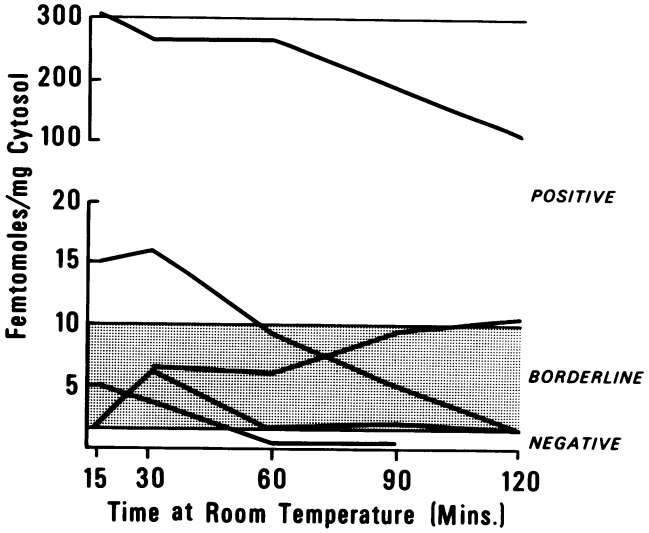

FIG. 4. Time-decay estrogen receptor studies, mastectomy specimens. Changes in estrogen receptor levels in postmastectomy tumor samples with increasing time at room temperature. Only those showing change are included. Note scale difference.

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.

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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-

pared, with removal of necrotic tissue and fat, and quickly frozen for storage. I do not believe the last word is in, however, and I do not think it is necessary for us to change our approach based on the information that is now available.

I would like to ask two questions. First, what is the effect of the excisional or incisional knife biopsy as opposed to needle biopsy or needle aspiration cytology (which we do on most of our patients) on the specimen which is analyzed?

Second, is the assay for both the biopsy specimen and the mastectomy specimen done at the same time? Both of these are variables that should be considered in evaluation of the results.

I think this is a very important concept. We should keep the time delay factor to a minimum and freeze the specimen as promptly as possible.

DR. SAMUEL A. WELLS, JR. (Durham, North Carolina): This paper makes a critical point, that an adequate sample of breast carcinoma tissue should be obtained from the primary tumor so that estrogen receptor determination can be performed by accepted methodology. This is important for two reasons.

The first is that if a patient develops a recurrence, it might not be possible to acquire an adequate sample of tissue, especially if it is in the bones or parenchymal lung. It has been clearly shown by several investigators that ER levels in the primary neoplasm and in cells from the first recurrence are almost always in the same range. That is, if the primary tumor is estrogen receptor rich, the metastatic lesion will be also. Therefore, it is possible in those patients who develop recurrence subsequent to mastectomy to base therapy on ER levels obtained in the primary tumor. If the metastasis is easily accessible, it should be biopsied for confirmation of the ER status. This is especially important in patients whose estrogen receptor levels are borderline positive. It has also been clearly shown that patients whose primary tumors are ER rich have a prolonged disease-free interval and an increased survival rate compared with patients whose primary tumors are ER negative.

(slide) I generally agree with the remarks which Dr. Newsome made, but my comments will be related primarily to methdology. The criteria for effective devascularization should be addressed first.

Regarding cellular integrity and viability, ultrastructure demonstrates intact mitochondria, lysosomes, and membranes in postmastectomy specimens from tumors remaining in devascularized breasts for up to two hours. The viability of the cells derived from the intact mastectomy specimen is demonstrated both by organ and cell culture techniques. Excision and/or mincing of tumors results in lysosomal disintegration and mitochondrial swelling which is usually apparent by fifteen minutes.

(slide) Retention of sex steroid receptors (premastectomy biopsy versus postmastectomy specimens) shows no change in the status of 11 of 14 patients. Three patients went from plus or minus to positive, or positive to negative, within the variation of the methodology of the assay by sucrose density gradient analysis. These data are those of our group: Dr. George Leight, Assistant Professor of Surgery, and Dr. Kenneth McCarthy, Jr., Director of the Oncology Endocrine Laboratory in the Departments of Medicine and Pathology. Minced, excised specimens show predictable decline from the time of excision to freezing within 10–15 minutes, which is highly dependent on the buffers used. The minimum requirements include a reducing agent (thioglycerol), a chelating agent (EDTA) for binding calcium since most of the proteases are calcium dependent, a proteolytic inhibitor (tyrosinol), and a translocation inhibitor (molybdate).

My first question to Dr. Newsome is: did the buffers used in this study contain such substances?

(slide) If one evaluates qualitative changes in the fourteen patients, again comparing premastectomy biopsy specimens with postmastectomy specimens, the ER changes from plus or minus to plus in two, and from plus to negative in one. In 11 patients, the ER value remained essentially the same. If one looks at quantitative changes, that is, less than 20%, there was no variability in ER level in nine patients. The ER value was quantitatively lower in three postmastectomy specimens and higher in two specimens. We have used sucrose density gradient analyses for determination of estrogen receptor proteins.

