Factors Affecting the Sensitivity of T-47D Human Breast Cancer Cells to Tamoxifen

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ABSTRACT

Cell proliferation kinetics during growth of an estrogen receptor-positive human breast carcinoma cell line, T-47D, was defined, and some factors which modify its response to tamoxifen were investigated in vitro. T-47D cells were estrogen responsive when grown in charcoal-stripped fetal calf serum, but the addition of 17β-estradiol did not fully restore the growth rate to that observed in the same concentration of fetal calf serum. Tamoxifen had both a low-dose, estrogen-reversible, growth-inhibitory effect and a high-dose, estrogen-irreversible, growth-inhibitory and cytotoxic effect on T-47D cells. Tamoxifen-induced growth inhibition was associated with a decrease in the percentage of S-phase cells and, to a lesser extent, G2-M-phase cells and an increase in G0-G1-phase cells. Plateau-phase cells were considerably less sensitive than were exponentially growing cells, and this was accompanied by a fall in unoccupied estrogen receptor content from 4407 ± 655 (S.E.) sites/cell in exponentially growing cultures to 1420 ± 315 sites/cell in plateau-phase cultures. T-47D cells were more sensitive to tamoxifen cytostasis when grown in fetal calf serum rather than charcoal-stripped fetal calf serum. However, with both types of growth medium, the sensitivity to tamoxifen was inversely related to the serum concentration, e.g., the 50%-inhibitory dose concentration increased 75-fold as the fetal calf serum concentration was increased from 0.25 to 10%. Addition of insulin to the culture medium had no effect on the growth rate, estrogen receptor content, or tamoxifen sensitivity of T-47D cells. These results illustrate that the conditions under which cells are cultured markedly affect their sensitivity to tamoxifen and highlight the need to specify these conditions when reporting effects of this drug.

INTRODUCTION

The nonsteroidal antiestrogen, tamoxifen, is in widespread clinical use for the therapy of human breast cancer. It is also a potentially useful experimental tool for the elucidation of hormonal control mechanisms in this disease. Although a small number of sex steroid hormone-responsive human breast carcinoma cell lines are now available (1, 4, 8, 16, 34), the effects of tamoxifen on cellular proliferation have been studied extensively only in the MCF-7 cell line (3, 6, 16, 20, 29, 30, 32) with less toxicity of concentrations of FCS (0.25 to 10%) or CS-FCS (1 to 10%). Removal of 17β-estradiol from FCS was monitored by the addition of a 0.22-μm filter (Falcon Plastics, Oxnard, CA). Removal of 17β-estradiol from FCS was monitored by the addition of 17β-[3H]estradiol (Amersham International, Amersham, Buckinghamshire, United Kingdom), and in a typical preparation, <1% radioactivity remained after the second dextran:charcoal treatment. Cell Growth Experiments. Cellular proliferation kinetics was studied by inoculating 2 × 10⁶ cells from stock cultures of Days 4 to 7 (see Chart 1A) into 150-sq cm plastic flasks containing 50 ml medium and incubating at 37°C. For some flasks (*closed*), the medium was replaced daily from Day 1. One or more flasks were harvested with 0.05% trypsin/0.02% EDTA each day until Day 10, and twice weekly thereafter. Viable cell counts were obtained with a hemocytometer and phase-contrast microscopy. Regrowth experiments were commenced twice weekly by reinoculating 1.7 × 10⁶ harvested cells into 25-sq cm flasks containing 8 ml medium. Triplicate flasks were harvested daily for 1 week thereafter for cell counts and/or flow cytometry. The effects of FCS and CS-FCS, with or without 10⁻⁸ M 17β-estradiol, on cell proliferation were investigated by preparing media with a range of concentrations of FCS (0.25 to 20%) or CS-FCS (1 to 10%). 17β-
estradiol was obtained and prepared as described previously (30). Cells
(1 \times 10^6) were inoculated into 25-sq cm flasks containing 5 ml medium.
The cells inoculated into media containing CS-FCS had 2 prior weekly
passages in medium containing 5% CS-FCS. Triplicate flasks were
harvested by trypsinization after 7 days, and cell counts were
obtained with a Model ZBI Coulter Counter (Coulter Electronics, Harpen-
den, Herts, United Kingdom). Cells grown in media containing FCS were
also stained for flow cytometry. Mean population doubling times were
calculated from the initial and final cell numbers.
Flow Cytometry. Cells were stained for DNA with ethidium bro-
mine:mithramycin, and DNA histograms were obtained by flow cytometry
as described previously (31). The proportion of cells in the G0-G1, S, and
G2-M phases of the cell cycle was analyzed planimetrically (17).
ICRF 159 (Razozone) Treatment. The percentage of cells remaining
in the G0-G1 phase of the cell cycle after 48-hr treatment with an inhibitor
of cytokinesis, ICRF 159, was determined by DNA flow cytometry. ICRF
159 was obtained and prepared as described previously (30) and added
to flasks of cells prepared as described above for cell growth experi-
ments. In 2 such experiments, the medium of fed flasks was replaced
by medium containing ICRF 159 (100 µg/ml), and the cells were har-
vested after 48 hr; in another experiment, the medium was replaced
twice at 24-hr intervals with medium containing ICRF 159 (50 µg/ml),
and the cells were harvested after a total of 48-hr drug treatment. Both
methods of treatment had no significant effect on cell numbers after 48
hr and yielded similar results for the proportion of G0-G1-phase cells.
ICRF 159 was added directly to "unfed" flasks to give a final concen-
tration of 100 µg/ml, and cells were harvested after 48 hr. Harvested cells
were subjected to DNA flow cytometry as described above, and the
proportion of cells remaining in G0-G1 was calculated.
Tamoxifen Treatment. Tamoxifen [trans-1-{4-/3-dimethylaminoethox-
yphenyl)-1,2-diphenylbut-1-one] was obtained and stored in ethanol as
described previously (30) and added to medium such that the final ethanol
concentration was 0.1%. The sensitivity of T-47D cells to tamoxifen
was tested by adding 1 \times 10^6 cells to 8 ml medium containing a range of
tamoxifen concentrations (0.1 to 10 µM) in 25-sq cm flasks. Control
flasks had 0.1% ethanol vehicle instead of tamoxifen. Flasks were
harvested by trypsinization, and cell counts were obtained in triplicate
with a Coulter Counter or with a hemocytometer if there were < 1 to 2
\times 10^5 cells/flask. Results were expressed as cells/flask or as doubling
(proliferation) rate (percentage of control). In some cases, cells were also
stained for flow cytometry.

