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HORMONE DEPENDENCY IN BREAST CANCER

ELWOOD V. JENSEN, SYLVIA SMITH and EUGENE R. DESOMBRE
Ben May Laboratory for Cancer Research, University of Chicago, Chicago, IL 60637, U.S.A.

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SUMMARY

On the basis of the minimum estrophilin levels required for response in 123 patients undergoing endocrine therapy for advanced breast cancer, about 70% of the 1200 women whose primary and/or metastatic tumors have been analyzed may be classified as estrophilin-poor and 30% as estrophilin-rich. Since the receptor-poor group has little chance of benefit from endocrine therapy, whereas nearly two-thirds of the receptor-rich group show objective response, determination of the estrophilin content of excised tumor specimens can suggest the proper type of therapy for 85–90% of women with advanced breast cancer.

More than 700 primary breast cancers have been analyzed from patients without evident metastases, who are being followed for recurrence. So far 3 of 4 patients with estrophilin-rich cancers responded to endocrine treatment 14–26 months later, whereas 10 of 11 patients with estrophilin-poor primary tumors failed to respond 4 months to 5 years later.

Failure of some receptor-positive patients to respond may result from various causes. Tumor heterogeneity or the presence of both receptor-rich and receptor-poor metastases in the same patient probably is responsible in some instances, emphasizing the need for multiple specimens whenever possible. Studies of induced rat mammary tumors demonstrate that an occasional autonomous tumor will contain a substantial amount of estrophilin, even though the RNA polymerase system of its nuclei is insensitive to stimulation by estrogen–receptor complex. This finding suggests that a simple test to evaluate the susceptibility of tumor nuclei to stimulation *in vitro* might provide a more direct indication of hormone dependency than does the receptor content.

INTRODUCTION

It has long been recognized that some human breast cancers are 'hormone-dependent' in that their growth is influenced by fluctuations in the levels of steroid sex hormones and they undergo regression after surgical removal of glands responsible for production of supporting hormones. As early as 1836, Cooper[1] observed a correlation between tumor growth and the menstrual cycle, and in 1896 Beatson[2] reported the regression of metastatic lesions following oophorectomy in premenopausal women with advanced breast cancer. The modern era of endocrine therapy for mammary cancer began in 1952 when Huggins and Bergenstal[3] reported that bilateral adrenalectomy can effect striking remission of advanced breast cancer in postmenopausal women; later it was shown by Luft[4] and by Pearson and Ray[5] that similar remissions are observed after hypophysectomy.

Hormone deprivation, by the surgical ablation of endocrine glands, affords the most effective treatment presently available for advanced breast cancer in those patients whose tumors are of the hormone-dependent type. Unfortunately, less than one half of the premenopausal patients and even a smaller fraction of postmenopausal patients respond to endocrine ablation. Thus, there is need for some means of predicting which breast cancers are of the hormone-dependent type, so that endocrine therapy can be restricted to persons it can help and the majority of patients can be spared the trauma of useless surgery and placed directly on chemotherapy. Studies during the past ten years have established that the estrogen receptor or estrophilin content of an excised specimen of the tumor can provide information useful in the selection of optimal therapy for most patients with advanced breast cancer.

The rationale of estrogen receptor determination originated in observations that estrogen-responsive or 'target' tissues of laboratory animals (uterus, vagina, anterior pituitary) contain characteristic estrogen-binding components as indicated by their striking uptake and retention of tritiated hexestrol[6] or estradiol[7] administered *in vivo*. Early studies by Folca, *et al.*[8] demonstrated that, when injected with tritiated hexestrol, patients with breast cancer who responded favorably to adrenalectomy incorporated more radioactivity into their tumors than those who did not respond. With the subsequent development of techniques for measuring estrogen binding by mammalian tissues *in vitro*, and, later, for determining the actual content of the receptor protein responsible for the binding, it became possible to examine excised specimens of breast cancers for correlation of estrophilin level with clinical response[9]. Our early findings[10, 11] that patients whose mammary tumors lack noteworthy amounts of estrophilin have little chance of responding either to endocrine ablation or to hormone administration, but that most patients with receptor-containing cancers receive benefit from such treatments, were soon confirmed by similar results from other laboratories[12–14]. In 1974, a

workshop was held under the auspices of the Breast Cancer Task Force of the U.S. National Cancer Institute, in which the results of fourteen different laboratories were in general agreement with the foregoing conclusions[15].

