Sampling Procedures in Estrogen Receptor Determinations

JAMES F. NEWSOME, M.D.,* FREDERICK P. AVIS, M.D.,* JOHN E. HAMMOND, PH.D.,† SUSAN SHERWOOD, B.S.‡

To determine the significance of proteolysis and delayed freezing of tumor samples on estrogen receptor levels, values from 19 of 31 biopsy specimens were compared with that in remaining tumor at the completion of mastectomy. There was a 15–100% decrease in receptor content. Time-decay studies on selected postmastectomy samples showed a further decrease in estrogen receptor content inversely proportional to the time it was exposed to room temperature. Factors that govern the valid measurement of receptor levels include tumor cell concentration, tumor necrosis, and time between devascularization of the specimen to freezing. A carefully procured histologically confirmed sample of *fresh* tumor is necessary for reliable estrogen receptor values.

From the Departments of Surgery, Pathology and Hospital Laboratories, University of North Carolina School of Medicine, Chapel Hill, North Carolina

THE ROLE OF STEROID hormone receptors in the management of hormone-dependent tumors is well-established. Among the various parameters that may influence the measurement of receptors in tumor tissue are the thermal lability and the proteolytic susceptibility of the receptor hormone complex.[4] To evaluate the influence of the time of sample procurement and the significance of time lapsed between devascularization of the tumor to specimen freezing, the following study was undertaken. Estrogen receptor levels were determined from histologically confirmed adenocarcinoma of the breast frozen immediately after biopsy and compared with levels taken from the same cancer at the completion of the mastectomy.

Procedure and Methods

An appropriate sample of breast tumor was procured at open biopsy under 1% lidocaine anesthesia, administered locally, in the Outpatient Department. The presence of cancer was confirmed by immediate frozen section. Following the removal of fat, grossly necrotic and hemorrhagic tissue, the remaining sample was rapidly frozen in liquid nitrogen and submitted for determination of estrogen receptor concentration. These results were compared with those determined from samples of the same tumor taken at the time of completion of the mastectomy. After the determination of a significant decrease in receptor levels in four of five mastectomy specimens when compared with biopsy specimens (Table 1), time–decay studies of the mastectomy specimens were undertaken. The initial study yielded inconsistent values, presumably the result of varying tumor density[1] within the specimen studied (Table 2). Subsequently, the remaining tumors in the mastectomy specimens were minced into 1–2 mm portions, thoroughly mixed, and random samples frozen at 15 minute intervals for subsequent measurement of receptor levels (Fig. 1).

Tissue specimens were stored at −70 C until the time of analysis. On the day of analysis the specimens were thawed, freeze fractured and homogenized. Cellular debris were removed by ultracentrifugation and the resulting cytosol was diluted to a final protein concentration of approximately 2.0 mg/ml. A five-point dextran-coated charcoal saturation analysis was performed. Scatchard data analysis was done and the best line was obtained by least squares regression analysis. All specimens showed dissociation constants (Kd) of less than 10^{-10} moles/L and binding capacity was expressed as femtomoles (10^{-15} moles) of ^{3}H estradiol per milligram of cytosol protein. The data presented in this paper show changes in estrogen receptor content between multiple specimens analyzed in the same assay. We find that the intra-assay and interassay coefficients of variation are approximately ±10 and ±15%, respectively.

In our laboratory, levels above 10 femtomoles per milligram of cytoplasmic protein are considered positive, 3–10 femtomoles, borderline, and below 3 femtomoles, negative. Those tumors found at initial biopsy

Presented at the Annual Meeting of the Southern Surgical Association, December 8–10, 1980, The Breakers, Palm Beach, Florida.

* Department of Surgery.

† Departments of Pathology and Hospital Laboratories.

‡ Hospital Laboratories.

Supported in part by PHS Grant CA 17973, National Cancer Institute, DHHS and the Kurt Appert Cancer Research Fund.

Reprint requests: James F. Newsome, M.D., Department of Surgery, University of North Carolina School of Medicine, Chapel Hill, North Carolina 27514.

