The E-Screen Assay: A Comparison of Different MCF7 Cell Stocks

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MCF7 human breast cancer cells have been studied extensively as a model for hormonal effects on breast cancer cell growth and specific protein synthesis. Because the proliferative effect of natural estrogen is considered the hallmark of estrogen action, it was proposed that this property be used to determine whether a substance is an estrogen. The E-screen assay, developed for this purpose, is based on the ability of MCF7 cells to proliferate in the presence of estrogens. The aim of our study was to characterize the response of four MCF7 cell stocks (BUS, ATCC, BB, and BB104) and determine which of them performed best in the E-screen test. The four stocks assayed were distinguishable by their biological behavior. In the absence of estrogen, MCF7 BUS cells stopped proliferating and accumulated in the G_0/G_1 phase of the cell cycle; estrogen receptors increased, progesterone receptors decreased, and small amounts of pS2 protein were secreted. Of all the MCF7 stocks tested, MCF7 BUS cells showed the highest proliferative response to estradiol-17ß: cell yields increased up to sixfold over those of nontreated cells in a 144-hr period. The differences between estrogen-supplemented and nonsupplemented MCF7 BUS cells were due mostly to G_0/G_1 proliferative arrest mediated by charcoal dextran-stripped serum. MCF7 BUS cell stocks and others showing a similar proliferative pattern should be chosen for use in the E-screen test, or whenever a proliferative effect of estrogen is to be demonstrated. *Key words*: bisphenol-A, cell type-specific proteins, estrogen sensitivity, hormone receptors, MCF7 cell variants, *p*-nonylphenol. *Environ Health Perspect* 103:844–850 (1995)

The use of pesticides in agriculture and the release of chemical compounds from manufacturing industries are common in southern Europe. Evidence of the estrogenic effects of some pesticides (*1*), alkylphenols (*2*), and plastic monomers (*3*) has raised concerns about environmental contamination by these chemicals. We became interested in the use of an estrogenicity test to assess the environmental and human health effects of estrogenic xenobiotics and to discriminate between estrogenic and nonestrogenic chemicals. Tests based on increased mitotic activity in tissues of the genital tract of female rodents after administration of chemicals have been proposed (*4*); but, although reliable, these methods are not suitable for large-scale screening of suspected estrogenic chemicals or for measuring the total estrogenic burden in human samples. We therefore adopted the biologically equivalent, easily performed E-screen assay described by Soto et al. (*5*). This bioassay compares the cell yield between cultures of breast tumor-derived MCF7 cells treated with estradiol and cultures treated with different concentrations of xenobiotics suspected of being estrogenic.

MCF7 cells were recommended as target cells because of their widely acknowledged estrogen-sensitivity (*6*).This cell line was initially established by Soule et al. (*7*) from a metastatic pleural effusion from a postmenopausal patient with metastatic, infiltrating ductal carcinoma of the breast: the patient was previously treated with radiotherapy and hormones. Although long-term established MCF7 cells are used worldwide, several MCF7 cell stocks with different sensitivities to estrogens have been developed during the last 20 years (*8*).

The purpose of this study was to characterize the response of four MCF7 cell stocks routinely held by different laboratories to assess which of them performs best in the E-screen test. We investigated the proliferative pattern and rate of estrogen-induced synthesis of cell type-specific proteins and the effects of *p*-nonyl-phenol and bisphenol-A on the four cell stocks.

Methods

Cell lines and cell culture conditions. Four stocks of MCF7 cells were used: MCF7 BUS cells were a gift from C. Sonnenschein (Tufts University, Boston), who cloned the cells (C_7MCF7) from passage 173 of the original MCF7 cells, received from C. McGrath of the Michigan Cancer Foundation; they were at post-cloning passages 70–103 at the time of our study. MCF7 ATCC cells at passage 147 were from the American Type Culture Collection (freeze no. 8655). MCF7 BB cells used at passages 580 to 595 were obtained in 1984 from G. Leclercq (Institut Jules Bordet, Brussels, Belgium), who received them from M. Rich of the Michigan Cancer Foundation. MCF7 BB104 cells were derived in our laboratory from MCF7 BB cells by keeping them in an estrogen-free medium for more than 24 months and were used at passages 12 to 21. For routine maintenance, cells were grown in Dulbecco's modification of Eagle's medium (DME) supplemented with 5% fetal bovine serum (FBS; PAA Labor und Forschungs Ges, MBH, Linz, Austria) in an atmosphere of 5% $C0_2$/95% air under saturating humidity at 37°C, except for MCF7 cells BB104, which were routinely maintained in 10% charcoal dextran-treated human serum (CDHS)-supplemented phenol red-free DME medium, prepared as described below.

Plasma-derived human serum was prepared from expired plasma by adding calcium chloride to a final concentration of 30 mM to facilitate clot formation. Sex steroids were removed from serum by charcoal-dextran stripping (*6*).

Cell proliferation experiments. We used MCF7 cells in the E-screen test according to a technique slightly modified from that originally described by Soto et al. (*5*). Briefly, cells were trypsinized and plated in 24-well plates (Limbro, McLean, Virginia) at an initial concentration of 10,000 cells per well in 5% FBS in DME. BB104 cells were seeded in 10% CDHS supplemented medium. The cells were allowed to attach for 24 hr, then 10% CDHS-supplemented phenol red-free DME was substituted for the seeding medium. A range of concentrations of the test compound were added, and the assay was stopped after 144 hr by removing the medium from wells, fixing the cells, and staining them with sulforhodamine-B (SRB).

