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PHARMACOLOGICAL RECEPTOR DETERMINATION IN ENDOCRINE THERAPY OF BREAST CANCER

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Exceedingly small amounts of estrogen are required to produce physiologic effects. Until relatively recently, little was known about the tissue distribution of estrogen because assay methods were not available. Glascock & Hoekstra (1), using tritium-labeled hexestrol in physiologic amounts, were able to show that, in female kids and lambs, the hexestrol accumulated in the target organs for estrogen (the uterus and vagina, kidneys, liver, ovaries, and mammary glands). The liver and kidneys accumulated a disproportionately large amount of radioactivity because the hexestrol was also in the bile and urine.

Using tritiated hexestrol of high specific activity, Folca, Glascock & Irvine (2) gave a group of ten patients physiologic amounts of hexestrol intravenously 6 hr before surgery for advanced breast cancer. In 6 of the 10, substantially more hexestrol was concentrated in the tumors than in either muscle or blood. Of these six patients, four subsequently responded favorably to bilateral adrenalectomy and oophorectomy. Of the four patients who showed no increased gradient not one responded favorably to the ablative therapy.

At the 1961 Laurentian Hormone Conference, Jensen & Jacobson (3) reported finding
tradiol 17-β in the target tissues. They predicted that an addition product

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was formed between the tritiated estradiol 17-β and a proteinaceous material in the cytosol of the target organs. This product was particularly concentrated and was demonstrated in the immature rat uterus. They also were able to show that the bound and apparently biologically active material in the uterus remains as estradiol 17-β and is not oxidized to estrone. This paved the way for the in vitro measurement of the ability of mammary tumors to bind tritium-labeled estradiol 17-β and an attempt to correlate the binding with the response of such tumors to hormonal manipulation.

The most comprehensive effort of which I am aware to bring together the results of such studies in human beings was under the aegis of the National Cancer Institute (NCI) (4). In an effort to equate the quality of the clinical evaluations with the quality of the biochemical measurement of receptors, the NCI requested that investigators submit their data to two well-trained extramural reviewers who reviewed the cases according to the criteria of the Cooperative Breast Cancer Group. These extramurally reviewed results drew the first substantial correlations between the presence of estrogen receptor (ER) protein in human tumors and their response to endocrine manipulation. This method of study does not mean that all of the patients were treated similarly or were entered into their endocrine therapy in a similar fashion. However, it does mean that the judgment as to the patient's objective responses was made by the same two reviewers and that cases not fulfilling

ted. These data supported a correlation between the presence of cytoplasmic ERs in mammary cancers and their ability to respond to hormonal manipulation, whether it be ablative or additive.

This study also revealed that methods for measuring ERs vary from institution to institution, that the means of expressing the results vary greatly, and that the level of ERs thought to be effective varies from institution to institution. Unfortunately, most other studies correlating ER levels and clinical response have not gone to this length to assure uniformity of clinical evaluations.

Receptor analysis is a difficult task, starting with the proper attainment and handling of tissue. Holt et al (5) pointed out the effort that goes into the handling of the specimens at their institution. Rich et al (6) reported that the ER levels in human breast cancers were stable for 6 hr on wet ice but that significant

that their ability to replicate the measurement of ER activity in numerous specimens of rabbit uterus or rat mammary tumors was excellent with a variance of only 16% to 17% on a wet weight basis, but when they measured the receptor activity in human tumors, the results varied considerably in different samples from the same tumors (a wet weight variance of from 22% to 125%).

Wittliff & Savlov (8) reported that ER content decreased 75% when human tumor tissue was frozen for one month. However, they found that the two molecular forms of ERs in cytosol (8–9S or 4–5S) did not change. Namkung, Moe & Petra (9) studied three human tumors containing both 8S and 4S species of cytosol ERs and found that there was greater loss of 8S (75%) than of 4S (51%) activity. Because objective response to hormonal therapy has been reported to correlate better with 8S content (see below) than with 4S, the inclusion of the 4S receptors in the receptor determination penalizes the accuracy of the receptor measurements for predicting response to hormonal change. The dextran-coated charcoal method without ultracentrifugation does not differentiate between 4S and 8S, and would therefore produce more false-positive values after prolonged frozen storage of tissue.

Receptors are of high affinity, saturable, highly specific,
some physiologic function. Affinity is measured as having a K_a greater than 10^7. $K_a = B/R \times L$, where B is the bound complex, R is the concentration of the receptor, and L of the ligand. K_a is thus a measure of how tightly the receptor holds the ligand.

