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Pathological Review of Breast Lesions Analyzed for Estrogen Receptor Protein[1]

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SUMMARY

This report provides a detailed pathological review of 333 specimens analyzed for estrogen receptor protein (ERP) and correlates a series of morphological features with ERP results. Included were 147 primary breast carcinomas, 78 metastases, 27 fibroadenomas, and 81 nonneoplastic tissues, all from women. ERP in cytosols was assayed by incubation with [^{3}H]estradiol in the presence and absence of "cold" estradiol followed by dextran-charcoal treatment. Results were summarized as positive (>60% inhibition by nontritiated estradiol, >10 fmoles/mg protein), negative (<60% inhibition by nontritiated estradiol, <10 fmoles/mg protein), or intermediate borderline combinations. ERP in primary tumors ranged from 0.2 to 358 fmoles/mg protein (54.4% positive, 35.4% negative, 10.2% borderline). New findings are: (*a*) a high frequency of positive ERP in invasive lobular carcinoma (12 of 13, 92.3%) compared to typical ductal tumors (64 of 117, 54.7%); and (*b*) low frequency of positive ERP (5 of 21, 23.8%) in tumors with a prominent local lymphocyte reaction. Three ERP-positive noncarcinomatous specimens were fibroadenomas of high epithelial cellularity from patients under 30 years. No statistically significant relationship existed between ERP and any other morphological features that were examined.

INTRODUCTION

Since the demonstration of ERP[3] (8) in primary and metastatic mammary carcinoma, several investigators have studied the relationship between ERP in neoplastic breast tissues and the effectiveness of hormonal therapy as a method for controlling metastatic carcinoma (3, 6, 18, 19, 24, 29). Although virtually all breast tissues have some measurable ERP, only those with more than specified amounts are termed ERP positive. Those with lesser ERP levels are designated as ERP negative. It has been observed that ERP-positive metastatic lesions are more likely to respond to hormonal therapy than are those that are ERP negative. However, not all ERP-positive lesions are responsive when judged by clinical evaluation.

When morphological characteristics of the tumors were examined in prior reports, there appeared to be no consistent relationship between specific histopathological features of the tissues and the level of ERP (1, 8, 14, 21-23, 28, 29, 31, 35). Unfortunately, the various investigators did not examine the same morphological characteristics or use uniform terminology. Methods of ERP analysis differed from one study to another. Consequently, it is difficult to compare the results of these studies or to combine them in developing a more comprehensive view. For these reasons and also the fact that some pathological features of mammary carcinoma that seem to be of prognostic significance have not yet been correlated with ERP, we have prepared the following report.

As part of an ongoing study of ERP in mammary tissues we undertook a detailed pathological examination of 333 specimens that were analyzed between 1972 and 1974. Our purpose was 2-fold: (*a*) we wished to know whether any morphological features of the tissues related in a statistically significant manner to the ERP level; and (*b*) it was felt that 1 or more morphological observations might subsequently prove useful when combined with data about ERP levels in more accurately predicting the responsiveness of metastases to hormonal therapy. This paper presents only the results of relating ERP levels and morphology of the tissues studied. Data regarding therapy will be the subject of a later report.

MATERIALS AND METHODS

The following specimens were examined: 147 primary breast carcinomas, 78 metastatic lesions in various anatomic sites, 81 specimens of non-neoplastic breast tissue from patients with or without cancer, and 27 fibroadenomas. All specimens were from women.

Most samples for ERP analysis were selected at the time of surgery by a pathologist who received the tissue for frozen section. Small numbers of metastatic lesions were delivered to the biochemistry laboratory directly from the operating room by messenger. All tissues were received

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[3] The abbreviations used are: ERP, estrogen receptor protein; TES, *N*-Tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid.

