Influence of Cell Proliferation and Cell Cycle Phase on Expression of Estrogen Receptor in MCF-7 Breast Cancer Cells


Medicine and Clinical Pharmacology Branches, National Cancer Institute, Bethesda, Maryland 20205

ABSTRACT

In the present study, the effects of cell cycle phase and proliferation rate on the expression of specific estrogen binding activity were explored in hormone-dependent human breast cancer cells. A technique was developed to alter the proliferative rate of MCF-7 cells by plating at different densities. The doubling time ranged from 20 to 48 hr, showing a negative relation to the number of plated cells. Slowly proliferating cells had accumulated more than twice as much estrogen receptor (ER) activity as did fast-proliferating cells. Exposure of exponentially growing cells to isoleucine-deficient medium resulted in decreased thymidine incorporation and disappearance of detectable cellular ER activity. Overall protein synthesis was reduced by only 30% in cells growing in isoleucine-free medium. At 24 hr after release from isoleucine deprivation, ER levels are fully restored, although thymidine incorporation does not resume for an additional 6 to 8 hr, and increases in cell number are not seen for 24 hr. Exposure of exponentially growing cells to 2 mM thymidine for 24 hr produced partially synchronized MCF-7 cells (~70%). Six hr after release from excess thymidine, cells reached S phase; after 9 hr, G1; and after 18 hr, G2. ER levels immediately and, 6 hr after release, remained unchanged, showed a slight increase at 9 hr, and showed an increase of about 50 to 60% at 18 hr. These data suggest that: (a) ER binding activity and DNA synthesis can be dissociated; (b) ongoing protein synthesis is necessary for maintenance of cellular ER activity; and (c) ER is apparently synthesized throughout the cell cycle, with some evidence that this is predominantly in G1 and G2.

INTRODUCTION

It is well-documented that the growth rate of MCF-7 cells, originally derived from a pleural effusion of a patient with metastatic breast cancer (32), can be modulated by a variety of hormones (16-18). The effects of these hormones are mediated by hormone receptors (3, 13). Important influences of serum and hormones (16-18). The effects of these hormones are mediated by hormone receptors (3, 13). Overall protein synthesis was reduced by only 30% in cells growing in isoleucine-free medium. At 24 hr after release from isoleucine deprivation, ER levels are fully restored, although thymidine incorporation does not resume for an additional 6 to 8 hr, and increases in cell number are not seen for 24 hr. Exposure of exponentially growing cells to 2 mM thymidine for 24 hr produced partially synchronized MCF-7 cells (~70%). Six hr after release from excess thymidine, cells reached S phase; after 9 hr, G1; and after 18 hr, G2. ER levels immediately and, 6 hr after release, remained unchanged, showed a slight increase at 9 hr, and showed an increase of about 50 to 60% at 18 hr. These data suggest that: (a) ER binding activity and DNA synthesis can be dissociated; (b) ongoing protein synthesis is necessary for maintenance of cellular ER activity; and (c) ER is apparently synthesized throughout the cell cycle, with some evidence that this is predominantly in G1 and G2.

MATERIALS AND METHODS

Materials. [3H]Thymidine (25 Ci/mmol), [3H]leucine (40 Ci/mmol), and 17β-[2,4,6,7-3H]estradiol (91 Ci/mmol) were purchased from New England Nuclear (Boston, MA). Unlabeled thymidine was purchased from Sigma Chemical Co. (St. Louis, MO), and unlabeled 17β-estradiol was obtained from Steraloids (Pawling, NY) and prepared in absolute ethanol. Crystalline trichloroacetic acid was purchased from the Baker Chemical Co. (Phillipsboro, NJ).

