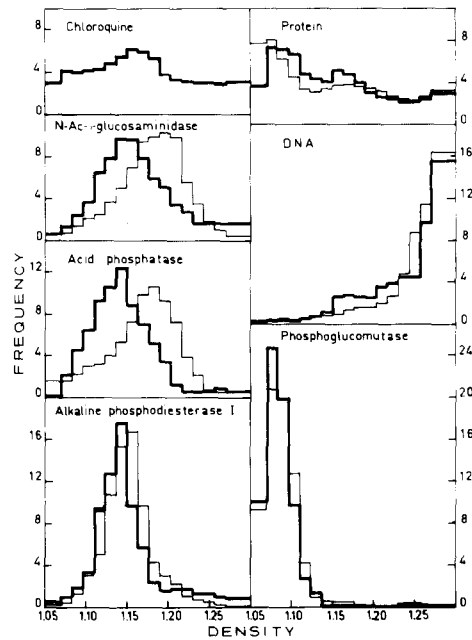


FIGURE 10 Density equilibration of homogenates of asynchronous cells incubated 1.5 h at 37°C in the presence (thick line) or the absence (thin line) of 100 μM chloroquine. Cells were homogenized and submitted to density equilibration as previously described (4). Presentation as in Fig. 7. Recoveries ranged between 87.1 and 118.5%.
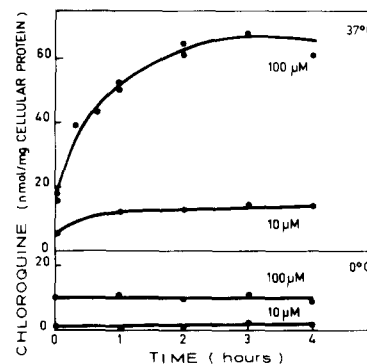


FIGURE 11 Chloroquine accumulation by asynchronous culture. Cells were incubated in the presence of 10 or 100 μM chloroquine at 37°C and 0°C.
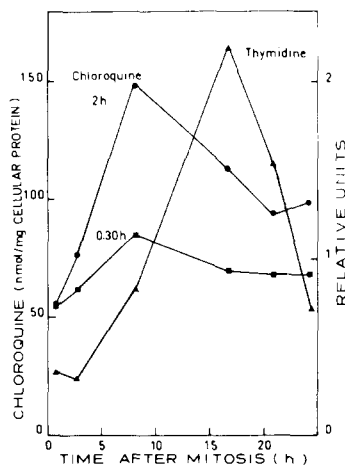
FIGURE 12  Chloroquine accumulation and thymidine incorporation in synchronized cells. Accumulation of chloroquine during 30 min (■) and 2 h (●) was measured at various times after mitosis. For thymidine incorporation measured over ½ h, unity represents 12.93 pmol/h/mg cellular protein.

## Dextran Uptake

In the dextran uptake experiments, the polysaccharide is largely recovered in soluble form upon homogenization and its transfer to lysosomes is much slower than that of peroxidase. The solubilization of dextran is not the result of a labilizing effect on the phagosomal membrane: we have verified that after simultaneous uptake of dextran and HRP, the solubilization pattern of the two macromolecules is essentially the same as in Fig. 6. To explain our experimental data, two different pathways for dextran uptake can be proposed. The first and most likely one involves the adsorption of dextran to the pericellular and phagosomal membranes, resulting in a slow transfer into lysosomes when fusion occurs between phagosome and lysosome. According to the current membrane recycling concept (14, 32, 34, 35), this fusion is only temporary and the phagosomal membrane is retrieved at the cell periphery. A long incubation period implicating several membrane cycles would therefore be needed (Fig. 9) before a significant dextran concentration is reached in lysosomes. The large amount of soluble dextran found after homogenization would in this case be explained by its release from the pericellular membrane owing to the mechanical stirring inherent in the homogenization procedure. We have indeed observed that when cells are incubated at 0°C in the absence of significant interiorization (34), the dex-

tran bound to the cells is found largely soluble after homogenization. At this stage, it should be mentioned that in a study of the intracellular fate of membrane retrieved from the luminal surface of secretory cells, Herzog and Farquhar (19) observed that after its initial uptake via coated pits dextran is found not only in lysosomes but also in other cell compartments including Golgi cisternae and condensing vacuoles. A second interpretation of our results would be to assume that dextran is interiorized in distinct phagosomal vesicles more fragile than those containing HRP. This cannot be ruled out but would imply a complex and yet undescribed mechanism of segregation of macromolecules during endocytosis.

The variations in dextran uptake during the cell cycle display the same trends as those observed for peroxidase. In our opinion, they reflect changes both in the adsorptive properties of the pericellular membrane and in the endocytic uptake rate.

