Effects of Inhibition of Microtubule Assembly on Bone Mineral Release and Enzyme Release by Human Breast Cancer Cells

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ABSTRACT When supernates from the established human breast cancer cell line MCF-7 were applied to fetal rat long bones that had been labeled with ^{45}Ca and devitalized to remove endogenous bone cells, mineral was released from the bones. The release of bone mineral by MCF-7 supernates was associated with increased basal release of hydrolytic enzyme activity by the tumor cells. The basal release of lysosomal enzymes and collagenolytic activity by MCF-7 cells was approximately twice that of mouse 3T3 cells, which did not cause mineral release by the fetal rat bones. Release of hydrolytic enzymes and bone mineral-releasing activity was increased by colchicine and vinblastine, drugs that inhibit microtubule assembly, but not affected by lumicolchicine. Time-course experiments performed on MCF-7 cells with or without colchicine showed that release of cathepsin D and collagenolytic activity was associated more closely with release of bone mineral and degradation of bone matrix than was the release of *N*-acetylglucosaminidase. The release of previously incorporated [^{3}H]proline from the bones exposed to MCF-7 cell cultures was more closely associated with release of collagenolytic activity by MCF-7 cells than with release of cathepsin D or *N*-acetylglucosaminidase. These data suggest that breast cancer-mediated bone resorption in vitro is positively correlated with release of hydrolytic enzymes by the tumor cells, and release of these enzymes is enhanced by disassembly of microtubules.

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Received for publication 23 April 1979 and in revised form 29 August 1980.

INTRODUCTION

Osteolytic bone metastases are a frequent complication of breast cancer. Recently we found that breast cancer cells are capable of directly resorbing fetal rat long bones in vitro (1). This effect of the breast cancer cells on bone occurred independent of endogeneous bone-resorbing cells.

Osteoclast-mediated bone resorption has been correlated closely with release of hydrolytic enzymes (2). When bone resorption is stimulated in vitro by parathyroid hormone or prostaglandin E_2, there is increased release of lysosomal enzymes and when resorption is inhibited by calcitonin or cortisol, lysosomal enzyme release is diminished (3). Colchicine, which inhibits microtubular assembly, is also a potent inhibitor of osteoclastic bone resorption. The purpose of this study was to examine the effects of drugs that inhibit microtubule assembly on the capacity of cultured human breast cancer cells to cause bone mineral release and release of lysosomal enzymes and collagenolytic activity.

METHODS

Cell cultures. The MCF-7 cell line is an established epithelial human breast cancer cell line derived from the pleural effusion of a patient with advanced breast cancer (4). The cells had been growing in a monolayer culture for over 5 yr. Our cells were obtained from E.G. and G. Mason Research Institute, Rockville, Md. 3T3 nontransformed mouse fibroblasts were obtained from the American Type Culture Collection, Rockville, Md. Supernates of cell cultures used in these experiments were taken from cultures that were near confluency. The MCF-7 and 3T3 cells were cultured in Dulbecco's modified Eagle's medium with 10% fetal calf serum and 10 μg/ml insulin. These cultures were routinely split every 3 d. Cell culture media were centrifuged for 10 min at 1,000 *g*. The cell-free supernates were then adjusted to pH 7.4 with 1 M $NaHCO_3$, and sterilized by passage through

Millipore filters (pore size, 0.22 μm; Millipore Corp., Bedford, Mass.).

Assay for bone resorption. We have described a similar bioassay for bone resorption (1, 5). Pregnant mice on the 16th d of gestation were injected subcutaneously with 0.04 mCi of ^{45}Ca. The next day, the fetal radius and ulna were dissected free of the cartilagenous ends and surrounding soft tissue. Some pregnant mice were injected with 0.25 mCi of [^{3}H]proline on the 16th, 18th, and 20th d of gestation. In these experiments the radius and ulna were obtained from the new-born animals. The bones were devitalized by wet exposure overnight to ultraviolet light to kill endogenous bone cells and leave a calcified acellular bone matrix. We checked that endogenous bone cells were killed by adding parathyroid hormone to the bones and measuring release of ^{45}Ca and incorporation of [^{3}H]thymidine. There was no release of ^{45}Ca or incorporation of [^{3}H]thymidine by these bones. The bones were then washed in one change of fresh media to remove hydrolytic enzymes released by the dead bone cells. The devitalized bones were cultured with chemically defined medium developed by Biggers et al. (6) for 24 h to allow for exchange of loosely complexed ^{45}Ca or [^{3}H]proline released during this period of culture from individual bones, or as the ratio of ^{45}Ca medium or [^{3}H]proline released from bones treated with the cell culture supernates compared with paired bones treated with control media. Statistical differences were determined using Student's *t* test.

