Specific Estrogen-binding Capacity of the Cytoplasmic Receptor in Normal and Neoplastic Breast Tissues of Humans

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SUMMARY

Carcinomas and nonmalignant breast tissues from 89 women undergoing surgery were examined for the presence of specific estrogen-binding substances by a radioligand binding technique in vitro. Following incubation of the 105,000 X g supernatant fraction with estradiol-17β-6,7,3H of high specific radioactivity, in the presence or absence of a known antiestrogen, specific receptors for estradiol-17β-3H were identified by isotopic profiles from sucrose gradient analyses. Cytoplasmic estrogen receptors from breast carcinoma migrated with a sedimentation velocity coefficient of approximately 8 to 9 S. Cytosols from 29 primary carcinomas were classified as positive, exhibiting an average binding capacity of 43.0 ± 5.3 fmoles/mg protein, with a range of 10.3 to 137.6 fmoles/mg protein. An additional 10 carcinomas were considered borderline on the basis of an average binding capacity of 5.6 ± 0.4 fmoles/mg protein, with a range of 3.3 to 7.4 fmoles/mg protein. Thirty-six of 75 tumors displayed insignificant specific estrogen binding. Inhibition of hormone binding by the competitive inhibitor, CN-55,945-27, averaged 87 ± 3% for the tumor cytosols exhibiting elevated binding; specific binding by cytosols with low receptor capacity was inhibited by 81 ± 5%. In addition to these neoplasms, 16 specimens of normal breast, 5 of fibrocystic disease, and 1 of fibroadenoma were analyzed; all but one of these exhibited insignificant specific binding. These data confirm the presence of specific estrogen-binding substances in certain infiltrating ductal carcinomas of the human breast. Twenty-three of the 29 carcinomas exhibiting specific estradiol-17β-binding capacity were from postmenopausal women 55 years of age or older. Specific estradiol-17β-binding capacity of a tumor was apparently not related to the presence of metastases, nor was it related to the estimated percentage of carcinoma cells in the specimen examined.

INTRODUCTION

A biochemical basis for the distinction between human breast carcinomas that are responsive to hormone therapy or 1

SUMMARY

specific binding of estradiol to its receptor. 2

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2 American Cancer Society Research Professor RP-42.

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Interference with endogenous synthesis of estrogen in postmenopausal women has been suggested as the basis for the beneficial results observed following adrenalectomy or hypophysectomy (15–17, 25). As early as 1961, Folca et al. (10) observed that, following injection of tritiated hexestrol into patients with breast cancer, the estrogen was taken up to a greater extent by the neoplasms from 4 patients who later experienced remission than it was by tumors from 6 patients who did not respond to ablative surgery. Reports from a number of laboratories (3, 6, 7, 12, 13, 16, 19, 20, 22, 26–28) have shown that certain primary carcinomas and metastatic lesions of the human breast have specific estrogen-binding capacity, as determined by methods in vivo and in vitro. Of these, Jensen et al. (16) have attempted to correlate the specific accumulation of estrogen with clinical data of the patients. Preliminary evidence suggested that remission of breast cancer after adrenalectomy occurred more often in patients having breast tumors with estrogen receptors than in those patients whose carcinomas did not contain measurable estrogen-binding proteins.

As part of a long-term study to establish the relationship between the presence of specific cytoplasmic receptors (8 S) for estradiol-17β in human mammary carcinomas and in metastatic lesions and the patients’ responses to endocrine organ ablative surgery and those that are not responsive has been difficult to achieve. Since one anticipates that about 25% of breast cancers will regress after removal of endogenous estrogen (e.g., oophorectomy or adrenalectomy), information on the hormonal characteristics of a tumor, i.e., dependent or independent, would be invaluable for predicting the response of the neoplasm in the patient.

It is accepted currently that an important characteristic of an estrogen-target organ, such as the uterus, vagina, or anterior pituitary, is the capacity to bind estradiol-17β specifically with high affinity (11, 17, 18, 33). In the uterus, for instance, binding of the steroid hormone to specific entities, termed “estrogen-binding proteins” or “estrogen receptors,” precedes alterations in the rates of synthesis of nucleic acids and proteins. Administration in vivo of certain antiserotoninergic substances, such as MER25, UI 1100A, or CN-55,945-27, prevents the normal response of the uterus to estrogen (5, 6, 8, 23), presumably by inhibiting the binding of estradiol-17β to its receptor.

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organ ablative surgery or hormone therapy, this report presents the results of our investigation of 97 benign and malignant tissues from patients undergoing breast surgery. Clinical information at the time of operation is presented for each patient. A preliminary report of this work has been presented (34).