This paper describes the present status of our studies of the correlation between tumor estrophilin content and clinical response to endocrine therapy in patients with advanced breast cancer as well as the use of a specimen of the primary cancer, obtained at mastectomy, to predict hormone dependency in metastases that appear at a later time.

EXPERIMENTAL PROCEDURES

For the first ten patients studied, 0.5 mm slices of their tumors were examined for uptake of radioactivity after being stirred for various time periods at 37°C in 300 ml of a 0.1 nM solution of tritiated estradiol in Krebs–Ringer–Henseleit buffer, pH 7.3, in the presence or absence of an inhibitor of specific binding, such as nafoxidine or Parke Davis CI-628[9–11]. Although the slice-uptake technique has the advantage that any endogenous estrogen present in that tumor undergoes exchange with radioactive estradiol, it has the limitations that a rather large tumor sample (>0.5 g), is required and that it can not be used with frozen tissue specimens. When it was recognized[16, 17] that the estrogen-receptor complex that accumulates in the nuclei of target cells is derived from an extranuclear complex that can be formed simply by adding tritiated estradiol to the supernatant or cytosol fraction of a tissue homogenate[18, 19], it was possible to devise a more sensitive procedure for determining estrogen binding by a breast cancer specimen. This procedure, involving the estimation of the cytosol estradiol–receptor complex by its sedimentation in a sucrose gradient, was employed for the evaluation of subsequent patients[10, 11, 20–22].

The weighed tumor specimen is immersed in liquid nitrogen and shattered in a Thermovac Autopulverizer (Thermovac Industries, Copiague, N.Y.). The residual tissue powder is homogenized with efficient cooling in four vol. of 10 mM Tris buffer, pH 7.4, containing 0.5 mM dithiothreitol using a Polytron PT-10 tissue disintegrator (Brinkmann Instruments, Inc.) with two or three 10-s homogenization periods, each followed by a 50-s cooling period. The homogenate is centrifuged at 2°C for 30 min at 210,000 *g* to precipitate the particulate matter. Two 150-μl portions of the cytosol fraction are removed and treated with 50 μl of either buffer alone or of buffer containing 1 μM Parke-Davis CI-628. After 10 min, 50 μl of buffer containing 2.5 nM tritiated estradiol is added to each mixture. In earlier experiments our own [6,7-^{3}H]-estradiol of S.A 57 Ci/mmol was used; recent experiments employed [2,4,6,7-^{3}H]-estradiol of S.A 109 Ci/mmol (New England Nuclear Corp.). After mixing and standing for 60 min in the cold, a 200-μl portion of each mixture is layered on 3.4 ml of a 10 to 30% sucrose gradient containing 10 mM KCl and 1 mM EDTA in 10 mM Tris buffer, pH 7.4, and centrifuged at 2°C for 16 h at 250,000 *g*. Successive 100-μl fractions are collected from the bottom of the polyallomer tube via an 18-gauge needle by displacement with paraffin oil in a fraction collector of our own design. Radioactivity is determined in a toluene–Triton X-100 scintillation medium.

As illustrated in Fig. 1, some receptor-containing tumor specimens exhibit only 8*S* estradiol–receptor complex, whereas others show various amounts of specific binding in the 4*S* region as well; both the 8*S* and 4*S* binding are eliminated by the inhibitor, PD CI-628. Some but not all breast cancers contain substantial amounts of serum proteins that bind estradiol with lower affinity to form a complex that sediments at about 4.6*S* and is not sensitive to the inhibitor.

Specific binding in both the 8*S* and the 4*S* regions is calculated by the difference between the sedimentation patterns in the absence and presence of the inhibitor, Parke Davis CI-628. DNA is determined on the washed sediment from the tumor homogenate, so that the assay results can be expressed in terms of fmol of estradiol bound per 100 μg DNA. However, DNA was not determined on the earlier specimens, so to permit comparison of all the patients studied, the results in this paper are expressed in terms of fmol of estradiol bound per g fresh tumor weight.