(slide) If one looks at the shift of 8S and 4S again in the same 14 patients, a change is noted. There was no change in the ratio in six patients, a decrease in eight, and an increase in none. A shift appears to occur in 8S/4S proportions toward more 4S binding with time from devascularization.

The second question I would like to ask of Dr. Newsome is: has he noticed any degradation of progesterone receptors in his studies?

DR. JAMES E. PRIDGEN (San Antonio, Texas): We agree completely with what Dr. Newsome said.

About five years ago, in our San Antonio area, we organized the Breast Cancer Task Force. This was made up of 25 clinical surgeons from the community, who send their material through the pathologist. He handled it very much the way Dr. Newsome has described, freezing it as soon as they get it, in nitrogen, and then sending it to a central laboratory operated by our medical school, which is right across the street. This laboratory is run by Dr. W. L. McGuire, who has been interested in estrogen receptor positive studies since 1969 and has done about 5000 studies, half of which are from our San Antonio area, and the other half have been shipped in from other parts of the country.

Our estrogen receptor positive patients have enjoyed a prognosis better than estrogen receptor negative patients, as has been shown by many others. Especially is this true in Stage II malignancy. We feel that those that have estrogen receptor negative studies are probably better put on chemotherapeutic agents directly, even though the nodes are negative.

Another interesting point that's come out of this study, again from the community support of this breast tissue, is the tissue cultures that we have been able to make; this cloning effect has been successful in about 80% of the cases. We have tested this against specificity of certain combinations of chemotherapeutic agents, and found that one microscopic type has been effected very much by one particular type of drugs, whereas another microscopic type is affected very little. The tissue cultures have been done in the test tube so far; we hope to transpose this information into the *in vivo* state during the coming year.

We are pleased with this community effort in the study of breast cancer so far.

DR. BENJAMIN F. BYRD, JR. (Nashville, Tennessee): This paper raises some fascinating questions which I'm sure they will continue to work on, why these estrogen receptors actually become insensitive, whether it's saturation on cooling or whether it's destruction of the receptors or the change in tissue hydrogen ion concentration. Whatever characteristic produces this diminution in estrogen receptor activity can be reversed, or altered in such a fashion that estrogen receptor concentrations which have lain latent with the current testing technique can be developed.

Certainly, this presentation brings to light the fact that the two-stage procedure, the biopsy with subsequent mastectomy, has inherent risks in it. Particularly when its performed in an institution that doesn't furnish full pathology capabilities, with frozen section diagnosis, with the capability of freezing tissues immediately. Waiting for a mastectomy and a second section results all too often in a second section not amenable to estrogen receptor studies.

And then the final point—and I would like to ask what their attitude has been about this. Should hormonal manipulation be used in those women who have estrogen receptor negative reports on their tumors? About 10% of these women with ER negative reports have been found to react to hormonal manipulation, and certainly there is a very minor disturbance of their physical state to give them this opportunity of benefit.

I think that there is still very little doubt that the woman with an estrogen receptor negative tumor who is menstruating, who has a recurrence of her tumor, should be treated primarily by ablation of the ovaries. This is much too easy an effect to offer to neglect the 10% of the women who, even though they have ER negative reports, actually have tumors which will respond to hormonal manipulation.

DR. JAMES F. NEWSOME (Closing discussion): If I may take these in reverse order, Dr. Byrd, you have raised the question for which this study was designed. And that is: What is the significance of the reported response to endocrine therapy of receptor negative tumors? The rate varies in many institutions. We have not looked at our data as carefully as we should have, but my best judgment is that the response has been very small in those who are truly negative. In order to interpret that, it's quite clear that the adequate preparation of the sample is of utmost importance.

In point of fact, it has to do with the first question that you raised, and that is: What about the importance of sequence? The sequence, as I made reference to earlier, obviously is going to depend on the local circumstances. It's convenient for many people to get the biopsy first, and then do the mastectomy subsequently, which is what we do. It is not convenient to do this in some other institutions, for many reasons. My plea is, whatever the sequence, that somewhere someone must get an immediately frozen, good sample of the tissue.

Dr. Pridgen's and Dr. Vance's comments again come back to the point for which the study was done: we need to have those data which are, indeed, reliable.

In answer to Dr. Wells' question, no, we do not use buffers. It's the recommendation of our receptor laboratory director that it is really not helpful, and the question as to whether or not some of the buffers actually will interfere with the determination of receptor levels has been raised by several authors. We do not fractionate into 8S and 4S samples, so I cannot answer your question as to what effect it has on either one of those fractions. The reported experience is, as you well know, that the 8S fraction is much more sensitive than the 4S.

Dr. Horsely, the answer to your question—I do not know; that is, whether the needle aspiration cytology alters the receptor level, which I presume is the intent of your question. In that regard, as I mentioned, it would have been helpful, and, indeed, we are beginning to do just that—to look at the biopsy specimen in terms of a time decay study, to see if we can help answer that question.

I would suspect, particularly if it's a true-cut needle, or something of that order, yes, it may alter the receptor levels. But again, my fundamental plea is—and this is not new, attention being called to this by the people at Albany and others several years ago—please give the receptor laboratory an adequate chance to help you know what the receptor level is in the tumors of the patients you treat.