MATERIALS AND METHODS

Reagents and Chemicals. Sucrose (RNase-free) and purified human albumin were obtained from Schwarz/Mann, Orangeburg, N.Y. Sigma Chemical Company, St. Louis, Mo., supplied the Tris (Trizma base). Estradiol-17β-6,7-3H (40 Ci/m mole) and scintillation flor (Omniflour) were purchased from New England Nuclear, Boston, Mass. At intervals, the radiochemical purity of the steroid was checked by thin-layer chromatography with at least 2 solvent systems. The antiserum to estrogen receptor, CN-55.943-17β, also designated as “Cl-628,” was provided through the courtesy of Dr. Merritt Callentine of Parke, Davis & Co., Detroit, Mich. Goat antibody against purified human albumin was purchased from Kallestad Laboratories, Inc., Minneapolis, Minn.

Handling of Breast Specimens Postoperatively. Normal and neoplastic breast specimens were obtained from the surgical services of various hospitals in Rochester, N.Y. Following surgery, tissues were placed in beakers kept on ice and were quickly brought to the laboratory. In the case of a neoplasm, necrotic and connective tissue, fat, and skin were removed quickly and the tumor was cut into pieces of approximately 4 cu mm. Several pieces, weighing approximately 500 mg, were placed in a small polypropylene vial; the vial containing the tissue was placed immediately in liquid nitrogen. Usually, the tissue was kept on ice no longer than 20 to 30 min, and only 5 to 10 min were required once the tissue reached the laboratory. The human breast tissues were stored at −86°C in a Revco freezer until analyses were performed. No attempt was made to select tissues on the basis of the patients’ clinical histories, i.e., probability of responses to endocrine therapy.

Preparation of Cytosols. The preweighed frozen tissues were shattered to a powder under liquid nitrogen (−196°C) by a piston-driven pulverizer (Thermovac Industries Corp., Copia-gue, N.Y.). The frozen powder was homogenized (1/3, w/v) in cold 10 mM Tris-HCl, pH 7.4, containing 1.5 mM EDTA, in small Duall homogenizers (Kontes Glass Co., Vineland, N.J.) for short pulses (15 to 20 sec), with 30 to 45 sec elapsing between pulses. A small ice bath was kept around the homogenizer at all times. The homogenate was centrifuged at 105,000 x g (0°) for 30 mm, and the supernatant was removed without disturbing the lipid layer at the top of the tube. This supernatant fraction (cytosol) was stored briefly at 0° until the binding assay was performed. Cytosols of rat uterine tissue were prepared similarly.

Pathology. Paraffin sections, 5 to 7 μm thick, were made from samples of tissue obtained within 2 mm of the specimen used for estrogen-binding assays. These were stained with hematoxylin and eosin and were examined microscopically. A semiquantitative estimation of the various cellular components was made by photographing the entire section and determining by planimetry the area represented by carcinoma, fibrous tissue (collagen), adipose cells, etc. In an earlier study (14), the areas were cut out and weighed to determine the percentage represented by each cell type. Since results from both methods were in agreement, planimetry was used in this study. The amount of carcinoma in a specimen is given as the percentage of the area that was occupied by the carcinoma cells in the section examined microscopically. Such an estimate represents only that portion of the neoplastic lesion at the edge of the tissue block used for determination of estrogen-binding capacity.

Assay for Specific Estrogen-binding Proteins. The sucrose gradient procedure, originally described by Gorski and his coworkers (31, 32) for the rat uterine receptor and modified by Jensen et al. (16) for use with human tissues, was used for the measurement of the specific estrogen-binding capacity of the cytoplasmic receptor. Aliquots (200 μl) of tissue cytosols were reacted with 50 μl of 10 mM Tris-HCl (containing 1.5 mM EDTA) buffer alone or with 50 μl of buffer containing the antiserum to estrogen receptor, CN-55,943-27, for 10 min at 0°. The final concentration of the inhibitor in the reaction was 20 μM. After incubation, the entire mixture was added to a 2nd reaction vial containing sufficient tritiated estradiol-17β to make the final steroid concentration between 0.5 and 2.0 nM. A 200-μl portion of each reaction mixture was layered onto a cold, linear gradient of sucrose (10 to 30%) that also contained Tris-EDTA buffer, pH 7.4. A cytosol from several rat uteri, which had been treated identically to the tumor cytosols, was included in each centrifuge run as a procedural control. The gradients were centrifuged for 12 hr (0°) at 308,000 x g with a Spinco-SW-56 titanium rotor in a Beckman L2-65B ultracentrifuge. After centrifugation, we fractionated each gradient by puncturing the bottom of the tube and collecting 6 drops into each scintillation vial. Two ml of 99% ethanol and 10 ml of scintillation fluor, containing 4 g Omnifluor per liter toluene, were added to each vial, and the tritium content was measured in a Mark II liquid scintillation counter (Nuclear-Chicago Corp., Des Plaines, Ill.). The counting efficiency for tritium in the “cocktail” described was 40 to 44%.

