ENDOCYTOSIS IN CHANG LIVER CELLS

Quantitation by Sucrose-^{3}H Uptake and Inhibition by Cytochalasin B

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ABSTRACT

The addition of 0.08 M sucrose to a culture medium containing Chang-strain human liver cells causes intense cytoplasmic vacuolation. Electron microscopy of these cells grown in ferritin, time-lapse cinematography, and radioautography reveal that the vacuoles arise by endocytosis and that the sucrose is taken into the cell and localized in the vacuoles. Tracer studies demonstrate that sucrose-^{3}H provides a marker for quantitation of endocytosis and that it neither induces nor stimulates endocytosis. Electron micrographs of vacuolated liver cells show microfilaments in close proximity to the inside of the plasma membrane, in the pseudopodia, and to the cytoplasmic side of the membrane surrounding endocytosis vacuoles. Cytochalasin B (CB), a mold metabolite that inhibits various types of cell motility, has a dose-dependent inhibitory effect on the uptake of sucrose-^{3}H by these cells. This inhibition is accompanied by a cessation of the movement of ruffles and pseudopodia on the surface of the cells and the formation of blebs which arise from the cell's surface. These morphological changes are quickly reversible upon removal of CB. Alterations in the appearance and location of microfilaments are also observed in CB-treated cells.

INTRODUCTION

Endocytosis in mammalian cells has been quantitated by the enumeration of vacuoles (Lewis, 1931 and Cohn, 1966), the analysis of cinematographic records (Rose, 1957 and Gropp, 1963), and through the use of various tracers (Danes and Freshney, 1965; Michell et al., 1969; Cushman, 1970). The observations of Cohn and Ehrenreich (1969) suggest that radiolabeled sucrose would also be a suitable tracer for quantitation of endocytosis. Munro (1968) observed intense vacuolation of Chinese hamster fibroblasts after addition of 0.08 M sucrose to the culture medium. Time-lapse cinematography revealed that the vacuoles were formed by endocytosis. The impermeability of the cell membrane to sucrose and other disacharides (Maunsbach and Madden, 1962: Trump and Janigan, 1962; Brewer and Heath, 1963; Wattiaux, 1966; Cohn and Ehrenreich, 1969; Jaques, 1969) as well as the indigestibility of sucrose by liver (Jaques, 1964, 1969; Jaques and Bruns, 1965) and other cell types (Wattiaux, 1966; DeDuve and Wattiaux, 1966; Cohn and

Ehrenreich, 1969; Jaques, 1969; Fell and Dingle, 1969) has been well documented. Thus the rate of uptake of sucrose by the cell should be a direct measure of endocytosis, since it can enter the cell only by endocytosis and it is not metabolized once it has entered.

Phagocytosis of bacteria by leucocytes is inhibited by the mold metabolite Cytochalasin B (CB) (Davis et al., 1970). Since endocytosis of particulate matter and endocytosis of fluids have much in common (Jaques, 1969), we tested the effect of the drug on endocytosis of sucrose. We report here the results of our experiments which indicate that the uptake of sucrose-^{3}H correlates well with visual observation of endocytotic activity and that sucrose-^{3}H uptake is inhibited in the presence of 5–20 μg/ml of Cytochalasin B.

METHODS AND TECHNIQUES

Cell Cultures

Serially propagated Chang-strain adult human liver cells were obtained from Flow Laboratories Inc., Inglewood, Calif. and maintained at 37°C in monolayer cultures in Eagle's medium supplemented with 10% fetal calf serum, penicillin (50 U/ml), and streptomycin (50 μg/ml).

Light Microscopy

Cells were dissociated with 0.25% trypsin, pelleted at 100 *g* for 5 min, and resuspended in test medium. 1.5 ml of the suspension was incubated on a glass cover slip at 37°C for 30 min. After attachment of the cells to the cover slip, they were observed with a Zeiss photomicroscope equipped with phase contrast and Nomarski optics.

Time-Lapse Microcinematography

Trypsin-dissociated cells were placed in a narrow glass chamber and perfused with test media maintained at 37°C. The cells were photographed for 6–12 hr periods at 0.2 frames/sec with phase contrast and Nomarski optics.

Radioautography

Trypsin-dissociated cells were placed in normal media containing 2×10^{-5} mM sucrose-^{3}H (Amersham-Searle Corp., SA 760 mCi/mmole) and 80 mM unlabeled sucrose, and 3.0 ml samples of this suspension (approximately 2×10^5 cells/ml) were placed in plastic Petri dishes containing clean glass cover slips and incubated at 37°C for 3 hr. The cover slips with attached cells were washed several times in Eagle's medium containing 80 mM unlabeled sucrose and fixed in Zenker's fixative for 10 min. After two washes in cold tap water the cover slips were air dried for 24 hr, coated with Kodak NTB-2 nuclear track emulsion by the dipping method (Baserga, 1967), and allowed to dry in a vertical position. The cover slips were placed in a light-tight box containing Drierite (W. A. Hammond Drierite Co., Xenia, Ohio) and exposed for 6 days at room temperature. The radioautograms were developed in D-19, fixed with rapid fixer, and examined with phase contrast microscopy.

Electron Microscopy

Treated cell monolayers were trypsin-dissociated, fixed in 2.5% glutaraldehyde for 30 min, postfixed with OsO_4 for 30 min, dehydrated with acetone, and infiltrated with Epon. The Epon blocks were sectioned at 600 A with a Porter-Blum ultramicrotome and the sections were placed on fine-mesh (500) uncoated grids. The grids were poststained with uranyl acetate (10 min) and lead citrate (3 min) and examined with an Hitachi electron microscope.

Quantitative Tracer Studies

A homogeneous suspension of trypsinized cells (approximately 6×10^5 cells/ml) was proportioned in 1 ml samples into clean sterile Packard scintillation vials (Packard Instrument Co., Inc., Downers Grove, Ill.) and incubated for 1 hr at 37°C to allow attachment. 1 ml of the same suspension was retained and counted in a hemacytometer. After 1 hr, iodoacetate was added to the control vials to a final concentration of 10^{-2} M, and all of the vials were incubated for an additional 30 min. The media in all of the vials were replaced by Eagle's medium containing 2×10^{-5} m M sucrose-^{3}H (760 mCi/mmole) and 80 mM unlabeled sucrose. The control vials still contained 10^{-2} M iodoacetate. The cells were incubated for 20 min intervals, after which the radioactive media were removed and the monolayers were washed three times with 80 mM unlabeled sucrose in Eagle's medium. Following air drying, 0.2 ml Beckman Biosolve and 10 ml toluene scintillation fluid were added to each vial, and the vials were placed in a refrigerated Packard Tri-Carb scintillation spectrophotometer overnight and counted the next morning at 43% efficiency. Disintegrations per minute from iodoacetate-poisoned controls were subtracted from disintegrations per minute from experimental cells, and the difference represented uptake of sucrose-^{3}H.