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within 5 min after being excised and were stored in labeled clean Petri dishes on ice in a closed Styrofoam container in a refrigerator and collected daily for analysis. After being trimmed of fat, samples were weighed, minced, homogenized with a Polytron homogenizer in 4 volumes of 10 mM of TES-12 mM thioglycerol buffer (pH 8.0) containing 250 mM sucrose, and centrifuged at 105,000 × *g* for 1 hr. The homogenization was carried out in an ice bath using three 15-sec pulses with 45-sec pauses between pulses for cooling. Aliquots of the supernatant or cytosol were analyzed for ERP and protein concentration.

ERP was determined by a competitive inhibition assay in which the cytosol was incubated with [^{3}H]estradiol in the presence or absence of either unlabeled estradiol or an estrogen antagonist. Unbound [^{3}H]estradiol was removed from the mixture with dextran-coated charcoal. Radioactivity of the sample was measured in a Packard scintillation counter. The concentration of ERP was calculated from the binding data with 1 nM [^{3}H]estradiol in the absence and presence of 1 μM cold estradiol or 20 μM of the estrogen antagonist CI628 expressed as fmoles/mg of cytosol protein. The cytosol was also incubated with 2 and 5 nM [^{3}H]estradiol and a dissociation constant was obtained from a Scatchard plot. Results were summarized as positive (>10 fmoles/mg protein, $>60\%$ inhibition), negative (<10 fmoles/mg protein, $<60\%$ inhibition), or borderline (intermediate combinations). Protein concentration was determined on an aliquot of cytosol by the method of Waddell (34), which related protein concentration to the difference in UV absorption at 215 and 225 nm. Negative and borderline ERP results were grouped together for purposes of analysis. When analyzed separately, the small numbers of borderline cases did not significantly alter any of the correlations.

Pathological features of the lesions were reviewed by one of the authors (P. P. R.) who did not know the results of biochemical analysis. Observations were based on examination of the routine slides, including the frozen section made at the time a sample was selected for ERP analysis, and the gross description of the specimen. The following observations were recorded; age of the patient; anatomic source of specimen (breast or site of metastasis); measured size of primary tumor in cm; histological classification; grade as an estimate of differentiation on a scale of 1 (well differentiated) to 4 (undifferentiated); presence or absence of axillary metastases; number of lymph node metastases; size of metastases (17) in lymph nodes (micro, less than 2 mm; macro, greater than 2 mm in diameter in section); presence or absence of lymph node hyperplasia (markedly increased number and/or size of germinal centers); invasion of lymphatic spaces in the breast by primary carcinoma; contour of primary carcinoma (circumscribed or irregular); amount of lymphoid reaction in and around primary carcinoma estimated microscopically on a scale of 1 (few or no lymphocytes or plasma cells) to 4 (very dense infiltrate); and epithelial cellularity of the tissue (estimated as the percentage of area of tissue in available sections that was occupied by tumor cells, expressed in arbitrary units of 10 from less than 10% to 90% or more).

RESULTS

The results are summarized in Table 1. Data relating to specific observations are given in Chart 1, Tables 2 to 9, and the following text.

Age of Patient. The ages of patients from whom primary carcinomas were obtained for analysis ranged from 29 to more than 70 years. Positive tumor ERP levels were found in 46% of 46 specimens from patients less than 50 years of age, in 57% of 49 specimens from patients 50 to 60 years of age, and in 58% of 52 tumors from patients more than 60 years old. Overall, 54.4% of the 147 primary tumors were found to have a positive ERP level.

Size of Primary Carcinoma. The relationship between size of the primary tumor and ERP is shown in Chart 1. On analysis of the data, the relationship of these observations is