Calculation of Specific Estrogen-binding Capacity. Data (as cpm/mg) from sucrose gradients were printed out by a Western Union teletype writer and simultaneously punched (in ASCII code) onto a paper tape. With programs prepared previously (4), computations of the percentage of efficiency and concentration of tritiated estradiol-17β in each fraction were accomplished with an Olivetti Programma 101, interfaced to a solenoid deck (Nuclear-Chicago Corp.), and with a paper tape reader (Teletype Corp., Skokie, Ill.). Estrogen-binding substances were identified by isotopic profiles from sucrose gradients. Since each tumor cytosol was measured in the presence and absence of the specific competitive inhibitor, CN-55,945-27, specific binding was estimated as the difference in radioactivity bound only in the 8 S region of these gradients (usually Tubes 11 to 25). Specific estrogen-binding capacity is expressed as fmole estradiol-17β bound per mg cytosol protein. Protein was determined by the method of Lowry et al. (24).
RESULTS

For determination of the nature of the molecular species binding estrogen, cytosols from homogenates of 97 carcinomas and nonmalignant breast tissues were reacted in vitro with estradiol-17β-3H and were separated by sucrose gradient centrifugation. When the quantity of cytosol from a tumor permitted, 2 concentrations of estradiol-17β-3H were used in the same centrifuge run (Chart 1). This insured that the reaction was carried out at a saturating concentration (usually 1 to 2 nM) of steroid and that results were duplicative. Chart 1 illustrates a typical isotopic profile obtained after centrifugation of a cytosol from a primary tumor diagnosed as infiltrating ductal carcinoma. The profile showed 2 peaks binding the radioactive estradiol-17β: the radioactive peak around Tube 19 corresponded to a sedimentation velocity coefficient of 8 to 9 S. The 2nd peak containing radioactivity sedimented at approximately Tube 30, corresponding to an S value of 4 to 5. In the presence of a competitive inhibitor (CN-55,945-27) of estradiol-17β binding, the steroid bound by the 8 S receptor was diminished nearly to zero, while that bound by the 4 to 5 S substance remained elevated. Increasing the concentration of estradiol-3H did not alter the amount of steroid bound by the 8 S binding protein, although the 4 to 5 S species continued to bind estradiol. These data indicate that estradiol-17β is bound specifically and at low concentrations by the 8 S receptor, but the hormone is also bound nonspecifically and in a nonsaturating fashion by the species sedimenting at 4 to 5 S. Chart 2 summarizes the types of profiles observed in this study. The profile in Chart 2A, which is derived from an infiltrating ductal carcinoma, is similar to that presented in Chart 1. However, in several cytosols from infiltrating ductal carcinomas, a specific estrogen-binding substance was also observed in the 4 to 5 S region of the gradient (Chart 2B), as evidenced by the inhibition of binding by CN-55,945-27. A similar type of profile has been reported by Jensen et al. (16) for several human breast carcinomas. For comparative purposes, only the specific binding capacity of the 8 S receptor was calculated. Of the 39 tumor cytosols exhibiting specific estrogen-binding capacity, the majority gave results similar to that shown in Chart 2A.

Many cytosols of malignant tumors contained only the 4 to 5 S substances, which bind estradiol-17β nonspecifically (similar to profile shown in Chart 2D); other cytosols did not bind the steroid, specifically or nonspecifically (Chart 2C). Cytosol from specimens of fibrocytic disease did not bind estradiol-17β specifically (Chart 2D). All but 1 specimen of normal breast exhibited gradient profiles similar to those shown in Chart 2, E and F, indicating the absence of available specific receptor sites for estradiol-17β. This may be due to the relatively high concentration of adipose cells and connective tissue as well as the comparatively low number of epithelial cells in these specimens.

