****

Batch Name: 77511

Batch Creator: SWORD 1

Batch Creation Date: 11/23/2005

Batch Creation Time: 13:57:21

Number of Pages: 8 [All]

Printed by: SWORD 1

Print Date: 11/23/2005

Print Time: 13:59:50

****

77511

Received: from mulnx11.mcs.muohio.edu (mulnx11.mcs.muohio.edu [134.53.6.66])
by sigerson.sword.org (8.13.4/8.13.4/Debian-3) with ESMTP id jAMldBV6016083
for <requests@sword.org>; Tue, 22 Nov 2005 16:39:11 -0500
Received: from mulnx23.mcs.muohio.edu (mulnx23.mcs.muohio.edu [134.53.6.10])
by mulnx11.mcs.muohio.edu (Switch-3.1.6/Switch-3.1.6) with ESMTP id jAMLeXfm020000
for <requests@sword.org>; Tue, 22 Nov 2005 16:37:33 -0500
Received: from smsl.wright.edu (smsl.wright.edu [130.108.66.31])
by mulnx23.mcs.muohio.edu (Switch-3.1.6/Switch-3.1.6) with ESMTP id jAMLeXfY005689
for <requests@sword.org>; Tue, 22 Nov 2005 16:37:33 -0500
Received: from conversion-daemon.smsl.wright.edu by smsl.wright.edu
(iPlanet Messaging Server 5.2 HotFix 2.08 (built Sep 23 2005))
id <0IQD003011DDAN@smsl.wright.edu>
(original mail from fill@www.libraries.wright.edu)
for requests@sword.org; Tue, 22 Nov 2005 16:37:33 -0500 (EST)
Received: from staff150 ([130.108.169.150])
by smsl.wright.edu (iPlanet Messaging Server 5.2 HotFix 2.08 (built Sep 23
2005)) with ESMTP id <0IQD00INALELDT@smsl.wright.edu> for requests@sword.org;
Tue, 22 Nov 2005 16:37:33 -0500 (EST)
Date: Tue, 22 Nov 2005 21:37:33 +0000 (GMT)
From: Fordham Interlibrary Loan <fill@www.libraries.wright.edu>
Subject: Please fill request
To: requests@sword.org
Message-id: <0IQD003HILELAN@smsl.wright.edu>
Content-transfer-encoding: 7BIT
X-Real-ConnectIP: 130.108.66.31
X-Scanned-By: MIMEDefang 2.45
X-Scanned-By: MIMEDefang 2.52 on 134.53.6.10

7 pgs. scanned
11/23/05

This request has been forwarded from ILL by barb.

Please fill this request for FORDHAM HEALTH SCIENCES LIBRARY

77511

81 383 10 06 10 ?
09

Call Number: 8138X00607

Journal Title: Cancer Research
Journal Vol: 37
Journal Issue:
Journal Year: 1977
Article Title: Effects of estrone, estradiol, and estriol ono hormone-responsive human breaast cancer in long term tissue culture
Article Author: Lippman
Article Pages: 1901-1907

Customer Information:

Name: Glaser, Rebecca
Status: Faculty
Address: SOUTHVIEW (via Kettering Hosp),
Site:
E-Mail Address: rglaser@woh.rr.com
Phone: 937-865-4555
Department: School of Medicine

[CANCER RESEARCH 37, 1901-1907, June 1977]

Effects of Estrone, Estradiol, and Estriol on Hormone-responsive Human Breast Cancer in Long-Term Tissue Culture[1]

Marc Lippman, Marie E. Monaco, and Gail Bolan

MecMedical Breast Cancer Service, Medicine Branch, National Cancer Institute, Bethesda, Maryland 20014

SUMMARY

The effects of estrone, estradiol, and estriol on MCF-7 human breast cancer are compared. In this estrogen-responsive cell line, all three estrogens are capable of inducing equivalent stimulation of amino acid and nucleoside incorporation. Estriol is capable of partially overcoming antiestrogen inhibition with Tamoxifen (ICI 46474), even when antiestrogen is present in 1000-fold excess. Antiestrogen effects are completely overcome by 100-fold less estriol. Studies of metabolism of estrogens by MCF-7 cells revealed no conversion of estriol to either estrone or estradiol. All three steroids bind to a high-affinity estrogen receptor found in these cells. The apparent dissociation constant is lower for estradiol than for estrone and estriol, but all three bind to an equal number of sites when saturating concentrations are used. Tritiated estrogens used in binding studies were shown to be radiochemically pure. We conclude that estriol can bind to estrogen receptor and stimulate human breast cancer in tissue culture. Our data do not support an antiestrogenic role for estriol in human breast cancer.

INTRODUCTION

Breast cancer is frequently dependent upon either endogenous estrogens or pharmacologically administered natural or synthetic estrogens. Dimethylbenzanthracene-induced rat mammary carcinomas frequently regress after castration (28) and may be restimulated by administration of physiological concentrations of estrogen (34). In premenopausal women with metastatic breast cancer, objective regressions occur about one-third of the time following oophorectomy (26). One problem with both animal and human studies is that complex interactions of multiple hormones as well as their biotransformations and metabolism may make analysis of specific effects difficult. Recently, we have shown that some human breast cancer cell lines maintained in long-term tissue culture both contain specific estrogen receptors and are responsive to 17β-estradiol[2] or the synthetic non-steroidal estrogen, diethylstilbestrol (18). With the use of serum-free conditions to preclude the effects of other hormones, physiological concentrations of estrogen induced enhanced precursor incorporation into macromolecules, increased specific activities of some enzymes such as thymidine kinase, and accelerated cell division (19, 22). Thus, these cells provide an interesting model system for studying the interactions of estrogenic substances with human cells under carefully controlled, defined conditions.

