It has been known for many years that sex steroid hormones play a role in the development, growth, and behavior of certain tumors.1-5 Hormonal manipulation has been shown to be effective in the management of a significant number of patients with breast, prostate, and gynecologic malignancies.1-4,6-13 It is recognized that, in order for sex steroid hormones to exert their effect, specific receptors should be present.14,15 Thus, detection of sex steroid hormone receptors in hormone-dependent tumors has been important in our understanding of tumor biology and the mechanism of endocrine therapy.

Hormone receptor analysis is traditionally performed on surgically removed specimens. However, there are situations where surgical specimens are not available or suitable for such analysis. In these circumstances, cytologic material such as fine-needle aspirates, imprints, and exfoliated cells in malignant effusions can be utilized for estrogen and progesterone receptor analysis. Fine-needle aspiration biopsy (FNAB) offers a suitable alternative for hormone receptor analysis in several circumstances: first, in inoperable cases and metastatic or recurrent tumors in which the size and the accessibility to surgical biopsy presents a problem; second, in selected cases in which preoperative irradiation or presurgical therapy are the initial treatment options; and third, in advanced tumors where serial hormone receptor studies may potentially provide information regarding response to therapy. FNAB is an easy, cost effective, and nontraumatic procedure which permits sampling of several areas of the same tumor and allows better assessment of hormone receptor heterogeneity.16-20

Imprint preparations have also been utilized for hormone receptor analysis and are recommended for tumors that are too small or ill defined to permit conventional sampling for hormone receptor analysis.21-24 It has also been possible to show expression of hormone receptors in cytologic preparation obtained from malignant effusions.25-27

This review summarizes the characteristics of estrogen and progesterone receptors with emphasis on the application of cytochemical techniques in assessment of sex steroid hormone receptors in cytologic samples.

Definition and Characteristics of Estrogen and Progesterone Receptors

Estrogens, in common with other steroid hormones, regulate gene expression in target cells through their interaction with specific macromolecular binding proteins called receptors.28 Receptors are normally found in target tissue such as breast and female reproductive organs. Because receptors appear in the supernatant or cytosol fraction of tissue homogenates, it was originally thought that estrogen binds to its receptor in the cytoplasm and then enters the nucleus by a process termed translocation (Fig. 1).29 Recent studies have suggested that the estrogen-free receptor is localized predominantly in the nucleus,30,31 where it is loosely bound until its association with estradiol converts the receptor to an active form with the ability to bind tightly in the genome.32 Initially, the estrogen receptor (ER) associates with low affinity type I receptors within the cytoplasm and then enters the nucleus by virtue of the high affinity receptors in the nucleus. The activated ER complex is believed to bind with chromatin, resulting in specific changes in gene expression, leading to synthesis of new messenger RNA (mRNA) and protein characteristics of the hormone's cellular effect (Fig. 2).32 Activation of ER stimulates transcription of progesterone receptor (PgR) mRNA. Binding of PgR with progesterone then induces synthesis of up to 100 proteins, including growth and mitogenic factors.33

There are at least two ERs each with different affinities for estrogen.34,35 Type I, the "true" receptor, is routinely determined in specimens (cytosolic ER) and has the great-
Fig. 1. Old model for the sequence of events in the mechanism of steroid hormone action on a target. The difference is in the formation of the hormone/receptor complex within the cytoplasm followed by translocation into the nucleus. Within the nucleus, the rest of the events are similar to the new model. (From Masood S: Sex Associated Hormones in the book, Serological Cancer Markers, 115-192, 1992, Sell S, ed., reproduced by permission of Humana Press, Totowa, NJ.)

est affinity for estrogen. Type II, the “low affinity” receptor, is present in cytoplasm and nucleus and represents a different group of proteins which was first reported in the cytosol of the rat uterus in 1978. The functional significance of type II ER is not yet quite clear.

At the present time, it is not clear why some hormone-dependent tumors fail to express ER. Gene mutation, heterogeneity of receptors in tumors, and effects secondary to transfection with the ras oncogene are the suggested hypotheses.

History of Hormone Dependency of Tumors
The first published report of hormonal therapy of breast carcinoma appeared in 1896 when Beatson reported remission of metastatic breast cancer following oophorectomy. In the 1940s orchiectomy was introduced for the treatment of prostate cancer. Interest in this form of therapy has been sustained since reports by Huggins and Bergensalt in 1952 and Luft et al. in 1958 on the efficiency of adrenalectomy and hypophysectomy in the management of metastatic breast disease.