These characteristics are shared by 17-hydroxysteroid binding globulin (SBG), and in specimens contaminated with human serum this must be corrected for (10) or avoided by using a ligand that binds to the ER but not to SBG (11). Gustafsson et al (12) have developed a microanalysis of ER content of a specimen obtained by needle biopsy. They use isoelectric focusing and polyacrylamide gel combined with limited proteolysis. However, because the tumors are sucked into the syringe by vacuum and the samples are usually contaminated with blood, the synthetic estrogen R-2858 [Moxestrol; 11β-methoxy-17-ethinyl-estra-1,3,5(10)triene-3,17β-diol], which has no affinity for sex hormone binding globulin, is used as the ligand (11).

These properties of receptors are also associated with enzymes for which the ligand serves as a substrate. In general, enzymes have a lower K_a. However, it is of great importance that K. S. McCarty, Jr. et al (unpublished information) have recently shown that what has been reported to be ER in melanotic malignant melanoma is actually tyrosinase.

The principle presumed to govern hormone receptors is that receptors are contained in target tissues and bind the hormone with high specificity
affinity. All known binding sites appear to be associated with protein molecules. In the target tissues the binding sites are capable of receiving the hormone and translating it into the molecular action characteristic of the hormone or into a hormone effect by forming a hormone receptor complex. This complex, either unchanged or in altered form, initiates the activity that is seen as the recognizable hormonal effect. Most of the steroid hormone effects that we recognize come from the transportation of the hormone

receptor complex into the nucleus where the other activity is initiated. The specificity
that bind to the receptor have structures that are generally associated with that hormone and fail to bind with structures that are different. The nonspecific binding or nonspecificity
selectivity. For ERs particularly, there are compounds, known as antagonists, which associate strongly with the receptor but are either not transported with the receptor into the nucleus or fail to initiate the expected effect from the hormone. Both steroidal and nonsteroidal estrogen antagonists exist.

Nonspecific binding occurs with all steroids in all of the systems that have been studied and may be high enough to make the specific binding hard to detect. This problem is generally overcome by duplicate incubations in the presence of excessive amounts of the nonradioactive ligand or with a similar ligand. For example, a 100-fold concentration of either an antagonist (nafoxidine), estradiol 17-β itself, or an agonist (diethylstilbestrol) is used. The specific binding is then considered to be the difference between the curves developed for total binding and those developed in the presence of these ligands used to inhibit the nonspecific

Hähnel & Twaddle (13) demonstrated that ERs have high affinity, readily saturable binding sites for tritiated estradiol 17-β. They determined both the number of binding sites and the affinity. McGuire et al (14) and Rich et al (6) found no correlation between tumors positive for ERs and axillary node involvement with tumor. Rich et al (6), Hawkins et al (7), and many others have found that there is a relationship between patient age and the number of breast tumors that are positive for ERs. Others have correlated these factors with the level of receptors. McGuire, Pearson & Segaloff (15) have also reported an increase in levels with increase in age.

Rich et al (6) had five pathologists grade breast tumors into three degrees of differentiation. They found that there was indeed a correlation between tumor grade and the presence or absence of ERs—the less differentiated tumors contained a significantly smaller percentage of ERs.

Exactly what conditions constitute recurrence of cancer after mastectomy are difficult to establish, and opinion varies widely. The problem is typified by the following two studies. The Primary Therapy of Breast Cancer Study Group, a subgroup of the Cooperative Breast Cancer Group, carefully evaluated time to recurrence, adhering to an absolutely defined protocol for follow-up (16, 17). Any evidence of recurrence was considered a recurrence. Patients were seen and observations made every three months. If the patients were not observed appropriately and on time (within a statistically determined leeway), and consequently were not comparable, they were excluded from the study. At the other extreme are the criteria

followed by Maynard et al (18). Patients are seen at a postmastectomy clinic at three-month intervals for the first 18 months and at six-month intervals thereafter. However, in this study recurrence is defined
of tumor deposits requiring a major change of treatment. There is no prescribed set of skeletal roentgenograms taken to look for early recurrence in bone or any other such observations. A proven local recurrence that can be excised and treated with radiation therapy or a proven nodal recurrence that can be handled similarly is not considered a recurrence. Thus, the time to recurrence may vary greatly, depending upon the clinician's definition
recurrence.

Singhakowinta et al (19) have found that, generally, the first
recurrence of ER-positive tumors is in bone. Indeed, in one of their studies, 42% of the first
bone, whereas only 2% of ER-negative tumors first
cause erosion of cortical bone must be extensive before it can be detected on roentgenograms and because small local recurrences at the operative site are readily detected by the patient, even with careful follow-up the local recurrences would probably be noticed first.
interval for patients with ER-negative tumors should be shorter than that for patients with ER-positive tumors.