Table 1
Summary of results

Pathological observation	Apparent relationship with ERP
Histological type of primary	Higher frequency positive ERP in lobular than in duct or other types
Lymphoid infiltrate in primary	Frequency of positive ERP decreases as intensity of lymphoid reaction increases
Epithelial cellularity of primary	Sparsely cellular tumors rarely have very high ERP levels; otherwise unrelated
Cellularity of metastasis	Unrelated for skin and lymph nodes; too few specimens at other sites
Fibroadenomas	11% had positive ERP. Tend to be younger patients with more cellular fibroadenomas
Other "benign" tissues	All negative ERP
Grade of primary	Unrelated
Presence or absence of axillary metastases	Unrelated
Size of axillary metastases	Unrelated
No. of axillary nodes with metastases	Unrelated
No. of axillary nodes examined	Unrelated
Presence or absence of follicular hyperplasia of axillary nodes	Unrelated
Lymphatic invasion in breast	Unrelated
Margins of primary, circumscribed or infiltrating	Unrelated
Site of metastasis	Unrelated

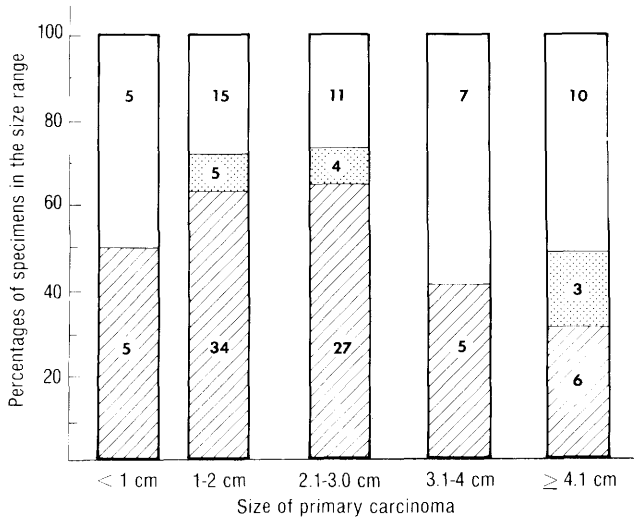

Chart 1. Relationship of size of primary breast carcinoma to ERP. *Numbers within bars*, absolute number of cases. ▨, ERP positive; ⊡, ERP borderline; □, ERP negative.

not statistically significant in terms of the categories of size shown.

Histological Type of Primary Carcinoma. Twelve of 13 specimens (92.3%) of lobular carcinoma had a positive ERP (Table 2). By contrast, a positive ERP was found in 64 of 117 specimens of infiltrating duct carcinoma (54.7%). The difference in the frequency of positive ERP between these types of carcinoma is statistically significant ($p < 0.05$). The few cases of medullary and colloid carcinoma, which are essentially ductal in origin, had a lower frequency of positive ERP than did lobular carcinomas. The medullary tumors also had a considerably lower frequency of positive ERP than did typical ductal carcinomas. Four specimens of tubular carcinoma, a special type of low grade, infiltrating duct carcinoma, had negative ERP levels. The numbers of cases in each of these groups of specialized carcinoma are too few for conclusive evaluation in regard to the relationship of ERP to tumor type. The age distribution among patients with duct and lobular carcinoma was not significantly different.

Histological Grade of Primary Carcinoma. As shown in Table 3, there was a tendency for a positive ERP to occur more often in low grade infiltrating ductal carcinomas, but the difference between low and high grade tumors was not statistically significant. Special types of carcinoma (lobular, medullary, colloid, tubular) were not graded and were not included in this correlation.

Lymphocytic Reaction to Primary Carcinoma. Data presented in Table 4 show that 64% of 117 lesions with a mild lymphocytic infiltrate (+ or ++) had a positive ERP compared to 19.2% of the 26 tumors in which the reaction was more intense (+++ or ++++). This inverse relationship was statistically significant when medullary carcinomas were included ($p < 0.001$) and remained so when the 8 medullary carcinomas were excluded ($p < 0.001$).

Epithelial Cellularity of Primary Carcinoma. The level of ERP activity was positive in 43% of tumors with low cellularity, in 60% with moderate cellularity, and in 52% of highly cellular tumors (Table 5). The differences were below the level of statistical significance. However, only 5% of lesions with low cellularity (less than 10%) had more than 25 fmoles/mg cytosol protein, which is 2.5 times the minimum required for positive. Among more cellular tumors, 37% to 55 % were found to contain more than 25 fmoles/mg cytosol protein.