Cell Culture. The MCF-7 cell line (32) was obtained from Dr. Marvin Rich, Michigan Cancer Foundation (Detroit, MI). The ER-negative MDA-MB-231 human breast cancer cell line established by Cailleau et al. (6) was provided by Dr. Ronald Herberman, National Cancer Institute, Bethesda, MD. Cell lines were grown in improved essential medium as modified by Richter et al. (27), supplemented with collagen (0.8 g/liter), gentamicin (40 ng/liter; Grand Island Biological Co., Buffalo, NY), 1 x 10^-7 M insulin (Eli Lilly, Indianapolis, IN), and 5% calf serum (Grand Island Biological Co.), which was treated with dextran-coated charcoal (16). This medium is defined in this paper as "supplemented IMEM." All experiments were performed with cells from replatedly plated, confluent monolayer cultures in T-150 tissue culture flasks (150 sq cm surface area). The cells were grown under these conditions for at least 10 passages before use. They are routinely passaged once per week and fed 3 times/week. They are passaged at a density of approximately 2 x 10^6 cells/flask. Exponentially growing subconfluent cells were used in all experiments. Flasks were left loosely capped in a humidified 5% CO2 atmosphere incubator, maintained at 37°. Experiments were performed in 4-well plastic dishes having 28-sq cm surface area.

Various experiments used cells growing at different densities. For this purpose, cells were harvested with 0.05% trypsin:0.02% EDTA (w/v) from confluent T150 flasks in 20 ml of supplemented IMEM. Cells from several flasks were pooled, and cell counts of this single-cell suspension were determined. This suspension was diluted with fresh supplemented IMEM to the appropriate cell number. One ml of the final dilution was added to 4-well dishes (Linbro, McLean, VA), each containing 6 ml of...
supplemented IMEM preincubated for 1 hr in a humidified 5% CO₂ incubator at 37°.

Synchronization Experiments. For synchronization experiments, cells from confluent flasks were harvested using 0.05% trypsin·0.02% EDTA (w/v) in PBS and plated replicately in 4-well dishes at densities indicated in chart legends. The medium was changed at indicated time points after plating into either supplemented IMEM (control) or to supplemented IMEM containing 2 mM thymidine (34). Twenty-four hr later, dishes were rinsed twice with IMEM, and cells were replaced in supplemented IMEM. Cells were harvested directly after medium change or at 6, 9, 12, 18, or 24 hr later. The exposure of exponentially growing cells to 2 mM thymidine for 24 hr reproducibly provided cultures that were approximately 70% synchronous, as described below. To evaluate the degree of synchronization and recovery from the block, we measured [³H]thymidine incorporation and cellular DNA content by flow cytometry. For other experiments, cells were replicately plated in 4-well dishes for 48 hr in supplemented IMEM and then exposed for 30 hr to isoleucine-deficient IMEM (Media Unit, NIH, Bethesda, MD), supplemented with 5% dialyzed fetal calf serum (Grand Island Biological Co.) and 1 x 10⁻⁷ M insulin. Control dishes were treated similarly, except that IMEM containing isoleucine was used. Cells were released from isoleucine deprivation by changing the medium to normal supplemented IMEM, after rinsing the plates once with supplemented IMEM. At time points indicated for each chart, cells were harvested and further processed as described.

Precursor Incorporation. Cells plated in 4-well dishes were pulsed with [³H]thymidine or [³H]leucine 1 ìCi/ml for 1 or 2 hr. Cells were harvested with 4 ml 0.04% EDTA in PBS after rinsing the plates 3 times with PBS. Two aliquots (500 µl) were dispersed in 10 ml of isoton (Coulter Electronics, Inc., Hialeah, FL) and counted in a cell counter (Particle Data, Inc., Elmhurst, IL). The remaining cells were collected in a high-speed serofuge (Clay Adams, Parsippany, NY). The pellet was stored at -20° until further use. Pellets were resuspended in 1 ml of PBS and disrupted with five 1-sec bursts of a sonicator at its lowest setting. Aliquots were taken for protein (20) and DNA quantification (4) and for determination of radioactivity in the total trichloroacetic acid-precipitable material on 0.45-µm Millipore filters. After drying, filters were solubilized in Ready-solv MP (Beckman, Fullerton, CA) and counted in a Beckman LS 7500 Liquid Scintillation Counter.