By the early 1960s, endocrine ablation for advanced breast cancer became the preferred treatment. However, clinical experience indicated that only 20%-30% of patients respond favorably to such treatment. This led to various investigations to distinguish between responsive and nonresponsive tumors. The work of Folca and associates in 1961 demonstrated greater binding of radioactive estrogen in patients who benefited from ablative surgery, pointing toward development of a practical method for prediction of response. Following description of ER protein in the rat uterus by Toft and Gorski, Jensen reported detection of ER in human breast tumors. Following this initial indication of a “receptor,” the correlation between ER content of carcinomas of the breast and their clinical responsiveness to endocrine therapy has become well established by many investigators.

Soon it was also recognized that PgR synthesis is usually an estrogen-dependent process and that the presence of PgR is an expression of a fully functional ER mechanism. Thus, it seemed reasonable to assume that the assessment of PgR may improve the predictive value of hormone receptor determination. Indeed, it has been shown clinically that PgR appears to be a more important prognosticator than ER.

In the past few years, hormone receptors have been well characterized. Monoclonal antibodies specific to both es-
Fig. 2. New model for the sequence of events in the interaction of steroid hormone with a target cell. The steroid hormone associates with low affinity receptors in the cytoplasm and then binds to high affinity receptors in the nucleus. Upon binding to nuclear chromatin, the ER complex stimulates RNA polymerase activity which leads to production of new messenger RNA (mRNA) and synthesis of new protein characteristics. (From Masood S: Sex Associated Hormones in the book, Serological Cancer Markers, 115-192, 1992, Sell S, ed., reproduced by permission of Humana Press, Totowa, NJ.)

trogen and progesterone have been developed. 30,54 Human genes for the receptors have been cloned.55,56 The ER gene, on chromosome 6, produces two 65-kd subunits. The PgR gene, on chromosome 11, transcribes two dissimilar subunits of 95 and 120 kd. Each cell contains about 10,000 ERs and 50,000-100,000 PgRs. With these new developments, we can more precisely study the expression of sex steroid hormone receptors in managing hormone-dependent tumors.

Pattern of Expression and Prognostic Value of Hormone Receptors in Breast and Gynecological Malignancies

Among many tumors tested for the presence of ER, PgR, and other steroid hormone receptors, breast and gynecological tumors have shown the most consistent pattern of expression. The prognostic, diagnostic, and therapeutic implications of hormone receptors in the management and follow-up of patients with breast and gynecological malignancies are also well established.7-12,57-63

Breast

In American women with breast cancer, expression of ER is seen in 77% of cases, while the frequency of PgR positivity is 55%.64 Basically, tumors that are both ER+ and PgR+ are regarded as being hormone responsive, while cancers that are hormonally nonresponsive are often ER− and PgR−. Tumors that are of a more dubious hormone responsive nature are either ER+ and PgR− or ER− and PgR+.65

Determination of hormone receptors in breast tumors also provides valuable information for predicting the risk of recurrence and metastasis in primary breast tumors and for choosing both adjuvant regimes and hormone therapy for patients with disseminated disease (Fig. 3).

Other prognostic factors in breast cancer are tumor size, nuclear grade, and lymph node involvement. Hormone receptor positive tumors tend to be smaller and often show a high degree of histological differentiation.66-68 The relationship between hormone receptor status and lymph node involvement is not significant.64

Despite the implementation of these well-established prognostic indicators, approximately 30% of patients defined as low risk for recurrent disease demonstrate progression of the disease within 5 yr.66 Thus, other prognostic factors must be found. DNA ploidy study, assessment of proliferative index, and detection of epidermal growth factor have been utilized recently in predicting the out-
CHOOSING THE THERAPEUTIC APPROACH

Fig. 3. Schematic presentation of choosing treatment options for patients with breast cancer based on ER status of the tumor. (From Masood S: Sex Associated Hormones in the book, Serological Cancer Markers, 115–192, 1992, Sell S, ed., reproduced by permission of Humana Press, Totowa, NJ.)

Of patients with breast cancer. In addition, expression of HER-2/neu oncogene and high concentration of a protease, 52K cathepsin-D, have been found to be associated with poor prognosis in breast cancer patients.

Ovary

In the last few years, it has been recognized that many primary ovarian carcinomas and their metastasis are rich in ERs and PgRs. Since then, the prognostic and therapeutic value of sex steroid hormone receptors in ovarian tumors have been under intense investigation. However, the results of these studies have been controversial and no clear guidelines are yet available. Nevertheless, cooperative studies with a large number of patients are required to definitively evaluate the role of ERs and PgRs in the management of patients with ovarian cancer.

Meanwhile, assessment of hormone receptors may be of some value in determining the primary site of tumors of unknown origin as well as providing a basis for possible new therapeutic modalities such as the use of radiolabeled ER ligands for imaging and treatment of hormone receptor rich tumors.