The fixation and staining technique was modified from that described by Skehan et al. (*9*). Briefly, cells were treated with cold 10% trichloracetic acid and incubated at 4°C for 30 min. Then the cells were washed five times with tap water and left to dry. The fixed cells were stained for 10 min with

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0.4% (w/v) SRB dissolved in 1% acetic acid. Wells were rinsed with 1% acetic acid and air dried. Bound dye was solubilized with 10 mM Tris base (pH 10.5) in a shaker. Finally, aliquots were transferred to a 96-well plate to be read in a Titertek Multiscan apparatus (Flow, Irvine, California) at 492 nm. We evaluated linearity of the SRB assay with cell number for each MCF7 cell stock before each cell-growth experiment. Alternatively, cells were lysed and nuclei counted on a ZM Coulter Counter apparatus (Coulter Electronics, Luton, England) according to a previously described technique (*6*).

We used the E-screen test to determine, for all four MCF7 cell stocks, the relative proliferative potency (RPP), defined as the ratio between the minimum concentration of estradiol-17ß needed for maximal cell yield and the minimum dose of the test compound needed to obtain a similar effect, and the relative proliferative effect (RPE); that is, the ratio between the highest cell yield obtained with the chemical and with estradiol-17ß × 100 (*5*).

Results are expressed as the means plus or minus standard deviations. In proliferation yield experiments, each point is the mean of three counts from four culture wells. Mean cell numbers were normalized to the steroid-free control, equal to 1, to correct for differences in the initial plating density. Differences between the diverse groups were calculated with Student's *t*-test.

Estrogen and progesterone receptor measurements. We seeded MCF7 cells in T-25 flasks in 5% FBS-supplemented DME. The next day, the medium was changed to 10% CDHS-supplemented DME medium, and estradiol-17ß or the chemicals to be tested were added. One group of cells received vehicle alone. After 72 hr, the culture medium was discarded and cells were frozen in liquid nitrogen. To extract receptor molecules, cells were incubated at 4°C for 30 min with 1 mL of extraction buffer (0.5M KCl, 10 mM potassium phosphate, 1.5 mM EDTA, and 1 mM monothioglycerol, pH 7.4) according to a previously described technique (*10*). The cell debris were pelleted, and estrogen receptors and progesterone receptors were measured in a 100-µL extract aliquot by enzyme immunoassay using the Abbott estrogen receptor and progesterone receptor-enzyme immunoassay monoclonal kits (Abbott Diagnostic, Wiesbaden, Germany) according to the manufacturer's instructions.

Cell type-specific proteins and compounds tested. Cathepsin-D and pS2 proteins were measured in culture media with the ELSA-CATH-D and ELSA-pS2 immunoradiometric assays (CIS BioInter-

Table 1. Cell cycle distribution and estimated doubling time (T_D) of the four MCF7 cell stocks[a]

Treatment/stock	Distribution (% total cells) G_0/G_1	S	G_2/M	$T_{D\,(hr)}$
10% FBS–DME				
MCF7 BUS	69.0 ± 2.6	19.2 ± 2.1	11.9 ± 1.4	32 ± 1.7
MCF7 ATCC	59.4 ± 3.9	32.4 ± 3.6	7.4 ± 2.5	49 ± 2.7
MCF7 BB	66.2 ± 3.4	22.3 ± 1.8	11.5 ± 1.6	27 ± 1.7
CDHS–PhR-DME				
MCF7 BUS	93.5 ± 4.2	4.0 ± 1.2	2.6 ± 0.5	46 ± 3.0
MCF7 ATCC	68.1 ± 4.7	19.7 ± 3.0	12.9 ± 2.0	59 ± 3.1
MCF7 BB104	60.8 ± 4.1	36.6 ± 2.7	4.2 ± 1.9	37 ± 2.2

Abbreviations: FBS, fetal bovine serum; DME, Dulbecco's modification of Eagle's medium; CDHS, charcoal dextran-treated human serum; PhR, phenol red-free.
[a]All data are means ± SD of at least three separate determinations.