Distribution of Axillary Lymph Node Metastases. There was no relationship between the presence or absence of axillary lymph node metastases and the ERP level in the primary carcinoma. Axillary lymph nodes were available for histological review in 140 of the cases. The ERP was positive in 53% of the 71 cases with negative axillary nodes and in 56% of the 69 cases with axillary metastases. Positive ERP was found in primary carcinomas from 52% of patients with metastases at level I in the axilla (lateral to pectoralis minor muscle), in 53% with level II metastases (beneath pectoralis minor), and in 65% when metastases were present at level III (medial to pectoralis minor muscle).

Characteristics of Axillary Metastases. Three features of axillary metastases were analyzed: (*a*) size of largest metastasis. Positive levels of ERP were found in primary tumors from 56.3% of 64 women with no metastases, 55.6% of 27 women in whom all metastases were less than 2 mm in diameter (micrometastasis), and in 53.2% of 47 patients in whom the largest metastasis was more than 2 mm in

Table 2

Relationship of histological type of primary carcinoma and results of ERP analysis of tissue

Histological type	No. of cases	ERP			
		Positive		Negative	
		No.	%	No.	%
Duct[a]	117	64	54.7	53	45.3
Lobular[b]	13	12	92.3[c]	1	6.7
Medullary	8	2	25	6	75
Colloid	5	2	40	3	60
Tubular	4	0	0	4	100
	147				

[a] One case intraductal; others infiltrating.
[b] All infiltrating.
[c] $p < 0.05$.

Table 3

Relationship of histological grade of primary carcinoma and results of ERP analysis of tissue

Grade	No. of cases	ERP			
		Positive		Negative	
		No.	%	No.	%
1–2	65	40	62	25	38
3–4	55	26	47	29	53
	120				

Table 4
Relationship of lymphocytic infiltrate in primary carcinoma and ERP analysis of tissue

Lymphocytic reaction to primary carcinoma		ERP level			
		Positive		Negative	
Intensity	No. of cases	No.	%	No.	%
+ and ++[a]	117	75[b]	64	42	36
+++ and ++++	26 (18)[c]	5[b] (3)	19.2	21 (15)	79.8

[a] + and ++, minimal and mild reaction; +++ and ++++, moderate and intense reaction.
[b] $p < 0.001$.
[c] Numbers in parentheses, figures when medullary carcinomas are excluded; $p < 0.001$.

Table 5
Relationship of estimated epithelial cellularity of primary carcinoma and ERP analysis of tissue

Estimated epithelial cellularity		ERP			
		Positive		Negative	
%	No. of cases	No.	%	No.	%
<30	28	12	43	16	57
30-69	91	54	60	37	40
70-100	27	14	52	13	48

diameter (macrometastasis); (*b*) number of lymph nodes containing metastases. When patients were ranked according to the number of lymph nodes containing metastases, the frequency of positive ERP in the primary tumor ranged from 25 to 71.4%, but no pattern was observed relating ERP to number of lymph nodes containing metastatic carcinoma; (*c*) number of lymph nodes isolated from axillary dissection. Among patients ranked by the total number of axillary lymph nodes, the pecentage with positive ERP levels varied from 44.4 to 60%. No consistent relationship between ERP and the number of axillary lymph nodes was observed.

Lymph Node Hyperplasia. Marked follicular hyperplasia of lymph nodes was present in 26 cases. Eleven or 42.3% had primary tumors that were ERP positive. Fifteen (57.7%) had ERP-negative tumors. Follicular hyperplasia was present in 13.7% of 80 patients with a positive ERP level and in 22.3% of 67 women with a negative tumor ERP.

Lymphatic Invasion in Breast. Invasion of lymphatic spaces within the breast was found in 21% of mastectomies. It was observed in 18.8% of 80 patients with a positive tumor ERP level and in 23.9% of the 67 with negative tumor ERP levels. Among the 31 specimens in which intralymphatic tumor was found, 48.4% had a positive ERP compared to 56.5% of 115 carcinomas with no demonstrable intralymphatic tumor.