Considerable controversy has arisen over the role of various estrogenic steroids in breast cancer and other tissues (37). Epidemiological studies have suggested that women with a relatively high excretion of estriol relative to estrone and estradiol have a decreased incidence of breast cancer (23). In immature female rodents, under certain conditions, estriol is incapable of inducing maximal estrogenic effects and has been termed an "impeded" estrogen (14). Lemon *et al.* (16, 17) have shown that estriol, but not estradiol or estrone, can protect rats against the carcinogenic effects of dimethylbenzanthracene. They further suggested that estriol should be considered for clinical trials in women as a potential agent to reduce breast cancer. On the other hand, recent studies by Anderson *et al.* (3) have suggested that estriol may be a completely active estrogen in rodents when administered under more physiological conditions. Furthermore, Rudali *et al.* (32) have shown that estriol has a carcinogenic potential equal to estradiol in mice. For these reasons, it seemed worthwhile to define the relative estrogenic activities of estrone, estradiol, and estriol in human breast cancer. In these studies, the effects of these steroids on the MCF-7 human breast cancer cell line were examined and compared directly with binding to the estrogen receptor in these cells. Purity of labeled and unlabeled estrogens was verified, and interconversion of these steroids during incubations with cells was monitored. Our studies suggest that, at least in tissue culture, estriol is capable of stimulating macromolecular synthesis in human breast cancer.

MATERIALS AND METHODS

Cells. MCF-7 is a human breast cancer cell line (35) that was generously provided by Marvin Rich of the Michigan Cancer Foundation. The human and mammary nature of this cell line has been previously described (18). The cells were grown in IMEM (NIH Media Unit) (31) supplemented with antibiotics and 5% fetal calf serum (North American

[1] This is Paper 5 in a series on the effects of hormones on human breast cancer cell lines in tissue culture.

[2] The trivial names used in this paper are: estradiol, 1,3,5-(10)-estratriene-3,17β-diol; estrone, 3-hydroxy-1,3,5(10)-estratriene-17-one; estriol, 1,3,5-(10)-estratriene-3,16α,17β-triol; 2-hydroxyestrone, 1,3,5(10)-estratriene-2,3-diol-17-one. The abbreviation used is: IMEM, improved minimal essential medium.

Received December 13, 1976; accepted March 14, 1977.

Biological, Miami, Fla.). Cells were repeatedly shown to be free of mycoplasm contamination (Litton Bionetics, Rockville, Md.). Cells were grown as monolayers in plastic roller bottles or flasks.

Steroid Receptor Studies. Cells grown as described above were changed to serum-free IMEM at least 24 hr prior to harvesting. Cytoplasmic extracts were prepared and specific receptor binding was assessed by minor modifications (21) of a dextran-coated charcoal assay described by McGuire and DeLaGarza (27). Nuclear-associated receptor was extracted by homogenizing the nuclear pellet in a tight-fitting Dounce homogenizer in a buffer of 0.6 M KCl, 10 mM Tris-HCl, 1 mM EDTA, and 1.5 mM dithiothreitol (pH 7.4). After standing for 1 hr at 4°, the cells were rehomogenized and the supernatant fraction was collected following centrifugation at 800 × *g* for 10 min. This was diluted with the above buffer without KCl, 1:10 (v/v), and then assayed by the protamine sulfate technique, using exchange conditions (18 hr at 20°) (21). In competition studies, mixtures of 1.25×10^{-9} M [^{3}H]estradiol with or without various concentrations of unlabeled estrogens, as shown in the chart legends, were added to cytoplasmic extracts or protamine precipitates of nuclear-associated receptor. Cytoplasmic competition assays were carried out for 18 hr at 4°. Methods of separating bound from free [^{3}H]estradiol are outlined elsewhere (21). Scatchard analyses (33) of binding data were prepared by computer-assisted methods (2).

Purity of Unlabeled Steroids. Estrone, 17β-estradiol, and estriol (Steraloids, Pauling, N. Y.) were checked for purity by melting point analyses with a Fisher melting point apparatus. All 3 steroids melted within the range described for the authentic compounds.

Precursor Incorporation Experiments. Cells growing in IMEM supplemented with 5% fetal calf serum were harvested with trypsin-EDTA solution and replicately plated into multiwell plastic dishes. After cells had attached to the dish surface, the medium was changed to serum-free IMEM. Twenty-four hr later, the medium was removed and fresh serum-free IMEM was added. Steroids were then added as freshly prepared 1000-fold concentrates in ethanol. The final concentration of ethanol (0.1%) has no detectable effect on macromolecular synthesis. After 36 hr of further incubation in a 37° humidified 5% CO_2 incubator, 0.5 μCi of radiolabeled [^{14}C]*l*-serine (specific activity, 174 mCi/mmole) or 1 μCi of [^{3}H]thymidine (specific activity, 47 Ci/mmole) (Amersham/Searle Corp., Arlington Heights, Ill.) diluted in IMEM, was added to each well. Two hr later, cells were washed free of medium, harvested, and incorporation of precursor into trichloroacetic acid-insoluble material and protein content were assessed as previously described (18).

