IMMUNOCYTOCHEMICAL LOCALIZATION OF ESTROGEN AND PROGESTERONE RECEPTORS IN NORMAL, HYPERPLASTIC AND NEOPLASTIC HUMAN ENDOMETRIA

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Short Title of Thesis:

Estrogen and progesterone receptors in human endometria

ABSTRACT

Estrogen and progesterone receptors were localized in fresh frozen sections of human endometrial tissues, in both health and disease, using the ER-ICA kit and a mouse monoclonal antiprogesterone receptor antibody (αPR6), respectively. Estrogen and progesterone receptors were detected with immunohistochemical method, exclusively in the nuclei of epithelial and stromal cells of the endometrium. Their highest levels in both components were found during the late proliferative phase of the normal menstrual cycle. Estrogen receptors decreased faster in the stroma than in the epithelium throughout the post ovulatory phase, whereas progesterone receptors decreased more rapidly in the epithelium during the mid and late secretory phases. Estrogen and progesterone receptor levels were high in the epithelium of hyperplasia without cytologic atypia. They were low in the epithelium of endometrial intraepithelial neoplasia (hyperplasia with cytologic atypia) and invasive carcinomas. The stroma contained relatively high estrogen and progesterone receptors levels, irrespective of whether the epithelium was hyperplastic or neoplastic

RESUME

Le kit ER-ICA et l'anticorps monoclonal de souris contre les récepteurs de la progesterone (αPR6) ont permis de detecter les récepteurs des estrogènes et de la progesterone sur des tissus congeles d'endomètres humains, normaux et pathologiques. En utilisant des méthodes immunohistochimiques, ces récepteurs sont présents uniquement dans les noyaux des cellules epitheliales et stromales de l'endomètre. C'est au terme de la phase proliferative du cycle menstruel que le nombre de ces recepteurs est le plus elevé dans ces deux composantes Au cours de la phase post-ovulatoire, les recepteurs des estrogènes diminuent d'abord dans le stroma, à l'inverse, ceux de la progestérone disparaissent plus rapidement dans l'épithelium, au cours de la phase sécrétoire intermediaire et tardive. Ces deux types de récepteurs sont nombreux dans l'epithelium des hyperplasies sans atypie cytologique, leur nombre est limité dans l'epithelium des neoplasies intraépithéliales (hyperplasies avec atypie cytologique) et des cancers envahissants. Indépendamment de la nature de l'épithelium, hyperplasique ou néoplasique, leur nombre est relativement élevé dans le stroma.

PUBLICATIONS

Included as part of this thesis and appropriately modified, are texts and figures of original papers published in the following peer reviewed journals:

CHAPTER II:

Bergeron C., Ferenczy A., and Shyamala G. Distribution of estrogen receptors in various cell types of normal, hyperplastic and neoplastic human endometrial tissues. Lab. Invest. 1988, 58: 338-345.

CHAPTER III:

Bergeron C., Ferenczy A., Toft D.O., Schneider W., and Shyamala G. Immunocytochemical study of progesterone receptors in the human endometrium during the menstrual cycle. Lab. Invest. 1988, 59:862-869.

CHAPTER IV:

Bergeron C., Ferenczy A., Toft D.O., and Shyamala G. Immunocytochemical study of progesterone receptors in hyperplastic and neoplastic endometrial tissues. Cancer Res. 1988, 48:6132-6136.

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1) The 78th Annual Meeting, American Association for Cancer Research, Atlanta, Georgia, May 20-23, 1987.

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In the course of these investigations, the candidate was responsible for collection of all tissues, performance of all experiments except for the biochemical assays in Chapter II and immunoblot analyses in Chapter III. She was responsible also of evaluation and quantitation of all cases and writing of all papers published.

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Chapter I

INTRODUCTION AND REVIEW OF LITERATURE

I-MECHANISMS OF STEROID HORMONE ACTION

Intracellular estrogen receptors (ER) and progesterone receptors (PgR) are necessary for the expression of sex steroid hormonal effects in target tissue (Jensen *et al*, 1967, 1968; Baulieu, 1975; O'Malley and Schrader, 1976; Clark and Peck, 1979; Jensen *et al*, 1982). These receptors are steroid specific proteins and can be inhibited only by the same class of hormones. They have a high affinity (K_d of 0.1 mM for ER) and low capacity to bind estrogens and progesterone, respectively (Katzenellenbogen, 1980) and can be saturated with increasing amounts of hormone. After binding, the steroid-receptor complex becomes activated and interacts with the genome in such a way that the synthesis of specific RNA's is stimulated. These, in turn, are involved in the synthesis of new proteins that affects target cell functions and growth (Gorski *et al*, 1968; Jensen *et al*, 1968).

i) Historical Background.

It was not until 1959 (Glascock and Hoeskstra, 1959) that tritiated sex-steroids such as hexestrol and tritiated estradiol of high specific activity became available as means of studying the tissue distribution and metabolism of estrogens. Early studies (Jensen and Jacobson, 1960) demonstrated that estrogen target tissues, and especially the uterus, take up and retain larger amounts of estradiol than their non-target counterparts. These observations suggested that target cells contained

binding components. However, it was not until 1967 that the first estrogen receptors were demonstrated in the cytoplasmic fraction of rat uterine tissue (Toft *et al*, 1967). In 1968, Gorski *et al* (1968) and Jensen *et al* (1968) reported on the intracellular mechanism of action of estradiol, the so-called "two-step" model. In this model, the steroid receptors were thought to be cytoplasmic proteins. The steroid-receptor complexes were then transported into the nucleus by a temperature sensitive process and associated with the chromatin to initiate a series of sequence of molecular and metabolic events. These observations evolved exclusively from cell homogenization and biochemical analysis of receptors under cell free conditions (Toft *et al*, 1967; Gorski *et al*, 1968; Jensen *et al*, 1968).

The need for a better understanding of the basic mechanism(s) of receptor action resulted in the development and application of morphological methods for identifying ER in tissue sections. Autoradiography both in vivo and in vitro were the first methods to be employed extensively for ER localization in rat uterus (Stumpf, 1968a) and other target tissues (Stumpf, 1968b, 1969). Dry and thawn mount autoradiography in vivo after single injection of ^{3}H estradiol and in vitro after slice incubation with ^{3}H estradiol showed that the latter was preferably localized over the nuclei of target cells. According to the two-step model, ^{3}H estradiol bound to ER was rapidly translocated to the nucleus at physiologic temperatures. However, ^{3}H estradiol bound to ER at 2°C failed to translocate to the nucleus. Early studies (Jensen *et al*, 1968) on excised rat uterus incubated in ^{3}H estradiol containing medium at 2°C showed most of the radioactivity of ^{3}H estradiol to be extranuclear. However, later studies (Sheridan *et al*, 1979; Martin and

Sheridan, 1982) using the same experimental model found only nuclear staining. Using a different approach with liquid emulsion autoradiography after in vitro uterine section incubation with ^{3}H estradiol, Tchernitchin *et al* (1973) found radioactivity in the cytoplasm of eosinophiles while all other cell types remained unlabelled. His results, however, were never confirmed by comparative in vivo autoradiographic experiments. Even when autoradiography was prepared and read with extreme care (Stumpf and Roth, 1969), it suffered from several technical and conceptual pitfalls. These included, possible dilution of the radioligand in vivo by circulating endogenous estrogens, possible occupation of receptors by endogenous estrogens, difficulty to obtain autoradiograms with low background and long exposure periods due to the relatively low specific activity of ^{3}H estradiol.

As a logical extension of and alternative to autoradiography, histochemical techniques were developed with fluorescent estradiol conjugates (Dandliker *et al*, 1977; Rao *et al*, 1980; Lee, 1981) or estradiol antibodies (Nenci *et al*, 1976; Pertschuk *et al*, 1976). In the former case, the ligand used was fluorescent 17β-estradiol or 17β-estradiol fluoresceinated bovine serum albumin isothiocyanate (Pertschuk *et al*, 1980). Variable staining reaction was observed in either the cytoplasm or the nucleus or both. While Lee (1978) and Panko *et al* (1982) found no correlations with biochemical assays, Pertschuk *et al* (1979) obtained 91% correlation. In the latter case, investigators (Pertschuk *et al*, 1976; Nenci *et al*, 1976; Mercer *et al*, 1980) used polyestradiol phosphate or estradiol as a ligand which was localized with immunofluorescent anti-estradiol antibodies. Pertschuk *et al* (1978) found 90% correlation with biochemical assays. However, the methodology

and ligand used in these two studies were severely criticized as to their validity (Chamness *et al*, 1980, Mc Carthy *et al*, 1980, Morrow *et al*, 1980) Firstly, the true affinity of the fluorescent or the polyestradiol phosphate ligand for ER could not be validated; no acceptable competition with ^{3}H estradiol for ER was found and nuclei of non target tissues such as in the rat diaphragm also showed positive staining reaction with polyestradiol phosphate as a ligand Secondly, neither Morrow *et al* (1980) nor Mc Carthy *et al* (1980) were able to correlate the results of these assays with biochemical assays. Thirdly, when estradiol was used as the ligand, the true affinity of estradiol for ER could only be achieved with very high concentrations of estradiol and the staining observed was suspected to be due to cytoplasmic type II or type III binding sites of estradiol rather than to estradiol receptor complexes (Chamness *et al*, 1980, Morrow *et al*, 1980). Type II binding sites even if they have steroid specificity and limited capacity, differ from true ER binding sites The former have lower affinity for estradiol, are not depleted from the cytoplasm when the tissue is exposed to estradiol (Clark *et al*, 1978, 1981) and are unable to initiate trancription-related hormonal effects. Type III binding sites are soluble molecules like albumin. These molecules have low affinity but very high capacity for estradiol. As a result, with high concentrations of estradiol, binding to these sites may become substantial (Chamness *et al*, 1980; Morrow *et al*, 1980; Mc Carty *et al*, 1980; Stumpf *et al*, 1982).