Since a number of tumor cytosols contained significant amounts of the 4 to 5 S substance, which binds estradiol-17β nonspecifically, it was of interest to determine whether the presence of this substance or any similar molecules would reduce the binding capacity of the 8 S receptor. Presence of nonspecific steroid binding to soluble protein in the uterus and other estrogen target tissues has been reported by several workers (1, 16, 18, 31, 32), and Jensen et al. (16) have suggested that these low-affinity sites may be attributed to plasma protein contamination of human breast carcinomas. Since human breast carcinoma specimens were more difficult to obtain, the cytoplasmic estrogen receptor of the rat uterus was used. Chart 3 presents the results of a representative experiment. The soluble-estrogen receptor was separated by centrifugation on a 10 to 30% linear gradient of sucrose containing the Tris-EDTA buffer previously described. As seen in Chart 3A, the radioactivity bound in the region of Estrogen Receptors in Human Breast Carcinoma

![Chart 1](chart1.png)

**Chart 1.** Isotopic profile of a specific estrogen receptor (8 S) from a cytosol of an infiltrating ductal carcinoma of the breast. Separation was carried out on a 10 to 30% sucrose gradient centrifuged at 308,000 × g for 12 hr at 0–3°C. Human serum albumin (4.6 S) serves as a marker of migration. Note that the binding to the 8 S species was not increased with an increase in the concentration of estradiol-17β-3H from 1 nM to 1.7 nM.
Attempts to analyze the kinetics of estradiol-17β binding to the specific cytoplasmic receptor have been hampered by the lack of adequate tissue from each tumor, particularly when a cytosol displaying elevated estrogen-binding capacity was observed. However, titration curves have been obtained on 3 infiltrating ductal carcinomas by adding increasing amounts of estradiol-17β-3H (usually 0.25 to 5.00 nM) to a constant volume of cytosol. Separation of bound from free steroid was accomplished by the sucrose gradient procedure, and the values obtained were plotted according to the method of Scatchard (29). A representative Scatchard plot, Chart 4, gave a single straight line, indicating 1 type of binding site, and provided an estimate of the dissociation constant (Kd) for the binding of estradiol-17β to the specific receptor. The Kd determined on this sample was approximately 9 × 10^{-10} M, indicative of a high binding affinity for the steroid ligand; this estimate agrees well with that reported by Feherty et al. (9).

Table 1 provides a comparison of the clinical and histological data on 39 breast cancer patients whose malignant tumors exhibited specific receptors of estradiol-17β-3H. Although all but 2 of the tumors examined were diagnosed as infiltrating ductal carcinoma, the 1 specimen of medullary carcinoma and that of colloid carcinoma also contained measurable amounts of the cytoplasmic estrogen receptor. The specific estrogen-binding capacity of all tumors exhibiting receptor ranged from 3.3 to 137.6 fmoles/mg cytosol protein. However, we have arbitrarily separated the tumors into 3 groups on the basis of the estrogen-binding capacity. Groups designated “+” and “±” were characterized by a high degree of inhibition of the steroid by the specific antiestrogen. A 3rd group of tumors was called “−” or “zero” with regard to the estrogen receptor because their low level of binding was not specifically inhibited by CN-55,945-27. Twenty-nine of the cytosols designated as positive (+) exhibited a binding capacity, with a range of 10.3 to 137.6 fmoles/mg protein and a mean of 43.0 fmoles/mg cytosol protein. A 2nd group of 10 cytosols contained lower levels of receptors (designated ±), with a range of 3.3 to 7.4 fmoles/mg protein and a mean of 5.6 fmoles/mg cytosol protein. The binding of estradiol-17β to the cytoplasmic receptors of the tissues exhibiting high binding capacity was depressed by the competitive inhibitor, CN-55,945-27, with a mean of 87%. In 4 carcinomas classified as +, the inhibition of ligand binding by CN-55,945-27 was less than 80% (27, 56, 60, and 60%). The low level of inhibition was attributable not to a lack of sufficient estradiol-3H to saturate the cytoplasmic receptors but rather to an elevated level of radioactivity in the denser portion of the gradient. However, problems with an elevated base line were
Estrogen Receptors in Human Breast Carcinoma

infrequently encountered. The inhibitor also diminished the binding of estradiol-17β-3H to the 8 S receptor from the tissues designated ± to the extent of 81%. Malignant tissues from 36 additional patients did not exhibit specific 8 S cytoplasmic receptors for estradiol-17β-3H (Table 2). There was no apparent relationship between the percentage of carcinoma cells in tumors displaying estrogen-binding capacity and the actual amount of receptor present (Tables 1 and 2). The distribution of the percentage of carcinoma cells was also similar in the tissues examined, whether or not the receptor was present. These data suggest that the number of carcinoma cells in a tumor is not a critical factor concerning the presence or absence of specific estrogen-binding proteins.

The presence of metastases at the time of operation was noted for tumors exhibiting (Table 1) and lacking (Table 2) specific receptors of estradiol. The data from 75 patients with malignant breast disease indicate that there was no apparent relationship between presence of receptors and presence of metastases.