Chromatographic Profiles of Labeled Steroids before and after Incubation with Cells. Labeled estrone (specific activity, 82 Ci/mmole), estradiol (specific activity, 96 Ci/mmole), and estriol (specific activity, 77 Ci/mmole) were obtained from Amersham/Searle. Their purity was checked by chromatography on Sephadex LH-20 (Pharmacia Fine Chemicals, Piscataway, N. J.) in a solvent system containing chloroform:benzene:methanol (3:6:1) (7). To determine the extent to which each of these estrogens was metabolized by MCF-7 cells, the following incubations were carried out. Cells were plated as described above for incorporation experiments. Labeled estrogen at a concentration of 5×10^{-9} M was incubated with the cells for 24 hr. At the end of the incubation period, carrier [^{14}C]estradiol (specific activity, 57 mCi/mmole) was added to each sample, and the cells and media were extracted 3 times with 3 volumes of chloroform. The solvent was evaporated under a stream of nitrogen, and the residue taken up in the solvent system was used for chromatography. Identification of labeled steroids was effected by chromatography on Sephadex LH-20 in the solvent system described above. The 15- x 0.5-cm column volume was 5 ml, and 0.5-ml fractions were collected.

RESULTS

Competition of Unlabeled Estrogens with [^{3}H]Estradiol for Cytoplasmic and Nuclear Receptor Sites. In Chart 1, the ability of various concentrations of unlabeled estrogens to compete with [^{3}H]estradiol (1.46×10^{-9} M) is shown. Note that one-half of maximal competition by unlabeled 17β-estradiol occurs at approximately 1.5×10^{-9} M, the expected result. One-half of the maximal competition by unlabeled estrone and estriol occurs at concentrations equal to 6×10^{-9} and 1×10^{-8} M, respectively. Although estrone and estriol are somewhat less potent in this competition assay, all 3 compete for an equivalent number of binding sites. Although only competition for specifically bound [^{3}H]-estradiol is shown in Chart 1, more than 80% of the total [^{3}H]estradiol bound can be competed by an excess of unlabeled estrogen when labeled estradiol is present at 1.46×10^{-9} M. This suggests that estriol is not competing for nonspecific binding sites.

It is possible that not all competible binding sites in the cytoplasmic fraction are true receptors. Nuclear translocation of receptor sites is one of the tasks of a functional receptor (3). We therefore examined competition of estradiol and estriol with [^{3}H]estradiol for receptor sites associated with the nuclear fraction of the cell (Chart 2). These results are very similar to those shown in Chart 1 and

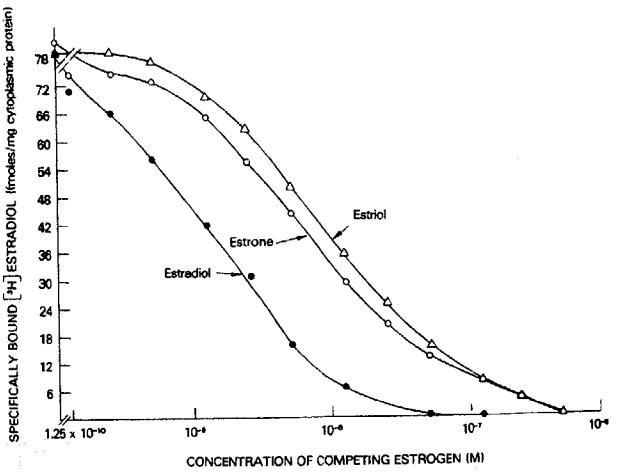

Chart 1. Ability of various concentrations of unlabeled estrogens to compete with 1.46×10^{-9} M [^{3}H]estradiol for specific binding sites in cytoplasmic extracts from MCF-7 human breast cancer cells. Results are means of triplicate determinations; standard deviations are generally less than 7.5% of the values shown. Incubation of cytoplasmic extract and steroid was for 18 hr at 4°. Details are supplied in "Materials and Methods."

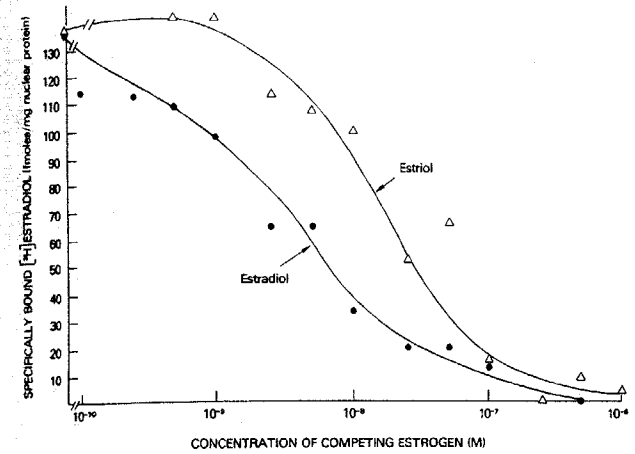

Chart 2. Ability of various concentrations of unlabeled estrogens to compete with 3.0×10^{-9} M [^{3}H]estradiol for specific binding in nuclear-associated receptor sites. Results are means of triplicate determinations; standard deviations are generally less than 10% of the values shown. Incubation of protamine sulfate precipitates of nuclear-associated receptor was for 18 hr at 20°. Details are supplied in "Materials and Methods."

suggest that estriol competes with an equivalent number of salt-extractable, nuclear-associated receptor sites as estradiol. One-half of maximal competition by unlabeled estrogens occurs at a concentration of 3.6×10^{-9} M when 17β-estradiol is the competitor and at 1.8×10^{-8} M when estriol is the competitor. In this experiment, [^{3}H]estradiol is present at a concentration of 3.0×10^{-9} M so that competition by unlabeled estradiol is as predicted.