The effect of growth phase on sensitivity to tamoxifen was tested by
replacing the medium of flasks containing exponentially growing or fed
plateau-phase cells with medium containing 5% FCS and 5 to 15 µM
tamoxifen or ethanol vehicle, repeating the medium change after 24 hr,
and harvesting the flasks after a further 24 hr for cell counts and cell
cycle kinetic analysis by DNA flow cytometry. The effect of growth phase
was also assessed by carrying out tamoxifen dose-response experi-
ments in medium containing 5% FCS with cells replated from Day 3, Day
21 fed and Day 21 unfed cultures (see Chart 1A).

Tamoxifen dose-response assays were also carried out in the presence
of various concentrations of insulin (10 µg/ml, 1 µg/ml, 10 ng/ml,
or no added insulin) in medium containing 10% FCS, in different concen-
trations of FCS (0.25, 0.5, 1, 2.5, and 10%), and CS-FCS (1, 2.5, and
10%). The cells utilized for these experiments had been passaged
routinely (in medium containing 10% FCS and 10 µg insulin/ml), but, in
the case of experiments with CS-FCS, cells which had been passaged
weekly for 4 weeks in 5% CS-FCS medium were also used, in view of
evidence that estrogens may be retained for prolonged periods in human
breast cancer cells (27). In the case of experiments with different concen-
trations of insulin, cells which had been passaged weekly for 4 weeks in
medium with the corresponding insulin concentrations (1 µg/ml, 10
ng/ml, or no added insulin) were also used.