Enzyme assays. Cell culture supernates were measured for the following enzyme activities: β-glucuronidase, *N*-acetylglucosaminidase, cathepsin D, lactic dehydrogenase, neutral proteases, and collagenolytic activity. The lysosomal enzymes β-glucuronidase and *N*-acetylglucosaminidase were assayed by measuring the hydrolysis of the appropriate glycoside of phenolphthalein or *p*-nitrophenol. Cathepsin D was assayed by measuring the degradation of [^{3}H]acetylhemoglobin at acid pH. Each of these assays were performed as described by Barrett (7). Lactic dehydrogenase was assayed by measuring the initial rate of oxidation of $NADH_2$ (8). Collagenolytic activity in the medium was assayed by the method of Gisslow and McBride (9). Acid soluble rat collagen, ^{14}C-acetylated, was gelled at 37°C and used as a substrate for collagenase assays (9). Neutral proteases were assayed by measuring the degradation of [^{3}H]acetylhemoglobin in 0.1 ml Tris-HCl buffer pH 7.6. Cells were washed with phosphate-buffered saline at 37°C, and then lysed with Triton X-100 (0.1%). Cell-soluble protein was measured by the method of Lowry et al. (10).

Materials. Colchicine, vinblastine, cytochalasin B, insulin, and the enzyme substrates were all of reagent grade and were purchased from the Sigma Chemical Co., St. Louis, Mo. Lumicolchicine was prepared by irradiation of colchicine (10 M) in 95% (vol/vol) ethanol for 1 h at 315 nm (11). ^{45}Ca was purchased from New England Nuclear, Boston, Mass.

RESULTS

Extracellular enzyme activity and bone mineral-releasing activity in the conditioned media from cultured human breast cancer cells (MCF-7) and 3T3 mouse fibroblasts is tabulated (Tables I and II). Under basal conditions MCF-7 conditioned media caused release of bone ^{45}Ca, whereas 3T3 conditioned media did not. There was increased extracellular activity of each of the enzymes that were measured in the MCF-7 cell culture supernates compared with the 3T3 cell culture supernates in the basal state. When colchicine (0.1 μM) was incubated with MCF-7 cells, there was increased release of all of these enzymes except for lactic dehydrogenase (Table II). The most marked increases occurred in *N*-acetylglucosaminidase, cathepsin D, and collagenolytic activities. There was also increased release of bone mineral by supernates from colchicin-treated MCF-7 cell cultures compared

TABLE I

Release of Bone Resorbing Activity and Enzyme by Cultured Human Breast Cancer Cells (MCF-7) and Mouse 3T3 Fibroblasts Incubated with and without Drugs that Depolymerize Microtubules

	Bone resorbing activity*	Extracellular lysosomal enzyme activity‡		Total cell culture protein
	^{45}Ca Release	*N*-Acetylglucosamidase	Cathepsin D	
	treated/control ratio	*nM/mg/h*	*cpm/mg/h* × 10^{-5}	*mg*
MCF-7 cells	1.84±0.05	2.21±0.22	8.71±0.52	0.69±0.35
+ Colchicine (0.1 μM)	2.50±0.15§	3.22±0.20§	15.72±1.02§	0.54±0.41
+ Lumi-colchicine (0.1 μM)	1.95±0.10	2.33±0.11	9.01±0.41	0.61±0.32
+ Vinblastine (0.1 μM)	2.48±0.10§	2.92±0.13§	12.23±1.00§	0.56±0.31
3T3 cells	1.10±0.03	0.81±0.12	3.00±0.31	0.79±0.61
+ Colchicine (0.1 μM)	1.18±0.02	0.92±0.11	3.61±0.23	0.62±0.42

The bones were devitalized by overnight exposure to ultraviolet light, washed, and then co-cultured with media harvested from cells that were growing as monolayers and near confluency. Enzyme activity is expressed as extracellular enzyme activity per milligram of cell culture protein. The media were incubated with the bones for 48 h. See Methods section for details.