Binding of Tritiated Estrogens to Cytoplasmic Receptor in MCF-7 Cells. The competitive studies just described suggest binding of all 3 estrogens to an equivalent number of binding sites with a lower apparent dissociation constant for estrone and estriol. Binding affinities were examined directly by studying binding of tritiated steroids to receptors. Chart 3 shows Scatchard plots of binding of tritiated estrone, estradiol, and estriol to a cytoplasmic extract from MCF-7 human breast cancer. The straight line obtained for each steroid is consistent with binding to a single class of receptor sites of uniform affinity for that steroid. The similar X intercept for each steroid suggests that, at infinite steroid concentration, estrone, estradiol, and estriol bind to an approximately equal number of receptor sites. This similarity between total number of sites for all 3 steroids is a point in favor of binding to a common estrogen receptor. The marked difference in affinity for estradiol, as compared with estrone and estriol, is noteworthy. The dissociation constant (K_d) for estradiol is 3.0×10^{-10} M, whereas that for estrone and estriol is 2.3×10^{-9} M. This value for the dissociation constant for estradiol is in good agreement with our previous results (19). The correlation coefficients (r values) were all greater than 0.96. These results are similar to those obtained in the previously described competition studies.

Effects of Estrogens on Precursor Incorporation. We next examined the ability of estrone, estradiol, and estriol to stimulate protein synthesis in the MCF-7 cell line. The results are shown in Chart 4. Estrone, estradiol, and estriol all stimulate [^{14}C]serine incorporation into acid-insoluble material 150% above controls in the experiment shown. Estrone and estradiol appear to be about equally active, and both are about 5 to 10 times as potent as estriol. The

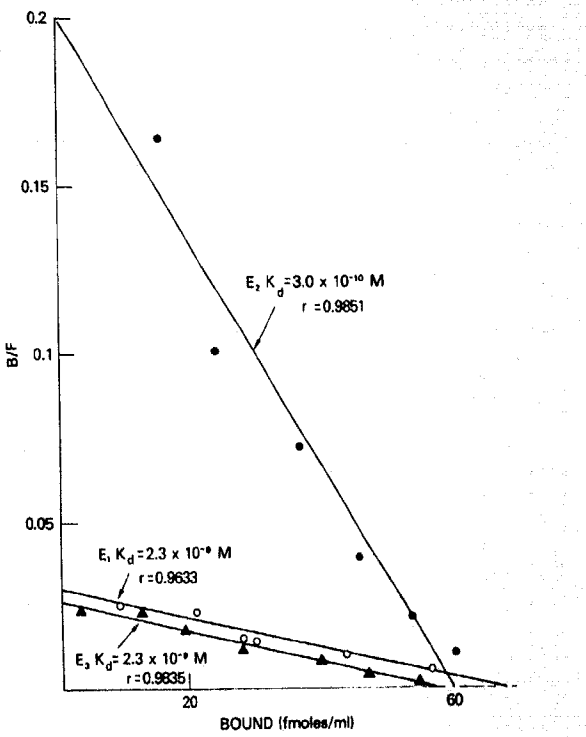

Chart 3. Binding of tritiated estrogens to estrogen receptor in MCF-7 human breast cancer. The binding data have been plotted by means of the Scatchard technique (32). The *straight lines* obtained are derived from a least-squares analysis of the binding data performed by computer (2). Binding techniques are supplied in "Materials and Methods." r = the least-squares-derived correlation coefficient.

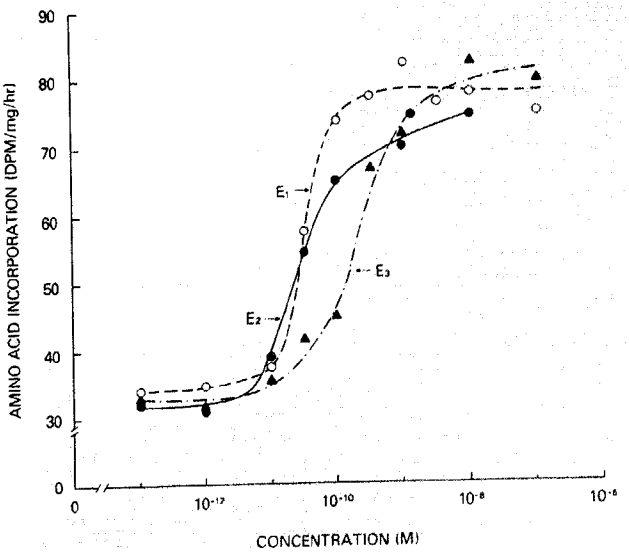

Chart 4. Stimulation of [^{14}C]serine incorporation into trichloroacetic acid-insoluble material as a function of increasing estrogen concentration. *Points*, mean of triplicate determinations. Standard deviations are generally less than 10% of any value. Techniques used including incubation times and labeling periods are given in "Materials and Methods." E_1, estrone; E_2, estradiol; E_3, estrone.

concentration of estradiol needed to minimally stimulate these cells, about 3×10^{-11} M, is identical to those we have previously reported, and emphasizes the sensitivity of these cells to estrogen (18 to 20).

Similar effects of these estrogens on [^{3}H]thymidine incorporation were detected (Table 1). Estriol is about 4 times less potent than estrone, which is about 4 times less potent than estradiol in stimulating [^{3}H]thymidine incorporation. Small changes in relative potency may be explained by the metabolism studies described below. Maximally effective concentrations of each induced similar increases in precursor incorporation. Thus, the continuous presence of estrone and estriol seem capable of inducing stimulation of macromolecular synthesis equivalent to that produced by estradiol. Effects of glucocorticoids, progestogens, androgens, and prolactins are described elsewhere (22).