The effects of 17β-estradiol on the sensitivity of T-47D cells to
tamoxifen were assessed as follows. Cells (5 \times 10^5) were inoculated into
25-sq cm flasks containing 5 ml medium with 10% FCS. When cell
numbers reached 1 \times 10^6, the medium was replaced with 8 ml medium
containing 2.5 to 20 µM tamoxifen with or without 17β-estradiol at a 17β-
estriadiol:tamoxifen concentration ratio of 1:1000, 1:100, 1:10, or 1:1 for
each tamoxifen dose tested. Flasks were harvested after 5 population
doublings of control cultures, cells were counted, and an aliquot was
stained for DNA flow cytometry.

Statistics. The Student t test was used to test the difference between
the means of experimental groups.

ER Assays. T-47D cells in different phases of the growth cycle and
cells which had been cultured in CS-FCS or FCS containing different
concentrations of insulin were washed once with 1 ml EDTA in Dulbecco's
phosphate-buffered saline (140 mM NaCl:2.7 mM KCl:8.1 mM
NaH2PO4:1.5 mM KH2PO4, pH 7.3) and then harvested with 3 ml of the
same solution. Cells were collected by centrifugation at 800 \times g for 10
min, and the cell pellet was washed once with TE buffer containing 0.25
m sucrose. The cells were homogenized in TE buffer containing 1 µl NaCl
(3 \times 10^7 cells/ml) using a Teflon-glass homogenizer, and a cell extract
was prepared by centrifugation at 135,000 \times g for 1 hr. This extract
contained the total extractable ER, i.e., cytoplasmic ER plus salt-extract-
able nuclear ER.

Endogenous unbound estrogens were removed from the extract by a
30-min incubation with 0.1 volume 5% charcoal:0.5% dextran followed
by a 10-min centrifugation at 1500 \times g.

Saturation analysis of 17β-estradiol binding to ER using increasing
concentrations of 17β[3H]-estradiol (specific activity, 91 to 100 Ci/mmol;
0.05 to 2 nmol) was performed by incubating 100 µl charcoal-treated cell
extract with 50 µl isotope and 50 µl buffer or 50 µl unlabelled 17β-
estriadiol (4 µM) for 16 to 20 hr at 4°. Bound and unbound ligands were
separated by a 30-min incubation at 4° with 500 µl 0.5% charcoal:0.05%
dextran in TE buffer:0.5 µl NaCl, followed by centrifugation at 1500 \times g
for 10 min at 4°. A 500-µl portion of the supernatant was removed,
added to 10 ml scintillant (ACS; Amersham International), and counted
in a liquid scintillation counter. Bound and unbound 17β-estradiol con-
centrations were calculated, and the data were analyzed by the method

![Chart 1. Changes in T-47D cell number and cell cycle kinetic parameters with
time in culture. Cells (2 \times 10^4) in 50 ml medium containing 10% FCS were inoculated
into 150-sq cm flasks on Day 0. Fed cultures had medium changes from Day
7. In A, cell counts were made on the days indicated for both fed (W) and
unfed (C) cultures. Points with error bars, single flasks; those with error bars, mean ±
S.E. (n = 3 to 5) of flasks from 3 separate experiments. The cell cycle parameters
(percentage of cells in G0-G1, S, and G2-M phases of cell cycle) shown in the inset
were calculated from DNA histograms obtained by flow cytometry as described in
"Materials and Methods." Bar histograms represent mean values for exponential
phase (Days 1 to 7, n = 13), fed plateau phase (Days 14 to 24, n = 13), and unfed
plateau phase (Days 10 to 24, n = 16). In all cases, S.E. was < 1.2%. The
differences between percentage of S phase of exponentially growing and both fed
and unfed plateau-phase cultures, and between percentage of G2-M phase of
exponentially growing and unfed plateau-phase cultures were significant (all p <
0.001). In B, cells remaining in G0-G1 after 48-hr treatment with the inhibitor of
cytokinesis, ICRF 159, are shown. Cells at various times on the growth curve
illustrated in Chart 1A were treated with ICRF 159 for 48 hr as described in
"Materials and Methods," then harvested, and subjected to DNA flow cytometry
to estimate the number of cells remaining in G0-G1. Points, mean; bars, S.E.; n = 3 to
5.
RESULTS