Recent receptor purification studies have characterized ER and PgR using high affinity chromatography (Greene *et al*, 1980; Schrader *et al*, 1981; Loosfelt *et al*, 1984; Horwitz *et al*, 1985). The development of

specific monoclonal antibodies against ER (Greene *et al*, 1980) has enabled the localization of ER in nuclei of target cells in tissue sections (King and Greene, 1984) and confirmed previous autoradiographic studies (Sheridan *et al*, 1979; Martin and Sheridan, 1982) which traced ER mainly in the nucleus. The data were further supported by biochemical assays, Welshons *et al* (1984) found that more than 90% of unoccupied ER was in the nucleoplast fraction of cytochalasin enucleated GH_3 rat pituitary adenoma cells. At 25°C, without freezing or cooling the tissue homogenate at any step of the cytosol preparation, Molinari *et al* (1985) showed an almost complete recovery of the ER in the fraction containing the cell nuclei.

ii) A new model for receptor mechanism.

Based on recent data, a new hypothesis on the mechanism of sex steroid hormone action has been proposed: the majority of the native receptors seems to be preferentially localized within the nuclear compartment where it is loosely bound and is in equilibrium with small amounts of extranuclear receptor. This extranuclear receptor serves to take up the hormone into the target cell. In the living cell, most of the hormone-receptor complex probably enters the nucleus in the native state and undergoes activation within the nuclear compartment. The activated form of the steroid receptor complex binds tightly to acceptor sites in the chromatin removing it from solution and providing force for more unoccupied receptor to enter the nucleus and to undergo activation (Jensen *et al*, 1984) Several recent immunohistochemical studies with monoclonal antibodies against ER and PgR have consistently demonstrated

the almost exclusive nuclear localization of ER and PgR (Press *et al*, 1984; Mc Carthy *et al*, 1985; Perrot-Applanat *et al*, 1985; Budwit *et al*, 1986). The identification of unbound receptors in the cytosol fraction by biochemical assays was in retrospect probably artifactual, due to extraction of nuclear receptors into the cytoplasm. Indeed, unbound receptors are loosely bound to nuclear structures and easily lost when the nucleus is isolated under strong homogenization conditions. After DNA transformation, the receptor binds more tightly to the chromatin/DNA and does not leak out so easily from the nuclear fraction. However, these modifications of the traditional two-step model of receptor functions have not changed the basic concept of receptor activity and steroid hormonal action, i.e. interaction with DNA and expression of hormonal effect on steroid target tissues.

II-HORMONAL REGULATION OF THE NORMAL CYCLIC ENDOMETRIUM

i) Morphological response to steroid hormones during the menstrual cycle.

The human endometrium is characterized by constant regeneration and cyclic changes of cell proliferation, differentiation and death. The basalis layer, which persists throughout every cycle, constitutes the matrix for regeneration of the post menstrual endometrial functionalis (Ferenczy, 1976,1977, Ferenczy *et al*, 1979a). Proliferation and secretory differentiation of the endometrial functionalis layer are mediated by the influence of estrogens (E_2) and progesterone,

respectively (Clark and Peck, 1979; Katzenellenbogen, 1980), and can be related to cyclic plasma levels of E_2 and progesterone during the menstrual cycle.

During the first part of the cycle, E_2 promotes proliferation of endometrial cells. These acquire an increased and sustained synthesis of RNA and proteins (Clark *et al*, 1985). This, in turn, results in cellular hypertrophy, DNA synthesis and cell replication. Autoradiography with radiolabelled thymidine shows an elevated labelling index (Ferenczy *et al*, 1979b) and histology numerous mitoses in the nuclei of gland cells, fibroblasts and vascular endothelium during the normal proliferative phase of the menstrual cycle (Ferenczy, 1987). The maximum number of cells engaged in DNA synthesis is seen between cycle days 8 and 10 and corresponds to peak plasma E_2 levels. Ultrastructurally, estrogens stimulate the formation of free and bound ribosomes, Golgi, mitochondria, primary lysosomes and intermediate filaments (Ferenczy and Richart, 1974). Scanning electron microscopy demonstrates a high number of cilia and alkaline phosphatase rich surface microvilli, a reflection of estrogenic stimulation. They presumably facilitate mobilization and distribution of endometrial secretions and increase the overall cell surface, respectively. This situation enhances excretory, secretory and absorptive functions of gland cells (Ferenczy and Richart, 1974).

After ovulation, progesterone modifies cellular growth and the biosynthetic activity of the E_2-primed uterus. It inhibits further proliferation of the endometrium and converts the E_2-primed endometrium to a secretory type tissue (Ferenczy and Richart, 1974; Clark *et al*, 1985). The endometrial glands become irregular and convoluted and accumulate intracytoplasmic glycogen. DNA synthesis and cell divisions

in epithelial cells are arrested at the same time as apocrine secretory activity begins (Ferenczy *et al*, 1979a). At the microstructural level, giant mitochondria and basket-like nucleolar channel systems appear. They are typical of and unique to the post-ovulatory human endometrium and are likely to be related to glycoprotein synthesis (Ferenczy and Richart, 1974; Wynn, 1979). Indeed, nucleolar channel systems can be produced both in vivo and in vitro by progesterone or its synthetic variants (Pryse *et al*, 1979). Progesterone, furthermore, induces predecidualization of spindle shaped stromal fibroblasts. These are transformed into plump liver-like cells. Predecidual cells represent precursor forms of gestational decidual cells. The latter have several metabolic functions which include 1) the control of the invasive nature of the normal trophoblast (Kirby and Cowell, 1968), 2) protection of the fetus against immunologic rejection by suppressing mixed lymphocyte reaction (Kirkwood *et al*, 1981) and 3) synthesis of prolactin (Rosenberg *et al*, 1980; Huang *et al*, 1987). Endometrial prolactin during gestation presumably stimulates amniotic fluid metabolism (Healy *et al*, 1983) and synthesis of prostaglandins E_2 and $F_{2\alpha}$, the latter mediating endometrial vascular permeability (Kennedy, 1980). Gestational decidua has probably also a nutritive role by synthesizing substances which are vital for the embryo prior to the development of the fetal circulatory system (Handwerger and Freedmark, 1987). If conception does not occur, predecidual cells contribute to menstrual breakdown of the endometrial stroma via their phagocytotic properties (Lawn *et al*, 1971; Ferenczy, 1980).

From cycle day 25, the endometrium undergoes involution and degeneration. Progesterone during the post ovulatory phase, stabilizes

lysosomal membranes (Weissman, 1964). With the fall of plasma progesterone, lysosomal membrane integrity is no longer maintained. The acid phosphatases synthetized during the pre-ovulatory phase in primary lysosomes by E_2 are released into various cellular and intercellular compartments resulting in digestion and destruction of the glandular epithelium, stromal cells and the vascular system. Endothelial membrane injury promotes platelet deposition and release of thromboxane, leading to vascular thrombosis. Parallel to these cellular events, a gradual increase in prostaglandins, particularly $PGF_{2\alpha}$, results in vasoconstriction of spiral arterioles (Abel, 1979), leading to ischemic tissue necrosis The menstrual period is characterized by tissue shedding and regeneration, both of which appear independent of hormonal influence (Ferenczy, 1980). Indeed, in the post menstrual phase, despite increased DNA synthesis, plasma levels of E_2 and progesterone are low, unchanged from the premenstrual values. The increased nuclear DNA and nucleolar RNA turnover is a reflection of initial tissue response to repair the lost substance. The marked increase in DNA synthesis and mitotic activity in all the cell components of the regenerated human endometrium coincide with the increase in plasma E_2 and reflect target cell sensitivity and response to E_2.

ii) Steroid receptors in the normal menstrual cycle.

It is well established that the interaction of the steroid with the intracellular receptor protein is the primary event which triggers specific hormonal responses within target tissues. After induction of sex steroid effect, the receptor and steroid are processed or

desactivated by an unknown mechanism and leave the nucleus. Studies with inhibitors of RNA and protein synthesis (actinomycin D and cycloheximide) showed that 1/3 of ER that reappear in the uterine cytosol after their initial depletion by an E_2 injection do not depend on de novo RNA or protein synthesis and most likely arise from recycling of ER from the nucleus (Jensen *et al*, 1969; Sarff and Gorski, 1971; Mester and Baulieu, 1975). The remaining is presumed to be newly synthesized, hence the receptor replenishement involves both recycling and synthesis of new receptors.

Receptors for E_2 (ER) and progesterone (PgR) as identified by conventional biochemical analyses and immunohistochemical analysis, have been found high during the late proliferative phase and low during the secretory phase (Tseng *et al*, 1972; Evans *et al*, 1974; Bayard *et al*, 1978; Clark and Peck, 1979; Levy *et al*, 1980; McCarthy *et al*, 1983; Press *et al*, 1984). The effects of E_2 and progesterone are consistent with these considerable fluctuations of receptor levels observed physiologically during the normal menstrual cycle The sequential presentation of E_2 and progesterone in the uterus is accompanied by changes in sensitivity of the responding tissue to hormones. ERs in the uterus are partly maintained at a constitutive level (Clark and Peck, 1979); Indeed, E_2-responsive cells of the uterus in a castrated rat maintain levels of receptors that enable it to respond to administred E_2 (Clark and Peck, 1979). However, administration of E_2 promotes new synthesis of ER and PgR in the uteri of animals and in human endometrium (Clark and Peck, 1979). The uterus is relatively insensitive to progesterone unless first exposed to E_2, and progesterone treatment in a nonestrogenized uterus will not produce a secretory uterine

transformation. These observations are explained by the fact that E_2 promotes the synthesis of PgR (Milgrom *et al*, 1973; Jänne *et al*, 1975; Leavitt *et al*, 1977) thereby enhancing the ability of the uterus to respond to progesterone. ER and PgR concentrations are the highest in the proliferative phase and correlate with peak plasma levels of E_2. Progesterone antagonizes E_2 action mainly by interfering with ER mechanisms (Hseuch *et al*, 1975, 1976; Takeda and Leavitt, 1986). It decreases ER concentration by inhibiting synthesis of de novo receptors or required protein factors (Bhakoo *and Katzenellenbogen*, 1977). Also, progesterone antagonizes the action of E_2 by converting E_2 into estrone (E_1) through the action of the progesterone specific enzyme 17 estradiol β-hydroxydehydrogenase (E_2-DH) (Tseng and Gurpide, 1975; Gurpide and Marks, 1981). The activity of the latter, however, is restricted to the glandular epithelium (Satyaswaroop *et al*, 1982). E_1 is able to bind to ER but it is only a weak binding which does not significantly stimulate the nuclei of target cells (Gurpide, 1978). And paradoxically, progesterone causes a rapid fall in the levels of uterine PgR (down regulation)(Milgrom *et al*, 1973) by antagonizing E_2-stimulation from which PgR synthesis is mostly dependant.