Endometrium

Clinical evidence reported in the literature supports the concept that response to hormonal therapy and prognosis in endometrial cancer are related to the steroid hormone receptor status of these tumors.

While some investigators have demonstrated a better prognosis for ER+ and PgR+ endometrial cancer, Ehrlich and associates in 1988 reported a significant relationship only between PgR content and survival. The role of PgR in the management of advanced or recurrent endometrial cancer is also supported by others. In the cumulative world literature, the response rate to progesterin for PgR+ endometrial cancer has been reported to be 72%, while the response rate for PgR− endometrial cancer is reportedly 12%.

Cervix

ERs and PgRs have been demonstrated in normal, pre-malignant lesions, and neoplastic processes of the cervix. Sporadic reports in the literature regarding the prognostic significance of hormone receptors in cervical cancer have shown mixed results. Nevertheless, Potish et al. in 1986 suggested that clinical stage and hormone receptor
level independently predict survival in patients with cervical cancer. Despite the independent prognostic value of receptors in cervical cancer, their therapeutic utility remains to be proven. Generally, cervical cancer is refractory to endocrine therapy. Thus, the value of ER and PgR determination in cervical cancer may be limited to select poor prognostic groups of patients for inclusion into a more aggressive chemotherapy regimen.

**Procedures Used in Hormone Receptor Analysis**

**Biochemical Assays**

Biochemical assays were among the first to be developed and are now the “standard” by which other methods are compared. These assays utilize a tumor extract in which radioligands combine with receptors to form a steroid-receptor complex. Following separation of bound from unbound radioligands, the bound hormone is measured and the quantity of hormone receptor calculated. Separation may be accomplished either by absorption to dextran-coated charcoal (DCC) or by sucrose density gradient centrifugation. The DCC technique is the most commonly used. Current biochemical assays suffer from some inherent disadvantages. Interlaboratory variation in biochemical assays may be considerable, factors such as dissociation of the noncovalently bound marker, contamination by traces of heavy metal, or degradation of the receptor in storage or processing may influence the procedure. Furthermore, anti-estrogen therapy, high dose estrogen therapy, nonspecific binding, and exogenous estradiol may alter the results.

Additionally, the biochemical methods require ultracentrifugation equipment, scintillation counters, and radiolabeled hormones. Thus, they are suitable only for large laboratories. Furthermore, biochemical assays cannot accurately assess tumor heterogeneity and may be completely unsuitable for small lesions or cases where only a small amount of tumor tissue is available. Above all, the predictive value of the biochemical assays is less than sterlil, since only 55%–60% of patients with biochemical receptor positive tumors and about 10% of patients with receptor negative tumors respond to endocrine therapy. Thus, the need for practical tests that are simpler, less expensive, and less sensitive to variation than the commonly used biochemical assays has been well recognized.

**Immunohistochemical Assays**

Immunohistochemical assays may be performed by either the immunoperoxidase or the immuno fluorescence technique. In these methods, tissue sections or cells are incubated with estradiol, and anti-estadiol antibody is added. Detection is accomplished with either a fluorescein-labeled anti-IgG or an unlabeled antibody binding to a peroxidase-antiperoxidase complex.

In addition to tissue sections or isolated cells, tissue homogenates may be utilized with employment of immunoradiometric or enzyme-linked immunoassays. In both of these procedures, a polystyrene bead coated with an anti-estrophilin antibody is incubated with tissue homogenates. Binding of the tissue receptor immobilizes the receptor on the bead. After several washings, a second labeled antibody is added which for immunometric assay is labeled with I-125 and for enzyme-linked immunoassay is labeled with peroxidase. Although good correlation with sucrose density gradient values has been demonstrated, unresolved issues concerning these latter assays include the availability of receptor bound estradiol to antibody, the detection of estradiol receptor for detection, and potential nonspecific binding.

**Fluorescent Technique**

The fluorescent technique introduced by Lee in 1978 employs estradiol linked to bovine serum albumin (BSA), coupled to fluorescein isothiocyanate (FITC). Progesterone is linked to BSA and coupled to tetramethylrhodamine isothiocyanate (TMRITC). This procedure is now patented as FluoroCep (Zeus Technologies, Inc., Raritan, NJ). In a number of studies, the fluorescent technique has shown good correlation with the biochemical assay. Indeed, reported sensitivity, specificity, as well as positive and negative predictive values for the clinical response rate are almost comparable to those reported for the biochemical assay.