Cell Proliferation Kinetics. Since one of the major objectives of this study was to investigate the influence of growth phase on the sensitivity of T-47D cells to tamoxifen, it was first necessary to define the basic cellular kinetic behavior of this cell line during unperturbed growth in monolayer culture. T-47D cells grown in medium supplemented with 10% FCS had a mean population doubling time of 40 hr during the exponential growth phase, and when the medium was replenished daily, a plateau-phase density of 1.87 ± 0.07 x 10^6 cells/150-sq cm flask (mean ± S.E., n = 12) was reached by Day 17 when the initial inoculum was 2 x 10^5 cells on Day 0 (Chart 1A). Phase-contrast microscopy revealed that, when this cell density was reached, the cells grew as a multilayered sheet; from about Day 21, this sheet of cells was readily detached from the plastic substrate by minor mechanical trauma. Unfed cultures had a maximum density of 5.49 ± 0.38 x 10^7 cells/150-sq cm flask (n = 12) at Days 10 to 17 with a gradual decrease thereafter (Chart 1A).

The cell cycle kinetic parameters (Chart 1A, inset) changed from exponential growth phase to fed plateau phase with a fall in the percentage of S-phase cells (from 18.7 ± 0.4% to 13.8 ± 1.2%) and a corresponding increase in the percentage of G0-G1 cells. The progression from exponential growth phase to unfed plateau phase was accompanied by more marked cell cycle kinetic changes with significant reductions in both the percentage of S-phase cells (from 18.7 ± 0.4% to 8.3 ± 0.8%) and the percentage of G2-M cells (from 12.9 ± 1.0% to 8.5 ± 0.7%). There was a correspondingly greater increase in the percentage of G0-G1 cells.

Treatment of cells with ICRF 159, an inhibitor of cytokinesis (9), prevents the return of cycling cells to G0-G1 phase, giving an indication of the proportion of cells which are either not cycling or cycling slowly (30). The proportion of cells leaving G0-G1 phase during the 48 hr following ICRF 159 treatment, i.e., the proportion of "rapidly cycling" cells (30), decreased markedly with increasing time in culture from 8.5 ± 2% for Day 3 cultures to 68.0 ± 1.5% for Day 21 fed cultures (Chart 1B). Assuming 8.5% slowly or non-cycling cells throughout exponential growth phase and a population doubling time of 40 hr, the lengths of the cell cycle phases were approximately 26, 8, and 5.6 hr for G0-G1, S, and G2-M, respectively.

When T-47D cells were harvested at various times on the growth curve presented in Chart 1A and replated at a lower density (1.7 x 10^5 cells/25-sq cm flask) in 8 ml fresh medium supplemented with 10% FCS, their regrowth kinetics was very similar to that reported previously for MCF-7 cells (see Chart 2 of Ref. 30). Cells replated from Day 3 cultures grew exponentially without any lag, while cells replated from Day 10 cultures had an initial lag or fall in cell number depending on whether they were from fed or unfed cultures, respectively. Following this lag period, Day 10 cells entered exponential growth phase by 2 days after replating. When cells were regrown from plateau-phase cultures (Days 14 to 24), there was always an initial decline in cell numbers which was greater for unfed cultures (45 ± 5%, n = 14, percentage decline at Day 1 from inoculum) than for fed cultures (19 ± 5%, n = 16). These cells also reentered exponential growth phase by 2 days after replating (data not shown).

The accompanying changes in cell cycle kinetic parameters upon replating at low density are illustrated in Chart 2. For cells replated from midexponential growth phase (Day 3), the percentage of S-phase cells remained constant until Day 5, when there was a slight decline as confluence was approached. For fed plateau-phase cells (e.g., Day 17), replating led to an initial large increase in the percentage of S-phase cells at Day 1, consistent with a semisynchronous exit of cells from G0-G1. This was followed by a gradual decrease in the percentage of S-phase cells to the level seen in exponential phase cultures by Day 7. A similar pattern was seen upon regrowth of 6 such fed plateau-phase cultures in 2 separate experiments. Unfed plateau-phase cells when replated into fresh medium showed a much more gradual rise in the percentage of S-phase cells, only reaching a maximum at Day 4 (Chart 2). This maximum, however, was significantly higher than that seen in exponentially growing populations (25.9 ± 0.5%, n = 12 for cells from Day 17 unfed cultures; cf. 18.9 ± 0.4%, n = 21 for cells from Day 3 cultures, p < 0.001). Again, the example shown in Chart 2A is representative of regrowth of unfed plateau-phase cultures in 2 separate experiments. To assess whether the process of replating cells was selecting out cell populations with permanently different cell cycle parameters, cells from Day 7 of each of the regrowth curves shown in Chart 2 were replated in triplicate at 1.7 x 10^5/25-sq cm flask, and cell numbers and cell cycle parameters were measured after 3 days. No significant differences were found in either of these measurements, making it unlikely that any such selection had occurred (data not shown).