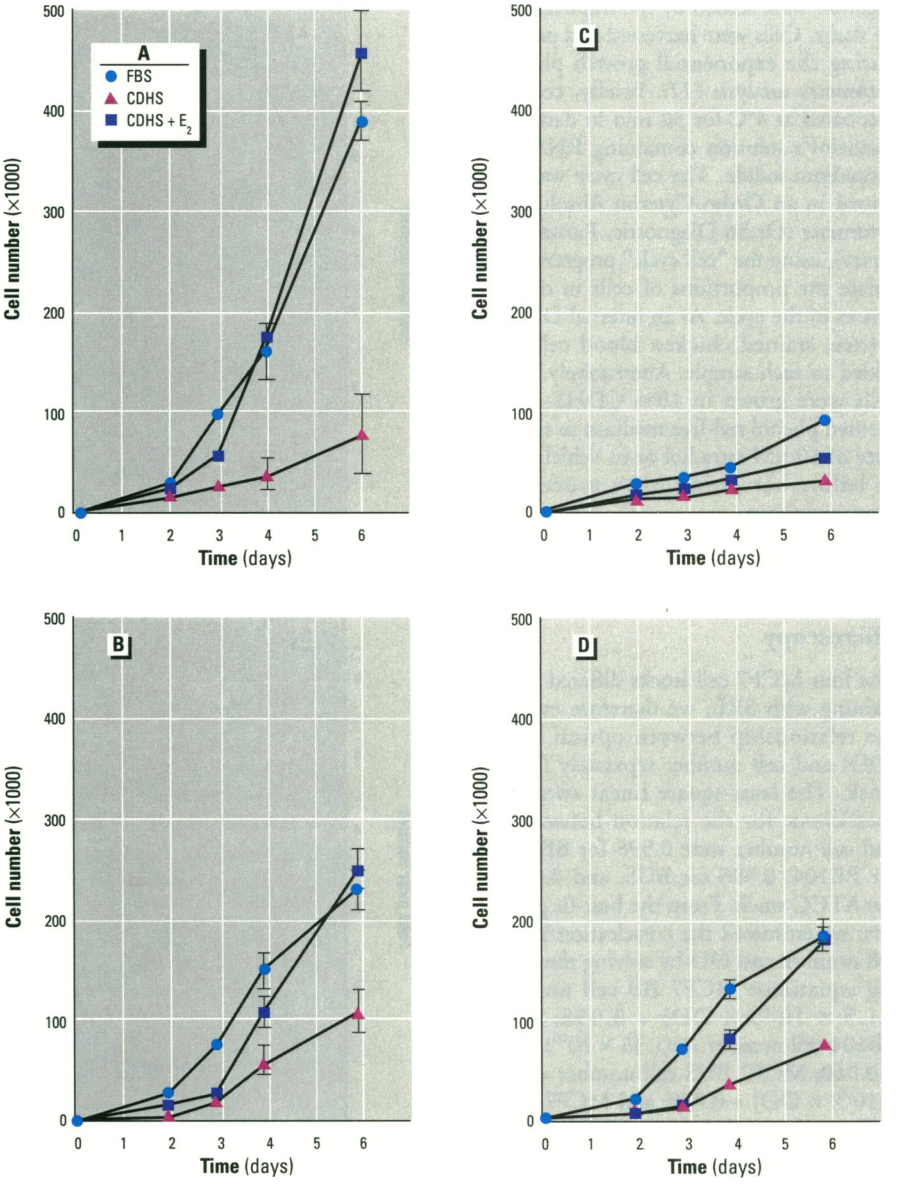

Figure 1. Growth curves of MCF7 cells cultured in the presence of 10% fetal bovine serum (FBS), 10% charcoal dextran-treated human serum (CDHS), or 10% CDHS plus 1 nM estradiol-17ß (CDHS + E_2). Each point is the mean of three counts from four culture wells; bars indicate SDs. (A) BUS cells, (B) BB cells, (C) ATCC cells, (D) BB104 cells.

national, Gif-sur-Yvette, France). The culture medium was centrifuged at 1000*g* for 10 min to eliminate floating and detached cells. Samples were kept frozen at -80°C until the assays were done.

Estradiol-17ß was obtained from Sigma (St. Louis, Missouri). Bisphenol-A (BPA) and *p*-nonyl-phenol (NP) were obtained from Aldrich-Chemie (Albuch, Germany). Chemicals were dissolved in ethanol to a final concentration of 1 mM and stored at -20°C; all were diluted in phenol red-free DME immediately before use. The final ethanol concentration in the culture medium did not exceed 0.1%.

Flow cytometry studies. MCF7 cells grown in 10% FBS-supplemented DME medium were seeded by quintuplicate in T-25 flasks. Cells were harvested and processed during the exponential growth phase for cytometry analysis (*11*). Briefly, cells were incubated at 4°C for 30 min in darkness in Vindelov's solution containing RNAse and propidium iodide. The cell cycle was determined in an Ortho Cyteron Absolute flow cytometer (Ortho Diagnostic, Raritan, New Jersey), using the "cell cycle" program to calculate the proportions of cells in different phases of the cycle. As an internal DNA reference, stained chicken blood cells were added to each sample. Alternatively, MCF7 cells were grown in 10% CDHS-supplemented phenol red-free medium in the presence of 10 nM estradiol or its vehicle for 72 hr before harvesting, then processed as described above.

Results

Growth Characteristics and Light Microscopy

The four MCF7 cell stocks differed in their staining with SRB; we therefore evaluated the relationship between optical density (OD) and cell number separately for each stock. The least-square linear correlation coefficients for the relation between OD and cell number were 0.998 for BB, 0.996 for BB104, 0.996 for BUS, and 0.996 for the ATCC stock. From the best-fit parameters, we estimated the correleation between cell number and OD by solving the following equations: MCF7 BB cell number = $[(1.5 \times 10^{-6}) \times OD] + 0.038$; MCF7 BB104 cell number = $[(1.38 \times 10^{-6}) \times OD] + 0.040$; MCF7 BUS cell number = $[(1.31 \times 10^{-6}) \times OD] + 0.048$; and MCF7 ATCC cell number = $[(2.45 \times 10^{-6}) \times OD] + 0.020$. Sulforhodamine-B staining was clearly more intense in MCF7 ATCC cells than in the other three cell stocks.