III-ENDOMETRIAL HYPERPLASIA AND CARCINOMA: MORPHOLOGIC AND PATHOLOGIC CONSIDERATIONS.

Non invasive endometrial proliferations constitute a group of heterogenous lesions with an increase in the number and/or volume of glands and stroma displaying a variety of cytologic and architectural alterations. These lesions have been studied for many years (Gusberg,

1947; Hertig and Sommers, 1949; Hertig *et al*, 1949; Mc Bride, 1959; Gusberg and Kaplan, 1963; Beutler *et al*, 1963; Vellios, 1972; Tavassoli and Kraus, 1978, Fox and Buckley, 1982; Ferenczy *et al*, 1983; Kurman *et al*, 1985; Ferenczy and Gelfand, 1989). Yet, their relationship to carcinoma remains unsettled, as are their appropriate treatments. Attempts to establish the relationship, if any, between endometrial hyperplasia and the subsequent development of endometrial carcinoma have involved mainly retrospective studies of prior biopsies of patients who subsequently developed endometrial carcinoma (Novak and Rutledge, 1948; Hertig and Sommers, 1949; Speert, 1959, Beutler *et al*, 1963). Most retrospective studies suffered, however, from patient selection bias. Indeed, only women with abnormal bleeding were studied without controls, ie. patients with cancer but without a history of prior bleeding. As a result, conclusions drawn from these studies apply to a highly selected and relatively small proportion of women with endometrial carcinoma. Many of the earlier "prospective" studies suffered also from several drawbacks in design. For example, the initial diagnosis was made by curettage (Hertig *et al*, 1949; Mc Bride, 1959; Gusberg and Kaplan, 1963; Buehl *et al*, 1964; Wentz, 1966; Chamlian and Taylor 1970). This procedure removes, and many times "treats" the pathologic endometrium and artificially reduces the risk of disease progression. Conversely, curettage does not necessarily sample the entire endometrial cavity and may miss areas of early focal carcinoma. In such cases, the endometrial sample initially obtained is histologically underdiagnosed and incorrectly interpreted as progressive lesions when on follow-up the undetected disease is discovered. Another pitfall of some of the prospective studies of presumed cancer precursors was the use of

radiation therapy. Twenty of 32 patients followed by Hertig *et al* (1949) and 6 of 8 patients followed by Gusberg and Kaplan (1963) received radiation treatment to control bleeding associated with hyperplasia prior to the development of corpus carcinoma. This is not surprising in view of the well known ionizing radiation carcinogenic effect of human tissue including the endometrium. This, in turn, may result in an artifically high rate of neoplasia in otherwise non-carcinoma risk lesions.

These methological difficulties were compounded by a total lack of a uniformly accepted and used terminology for presumed precursors of endometrial carcinoma. The terminological "melting-pot" resulted in confused knowledge as to the types of "hyperplasia" being documented and thus a poor understanding of their precise natural history. Welch and Scully (1977) came to the depressing conclusion that "the lack of a uniform terminology throughout the literature has made it impossible to render even a scientific estimate of the proportion of cases of precancerous hyperplasia that are destined to progress to cancer if untreated".

Nevertheless, until recently, it was generally believed that some or perhaps most endometrial carcinomas evolve from normal endometrium through a continuing and progressive sequence of subsets of hyperplasia ranging from simple or cystic glandular hyperplasia to atypical complex or adenomatous hyperplasia with severe atypical architectural and cellular patterns (Hertig *et al*, 1949; Gusberg and Kaplan, 1963; Beutler *et al*, 1963; Vellios, 1972; Welch and Scully, 1977). This continuum concept seemed to be supported by the evaluation of coexistent association studies on invasive carcinoma and hyperplasia of the

endometrium. Green *et al* (1959) and Gray *et al* (1974) found concomittant hyperplastic changes in 10% and 42% of adenocarcinoma, respectively. Conversely, Gusberg and Kaplan (1963) and Tavassoli and Kraus (1978) found concomittant adenocarcinoma in uteri with hyperplasia in 20% and 25% of the cases, respectively. Also, hyperplasia preceded experimentally induced endometrial carcinoma by long term estrogen administration (Meissner *et al*, 1957) or methylcholanthrene (Merriam *et al*, 1960). However, the concept of continuous progression from simple hyperplasia to carcinoma has never been confirmed by well-designed and bias-free prospective studies of patients with various forms of endometrial hyperplasia. As a result, the carcinoma risk rates as related to morphologic modifications in hyperplasia are not known.

In recent years, attempts have been made to simplify terminology by replacing the vagaries of terms by a nomenclature that can be related to clinical management and prognosis (Fox and Buckley, 1982; Norris *et al*, 1983; Kurman *et al*, 1985; Ferenczy, 1988). It has been suggested that endometrial proliferations traditionally named as anovulatory persistent proliferative endometrium, cystic glandular hyperplasia, simple or mild hyperplasia and adenomatous hyperplasia, moderate hyperplasia or complex hyperplasia be named by a unifying generic name endometrial hyperplasia (Ferenczy, 1988) or according to the Committee on Endometrial Terminology of the International Society of Gynecologic Pathologists, simple and complex hyperplasia. Irrespective of architectural glandular alterations, by definition, the lining epithelium of the glands lacks cytologic atypia and is usually formed of pseudo-stratified tall, columnar cells, many of which are of the ciliated variant. These lesions represent essentially an exaggeration of the normal, cyclic

proliferative endometrium. In great contrast, endometrial proliferations with cytologic atypia are characterized by gland cells with nuclear enlargement, rounding, pleomorphism, loss of polarity and often with clumped or coarse nuclear chromatin with macronucleoli. The cytologic characteristics of gland cells are indistinguishable from those found in well differentiated adenocarcinomas More often than not, cytologic atypia is associated with architectural alterations as well. Based on their cytologic similarities to carcinoma cells, they are considered to be carcinoma precursors. They were traditionally referred to as atypical adenomatous hyperplasia, severe hyperplasia, hyperplasia with cytologic atypia, atypical complex hyperplasia and carcinoma in situ. The unifying generic term endometrial intraepithelial neoplasia (EIN) has been suggested for these by some (Ferenczy, 1988) and atypical hyperplasia by the Committee of the International Society of Gynecologic Pathologists. By definition, endometrial stromal or myometrial invasion distinguishes EIN from well differentiated carcinoma (Kurman and Norris, 1982; Ferenczy, 1988) Myometrial invasion can be recognized relatively easily in hysterectomy specimens but is only seldom seen in the curettings. In the latter, one has to evaluate whether there is evidence of endometrial stroma invasion. Until recently, there was no well defined criteria for stromal invasion but only vague imprecise and subjective guidelines (Buehl *et al*, 1964; Vellios, 1972; Shanklin, 1978) for distinguishing well differentiated carcinoma from extremes of "atypical hyperplasia" or "carcinoma in situ". Kurman and Norris (1982) in a careful study compared a variety of histologic features in curettings and hysterectomy specimens, and have arbitrarily defined criteria for stromal invasion. These are 1) replacement of large portions of endometrial stroma by

glands with a cribriform pattern 2) inflammation and/or necrosis or desmoplasia of the stroma, 3) complex papillary pattern and 4) replacement of stroma by masses of squamous epithelium. These histologic criteria delineate biologically significant lesions having a greater likelihood of metastasis as compared to those in which invasion is absent.

Recent methods of investigation, including transmission and scanning electron microscopy (Ferenczy and Richart, 1974; Fenoglio *et al*, 1982; Ferenczy *et al*, 1983), morphometry (Baak *et al*, 1981; Colgan *et al*, 1983; Aausems *et al*, 1985; Oud *et al*, 1986; Roberts *et al*, 1986; Fu *et al*, 1988; Baak *et al*, 1988) and in vitro DNA histoautoradiography (Ferenczy, 1983), support also the concept that proliferations with cytologic atypia are the immediate precursors of invasive carcinoma. Electron microscopy demonstrated that "hyperplasias" with cytologic atypia compared to those without atypia, contain less estrogen related cellular alterations, and contain organellar pleomorphism, similar to that of well differentiated carcinoma. Morphometric analysis showed nuclear perimeters, mean maximal nuclear diameter, standard deviation of nuclear area and of shortest nuclear axis and in vitro DNA histoautoradiography showed DNA phase duration and cell doubling time values in hyperplasias with cytologic atypia similar to values found in well-differentiated carcinoma but not in normal or hyperplastic endometria without cytologic atypia. Measurements of DNA content by Feulgen microspectrophotometry and flow cytometry are not useful in distinguishing cellular proliferations with or without invasive potential. Earlier observations of nuclear aneuploidy (a specific feature of cancer in general) in endometrial carcinoma precursors

(Wagner *et al*, 1967) have not been confirmed by subsequent investigators (Sachs *et al*, 1974; Feichter *et al*, 1982; Iversen, 1986; Lindahl *et al*, 1987) It seems that most well differentiated endometrial carcinomas and EIN/atypical hyperplasias have diploid nuclear DNA content indistinguishable from normal endometrium or hyperplasia without cytologic atypia (Sachs *et al*, 1974; Feichter, 1982; Iversen, 1986; Lindahl *et al*, 1987). The diploid DNA values in these lesions correspond to subtle numerical (trisomy 1q or 10) rather than structural aberrations of the chromosomes (Katayama and Jones, 1967; Couturier *et al*, 1986, Gibas and Rubin, 1987) Although, one cytogenetic study found, similar structural alterations in D group chromosomes in both endometrial carcinoma and "hyperplasia" (Trent and Davis, 1979), the authors provided no information on the presence or absence of cytologic atypia in their hyperplastic lesions.