Margins of Primary Carcinoma. Of 49 tumors with circumscribed smooth margins, 51% had positive ERP levels; 59.1% of 93 tumors with infiltrative margins had positive tumor ERP levels. Well circumscribed or smooth margins were found in 31.3% of 80 tumors with positive ERP levels and in 39.9% of 67 lesions with negative ERP levels.

Site of Metastases and ERP in Metastatic Carcinoma. Positive ERP levels were found in 34 of 78 (43.6%) of the metastases analyzed. Substantial numbers of specimens were available only for lymph node (29) and skin (30) metastases, and in these the frequency of positive ERP was very similar (44.8% in lymph nodes and 40.0% in skin). Other sites from which metastases were obtained for analysis were bone (1), breast (4), omentum (3), ovary (4), lung and pleura (3), noncutaneous soft tissue (1), liver (2), and adrenal (1).

Cellularity of Tumor in Metastases. As shown in Table 6, metastatic lesions estimated to be composed of less than 30% tumor cells had a low frequency of positive estrogen-binding level (6.3% of 16 specimens). A higher frequency of positive ERP was observed in more cellular metastases, ranging from 52% in tissues estimated to contain 30 to 69% tumor cells to 55% in those judged to consist of more than 69% tumor cells.

Fibroadenomas. Positive ERP levels were found in 3 of 27 (11.1%) fibroadenomas analyzed. None of the ERP positive fibroadenomas was among the 20 tumors estimated to have less than 30% epithelial cellularity. One fibroadenoma with positive ERP level was among the 3 rated as having more than 70% epithelial cellularity. Two had a 20% and 69% cellularity. All 3 ERP-positive fibroadenomas were among the 9 obtained from patients less than 25 years of age. None of the 18 fibroadenomas from patients more than 25 years old was ERP positive.

Other Noncarcinomatous Specimens. None of the other

Table 6
Relationship of estimated epithelial cellularity of metastases and ERP analysis of tissue

Estimated epithelial cellularity		ERP level			
		Positive		Negative	
%	Total	No.	%	No.	%
<30	16	1	6.3	15	93.7
30-69	29	15	52.0	14	48.0
70-100	33	18	55.0	15	45.0

81 noncarcinomatous specimens had a positive ERP level. These were histologically "benign" tissues from the same or opposite breasts of women with breast carcinoma and specimens from women with no carcinoma. Included were 6 with atypical duct hyperplasia and 3 with atypical lobular hyperplasia. Fibroadenomas, described above, are not included in this category.

Relationship of Specimen Weight and Protein Concentration of Cytosol to ERP. The weight of individual trimmed specimens of carcinoma, either primary or metastatic, varied from 40 mg to 12.5 g. Table 7 shows the relationship between ERP and specimen weight. The majority of specimens (76%) for which weights are available weighed less than 2 g. ERP-positive specimens were especially frequent among those that weighed 1 g or less.

The relationship of cytosol protein concentration to ERP is shown in Table 8. Four of 10 (40%) specimens with protein concentration less than 2.1 mg/ml were ERP positive. Among specimens with a protein concentration greater than 2.1 mg/ml, 116 of 243 (47.5%) were ERP positive.

DISCUSSION

Successful treatment of mammary carcinoma in postmenopausal women by adrenalectomy was reported nearly 25 years ago (15), and it has been known since 1896 that premenopausal patients may benefit from oophorectomy (2). Because patients with similar clinical situations do not always respond to appropriate ablative or hormonal treatment, there has been considerable interest in finding methods that would permit more accurate selection of therapy for individual patients.