Effects of FCS Concentration and 17β-Estradiol on Proliferation Rates. Maximal growth of T-47D cells was sustained by FCS over the concentration range of 2.5 to 20% (Chart 3). 17β-Estradiol (10^-9 M) had no significant mitogenic effect in the presence of 0.25 to 20% FCS, and in a similar experiment with medium containing 10% FCS, 17β-estradiol concentrations in the range of 10^-11 to 10^-8 M produced no increase in cell numbers compared with control (data not shown). The population doubling time increased at FCS concentrations below 2.5%, with a marked rise at 0.25%. This prolonged doubling time seen with 0.25%
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FCS was accompanied by a significant rise in the percentage of G2-M cells (18.3 ± 0.9%, n = 13 for 0.25% FCS, cf. 12.4 ± 0.3%, n = 21 for >0.25% FCS, p < 0.001), suggesting that serum deprivation below a critical concentration caused a "block" of T-47D cells in the G2-M phase of the cell cycle.

When cells were grown in the presence of various concentrations of CS-FCS, the population doubling times were prolonged when compared with the same concentration of FCS. Addition of 10^(-9) M 17β-estradiol to CS-FCS markedly decreased the doubling times (Chart 3), but at all CS-FCS concentrations studied, they were still significantly greater than those seen in the presence of the same concentrations of FCS (for 2.5% serum, p < 0.025; for 5 and 10% serum, p < 0.001). Two similar experiments with medium containing 10% CS-FCS showed no further decrease in the doubling time when 17β-estradiol was added over the concentration range of 10^(-10) to 10^(-6) M. These data indicate that charcoal treatment of FCS removes factors other than 17β-estradiol which are mitogenic for T-47D cells.

Effect of Tamoxifen on Exponentially Growing T-47D Cells; Cell Cycle Changes and 17β-Estradiol Reversibility. The effects of tamoxifen on the growth and cell cycle kinetic parameters of exponentially growing T-47D cells were tested in medium supplemented with 10% FCS, and the data are presented in Chart 4. Tamoxifen induced a dose-dependent decrease in cell growth rate with a dose of 10 µM almost completely arresting the increase in cell numbers (Chart 4A). At 20 µM, the drug was cytotoxic, since cell numbers after 6 days of treatment were greater than 10-fold lower than the inoculation density (Chart 4A, inset).

The effect of 2.5 µM tamoxifen on T-47D cell numbers was completely reversed by the simultaneous addition of 17β-estradiol at 17β-estradiol: tamoxifen concentration ratios of 1:100, 1:10, and 1:1 and was partially reversed at 17β-estradiol: tamoxifen ratio of 1:1000 (Chart 4A). The effect of higher doses of tamoxifen (5 to 10 µM) on cell number was only partially reversed by 17β-estradiol at all 17β-estradiol concentrations tested, whereas 20 µM tamoxifen-induced cytotoxicity could not be reversed by 17β-estradiol and was augmented by 2 or 20 µM 17β-estradiol (Chart 4A, inset). Treatment with 10^(-10) to 10^(-6) M...
17β-estradiol alone caused no significant change in cell numbers, but $10^{-3} \text{ M}$ and $2 \times 10^{-3} \text{ M}$ 17β-estradiol caused 17 ± 1% and 54 ± 3% ($n = 3$) decreases below control, respectively. Tamoxifen caused a dose-dependent change in the cell cycle kinetic parameters of exponentially growing T-47D cells, namely, a decrease in the proportion of cells in S and, to a lesser extent, G2-M phases, accompanied by an increase in the proportion of G0-G1 cells (Chart 4B). For tamoxifen concentrations ≤ 5 μM, these changes were completely reversed by the simultaneous administration of a 10-fold-lower dose of 17β-estradiol. At higher tamoxifen doses (10 and 12.5 μM), 17β-estradiol caused partial reversal of the cell cycle changes. 17β-Estradiol alone (10⁻¹⁰ to 10⁻⁸ M) had no significant effect on the cell cycle kinetic parameters of T-47D cells grown under these conditions (data not shown).