DNA Histograms. Cells were grown in 4-well dishes and harvested with 1.5 ml 0.04% EDTA in PBS after rinsing the plates twice with PBS. Cells were pooled from 2 identically treated wells, fixed in cold 95% ethanol, and stained with mithramycin (100 µg/ml) in 15 mM MgCl₂ and 0.15 M NaCl. Nuclear fluorescence was measured with a Los Alamos Cell Sorter. Data were recorded, stored, and analyzed with a DEC 11/40 computer system, using software developed at the Los Alamos Scientific Laboratory (Los Alamos, NM). At least 20,000 cells were measured per sample. All DNA content distributions were normalized with respect to total cell number per sample. The G₂ peak was set in Channel 60 of 256. Unperturbed DNA histograms were analyzed by the method of Ritch et al. (28). Perturbed DNA histograms were divided into bounded regions as follows: G₀, Channels 50 to 70; S, 79 to 99; and G₂ M, 100 to 140; and the fraction of cells in each region was determined.

ER Determinations. ER activity was measured using whole-cell equilibrium binding assays. In brief, cells were replicately plated in 4-well dishes as indicated for each experiment and incubated for 1 hr with 5 concentrations of 17β-estradiol [³H] (0.1 to 3 nM) with or without a 200-fold excess of unlabeled 17β-estradiol at 37°. Following incubation, cells were washed 3 times with PBS and harvested in 3 ml of 0.04% EDTA in PBS. One aliquot (500 µl) was taken for cell count, and 2 aliquots (500 µl) were transferred directly to scintillation vials and counted for radioactivity. Results were analyzed by Scatchard plot analysis (29) and expressed in specific binding sites per cell, or per unit DNA or protein, using a computer-assisted method after subtracting the nonspecific binding (1).

Correlations were determined by the method of least-square analysis using the DEC-10 Computer Statistical Package “Bright.”

RESULTS

Relation between Cell Density, Growth Rate, and ER Levels. We wished to develop a model for influencing the proliferative rate of MCF-7 cells by plating cells at different densities in a drug-free system without the need of hormonal perturbation. Chart 1A shows the growth curves of cells plated at 5 x 10⁶ to 1 x 10⁷ cells/dish (1.8 x 10⁶ to 35.7 x 10⁶/sq cm). Cells plated at the highest density reached plateau phase by 96 hr after plating (data not shown). The slopes of the other growth curves did not change between 24 hr (Chart 1, time point 0) and 96 hr after plating. We found an obvious difference in the slope of these curves. The doubling times calculated from 3 different experiments (20 to 48 hr) were plotted against the log number of plated cells (Chart 1B). We found a highly significant (r = 0.98) negative correlation between the number of plated cells and the doubling time. Results from flow cytometry studies are depicted in Table 1, showing a similar percentage of cells in S phase at 48 hr after being plated at different densities. We conclude that, despite alterations in growth rate induced by plating cells at varying densities, the ratio of S-phase duration to the duration of the total cell cycle did not change.

We then determined the ER levels in cells proliferating at different rates and compared these with the [³H]thymidine incorporation (Chart 2). We found a more than 2-fold increase of ER binding sites in cells plated at low density, as compared to more rapidly proliferating cells. The K₀ ranged from 1.84 to 4.05 x 10⁻¹⁰ M for 17β-[³H]estradiol and showed no specific relation to proliferation rate or receptor concentration (data not shown). As expected from the growth curve, rates of precursor incorporation increased with increasing proliferation rates associated with higher initial numbers of plated cells. These data clearly show an inverse relation between proliferative rate and ER levels in MCF-7 cells.