Recognizing that organs that normally respond to estrogens also concentrate the hormone, Folca *et al.* (9) studied the *in vitro* accumulation of tritiated estrogen in human mammary carcinoma and correlated their findings with the clinical response of metastatic carcinoma to treatment. Four patients in whom the concentration of tritiated hormone in tumor was at least 3 times greater than that in their own muscle or blood improved after ablative treatment. No improvement was seen in 6 others whose tumors did not concentrate radioactive hexestriol to the same extent. Subsequent studies showed that estrogen and other steroid hormones are bound by receptor proteins (ERP) that can be recovered in the supernatant or cytosol obtained after ultracentrifugation of tissue homogenates. Steroid hormones differ in this regard from polypeptide hormones, which are bound by membrane-associated rather than cytoplasmic receptors.

Initial observations relating the ERP content of tumor tissue with response to hormone therapy (20) suggested that the vast majority of patients whose carcinomas were ERP positive would have a favorable response to treatment for recurrent or metastatic carcinoma. Little or no improvement was observed in those with an ERP-negative carcinoma. As additional experience has accumulated, the latter conclusion has remained true, but it now appears that the favorable response rate among patients with ERP-positive tumors is somewhat lower than initially thought and varies from 50 to 60% (18).

The ERP determination can be helpful in determining appropriate treatment for patients with breast carcinoma. It is especially important to know that the ERP is negative, since these patients are not likely to benefit from hormonal therapy and may be treated initially by chemotherapy or radiation.

Table 7

Relationship of weight of specimen with result of analysis for ERP

	Specimen wt (g)													
	<0.5 (61)[a]		0.5–1.0 (57)		1.1 1.9 (52)		2.0–3.0 (26)		3.1–4.0 (15)		4.1–5.0 (7)		>5.0 (6)	
ERP	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Positive	35	57	29	51	24	46	8	32	6	40	2	30	3	50
Negative	18	30	22	39	25	48	17	65	8	53	5	70	2	33.3
Borderline	8	13	6	10	3	6	1	3	1	7	0		1	16.6

[a] Numbers in parentheses, total number of cases.

Table 8

Relationship of ERP to protein concentration of cytosol

	Cytosol protein concentration (mg/ml)									
	<2.1 (9)[a]		2.1–3.0 (19)		3.1–4.0 (15)		4.1–5.0 (25)		>5.1 (165)	
ERP	No.	%	No.	%	No.	%	No.	%	No.	%
Positive	4	44	8	42	7	46	17	61	74	45
Negative	4	44	7	37	4	27	6	24	82	49
Borderline	1	12	4	21	4	27	2	15	9	6

[a] Numbers in parentheses, total number of cases.

Studies of hormone therapy for metastatic mammary carcinoma have not revealed a consistent relationship between the histological characteristics of the tumor and response to ablative treatment (10 12). While 2 studies failed to find any correlation between histological type of the primary tumor and response to adrenalectomy (12) or oophorectomy (11), a 3rd revealed an improved response rate (57%) associated with medullary carcinoma when compared to lobular (32%) and duct (about 31%) carcinomas (10).

A review of the available reports, summarized in Table 9, failed to show a relationship between morphological features of a primary carcinoma and its ERP content. However, our own data indicated a highly significant association between positive ERP and invasive lobular carcinoma. This uncommon, histologically distinctive type of carcinoma that develops in the terminal duct and lobular portion of the mammary gland epithelium constituted 9% of all carcinomas studied. Patients with lobular carcinoma have not shown a particularly favorable response to ablative hormone therapy (10-12).

The high frequency of positive ERP in invasive lobular carcinoma may reflect a special selective sensitivity of this distal segment of the duct to estrogens and other hormones. In ultrastructural studies, the cells of lobular carcinoma are found to have numerous microfilaments, a feature associated with myoepithelial cells rather than with ductal carcinoma cells (4, 32). It is in this portion of the duct that the physiological lobular hyperplasia associated with lactation occurs (26). Evidence for a selective sensitivity of this region of the duct system can be drawn from morphological studies by Huseby and Thomas (16) of breast tissue of postmenopausal women who received high-dose estrogen therapy for breast cancer and of men with prostatic carcinoma who were treated with synthetic estrogens (30). In both situations acinar differentiation is a prominent aspect of the epithelial hyperplasia that occurs. A less marked effect has been observed in women receiving lower doses of estrogen for menopausal or postmenopausal symptoms (7).