A few earlier and more recent, relatively bias-free prospective studies (McBride, 1959; Kurman *et al*, 1985; Ferenczy and Gelfand, 1989) on over 200 patients with various subsets of endometrial hyperplasia also support the concept that cytologic atypia is the morphologic predictor of biologic behavior. The results of these studies revealed that hyperplasia without cytologic atypia failed to carry greater carcinoma risk than is observed in an age-matched normal women population, whereas hyperplasia with cytologic atypia progressed to carcinoma in 23% (Kurman *et al*, 1985) to 25% of the cases (Ferenczy and Gelfand, 1989) with a mean of 5.5 years progressive transit time. The efficacity of progestin therapy in obtaining regression of endometrial hyperplasia has been evaluated in a number of publications (Kistner, 1959; Wentz, 1966; Eichner and Abellera, 1971; Gusberg *et al*, 1974; Kurman *et al*, 1985;

Gal, 1986; Ferenczy and Gelfand, 1989). However, it is difficult to understand which types of hyperplasia respond better than others because of the confusing terminology used in many studies on hyperplasias. Some investigations obtained a very high rate (100% and 93%) of regression to normal with any kind of hyperplasia (Wentz, 1966; Eichner and Abellera, 1971; Gal, 1986) while others (Ferenczy and Gelfand, 1989) obtained 25% and 80% long term cure rates in hyperplasia with and without cytologic atypia, respectively. Similar experience has been published by Kurman *et al* (1985). The recurrence rate of hyperplasia after discontinuation of or during progestational treatment is also difficult to ascertain because in certain series, investigators have not related recurrence rates to the presence or absence of cytologic atypia. Wentz (1966) found no case with recurrence, while Eichner and Abellera (1971) and Gal (1986) reported 60% and 10% recurrences, respectively. On the other hand, in a series of 85 cases followed prospectively by Ferenczy and Gelfand (1989) 50% and 13% of hyperplasia with and without cytologic atypia, respectively have recurred. Experience of Kurman *et al* (1985), has been similar. Why some hyperplasia fail to respond to progestogen is not clear. Progesterone receptors seem to be mandatory for action of progesterone but none of the above clinical studies have studied the tissue receptor content and variation of, according to the type of lesionnal tissue.

IV-STEROID RECEPTORS IN ENDOMETRIAL HYPERPLASIA AND CARCINOMA.

To further characterize the two disease concept, a series of

comparative steroid receptor measurements have been performed in both normal and abnormal endometria.

i) Steroid receptors in endometrial hyperplasia.

Many of the morphological alterations in endometrial hyperplasia are consistent with hyperestrogenism from both endogenous and exogenous sources and may be induced in the rabbit by administration of high doses of stilbestrol (Meissner *et al*, 1957). Therefore, it was suggested that endometrial hyperplasia includes a spectrum of epithelial changes induced by E_2 stimulation in the absence of biologically active progesterone (Brush *et al*, 1975; Rome *et al*, 1977).

Studies using charcoal-coated receptor binding assays found both ER and PgR elevated in hyperplasia (Terenius *et al*, 1971; Haukkamaa *et al*, 1971; Evans *et al*, 1974; Mac Laughin and Richardson, 1976; Tseng *et al*, 1977; Rodriguez *et al*, 1979; Jänne *et al*, 1979; Shyamala and Ferenczy, 1981; Ehrhich *et al*, 1981). Levels were comparable to those found in normal proliferative endometria and higher than those found in endometrial carcinoma. Most of the time, however, these methods failed to provide information on the types of hyperplasia studied and in particular on the presence or absence of cytologic atypia. These methods, furthermore, used tissue homogenates and then failed to give information on the distribution of receptors in the various cell components of the endometrium.

Jänne *et al* (1979) measured ER and PgR in hyperplastic endometria prior to medroxyprogesterone acetate (Provera$^{(R)}$) treatment. After treatment, the decrease in receptors levels ranged from 10% to 30% of

the initial values. This response seems similar to that observed in the normal secretory (progestional phase) endometrium. Therefore endometrial hyperplasias with high levels of PgR would be expected to respond to exogenous progestins. On the other hand, the decrease of progesterone receptors induced by progesterone should diminish its therapeutic effectiveness, i.e. inducing secretory conversion after relatively short exposures. Clinical results, however, indicate that progesterone effect is usually maintained over many years of treatment. In progestagin-resistent endometria with PgR, failure to respond or recurrence after discontinuation of treatment may indicate the presence of non-fonctionnal receptors. An alternative situation may be that high PgR containing cells have a focal and limited rather than diffuse endometrial distribution or that PgRs are concentrated in the stroma rather than in the glandular epithelium.

ii) Steroid receptors in endometrial carcinoma.

Endometrial carcinoma is the most frequent gynecologic malignancy with approximately 34000 and 4300 new cases diagnosed every year in the United States (Silverberg and Lubera, 1988) and Canada (Hill *et al*, 1988), respectively. Although the majority of cases are diagnosed in early clinical stages (cancer confined to the uterus Stage I), approximately 3000 North-American women die yearly with advanced or recurrent disease. Malignancies of endocrine target tissues often share biologic properties with their benign counterparts but knowledge of the biology of endometrial carcinoma including its control by sex-steroid hormones remains limited. Carcinogenesis in general involves initiation

and promotion of cells in a given organ. The initiator(s) induces chemical alterations (mutations) in the cells which, if stimulated by a promoter(s), may develop into clinical cancer. While initiators of human endometrial carcinogenesis are unknown, estradiol is considered to be a potent promoter of normal and possibly neoplastic growth of the endometrium.

Numerous epidemiologic studies indicate an estrogenic milieu unopposed by biologically active progesterone in most patients with well-differentiated endometrial adenocarcinoma (Smith *et al*, 1975; Ziel and Finkle, 1975; Mc Donald *et al*, 1977; Hulka, 1987). Indeed, most endometrial cancer risk indicators such as obesity, nulliparity, late menopause, long term estrogen alone replacement therapy, granulosa tumor of the ovary and Stein-Leventhal syndrome are all related to hyperestrogenism (Gusberg, 1976; Horwitz and Feinstein, 1986). However, a substantial number of patients with endometrial carcinoma lack the above risk indicators and in these cases the disease may not be hormonodependent. Literature review indicates that 74% of patients with adenocarcinoma of the endometrium are not obese, 58% are not nulliparous, 22% experience menopause before age 49 and 43% to 89% are not exposed to estrogen alone replacement therapy (Ziel *et al*, 1975; McDonald *et al*, 1977; Richardson, 1978). In a prospective study of 133 patients with bilateral oophorectomy followed from 5 to 30 years without hormone replacement therapy 6.5% developed carcinoma of the endometrium (Lucas, 1974). The presently available data suggest that corpus carcinoma have two distinct metabolic backgrounds, one estrogenic and the other non-estrogenic (Bokhman, 1981; Boronow *et al*, 1984; Deligdish and Cohen, 1985). Typically, carcinoma associated with a hyperestrogenic

background is seen in patients younger than 55 years old, is associated with endometrial hyperplasia, is well differentiated and has nearly 100% 5-year survival rates. They contrast with their "non-estrogenic" counterparts which are diagnosed commonly in elderly women (60 years or older) who typically show signs of hypoestrogenism at the time of diagnosis and have atrophic rather than hyperplastic endometrium adjacent to poorly differentiated carcinoma. The 5-year survival rate in these women is dismal (25%).

Knowledge of the endometrial response to sex-steroids led some to use hormone therapy for hyperplasia and carcinoma. Kistner, (1959) was the first to report the use of progestagens in patients with endometrial hyperplasia and Kelly and Baker (1960) demonstrated response to progestagens in 35% of patients with metastatic carcinoma of the endometrium Varga and Henricksen (1961) and Kennedy (1963) soon showed similar results. Apparently, effective cancer therapy was being achieved without toxicity, and progestational steroid hormones were given freely. However, later experiences failed to confirm the initial reports (Mac Donald *et al*, 1988) and it became evident that some endometrial carcinoma were hormone insensitive. Since then, attention has been focused on steroid sensitivity of endometrial carcinoma and the development of predictive tests for hormonal therapy. Determination of steroid receptor levels in carcinoma of the breast became routine practice and has repeatedly been shown to be of clinical value in the management of breast cancers patients (McGuire *et al*, 1975) For example, breast tumors positive for ER and PgR were more likely to respond to hormone therapy than tumors lacking receptors (McGuire, 1978). Encouraged by these reports of breast carcinoma, determination of

steroid receptors has been applied to endometrial carcinoma. The most constant positive association of receptor content has been with the histological differentiation of endometrial carcinoma. There is a trend for higher ER and/or PgR positivity and concentrations in well differentiated (Grade 1) carcinoma than in their less differentiated counterparts (Terenius *et al*, 1971; Jänne *et al*, 1979; McCarty *et al*, 1979; Creasman *et al*, 1980; Ehrlich *et al*, 1981; Kauppila *et al*, 1982; Creasman *et al*, 1985; Geisinger *et al*, 1986a). This correlation cannot be generalized, however, as some highly differentiated carcinoma have low levels of ER and high levels of PgR and some poorly differentiated carcinoma have significant ER or PgR concentrations (Martin *et al*, 1979; Pollow *et al*, 1975). Also, some investigators found no relationship between receptors content and morphologic differentiation of corpus carcinoma (Haukkamaa *et al*, 1971; Rodriguez *et al*, 1979; Prodi *et al*, 1980; Benraad *et al*, 1980). The variability in results has been explained in part by tumor cell heterogeneity with respect to receptor content and histologic differentiation and the association of normal/hyperplastic with cancerous tissue in the same specimen (Mortel *et al*, 1984). Receptor contents failed to correlate with the clinical stage of carcinoma or depth of myometrial invasion when controlled for their histological grade (McCarty *et al*, 1979; Creasman *et al*, 1980). On the other hand, several studies have identified presence of receptors, particularly PgR content in corpus carcinoma to be an excellent discriminator between good and poor prognosis groups (Creasman *et al*, 1985; Kauppila *et al*, 1986; Liao *et al*, 1986; Geisinger *et al*, 1986b).