In addition to the samples of malignant breast tissue, 16 specimens of normal breast, 5 of fibrocystic disease, and 1 of fibroadenoma were analyzed for cytoplasmic proteins binding estradiol-17β-3H specifically. The results, as well as the clinical and histological data, are presented in Table 3. The cytosols from all but 1 of these tissue samples displayed insignificant binding capacity. That cytosol from Patient A. W. exhibited a specific estrogen-binding capacity of 9.4 fmol/mg protein and a defined peak of radioactivity at approximately 8 S, similar to the profile seen in Chart 2A. The binding was reduced by 82.8% in the presence of the inhibitor CN-55,945-27. Histological examination of a portion of this tissue revealed approximately 10% normal epithelial cells. Several of the other nonmalignant tissues exhibited higher concentrations of normal cells, although they did not show measurable quantities of estrogen receptors.

A summary of the results of our findings on the estrogen-binding capacity of 97 tissue specimens is presented

Chart 3. A, isotopic profiles of the estrogen-binding substances in rat uterine cytosol showing the presence of the 8 S receptor. B, isotopic profile of estrogen-binding substances in a mixture of rat uterine cytosol and purified human albumin. Note that, in the presence or absence of the binding inhibitor, CN-55,945-27, the estradiol-17β bound by the substance sedimenting in the 4 to 5 S region is elevated. C, isotopic profile of the binding of estradiol-17β-3H to purified human albumin. See text for details.

Chart 4. Scatchard analysis of the titration of specific estrogen receptors with increasing concentrations of estradiol-17β-3H. The 8 S estradiol-receptor complex from the cytosol of a human breast carcinoma was separated at 0° by the sucrose gradient assay. The slope of the line is equal to −1/Kd, where Kd is the dissociation constant of the estradiol-receptor complex. The value of Kd obtained from this plot is 9 × 10−10 M.
in Table 4. From these data, it was demonstrated that approximately 50% of the patients with infiltrating ductal carcinoma of the breast have tumors containing specific estrogen receptors. To ascertain if there was a correlation between the age of the patient at the time of mastectomy and the capacity of the tumor cytosols to bind estrogen, we made a plot of these data (Chart 5). The graph was arbitrarily divided into 2 portions, with age 55 years, an age at which virtually every woman has completed the menopause, as the point of division. Although measurements of binding capacity of cytosols were made on tumors removed from 26 women under 55 years of age, only 9 exhibited specific estrogen receptors. Two of these patients (J. S. and D. L.) were clinically postmenopausal; the tumor from J. S. (49 years old) contained a high level of estrogen-binding protein (51.9 fmoles/mg protein), whereas the tumor from D. L. (45 years old) did not contain specific estrogen receptors. Tumors from 2 premenopausal patients, R. B. and R. S., contained significant amounts of estrogen receptors, 23.5 and 16.9 fmoles/mg cytosol protein, respectively. Specimens removed from the remaining 5 premenopausal patients exhibited varied binding (Table 1; Chart 5).

Nevertheless, 30 of 49 tumors from patients 55 years of age or older contained significant levels of estrogen-binding proteins (Table 1; Chart 5). These data indicate that in tumors from patients who have completed the menopause there is a
higher capacity to bind estradiol than in those from premenopausal patients.

DISCUSSION

The classical report by Beatson (2) and the reports of Huggins and Bergenstal (15) and Luft and Olivecrona (25) were instrumental in establishing the premise that the growth of cancer cells can be deterred by modifications of the endocrine state of the host, e.g., oophorectomy, adrenalectomy, and hypophysectomy. Additionally, the report of Folca et al. (10) on the remissions after adrenalectomy of 4 patients whose metastases demonstrated the ability to concentrate tritiated hexestrol suggested that information on the estrogen-binding capacity of a neoplasm may be useful in the design of rational therapy for advanced breast disease. Although the utility of such information on the hormonal nature of a tumor, i.e., dependent, responsive, or independent, in predicting the response of the patient to ablative surgery, has not been well established, Jensen et al. (16) have suggested that breast cancer patients whose tumors lack the estrogen receptor have little chance of responding to ablative endocrine therapy, i.e., adrenalectomy. These studies were initiated to ascertain the relationship between the presence of specific receptors of estradiol-17β in human mammary carcinomas and metastatic lesions and the patient's response to endocrine organ ablative surgery and/or hormone therapy. Our results from the examination of 97 tissues from patients undergoing breast surgery confirm earlier reports (13, 16, 19, 20) that there are distinct differences in the ability of breast tumors to bind estradiol-17β3H. In this series of patients, approximately one-half of the 75 samples of infiltrating ductal carcinomas contained specific receptors for estradiol; specificity was determined by use of the inhibition of binding in the presence of 25—80% free estrogen.