In Huseby and Thomas' study of women with breast carcinoma who were treated with estrogens (16), there was

Table 9

Summary of prior reports correlating histopathology and ERP in primary breast carcinomas

Author(s) and year reported	Histopathology-ERP Correlation					
	Histopathology of primary tumor	Cellularity of tissue	Grade	Stage nodes (metastases present or absent)	Benign tissues	Age
Sander, 1968 (28)	25 primary: NC[a]					NC
Korenman and Dukes, 1970 (22)	14 duct { 4 (+)[b] / 1 (+/−) / 7 (−) } 1 colloid: (+/−)					All (+)PM[b]
Johansson *et al.*, 1970 (21)	Primary: NC		NC	NC		NC
Feherty *et al.*, 1971 (8)	Primary: NC	NC	NC		3 of 12 FA(+); histology not different	Higher %(+)PM
Wittliff *et al.*, 1971 (35)	73 duct 1 colloid 1 medullary } NC	NC		NC		Higher %(+)PM
Hahnell *et al.*, 1971 (14)	Primary: NC		NC	NC		Higher %(+)PM
LeClerq *et al.*, 1973 (23)	65 duct 7 lobular 3 Paget 1 medullary 1 intraductal } NC			NC		NC
Terenius *et al.*, 1974 (31)	Higher ERP, in "ductal" *versus* "adenocarcinoma": NC	Higher ERP in medium *versus* high and low cellularity	NC	NC	3 of 12 FA(+); histology not different	Higher %(+)PM
Aspegren and Hakansson, 1974 (1)	Primary: NC			NC		NC

[a] NC, author reported no correlation; PM, postmenopausal; FA, fibroadenoma.

[b] +, positive ERP; −, negative ERP; (+/−), borderline.

no correlation between the degree of differentiation of nonneoplastic lobules and regression of tumor. Lobular proliferation in the breasts of women whose carcinomas regressed with therapy did not differ appreciably from lobular growth in women with unresponsive tumors. It appeared that doses of estrogenic steroids that were substantially above physiological levels caused regression of carcinoma while simultaneously producing lobular proliferation in the same individual. Although a number of hypotheses have been suggested,[1] this seeming contradiction remains unexplained.

It is not unreasonable to speculate that there may be a gradient of ERP in the normal lobule-duct structures of the breast with the maximal concentration/cell at the lobule end. Since carcinomas can arise from or involve different segments of the ducts and lobules, variable sites of origin could influence the ERP content of neoplastic cells in a given tumor.

Since the majority of ERP positive carcinomas respond to hormonal therapy, it is generally assumed that ERP is somehow involved in hormone-related regression of carcinoma. On the other hand, the same receptor protein, or a similar one, is undoubtedly present in normal tissues that proliferate in response to estrogenic stimulation. The differences in response may therefore not simply depend on the absolute level of ERP in the tissue as a whole but also on the interaction of this system with other hormones (5, 27) or with other binding proteins (33) in individual cells.

Another factor that correlated in a statistically significant way with ERP was the intensity of lymphoid infiltration in or around the primary carcinoma. Tumors with the least lymphoid infiltrate most often had a positive ERP. Since all lobular carcinomas had little or no lymphoid reaction and had a high frequency of ERP positivity, the factor or factors responsible for the amount of lymphoid reaction could be partly responsible for the ERP findings in lobular carcinomas.

There was no readily apparent explanation for the observation that tumors with the most intense lymphoid reaction had the lowest frequency of ERP positivity. This does not simply reflect a bias that might be due to inclusion of medullary carcinomas, since the significance of the relationship remained unchanged even when medullary tumors were excluded. Conversely, the fact that only 25% of the medullary carcinomas were ERP positive, compared to 55% of infiltrating duct carcinomas, probably reflects the abundant lymphoid component of medullary tumors. On the basis of a small group of carcinoma specimens in which ERP was measured with and without admixed grossly normal axillary lymph nodes from the same patient, it appears that lymphocytes *per se* do not interfere with the measurement of ERP.