* Mean±SEM for pooled data from seven separate experiments (four bone cultures for each treatment group in each separate experiment).

‡ Mean±SEM for pooled data from seven separate experiments (four bone cultures for each treatment group in each separate experiment).

§ Significantly greater than activity produced by cells in basal state, $P < 0.05$.

TABLE II

Release of Bone Resorbing Activity and Enzyme by Cultured Human Breast Cancer Cells (MCF-7) and Mouse 3T3 Fibroblasts Incubated with and without Drugs that Depolymerize Microtubules

	Bone resorbing activity*	Extracellular enzyme activity‡			Total cell culture protein
	^{45}Ca Release	Neutral protease	Collagenolytic activity	Lactic dehydrogenase	
	treated/control ratio	*cpm/mg/1 h* $\times 10^{-4}$	*cpm/mg/h* $\times 10^{-2}$	*U/mg* $\times 10^{-2}$	*mg*
MCF-7 cells	1.86±0.06	1.61±0.24	4.90±0.20	15.7±1.5	0.71±0.20
+ Colchicine (0.1 μM)	2.47±0.11§	2.36±0.23§	8.10±0.33§	16.9±1.1	0.52±0.30
+ Lumi-colchicine (0.1 μM)	1.94±0.10	1.90±0.11	6.13±0.21	15.2±1.7	0.66±0.45
+ Vinblastine (0.1 μM)	2.51±0.10§	2.55±0.21§	9.31±0.53§	15.4±1.3	0.58±0.35
3T3 cells	1.10±0.04	0.85±0.12	2.03±0.10	9.0±0.61	0.81±0.60
+ Colchicine (0.1 μM)	1.20±0.05	1.09±0.43	2.43±0.21	10.4±0.92	0.64±0.50

The bones were devitalized by overnight exposure to ultraviolet light, washed, and then co-cultured with media harvested from cells that were growing as monolayers and near confluency. Enzyme activity is expressed as extracellular enzyme activity per milligram of cell culture protein. The media were incubated with the bones for 48 h. See Methods section for details.

* Values are mean±SEM for pooled data from seven separate experiments (four bone cultures for each treatment group in each separate experiment).

‡ Values are mean±SEM for pooled data from seven separate experiments (four bone cultures for each treatment group in each separate experiment).

§ Significantly greater than activity produced by cells in basal state, $P < 0.05$.

with the basal state. Similar effects on MCF-7 cells were produced by vinblastine (0.1 μM). Lumicolchicine, which is an analog of colchicine without effects on microtubule assembly, did not increase release of bone mineral nor did it have any significant effect on enzyme release. Colchicine (0.1 μM) caused minimal increases in bone mineral release and release of enzymes by 3T3 cells (Tables I and III).

The effects of colchicine (0.1 μM) on cell viability and on [^{3}H]deoxyglucose uptake by the MCF-7 cells were tested after the cells had grown to confluency. There was no change in the percentage of cells excluding trypan blue after the addition of colchicine. Deoxyglucose uptake was also unaffected by colchicine. [^{3}H]deoxyglucose (0.1 μCi) was added to each of six separate cultures (three treated with colchicine, three without colchicine) and incubated for an additional 30 min. Radioactivity present in cell lysates was the same in cells treated with colchicine as cells without colchicine. In separate experiments, we found that after incubation of MCF-7 cells with 2-deoxyglucose, 75% of the total deoxyglucose was in the form of 2-deoxyglucose 6-phosphate (data not shown).

Supernates from MCF-7 cultures containing bone mineral-releasing activity and enzymatic activity were subjected to dialysis across Spectrapor membranes (assigned cut-off 3,500 daltons) and heat (Table III). Dialyzed supernates retained their resorptive and enzymatic activity, whereas heating the supernates at 75°C for 10 min reduced enzymatic and resorptive activity significantly.

Table IV shows the dose-response relationship of colchicine to the release of bone mineral and activity of the lysosomal enzyme *N*-acetylglucosaminidase by MCF-7 cells. There was a parallel inhibitory effect of colchicine on the release of bone mineral-releasing activity and the lysosomal enzyme. At 0.1 μM colchicine there was a clear increase in *N*-acetylglucosaminidase activity and bone mineral-releasing activity. There was no effect on either response by colchicine at 10 nM.