The fluorescent technique may identify the lower affinity type II cytoplasmic estrogen binding proteins rather than the high affinity type I nuclear ER measured biochemically. This latter issue has been raised as a strong point against the fluorescent technique. However, the good results of this assay as reported in the literature may be explained by the frequent coexistence of cytoplasmic and nuclear ERs, as demonstrated by Lee in 1989. He clearly showed that, for comparative purposes, both the cytoplasmic and nuclear ER assay appear to be useful for identification of ER+ tumors, demonstrating 94%–97% concordance.

**Immunoperoxidase Technique**

This technique, utilizing monoclonal antibody to nuclear ER or PgRs and peroxidase-antiperoxidase for visualization, is applicable to paraffin or frozen tissue sections and to cytologic preparations (Fig. 5). ER monoclonal antibodies are produced by hybridomas utilizing rats immunized with purified receptor from MCF-7 type human breast cancer cell. Typically, a rat monoclonal antibody binds to human estrophilin in tissue or cells on a microscope slide. A goat antibody forms a bridge between...
Ab, attached to ER molecule at second binding site. The antibody is tagged with either radioactive label or peroxidase enzyme

The anti-estrophilin monoclonal antibody and a rat antibody bound to horseradish peroxidase. After washing, the bound enzyme-bearing complex reacts with a chromogenic substance to form a colored substance at the receptor site, yielding brown nuclear staining. This nuclear staining may be eliminated by preincubation of the anti-estrophilin with either purified ER or receptor-rich cytosol. Other evidences supporting specificity of this assay include absent staining with control antibodies, identical patterns of staining with different ER monoclonal antibodies, excellent correlation with the biochemical procedure, and appropriate tissue specificity of the staining.118-122 This procedure is available through a commercial kit [estrogen receptor immunocytochemical assay. (ER-ICA), Abbott Laboratories, Chicago, IL]. More recently, a similar kit has been made available for progesterone receptor (PgR-ICA). Data concerning concordance of PgR-ICA with the biochemical procedure are limited but appear promising.123-125

The immunoperoxidase technique is attractive not only for its binding to the nuclear ERs or PgRs but also for the ability to utilize fresh, frozen, or formalin-fixed paraffin-embedded specimens. This technique is also easily adaptable to cytologic material.

The use of paraffin block material is well suited for retrospective studies. For example, utilizing protease and paraffin-embedded material, Barnes and Masood126 demonstrated a strong correlation between ER patterns in situ and invasive carcinoma within the same breast. This method also offers a reliable alternative when tissue

Fig. 4. Schematic presentation of immunometric and enzyme-linked immunoassay for detection of ER. (From Masood S: Sex Associated Hormones in the book, Serological Cancer Markers, 115-192, 1992, Sell S, ed., reproduced by permission of Humana Press, Totowa, NJ.)
ESTROGEN AND PROGESTERONE RECEPTORS IN CYTOLOGY

Frozen Section, Fine-Needle Aspirate, Imprint Preparation, Permanent Section, Cytospin Preparation

Chromogen

Rat-PAP

Goat Anti-Rat

Monoclonal Rat Anti-ER

Estrogen

ER

Fig. 5. (A) ER-ICA can be applied to frozen sections, fine-needle aspirates, imprint preparations, permanent sections, and cytospin preparations. (B) Schematic presentation of peroxidase-antiperoxidase technique utilizing specific monoclonal antibody directed against estrogen. (C) ER expression is seen exclusively as brown nuclear staining. (From Masood S: Sex Steroid Hormone Receptors in Cytologic Material, in Cytopathology Annual 1992:4:83, reproduced by permission of Williams & Wilkins, Co., Baltimore.)

is unavailable or not suitable for biochemical assay or conventional immunocytochemical assay on frozen tissue.

Use of Cytologic Preparations in Hormone Receptor Assays

Imprint Preparations

Imprint cytology, introduced by Dudgeon and Patrick in 1927, has been applied to a wide variety of organs and tissues. The value of this technique is now firmly established and it is utilized in some institutions as an adjunct to frozen sections. Steroid hormone receptors can be analyzed in imprint preparations with either the fluorescent or immunoperoxidase technique. It is already demonstrated that assessment of ER and PgR using imprint preparation is comparable to that obtained from conventional cytochemical techniques on frozen sections as well as with DCC (Table I, Fig. 6). The first report by Masood and Johnson on the value of imprint cytology in cytochemical detection of steroid hormone receptors in breast cancer appeared in 1987. They utilized the fluorescent technique.