Table 2
Summary of characteristics of patients whose breast tumors lack specific estrogen-binding capacity

<table>
<thead>
<tr>
<th>Age (range in yr)</th>
<th>No. of patients</th>
<th>Av. % carcinoma in specimen</th>
<th>No. of patients with metastases</th>
</tr>
</thead>
<tbody>
<tr>
<td>30—40</td>
<td>2</td>
<td>48</td>
<td>(40—55)a</td>
</tr>
<tr>
<td>41—50</td>
<td>9</td>
<td>23</td>
<td>(5—60)</td>
</tr>
<tr>
<td>51—60</td>
<td>11</td>
<td>36</td>
<td>(15—90)</td>
</tr>
<tr>
<td>61—70</td>
<td>6</td>
<td>26</td>
<td>(10—50)</td>
</tr>
<tr>
<td>71—80</td>
<td>4</td>
<td>19</td>
<td>(1—30)</td>
</tr>
<tr>
<td>&gt;80</td>
<td>4</td>
<td>58</td>
<td>(25—80)</td>
</tr>
</tbody>
</table>

a Numbers in parentheses, range.
b One of these patients had recurrent disease.
c Two of these patients had recurrent disease.

Table 3
A comparison of clinical and histological data with the specific estrogen-binding capacity of nonmalignant and normal tissues from breast cancer patients

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yr)</th>
<th>Pathology</th>
<th>% normal cells in specimen</th>
<th>Remarks</th>
<th>% specific binding by 8 S receptor</th>
<th>Femoles estradiol 17β/mg protein</th>
<th>Rating</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. C.</td>
<td>20</td>
<td>Fibroadenoma</td>
<td>15</td>
<td></td>
<td>12</td>
<td>&lt;1</td>
<td>-</td>
</tr>
<tr>
<td>M. F.</td>
<td>47</td>
<td>FCD</td>
<td>25</td>
<td></td>
<td>38</td>
<td>1.0</td>
<td>-</td>
</tr>
<tr>
<td>F. U.</td>
<td>41</td>
<td>FCD</td>
<td>15</td>
<td>IDC present</td>
<td>8</td>
<td>&lt;1</td>
<td>-</td>
</tr>
<tr>
<td>A. M.</td>
<td>63</td>
<td>FCD</td>
<td>5</td>
<td></td>
<td>14</td>
<td>&lt;1</td>
<td>-</td>
</tr>
<tr>
<td>M. M.</td>
<td>71</td>
<td>FCD</td>
<td>1</td>
<td>IDC present</td>
<td>1</td>
<td>&lt;1</td>
<td>-</td>
</tr>
<tr>
<td>E. M.</td>
<td>60</td>
<td>FCD</td>
<td>5</td>
<td>IDC present</td>
<td>55</td>
<td>1.4</td>
<td>-</td>
</tr>
<tr>
<td>M. E.</td>
<td>76</td>
<td>NB</td>
<td>10</td>
<td>IDC present</td>
<td>1</td>
<td>&lt;1</td>
<td>-</td>
</tr>
<tr>
<td>R. D.</td>
<td>82</td>
<td>NB</td>
<td>10</td>
<td>IDC present</td>
<td>1</td>
<td>&lt;1</td>
<td>-</td>
</tr>
<tr>
<td>A. W.</td>
<td>71</td>
<td>NB</td>
<td>10</td>
<td></td>
<td>83</td>
<td>9.4</td>
<td>+</td>
</tr>
<tr>
<td>R. F.</td>
<td>45</td>
<td>NB</td>
<td>61</td>
<td></td>
<td>1</td>
<td>&lt;1</td>
<td>-</td>
</tr>
<tr>
<td>M. F.</td>
<td>78</td>
<td>NB</td>
<td>20</td>
<td></td>
<td>100</td>
<td>&lt;1</td>
<td>-</td>
</tr>
<tr>
<td>E. D.</td>
<td>61</td>
<td>NB</td>
<td>20</td>
<td>IDC present</td>
<td>15</td>
<td>&lt;1</td>
<td>-</td>
</tr>
<tr>
<td>V. M.</td>
<td>45</td>
<td>NB</td>
<td>10</td>
<td></td>
<td>23</td>
<td>&lt;1</td>
<td>-</td>
</tr>
<tr>
<td>E. P.</td>
<td>51</td>
<td>NB</td>
<td>90</td>
<td></td>
<td>44</td>
<td>1.1</td>
<td>-</td>
</tr>
<tr>
<td>L. B.</td>
<td>45</td>
<td>NB</td>
<td>15</td>
<td></td>
<td>7</td>
<td>&lt;1</td>
<td>-</td>
</tr>
<tr>
<td>F. H.</td>
<td>80</td>
<td>NB</td>
<td>1</td>
<td></td>
<td>56</td>
<td>1.4</td>
<td>-</td>
</tr>
<tr>
<td>J. B.</td>
<td>33</td>
<td>NB</td>
<td>15</td>
<td></td>
<td>30</td>
<td>&lt;1</td>
<td>-</td>
</tr>
<tr>
<td>R. H.</td>
<td>41</td>
<td>NB</td>
<td>1</td>
<td></td>
<td>1</td>
<td>&lt;1</td>
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<td>D. B.</td>
<td>41</td>
<td>NB</td>
<td>10</td>
<td></td>
<td>1</td>
<td>&lt;1</td>
<td>-</td>
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<td>M. G.</td>
<td>42</td>
<td>NB</td>
<td>5</td>
<td></td>
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<td>-</td>
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<tr>
<td>R. F.</td>
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<td>NB</td>
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<td></td>
<td>17</td>
<td>1.9</td>
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<tr>
<td>J. M.</td>
<td>46</td>
<td>NB</td>
<td>10</td>
<td>Lobular carcinoma in situ</td>
<td>11</td>
<td>&lt;1</td>
<td>-</td>
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</table>