We measured the growth rate and cell cycle distribution for MCF7 cells grown in 10% FBS-supplemented DME medium. Table 1 shows the distribution of phases in the cell cycle and the estimated doubling time (T_D) for all clones studied.

MCF7 BUS and ATCC cells were easily distinguishable from BB and BB104 cells by light microscopy. The first two stocks had rounded edges, and were smaller and more refractive than the latter two. Cells from BB and BB104 stocks had extensive intercellular contacts, showed greater cell density at confluence, and attached more strongly to the plastic surfaces.

Proliferative Patterns

MCF7 cells maintained for 6 days in 10% CDHS-supplemented DME behaved differently depending on the stock tested. MCF7 BUS cells underwent two doublings and then stopped proliferating. In contrast, the other three MCF7 clones either slowed their proliferation rate (MCF7 BB104 and BB cells) or were not disturbed at all (MCF7 ATCC cells) in estrogen free-medium (Fig. 1). Flow cytometry studies confirmed the high proportion of arrest in MCF7 BUS cells cultured for 72 hr in estrogen-depleted medium. Switching ATCC cells to an estrogen-free medium did not significantly modify the distribution of cell cycle phases (Table 1).

The addition of estradiol-17ß to CDHS-supplemented medium increased

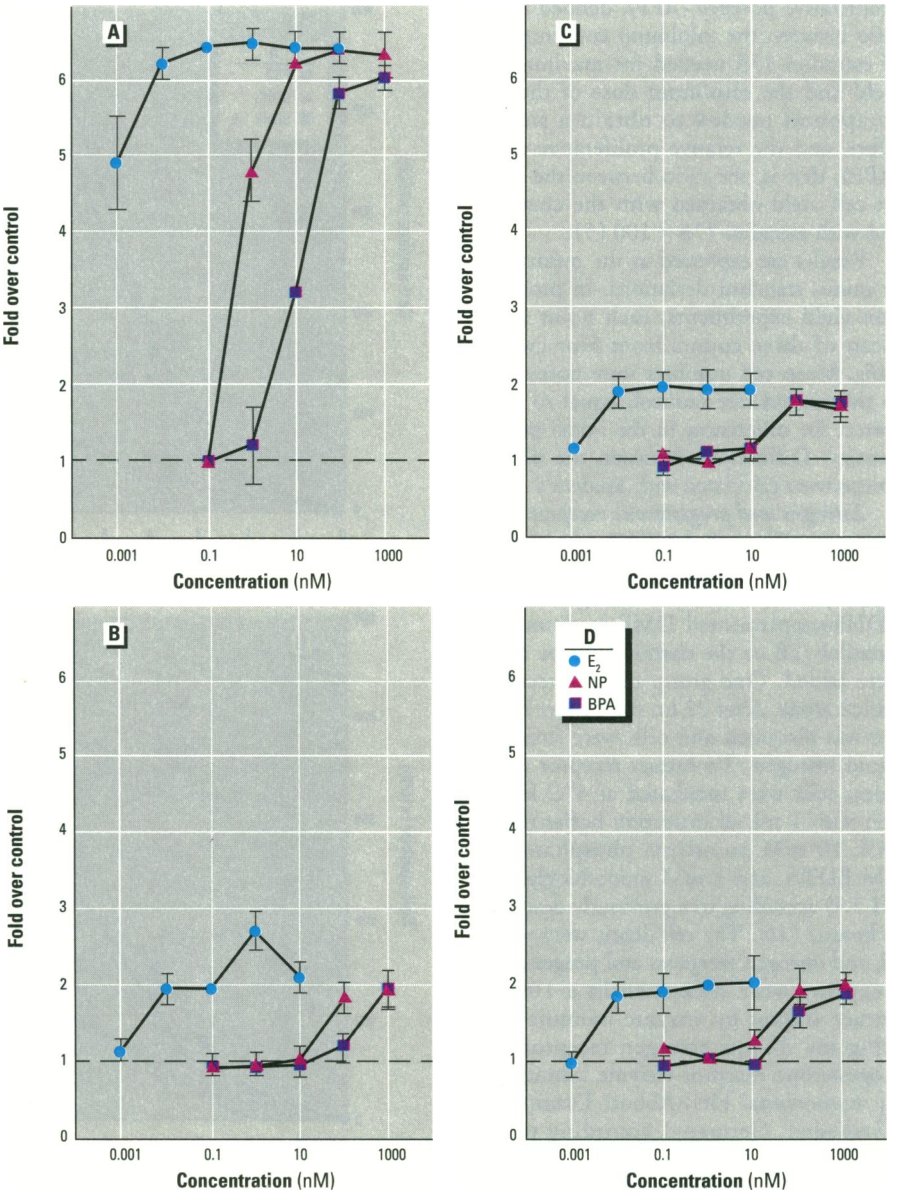

Figure 2. Cell proliferation yields of MCF7 cells. (A) BUS, (B) BB, (C) ATCC, and (D) BB104 cell stocks growing in 10% charcoal dextran-treated human serum supplemented medium were exposed for 144 hr to different amounts of estradiol-17ß (E_2), *p*-nonyl-phenol (NP), and bisphenol-A (BPA). Each point is the mean of three counts from four culture wells; bars indicate SDs.