Oxygen Consumption Studies

3.0 ml of cells suspended in test medium (approximately 6×10^5 cells/ml) was placed in the stirring chamber of a biological oxygen monitor. Depletion of oxygen from the medium by the cells was monitored for 100 min and was recorded as per cent saturation of oxygen in the medium.

Viability Stain

Monolayers of treated and untreated cells were placed in fresh media containing 0.2 mg/ml nigrosine stain. Counts of stained and unstained cells per field (160 ×) were taken at 10 min after termination of treatment and were recorded as per cent viability.

RESULTS

Morphology of Sucrose Treated Cells

The cytoplasm of Chang liver cells in monolayer cultures was intensely vacuolated 2–3 hr after addition of 0.08 M sucrose to the culture medium (Fig. 1). Time-lapse cinematography revealed that the vacuoles arose by endocytosis which was dependent on ruffling of the plasma membrane. Many of the newly-formed vacuoles appeared to swell as they moved to the perinuclear region, and larger vacuoles also resulted from vacuolar fusion. Control cells without sucrose also exhibited endocytosis but the vacuoles formed remained small and, after the same period of time (2–3 hr), only a few vacuoles were noticeable in the cytoplasm.

Radioautography with sucrose-^{3}H revealed that the highest concentration of reduced silver grains was located directly over the area of vacuolar accumulation (Figs. 2 and 3), indicating that the sucrose taken into the cells was localized in the vacuoles.

Electron microscopy of trypsinized cells previously treated with 0.08 M sucrose for 2 hr showed that the vacuoles were membrane-bounded (Fig. 4). Cells treated with 0.08 M sucrose and 10% Cd-free ferritin for 2 hr had ferritin localized within the vacuoles (Fig. 5), indicating that the vacuoles arose by endocytosis. A network of structures resembling microfilaments was observed subjacent to the inside of the plasma membrane and in the pseudopodia (Fig. 6). Similar microfilaments were also observed in close proximity to the cytoplasmic side of the membranes surrounding some endocytosis vacuoles (Fig. 7). Actual attachment of the microfilaments to the vacuolar membrane is not discernible due to the grazed section through the membrane.

Quantitative Tracer Studies

The uptake of sucrose-^{3}H by the cells was completely inhibited by treatment with 10^{-2} M iodoacetate (Fig. 8), a glycolytic poison and inhibitor of endocytosis (Yokomura et al., 1967). Subsequent measurements were computed as dpm above that measured from iodoacetate-poisoned controls. This corrected for any sucrose-^{3}H adsorbed to the surface of the cells or to the bottom of the vials and for any possible passive movement of sucrose into the cells. The amount of sucrose-^{3}H taken up within 4 hr was linearly proportional to the cell number up to 4×10^6 cells/ml, then approached a constant value and, finally, decreased as cell number increased (Fig. 9). All subsequent uptake studies were carried out using $3–6 \times 10^5$ cells/ml.

Cells treated with 2×10^{-5} mM sucrose-^{3}H took up sucrose at a constant rate for 4 hr, which thereafter decreased slightly. Cells treated with 2×10^{-5} mM sucrose-^{3}H plus 80 mM unlabeled sucrose took up sucrose at the same rate for the first 3 hr but uptake ceased thereafter (Fig. 10). This cessation of uptake occurred concomitantly with accumulation of a large number of vacuoles in the perinuclear region and the surface of the cells was no longer ruffling (Fig. 1).

Inhibition of Sucrose-^{3}H Uptake by Cytochalasin B

Cytochalasin B had a dose-dependent inhibitory action on the uptake of sucrose-^{3}H by the cells, and a CB concentration of 20 μg/ml completely inhibited uptake (Figs. 11 and 12). Dimethyl sulfoxide (DMSO), which is needed to solubilize CB, had no effect on the rate of uptake when tested at the same concentrations used in the CB solutions.

Morphological Effects of Cytochalasin B

CB-inhibited cells underwent drastic morphological alterations. After treatment for 2 hr with 20 μg/ml CB, the pseudopodia, which had transient existence in untreated cells, became fixed in position and blebs began to arise from them (Figs. 13 a and 14 b). Cessation of membrane ruffling was also evident. These morphological effects were manifested within minutes after exposure to CB (Figs. 14 a and 14 b) and were as swiftly reversible upon removal of CB (Figs. 13 a, 13 b, 14 b, and 14 c). Ultrastructurally, these stalked clusters of blebs often contained membranous structures such as endoplasmic reticulum (*ER*) (Fig. 15). Microfilaments, located subjacent to the plasma membrane and in the pseudopodia of control cells (Fig. 6), were found primarily in