Tamoxifen, ICI 46474, a triphenylethylene derivative, is an antiestrogen that strongly inhibits macromolecular synthesis in MCF-7 cells (19, 20, 22). We have also observed that simultaneous estradiol administration blocks the antiestrogen effect, and sequential addition of estradiol to cells incubated in Tamoxifen for up to 48 hr reverses inhibitory effects. We therefore examined the interactions of estriol and Tamoxifen (Chart 5). Estradiol, at 10^{-8} M, stimulates thymidine incorporation about 70% above control in the experiment shown, and 10^{-7} M Tamoxifen inhibits thymidine incorporation to about 50% of control. With this same inhibitory concentration of Tamoxifen, 10^{-7} M, replicate dishes of cells were incubated concurrently with increasing concentrations of estriol. As shown, 10,000-fold less estriol has no effect on Tamoxifen inhibition, 1,000-fold less estriol can reverse antiestrogen effects partially, and 100-fold less estriol is capable of reversing nearly all antiestrogenic effects on thymidine incorporation. Thus, by this test as well, estriol appears to function as an estrogen. The maximal extents of estradiol and estriol stimulation are essentially equivalent.

Purity of Labeled and Unlabeled Steroids. Because of the significance of concluding that estriol is a potent estrogen in this system, we carefully examined the purity of the steroids used for stimulation and binding studies to guarantee authenticity. Obviously, metabolic transformations might compromise interpretation of the results.

Radioactive estrone, estradiol, and estriol used in the binding studies shown in Charts 1 to 3 were checked for radiochemical purity by Sephadex LH-20 chromatography (Chart 6). Separate chromatographic runs for the 3 estrogens have been presented in composite form. None of the radiolabeled estrogens contain radioactivity migrating in the position of either of the other 2 estrogens. An unlabeled estrogenic contaminant might effectively reduce the specific activity of one of the labeled estrogens used in the binding studies. However, an alteration in specific activity would affect only the dissociation constant but not the total number of binding sites observed. Thus a nonlabeled contaminant obviously would not alter our observation that [^{3}H]estriol binding is equivalent in magnitude to that of the other estrogens.

As outlined in "Materials and Methods," unlabeled steroids used were checked for purity by melting point analyses before use in stimulation experiments. The melting point for estriol (272–280°) is above that for estrone (254–258°) and estradiol (173–179°). The fact that each melted at the appropriate temperature suggests that, at least initially, estriol was not contaminated significantly by the other two.

Metabolic Interconversion of Estrogens. Such purity studies cannot, however, rule out significant steroid interconversion during the incubation of cells with steroid. This issue was examined directly by incubating cells with tritiated steroids for times and at concentrations equivalent to those used to study hormone-mediated stimulation of macromolecular synthesis. Following incubation, steroids were extracted and analyzed by Sephadex LH-20 chromatography (Chart 7). Chart 7, *A* to *C*, represents, respectively, incubations of cells with either estrone, estradiol, or estriol.

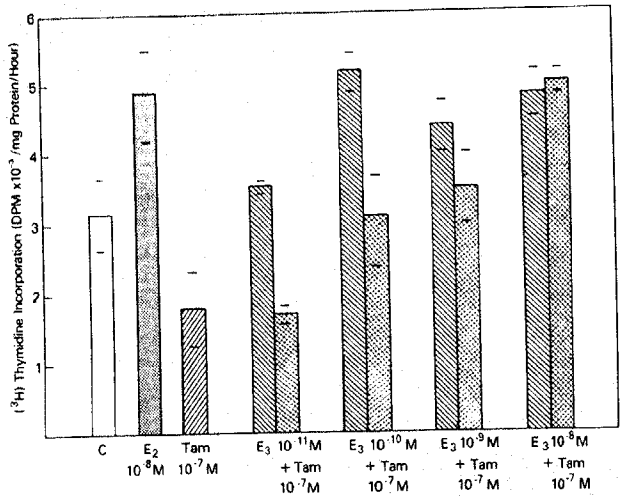

Chart 5. Effects of estradiol (E_2), Tamoxifen (*Tam*), and estriol (E_3) on [^{3}H]thymidine incorporation into trichloroacetic acid-insoluble material as compared with control (*C*). *Points*, mean of triplicate determinations ± S.D. The techniques used, including incubation times and labeling periods, are given in "Materials and Methods."

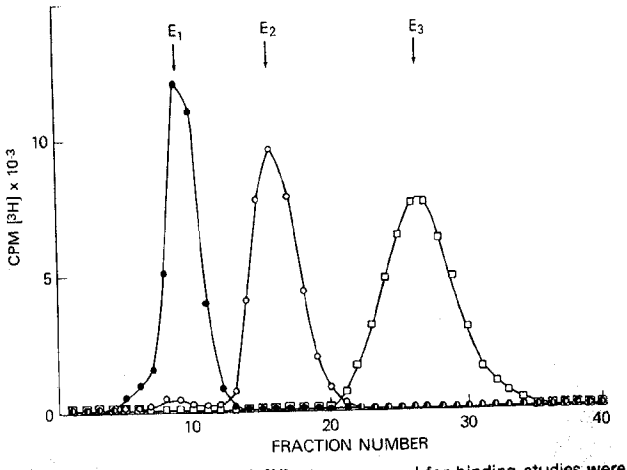

Chart 6. Chromatograms of [^{3}H]estrogens used for binding studies were run separately on a Sephadex LH-20 column. Steroids were eluted in a solvent system consisting of chloroform:benzene:methanol, 3:6:1. Exact conditions are supplied in "Materials and Methods." E_1, estrone; E_2, estradiol; E_3, estrone.