Knight and associates (20) and Rich and associates (6) reported that, if patients are classified solely on the basis of having ER-positive or -negative tumors, fewer recurrences and fewer deaths occur in patients with ER-positive tumors, at least in the early course of the disease. Knight et al (20) suggested that this is an independent prognostic factor, not related to any other.

Block et al (21) reported similar findings. However,
sented data on 112 women who had primary mastectomies and no intervening therapy. At the time of the report, 50 patients already had had a recurrence. These authors found no correlation of the free period with ER status, but only with the occurrence of metastatic tumor in the axillary nodes. Premenopausal, node-positive patients had the shortest time to recurrence; postmenopausal, node-negative women had the longest free period. Leclercq & Heuson (23) also were unable to find
ER positivity and the free period. These studies are illustrative of many. Any correlation found between ER status and free period is dependent upon the investigators and the patients studied. Metastatic cancer in axillary nodes is the finding
and early recurrence.

Several other prognostic indicators have been defined:
survival rather than time to recurrence, reported that differences in survival can be predicted by, for example, tumor size, skin involvement, and whether

or not the ipsilateral nodes are palpable and/or pathologically involved. When one considers that ER-positive tumors appear to be better differentiated and to incorporate less thymidine than ER-negative tumors, the absence of early recurrence is not surprising, as these factors are generally associated with a better prognosis.

When Bonadonna, Di Fronzo & Tancini (25) extended their study retrospectively to 224 women with operable breast cancer who had positive lymph nodes and were given adjuvant chemotherapy [Cytoxan®, methotrexate, and 5-fluorouracil (CMF)], there was no statistical difference between the patients who were premenopausal and those who were postmenopausal or between those who were given 6 and those who were given 12 cycles of CMF. There was also no significant
the premenopausal patients who became amenorrheic and those who did not. In addition, in the patients with operable tumors, the disease-free period was influenced
presence or absence of ERs in the tumors.

Although the objective regressions reported by Wittliff et al (26) have not been reviewed by extramural reviewers, and the patient population may or may not be homogeneous, there does appear to be a greater response rate to all sorts of hormonal manipulation in those breast cancers that have either 8–9S ERs or both 8–9S and 4–5S ERs. In those tumors in which only 4–5S receptors were detectable, only 4 of 23 responded objectively; in those tumors in which 8–9S receptors were detectable, 33 of 44 tumors responded with objective regressions.

McGuire (27) reviewed many reported findings
explanations for the different results among the various studies. He emphasized that ER positivity seems to be an independent factor predicting a prolonged free period after the primary tumor is removed. However, he also pointed out that a partial explanation for the effectiveness of premenopausal administration of adjuvant chemotherapy is that cytotoxic ovarian failure is induced and the patients become postmenopausal, with a better prognosis. As evidence of the greater aggressiveness of undifferentiated tumors, he cited the experience of Meyer et al (28). McGuire noted the inconsistency of studies reporting that ER-negative tumors may respond better to chemotherapy in contrast to the generally held belief that more rapidly dividing and differentiated tumor cells are more responsive to chemotherapy. This discrepancy is not easily reconciled.

Regarding the controversy about the response of ER-negative tumors to cytotoxic chemotherapy, if the data of Lippman et al (29) are accepted, then there is a clear answer: Receptor-positive breast cancer responds well to hormonal manipulation, whereas receptor-negative breast cancer responds

well to cytotoxic chemotherapy. On the other hand, if others, such as Kiang et al (30), are right and ER-positive tumors are not only particularly responsive to hormonal manipulation but, in patients matched for disease extent and site of involvement, they also tend to respond well to cytotoxic therapy, then we are left with something akin to Hobson's choice. That is, we have selected a group of patients who will respond well to any of our modalities and are left with the difficult decision of what to do with the remaining patients who have a poor prognosis. The preliminary report of the NIH Consensus Development Conference ("Steroid Receptors in Breast Cancer," June 27–29, 1979, Bethesda, Md.) states that there is no demonstrable correlation between ER positivity and response to cytotoxic chemotherapy and that much study is yet required.

Unless we have something advantageous to offer the breast cancer patients that we have selected as having a favorable prognosis on the basis of ER measurement, we are depriving the other patients of hope without actually accomplishing anything. Of course, the percentage figures will look much better. But we must treat all patients, regardless of prognosis. If there appears to be no reasonable therapy for a certain patient, an experimental form of therapy that can provide some hope should be suggested.

What does ER positivity in tumors actually represent? Is it merely an indication that those tumors that retain measurable amounts of ER protein (estrophilin) are more differentiated than the other tumors? That is to say, is the ER merely a measure of the departure of the neoplastic cell from its parent nonmalignant cell and therefore an index of invasiveness, growth rate, etc?

At the recent Consensus Conference, J. L. Hayward of Guy's Hospital asked whether ER measurement is not merely an expensive and complex way of grading tumors into their degree of differentiation. It is interesting that there was no satisfactory refutation.