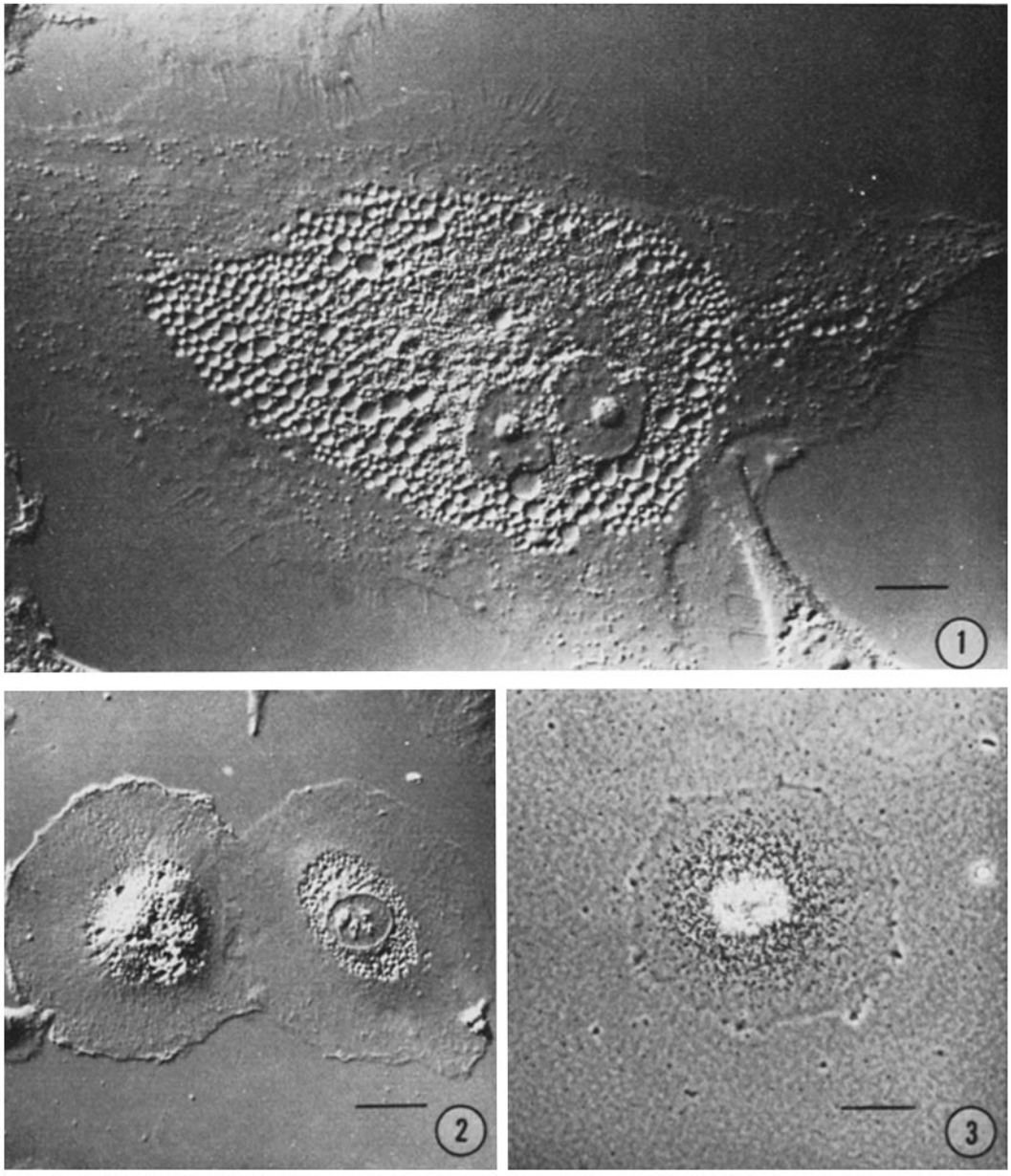
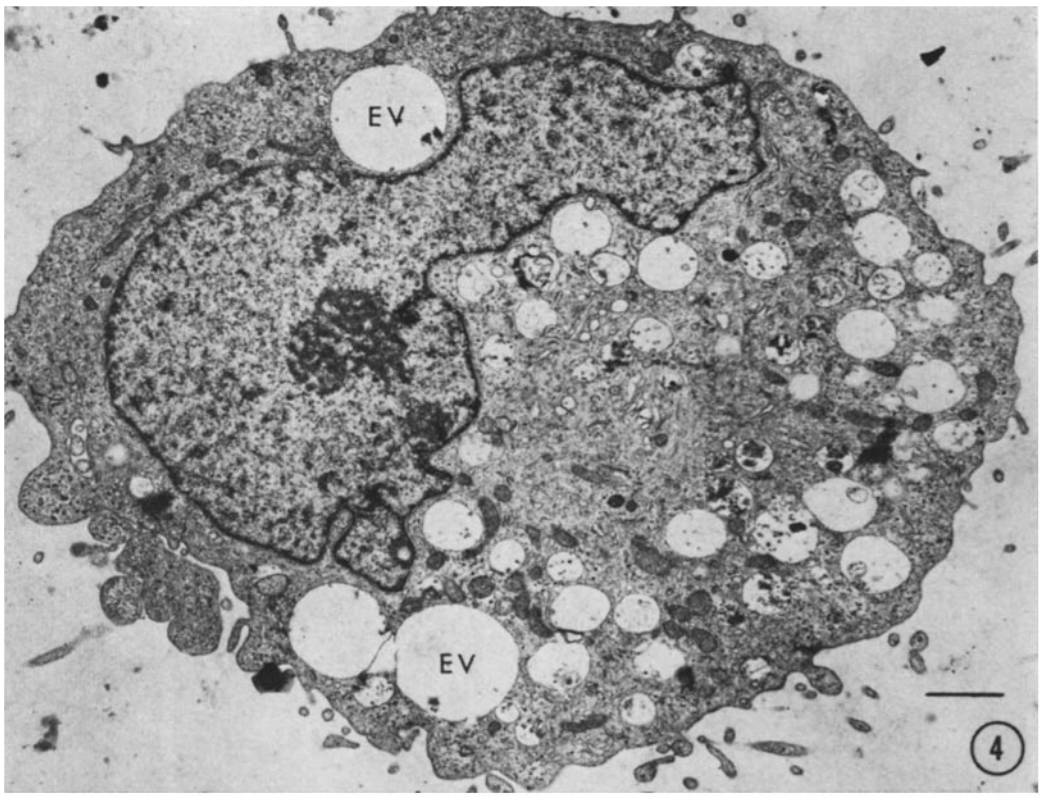


FIGURE 1 Micrograph (Nomarski optics) of a Chang Liver cell exhibiting intense cytoplasmic vacuolation after treatment for 3 hr with 0.08 M sucrose. × 800. Scale marker, 12 μ.

FIGURE 2 Micrograph (Nomarski optics) of two cells showing accumulation of vacuoles in the perinuclear region. × 640. Scale marker, 15 μ.

FIGURE 3 Radioautogram of a cell treated for 3 hr with 2×10^{-5} mM sucrose ^{3}H. The highest concentration of reduced silver grains is located directly over the area of vacuolar accumulation, suggesting that the sucrose taken into the cell became localized in the vacuoles (phase contrast). × 640. Scale marker, 15 μ.