cell yields in all MCF7 stocks. In MCF7 BUS cells, the proliferative effect was greatest with ≥0.01 nM estradiol-17ß (Fig. 2). The cell yield was sixfold greater than in controls (6.67 ± 1.21; $p < 0.001$). Estradiol-17ß also increased cell yield in BB and BB104 cells by up to twofold compared to controls ($p < 0.05$). In ATCC cells, the effect of estradiol-17ß was almost negligible (<1.5-fold increase, not significant). As expected from the preceding data, estradiol-17ß treatment also modified the proliferation of these cells differently. When 0.1 nM estradiol-17ß was added to 10% CDHS-supplemented DME medium, MCF7 BUS cells showed the shortest doubling time (T_D = 21 ± 3.8 hr) and ATCC cells the longest T_D (54 ± 4.2 hr).

In all cell stocks, NP and BPA increased cell yields to values similar to those obtained with estradiol-17ß. However, NP and BPA were much less potent than estradiol-17ß (i.e., MCF7 BUS cells showed maximal proliferation at concentrations of nonylphenol of 10 nM and higher (Fig. 2). The RPP values are shown in Table 2.

We also studied the response of MCF7 cells to estradiol-17ß in medium supplemented with different amounts of serum. MCF7 BUS and BB104 cells were cultured in DME medium with 5–50% CDHS. Figure 3 shows the effect of 0.1 nM estradiol-17ß on cell yield. In MCF7 BB104 cells; estradiol-17ß consistently increased proliferation (approximately twofold over control values), regardless of the concentration of CD serum added. In MCF7 BUS cells, differences in cell yield between estradiol-17ß-treated and nontreated cells decreased as the concentration of serum increased. The effect of 0.1 nM estradiol-17ß was maximal when 10% serum was added to the medium. At 50% of serum replacement, 0.1 nM estradiol-17ß had no apparent effect on MCF7 BUS cells.

Cathepsin-D and pS2 Secretion

Cathepsin-D and pS2 protein accumulation in the culture medium reflected increases in cell number (Fig. 4). MCF7 BB and BB104 cells secreted the largest amounts of pS2 after 144 hr of subculture in estrogen-free medium (BB, 508 ± 101 ng/10^6 cells; BB104 612 ± 133 ng/10^6 million cells). Estradiol had little effect on the secretion of pS2 in both cell stocks (~1.7 increase over control values in BB104). However, pS2 secretion by MCF7-BUS cells was significantly increased by concentrations of estradiol-17ß 0.1 nM and higher (~3.5-fold increase over controls). Interestingly, MCF7-BUS cells showed the lowest basal levels (53.9 ± 16.7 ng/10^6 cells) of protein secretion and the greatest effect of estradiol-17ß on pS2 secretion (Fig. 4A). Differences in cathepsin-D protein secretion between the four MCF7 cell stocks were smaller; basal levels ranged from 8.9 ± 4.0 pmol/10^6 cells in the BUS stock to 19.5 ± 7.8 pmol/10^6 cells in the BB104 clone. Estradiol-17ß treatment slightly increased cathepsin-D accumulation in the culture medium. The greatest effect was seen in BB cells, in which 1 nM estradiol-17ß raised cathepsin-D protein levels 1.8-fold (Fig. 4B).

Table 2. Estrogenic response of MCF7 cells to estradiol-17ß (E_2), *p*-nonylphenol (NP) and bisphenol-A (BPA)

Cell stock	Compound	Concentration (nM)[a]	PE[b]	RPE (%)[c]	RPP (%)[d]
BUS	E_2	0.01	6.7	100	100
	NP	10	6.8	103	0.001
	BPA	100	6.3	97	0.0001
ATCC	E_2	0.01	1.6	100	100
	NP	100	1.6	100	0.0001
	BPA	100	1.5	95	0.0001
BB	E_2	0.01	2.2	100	100
	NP	100	2.0	95	0.0001
	BPA	1000	1.9	90	0.00001
BB104	E_2	0.01	2.3	100	100
	NP	100	2.1	98	0.0001
	BPA	1000	2.1	98	0.00001

[a]Lowest concentration needed for maximal cell yield.
[b]Proliferative effect: ratio between the highest cell yield obtained with the chemical and the hormone-free control.
[c]Relative proliferative effect: (PE of the test compound/PE of E_2) 100.
[d]Relative proliferative potency: (dose of E_2/dose of test compound needed to produce maximal cell yield)100.