Table I. Summary of Concordance Between the Cytochemical Hormone Receptor Assay on Imprint Preparations and the Biochemical Assay of the Excised Surgical Specimen

<table>
<thead>
<tr>
<th>Reference</th>
<th>No. of cases</th>
<th>Site</th>
<th>ER</th>
<th>PgR</th>
<th>Technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>Masood and Johnson</td>
<td>42</td>
<td>Breast</td>
<td>90</td>
<td>90</td>
<td>Fluoro-Cep</td>
</tr>
<tr>
<td>Masood</td>
<td>45</td>
<td>Ovary</td>
<td>91</td>
<td>93</td>
<td>Fluoro-Cep</td>
</tr>
<tr>
<td>DeRosa et al.</td>
<td>47</td>
<td>Breast</td>
<td>92.3</td>
<td>92.3</td>
<td>ER-ICA</td>
</tr>
<tr>
<td>Masood</td>
<td>214</td>
<td>Breast</td>
<td>95</td>
<td>94</td>
<td>ER-ICA</td>
</tr>
</tbody>
</table>

Total 348 92 92

4ER, estrogen receptor; PgR, progesterone receptor; Fluoro-Cep, fluorescent cytochemical technique; ER-ICA, estrogen receptor immunocytochemical assay; PgR-ICA, progesterone receptor immunocytochemical assay.

(From Masood S: Sex Steroid Hormone Receptors in Cytologic Material, in Cytopathology Annual 1992:4:83, reproduced by permission of Williams & Wilkins, Co., Baltimore.)

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cent cytochemical method to study ERs and PgRs in frozen sections and imprint preparations. The imprint method yielded a 90% concordance with the DCC biochemical method and in this respect was superior to the frozen sections. Another study demonstrated good concordance with the DCC biochemical method using imprint preparation in ovarian cancer.22 These studies demonstrated that cytologic material is adequate to depict the presence of hormone receptors using the fluorescent technique.

More recently, good results have been demonstrated with the immunoperoxidase technique for ER and PgR assays in touch preparations.23,24 It is also shown that storage at −80°C for up to 56 wk is possible without loss of reactivity, potentially making assays more flexible and economical.23

Thus, imprint preparations are suitable for sex steroid hormone receptor analysis. Imprint preparations may be particularly useful for small specimens such as clinically early or occult breast carcinomas and for small metastatic lesions. In such cases it may not be possible to submit a portion of the biopsy specimen for hormone receptor analysis without jeopardizing the histologic diagnosis.

With increasing emphasis on the diagnosis of clinically occult breast carcinomas as the result of refinements in mammography, the value of cytochemical hormone receptor assays in imprint preparations will become more recognized.

**FNAB**

Over the past several years, FNAB has been increasingly recognized as a valuable tool for the diagnosis of cancer. Although FNAB does not eliminate the need for surgical biopsy, therapy in some cases may be based on FNAB results without either open biopsy or frozen section. In addition to the application of established cytologic crite-
ria, the ability to identify specific markers has enhanced the diagnostic and therapeutic utility of FNAB. Because of the small quantity of material obtained, biochemical DCC analysis cannot be performed on FNAB material. Isoelectric focusing has been applied to ER measurement in FNAB. This procedure is based on electrofocusing of tritiated estradiol-bound receptors in slices of polyacrylamide gel. Silversward and Humlia in 1980 reported promising results using this technique. However, this procedure requires a highly cellular aspirate and is relatively difficult to perform. More recently cytochemical hormone receptor assays have been employed in FNAB material. Good correlation with the biochemical method performed on tissue adjacent to or near the FNAB site has been demonstrated for both the fluorescent and the immunoperoxidase techniques (Table II). These studies suggest that through cytochemical hormone receptor assays, using fluorescent conjugate or monoclonal antibodies against ER and PgR, cytologic preparations of aspirated cells can be evaluated for presence or absence of hormone receptors. With the fluorescent method, heterogeneity of cytoplasmic staining is frequently prominent and tumors interpreted as positive should demonstrate bright green and red fluorescence for ER and PgR in 30% of cells examined (Figs. C-1–C-3). With the immunoperoxidase technique, nuclear staining in 20% of tumor cells is regarded as positive (Figs. C-4, C-5). Although the fluorescent method may be more economical, the immunoperoxidase technique offers great flexibility in that it may be successfully applied to direct smears, cytospin preparations, and paraffin-embedded cell block material.