It should be noted that the concentration of tritiated estradiol added to the tumor cytosol in these studies was chosen at a time when the main consideration was to permit the sensitive detection of the presence of 8*S* and 4*S* complexes characteristic of hormone-dependent normal tissues. A total estradiol concentration of 0.5 nM was found to be favorable for this purpose, superior to 2 and 5 nM concentrations where the large excess of unbound hormone at the top of the gradient often obscures the specific binding,

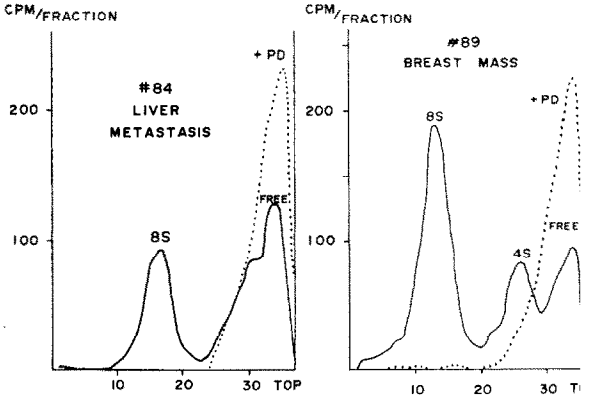

Fig. 1. Typical sedimentation patterns in 10–30% sucrose gradients of receptor-rich human breast cancer cytosols containing 0.5 nM tritiated estradiol in the absence (solid line) or presence (dotted line) of 0.2 μM Parke-Davis CI-628 (PD). From Jensen *et al.*[10].

especially in the 4*S* region, and also gives much larger amounts of non-specific binding. When it was found[21] that receptor-containing breast cancers with estrophilin levels below a certain value do not respond to endocrine therapy, the importance of quantitative receptor measurement was recognized. It also was evident that a total estradiol concentration of 0.5 nM does not completely saturate the binding capacity of a receptor-containing tumor cytosol.

Because the results obtained with estradiol concentrations of 0.5 nM are entirely self-consistent, they will be used as the basis of this presentation. However, the total binding capacity of each tumor cytosol can be readily calculated[23] by applying a correction factor based on the apparent dissociation constant for breast cancer estrophilin under the conditions of analysis (an average K_D value of 1 nM was obtained from the slopes of Scatchard plots[24] for 60 tumor cytosols in which sedimentation analysis was carried out at several estradiol concentrations) and the amount of bound and unbound estradiol. As indicated in the next section, for tumor cytosols with receptor content near that of the critical level for response, this factor is approximately 3.

RESULTS AND DISCUSSION

Clinical correlations

So far the correlation of estrogen binding with response to endocrine therapy has been carried out for 133 women with advanced breast cancer. All but the first ten were characterized by the quantitative sucrose gradient ultracentrifugation procedure. As shown in Fig. 2, these 123 cancer specimens varied widely in estradiol binding capacity from undetectable levels to more than 3000 fmol g^{-1} of tumor, under the conditions employed. With two exceptions, no patient without ovarian function whose tumor bound less than 750 fmol g^{-1}, and no premenopausal patient showing a level of less than 300 fmol g^{-1} responded to any type of endocrine therapy. Until results with larger numbers of patients permit a more precise assignment of values, we have defined the critical estrophilin level for a receptor-rich tumor as > 750 fmol g^{-1} in the postmenopausal or castrate patient and > 250 in the premenopausal patient. Levels of 400–750 for postmenopausal and 150–250 for premenopausal patients are classified as borderline, and values below these ranges are called negative; both borderline and negative tumors are called receptor poor. The fact that the estrophilin concentration required for response appears to be lower in tumors of premenopausal women presumably is due to the fact that these patients are producing larger amounts of endogenous estrogen that not only masks part of the receptor present in the cytosol but also has caused some of it to have moved into the tumor cell nuclei.

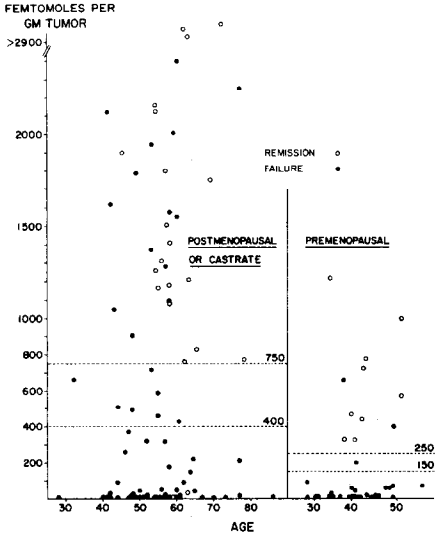

Fig. 2. Correlation of tumor estrophilin content with response to endocrine therapy for 123 patients with advanced breast cancer. From Jensen[22].