Cytochalasin B is an agent that inhibits microfilament function. To ascertain the effects of alterations of microfilament function on the release of bone resorbing activity and enzymes by the MCF-7 cells, we added cytochalasin B (2 μM) to MCF-7 cultures near confluency, harvested the media after 48 h and added the

TABLE III

Effects of Dialysis and Heat (75°C, 10 min) on Bone Mineral-releasing Activity and Activity of Extracellular N-acetylglucosaminidase in MCF-7 Cell-conditioned Media

	^{45}Ca Release*	*N*-Acetylglucosaminidase‡
	treated/control ratios	*mM/mg protein/h*
MCF-7 cells conditioned media	1.56±0.04	0.77±0.05
+ dialysis	1.47±0.08	0.71±0.04
+ heat	1.12±0.04§	—§

Dialysis was for 24 h against two changes of 1,000 vol of Tris buffer using Spectrapor 3 tubing (assigned cut-off 3,500 daltons).

* Values are mean±SEM for four bone cultures.

‡ Values are mean±SEM for four cell cultures.

§ Significantly less than untreated supernates, $P < 0.05$.

TABLE IV

Dose-response Relationship of Colchicine on Release of Bone Mineral and Production of N-acetylglucosaminidase by Human Breast Cancer Cells (MCF-7)

	^{45}Ca Release*	*N*-Acetylglucosaminidase‡
	treated/control ratios	*mM/mg protein*
MCF-7 cells	1.42±0.03	1.7±0.1
+ 1 μM colchicine	2.15±0.22§	3.1±0.1§
+ 0.1 μM colchicine	1.88±0.13§	2.8±0.1§
+ 10 nM colchicine	1.36±0.11	2.0±0.1

* Values are mean±SEM for four bone cultures.
‡ Values are mean±SEM for four cell cultures.
§ Significantly greater than cells incubated without colchicine, $P < 0.05$.

TABLE V

Effects of Cytochalasin B (2 μM) on Bone Mineral Release and Production of Extracellular N-acetylglucosaminidase Activity by Human Breast Cancer Cells (MCF-7)

	^{45}Ca Release*	*N*-Acetylglucosaminidase‡
	treated/control ratios	*mM/mg protein*
MCF-7 cells	1.46±0.05	0.67±0.08
+ cytochalasin B in dimethylsulfoxide	1.82±0.10§	1.11±0.03§
+ dimethylsulfoxide	1.33±0.07	0.74±0.05

* Values are mean±SEM for four bone cultures.
‡ Values are mean±SEM for four cell cultures.
§ Significantly greater than cells alone, $P < 0.05$.

media to devitalized bones labeled with ^{45}Ca. Media was also taken from identical MCF-7 cultures not treated with cytochalasin B and from cultures treated with dimethylsulfoxide. There was an increase in both *N*-acetylglucosaminidase and bone mineral releasing activity by the cells cultured with cytochalasin B compared with those cultured without cytochalasin B (Table V). This suggests that inhibition of microfilament function also increases production of enzymes and bone mineral-releasing activity by the MCF-7 cells.

Fig. 1 is the time-course of the release of ^{45}Ca from devitalized bones by culture media harvested from MCF-7 cells after 48 h of culture. Most of the mineral that was released by the MCF-7 supernates appeared in the first 24 h. At all time-points there was increased mineral released by the bones that were incubated with supernates harvested from cells cultured with colchicine.

Figs. 2 and 3 represent the time-courses for the appearance of collagenolytic activity, cathepsin D, and *N*-acetylglucosaminidase as well as bone mineral and matrix resorbing activity in the supernates of MCF-7 cultures. Collagenolytic activity peaked slowly, reaching maximal activity at 36–48 h. Colchicine caused an increase in the release of collagenolytic activity. Cathepsin D activity in the culture media peaked at