Response to hormonal therapy of endometrial carcinoma and steroid receptor levels have been investigated. Unfortunately, most of the

studies have been hampered by different methods of measuring receptors and criteria for defining receptor "positive" lesions as well as by small series of cases. Nevertheless, the presence of PgR seems to correlate with a favorable clinical response to progestagin therapy (Martin *et al*, 1979; Benraad *et al*, 1980; Creasman *et al*, 1980; Ehrlich *et al*, 1981, Kauppila *et al*, 1982). Furthermore, several studies have attempted to relate receptor levels with hormonal manipulation in vivo or after transplantation of endometrial carcinoma in nude mice. They noted a decrease of PgR following treatment by progestin (Jänne *et al*, 1979; Martin *et al*, 1979) and a significant increase of PgR following treatment by the anti-estrogen Tamoxifen (Mortel *et al*, 1981). In a nude mouse model, Satyaswaroop *et al* (1983) and Zaino *et al* (1984) have demonstrated that, receptor positive-transplanted endometrial carcinoma responds to hormonal stimulation, with an increase in PgR content under the action of E_2 or Tamoxifen and inhibition of tumor growth under Tamoxifen plus progestin (Clarke *et al*, 1987b).

V- IMMUNOHISTOCHEMICAL ANALYSIS.

The development of monoclonal antibodies specific of human ER (Greene and Jensen, 1982) made immunocytochemical localization of ER feasible. This method selectively and specifically localizes ER and provides the first direct access to the receptor molecule, independant of its occupancy by the hormonal ligand. Besides, the technique contains insignificant staining for non-specific binding proteins (King and Greene, 1984; McClellan *et al*, 1984) avoiding problems encountered in immunohistochemical and fluorescent cytochemical methods previously

mentioned (see historical background). The first monoclonal antibodies were developed against MCF.7 human breast cancer ER. They are capable of recognizing receptors whether or not steroid combining site is occupied. They recognize unique epitopes on the receptor molecule. Competition studies show that ER in MCF.7 cytosol prevents monoclonal antibodies from binding to their respective antigenic determinants in tissue sections. The first report showed the receptor in the cytoplasm of Bouin-fixed (picric acid), paraffin embedded sections of cultured cells (Greene and Jensen, 1982). Later, when frozen tissues were assayed, the majority of the cellular pool of ER was shown exclusively in the nucleus of MCF.7 cells (King and Greene, 1984) and then in primate reproductive tract (McClellan *et al*, 1984), human uterus (Press *et al*, 1984) and breast tumors (King *et al*, 1985; Pertschuk *et al*, 1985; McCarty *et al*, 1985). These results diverged from previous histochemical (Nenci *et al*, 1976: Dandliker *et al*, 1977) and immunohistochemical (Raam *et al*, 1983; Coffer *et al*, 1985) methods which found ER in the cytoplasm of human endometrium and breast cancer. As discussed earlier (see historical background), the previous histochemical methods employed estrogens as the ligand with questionable specificity and binding affinity for ER. Similarly, the earlier immunohistochemical studies employed polyclonal rather than monoclonal antibodies against ER (Raam *et al*, 1983) that might have been contaminated by non-receptor antibodies. In the Coffer *et al* (1985) studies, however, the antibody was a specific monoclonal antibody raised against a M 36,000 cytosolic ER component from human endometrium. This antibody may recognize an epitope or the estradiol-binding unit different from that recognized by the antibodies of Greene and Jensen (1982). On the other hand, ER antibodies developed

against calf uterus, but cross-reacting with human ER (Marchetti *et al*, 1987) have shown both nuclear and cytoplasmic staining in formalin fixed paraffin embedded sections. The discrepancy in results may be explained by a possible artifactual redistribution of receptors induced by the fixation and embedding of tissues studied.

To amplify receptor detection (Sternberger, 1979) in frozen tissues, immunohistochemical studies with Greene's antibodies were carried out on breast carcinoma. Excellent correlation was found between the immunohistochemical method and quantitative biochemical assays, the latter being considered until now as the gold standard method (McCarthy *et al*, 1985; King *et al*, 1985; Pertschuk *et al*, 1985). In parallel to the breast studies, the human endometrium was assayed also by means of immunohistochemistry with monoclonal ER antibodies (Press *et al*, 1984). The results provided unique information which was not available prior to breakthoughs in monoclonal antibody technology. Indeed, ER was observed exclusively in the nuclei of the vast majority of epithelial and stromal cells in both premenopausal and postmenopausal uteri (Press *et al*, 1984). The endometrial data confirmed the concept that most of the receptors have an intranuclear location. The staining intensity varied with the endometrial cell type during the menstrual cycle and compared favorably with the conventional steroid binding assays of human endometrial receptor content. Immunoelectron microscopic tracing of ER showed, furthermore, positive staining in the euchromatin and absence of any specific cytoplasmic localization (Press *et al*, 1985). More recently, monoclonal antibodies against rabbit, chick, avian and human PgR have been developed (Logeat *et al*, 1983; Schrader *et al*, 1981; Tuohimaa *et al*, 1984; Sullivan *et al*, 1986; Clarke *et al*, 1987a). The

antibodies against rabbit and avian PgR were shown to recognize human PgR as well (Logeat *et al*, 1983; Sullivan *et al*, 1986; Wei *et al*, 1987, Estes *et al*, 1987). Using these antibodies with immunocytochemistry, PgR was shown exclusively in the nucleus of chick oviduct (Gasc *et al*, 1984; Isola *et al*, 1987a), and ovary (Isola *et al*, 1987b), rabbit uterus, oviduct, cervix, vagina (Perrot-Applanat *et al*, 1985) as well as human neoplastic breast and normal uterine cells (Perrot-Applanat *et al*, 1987; Clarke *et al*, 1987a). In the human breast cancer model there was a high correlation between the immunohistochemical method and biochemical assays (Perrot-Applanat *et al*, 1987). When we began our study, these PgR antibodies have been used only in the normal proliferative phase endometrium (Clarke *et al*, 1987a) and PgR were found in the vast majority of epithelial and stromal cells. The post ovulatory phase endometrium was not studied.

The application of immunohistochemical techniques for tracing sex-steroid receptors in pathologic endometria may provide important information as to their precise localization and may avoid many drawbacks encountered in the standard biochemical assays: 1) the amount of tissue required for immunohistochemistry is considerably less than for biochemical assays and 2) contamination of specimens by the surrounding benign but receptor-rich endometrial or myometrial components can be excluded as can the presence of receptors in the stromal component which may contribute to total steroid binding. Furthermore, the localization of receptors in the endometrial stroma surrounding hyperplastic and neoplastic gland cells may permit a better understanding of epithelial-stromal interactions that may influence normal and abnormal endometrial proliferations. Indeed, the growth of

normal endometrial epithelium is suspected to be initiated and maintained by the surrounding mesenchymal tissue (Cunha *et al*, 1983). It is therefore conceivable that disturbances in epithelial-stromal interactions, may play a part in the induction and growth of epithelial tumors (Cunha *et al*, 1983). When we began our study, only a few immunohistochemical studies on the ER content of corpus carcinoma have appeared with biochemical correlations (Mc Carty *et al*, 1985; Budwit *et al*, 1986; Pertschuk *et al*, 1986). Endometrial hyperplasia, however, has not been investigated by means of immunohistochemistry with respect to its sex-steroid receptor content. Immunolocalization of sex-steroid receptors in both the epithelial and stromal components of hyperplastic or neoplastic endometrial cells may provide a more precise estimation of their receptor content and distribution. Such studies may help in the identification of hyperplastic and malignant growths which are more likely to respond to exogenous progestin therapy, and to determine the hormonal relationship between hyperplasia and neoplasia. We have used immunohistochemistry to assess the relative quantitative distribution of ER and PgR in normal, hyperplastic and neoplastic gland cells and stromal fibroblasts and the results are described in Chapter II,III and IV.

VI-INTRODUCTION TO THE EXPERIMENTAL SECTION.

i) Tissue samples.

Recent studies show that ERs are detectable in formalin fixed paraffin embedded breast tissues (Shinada *et al*, 1985; Shintaku and

Said, 1987; De Rosa *et al*, 1987; Cheng *et al*, 1988). Some of the methods require the use of special fixatives or refrigeration during fixation. Such methods are very useful when cases are to be studied after the tissue has been processed and embedded in paraffin. This enables the retrospective study of archival material. However, these methods were not available when we began the study of the first cases in Chapter II. Also, in order to evaluate and compare our results on PgR which can be detected only on frozen sections, with these previously published, we decided to use only snap frozen tissues. The diagnosis was made in all cases before the immunohistochemical (IHC) study and the specimens were recovered for IHC studies in the operating room immediatly after removal of the uterus.

The avian PgR antibody we have used had never been tested in the human endometrium or in the breast with IHC techniques. Human studies published with other antibodies against PgR using IHC techniques used only snap frozen tissue (Perrot-Applanat *et al*, 1987; Clarke *et al*, 1987a). Then, to develop a reproducible technique and to test the specificity of avian PgR antibody in human endometrial tissue, we decided to use snap frozen specimens. When antibodies to PgR become commercially available, methods will probably be published with formalin-fixed paraffin embedded tissues.