a FCD, fibrocystic disease; IDC, infiltrating ductal carcinoma; NB, normal breast.
of a competitive inhibitor, CN-55,945-27. Similar results were reported earlier by Korenman and Dukes (20), who used a charcoal adsorption assay, by Johansson et al. (19), who used a tissue slice procedure, and by Jensen et al. (16), who used sucrose gradient analyses of supernatants from homogenates of tumors. Thus, exclusive of the data reported here, specific estrogen receptors have been found in 67 of a total of 130 primary carcinomas of the breast. In contrast to the results just quoted and to the data reported here, Hähnel et al. (13) observed only 13 of 71 malignant tumors exhibiting "high binding" of estradiol-3H. The majority of the malignant tumors reported in their study displayed binding capacities that were similar to the levels found in benign breast disease. These measurements were made by use of a tissue slice technique and a charcoal adsorption procedure (12). Recently, Feherty et al. (9), utilizing a charcoal adsorption assay, reported that 37 of 53 biopsies of breast carcinomas contained high-affinity estradiol receptors in the 1000 X g supernatant preparation of these tissues. Although the patient population comprising all of these studies is still small, a summary of the reported data indicates that 158 of 329 (48%) primary breast tumors examined contain specific estrogen receptors. The results reported by Hähnel et al. (12) and by Feherty et al. (9) may have been influenced by the use of low-speed cytosols, 3500 X g and 1000 X g, respectively, which probably contained microsomes and mitochondria.

Although many studies (see Ref. 33) have indicated the presence of specific estrogen receptors in human breast carcinoma, the study of Jensen et al. (16) and this report present evidence that certain malignant tumors contain estrogen-binding species sedimenting at 8 to 9 S in sucrose gradients. The properties of receptors in breast cancer tissue are similar to those of the binding protein(s) found in rodent uterus (11, 18) and in the mammary tumors induced in the rat by 7,12-dimethylnzen(a)anthracene (21). Our results indicate that certain benign and malignant human breast tumors contain high concentrations of albumin, as identified by reaction against antialbumin (human) prepared in goats. We demonstrated that, although the dissociation constant of albumin is higher (10^-3 to 10^-6 M) than that of the estrogen receptor (10^-9 to 10^-10 M), its presence in large amounts in cytosol preparations may depress specific binding to the cytoplasmic receptor. As shown, cytosols from specimens of nonmalignant disease and normal breast did not contain defined peaks indicative of specific estrogen receptors when separated on sucrose gradients.

Steggles and King (30) reported the presence of a 4 S estrogen receptor in addition to the 8 S receptor in the cytoplasm of uteri from mature rats. The binding of estradiol-17β-3H to the 4-S receptor was inhibited to a lesser degree by the addition of either unlabeled estradiol-17β or U11100A than that to the 8 S receptor from uteri. As shown in the results, a few of the cytosols of breast carcinomas demonstrated binding of estradiol-17β-3H to macromolecules, sedimenting with a coefficient of 4 S to 5 S; binding was inhibited partially by CN-55,945-27. Similar results were shown by Jensen et al. (16); however, the presence of an 8 S receptor in the absence of a 4 S receptor occurred most frequently in their study and our own. The estimate of estrogen-binding capacity reported here is considered to be minimal, since one can measure only the available receptor sites by the methods used.