It has been suggested that the intensity of the lymphoid and plasmacytic reaction in or around a primary mammary carcinoma may represent an immunological reaction to tumor antigens. More prominent lymphoid reactions exemplified by medullary carcinoma have been associated with a favorable prognosis, and in at least 1 study (9) patients with advanced medullary carcinoma had a better than average response to ablative hormone therapy. Conceivably, an immunological reaction the intensity of which is reflected by the specific lymphoid response in a given tumor interferes with measurement of ERP, but the role that ERP itself may play in the immunology of breast cancer is obscure and there is as yet no information on the relationship of ERP to prognosis.

We did not find a significant relationship between ERP and the following morphological factors of prognostic significance: size of the primary tumor, histological grade or degree of differentiation, presence or absence of axillary lymph node metastases, the number or size of axillary nodal metastases, the level of lymph node spread, the presence or absence or lymphatic vessel invasion in the breast itself, follicular hyperplasia of axillary lymph nodes, and tumor margins, whether well circumscribed or invasive.

Since most or all ERP probably resides in epithelial rather than stromal cells, it would be reasonable to expect some correlation between the epithelial cellularity and the ERP content of a tumor. If true, this would provide a convenient explanation for the very low concentrations of ERP found in nonneoplastic tissues (13, 14), since they invariably have a low epithelial cellularity when compared to carcinomas. In our material the only noncarcinomatous tissue with ERP levels sufficient to be classified as positive were 3 fibroadenomas. These were obtained from younger women and tended to have an abundant epithelial component. Although other specimens from equallly cellular fibroadenomas were ERP negative, the findings suggest that epithelial cellularity and ERP content may be related in noncarcinomatous tissues or at least in fibroadenomas.

If the same relationship were true for carcinomas, scirrhous tumors should, on average, have less ERP/volume of cytosol than very cellular ones. Terenius *et al.* (31) found that more cellular tumors tended to have a higher estrogen-binding capacity but that the difference in binding between tumors of high and low cellularity was not statistically significant. Using more elaborate methods, including planimetry of magnified tissue sections, Wittliff *et al.* (35) failed to find a consistent relationship between the ERP content and epithelial cellularity of mammary carcinomas. In general, our observations support this latter conclusion. Since it appears that cellularity does not influence the overall classification of ERP activity as positive or negative, in most cases it is possible that the average ERP/cell is higher in less cellular specimens. However, the low frequency of ERP positivity in very hypocellular metastases may be an indication that, at this extreme of the spectrum of cellularity, the scarcity of tumor cells could be responsible for a falsely negative assay.

One morphological characteristic of many carcinomas is cellular pleomorphism. It would not be surprising to find that cells comprising a carcinoma are heterogeneous in biochemical functions, including ERP. Methods of analysis based on the ERP content in the cytosol obtained from homogenized carcinomatous tissue mask any variation in the distribution of ERP among individual cells. Such variation seems probable in view of the observation that, in a small percentage of patients, there may be substantial

differences in the ERP content of various metastases or between the primary and metastases (31). A clearer understanding of the distribution of receptor proteins within individual cells is of more than academic interest (25), since this variability might be 1 reason for the variable response to therapy obtained even with ERP-positive tumors.

The suggestion that a low cytosol protein concentration may cause false negative ERP determinations (23) led us to relate the ERP data obtained in this study to specimen weight and cytosol protein concentration. Since there was no correlation between the size of primary carcinomas and ERP, the decreasing percentage of ERP-positive specimens among larger samples suggests that some benign tissue may have been incorporated into and intermixed with the carcinomatous tissue. Despite this trend in specimen weight, the frequency of ERP positivity did not seem to be affected by cytosol protein concentration.

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