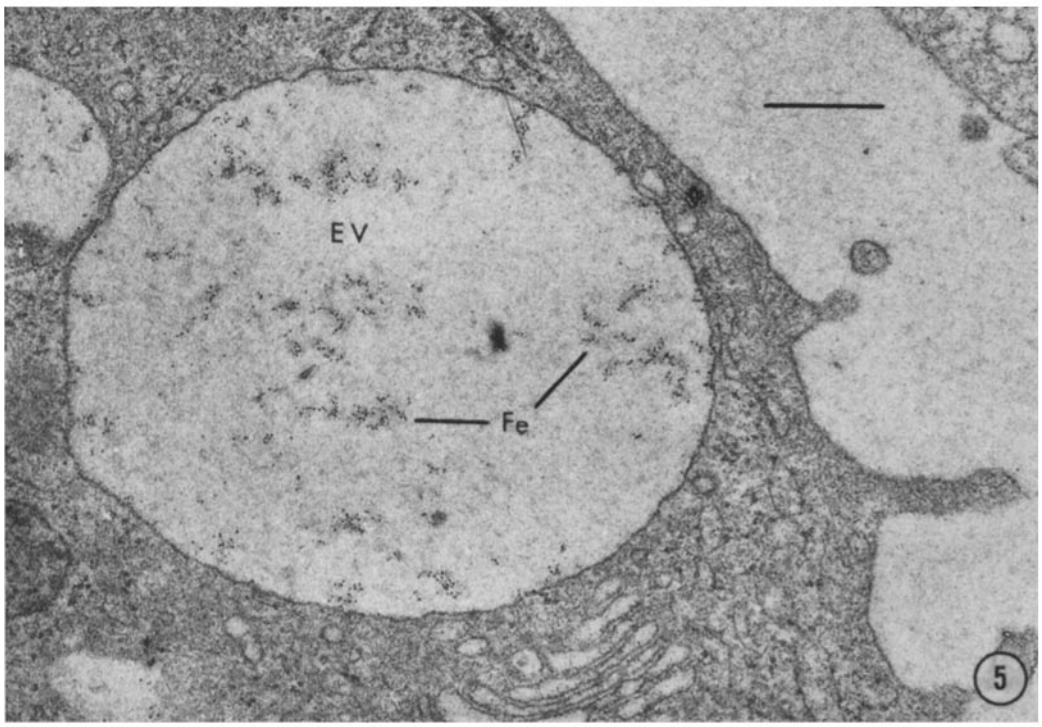


FIGURE 4 Electron micrograph of a cell treated for 3 hr with 0.08 M sucrose. Endocytosis vacuoles (*EV*) are membrane-bounded. × 5260. Scale marker, 2 μ.

FIGURE 5 Vacuole in a cell treated for 3 hr with 0.08 M sucrose and 10% ferritin. Ferritin (*Fe*) is localized in the vacuole, indicating that it is an endocytosis vacuole. *EV*, endocytosis vacuoles. ×32,500. Scale marker, 0.5 μ.

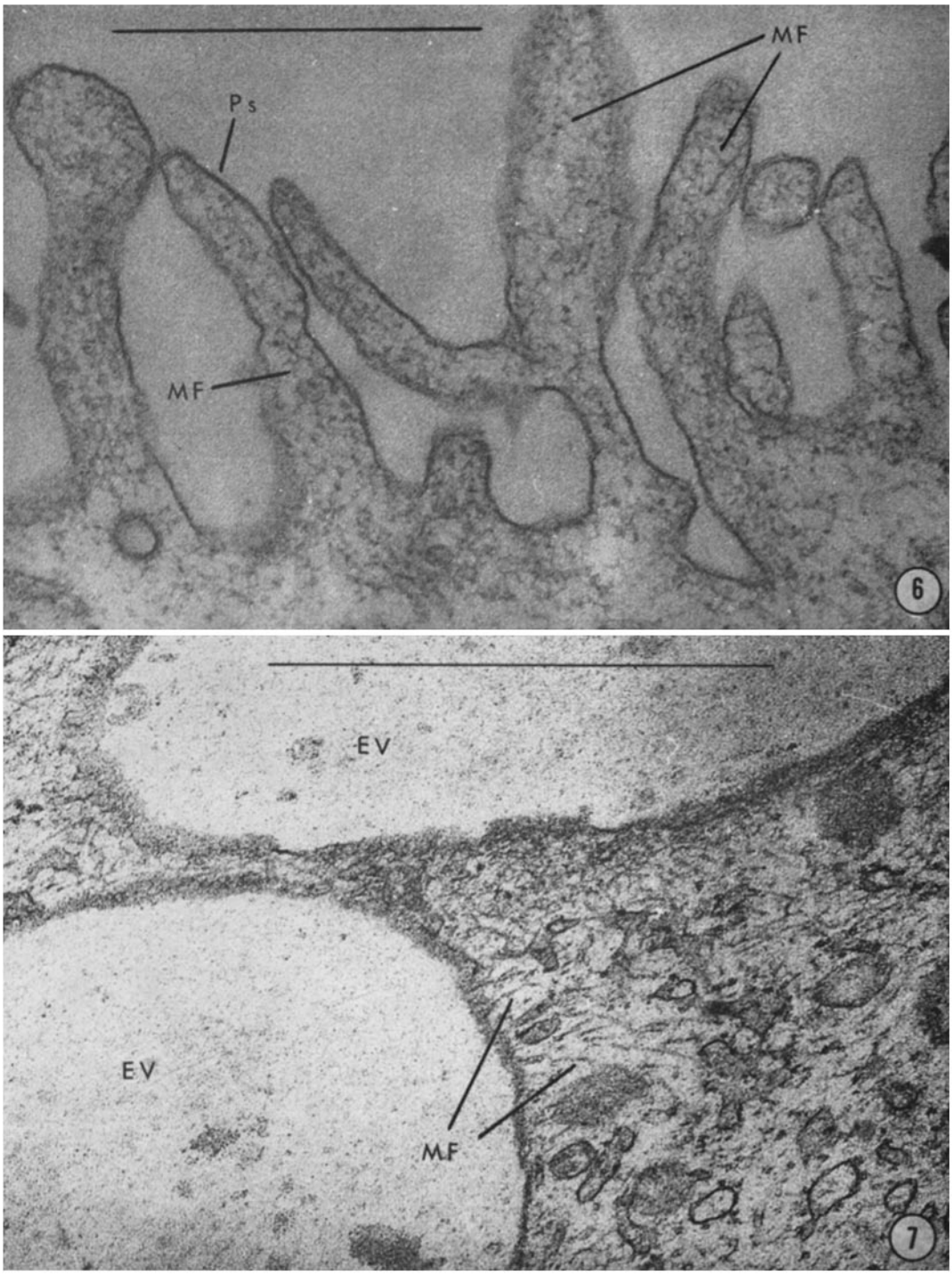


FIGURE 6 A network of structures resembling microfilaments (*MF*) is located subjacent to the plasma membrane and in the pseudopodia (*Ps*) of sucrose-treated cells. × 220,000. Scale marker, 0.25 μ.