Although normal breast tissue from humans is known to concentrate estradiol-17β-3H (7), the levels of isotope bound are approximately one-third of that found in tumor tissue. One possible explanation for this reduced binding is that normal breast tissue contains fewer epithelial cells and a greater predominance of adipose cells, when compared to tumor tissue. Our results suggest that the simple explanation of a lack of cellularity may not be the answer. Although several of the samples of the normal breast examined here contained 20 to 90% epithelial cells, only 1 of these contained specific estrogen receptors, i.e., a single specimen of normal breast tissue from A. W. exhibited a specific estrogen-binding capacity of 9.4 fmoles/mg protein, and the bound radioactivity in the region of the 8 S receptor was reduced in the presence of the antiestrogen by 82.8%.
breast exhibited a gradient profile containing an 8 S cytoplasmic receptor with binding specificity. Likewise, Johansson et al. (19) found positive estrogen binding in only 2 of 26 samples of benign tumors of the breast.

The absolute level of receptor in a tissue may change as a function of a number of variables, such as length of time for transport from operating room to laboratory and storage time and temperature, as well as the level of endogenous estrogen. In all cases, we assumed that the level of receptor reported for a tissue was probably less than the actual level in situ. The breast tissues used in this study were handled quickly and retained on ice until frozen in liquid nitrogen, as outlined in the text. Johansson et al. (19) presented evidence that storage of breast tumor tissue on ice did not significantly alter the binding capacity within 24 hr of excision. However, they mentioned that storage at −20° or −81° for several days was deleterious to the receptor capacity. Hähnel et al. (13) observed that freezing of tissue or tissue extracts gave variable levels of receptors in tumors known to bind estradiol-17β. Our own experience, with several samples reexamined at intervals up to 2 months, indicated no significant loss in estrogen-binding capacity in tissues that were quick-frozen and stored at −86°. In addition, all steps in the gradient assay were conducted at 0°—3° with a 10 to 30% sucrose gradient. As suggested by Jensen et al. (16), less decomposition of the hormone-receptor complex occurs with these sucrose concentrations.

Circulating levels of estradiol-17β in premenopausal women has been considered in the determination of specific receptor concentrations (20). After measuring the levels of endogenous estrogen in cytosols of several breast tumors, these workers concluded that tissue fractions from women actively menstruating contained no more estrogen than those from postmenopausal patients. However, all of the tumors exhibiting estrogen-binding capacity were excised from postmenopausal women. Similar results were reported by Hähnel et al. (13) for 12 of 13 tumors containing significant quantities of receptor that were removed from postmenopausal patients. Contrasted with these data, Johansson et al. (19) did not report a correlation between estradiol-17β-binding capacity of a tumor and the menopausal state of the patient. Our results indicate that 9 of 26 (35%) tumors removed from premenopausal women contained significant levels of the specific estrogen receptor, whereas at least 60% of the tumors removed from women 55 years of age or older demonstrated elevated estrogen-binding capacity. Data such as those presented in Chart 5 indicate that there appears to be a relationship between the level of specific estrogen receptors in infiltrating ductal carcinoma and the age of the patient at the time of surgery. Whether these results reflect endogenous levels of estrogen will have to await additional analyses. However, since a considerable number (19 of 49) of tumors from postmenopausal women did not demonstrate significant binding capacity, the presence of the receptor may be the expression of a phenotype characteristic of hormonally responsive tissues. A relationship of specific receptor concentration of a tumor with the presence of metastases at the time of surgery was not obvious, nor was there a relationship between binding capacity and the percentage of carcinoma cells in the specimen examined.

One of the major purposes of these studies is to seek correlation between the presence of specific estrogen receptors and clinical course so as to test the hypothesis that prediction of responsiveness may be made on the basis of each individual patient. If a predictive test is to be established, one would anticipate a relatively high success rate (regression) in those patients whose neoplasms were categorized as hormone dependent, in contrast to those whose tumors were predicted to be hormone independent. According to Jensen et al. (16), the 36 patients presenting with carcinomas lacking specific receptors should, in all probability, not be subjected to endocrine organ ablative surgery. However, a large portion of the 39 patients whose tumors demonstrated the capacity to bind estradiol-17β specifically should receive benefit from such procedures. In a later publication, we will report the clinical course of these patients, in addition to the levels of estrogen receptors in recurrent disease, an event which will ensue as the time from the original surgery approaches 2 years or more.

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Specific Estrogen-binding Capacity of the Cytoplasmic Receptor in Normal and Neoplastic Breast Tissues of Humans

James L. Wittliff, Russell Hilf, William F. Brooks, Jr., et al.


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