### Table II. Summary of Concordance Between the Cytochemical Hormone Receptor Analysis on Fine-Needle Aspirates and the Biochemical Assay of the Corresponding Surgical Specimen

<table>
<thead>
<tr>
<th>Reference</th>
<th>No. of cases</th>
<th>ER</th>
<th>PgR</th>
<th>Technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>Azevedo et al.136</td>
<td>31</td>
<td>90%</td>
<td>87</td>
<td>ER-ICA</td>
</tr>
<tr>
<td>Curtin et al.138</td>
<td>41</td>
<td>83%</td>
<td>87</td>
<td>Fluoro-Cep</td>
</tr>
<tr>
<td>Flowers et al.139</td>
<td>33</td>
<td>85%</td>
<td>87</td>
<td>ER-ICA</td>
</tr>
<tr>
<td>Keshgegian et al.139</td>
<td>58</td>
<td>76%</td>
<td>92</td>
<td>ER-ICA</td>
</tr>
<tr>
<td>Lozowski et al.140</td>
<td>25</td>
<td>92%</td>
<td>92</td>
<td>ER-ICA</td>
</tr>
<tr>
<td>Lundby et al.19</td>
<td>96</td>
<td>87.5</td>
<td>91.6</td>
<td>ER-ICA</td>
</tr>
<tr>
<td>Masood141</td>
<td>62</td>
<td>92%</td>
<td></td>
<td>ER-ICA</td>
</tr>
<tr>
<td>Masood141</td>
<td>71</td>
<td>89%</td>
<td>86</td>
<td>PgR-ICA</td>
</tr>
<tr>
<td>Masood135</td>
<td>56</td>
<td>89%</td>
<td>86</td>
<td>Fluoro-Cep</td>
</tr>
<tr>
<td>Katz et al.137</td>
<td>27</td>
<td>94%</td>
<td></td>
<td>ER-ICA</td>
</tr>
<tr>
<td>Total</td>
<td>500</td>
<td>87.6</td>
<td>88.4</td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations as defined in Table I.

(From Masood S: Sex Steroid Hormone Receptors in Cytologic Material, in Cytopathology Annual 1992:4-83, reproduced by permission of Williams & Wilkins, Co., Baltimore.)

Using a simple score based on heterogeneity and staining intensity it is also possible to show statistically significant correlation between receptor levels assayed biochemically and those estimated by cytochemical hormone receptor assay (Fig. 6). Thus, semiquantitation of hormone receptor values may be possible. For breast cancer patients in whom surgery might not be performed, such as in advanced localized and recurrent or metastatic disease, sex steroid receptor analysis of FNAB material can eliminate the need for open biopsy. These techniques also may be applied to mammographically guided fine-needle aspirates. Provided that adequate and representative material is obtained, FNAB may be utilized to identify breast cancer patients most likely to respond to hormone therapy.

### Malignant Effusions

The value of ER and PgR as important predictors of response to hormone therapy has been established for both breast and gynecological malignancies. In advanced disease, however, receptor analysis may be more difficult due to the inaccessibility of metastatic tumor. Patients with advanced disease, however, may have pleural or peritoneal effusions in which cytologic material is readily obtainable. Quantitative biochemical hormone receptor assays have been employed in detection of hormone receptors in tumor cells exfoliated in malignant effusion. However, these studies are sparse and report only a small number of cases. Quantitative biochemical assay on malignant effusion is also limited by a need for highly cellular material. Immunocytochemical assays require less cellular material and are well suited for assessment of hormone receptors in the cells derived from malignant effusion.

Hormone receptor analysis has been performed on tumor cells obtained from patients with malignant effusions using both the fluorescent and immunoperoxidase techniques. In studies where biochemical values of primary tumors are available, a satisfactory correlation was obtained between the two results (Table III).

Direct smears, cytospin preparations, or cell block material may be utilized for hormone receptor analysis. In our experience, Cytospin (Shandon, Inc., Pittsburgh, PA) preparation technique allowed use of effusions with a small volume or limited number of tumor cells. In none of the cases studied was there problem obtaining adequate cellular material for hormone receptor analysis (Figs. C-6, C-7).

Assessment of ER and PgR in malignant effusions is clinically significant especially when the hormone receptor status of the primary tumor is unknown. In ovarian tumors, ER expression continues in patients with advanced disease and even after chemotherapy, whereas PgR expression is lower if the patient has received chemo-
Fig. C-1. Imprint smear of primary breast carcinoma. Malignant cells show loss of cohesion (Diff-Quik, ×250). (From Masood S: Sex Steroid Hormone Receptors in Cytologic Material, in Cytopathology Annual 1992:4-86, reproduced by permission of Williams & Wilkins, Co., Baltimore.)

Fig. C-2. Fluorescent photomicrograph of ER+ tumor cells in an imprint smear of carcinoma of the breast (original magnification ×400). (From Masood S: Sex Steroid Hormone Receptors in Cytologic Material, in Cytopathology Annual 1992:4-86, reproduced by permission of Williams & Wilkins, Co., Baltimore.)