FIGURE 7 Microfilaments (*MF*) are in close proximity to the cytoplasmic side of the membranes surrounding endocytosis vacuoles (*EV*). × 75,000. Scale marker, 1 μ.

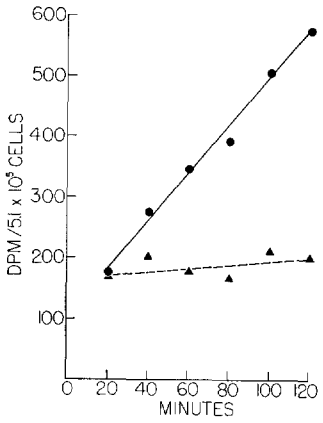


FIGURE 8 Effect of Iodoacetate on the uptake of sucrose-^{3}H by Chang cells. (– – –▲– – –, cells treated with 10^{-2} M iodoacetate; – – –●– – –, control cells). Iodoacetate, a glycolytic poison, inhibits endocytosis, a metabolically dependent process.

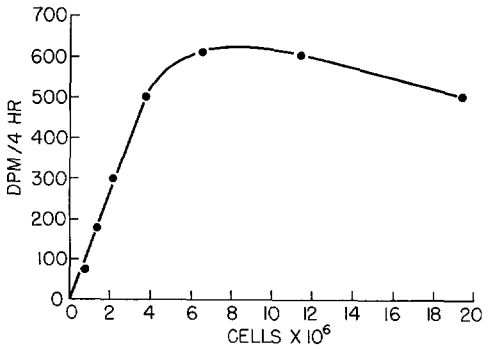


FIGURE 9 Effect of cell number on the quantity of sucrose-^{3}H taken up by the cells. The amount of sucrose-^{3}H taken up is linearly proportional to cell number up to 4×10^6 cell/ml, then approaches a constant value and finally decreases as cell number increases. (10^6 cells/ml, confluence.)

the stalks and blebs of CB-treated cells, and few were seen subjacent to the plasma membrane proper (Figs. 15 and 16). These morphological alterations were not seen in cells treated with 2% DMSO for 2 hr.

Oxygen Consumption Studies

Measurements of rates of oxygen consumption by CB-treated cells for 100 min indicated that 20 μg/ml CB had no appreciable effect on cellular respiration and did not inhibit endocytosis by acting as a respiratory inhibitor (Fig. 17).

Viability Staining

The average viability of cells treated with 20 μg/ml CB for 2 hr was 95%. Untreated controls had an average viability of 97%.

DISCUSSION

Our observation of intense cytoplasmic vacuolation of cells treated with 0.08 M sucrose is in agree-

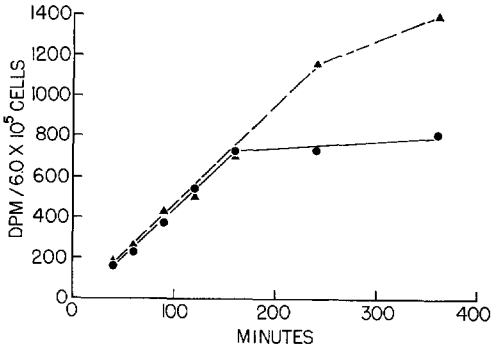


FIGURE 10 Effect of sucrose concentration on the uptake of sucrose-^{3}H by the cells (– – –▲– – –, cells treated with 2×10^{-5} mM sucrose-^{3}H; – – –●– – –, cells treated with 2×10^{-5} mM sucrose-^{3}H plus 80 mM unlabeled sucrose). Cells treated with 2×10^{-5} mM sucrose-^{3}H take up sucrose at a constant rate for 4 hr, which thereafter decreases slightly. Cells treated with 2×10^{-5} mM sucrose-^{3}H plus 80 mM unlabeled sucrose take up sucrose at the same rate for the first 3 hr, but uptake ceases thereafter.

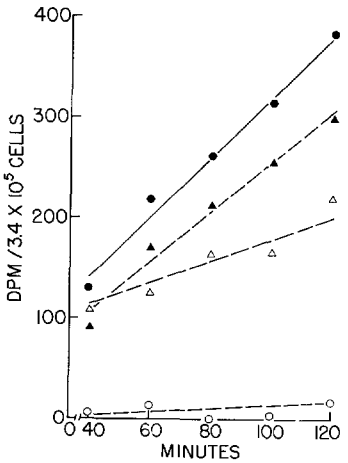


FIGURE 11 Effect of cytochalasin B on the uptake of sucrose-^{3}H (– – –●– – –, control cells; – – –▲– – –, 5 μg/ml CB; – – –△– – –, 10 μg/ml CB; – – –○– – –, 20 μg/ml CB). 20 μg/ml CB causes complete inhibition of endocytosis.

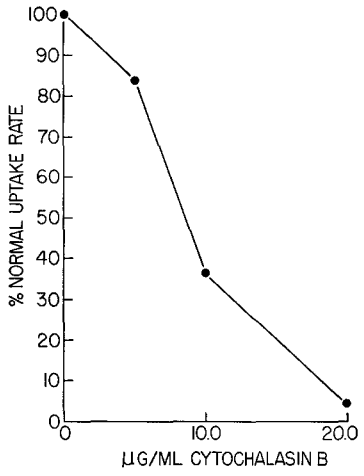


FIGURE 12 Dosage effect of Cytochalasin B on the per cent normal uptake of sucrose-^{3}H. Dosage response of the cells to CB is nearly linear.

ment with the results of Munro (1968). Electron microscopy of these cells grown in media containing ferritin, time-lapse cinematography, and radioautography with sucrose-^{3}H reveal that the vacuoles arise by endocytosis and that the sucrose is taken into the cells and is localized in the vacuoles.

The cells used in this study normally exhibit endocytosis in the absence of sucrose. The uptake of sucrose-^{3}H (2×10^{-5} mM) during the first 3 hr is the same as the rate observed in the presence of 80 mM unlabeled sucrose (Fig. 10). These data indicate that the extensive vacuolation of sucrose-treated cells is not due to induction or stimulation of endocytosis by sucrose (Munro, 1968).