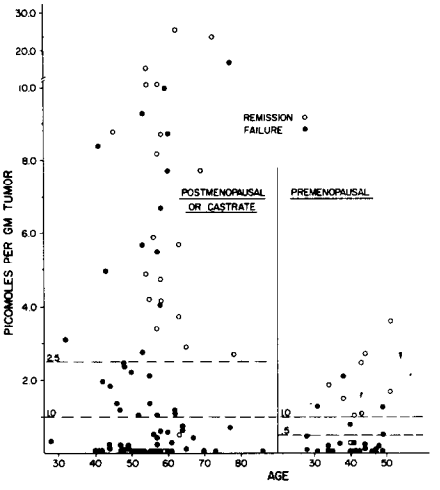

Fig. 3. Correlation of tumor estrophilin content, corrected to saturation, with response to endocrine therapy for the patients of Fig. 2. From DeSombre and Jensen[23].

As discussed in the section on Experimental Procedures, the estrophilin levels indicated in Fig. 2, obtained with 0.5 nM estradiol, do not represent the total binding capacity of the tumor cytosols. When these values are corrected to total binding capacity (Fig. 3), essentially the same relative pattern is seen (except that one responding cancer in a premenopausal patient now falls in the receptor-poor category). For total binding, the critical level for response is about three times the value obtained under the experimental conditions of Fig. 2. Although the results of Fig. 2 provide a self-consistent comparison of the relative estrophilin content of various breast cancers, the values of Fig. 3 are more comparable to those obtained by other analytical procedures, such as the Dextran-coated charcoal technique, where total binding capacity is calculated by the extrapolation of a Scatchard plot.

Table 1. Objective remissions to endocrine therapy

Treatment	Receptor test		
	Positive	Borderline	Negative
Ablation-104			
Adrenalectomy	2/5	0/4	0/14
Adrenalectomy + oophorectomy	13/19	0/5	1/11
Hypophysectomy	2/4		0/9
Oophorectomy	6/8		0/25
Hormone-29			
Androgen	0/1		0/3
Estrogen	2/2	0/1	1/7
Antiestrogen	1/2		0/1
Estrogen + progestin	3/5		0/7
Total cases-133	29/46 (63%)	0/10	2/77

The 133 patients that have been evaluated are summarized in Table 1. Of these, 104 were treated with ablative procedures and 29 received some type of hormone administration. Of the 87 women whose tumors gave negative or borderline tests (receptor poor), only two showed objective response to the endocrine therapy employed, in marked contrast to remissions in 29 of 46 or 63% of the receptor-rich cancers. In early 1974, the clinical records of 68 of these patients were subjected to independent evaluation by review team from the Breast Cancer Task Force of the National Cancer Institute. The conclusions obtained from these reviewed patients are essentially the same as those from the total cases. It would appear that nearly two-thirds of the patients whose cancers contain significant amounts of estrophilin can expect benefit from some type of endocrine therapy. But if the tumor estrophilin level is below a critical value, that patient has little if any chance of response to endocrine therapy and probably is better treated directly with chemotherapeutic agents.

Evaluation of mastectomy specimens

In the foregoing studies, nearly all the breast cancer specimens, both primary and metastatic, were obtained from patients with advanced disease who were already scheduled to undergo some type of endocrine therapy. In most cases, the results of the receptor assay were not revealed until a decision had been reached concerning the clinical response of the patient. In patients undergoing adrenalectomy or oophorectomy, the specimen of metastatic cancer was obtained during the course of the surgical operation.

If the estrophilin assay is to be used to predict whether or not the patient should undergo endocrine ablation, a problem arises as to the availability of the tumor specimen, for many patients, who have had previous mastectomy, do not have metastases that are conveniently accessible. Because one can nearly always obtain a satisfactory sample of the primary tumor, it is of considerable interest whether the estrophilin level of a primary tumor, determined at the time of mastectomy, can predict subsequent response to endocrine therapy if metastases appear at a later time. Although only a limited number of such patients have been studied, the results so far appear promising. As summarized in Table 2, of four patients with receptor-rich primary breast cancers, three showed objective remission to endocrine therapy one to two years later, and the fourth experienced a prolonged subjective remission of more than one year. Of eleven patients with receptor-poor tumors, all but one failed to respond to the endocrine treatment when metastases appeared from 4 months to more than five years later. Although many more patients must be studied before definitive conclusions can be drawn, these preliminary results suggest potential value in routinely characterizing primary breast cancers at the time of mastectomy so that this information will be available as a guide to therapy in case of recurrence. We have analyzed present primary tumors from more than 700 mastectomy patients, who are being followed for recurrence and response to endocrine therapy if and when metastases appear.