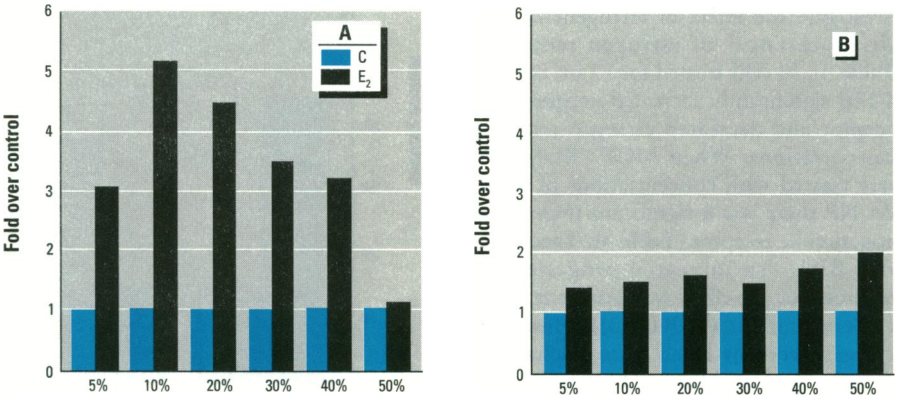

Figure 3. Effect of increasing concentrations of charcoal dextran-treated human serum on the growth of MCF7 (A) BUS and (B) BB104 cells. MCF7 cells were grown for 6 days in estrogen-depleted medium supplemented with different concentrations of charcoal dextran-treated human serum (from 5% to 50% as indicated along the X-axis). Estradiol-17ß (0.1 nM) was added to cultures (E_2); controls (C) received vehicle alone.

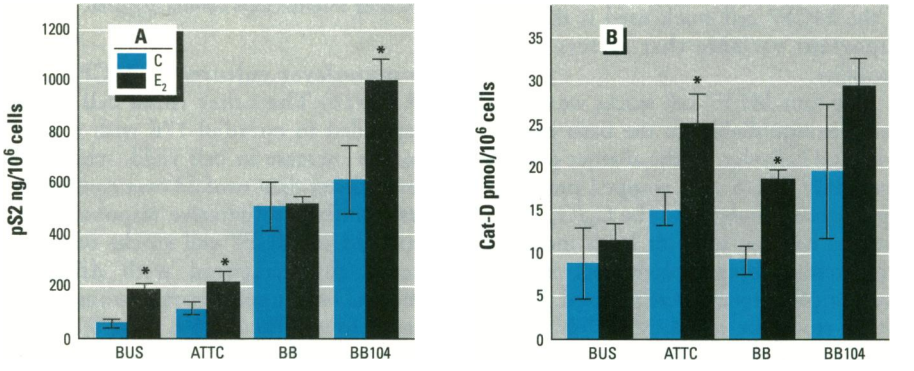

Figure 4. pS2 (A) and cathepsin-D (B) accumulated in the culture medium. MCF7 BUS, ATCC, BB, and BB104 cell stocks were grown in 10% charcoal dextran-treated human serum-supplemented medium (C) and exposed for 144 hr to 1 nM estradiol-17ß (E_2). *Values significantly different from control ($p < 0.05$). Bars indicate SDs.

Hormone Receptors

MCF7 cells bear receptors for estradiol-17ß and progesterone. The highest value for estrogen receptor (400 ± 55 fmol/mg protein) was found in the BB104 stock; these cells are routinely kept in estrogen-free medium. In BUS cells, estrogen receptor expression was 183 ± 29 fmol/mg of extracted protein. Treatment with estradiol-17ß decreased estrogen receptor levels and increased progesterone receptor levels. The lowest basal progesterone receptor value (7.9 ± 1.3 fmol/mg protein), which approached the lower limit of detection of the monoclonal antibody assay, was observed in the MCF7 BUS stock, which also showed the largest estradiol-mediated increase in progesterone receptor (~12-fold increase) (Fig. 5). Basal levels of progesterone receptor were 24 ± 7, 75 ± 12, and 83 ± 7 fmol/mg of protein in BB104, BB, and ATCC cells, respectively. In all the three stocks, estradiol-17ß increased progesterone receptor levels in a dose-dependent manner; however, the effect was smaller than that observed in BUS cells (Fig. 5).

We did another set of experiments to investigate the effect of estrogens on the "disappearance" of estrogen receptors (Table 3). In cells treated for 72 hr, estradiol-17ß significantly increased progesterone receptor and decreased estrogen receptor concentrations. When MCF7 BUS cells were treated with concentrations of ≥100 nM NP there was a significant increase in progesterone receptor (Table 3). Treatment with BPA also increased progesterone receptor, but the effect was weaker at higher concentrations (>1000 nM). However, estrogen receptor levels were unchanged when the medium contained NP or BPA.