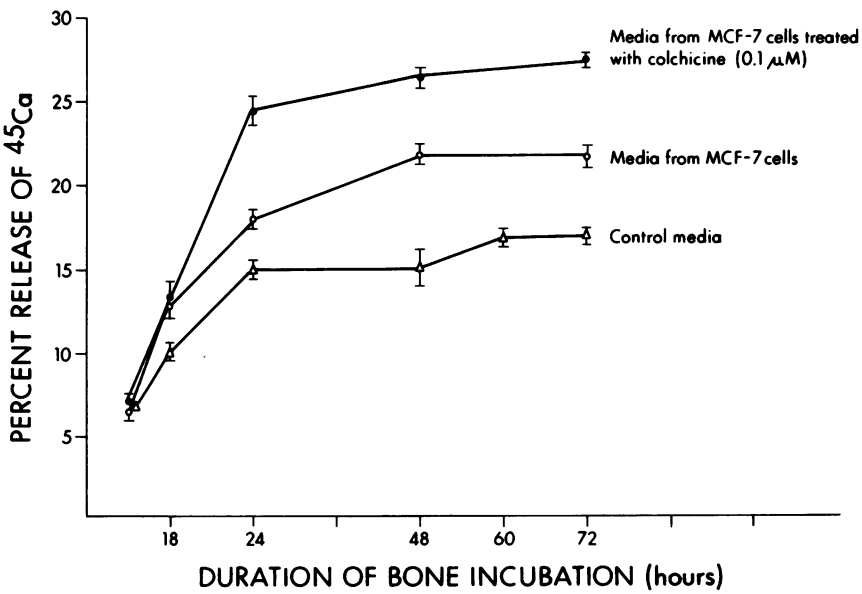

FIGURE 1 Time-course for release of ^{45}Ca from devitalized bones by MCF-7 culture media. Media were harvested from MCF-7 cells after 48 h of culture as they were nearing confluency. Some of the MCF-7 cultures were incubated with colchicine (0.1 μM). Values are mean±SEM for four bone cultures.

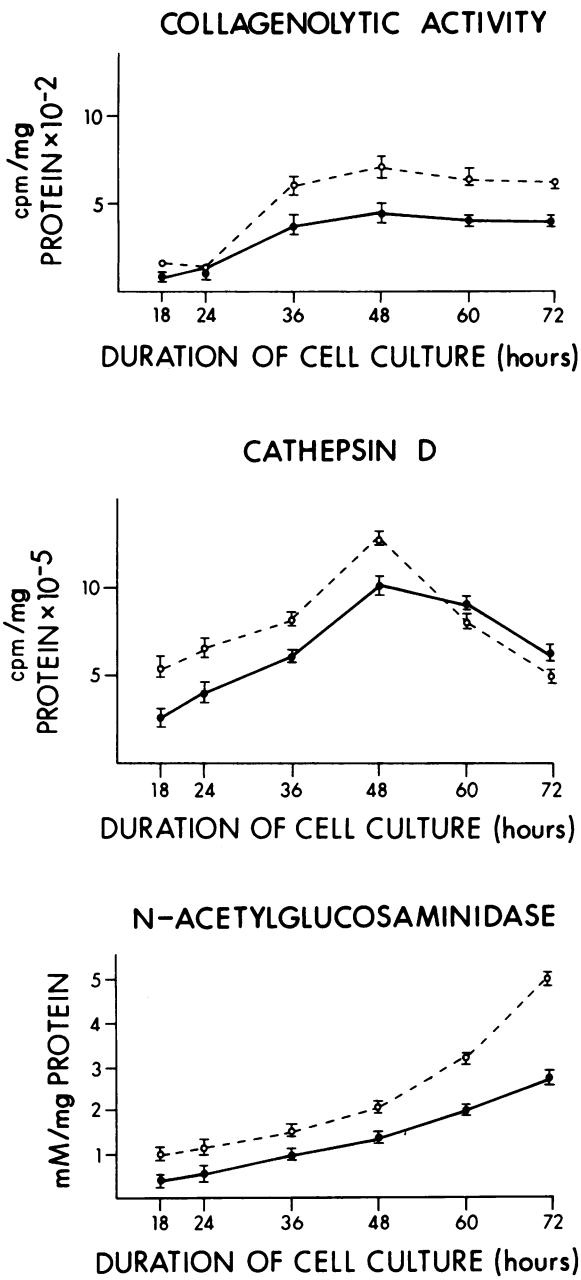

FIGURE 2 Time-course for appearance of hydrolytic activity in culture media harvested from MCF-7 cells. Each time point represents mean±SEM for four cell cultures. Cell cultures incubated with colchicine (0.1 μM) are represented by broken lines. Cell cultures incubated without colchicine are represented by solid lines. Methods of assay for enzyme activity are given in the Methods section.