Behavior of MCF-7 Cells during and after Isoleucine Deprivation. To study the influence of cell cycle cell phase on ER levels, we tried to synchronize MCF-7 by exposure to isoleucine-deficient medium for 30 hr (15). We found that this metabolic block of exponentially growing cells led to a rapid decrease of [³H]thymidine incorporation. By 30 hr of isoleucine deprivation, [³H]thymidine was only 15% of control values (Chart 3A). The percentage of cells in S phase dropped from 28 to 10% in 3 different experiments. Changing medium to release cells from isoleucine deprivation by medium change did not lead to a rapid recovery of proliferative rate. Instead, [³H]thymidine incorporation continuously decreased for an additional 14 hr after isoleucine replenishment. [³H]Thymidine incorporation rapidly increased 24 hr after release from isoleucine-deficient medium, reaching a maximum after 48 hr. The percentage of cells in S phase paralleled this distribution, reaching a peak at 38 hr after release. Chart 3b depicts the growth curve during and after release from isoleucine deprivation. We observed no cell replication during the time of exposure to isoleucine-deficient medium. The cells started proliferating exponentially again at 72 hr after release from isoleucine deprivation and reached a plateau phase after 120 hr. We observed this growth pattern consistently in 3 different experiments. Chart 4 shows the [³H]leucine incorporation and total protein content during isoleucine deprivation. Immediately after exposure to isoleucine-deficient medium, protein synthesis dropped by about 30% and remained at that level.
Cell Proliferation, Cycle Phase, and ER

Chart 1. Relation between density and plated cells and doubling time. MCF-7 cells were plated for 24, 48, 72, and 96 hr at densities of 5 x 10^6; 1 x 10^6; 2 x 10^5; and 1 x 10^5 (a). At these time points, cells were harvested, and cell counts were determined as indicated in "Materials and Methods." Points, mean of 3 different experiments; each experiment was performed with six 4-well dishes.

Throughout the period of the block. This may indicate that cells have some alternative sources of isoleucine by which to maintain protein synthesis. Six hr after release from isoleucine deprivation, protein synthesis again increased, reaching control levels 28 hr after release from isoleucine deprivation. This level is maintained for the rest of the observation period. As expected from these results, the total protein content per cell decreased during isoleucine deprivation by about 30% and remained at that level for 30 hr after release. Interestingly, when cells started proliferating again, cellular protein further decreased to about 30% of the level before exposure to isoleucine-deficient medium.

The quantity of detectable ER binding sites during and after release from isoleucine deprivation is depicted in Chart 2. In 3 different experiments, we consistently found a rapid decrease in the first 12 hr of isoleucine deprivation from 1000 to 400 fmol/mg DMA (Chart 5). The decrease in ER binding capacity substantially exceeded the decrease of total protein synthesis (Chart 4). ER levels were unmeasurable after 30 hr of isoleucine deprivation. In the course of decline of ER activity during isoleucine deprivation, receptor affinity constant values obtained from Scatchard analysis did not differ from control values at 1 to 2.2 x 10^-10 m. Whereas, in the first 12 hr after release from isoleucine deprivation, ER activity increased moderately, a full recovery was not observed until 24 hr after release. Subsequently, ER reached a maximum at 38 hr. It should be noted that recovery of ER happened during a time period when [as shown by [3H]thymidine incorporation data summarized in Chart 3A and cell numbers (Chart 3B)] cells are apparently not proliferating at all. At the time point of maximal thymidine incorporation (48 hr after release from isoleucine-deficient medium), ER activity started decreasing again. We conclude, from these experiments, that...
ongoing protein synthesis is probably required for maintenance of ER activity in MCF-7 cells. This question cannot be addressed unequivocally without means of assaying receptor protein independent of binding activity. ER recovery after release from isoleucine deprivation precedes the start of thymidine incorporation by about 18 hr.