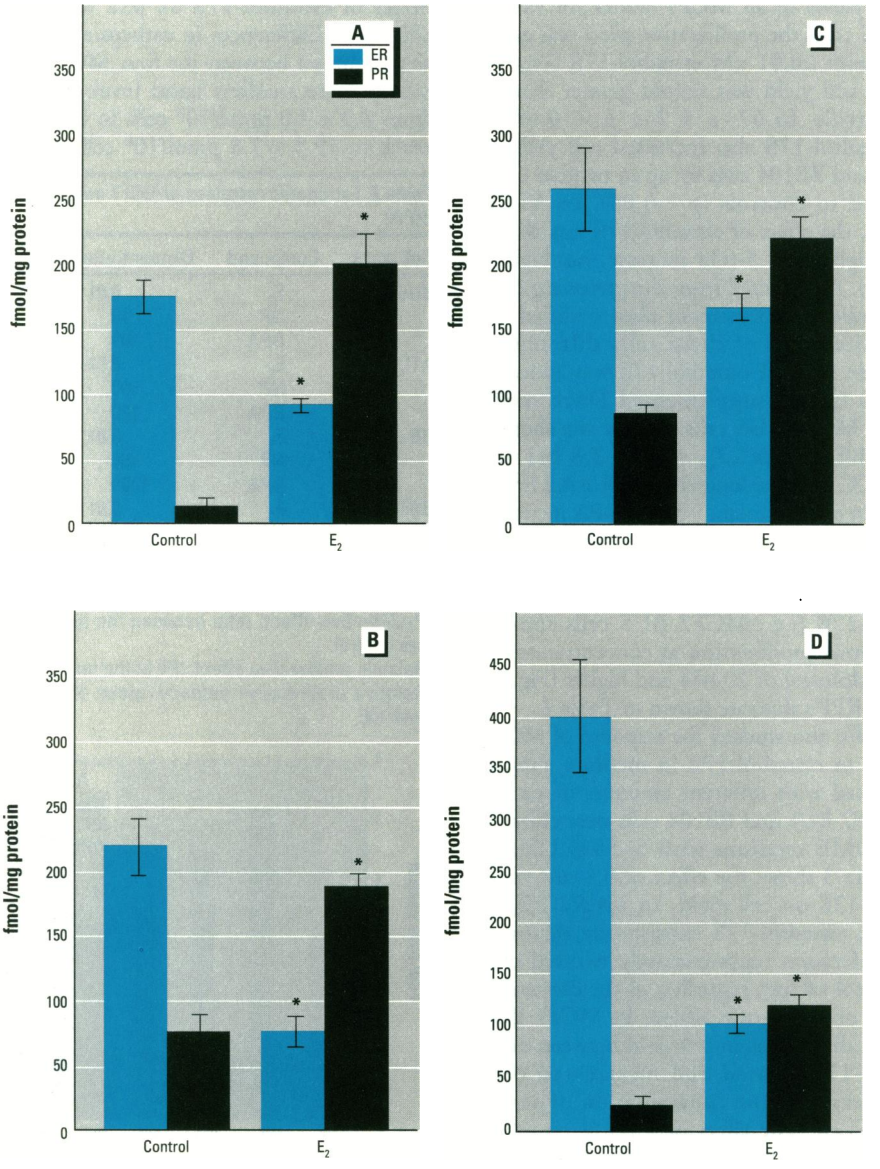

Figure 5. Estrogen and progesterone receptors in MCF7 cells: (A) BUS, (B) BB, (C) ATCC, (D) BB104. Cells in T-25 flasks were incubated in 10% charcoal dextran-treated human serum for 72 hr with 1 nM of estradiol-17ß (E_2). Controls received the vehicle alone. Estrogen receptors (ER) and progesterone receptors (PR) were measured in the extracted cells with the monoclonal antibody technique as deccribed in Methods. Results are expressed as femtomole per milligram of extracted protein ± SD (bars). *Values significantly different from control ($p < 0.05$).

Discussion

A bioassay can be effectively assessed only with the help of a standardized set of parameters that measure reproducibility. In evaluations of the E-screen test, uniformity of the MCF7 cell stock used is the most important variable that affects reproducibility.

The four MCF7 cell stocks we assayed were distinguishable on the basis of their biological behavior. In the absence of estrogen, MCF7 BUS cells stopped proliferating; they accumulated in the G_0/G_1 phase, estrogen receptor receptor levels increased, progesterone receptor decreased, and low levels of pS2 protein were secreted. Of the MCF7 stocks we tested, MCF7 BUS cells showed the highest proliferative response to estradiol-17ß, with cell yields increasing up to sixfold over nontreated cells in a 144-hr period. This increase was of the same order of magnitude as that described previously in monolayer cultures of MCF7 cells (*5,12–16*).The other three cell stocks responded to estradiol-17ß with a much smaller increase in cell yield, which was never higher than twofold over control values. Similar proliferative responses were reported in MCF7 cell stocks tested in media supplemented with different amounts of charcoal dextran serum, which ranged from 20% to 0.5% (*17–30*), and in serumless medium (*31–33*). Poor proliferative responses to estradiol-17ß were described when nonstripped, serum-supplemented media were used (*34–36*).

Differences in culture condition (*29–37*), the type of serum-supplemented medium (*17,18*), serum lots (*17*), the presence of phenol red (*38*), insulin (*27,39,40*), cell passage (*41*), and cell density or culture matrices (*20,33,41*), may explain the poor proliferative effect of estradiol-17ß in some MCF7 cell stocks. The disparate effects of estradiol-17ß, other so-called mitogens, and growth inhibitors on cell proliferation have also been attributed to heterogeneity of the uncloned cells (*39,42,43*) or to differences in the MCF7 cells used (*8,13,16,25,44,45*). The four cell stocks we assayed were cultured in the same medium (phenol red-free DME), which was supple-

Table 3. Effect (means ± SD) of estradiol-17ß (E_2), *p*-nonyl-phenol (NP), and bisphenol-A (BPA) on estrogen and progesterone receptors in MCF7 BUS cell stock