Fig. C-3. Fluorescent photomicrograph of PgR tumor cells in an imprint preparation of carcinoma of the breast. Fig. C-4. Smear from FNAB of an infiltrating duct cell carcinoma showing clusters of neoplastic epithelial cells (Diff-Quik, ×250). (From Masood S: Use of Monoclonal Antibody in Assessment of Estrogen Receptor Content in Fine Needle Aspiration Biopsy Specimen from Patients With Breast Cancer. Arch Pathol Lab Med 1989;113:26–30, reproduced by permission, copyright 1989, American Medical Association.)

Fig. C-5. Fine-needle aspiration smear from a patient with primary breast carcinoma showing strong nuclear staining for ER with monoclonal antibody technique (×250). (From Masood S: Use of Monoclonal Antibody in Assessment of Estrogen Receptor Content in Fine Needle Aspiration Biopsy Specimen from Patients With Breast Cancer. Arch Pathol Lab Med 1989;113:26–30, reproduced by permission, copyright 1989, American Medical Association.)

Fig. C-6. Clusters of neoplastic epithelial cells seen in cytospin preparation of ascitic fluid from a patient with primary ovarian carcinoma (Diff-Quik, ×400). (From Masood S: Sex Steroid Hormone Receptors in Cytologic Material, in Cytopathology Annual 1992:4-87, reproduced by permission of Williams & Wilkins, Co., Baltimore.)

Fig. C-7. Metastatic tumor cells in ascitic fluid from a patient with ovarian carcinoma showing nuclear staining for ER utilizing monoclonal antibody (×250). (From Masood S: Sex Steroid Hormone Receptors in Cytologic Material, in Cytopathology Annual 1992:4-87, reproduced by permission of Williams & Wilkins, Co., Baltimore.)
The prepared slides are then processed in accordance with either fluorescent cytochemical technique, (Fluoro-Cep) or estrogen and progesterone receptor immunocytochemical assay (ER-ICA and PGR-ICA).

Fig. 7. Specimen preparation for cytochemical hormone receptor assays in cytologic material. (From Masood S: Sex Steroid Hormone Receptors in Cytologic Material, in Cytopathology Annual 1992;4:93, reproduced by permission of Williams & Wilkins, Co., Baltimore.)
therapy. Thus, assessment of ER status in patients with ovarian tumors that have been treated medically may provide the basis for possible new therapeutic modalities. Overall, with a defined hormone receptor status, appropriate therapy can be initiated with enhanced confidence of response to hormone therapy.

In addition to prognostic or therapeutic significance, assessment of hormone receptors may be of diagnostic assistance in patients presenting with unknown primary cancers or with metastatic undifferentiated tumors.

**Specimen Preparation (Fig. 7)**

**Imprints**

Soon after the fresh tumor tissue is received, a portion of tissue is selected for imprint preparation. The cut surface of the portion of the lesion selected is touched with a clear glass slide to make multiple imprints. Sparsely cellular lesions are scraped gently with a scalpel blade and direct smears are made. Some of the imprints should be stained with Diff-Quik or hematoxylin-eosin (H&E) to determine adequacy.

**Fluorescent Technique**

The imprints are placed at 4°C for 1 hr in a frost-free refrigerator to dry. They are either processed immediately or stored at −70°C for later processing. The exact test procedure is as suggested by the supplier.

**Immunoperoxidase Technique**

Polylysine-treated slides are used for smears or imprint preparations and are fixed immediately in 3.7% formaldehyde-phosphate buffered saline (PBS) for 15 min. After a minimum 5-min wash in 0.01 M, pH 7.4 PBS, slides are placed in −10°C methanol and acetone for 3 min and then rinsed in cold PBS for 5 min. Slides may then be stored at −20°C in PBS-glycerol prior to immunostaining. The rest of the procedure is as outlined by the supplier.

**FNAB**

FNAB is performed, utilizing standard procedure. A Diff-Quik stain is employed for immediate evaluation. If malignant cells are present on the Diff-Quik smears, a portion of the aspirate is used for hormone receptor assay, prepared in one of two ways:

1. **Direct smear preparation**—Polylysine-treated slides are utilized for the immunoperoxidase technique and regular glass slides for the fluorescent technique. Fixation and storage procedures are the same as for imprint preparation.

2. **Cytospin preparation**—For the immunoperoxidase technique, fine-needle aspirates are rinsed into a conical 50-ml centrifuge tube containing about 20 ml of fresh 3.7 formaldehyde-PBS at room temperature. Within 15 min the fixed material is centrifuged at 2,400 rpm for 10 min. The supernatant is removed leaving 1–2 ml liquid in which the pellet is resuspended. Two to four cytospin slides are prepared, using about five to seven drops of suspension per slide. The slides require tissue adhesive as specified in the ER-ICA or PgR-ICA kit.