We have described in Chapter II, III, IV (Materials and Methods section) the way the tissue was snap frozen. However, it should be pointed out that the specimen was very rapidly frozen. It was never left in the hands of surgeons or operating room personnel but always handled by the same person (CB). Specimens were cut perpendicularly in order to sample a maximum of uterine mucosa, whether it was normal, hyperplastic

or neoplastic. Each specimen was placed in a labelled small plastic container and frozen immediatly in the operating room. The labelled container was kept after in the freezer at 70°C until it was processed for immunohistochemistry.

ii) Immunohistochemical techniques.

As mentionned in the above immunohistochemical analysis section, the detection of ER and PgR by IHC presents advantages over the conventional biochemical method. The more rational approach appears to be the detection of the receptor by the use of monoclonal antibodies (Mabs) specifically prepared, against the receptor molecule (see historical background). Among these, the first Mab to ER was developed by Greene et Jensen (1982) and is now available commercially (ER-ICA kit, Abbott labs). The high degree of specificity and sensitivity of this Mab for ER has been mentionned before (see Immunohistochemical analysis). It explains why it was selected for our studies to the demonstration of human endometrial ER (Chapter II).

Several monoclonal antibodies to PgR have been prepared (see Immunohistochemical analysis). None of them was commercially available at the time we began our study. However, thanks to Dr Toft and collaborators, Mayo Clinic, Rochester, we had the opportunity to test with IHC method, the avian antibody αPR6 shown to cross react with human PgR (Sullivan *et al*, 1986). The specificity of αPR6 against endometrial PgR will be described in Chapter III.

We have used the peroxidase-antiperoxidase technique (PAP) to detect ER exactly the same way as described in the booklet provided by

Abbott labs with the kit. This method is very sensitive and reproducible. All the antibodies and the basic immunostaining ingredients for the PAP method are prepackaged. However, another technique using the avidin-biotin complex (ABC) may be employed if the primary antibody becomes available in the future. The choice is mainly a matter of preference and experience of the investigator.

We have used the ABC method to detect PgR. This method (Swanson *et al*, 1987) is very sensitive and easily reproducible. The biotinylated antibody and avidin-biotin peroxidase complexes are commercially available (Vector laboratories) and the author (CB) was very familiar with this technique. A limiting dilution and positive and negative controls which have to be performed with a new antibody, are described in Chapters III and IV.

Several methods have been employed to interpret the results of immunohistochemistry for ER or PgR in an attempt to facilitate comparison with the cytosol technique. These range from simply estimating the percentage of cell nuclei immunostained to the use of computerized cell analysis (Charpin *et al*, 1986). The latter system was not available to us. As a result, a semiquantitative method for ER and PgR was chosen evaluating visually the percentage of positive cells and staining intensity in calibrated microscopic fields and giving a total score, as described in Materials and Methods of Chapters II, III and IV.

REFERENCES

Aausems E.W.M.A., Vanderkamp J.K. and Baak J.P.A. Nuclear morphometry in the determination of the prognosis of marked atypical endometrial hyperplasia. Int. J. Gynecol. Pathol. 1985, 4:180-185.

Abel M.N. Production of prostaglandins by the human uterus- Are they involved in menstruation ? Res. Clin. For. 1979, 1:33-37.

Baak J.P.A., Kurver P.H.J., Diegenbach P.C., Delemarre J.F.M., Brekelmans E.C.M. and Nieuwlaat J.E. Discrimination of hyperplasia and carcinoma of the endometrium by quantitative microscopy, a feasibility study. Histopathology 1981, 5:61-68

Baak J.P.A., Nauta J.J.P., Wiss-Brekelmans E.C.M. and Bezener P.D. Architectural and nuclear morphometrical features together are more important prognosticators in endometrial hyperplasias than nuclear morphometrical features alone. J. Pathol. 1988, 154:335-341.

Baulieu E.E. Steroid receptors and hormone receptivity. New approaches in pharmacology and therapeutics. J.A.M.A. 1975 ,234: 404-409.

Bayard F., Damilaro S., Robel P. and Baulieu E.E. Cytoplasmic and nuclear estradiol and progesterone receptors in human endometrium. J. Clin. Endocrinol. Metab. 1978, 46: 635-648.

Benraad T.H.J., Fubeig L.G., Koenders A.M.M. and Kullander S. Do

estrogens and progesterone receptors (E_2R and PR) in metastasing endometrial cancers predict the response to gestagen therapy ? Acta Obstetrica Gynecologica Scandinavica 1980, 59:155-159.

Beutler H.K., Dockerty M.B. and Randall L.M. Precancerous lesions of the endometrium. Am. J. Obstet. Gynecol. 1963, 86:433-443.

Bhakoo H.S. and Katzenellenbogen B.S. Progesterone modulation of estrogen stimulated uterine biosynthetic events and estrogen receptor levels. Mol. Cell. Endocrinol. 1977, 8:121-134.

Bokhman J.V. Two pathogenic types of endometrial carcinoma. Gynecol. Oncol. 1981, 15:10-17.

Boronow R.C., Morrow C.P. and Creasman W.T. Surgical staging in endometrial cancer: Clinical-pathologic findings of a prospective study. Obstet. Gynecol. 1984, 63:825-832.

Brush M.G., Milton P.J.D., Swain M.C. and Moore J.M. Endometrial cystic hyperplasia, a premalignant state or a complex endocrine disorder ? In: Gynaecological Malignancy (Brush M.G, Taylor R.W eds). London. Bailliere Tindall. 1975, 155-178.

Budwit-Novotny D.A., McCarthy K.S., Cose E.B., Soper J.T., Mutch D.G., Creasman W.T., Flowers J.L. and McCarthy K. Immunohistochemical analyses of estrogen receptor in endometrial adenocarcinoma using a monoclonal antibody. Cancer Res. 1986, 46:5419-5425.

Buehl I.A., Vellios F., Carter J.E. and Huber C.P. Carcinoma in situ of the endometrium. Am. J. Clin.Pathol. 1964, 42:594-601.

Chamlian D.L. and Taylor H.B. Endometrial hyperplasia in young women. Obstet. Gynecol. 1970, 36:659-665.

Chamness G.C., Mercer W.D. and Mc Guire W.L. Are histochemical methods for estrogen receptor valid ? J. Histochem. Cytochem. 1980, 28: 792-798.

Charpin C., Martin P.M., Lavaut M.N., Pourreau-Schneider N. and Toga M. Estrogen receptor immunocytochemical assay (ER-ICA) in human endometrium. Int. J. Gynecol. Pathol. 1986, 2, 119-131.

Cheng L., Binder S.W., Fu Y.S. and Lewin K.J. Methods in laboratory investigation: Demonstration of estrogen receptors by monoclonal antibody in formalin-fixed breast tumors. Lab. Invest. 1988, 58, 346-353.

Clark J.H., Hardin J.W., Upchurch S. and Eriksson H. Heterogeneity of estrogen binding sites in the cytosol of the rat uterus. J. Biol. Chem. 1978, 253:7630-7634.

Clark J.H. and Peck E.J. Jr. Female sex steroids receptors and fonction. Monog. Endocrinol. 1979, 14:4-36.

Clark J.H. and Markaverich B.M. Relationships between type I and II estradiol binding sites and estrogen induced responses. J. Steroid

Biochem. 1981, 15:49-54.

Clark J.H., Schrader W.T. and O'Malley B.W. Mechanisms of steroid hormone action. In: Textbook of Endocrinology. (Wilson J.D, Foster D.W. eds.). Philadelphia, WB Saunders Co. 1985, 33.

Clarke C.L., Zaino R.J., Feil P.D., Miller J.V., Steck M.E., Ohlsson-Wilhelm B.M. and Satyaswaroop P.G. Monoclonal antibodies to human progesterone receptor: Characterization by biochemical and immunohistochemical techniques. Endocrinology. 1987, 121:1123-1132.

Clarke C.L , Feil P.D. and Satyaswaroop P.G. Progesterone receptor regulation by 17 β-estradiol in human endometrial carcinoma grown in nude mice. Endocrinology. 1987, 121:1642-1648.

Coffer A.I., Lewis K.M., Brockas A.J. and King R.J.B. Monoclonal antibodies against a component related to soluble estrogen receptor. Cancer Res. 1985, 45:3686-3693.

Colgan T.J., Norris H.J., Foster W., Kurman R.J. and Fox C.H. Predicting the outcome of endometrial hyperplasia by quantitative analysis of nuclear features using a linear discriminant function. Int. J. Gynecol. Pathol. 1983, 1:347-352.

Couturier J., Vielh P.H., Salmon R. and Dutrillaux B. Trisomy and tetrasomy for long arm of chromosome 1 in near diploid human endometrial adenocarcinomas. Int. J. Cancer. 1986, 38:17-19.

Creasman W.T., Mc Carthy K.S. Jr, Barton T.K. and Mc Carthy K.S. Sr. Clinical correlates of estrogen and progesterone-binding proteins in human endometrial adenocarcinoma. Obstet. Gynecol. 1980, 55:363-370.

Creasman W.T., Soper J.T., Mc Carthy K.S. Jr, Mc Carty K.S. Sr, Hinchaw W and Clarke-Pearson D.L. Influence of cytoplasmic steroid receptor content on prognosis of early stage endometrial carcinoma. Am. J Obstet. Gynecol. 1985, 151:922-932.

Cunha G.R., Chung L.W.K., Shannon J.M., Taguchi O. and Fujii N. Hormone induced morphogenesis and growth. Role of mesenchymal-epithelial interactions. Rec. Prog. Horm. Res. 1983, 39: 559-598.