**Synchronization of MCF-7 Cells by Exposure to Excess Thymidine.** Since cells were not released from isoleucine deprivation as a synchronous cohort, we did not obtain information about the influence of cell cycle on ER binding sites. Therefore, we tried another established method for cell synchronization, exposure to excess thymidine for at least one doubling time (34). With this method, we achieved a partial synchronization of 60 to 70% (Table 2; Chart 6). We found that, at 6 hr after release from a 24-hr exposure to excess thymidine, 65% of cells were in S phase, reaching G₂ at 9 hr after release. Thus, cells blocked by this method resume cell cycle traverse within 6 hr after release from the block. Eighteen hr after release from excess thymidine, 75% of cells have completed a cell cycle and have reached G₁ again. The low fractions of cells in S at this time point as well as 6 hr later suggest a protracted decrease in the rate of DNA synthesis. Chart 7 depicts the time course of precursor incorporation into DNA and protein after release from 2 mM thymidine. The low value for thymidine incorporation directly after release indicates that DNA synthesis in these cells is essentially arrested. At 6 hr after release, maximal levels of [³H]thymidine incorporation were determined by Scatchard plot analysis, as described in "Materials and Methods." Control cells for [³H]leucine incorporation were treated similarly except they were never exposed to isoleucine-deficient medium (Ile⁻, isoleucine-deficient IMEM; Ile⁺, normal IMEM). Arrow, point at which cells were switched back to isoleucine-containing medium.

**Chart 4.** Protein synthesis during and after release from isoleucine-deficient medium. Cells (5 × 10⁵) were plated and processed as indicated for Chart 3. [³H]Leucine incorporation (Θ) and protein content determinations (Ο) were performed as described in "Materials and Methods." Control cells for [³H]leucine incorporation (Θ) were treated similarly except they were never exposed to isoleucine-deficient medium (Ile⁻, isoleucine-deficient IMEM; Ile⁺, normal IMEM). Arrow, point at which cells were switched back to normal IMEM. Bars, S.D.

**Chart 8 depicts the time course of ER binding sites after release from excess thymidine.** We did not find values different from control immediately or 6 hr after release from 2 mM thymidine. When ER levels were expressed per unit of DNA, we found a slight decrease after 6 hr. We observed an obvious increase of ER levels in cells predominantly in G₂ and a further increase when cells had mostly reached G₁. These data were consistently

---

**Chart 3.** Cell proliferation (A) and growth curve (B) of MCF-7 cells during and after isoleucine deprivation. Cells (5 × 10⁵) were plated for 48 hr and exposed for 30 hr to isoleucine-deficient medium (Ile⁻). At 30 hr, medium was changed to isoleucine-containing IMEM (Ile⁺). At indicated time points, cells were either incubated with [³H]thymidine (Δ) or harvested for cell counts or DNA histograms (Θ). [³H]Thymidine incorporation was determined as indicated in "Materials and Methods." Data represent 3 experiments. DNA histograms were performed in duplicate, and precursor incorporation represents at least 8 determinations performed in duplicate.

**Chart 5.** ER levels in MCF-7 cells during exposure and after release from isoleucine-deficient medium. Cells (5 × 10⁵) were plated as indicated in Chart 3. ER levels were determined by Scatchard plot analysis, as described in "Materials and Methods." Data are results from 3 experiments. S.E. (bars) was approximately 10% (Ile⁻, isoleucine-deficient IMEM; Ile⁺, normal IMEM). Arrow, time point at which cells were switched back to isoleucine-containing medium.
Table 2

Partial synchronization of MCF-7 cells by exposure to 2 mM thymidine

Cells (5 x 10^5) were plated for 48 hr and exposed for 24 hr to 2 mM thymidine. After rinsing the plate twice with supplemented IMEM, cells were harvested at indicated time points with PBS containing 0.04% EDTA and were fixed in 95% cold ethanol. Flow cytometry was performed as described in "Materials and Methods."

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>G_1</th>
<th>S</th>
<th>G_2</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>53 ± 6.2</td>
<td>84 ± 0.05</td>
<td>38.7 ± 4.3</td>
</tr>
<tr>
<td>6</td>
<td>20.7 ± 1.9</td>
<td>61.4 ± 2.4</td>
<td>18.3 ± 0.5</td>
</tr>
<tr>
<td>9</td>
<td>12.3 ± 0.08</td>
<td>17.4 ± 5.4</td>
<td>70.3 ± 4.6</td>
</tr>
<tr>
<td>18</td>
<td>74.4 ± 4.1</td>
<td>8.0 ± 0.05</td>
<td>18.3 ± 1.2</td>
</tr>
<tr>
<td>24</td>
<td>57.4 ± 1.3</td>
<td>15.5 ± 0.9</td>
<td>27.3 ± 2</td>
</tr>
</tbody>
</table>

* Mean ± S.D. from 3 different experiments.