Submitted for publication: December 30, 1980.

0003-4932/81/0500/0549 $00.80

TABLE 1. *Estrogen Receptor Content—Femtomoles/mg Cytosol*

Patients	Biopsy Specimens	Mastectomy Specimens
1	15.9	5.9
2	186.5	149.4
3	20.3	7.0
4	181.5	153.5
5	7.5	7.8

TABLE 2. *Time–Decay Study of Estrogen Receptor Concentration in Breast Cancer*

Estrogen Receptor Content—Femtomoles/mg Cytosol						
		Mastectomy specimens				
		(Minutes)				
Patients	Biopsy Specimens	15	30	60	90	120
6*	123	×	184	316	244	240
7	43	15	17	8	5	3
8	5	3	7	3	4	3
9	9	<3	<3	<3	<3	<3
10	171	11	—	(Inadequate Tissue)		—
11	89	17	7	7	9	11
12	218	305	267	268	182	112
13	56	<3	—	(Tissue Necrotic)		—
14	37	<3	<3	9	3	16
15	5	3	4	3	<3	<3
16	21	<3	<3	<3	<3	4
17	4	3	<3	<3	<3	<3
18	5	5	×	<3	<3	×
19	5	39	—	(Inadequate Tissue)		—

* Time studies without mincing technique.
×: Not done.

to be receptor-negative were not further studied. Both biopsy and mastectomy specimens were frozen within 15 minutes of the time of removal. Care was taken not to expose the tumor to electrocoagulation.[3] Most patients underwent a modified radical mastectomy from one to seven days following biopsy, with two exceptions, who, because of medical and personal reasons, underwent mastectomy 21 and 26 days, respectively, following biopsy. The mean time from the beginning of mastectomy to harvesting the specimen for receptor determination was approximately two and one-half hours.

Results

During the period of this study, 59 patients were proved at open biopsy to have previously untreated breast cancer. Thirty-one patients had adequate amounts of cancer remaining in the mastectomy specimen to be included in this study. Nineteen patients (61%) had receptor levels of 3 femtomoles or greater, seven (35%) of which were in the borderline range (3–10 femtomoles). Though the receptor concentration in this latter group is such as to question the results,