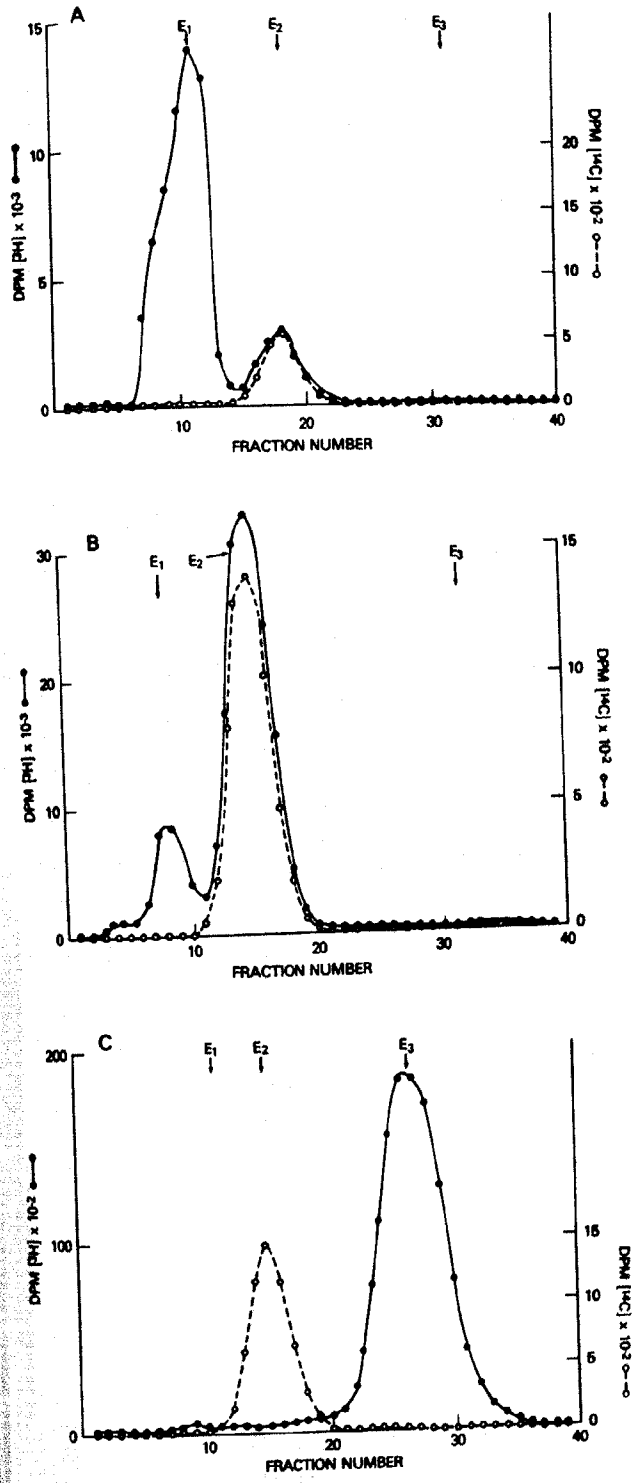

Chart 7. Chromatograms of [³H]estrogens after 24 hr of incubation with MCF-7 cells in monolayer cultures. Each steroid was used at a concentration of 5×10^{-9} M. Following incubation, cells and media were extracted 3 times with chloroform, the solvent was evaporated under a nitrogen stream, and the residue was taken up in the solvent system and run on an LH-20 column as described in "Materials and Methods." *A*, estrone; *B*, estradiol; *C*, estriol. O, [¹⁴C]estradiol added before extraction. E_1, estrone; E_2, estradiol; E_3, estriol. Exact details are supplied in "Materials and Methods."

After 24 hr, there is approximately a 20% conversion of estrone to estradiol. A similar incubation period resulted in about a 20% conversion of estradiol to estrone. These 2 results make it more difficult to interpret small differences in the biological activity of these 2 steroids shown in Chart 4 and Table 1. However, this might explain why estrone is more potent in stimulation studies than estriol, although both have the same dissociation constant. It is extremely unlikely that, under the conditions we use for binding (cytoplasmic extracts incubated for 18 hr at 0–4°), metabolism to this extent would occur.

As shown in Chart 7*C*, there is essentially no conversion of [³H]estriol to a compound migrating in the positions of either estrone or estradiol. In the incubation shown, 5×10^{-9} M estriol was used. We conclude that conversion of estriol to estrone or estradiol does not occur under the conditions we used. Thus, apparent binding of and stimulation by estriol is due to the authentic compound.

DISCUSSION

The role of estriol in human breast cancer is at present highly controversial (37). Epidemiological studies have attempted to establish a protective role for estriol. Cole and MacMahon (9) have concluded that the estriol ratio before age 30 significantly affects breast cancer risk for life. A protective effect of estriol has also been suggested by the interpopulation studies of MacMahon *et al.* (23) and Dickinson *et al.* (10), who showed that, for Asian women living in Hawaii and having an intermediate risk of breast cancer, estriol ratios were intermediate between Asian women in Asia and Caucasian women. Several within population studies have also supported a protective effect for estriol (4, 15, 17, 36). All of these studies found lower estriol ratios in patients with breast cancer, compared with those of controls. In contrast, other workers (6, 12, 24, 29, 30) have found higher estriol ratios in breast cancer patients compared with controls. The difficulties of such studies in terms of accuracy of measurement and suitable controls are manifold.