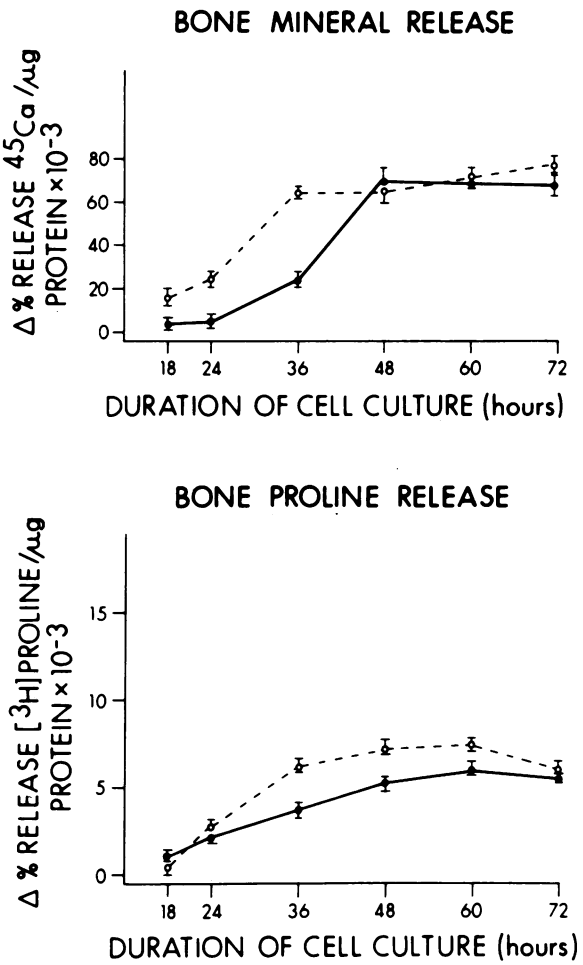

FIGURE 3 Time-course for appearance of bone mineral-releasing activity and bone proline-releasing activity in culture media harvested from MCF-7 cells. Each time point represents mean±SEM for four cell cultures. Cell cultures incubated with colchicine (0.1 μM) are represented as broken lines. Cell cultures incubated without colchicine are represented as solid lines. Methods of assessment of bone mineral-releasing activity and bone proline-releasing activity are given in the Methods section.

48 h and then slowly declined. Again there was increased enzyme in the media of cells cultured with colchicine. There was a significant increase in release of *N*-acetylglucosaminidase during culture up to a period of 74 h. At all time-points there was an increased release of *N*-acetylglucosaminidase by the MCF-7 cells cultured in the presence of colchicine. Release of bone mineral-releasing activity reached a peak at 48 h of cell culture (without colchicine) or 36 h (with colchicine) and did not increase after longer periods of culture. Bone matrix resorbing activity was represented by the ability of the MCF-7 culture supernates to release [^{3}H]proline from bones. This activity peaked later than mineral releasing activity and coincided more closely with the release of collagenolytic activity than with the other enzymes.

Fig. 4 shows the effect of dilutions of conditioned media from MCF-7 cells on bone mineral-releasing activity. Each of the lower dilutions caused decreased

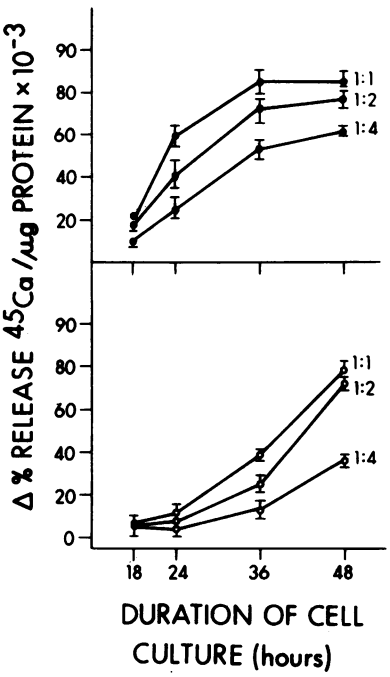

FIGURE 4 Effects of dilution of culture media with fresh unconditioned medium harvested from MCF-7 cells after 18, 24, 36, and 48 h of culture on release of previously incorporated ^{45}Ca from devitalized bones. Values are mean ±SEM for four cell cultures. The upper panel represents cells incubated with colchicine (0.1 μM). The lower panel represents cells incubated without colchicine.