Table 2. Use of mastectomy specimen in predicting subsequent response

	Treatment*	Time after mastectomy (months)	Response†
Receptor-rich	EP	26	R
	EP	26	F
	E	24	R
	Ado	14	R
Receptor-poor	E	67	R
	O	60	F
	Ord	16	F
	Ord	15	F
	Ado	12	F
	H	12	F
	O	11	F
	An	10	F
	Ado	8	F
	O	7	F
	E	4	F

* Ado, adrenalectomy plus oophorectomy; An, androgen; E, estrogen; H, hypophysectomy; O, oophorectomy; Ord, radiation castration; EP, estrogen plus progestin.
† R, objective remission; F, failure.

Non-responding receptor-rich cancers

From the foregoing results, it is evident that there are some patients with substantial estrophilin levels in their tumors who still do not respond to hormone therapy. The reason for these non-responding, receptor-rich cases is not entirely clear. In some instances, it appears that the patient may have a mixture of receptor-rich and receptor-poor metastases with a positive specimen obtained for study. The fact that many of the estrophilin-rich patients, who do not show objective remissions, do experience subjective remissions is consistent with the supposition that some but not all of their metastases are hormone-responsive. It has also been suggested[25] that, since its synthesis in target tissues is known to be dependent on estrogen, the progesterone receptor may be a marker protein for the action of estrogen in a tumor, so that hormone-dependent cancers will contain receptors for both estrogenic and progestational

hormones. Thus estrophilin-containing tumors that lack progesterone receptor may be the ones that do not respond to endocrine therapy. The preliminary report[25] of the correlation of response with the presence of both progesterone and estrogen receptors appears promising, although more patients must be studied before the usefulness of progesterone receptor measurements can be evaluated.

It is also possible that some tumors, which have escaped from homone-dependency during neoplastic transformation, may continue to produce estrophilin even though they do not need it. A characteristic of target tissues is that the RNA polymerase activity of their nuclei can be increased significantly by administration of estrogen to the animal *in vivo*[26] or by exposure of the isolated nuclei to estrogen–receptor complex *in vitro*[27, 28]. The susceptibility of nuclei to stimulation by estrogen-receptor complex is specific for hormone-dependent tissues (Fig. 4), which also contain much higher levels of estrophilin than do non-dependent tissues[29]. A similar relation is seen in the case of mammary tumors induced by dimethylbenzanthracene in the Sprague–Dawley rat[30]. As shown in Fig. 5, the RNA polymerase system in nuclei from hormone-dependent tumors that regress on ovariectomy resembles that of uterine nuclei in its sensitivity to stimulation by the estrogen–receptor complex of tumor cytosol, whereas the polymerase activity of nuclei from autonomous tumors that continue to grow in the ovariectomized animal tends to be higher than that of hormone-dependent nuclei and cannot be increased further by estrogen–receptor

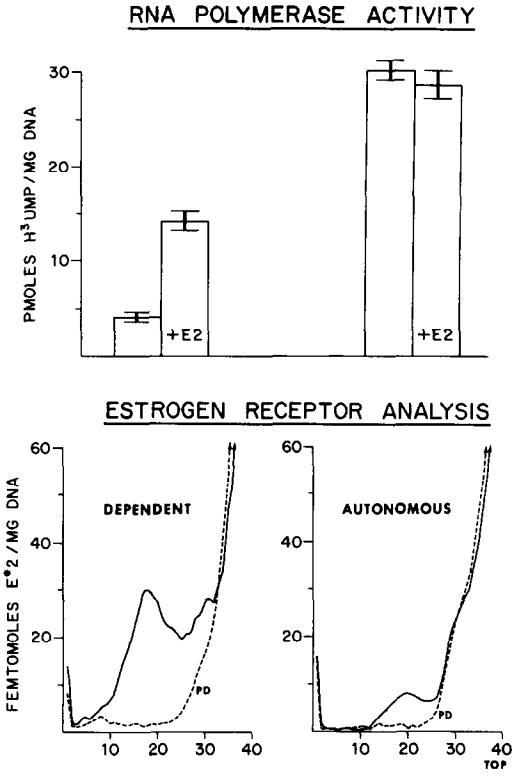

Fig. 5. RNA polymerase activity of isolated nuclei and cytosol estrophilin content (sedimentation pattern) of cytosol from DMBA-induced rat mammary tumors that either regress (dependent) or continue to grow (autonomous) in ovariectomized animals. Experimental details given in Arbogast and DeSombre[30].