Dandliker W.B., Hicks A.N., Levision S.A. and Brawn R.J. A fluorescein-labeled derivative of estradiol with binding affinity towards cellular receptors. Biochem. Biophys. Res. Commun. 1977, 74:538-544.

Deligdisch L. and Cohen C.J. Histologic correlates and virulence implications of endometrial carcinoma associated with adenomatous hyperplasia. Cancer 1985, 56:1452-1455.

De Rosa C.M., Ozzello L., Greene G.L. and Habif D.V. Immunostaining of estrogen receptor in paraffin sections of breast carcinomas using monoclonal antibody D75P 3g: Effects of fixation. Am. J. Surg. Pathol. 1987, 11: 943-950.

Ehrlich L.E., Young R.M., and Cleary R.E. Cytoplasmic progesterone and

estrogen receptors in normal, hyperplastic and carcinomatous endometria: Therapeutic implication. Am. J. Obstet. Gynecol. 1981, 141:539-546.

Eichner E. and Abellera M. Endometrial hyperplasia treated by progestagens. Obstet. Gynecol. 1971, 38: 739-741.

Estes P.A., Suba E.J., Lawler-Heavner J., Elashry-Stowers D., Wei L.L., Toft D.O., Sullivan W.P., Horwitz K.B. and Edwards D.P. Immunologic analysis of human breast cancer progesterone receptors. I. Immunoaffinity purification of transformed receptors and production of monoclonal antibodies. Biochemistry 1987, 26: 6250-6262.

Evans L.H., Martin J.D. and Hamel R. Estrogen receptor concentration in normal and pathological human uterine tissues. J. Clin. Endocrinol. Metabol. 1974, 38:23-32.

Feichter G.E., Hoffken H., Heep J., Haag D., Heberling D., Brandt H., Rummel H. and Goerttler K.L. DNA-flow-cytometric measurements on the normal, atrophic, hyperplastic, and neoplastic human endometrium. Virchows Arch. Pathol. Anat. 1982, 398:53-65.

Fenoglio C.M., Crum C.P. and Ferenczy A. Endometrial hyperplasia and carcinoma. Are ultrastructural, biochemical and immunocytochemical studies useful in distinguishing between them ? Path. Res. Pract. 1982, 174:257-284.

Ferenczy A. and Richart R.M. Female reproductive system: Dynamics of

scan and transmission electron microscopy. New York, Wiley J & Sons. 1974.

Ferenczy A. Studies on the cytodynamics of human endometrial regeneration. II. Transmission electron microscopy and histochemistry. Am. J. Obstet. Gynecol. 1976, 124:582-595.

Ferenczy A. Studies on the cytodyramics of experimental endometrial regeneration in the rabbit. Historadioautography and ultrastructure. Am. J. Obstet. Gynecol. 1977, 128:536-545.

Ferenczy A., Bertrand G. and Gelfand M.M. Proliferation kinetics of human endometrium during the normal menstrual cycle. Am. J. Obstet. Gynecol. 1979, 133:859-867.

Ferenczy A., Bertrand G. and Gelfand M.M. Studies on the cytodynamics of human endometrial regeneration. III. In vitro short-term incubation historadioautography. Am. J. Obstet. Gynecol. 1979, 134:297-304.

Ferenczy A. Regeneration of the human endometrium. In: Progress in Surgical Pathology, (Fenoglio CM and Wolff M eds.), New York, Masson Publishing USA. 1980, 157-173.

Ferenczy A. Cytodynamics of endometrial hyperplasia and neoplasia. Part II: In vitro DNA histoautoradiography. Human Pathol. 1983, 14:77-82.

Ferenczy A., Gelfand M.M., and Tzipris A. The cytodynamics of

endometrial hyperplasia and carcinoma. A review, Ann. Pathol. 1983, 3:189-202.

Ferenczy A. Anatomy and Histology of the Uterine Corpus, In: Blaustein's Pathology of the female genital tract, third edition (R. Kurman ed) New-York, Springer Verlag. 1987, 177-217.

Ferenczy A. Endometrial hyperplasia and neoplasia: A two disease concept, In: Contemporary issues in Obstetrics and Gynecology vol. 5: Gynecology Oncology (Cohen C. and Kale M.G eds). New York, Churchill Levenghoe Inc. 1988, 197-213.

Ferenczy A. and Gelfand M.M. The biologic significance of cytologic atypia in progestagen treated endometrial hyperplasia. Am. J. Obstet. Gynecol. 1989, 160:126-131.

Fox H. and Buckley C.H. The endometrial hyperplasias and their relationship to endometrial neoplasia. Histopathol. 1982, 6:493-510.

Fu Y.S., Ferenczy A., Huang I. and Gelfand M.M. Digital imaging analysis of normal hyperplastic and malignant endometrial cells in endometrial brushing samples. Analyt. Quant. Cytol. Hist. 1988, 10: 139-149.

Gal D. Hormonal therapy for lesions of the endometrium. Seminars in Oncol. 1986, 13:33-36.

Gasc J.M., Renoir J.M., Radanyi C., Joab I., Tuohimaa P. and Baulieu

E.E. Progesterone receptor in the chick oviduct: an immunohistochemical study with antibodies to distinct receptor components. J. Cell. Biol. 1984, 99:1193-1200.

Geisinger K.R., Marshall R.B., Kute T.E., and Homesley H.D. Correlation of female sex steroid hormone receptors with histologic and ultrastructural differentiation in adenocarcinoma of the endometrium. Cancer 1986, 58: 1506-1517.

Geisinger K.R., Homesley H.D., Morgan T.M., Kute T.E., and Marshall R.B. Endometrial carcinoma : A multiparameter clinicopathologic analysis including the DNA profile and the sex steroid hormone receptors. Cancer 1986, 58:1518-1525.

Gibas Z. and Rubin S.C. Well-differentiated adenocarcinoma of endometrium with simple karyotypic changes: A case report. Cancer Genet. Cytogenet. 1987, 25:21-26.

Glascock W.G. and Hoeskstra R.F. Selective accumulation of tritium-labelled hexoestrol by the reproductive organs of immature female goats and sheep. Biochem J. 1959, 72:673-682.

Gorski J., Toft D., Shyamala G., Smith D., and Notides P. Hormone receptors: studies on interaction of estrogen with the uterus. Rec. Prog. Horm. Res. 1968, 24:45-80.

Gray L.A., Robertson R.W. and Christopherson W.M. Atypical endometrial

changes associated with carcinoma. Gynecol. Oncol. 1974, 2:93-100.

Green R.R., Roddick J.W. and Milligan M. Estrogens, endometrial hyperplasia and endometrial carcinoma. Ann. NY Acad. Sci. 1959, 75:586-599.

Greene G.L., Nolan C., Engler J.P. and Jensen E.V. Monoclonal antibodies to human estrogen receptor. Proc. Natl. Acad. Sc. USA. 1980, 77:5115-5119.

Greene G.L. and Jensen E.V. Monoclonal antibodies for estrogen receptor detection and characterization. J. Steroid Biochem. 1982, 16:353-359.

Gurpide E. and Marks C. Influence of endometrial 17β-hydroxysteroid dehydrogenase activity on the binding of estradiol to receptors. J. Clin. Endocrinol. Metab. 1981, 52:252-255.

Gurpide E. Enzymatic modulation of hormonal action at the target tissue. J. Toxicol. Environ. Health. 1978, 4:249-268.

Gusberg S.B. Precursors of corpus carcinoma: estrogens and adenomatous hyperplasia. Am. J. Obstet. Gynecol. 1947,54:905-927.

Gusberg S.B. and Kaplan A.L. Precursors of corpus cancer IV Adenomatous hyperplasia or stage o carcinoma of the endometrium. Am. J. Obstet. Gynecol., 1963, 87:662-678.

Gusberg S.B., Chen S.J. and Cohen C.J. Endometrial cancer: factors influencing the choice of treatment. Gynecol. Oncol. 1974, 2:308-313

Gusberg S.B. The individual at high risk for endometrial carcinoma. Am J. Obstet. Gynecol. 1976, 126:535-542.

Handwerger S. and Freedmark M. Role of placental lactogen and prolactin in human pregnancy. In: Regulation of ovarian and testicular function (Malesh VB, Dhindsa DS, Anderson E, Kabra SB (Ed)), New York, Plenum Publishing Corp, 1987, 399

Haukkamaa M., Karjalainen O. and Luukkainen T. In vitro binding of progesterone by the human endometrium during the menstrual cycle and by hyperplastic, atrophic and carcinomatous endometrium. Am. J. Obstet. Gynecol. 1971, 111:205-210.

Healy D.L., Herington A.L and O'Herlihy C. Chronic polyhydramnios· evidence for a defect in the chorion laeve receptor for lactogenic hormones. J. Clin. Endocrinol. Metab. 1983, 56,520-525.

Hertig A.T. and Sommers S.C. Genesis of endometrial carcinoma. I Study of prior biopsies. Cancer 1949, 2:946-956.

Hertig A.T., Sommers S.C. and Bengloff H. Genesis of endometrial carcinoma. III. Carcinoma in situ. Cancer, 1949, 2·946-971.

Hill G.B., Mao Y., Laidlaw J., Sanscartier G.P., Silins J Canadian

Cancer Statistics. 1988.

Horwitz K.B., Wei L.L., Sedlacek S.M. and d'Arville C.N. Progestin action and progesterone receptor structure in human breast cancer: a review. Rec. Progr. Horm. Res. 1985, 41, 249-316.

Horwitz R.I. and Feinstein A.R. Estrogens and endometrial cancer Responses to arguments and current status of an epidemiologic controversy. Am. J. Med. 1986, 81:503-507.

Hseuch A.J.W., Peck E.J. and Clark J.H. Progesterone antagonism of the oestrogen receptor and oestrogen-induced uterine growth. Nature 1975, 254:337-338.

Hseuch A.J.W., Peck E.J.J., and Clark J.H. Control of uterine estrogen receptor levels by progesterone. Endocrinology 1976, 98:438-444.