DISCUSSION

It has been long known that, in nontransformed cells, proliferation and differentiation appear to bear a reciprocal relationship (11, 21). Recently, it has been shown that, in proadipocytes, growth arrest and differentiation are coupled at a distinct state in G_1 (30). Preliminary results with transformed cells revealed that some lost the ability to couple growth arrest and differentiation. Several methods have been reported: (a) plating at high density and waiting until confluency; (b) plating at low density in the presence of low serum concentrations; and (c) exposure to a metabolic block of cell replication (15, 34). We adapted these methods for our experimental design, which was to affect the growth rate of MCF-7 cells "physiologically." Since it is known that a serum concentration itself can change the sensitivity of MCF-7 cells to estrogen and antiestrogen (5), we choose not to change the serum concentration in our experiments. Bar, S.D.

reproduced in 3 separate experiments. Similar results were obtained in other single experiments in which different time points were chosen including times more closely corresponding to mitosis (~12 hr). Thymidine added at a 2 mM concentration just prior to the whole-cell assay for ER determination did not affect the binding capacity or affinity of ER (data not shown).

Thus, 3 sets of experiments involving experimental manipulation of cellular replication processes suggest that maximal estrogen binding capacity in MCF-7 cells is not associated with S phase. Data derived from cell density experiments can be interpreted as showing that a rise in ER is associated with a decrease in rate of cell proliferation. Under these conditions, no relative changes in cellular distribution over cell cycle phases were detected. Therefore, it appears probable that the absolute duration of S phase in rapidly dividing cells must have decreased. Moreover, experiments using isoleucine deprivation indicate that levels of ER binding rise in the absence of significant thymidine incorporation or cellular division. Finally, cell synchronization studies suggest that ER binding capacity is decreased during S phase and is predominantly linked to G_1 and G_2 cell cycle phases.
the proliferative rate. Holley and Kiernan (12) have reported that the depletion of growth nutrients, particularly of serum factors, may be responsible for inhibition of proliferation at high densities. By plating cells over more than a 10-fold range, we were able to create a model which produced a gradual change from slowly to more rapidly proliferating cells. The doubling time ranged from 20 to 48 hr. Interestingly, these cells, although growing at markedly different rates, did not show a different distribution throughout the cell cycle. This implies that the length of each phase changed in proportion to the length to the cell cycle time. One explanation for the observation that low-density cells are growing at a lower proliferative rate is that MCF-7 cells produce and possibly secrete certain factors into the culture medium, which stimulate their own growth. This explanation is strongly supported by experimental data from Vignon et al. (33) and from our own group,6 using "conditioned medium." Medium, conditioned by MCF-7 cells for several days and added to freshly plated cells, increased the growth rate and the [3H]thymidine incorporation significantly. The proliferative rate was found to be inversely correlated to the amount of ER. Slowly proliferating cells accumulate higher concentrations of receptor activity. This may be due to either increased synthesis or reduced degradation, or both. Alterations in receptor activity are also possible.

The mechanisms which affect the proliferation of normal or transformed cells are not entirely clear. Most previous attempts have focused on events occurring in the $G_1$ phase, and have been interpreted as showing that specific restriction points in the $G_1$ phase control proliferation (26). Despite the fact that the significance of affecting the proliferative rate of cancer cells by growth factors or nutrient deprivation is unknown, these observations fit clinical data from breast cancer specimens showing that the proliferative activity, measured by labeling index, and the ER content in breast cancer samples show an inverse relationship (2, 22, 31). Our experimental data strongly support the idea that the ER content in MCF-7 cells is not constant, but is strongly dependent on the proliferative state of the cells.