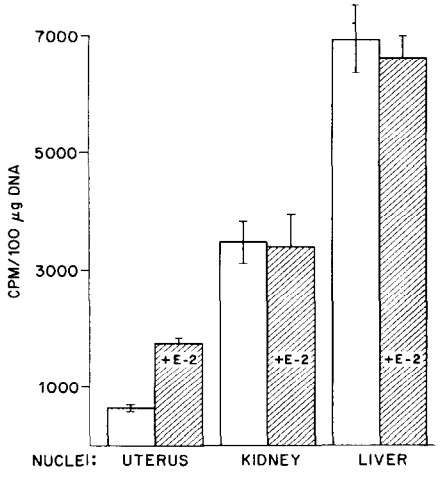

Fig. 4. Tissue specificity of the influence of estrogen-receptor complex *in vitro* on RNA polymerase activity of isolated nuclei. Nuclei, isolated from 2.2 M sucrose homogenates of various immature rat tissues, were incubated at 25°C for 30 min with rat uterine cytosol (in 2.2 M sucrose, 1 mM $MgCl_2$) in the presence and in the absence of 10 mM estradiol (E-2). The nuclei were then separated by centrifugation and resuspended in 0.32 M sucrose for assay of magnesium-dependent RNA polymerase by measuring the incorporation of tritiated UMP from UTP. Results are the mean values of seven replicate determinations. From Jensen *et al.*[28].

complex. Cytosol from hormone-dependent rat mammary tumors contains substantial amounts of estrophilin, whereas cytosol from autonomous tumors usually contains receptor in much lower amounts, suggestive of a critical level similar to the phenomenon in human breast cancers. However, one occasionally finds an autonomous tumor, with nuclei insensitive to stimulation, that contains a high level of cytosol receptor[30], suggesting that the turning off of estrophilin synthesis, that usually is coupled to the escape of the nucleus from hormone dependency, does not invariably take place and that some autonomous mammary tumors continue to synthesize large amounts of estrophilin, even though their nuclei do not require stimulation by hormone–receptor complex.

The foregoing results imply that the primary basis of hormone-dependency involves the RNA polymerase system of the nucleus and that the production of estrophilin is an associated phenomenon which usually but not always is correlated with the nuclear requirement for stimulation. If a similar situation obtains in the case of human breast cancer, giving rise to some estrophilin-rich autonomous tumors, such non-responding, receptor-positive patients might be identified by determining the sensitivity of their

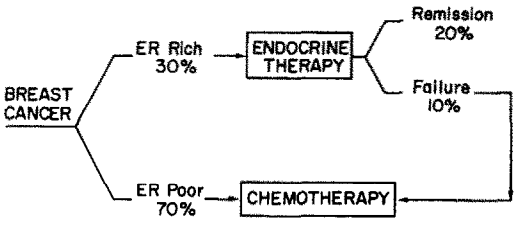

Fig. 6. Estrophilin assay as a guide to therapy.

tumor nuclei to stimulation by estrogen–receptor complex.

On the basis of the critical estrophilin level as defined in Fig. 2, more than 1200 women, whose primary and/or metastatic breast cancers have been assayed, were found to consist of about 30% classified as receptor-rich and 70% as receptor-poor (Fig. 6). From the results with 133 treated cases, nearly two-thirds of the receptor-rich patients can expect objective benefit from endocrine therapy, whereas few if any of the receptor-poor group will respond. Thus estrophilin determination of the tumor can aid in choosing optimal therapy in 85 to 90% of the total patients.

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DISCUSSION

King. I am puzzled about your switch from 30% receptor poor to 30% receptor rich. It seems to most of us that it's the other way round.

Jensen. There has not been any switch, sudden or otherwise. Only 30% are receptor rich by the definition I gave.

King. Could you explain this more fully?