Obviously, some test of the actions of various estrogenic compounds directly on human breast cancer would be more satisfactory. This is particularly emphasized by the fact that many illnesses such as myocardial infarction (5) and prostatic cancer (25) can also elevate the estriol ratio, and since breast tissue itself has been reported to have 16α-hydroxylase activity (1), it therefore should be capable of

Table 1

Effects of various estrogens on [³H]thymidine incorporation in MCF-7 human breast cancer

Estrogen	Concentration (M) of estrogen resulting in one-half maximal stimulation of thymidine incorporation	Maximum stimulation of thymidine incorporation (% of control)
Estrone	1.5×10^{-10}	250[a]
Estradiol	3×10^{-11}	260
Estriol	6×10^{-10}	255

[a] Results are means of triplicate determinations. Standard deviations do not exceed 10% for thymidine incorporation studies.

estriol production from estradiol. It must also be borne in mind that some of the apparent discrepancies between these studies may be due to an association between estriol and some other estrogen metabolite. For example, 2-hydroxyestrone, a principal urinary metabolite of estradiol, probably does not have any estrogenic activity and may even be antiestrongenic (37). In some settings, estriol might be correlated with 2-hydroxyestrone excretion, and thus measurements of estriol will suggest a protective function. In other settings, if estriol excretion alone is elevated, an increased risk may be observed.

Biological data have largely been derived from 2 observations in animal models. (*a*) Estriol not only has minimal uterotropic activity, but it can block the effects of estradiol (13, 14, 36). (*b*) Estriol does not induce mammary cancer (11) and can protect against the carcinogenic effect of dimethylbenzanthracene (16, 17). Recently, however, Anderson *et al.* (3) and Clark and Peck (8) have shown that estriol can be completely estrogenic in a rodent system and Rudali *et al.* (32) have shown that estriol may have the same carcinogenic potential as estradiol in inducing mammary cancer in mice.

We therefore examined the effects of estrone, estradiol, and estriol on an established line of human breast cancer in tissue culture. We have shown that estriol can stimulate macromolecular synthesis in these cells to the same extent as estrone and estradiol. Estriol completely antagonizes the activity of the antiestrogen, Tamoxifen. We have ruled out significant contamination of the estriol by other estrogens and also excluded metabolism of estriol to either estrone or estradiol. We conclude that under the conditions we use, estriol must be considered to be estrogenic for human breast cancer in tissue culture.

Two cautions are necessary. First, we measure overall effects on macromolecular synthesis. It is possible that these estrogens have different effects on other cellular processes. Second, it is possible that, in an alternative milieu containing other hormones, differences in response to various estrogens could be observed.

It is certainly possible that, by a process of selection in culture, the MCF-7 cell line is atypical of human breast cancer in general. However, binding affinities and specificities of the estrogen receptor in these cells are virtually identical to those found in fresh human tumor samples (20, 21). Furthermore, detailed characterization of the hormonal responsiveness of this cell line has suggested that it closely resembles breast cancer *in vivo* (22). Our studies would suggest the estriol administration to patients at risk of breast cancer may be contraindicated.