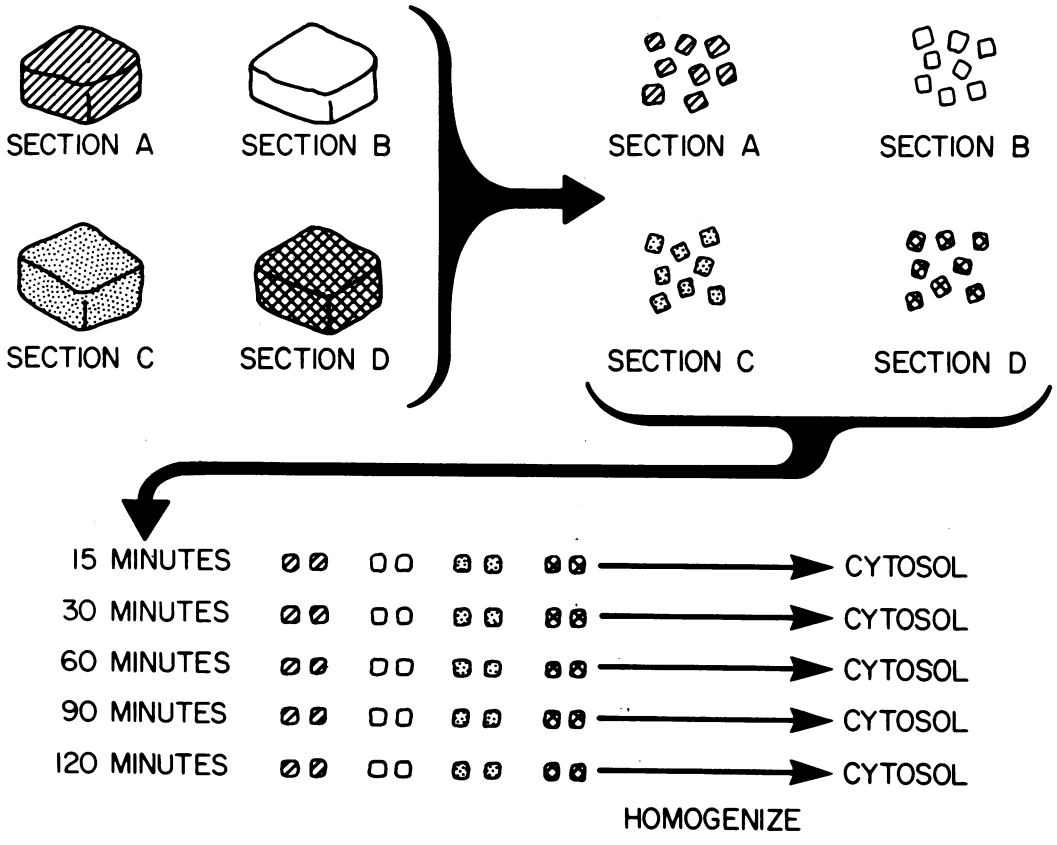

FIG. 1. Technique for preparation of postmastectomy tumor samples for time–decay studies.

they were, nevertheless, studied. The sample from one such patient (#19) was shown to have significant receptor concentration in the mastectomy specimen, though the biopsy specimen was low borderline. The results of the 19 studies are depicted in Tables 1 and 2.

Of the paired specimens which contained a biologically significant amount of estrogen receptors (greater than 10 femtomoles per milligram), ten (83%) showed a decrease and two (16.6%) increased in receptor content (Fig. 2). Because of technical reasons, the 15 minute postmastectomy receptor level in one (Number 6) was not determined and is thus not included. It was the variability of results in this time–decay study that suggested the mincing techniques used subsequently.

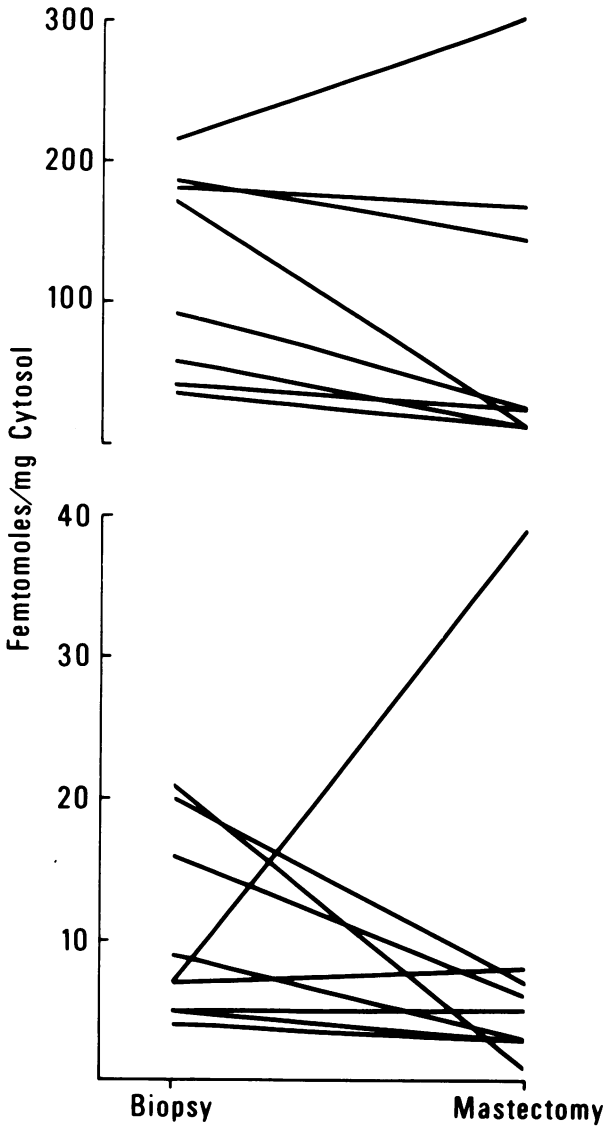

FIG. 2. Changes in estrogen receptor concentration, biopsy vs. mastectomy specimen in the 18 studied patients. (Patient number 6 not included; two samples had identical values.) Note the difference in scale of femtomoles per milligram of cytosol.