Jensen. That depends on how one defines rich and poor. We used to speak of positive and negative, depending on whether or not one could detect any receptor at all. After we began to express our results in terms of the amount of receptor present, it became apparent, as seen from Figs 2 and 3, that, with the exception of a few anomalous cases, responses are not seen with tumors containing less than a certain amount of receptor and that this "critical level" is lower in patients with ovarian function. Similarly DeSombre found that most autonomous rat mammary tumors contain small but definite amounts of receptor. So we have abandoned the absolute terms, positive and negative, and now define receptor rich and receptor poor on the basis of the minimum receptor level associated with response. In our experience, 70% of the more than 1200 human breast cancers we have assayed have receptor levels less than the empirically determined critical value and thus are classified receptor poor.

Lippmann. At least 2 years ago, with the sensitivity of the assays that investigators were using then, it seemed to be that if their assays could detect any significant amount of receptor usually it was the limit of their assay. I believe for Dr. McGuire it was 3 fmol/ml of cytoplasm. Once one got above that level, there did not appear to be an increasing response rate with increasing quantitative amounts of receptor and I wonder if other people could comment on this, because just like Dr. King, I am very puzzled by what appears to be a discrepancy between different people working in this field. I am feeling very confused about what really is going on.

Jensen. Looking again at the results with rat mammary tumor (Fig. 5) it is clear that this autonomous tumor, which continues to grow in the ovariectomized animal, has definitive receptor content. It just is lower than the level in the dependent tumor.

Kellie. Could I just ask at this particular point something about the properties of these DMBA induced rat mammary tumours. I understand that originally they were considered to be estrogen-dependent.

Jensen. About 85% will regress and 15% will continue to grow after ovariectomy.

Kellie. But I understand that this is not true in hypophysectomized animals and that in fact estradiol will not maintain them.

Jensen. That is another matter. The tumors that are hormone dependent regress in both the hypophysectomized and ovariectomized animals. Administration of estrogen will restore their growth in the ovariectomized but not in the hypophysectomized rat, indicating that, in addition to estrogen, there is some pituitary factor that is required for mammary tumor growth. This is not true for uterus which grows in the hypophysectomized rat on administration of estrogen alone.

Kellie. You don't think a pituitary compound is being activated by the estradiol and the administration of estradiol is causing the secretion for prolactin which is itself affecting the tumour.

Jensen. That has been proposed, but I believe that there is now evidence that estrogen can act on the tumor directly. Certainly the estrogen–receptor complex can stimulate RNA polymerase in isolated tumor nuclei.

McGuire. The question of receptor rich and receptor poor cutoff values has been raised. It is of interest that in the international workshop data there was a group of patients whose tumors fell into an equivocal area slightly higher than 3 fmol per mg of cytosol protein. It is of interest that even in this group the response rate was approximately 43%. In any case, I am not sure it is worth arguing whether one should use absolute values or receptor poor–receptor rich classification. If we are talking about a test that can be used in a clinical setting, then the most important thing is that it accurately predicts the outcome of therapy. On the other hand, if we are talking about a very basic biochemical research problem, then receptor rich and receptor poor could be misleading.

Jensen. Your value of 3 fmol is expressed in terms of cytosol protein so it is not comparable with levels I referred to, which are based on tumor weight.

Lidner. You stress the fact that the prognostic of the tumour behaviour is still less than perfect and that ablative surgery is a severe trauma that you would like to spare many patients. If the aim is to deprive the patients of steroids, of estrogens, is ablative surgery today the only way to achieve this? Should one not consider also active immunization in animals? For instance, today we can immunize the LRH deca peptide and have the gonads shrivel so that you can hardly find them. Ovariectomy is not such a serious procedure, but we would also like to eliminate adrenal androgens. If we could immunize against androstenedione which seems to be the main adrenal source of estrogen, could this not substitute for adrenalectomy?

Jensen. I certainly think it could.

Lidner. You could do it on your DMBA-induced rats first and see whether one can get regression of tumours.

McGuire. DMBA tumor bearing rats have been immunized with BSA-estradiol conjugates and tumor regression has occurred. Personally, I think a better approach is to use antiestrogen because you can start and stop the therapy as dictated by the clinical circumstances.

Jensen. In that regard, several studies of anti-estrogens have been carried out. The earlier experiments with nafoxidine suffered from the side effects that build up on prolonged administration. It is my understanding that tamoxifen, although it is of the same chemical class as nafoxidine and Parke Davis CI 628, is remarkably free from side effects. It has been studied most extensively by Heuson at the Institute Jules Bordet in Belgium, and his results are quite encouraging.