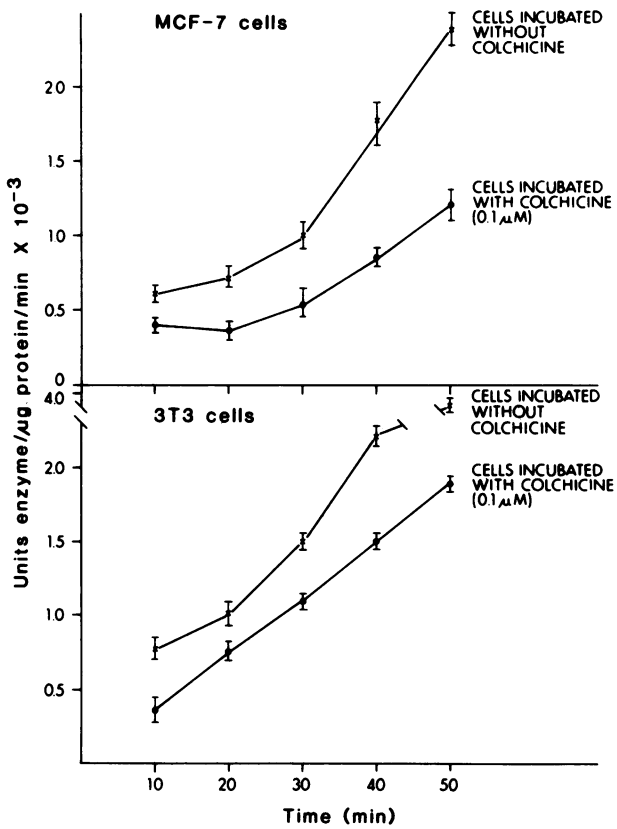

FIGURE 5 The effect of colchicine on uptake of horse radish peroxidase into MCF-7 and 3T3 cells. Four cell cultures for each treatment group were incubated until they reached confluency. Horse radish peroxidase uptake was assessed according to the method of Steinman et al. (12). Values are mean±SEM for each time point represented.

mineral release. This suggests that in this experimental system there is no limitation of available substrate for the enzymes, since decreased amounts of enzyme activity caused corresponding decreased release of bone mineral. If decreased substrate was a limiting factor greater dilutions would cause the same release of bone mineral.

The effect of colchicine to cause enhanced enzyme activity in conditioned media from MCF-7 cells could represent increased release of enzymes or a decreased rate of endocytosis caused by colchicine. To assess the role of endocytosis, we examined the accumulation of horse radish peroxidase by MCF-7 cells and 3T3 cells treated with colchicine (0.1 μM) (Fig. 5). In both instances there was a comparable inhibition of horse radish peroxidase accumulation in cells treated with colchicine. This suggests that the effect of colchicine on MCF-7 cells was different than that on 3T3 cells in that colchicine caused increased release of enzymes from the MCF-7 cells into the media.

DISCUSSION

In this study we have shown that bone mineral mobilization by human breast cancer cells is associated with enzyme release by the cells. This was demonstrated by measurement of lysosomal and cytoplasmic enzymes in cell culture supernates that release mineral from devitalized bone in vitro. All hydrolytic enzymes that were measured were released in increased amounts. There was no evidence for a specific effect on any particular type of hydrolytic enzyme. 3T3 fibroblasts had little effect on bone resorption and released lesser amounts of hydrolytic enzymes in the basal state than MCF-7 cells.

There was not a close quantitative correlation between the presence of the enzyme *N*-acetylglucosaminidase in the cell culture media and the extent of bone resorption in different experiments (Tables I, III, and IV). However, there were no experiments in which enhancement of bone resorption was not associated with an increase in medium hydrolytic enzyme activity. This lack of a close relationship between *N*-acetylglucosaminidase release and bone resorption may mean that this enzyme is not the key

enzyme for bone resorption. However, a more likely explanation for the variability of extent of bone resorption from experiment to experiment is that the measurements are derived from a bioassay and the measurements obtained from experiments performed on different occasions could not be expected to correlate perfectly.

This study was based on our recent observation that cultured human breast cancer cells are capable of releasing bone mineral and degrading the matrix of cultured fetal rat long bones in organ culture (1). This was found to be a direct effect of tumor cell products on bone and not mediated by osteoclasts. Histologic sections of the resorbing bones showed no evidence of osteoclast activity, and the resorbing effects of the tumor cells were not decreased by drugs that inhibit osteoclast activity such as cortisol and phosphate. Furthermore, the tumor cells and their supernates resorbed fetal rodent bones that had been devitalized by overnight exposure to ultraviolet light to kill the osteoclasts and leave a calcified acellular bone matrix.