Many authors have reported that patients with high levels of ERs have the greatest chance of objective response to hormonal manipulation. Heuson et al (31, 32) noted that over the years, with experience and improvement in methodology, the percentage of breast cancers with detectable ERs has been increasing. They suggested that all breast cancers may have some ERs.

Jensen, Smith & DeSombre (33) have suggested that breast cancers be referred to as estrophilin-rich or -poor, stressing that the values vary with methodology and means of expression. As have most workers, they found lower levels of ERs in premenopausal patients than in postmenopausal patients. When they correlated the menstrual status, the ER levels in the tumors, and the response to endocrine manipulation, they found that

premenopausal patients responded objectively (and favorably) at a lower level of ER than postmenopausal patients. They therefore proposed that tumors binding 750 fmol/g^{-1} be called estrophilin-rich in postmenopausal women, but that the level be lowered to 250 fmol/g^{-1} in premenopausal women. Usually a single, predetermined figure
positivity. The most frequently used is that of McGuire et al (34): at least 3 fmol/mg cytosol protein. This break point was set on the basis of study results showing the regression rate and percentage of positive progesterone receptor to be lowest in patients with an ER value less than 3 fmol/mg cytosol protein, slightly higher in patients with 3–10 fmol/mg cytosol protein, higher in the 11–100 fmol/mg range, and highest in patients who had over 101 fmol/mg cytosol protein. Others (35) say they cannot measure 10 fmol/mg cytosol protein or use an arbitrary level of 10 fmol/mg cytosol protein (29).

Antibodies against receptor protein (estrophilin) from calf uterus (36) and human breast cancer (37) have been prepared. It is hoped that these can be used to develop a clinically useful radioimmunoassay for estrophilin. Pertschuk et al (38) have used a fluorescent
breast cancers and have shown fluorescence which appears to correlate with the presence of ER. However, insufficient data are available to warrant the use of this technique as the sole index of ER presence.

McGuire and colleagues (39) reasoned that it would be helpful to look for other steps in the receptor mechanism, and they observed that a progesterone receptor (PgR) is normally induced in target cells by the action of estrogen. They believe that the ability of the cell to produce the PgR is probably an indication that there is a closer parallel to the parent cell. Indeed, the presence of PgR appears to correlate better with the clinical response of patients than does the ER.

Philibert & Raynaud (40) introduced a tritiated, highly active progestin, R5020 (17,21-dimethyl-19-nor-4,9-pregnadiene-3,20-dione) as a specific
gand for the measurement of PgR binding in the immature rat and mouse uterus. The progestin binds more tightly than progesterone and the number of binding sites does not differ significantly
McGuire (41) extended the use of this agent to human breast cancer. They demonstrated that it has an advantage over other ligands in that it does not bind to either the tissue glucocorticoid receptor or to the serum corticosteroid-binding globulin. McGuire et al (34) summarized the data on PgR determination in over 500 human mammary tumors. They pointed out that only the 8S peak should be considered as positive and then only when 2 fmol/mg cytosol protein are bound. In general, they found it more likely to see PgR positivity with higher values of ER. Of 54 patients in whom both ER and PgR were measured in the metastatic tumor, objective response to

endocrine therapy was seen in 0 of 11 (0%) patients with ER-negative tumors, 7 of 17 (41%) with ER-positive–PgR-negative tumors, and 13 of 16 (87%) with ER-positive–PgR-positive tumors.

Many investigators believe that the growth of mammary malignancies is dependent not only upon steroid hormones but also upon proteinaceous hormones. Although receptors for the pituitary hormone, prolactin, and for the ovarian hormone, relaxin, have been demonstrated, the presence of these receptors in tumor tissues is as yet not confirmed.

are able to demonstrate them and some are not. In addition, I know of no acceptable studies correlating the presence or absence of such receptors and the clinical response to hormonal manipulation.

What, then, is the present status of the correlation between steroid receptor determination and clinical response of breast cancer to therapy? The determinations are difficult and must be done meticulously from the first incision to the final

Despite changing attitudes about the overall therapy of metastatic and primary breast cancer, several things are clear. The presence of ERs in the primary tumor may be an independent determinant of a prolonged free period, but there is not sufficient information or unanimity on this point. Clearly, the presence of estrogen and/or progesterone receptors in the tumor cytosol should be an important factor in selection of hormonal therapy, but it should not be the sole determining factor. It does appear, however, that tumors containing either receptor, or most particularly, both receptors have the greatest chance of responding to hormonal therapy. The intermediate levels of receptor predict an intermediate chance for objective response, and tumors lacking such receptors have a lesser chance of responding (34). Many problems remain to be solved.

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