Observations of time-lapse movies indicate that the large size of the vacuoles results from both vacuolar swelling and fusion of smaller vacuoles. The persistence of the vacuoles probably stems from the lack of lysosomal enzymes necessary for the splitting and digestion of sucrose (Jaques, 1964; Jaques and Bruns, 1965; Munro, 1968; Fell and Dingle, 1969). The contents of the vacuoles thus remain hypertonic and they swell osmotically. The net result is accumulation of abnormally large vacuoles.

The accumulation of vacuoles inhibits further endocytosis after 3 hr exposure to 0.08 M sucrose (Fig. 10). The average size of a newly-formed endocytosis vacuole is approximately 10^{-12} cm^3. The measurement of uptake of sucrose-^{3}H allows for the direct calculation that approximately 10^3 vacuoles have been taken into the cell at the time of saturation (after 3 hr). Two reasons for this inhibition are suggested by our observations: The large volume of cytoplasm displaced by the vacuoles might interfere with normal cell functioning (i.e. endocytosis) or, since the total surface area of the vacuoles represents about one third of the surface area of the cell, the cells may be unable to replace their plasma membrane at the rate at which it is being depleted by vacuole formation and a compensatory reduction or cessation of endocytosis results. Another reason may be that, since some of the vcacuoles have microfilaments located in close proximity to the cytoplasmic side of their membranes, they may have depleted the microfilaments at the cell surface and thus deprived the plasma membrane of its mode of movement.

The uptake of sucrose-^{3}H decreases when the cell concentration exceeds 7×10^6 cells/ml (Fig. 9). Under our experimental conditions, the cells in the vial become confluent at approximately 10^6 cells/ml. Several mechanisms could account for the suppression of uptake when the cell concentration becomes too great, such as contact inhibition by other cells, accumulation of metabolic wastes in the medium, and reduction in the amount of nutrient per cell. O'Neill and Follett (1970) have observed as inverse relation between cell density and the number of microvilli on cultured cells. Since microvilli are involved in endocytosis, a reduction in their number might suppress endocytosis. Further studies are required to clarify this effect.

Cytochalasin B has an inhibitory effect on endocytosis (Figs. 11 and 12) that cannot be explained as a consequence of respiratory inhibition (Fig. 17) or of toxicity (viability stain). It has been shown that CB has no appreciable effect on the incorporation of protein precursors (Yamada et al., 1970; Shepro et al., 1970 and Estensen, 1970) or of DNA, RNA, and membrane precursors (Estensen, 1970). It therefore seems unlikely that the inhibitory effect of CB on endocytosis occurs via these macromolecular syntheses. The rapidity with which CB is effective and the swift recovery of the cells after the removal of CB indicate that CB may exert its influence at the cell surface or that CB enters the cell and is metabolized so rapidly that when exogenous CB is removed the cells immediately revert.

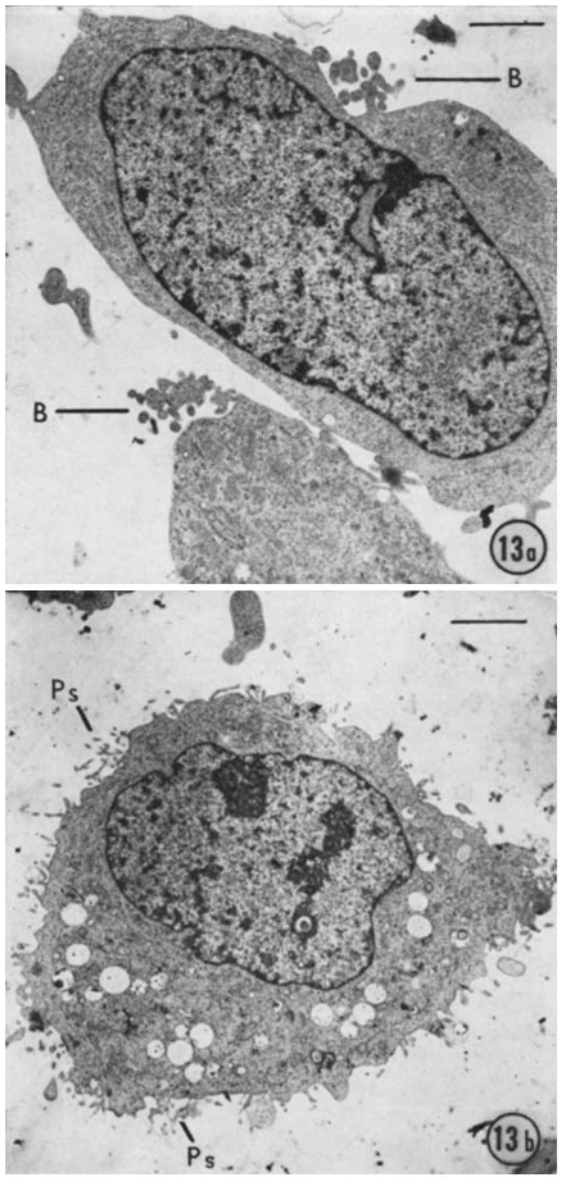


FIGURE 13 a Electron micrograph of cells treated for 2 hr with 20 μg/ml CB plus 80 mM sucrose. There is a paucity of endocytosis vacuoles, and pseudopodia are absent. Stalked clusters of blebs (*B*) are evident on the cells' surface. × 5000. Scale marker, 0.5 μ.

FIGURE 13 b Electron micrograph of a cell treated for 2 hr with 20 μg/ml CB plus 80 mM sucrose and then placed back in fresh media without CB for 30 min. Endocytosis vacuoles are present and the cells' surface is covered with pseudopodia (*Ps*). The stalked clusters of blebs have disappeared. × 5000. Scale marker, 0.5 μ.