## Chloroquine Accumulation

Our data on chloroquine accumulation in asynchronously growing HTC cells are in good agreement with those of Wibo and Poole for rat fibroblasts (40). Those authors consider that the shift in equilibrium density of lysosomes results from osmotic swelling induced by the high chloroquine concentration in these particles. In HTC cells, the distribution of particle-bound chloroquine appears very disperse. In our opinion, the presence of chloroquine in the high density region, not reported for fibroblasts (40), is a result of the use of the whole homogenate instead of postnuclear supernates. Association with the nucleus could result from interactions (in the intact cell or after homogenization) between chloroquine and DNA (10, 24). As in fibroblasts (40), the chloroquine distribution is different from that of lysosomal hydrolases in the low density region. A greater sensitivity of large chloroquine-filled vacuoles to homogenization could explain part of this difference. Fractionation data do not by themselves give conclusive evidence for a lysosomal localization of the drug. However, the shift in the equilibrium density of acid hydrolases, also observed in fibroblasts (40), is, in our opinion, highly significant and implicates the lysosomes in the accumulation of chloroquine. As pointed out by others for macrophages and L cells (15), and for fibroblasts (13, 40), association of part of the chloroquine with the Golgi complex or plasma membrane cannot be excluded.

The mechanisms proposed to explain the accumulation of weak bases in lysosomes (11, 18, 30) are all founded on permeation and protonation of the drug inside the lysosomes, preventing its diffusion back to the cytosol. Even if nonlinear kinetics of chloroquine accumulation preclude quantitative interpretation of the variations observed along the cell cycle, chloroquine uptake can nevertheless be considered as an indirect way of measuring the pH of lysosomes and their buffering capacity. Our observations show that these properties vary during the cell cycle more or less in parallel with the changes in the endocytic properties.

*Lysosomal Functions and the Cell Cycle*

From our results and the recent data of Berlin et al. (8), it is clear that uptake of HRP into phagosomes is periodically inhibited and triggered during the cell cycle. This means that after the G2 phase and mitosis, both characterized by low endocytosis, a large number of phagolysosomes will be produced during the second half of the G1 phase. This may lead to a decrease in the equilibrium density of lysosomes, probably because of a larger amount of water brought by the phagosomes (28). These changes are concomitant with an increase in the fractional volume of lysosomes at the middle of the cell cycle (6). The arrest of phagosome formation before mitosis might, if accompanied by a decreased utilization of the plasma membrane, explain the increase of microvilli at the cell surface described during the G2 phase (22, 25). On the other hand, the increase in microviscosity of the cell membrane reported during mitosis and G2 phase of neuroblastoma cells (12) suggests that a given degree of membrane fluidity might be required for endocytosis. The high rate of endocytosis occurring during G1 could also explain the enhanced pinocytosis activity characteristic of confluent cells which are probably mainly in G1 (or G0) phase (20, 36).

Some years ago, Allison and Mallucci (1) suggested that release of lysosomal enzymes may be a signal inducing cell division. We have not observed an increase of soluble or free activity of lysosomal enzymes around mitosis (28). Moreover, the changes in the distribution of lysosomes in HeLa cells reported by these authors may very well be a consequence of the slowing down of endocytosis during the G2 phase.

Our study also has implications for the penetration of pharmacological substances into cells through the lysosomal system and especial. macromolecular carriers of antimitotic drugs (38, 39): success in such application requires all cells to be exposed to the lysosomotropic complex at a time when endocytosis is sufficiently active. Barlogie et al. (2, 3) have shown that while free adriamycin acts identically at all stages of the cell cycle, the human lymphoma cells can escape the action of DNA-adriamycin complex at the beginning of G1 or during the G2 phase. This would be consistent with a low endocytic capacity at these stages of the cell cycle and would extend our results on hepatoma cells to another cellular strain.

The possible role of endocytosis in the nutrition and the growth of actively dividing cells can be evaluated from our data. If it is assumed that HRP uptake occurs by fluid endocytosis (34–36), we can calculate that 3–4% of the cellular volume are interiorized each hour. It can be computed from the culture medium composition that during one cycle, only 4% of the amino acids incorporated into the newly synthesized proteins can originate from serum protein and from free amino acid uptake during fluid endocytosis. This suggests that fluid endocytosis cannot be responsible for the nutrition of the cell. Without excluding that adsorptive endocytosis may be essential in the uptake of some specific molecules (see for example references 9, 16, and 41), it appears unlikely that it may enhance the efficiency of endocytosis for the bulk of serum proteins to account for a major role in cell nutrition.

CONCLUSION

The main conclusion of our work is that endocytosis and lysosomal functions are modified during the cell cycle. Actively dividing cells in culture are thus capable of regulating the processing of substances taken up from the surrounding medium, under the sole influence of intracellular events. This was observed in three different models. Peroxidase uptake can indeed be considered as reflecting primarily the endocytic function, while the uptake of dextran is likely to depend upon adsorption to the plasma membrane as well as upon endocytosis. In the third model, chloroquine which penetrates the cell by permeation gives some indirect indication about the cyclic variations of lysosomal pH or buffering capacity.

No evidence for the intervention of lysosomes in mitosis was obtained. Our observations of growing cells show that variations in endocytic capacity and in lysosomal functions should be taken into

consideration in the use of the lysosomal route for penetration of extracellular compounds (11, 38, 39), either in the form of complexes with macromolecules or in free form, penetrating by permeation and accumulating in lysosomes owing to their physical properties.

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