REFERENCES

1. Adams, J. B., and Wong, M. S. F. Paraendocrine Behavior of Human Breast Carcinoma: *In Vitro* Transformation of Steroids to Physiologically Active Hormones. J. Endocrinol., *41:* 41-52, 1968.
2. Aitken, S. C., and Lippman, M. E. A Simple Computer Program for Quantification and Scatchard Analysis of Steroid Receptor Proteins. J. Steroid Biochem., *8:* 77-93, 1977.
3. Anderson, J. N., Peck, E. J., and Clark, J. H. Estrogen Induced Uterine Responses and Growth. Relationship to Receptor Estrogen Binding by Urine in Mastopathy. Klin. Wochschr., *38:* 804-805, 1960.
4. Baigalupo, G., and Schubert, K. Studies on the Estrogen Excretion in Urine in Mastopathy. Klin. Wochschre., *38:* 804-805, 1960.
5. Bersohn, I., and Oelofse, P. J. Urinary Oestrogen Levels in Myocardial Infarction. S. African Med. J., *32:* 979-983, 1958.
6. Brown, J. B. Urinary Oestrogen Excretion in the Study of Mammary Cancer. *In:* A. R. Currie (ed.), Endocrine Aspects of Breast Cancer, pp. 197-207. Edinburgh: E. & S. Livingstone, Ltd., 1958.
7. Carr, B. R., Mikhail, G., and Flickinger, J. Column Chromatography of Steroids on Sephadex LH-20. J. Clin. Endocrinol. Metab., *33:* 358-363, 1971.
8. Clark, J., and Peck, E. Estriol: An Estrogen Agonist and/or Antagonist. Proceedings of the 58th Annual Meeting of the Endocrine Society, Abstract 79, p. 96, 1976.
9. Cole, P., and MacMahon, B. Oestrogen Fractions during Early Reproductive Life in the Etiology of Breast Cancer. Lancet, *1:* 604-606, 1969.
10. Dickinson, L. E., MacMahon, B., Cole, P., and Brown, J. B. Estrogen Profiles of Oriental and Caucasian Women in Hawaii. New Engl. J. Med., *291:* 1211-1213, 1974.
11. Hartwell, J. Survey of Compounds Which Have Been Tested for Carcinogenic Activity. USPHS Publication 149, pp. 425-426. Washington, D. C.: United States Government Printing Office, 1951.
12. Hellman, L., Zumoff, B., Fishman, J., and Gallagher, T. F. Peripheral Metabolism of [^{3}H]-Estradiol and the Excretion of Endogenous Estrone and Estriol Glucuronates in Women with Breast Cancer. J. Clin. Endocrinol., *33:* 138-144, 1971.
13. Hisaw, F. L., Velardo, J. T., and Goulsby, S. M. Interaction of Estrogens on Uterine Growth. J. Clin. Endocrinol., *14:* 1134-1143, 1954.
14. Huggins, C., and Jensen, E. V. The Depression of Estrone-Induced Uterine Growth by Phenolic Estrogens with Oxygenated Functions at Position 6 or 16; The Impeded Estrogens. J. Exptl. Med., *102:* 335-346, 1955.
15. Irvine, W. T., Sitken, E. H., Rendelman, D. H., and Folea, P. J. Urinary Oestrogen Measurements after Oophorectomy and Adrenalectomy for Advanced Breast Cancer. Lancet, *2:* 791-796, 1961.
16. Lemon, H. M. Estriol Prevention of Mammary Carcinoma Induced by 7,12-Dimethylbenzanthracene and Procarbazine. Cancer Res., *35:* 1341-1353, 1975.
17. Lemon, H. M., Wotiz, H. H., Parsons, L., and Mozden, P. J. Reduced Estriol Excretion in Patients with Breast Cancer Prior to Endocrine Therapy. J. Am. Med. Assoc., *196:* 112-120, 1966.
18. Lippman, M. E., and Bolan, G. Estrogen Responsive Human Breast Cancer in Continuous Tissue Culture. Nature, *256:* 592-593, 1975.
19. Lippman, M. E., Bolan, G., and Huff, K. Hormone Responsive Human Breast Cancer in Long Term Tissue Culture. *In:* J. C. Heuson, W. H. Mattheiem, and M. Rozencweig (eds.), Breast Cancer: Trends in Research and Treatment, pp. 111-139. New York: Raven Press, 1976.
20. Lippman, M. E., Bolan, G., Monaco, M. E., Pinkus, L., and Engel, L. Model Systems for the Study of Estrogen Action. J. Steroid Biochem., *1:* 1045-1052, 1976.
21. Lippman, M. E., and Huff, K. A Demonstration of Androgen and Estrogen Receptors in Human Breast Cancer Using a New Protamine Sulfate Assay. Cancer, *38:* 868-874, 1976.
22. Lippman, M. E., Osborne, C. K., Knazek, R., and Young, N. *In Vitro* Model Systems for the Study of Hormone Dependent Human Breast Cancer. New Engl. J. Med., *296:* 154-161, 1977.
23. MacMahon, B., Cole, P., Brown, J. B., Aoki, K., Lin, T. M., Morgan, R. W., and Woo, N. C. Urine Oestrogen Profiles of Asian and North American Women. Intern. J. Cancer, *14:* 161-167, 1974.
24. Marmorston, J., Fowley, L. G., Myers, S. M., Stern, E., and Hopkins, C. E. Urinary Excretion of Estrone, Estradiol and Estriol by Patients with Breast Cancer and Benign Breast Disease. Am. J. Gynecol., *92:* 460-467, 1965.
25. Marmorston, J., Lombardo, L. J., Myers, S. M., Gierson, H., Stern, E., and Hopkins, C. E. Urinary Excretion of Estrone, Estradiol and Estriol by Patients with Prostate Cancer and Benign Prostatic Hypertrophy. J. Urol., *93:* 287-295, 1965.
26. McGuire, W. L., Carbone, P. P., Sears, M. E., and Escher, G. C. Estrogen Receptors in Human Breast Cancer: An Overview. *In:* W. L. McGuire, P. P. Carbone, and E. P. Vollmer (eds.), Estrogen Receptors in Human Breast Cancer, pp. 1-8. New York: Raven Press, 1975.
27. McGuire, W. L., and DeLaGarza, M. I. Improved Sensitivity in the Measurement of Estrogen Receptor in Human Breast Cancer. J. Clin. Endocrinol. Metab., *36:* 548-552, 1973.
28. Mobbs, B. G., and Johnson, I. E. Estrogen Binding *in Vitro* by DMBA-induced Rat Mammary Tumors: Its Relationship to Hormone Responsiveness. European J. Cancer, *10:* 757-763, 1974.
29. Nissen-Meyer, P., and Sanner, T. The Excretion of Oestrone, Pregnanediol and Pregnanetriol in Breast Cancer Patients. I. Excretion after Spontaneous Menopause. Acta Endocrinol., *44:* 334-345, 1961.
30. Persson, B. H., and Risholm, L. Oophorectomy and Cortisone Treatment as a Method of Eliminating Oestrogen Production in Patients with Breast Cancer. Acta Endocrinol., *47:* 15-26, 1964.
31. Richter, A., Sanford, K. K., and Evans, V. J. Influence of Oxygen and Culture Medium on Plating Efficiency on Some Mammalian Tissue Culture Cells. J. Natl. Cancer Inst., *49:* 1705-1712, 1972.

32. Rudali, G., Apiou, F., and Muel, B. Mammary Cancer Produced in Mice with Estriol. European J. Cancer, *4:* 39–43, 1975.
33. Scatchard, G. The Attractions of Proteins for Small Molecules and Ions. Ann. N. Y. Acad. Sci., *51:* 660–672, 1949.
34. Simpson-Herren, L., and Griswold, D. P. Studies of the Kinetics of Growth and Regression 7,12-Dimethylbenz(a)anthracene-induced Mammary Adenocarcinoma in Sprague-Dawley Rats. Cancer Res., *30:* 813–818, 1970.
35. Soule, H. D., Vazquez, J., Long, A., Abert, S., and Brennan, M. A Human Cell Line from a Pleural Effusion Derived from a Breast Carcinoma. J. Natl. Cancer Inst., *51:* 1409–1413, 1973.
36. Wicks, A. E., and Segal, S. J. Time and Dose Relationship in Estriol-Estradiol Interactions. Proc. Soc. Exptl. Biol. Med., *93:* 270–273, 1956.
37. Zumoff, B., Fishman, J., Bradlow, H. L., and Hellman, L. Hormone Profiles in Hormone-dependent Cancers. Cancer Res., *35:* 3365–3373, 1975.