Endocytosis in mammalian cells in tissue culture is a metabolically dependent process (Sbarra and Karnovsky, 1959; Oren et al., 1963; Yokomura et al., 1967) and results from the action of "ruffling pseudopodia" which, by their undulations, come into contact and fuse to enclose a droplet of medium (Lewis, 1931). Goldman and Follett (1969) have proposed that ruffling of the mem-

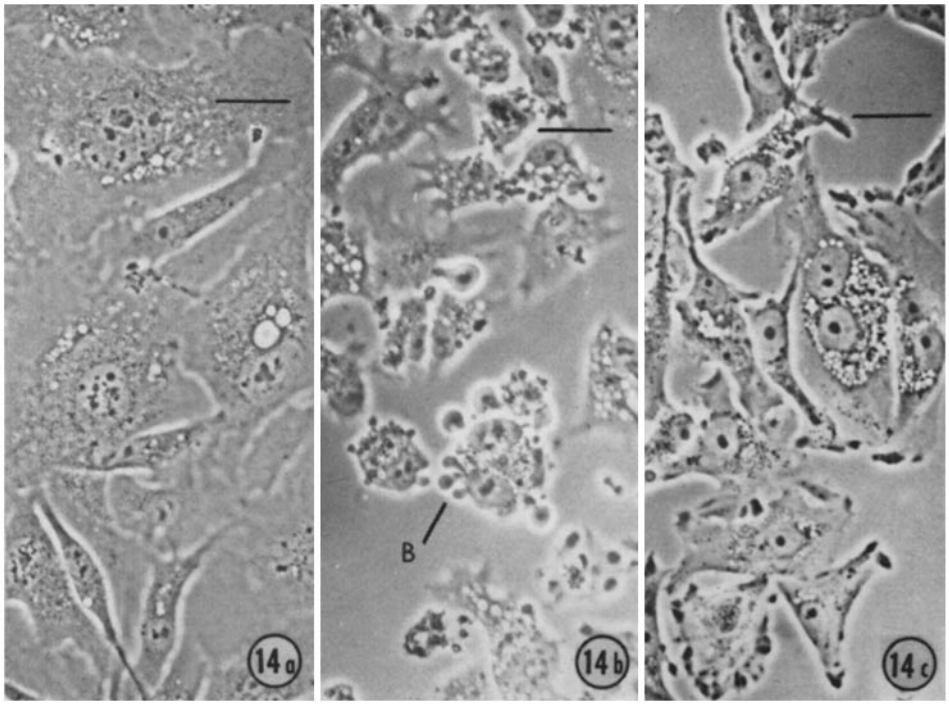


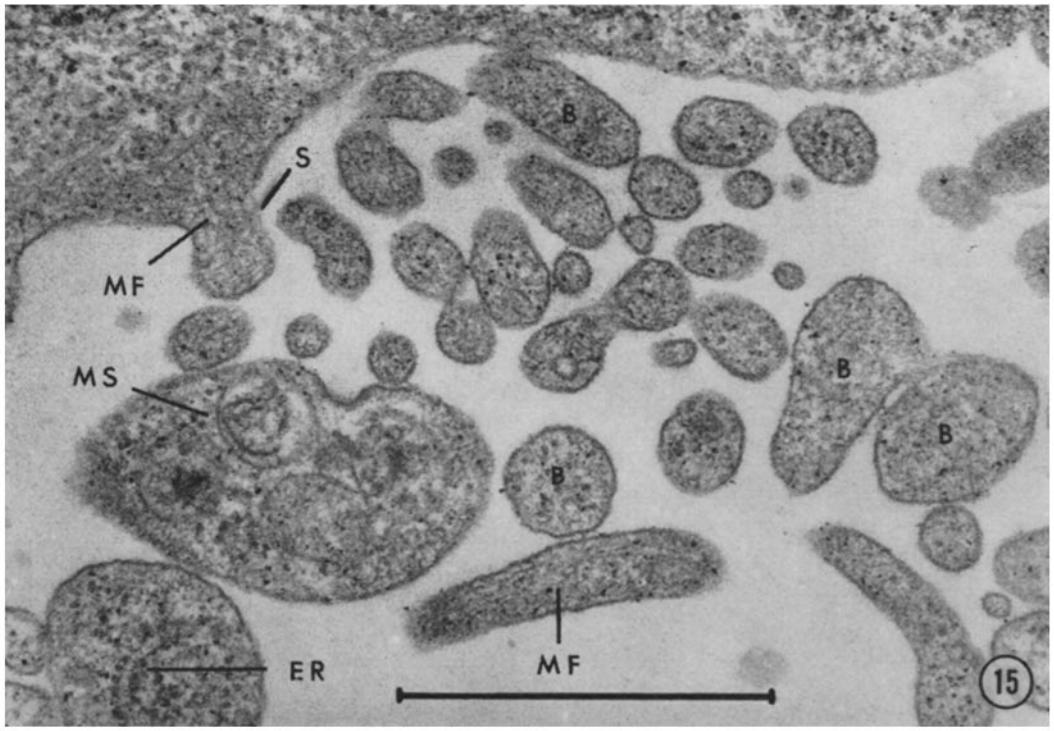
FIGURE 14 a Micrograph (phase contrast) of Chang cells treated with 0.08 M sucrose for 2 hr. The cytoplasm of the cells is vacuolated, and they are spread on the substrate in a normal fashion. × 284. Scale marker, 30 μ.

FIGURE 14 b Micrograph (phase contrast) of cells treated with 0.08 M sucrose for 2 hr and then placed in the same media containing 20 μg/ml CB for 30 min. The vacuoles persist but the cells appear constricted, and numerous blebs (*B*) are present on the cells' surface. × 284. Scale marker, 30 μ.

FIGURE 14 c Micrograph (phase contrast) of cells treated in the same manner as those in Fig. 14 b but after 30 min in 20 μg/ml CB the medium was replaced by fresh CB-free medium. After 30 min, the cells have reversed to their original morphology. × 284. Scale marker, 30 μ.

brane may arise by contraction of bundles of microfilaments localized just under the plasma membrane. Similar structures observed by glycerinated fibroblasts bind heavy meromyosin (HMM) in a manner characteristic of the arrowhead structure of actin-HMM complexes (Ishikawa et al., 1969). An actomyosin-like protein has also been claimed to be associated with cell membrane fractions (Neifakh et al., 1965; Jones, 1966). These microfilaments may be part of a contractile system in the cortical regions of cells (Goldman and Follett, 1969; Franks et al., 1969) and may be necessary for the membrane movements involved in endocytosis. The endocytotic process has been shown to be independent of microtubule integrity (Malawista and Bodel, 1967; Freed and LeBowitz, 1970), thereby strengthening the possibility of the dependence of the process on microfilaments.