**Effect of Growth Phase on Tamoxifen Sensitivity.** Upon replating into medium containing tamoxifen, cells from exponentially growing cells were significantly more sensitive to the growth-inhibitory effects of tamoxifen than were cells from plateau-phase cultures, and cells from unfed cultures were less sensitive than those from fed cultures except at 7.5 μM tamoxifen (Chart 5A).

When flasks of cells containing exponentially growing or fed plateau-phase cells were treated for 48 hr with 5 to 15 μM tamoxifen in medium containing 5% FCS, there were marked differences in response as assessed by changes in both cell numbers and cell cycle kinetic parameters (Chart 5B and C). For the exponentially growing cultures, 5 μM tamoxifen inhibited the increase in cell number by 71%, while 10 μM tamoxifen was cytostatic. The 15 μM dose of tamoxifen caused marked cytotoxicity with a 98% reduction in cell numbers after 48-hr treatment. In contrast, 5 to 15 μM tamoxifen had no effect on the cell numbers of fed plateau-phase cultures (Chart 5B). Similarly, whereas tamoxifen treatment caused marked changes in the cell cycle kinetic parameters of exponentially growing cultures (e.g., 82 ± 2.5% decrease in the percentage of S-phase cells for 10 μM tamoxifen), there were only minimal changes in these parameters with fed plateau-phase cultures (Chart 5C).

**Effect of FCS and CS-FCS Concentration on Tamoxifen Sensitivity.** The sensitivity of T-47D cells to the growth-inhibitory actions of tamoxifen varied inversely with the FCS concentration (Chart 6A). The tamoxifen concentration at which a 50% reduction in proliferation rate occurred varied over a 75-fold range (0.1 μM tamoxifen in 0.25% FCS, 7.5 μM in 10% FCS). For 0.1 μM tamoxifen, the only concentration at which there were surviving cells at all FCS concentrations tested, the relationship between drug effect (percentage of decrease in proliferation rate) and the reciprocal of the FCS concentration was linear (coefficient of determination, $r^2 = 0.95$), as would be predicted (12) if the sensitivity of the cells to tamoxifen was proportional to the unbound tamoxifen concentration (Chart 6A, inset).

Tamoxifen sensitivity was also inversely proportional to the concentration of CS-FCS (Chart 6B). However, at serum concentrations of 10 and 2.5%, cells grown in CS-FCS were significantly less sensitive to the growth-inhibitory effects of 0.1 to 2.5 μM tamoxifen than those grown in FCS, although the sensitivities tended to converge at the higher tamoxifen concentrations (5 to 10 μM). Similar results were seen in CS-FCS-containing medium.
when the cells had been passaged previously in medium containing FCS (data not shown). At 1% serum concentration, the effect of tamoxifen was similar when the medium was supplemented with either CS-FCS or FCS. The decreased tamoxifen sensitivity in 10% CS-FCS (cf. 10% FCS) was confirmed in 6 separate experiments. The small increase in doubling rate seen with 0.1 μM tamoxifen in medium containing 10% CS-FCS was significant (p < 0.025) and has been confirmed in subsequent experiments.³

Although the data are fewer for CS-FCS, there also appeared to be a linear relationship (coefficient of determination, r² = 0.96) between the percentage of decrease in proliferation rate and the reciprocal of CS-FCS concentration (Chart 6B, inset).

Effect of Insulin Concentration on Tamoxifen Sensitivity. Although it has been reported previously that the concentration of insulin in the culture medium has a major effect on the sensitivity of MCF-7 cells to tamoxifen (3), culturing T-47D cells in medium supplemented with 10 ng, 1 μg, or 10 μg insulin/ml or with no added insulin had no effect on their sensitivity to tamoxifen. This occurred regardless of whether the cells had been maintained previously in the routine concentration of 10 μg insulin/ml (data not shown) or had been cultured for 4 prior weekly passages in medium supplemented with the insulin concentration used in the tamoxifen sensitivity studies. It was also noted that changing the insulin concentration of the medium over this range had no effect on the doubling time of control cultures.