The question arises as to whether ER is produced throughout the entire cell cycle, or if ER is specifically produced in only one or in two cell cycle phases and increases in slowly proliferating cells because of the elongation of a cycle phase. There are innumerable examples in the literature, showing cell cycle-de- pendent or -independent production of macromolecular compo- nents (20). There is some evidence that hormone receptors are expressed cell cycle specifically. Cidlowski and Michaelis (8) showed that the glucocorticoid receptor in HeLa cells is increased during S phase and suggested that this increase results from alterations in RNA and protein synthetic processes (7). DeFeset al. (9) showed that the nuclear 3,5,3'-triiodothyronine receptor concentration increases during S phase in glucocorticoid cells.

We failed to synchronize the MCF-7 cells by exposure to isoleucine-deficient medium. Binding to ER was unmeasurable in cells which had stopped proliferating after 30 hr of isoleucine deprivation. Overall protein synthesis decreased in this time period by only about 30%. Interestingly, ER binding activity is recovered in resting cells which have not begun to incorporate [3H]thymidine. We believe that ER production in these resting cells probably occurs predominantly in the $G_1$ and $G_2$ phase, and it is not dependent on the presence of cells in S phase. Between 24 and 48 hr after release from isoleucine deprivation, cells start proliferating again. ER activity showed a moderate increase after 34 hr and a sharp decrease at the time point of maximal DNA synthesis at 48 hr. This is compatible with data shown in Chart 2, describing an inverse relation between proliferative rate and ER. These data are again consistent with an inverse relation between proliferative rate and ER content in MCF-7 cells. One may separate the time course of an isoleucine experiment into 3 phases: (a) exposure to isoleucine-deficient medium; (b) 24 hr of release characterized by resting cells; and (c) the next 24 hr characterized by rapid DNA synthesis and cell division.

We would suggest that, in Phase 1, ER degradation obviously is dominant and, in Phase 2, ER synthesis exceeds degradation. We have no information concerning the relation between degra- dation and synthesis in Phase 3, which would allow us to attribute the decrease of ER levels either to decreased ER synthesis or increased degradation. There are observations in the literature that degradation in rapidly proliferating, nontransformed cells may be either high (14) or low (10).

Our attempt to produce partially synchronized cells by exposing them to excess thymidine, a method originally described by Xeros (34), was more successful. We found, from analysis of DNA histograms, that the initial, approximately 70% synchrony was lost within 18 hr after release from excess thymidine and, additionally, that thymidine incorporation (DNA synthesis) markedly decreased over the 24-hr period.

Since the ER level immediately after, or 6 hr after, release from 2 mM thymidine did not change, we believe the S phase is not a predominant period for ER synthesis. ER levels started increasing again when cells reached the $G_2$ phase and showed, despite mitosis, a steady increase until most of the cells were in the $G_1$ phase. Therefore, $G_1$ and $G_2$ are more likely periods in the division cycle in which predominant net ER synthesis occurs. These data are compatible with results obtained from the isolateu- cine experiments. Obviously, since we measure only binding activity and not true receptor synthesis or degradation rates, final conclusions concerning receptor regulation within the cell cycle will await alternative means of analysis.

According to our results, shown in Chart 2, rapidly proliferating cells accumulate a lower amount of ER than do cells growing at a slower proliferative rate. The absolute duration of S phase in rapidly proliferating cells appears to be less than in slowly multiplying cells. Most probably, a combination of both processes influences ER changes after release from thymidine block.

We conclude, from these experiments, that the proliferative rate of MCF-7 cells strongly affects the ER content of the cells. Furthermore, we suggest that $G_1$ and $G_2$ may be more likely phases in the division cycle for predominant ER synthesis. Whether the latter observation is true for the majority of malignant breast tumors and whether in vivo manipulation of tumors, which takes cognizance of these variations in receptor and proliferative rate, will have any clinical applicability must await results of future studies.

REFERENCES

2. Bertuzzi, A., Daidone, M. D., Di Franza, G., and Silvestrini, R. Relationship among estrogen receptors, proliferative activity and menopausal status in...
Influence of Cell Proliferation and Cell Cycle Phase on Expression of Estrogen Receptor in MCF-7 Breast Cancer Cells