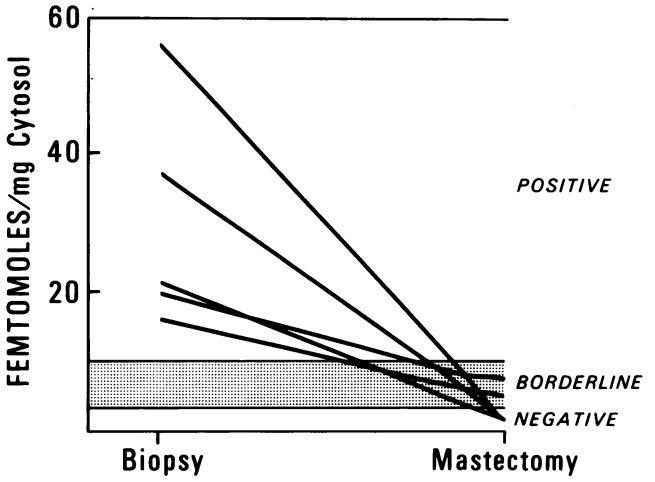

FIG. 3. Estrogen receptor concentration, biopsy vs. mastectomy specimen in selected patients. Five patients would have been judged borderline or negative had the postmastectomy values been relied upon.

Changes in estrogen receptor levels in tumor samples from 11 patients demonstrated a reduction from the biopsy to postmastectomy that ranged from 15 to 100%, with a mean of 70%. In one patient (Number 12) there was a significantly higher value for the mastectomy specimen. Five of the 11 samples (45%) would have been judged borderline or negative had the results of the postmastectomy specimen been relied upon (Fig. 3).

Of the 13 postmastectomy specimens submitted for time–decay studies, five had adequate receptor levels in the initial 15 minute sample to permit evaluation (Fig. 4). All time specimens were analyzed in one batch. Each of these specimens showed the expected decrease in receptor levels with time though there was some variability. This change may be due to analytic variation or to sampling error despite attempts to control for this.

Discussion

Analysis of the data shows a significant drop in tumor receptor content occurring in the interval between open biopsy and completion of mastectomy. While the time–decay studies suggest that proteolysis and/or thermal decay may result from separation of the tumor from its blood supply, other factors are, doubtless, important. Though the electrocautery was not employed at either the biopsy or mastectomy, it seems reasonable to attribute some of the difference in receptor levels to tissue necrosis resulting from ligatures, tumor manipulation, and from hematoma generated at the time of biopsy. Further work is necessary to document the stability of receptors under various conditions. In this re-

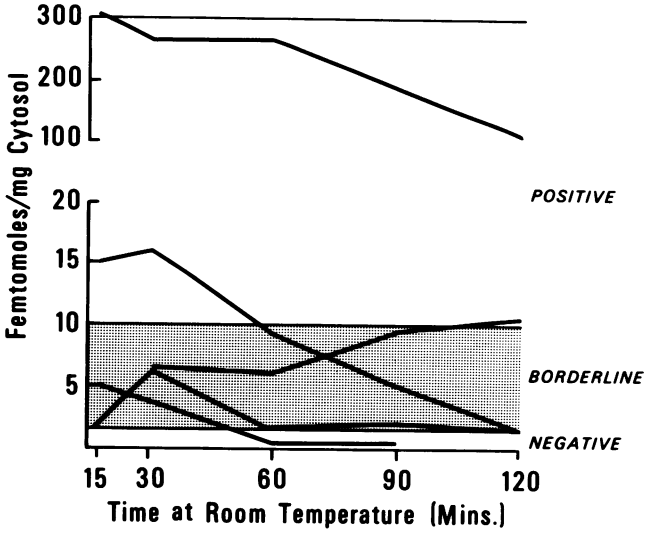

FIG. 4. Time-decay estrogen receptor studies, mastectomy specimens. Changes in estrogen receptor levels in postmastectomy tumor samples with increasing time at room temperature. Only those showing change are included. Note scale difference.

gard it would have been informative had time-decay studies been carried out on the biopsy sample.