Cytochalasin B is known to interrupt ruffling movements (Carter, 1967) as well as motility of cells (Carter, 1967), blood platelets (Shepro et al., 1970), and various morphogenetic movements (Wessells et al., 1971). The disappearance or disorganization of microfilaments had been shown to accompany the biological actions of CB on dividing *Arbacia* eggs and HeLa cells (Schroeder, 1969 and 1970), developing epithelial cells (Spooner and Wessells, 1970), and the growth cones of elongating axons (Yamada et al., 1970). Controversy still exists over the extent of microfilament structural alterations by CB in mammalian cells (Goldman, 1970). Our observations are not conclusive in this regard. Nonetheless,



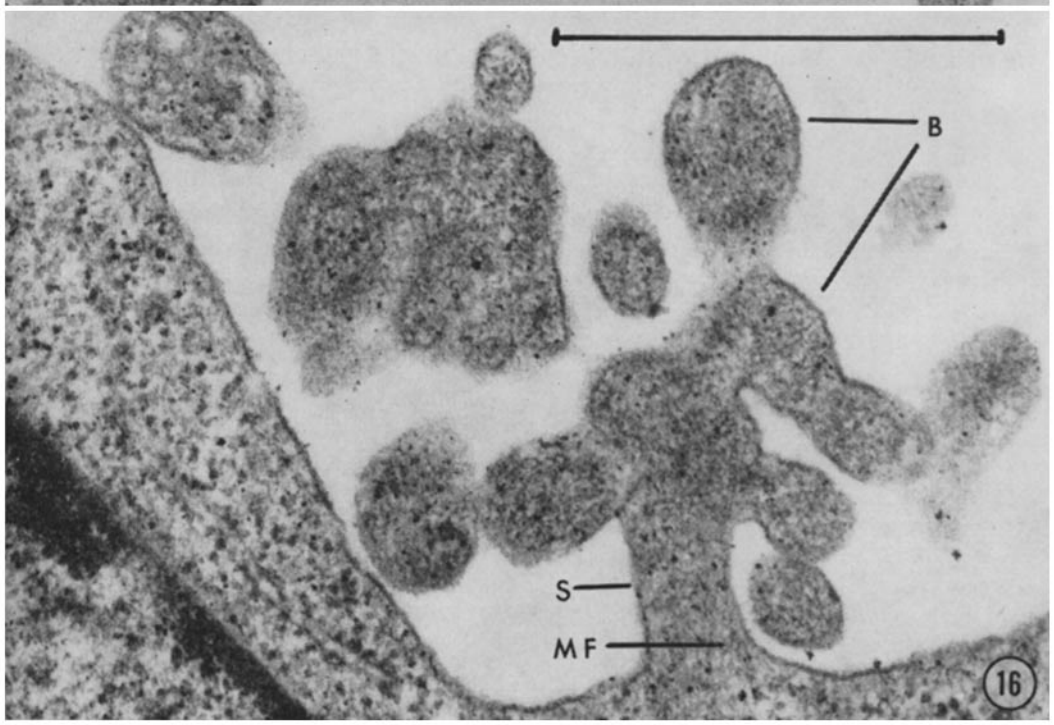


FIGURE 15 Electron micrograph of the periphery of a cell treated with 0.08 M sucrose and 20 μg/ml CB for 2 hr. Blebs (*B*) on the stalk (*S*) contain numerous membranous structures (*MS*) and endoplasmic reticulum (*ER*). Blebs and stalk contain microfilaments (*MF*), and no microfilaments are found subjacent to the plasma membrane proper. × 101,500. Scale marker, 0.5 μ.

FIGURE 16 Electron micrograph of the periphery of a cell treated with 0.08 M sucrose and 20 μg/ml CB for 2 hr. Microfilaments (*MF*) in the blebs (*B*) and stalk (*S*) appear condensed and amorphous. × 122,500. Scale marker, 0.5 μ.

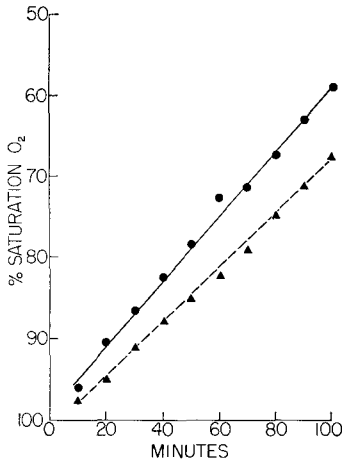


FIGURE 17 Effect of 20 μg/ml CB on oxygen consumption by Chang cells (– – –▲– – –, cells treated with 0.08 M sucrose and 20 μg/ml CB; – – –●– – –, control cells). CB at the same concentration that completely inhibits endocytosis has little effect on the rate of oxygen consumption in Chang cells.

surface similar to those observed by Schroeder (1970) in CB-treated HeLa cells. The morphological alterations of the microfilaments are less clear but can be best described as a concentration of microfilaments in the amorphous dense cytoplasm of the stalks and blebs and a paucity of microfilaments in regions subjacent to the plasma membrane proper (Fig. 18). The inhibition of membrane motility may result from a change in either the structure and function, or location of microfilaments in the cortical regions of the cell which precludes the concerted motion of the membrane necessary for endocytosis.

This investigation provides a quantitative method for the measurement of endocytosis and evidence that CB inhibits endocytosis. These results are consistent with the hypothesis that microfilaments are necessary for endocytosis.

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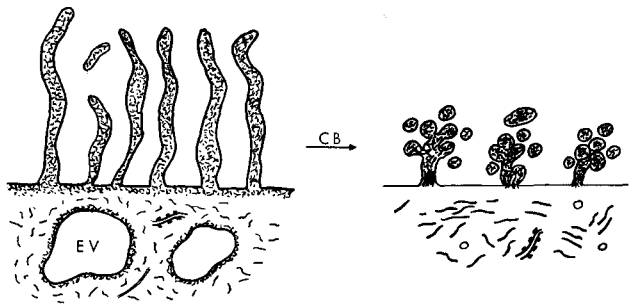

FIGURE 18 Schematic interpretation of the morphological changes occurring at the surface of Chang liver cells that have been treated with 20 μg/ml CB. The pseudopodia involved in the formation of endocytosis vacuoles (*EV*) are replaced by stalked clusters of blebs. Microfilaments that are found in the pseudopodia, and subjacent to the plasma membrane and the cytoplasmic side of membranes surrounding endocygosis vacuoles in the control cells, are found in the stalks and blebs of CB-treated cells, and few are found subjacent to the plasma membrane proper.

microfilaments may be part of a system that is necessary for membrane movements involved in endocytosis. It is therefore reasonable to suspect that an altered appearance of microfilaments in the presence of CB and the simultaneous inhibition of endocytotic activity are interrelated.

We have observed an inhibition of endocytosis in cells treated with CB and a simultaneous cessation of membrane movements involving pseudopodia and ruffles. These membrane processes (pseudopodia and ruffles) are replaced by clusters of blebs attached to stalks arising from the cell

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