Osteoclastic bone resorption is closely associated with hydrolytic enzyme production (2, 3, 13). It has not been possible to dissociate lysosomal enzyme release from bone resorption in tissue culture. In situations where osteoclastic bone resorption is stimulated by agents such as parathyroid hormone and prostaglandins E_2, lysosomal enzyme production is also increased (2, 3, 13). Parathyroid hormone and prostaglandin E_2 increase the release of lysosomal enzymes such as β-glucuronidase and cathepsin D from cultured bones but have little effect on collagenase activity in the bone culture medium at 48 h. Although the dose-response relations for β-glucuronidase release and ^{45}Ca release are similar, the increase in lysosomal enzyme release is proportionally greater and occurs earlier than the increase in ^{45}Ca release. In addition, agents that inhibit bone resorption such as calcitonin and cortisol also inhibit lysosomal enzyme release.

The mechanism by which bone mineral release and matrix resorption is linked to enzyme production is not clearly understood. There are a number of different ways in which hydrolytic enzymes produced by bone-resorbing cells could initiate bone resorption. These enzymes could be responsible for degrading the noncollagenous portion of the bone matrix that comprises glycoproteins, proteoglycans, and lipid. Hydrolytic enzymes could initiate mineral release from bone by degrading those matrix components that normally prevent mineral release. Hydrolytic enzymes released from lysosomes might also assist cytoplasmic collagenase in the degradation of collagen directly or be responsible for the activation of collagenase from precursor forms.

Microtubular function is closely related to secretory events in many different types of cells. Although depolymerization of microtubules is usually associated with inhibition of secretion (14, 15), it has been shown in cultured fibroblasts that disassembly of microtubules leads to stimulation of release of a number of lysosomal enzymes (16). Others have found that membrane fusion leading to lysosomal discharge into incubation media occur independent of microtubules (17). In osteoclasts, which are the major bone resorbing cells, depolymerization of microtubules by colchicine inhibits bone resorption and lysosomal enzyme release (3, 18) but simultaneously increases collagenase production (3). Colchicine causes similar alterations in enzyme production by monocytes (19). Lysosomal enzyme production by monocytes is decreased by colchicine, whereas collagenase production is increased. MCF-7 cells have an opposite response to microtubule depolymerization by colchicine. In this case colchicine stimulates release of hydrolytic enzymes as well as bone mineral and [^{3}H]proline by the MCF-7 cells.

Only about 30% of the total ^{45}Ca was released from the devitalized bones by the MCF-7 cultures. Longer exposures of the matrix to enzymes did not increase mineral release or matrix degradation, nor did media harvested from cell cultures incubated for longer periods. The time-course of enzyme release and the experiments where conditioned media were diluted suggest that the major factor limiting bone resorption is the amount and activity of enzymes released by the cells, rather than the availability of approachable bone matrix (or substrate). Because greater dilutions caused less mineral release up to 48 h of culture, it is unlikely that substrate availability was a limiting factor.

Bone matrix degradation was assessed by release of previously incorporated [^{3}H]proline. Previous experiments have shown that release of [^{3}H]proline corresponds very closely with release of [^{3}H]hydroxyproline and collagen degradation in this system (1). [^{3}H]proline release and release of collagenolytic activity occurred at later time-points compared with lysosomal enzyme release and mineral release (Figs. 2 and 3). This is consistent with the notion that collagenolytic activity is expressed after demineralization occurs, and the hypothesis that lysosomal enzymes are responsible for mineral release, and after removal of bone mineral collagenase degrades the collagenous bone matrix.

The present study provides further evidence that hydrolytic enzyme release is correlated closely with bone resorption. This correlation between hydrolytic enzymes and bone resorption occurs whether bone resorption is mediated by osteoclasts or by breast cancer cells. Clarification of the molecular mechanism of bone resorption in breast cancer is important because osteolytic bone metastases, bone destruction, and hypercalcemia occur frequently in patients with ad-

vanced breast carcinoma and are responsible for considerable morbidity and mortality in patients with this disease (20).

ACKNOWLEDGMENTS

This work was supported in part by grant CA-25500 from the National Cancer Institute, grant AM 21584 from the National Institutes of Health, and research grant CH-69B from the American Cancer Society.

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