Table 1

<table>
<thead>
<tr>
<th>Serum content of medium</th>
<th>Growth phase</th>
<th>Sites/cell</th>
<th>fmol/mg protein</th>
<th>fmol/mg DNA</th>
<th>Kᵦ (nM)</th>
<th>No. of determinations</th>
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<tr>
<td>10% FCS</td>
<td>Exponential</td>
<td>4,407 ± 655</td>
<td>48 ± 6</td>
<td>404 ± 47</td>
<td>0.10 ± 0.01</td>
<td>11</td>
</tr>
<tr>
<td>10% CS-FCS</td>
<td>Exponential</td>
<td>17,071 ± 1,565</td>
<td>151 ± 27</td>
<td>1,259 ± 134</td>
<td>0.06 ± 0.01</td>
<td>3</td>
</tr>
</tbody>
</table>


duced by charcoal treatment of FCS (Chart 3). Addition of 17β-estradiol to CS-FCS was not able to fully restore the doubling time to that seen with untreated FCS (Chart 3), which suggests that CS-FCS is also depleted of mitogens other than estrogens. A direct mitogenic effect of 17β-estradiol has been shown previously for one of 2 cloned sublines of T-47D (4) but not for a variant T-47D line (10). T-47D cells grown in athymic nude mice were shown to require both estrogen and pituitary factors for growth (14). The present study demonstrates 17β-estradiol mitogenicity for the uncioned “wild-type” T-47D cell line. There has been some uncertainty as to whether estrogens have such direct effects on cells in culture, and it has been proposed that estrogens may act in vivo via intermediate effector molecules termed estromedins (21, 23–25). The data presented here add to the growing body of evidence that at least some of the effects of estrogens on cell proliferation are mediated directly (2, 7, 13, 16, 33, 34).

T-47D cells exhibited a sensitivity to the growth-inhibitory effects of the antiestrogen, tamoxifen, which has been shown in studies from this laboratory to be comparable to that seen in other ER-containing human breast cancer cell lines.4 Tamoxifen had at least 2 effects on T-47D cell growth: 17β-estradiol-reversible growth inhibition associated with characteristic cell cycle parameter changes at lower doses, i.e., increased G0-G1 cells, and decreased S- and G2-M-phase cells; and 17β-estradiol-irreversible cytotoxicity at higher doses (Chart 4). The tamoxifen concentration at which cytotoxicity occurred depended on the FCS concentration (Chart 6). Cytostatic and cytotoxic components of the action of tamoxifen and its metabolites have also been observed in other human breast cancer cell lines4 (20, 29, 30).

T-47D cells were more sensitive to the cytostatic effects of tamoxifen when grown in medium containing FCS as compared to medium containing CS-FCS (Chart 6). This is in agreement with previous studies in this laboratory with MCF-7 cells (cf. Figs. 1 and 2 of Ref. 29 with Charts 6 and 7 of Ref. 30). These results are consistent with this action of tamoxifen being due to estrogen antagonism: maximum effect would be predicted where the 17β-estradiol concentration is higher (i.e., in FCS).

Cellular ER content has been shown previously to vary according to the conditions of growth in vitro. In a clonal rat pituitary cell line, C11R4AP, ER content correlated with serum concentration (26), and in MCF-7 human breast cancer cells, ER content was found to be increased by prolactin (22) and decreased by insulin (3), although the effect of insulin was not confirmed in another study (22). The finding that ER content is also markedly dependent on growth phase (Table 1) could be explained by a requirement for a serum factor (26) which the medium may be rapidly depleted of by the large numbers of cells present in plateau phase. Alternatively, ER content may be a direct correlate of growth phase. Clearly, the medium composition and proliferation phase should be specified when a cell line is assayed for ER.

FCS content of the medium had a marked effect on T-47D growth rate (Chart 3), confirming a previous report (15). The serum concentration also had a major effect on the sensitivity of these cells to tamoxifen (Chart 6). This appeared to be inde-

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REFERENCES


8. Engel, L. W., Young, N.A., Traika, T. S., Lippman, M. E., O’Brien, S. J., and
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