It is tempting to speculate that the variously reported objective responses to endocrine therapy of estrogen receptor negative tumors of 1-14% may reflect inadequate attention to sample procurement.[2]

In many centers, the traditional open biopsy-frozen section-mastectomy sequence has been replaced with schemes that result in hospital admission only after histologic confirmation of the presence of cancer, and the complete assessment of the extent of disease; procedures that in most instances have a sound medical, economic, and humane logic. Whatever the sequence employed, it is apparent that high priority should be given to the procurement of a fresh sample with the presence of adequate tumor histologically confirmed, and that any technique resulting in tumor necrosis must be assiduously avoided if the steroid hormone receptor levels are to be reliable.

We conclude that estrogen receptors are greatly altered by proteolysis and/or thermal decay and to a certain extent are inversely proportional to the time from separation of blood supply to freezing of the tumor specimen. To insure valid determinations of estrogen receptor levels in tumor tissue, we recommend that any sequence for the diagnosis and treatment of breast cancer include the rapid freezing of a carefully procured, histologically confirmed sample of *fresh* tumor.

Acknowledgments

The authors wish to acknowledge the aid of Gary Koch, Ph.D., and Ms. Judy McMillan for their statistical review of the data, and to Mrs. Randolph P. Martin for her technical assistance.

References

1. Hoehn JL, Plotka ED, Dickson KB. Comparison of estrogen receptor levels in primary and regional metastatic carcinoma of the breast. Ann Surg 1979; 190:69-71.
2. McGuire WL. Hormone receptors: their role in predicting prognosis and response to endocrine therapy. Semin Oncol 1978; 5(4):428-33.
3. Rosenthal LJ. Discrepant estrogen receptor protein levels according to surgical technique. Am J Surg 1979; 138:680-81.
4. Wittliff JL. *In* Wittliff and Dapunt (eds) Steroid Receptors and Hormone Dependent Neoplasia. New York, Masson. 1980. pp. 296-97.

DISCUSSION

DR. RALPH B. VANCE (Jackson, Mississippi): About 65% of the human breast tumor tissue contains measurable amounts of estrogen receptor protein, and about 60% of those tumors which are estrogen receptor positive will show tumor regression when treated with hormones alone. This means that about 39% of the patients with breast carcinoma can expect to respond to hormone treatment alone, and therefore we consider the importance of obtaining accurate measurements of the receptor from the assay without false negative results.

Probably the most important point in this well-done paper is the recognition of borderline positive samples which would otherwise have been missed. Specifically, Dr. Newsome's slide Samples 1 and 3 would yield false negative results if the mastectomy specimens alone were the only data base. At our institution, we have requested that the temperature of the specimen container be cooled to −20° in order to facilitate freezing as much as possible, and thereby to avoid decay. I think the point is well taken in this paper.

DR. J. SHELTON HORSLEY, III (Richmond, Virginia): I believe estrogen receptors are a very important piece of information. Certainly, today it is a vital determination in planning the treatment of a woman who has metastatic breast cancer. I think, in the very near future, it will play a major role in selecting the proper type of adjuvant therapy for women with primary breast cancer who have metastases in their axillary nodes.

There have been some disturbing findings with regard to the analyses for estrogen receptors. When the results of various laboratories performing these tests have been compared with standardized powders, an error in approximately 33% has been found. It is well known that there are different values found within the same specimen, as Dr. Newsome has pointed out; and now he has called our attention to the fact that there is a difference with regard to time delay.

We have done several of these studies and our variability is so great that we don't know exactly what to make of it. However, we should all be attentive to the point that as soon as possible upon completion of the mastectomy, the specimen should be carefully pre-