For the fluorescent technique, the material is rinsed in PBS solution and direct smears are made on regular glass slides.

**Malignant Effusions**

The fluids should be processed as soon as possible following thoracentesis or paracentesis. Following centrifugation, direct smears are processed and interpreted following staining with Diff-Quik. If adequate malignant cells are present, part of the sediment is rinsed into a 50-ml conical centrifuge tube and processed as described under cytospin preparation technique.

**Cell Block Preparations**

Cytologic material may also be prepared as cell block and processed for hormone receptor immunocytochemical assay using enzyme pretreatment as already described for formalin-fixed paraffin-embedded tissue.

**Advantages and Limitations of Cytochemical Hormone Receptor Assays**

The cytochemical assays greatly expand the potential for hormone receptor analysis on cytologic material, since they frequently can be performed on the limited sources obtained by cytologic techniques. The cytochemical assays correlate very well with the biochemical assays and are generally less expensive and easier to perform in the smaller laboratories. The immunoperoxidase technique has been successfully automated. Masood et al. applied the automated "code-on" immunology system (Fisher Scientific Corporation, Pittsburgh, PA) for assessment of

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**Table III. Summary of Concordance Between the Cytochemical Hormone Receptor Assay on Malignant Effusions and the Biochemical Assay of the Primary Tumors**

<table>
<thead>
<tr>
<th>Reference</th>
<th>No. of cases</th>
<th>ER</th>
<th>PgR</th>
<th>Technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>Masood</td>
<td>41</td>
<td>88</td>
<td>83</td>
<td>ER-ICA</td>
</tr>
<tr>
<td>Masood</td>
<td>28</td>
<td>82</td>
<td>90</td>
<td>PgR-ICA</td>
</tr>
<tr>
<td>Total</td>
<td>69</td>
<td>85</td>
<td>86.5</td>
<td>Fluoro-Cep</td>
</tr>
</tbody>
</table>

*Abbreviations as defined in Table I.*

(From Masood S. Sex Steroid Hormone Receptors in Cytologic Material, in Cytopathology Annual 1992;4:83, reproduced by permission of Williams & Wilkins, Co., Baltimore.)
hormone receptors and demonstrated the potential of this system to conserve monoclonal antibodies and to substantially reduce the cost of the test. Adaptability of the immunoperoxidase technique in detection of hormone receptor in formalin-fixed paraffin-embedded tumors is another attractive advantage. Furthermore, cytochemical assays provide information about tumor hormone receptor heterogeneity in contrast to biochemical assay. This information remains a worthwhile goal, not only with respect to understanding the biology of tumors, but also with regard to explaining the difference in response to endocrine therapy that is commonly observed in clinical practice.

Overall, special attention should be given to the results of hormone receptors by biochemical assays. These assays cannot distinguish between tumor tissue, stromal and benign epithelial components, whereas immunocytochemical assays tend to correct this deficiency. This is particularly important in gynecological malignancies since the stroma has considerable receptor activity. In our experience, often in situations with positive DCC and negative immunocytochemical assay, we have demonstrated the presence of hormone receptor expression in nonmalignant components of the lesion only. This, associated with other factors, may explain the reported discrepancies between the DCC and the cytochemical assays.

On the other hand, lack of adequate standardization and quantitation remain limiting factors in the use of cytochemical hormone receptor assays. As a result, many national cancer therapy protocol studies still require biochemical assays for determination of hormone receptor proteins. Only recently, immunocytochemical hormone receptor assay for ER and PgR has been started in the National Surgical Adjuvant Breast Project (NSABP).

Biochemical assays provide a quantitative value which is not yet available with cytochemical assays. Continuous efforts have been made to quantitate hormone receptor proteins in cytochemical assays; however, the interpretations are subjective and the measurements are at best semi-quantitative.68,151–153 Sporadic reports in the literature give promise to the use of a computerized image analysis system in quantitation of hormone receptors.154–156 Sklarz and Pertschuk156 in 1987 introduced a pattern-oriented approach called receptogram analysis to evaluate ER status or a potential response predictor to hormone therapy. Despite these efforts, difficulties with quantitation in cytochemical hormone receptor assays remain. Overall, it has not been shown that quantitative values beyond a defined level are helpful in selecting treatment options. However, many investigators feel that quantitation may have prognostic value.68,157,158

Nevertheless, currently the cytochemical hormone receptor assays are most adaptable to cytolologic material. Also, it seems reasonable to assume that with inherent advantages in the cytochemical hormone receptor assays, these procedures will continue to evolve and may eventually displace the biochemical assays.

Acknowledgment
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