	Concentration (nM)	fmol receptor/mg extracted protein: Estrogen receptors	Progesterone receptors
Control		174 ± 12 (100)[a]	17 ± 12 (100)
E_2	0.1	195 ± 35 (112)	131 ± 12 (770)*
	1	91 ± 11 (52)*	202 ± 21 (1188)*
	10	87 ± 15 (50)*	211 ± 15 (1241)*
	100	87 ± 07 (50)*	154 ± 70 (906)*
NP	1	179 ± 30 (103)	23 ± 10 (135)
	10	207 ± 12 (119)	29 ± 13 (171)
	100	189 ± 15 (109)	161 ± 35 (575)*
	1000	164 ± 13(94)	191 ± 11 (1123)*
BPA	1	164 ± 10 (94)	23 ± 21 (135)
	10	229 ± 18 (132)	23 ± 12 (135)
	100	228 ± 30 (131)	56 ± 33 (329)
	1000	167 ± 18 (96)	167 ± 21 (982)*

[a]Numbers in parentheses are the percentage of variation versus controls.
*Values significantly different from control ($p < 0.05$).

mented with equal concentrations of human serum (10%) from the same source (healthy voluntary donors). Experiments with all four cell stocks were always run in parallel. Obviously, we could not use exactly the same number of passages (*41*). Nevertheless, MCF7 BUS cells, which showed the greatest proliferative response to estradiol-17ß, were at passage 100 (+173 passages before cloning) and MCF7 ATCC cells, which showed the poorest response to estradiol-17ß, were at a similar number of passages (received at passage 148, tested after 150–170 passages in our laboratory). Our results therefore suggest that differences in the response cannot be attributed to culture conditions or to passage number.

In experiments designed to test the influence of serum concentration on the effect of 17ß-estradiol, serum seemed to counteract the effect of estradiol-17ß in MCF7 BUS cells. Increasing serum concentration from 5% to 50% reduced cell yield despite the presence of 0.1 nM of estradiol-17ß. We found it necessary to increase the concentration of estradiol-17ß to maintain the differences between treated and nontreated cells as serum supplementation increased.

Differences in cell yield between estradiol-17ß-treated and nontreated cultures were significantly higher in cell stocks that showed arrest of growth in serum-supplemented, estrogen-free medium. The differences between estrogen-supplemented and nonsupplemented MCF7 BUS cells were mostly due to arrest in G_0/G_1 mediated by CD serum in the absence of estrogen (Table 1).

The minimal effect of estradiol-17ß on MCF7 ATCC cell yields was notable; similar results have been described by others (*30,32,35*). Because ATCC cells are from a different patient than the one from which MCF7 cells originated (*8*), this stock should be used with caution in cell proliferation tests such as the E-screen bioassay.

Estradiol-17ß affected MCF7 BUS cell yield, cell cycle distribution, and specific protein synthesis. In this stock, the hormone reduced estrogen receptor content, increased the amount of measurable progesterone receptor, and increased pS2 protein secretion. We found no significant effect of estradiol-17ß on cathepsin-D protein synthesis (*25*). Although some MCF7 cell stocks respond to estradiol-17ß with a higher increase in cathepsin-D protein secretion than others (*46*), all four MCF7 cell stocks tested here showed the same poor response. In MCF7 BUS cells, the presence of estradiol-17ß in the culture medium had a net stimulatory effect on pS2 protein production, mainly because of the low amounts of this protein secreted in estrogen free-medium.

p-Nonyl-phenol and BPA were found to be estrogenic, increasing cell yield and progesterone receptor concentration in MCF7 cells (*2,3,47*). These compounds mimicked the proliferative effect of estradiol-17ß and increased progesterone receptor levels, albeit to a lower extent than did the hormone. However, the effects of estradiol-17ß and these chemicals on the disappearance of estrogen receptor differed. An increase in progesterone receptor levels was not associated with estrogen receptor downregulation. Interestingly, Schutze et al. (*19*) showed that catecholestrogens, which increase the rate of MCF7 cell proliferation and progesterone receptor levels, evoked estrogen receptor processing only during the first 8 hr after treatment; thereafter, estrogen receptor increased, reaching basal levels at 24 hr. We are now investigating whether differences in the ability to evoke processing are due to an early phenomenon occurring before 72 hr, when estrogen receptor was routinely evaluated, or whether these differences are related to the use of exchange assays or the immunological detection of estrogen receptor (*48*).

Validation of the RPE and RPP of the chemicals tested here seems to depend on the cell stocks used in the E-screen bioassay. Although RPE was only slightly different in MCF7 BUS, BB, and BB104 cells, it may not be easy to detect partial estrogen agonists with cells other than BUS. The differences of less than twofold between estradiol-17ß-treated cells and controls when MCF7 BB, BB104, and ATCC cells were used defined a narrow range of sensitivity. It seems evident that the limited ability of BB and BB104 cells to grow in the presence of NP and BPA resulted in underestimation of RPP. The ATCC cell stock seems to be the least appropriate for both purposes.

In summary, it is now clear that induction of cell proliferation is the hallmark of estrogen action. The effects of estrogens on cell type-specific protein synthesis (whether induction or downregulation) and cell hypertrophy are variable and may be evoked by nonestrogenic agents. Our results suggest that the ability of estrogens to make cells proliferate can be proved *in vitro* using an appropriate bioassay such as the E-screen test. MCF7 BUS cell stocks and others showing a similar proliferative pattern should be chosen for use in the E-screen test, or whenever a proliferative effect of estrogen is to be demonstrated.

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