Huang J.R., Tseng L., Bischof P. and Janne O.A. Regulation of prolactin production by progestin, estrogen and relaxin in human endometrial stromal cells Endocrinology 1987, 121:2011-2017.

Hulka B.S. Replacement estrogens and risk of gynecologic cancers and breast cancer. Cancer 1987, 60:1960-1964.

Isola J , Pelto-Huikko M. and Tuohimaa P. Immunoelectron microscopic localization of progesterone receptor in chick oviduct. J. Steroid Biochem. 1987, 26:19-23.

Isola J., Korte J.M and Tuohimaa P. Immunocytochemical localization of progesterone receptor in the chick ovary. Endocrinology 1987, 121:1034-1040.

Iversen O.E. Flow cytometric deoxyribonucleic acid index: a prognostic factor in endometrial carcinoma. Am. J. Obstet. Gynecol. 1986, 155:770-776.

Jänne O., Kontula K., Luukkainen T. and Vihko R. Estrogen induced progesterone receptor in human uterus. J. Steroid Biochem. 1975, 6:501-509.

Jänne O., Kauppila A., Kontula K., Syrjala P. and Vihko R. Female sex steroid receptors in normal, hyperplastic and carcinomatous endometrium. The relationship to serum steroid hormones and gonadotropins and changes during medroxyprogesterone acetate administration. Int. J. Cancer 1979, 24:545-554.

Jensen E.V. and Jacobson H.I. Fate of steroid estrogens in target tissues. In: Biological activities of steroids in relation to cancer. (G. Pincus, EP Vollmer. eds). New York, Academic Press. 1960, 161.

Jensen E.V., De Sombre E.R. and Jungblut P.W. Estrogen receptors in hormone responsive tissues and tumors. In: Endogenous factors influencing host tumor balance. (Wissler R.W, Dao T.L, Wood S.J.F eds), Chicago, The University of Chicago Press, 1967,15.

Jensen E.V., Suzuki T., Kawashima T., Stumpf W.E., Junglbut P.W. and De Sombre E.R. A two step mechanism for the interaction of estradiol with rat uterus. Proc. Natl. Acad. Sci. USA. 1968, 59,632-638.

Jensen E.V., Suzuki T., Nimata M., Smith S. and De Sombre E.R. Estrogen binding of target tissues. Steroids 1969, 13,417-427.

Jensen E.V., Greene G.C., Closs C.E., De Sombre E.R. and Nadji M. Receptors reconsidered: a 20-year perspective. Rec. Prog. Horm. Res. 1982, 38:1-40.

Jensen E.V. Intracellular localization of estrogen receptors: implications for interaction mechanism. Lab. Invest. 1984, 51:487-488.

Katayama K.P. and Jones H.W. Chromosomes of atypical (adenomatous) hyperplasia and carcinoma of the endometrium. Am. J. Obstet. Gynecol. 1967, 97·978-983.

Katzenellenbogen B.S. Dynamics of steroid hormone receptor action. Ann. Rev Physiol. 1980, 42:17-35.

Kauppila A., Jujanswi E. and Vihko R. Cytosol estrogen and progesterone receptors in endometrial carcinoma treated with surgery, radiotherapy and progestin: clinical correlates. Cancer 1982, 50:2157-2162.

Kauppila A.J.I., Isotalo H.E., Kivinen S.T. and Vihko R.K. Prediction of

clinical outcome with estrogen and progestin receptor concentrations and their relationships to clinical and histopathological variables in endometrial cancer. Cancer Res. 1986, 46:5380-5384.

Kelly R.M. and Baker W.H. Progestational agents in the treatment of carcinoma of the endometrium. N. Engl. J. Med. 1960, 264:216-222.

Kennedy B.J. A progestogen for treatment of advanced endometrial cancer. J.A.M.A. 1963, 184:758-761.

Kennedy T.G. Estrogen and uterine sensitization for the decidual cell reaction: role of prostaglandins. Biol. Reprod. 1980, 23:955-962.

King W.J. and Greene G.L. Monoclonal antibodies localize oestrogen receptor in the nuclei of target cells. Nature 1984, 307·745-747.

King W.J., De Sombre E.R., Jensen E.V. and Greene G.L. Comparison of immunocytochemical and steroid-binding assays for estrogen receptor in human breast tumors. Cancer Res. 1985, 45:293-304.

Kirby D.R.S. and Cowell T.P. Trophoblast- host interactions, In: Epithelial-mesenchymal interactions. (Fleischmajer R, Billingham R E eds) Baltimore, Williams and Wilkins, 1968.

Kirkwood K.J. and Bell S.C. Inhibitory activity of supernatants from murine decidual cell cultures on the mixed lymphocyte reaction. J Reprod. Immunol. 1981, 3:243-252.

Kistner R.W. Histologic effects of progestins on hyperplasia and carcinoma in situ of the endometrium. Cancer 1959, 12:1106-1110.

Kurman R J. and Norris H.J. Evaluation of criteria for distinguishing atypical endometrial hyperplasia from well-differentiated carcinoma. Cancer 1982, 49:2547-2559.

Kurman R.J., Kaminski P.T. and Norris H.J. The behavior of endometrial hyperplasia in 170 patients. Cancer 1985, 56:403-412.

Lawn A.M., Wilson E.W. and Finn C.A. The ultrastructure of human decidual and predecidual cells. J. Reprod. Fertil. 1971, 26:85-90.

Leavitt W.W., Chen T.J. and Allen T.C. Regulation of progesterone receptor formation by estrogen action. Ann. N.Y. Acad. Sci. 1977, 286:210-225.

Lee S.H. Cytochemical study of estrogen receptor in human mammary cancer. Am. J. Clin. Pathol. 1978, 70:197-203.

Lee S.H. The histochemistry of estrogen receptor. Histochemistry 1981, 71:491-500.

Levy C., Robel P., Gautray J.P., De Brux J., Herman V., Descomps B. and Baulieu E.E. Estradiol and progesterone receptors in human endometrium. Normal and abnormal menstrual cycles and early pregnancy. Am. J. Obstet.

Gynecol. 1980, 136:646-651.

Liao B.S., Twiggs L.B., Leung B.S., Yu W.C., Potish R.A. and Prem K A. Cytoplasmic estrogen and progesterone receptors as prognostic parameters in primary endometrial carcinoma. Obstet. Gynecol. 1986, 67:463-467.

Lindahl B., Alm P., Killander D., Langstrom E. and Trope C. Flow cytometric DNA analysis of normal and cancerous human endometrium and cytological-histopathological correlations. Anticancer Res. 1987, 7:781-790.

Logeat F., Vu Hai M.T., Fournier A., Legrain P., Buttin G. and Milgrom E. Monoclonal antibodies to rabbit progesterone receptor: cross reaction with other mammalian progesterone receptors. Proc. Natl. Acad. Sci. USA. 1983, 30:6456-6459.

Loosfelt H., Logeat F., Vu Hai M.T. and Milgrom E. The rabbit progesterone receptor. Evidence for a single steroid-binding subunit and characterization of receptor mRNA. J. Biol. Chem, 1984, 259:14196-14202.

Lucas W.E. Causal relationships between endocrine-metabolic variables in patients with endometrial carcinoma. Obstet. Gynecol. Survey. 1974, 29:507-528.

Mac Donald R.R., Thorogood J. and Mason M.K. A randomized trial of progestogens in the primary treatment of endometrial carcinoma. Br J Obst Gynecol. 1988, 95, 166-174.

Mac Laughlin D.T. and Richardson G.S. Progesterone binding by normal and abnormal human andometrium. J. Clin. Endocrinol. Metab. 1976, 52:667-678.

Marchetti E., Querzoli P., Moncharmont B., Parikh I., Bagni A., Marzola A., Fabus G. and Nenci I. Immunocytochemical demonstration of estrogen receptors by monoclonal antibodies in human breast cancers. Correlation with estrogen receptor assay by dextran coated charcoal method. Cancer Res. 1987, 47,2508-2513.

Martin P.M., Rolland P.H., Gammerie M., Serment H. and Toga M. Estradiol and progesterone receptors in normal and neoplastic endometrium correlations between receptors, histopathological examinations and clinical responses under progestin therapy. Int. J. Cancer 1979, 23:321-329.

Martin P M. and Sheridan P.J. Toward a new model for the mechanism of action of steroids. J. Steroid Biochem. 1982, 16:215-229.

Mc Bride J.M. Premenopausal cystic hyperplasia and endometrial carcinoma. J. Obstet. Gynecol. Brit. Emp. 1959, 66:288-296.

Mc Carthy K.S. Jr, Barton T.K., Fetter B.F., Creasman, W.T. and Mc Carthy K.S. Sr. Correlation of estrogen and progesterone receptors with histologic differentiation in endometrial adenocarcinoma. Am. J. Pathol. 1979, 96:171-184.

Mc Carthy K.S. Jr, Woodard B.H., Nichols D.E., Wilkinson W. and Mc Carthy K.S. Sr. Comparison of biochemical and histochemical techniques for estrogen receptor analyses in mammary carcinoma. Cancer 1980, 56:2842-2845

Mc Carthy K.S. Jr, Lubahn D.B. and Mc Carthy K.S. Sr. Estrogen and progesterone receptors: physiological and pathological considerations. Clinics in Endocrinology and Metabolism. 1983, 12:133-154.

Mc Carthy K.S. Jr, Miller L.S., Cox E.B., Konrath J and Mc Carthy K.S. Sr. Estrogen receptor analyses correlation of biochemical and immunohistochemical methods using monoclonal antireceptor antibodies. Arch. Pathol. Lab. Med. 1985, 109:716-721.

Mc Clellan M.C., West N.B., Tacha D.E., Greene G.L. and Brenner R.M Immunocytochemical localization of estrogen receptors in the macaque reproductive tract with monoclonal antiestrophilins. Endocrinology 1984, 114:2002-2014.

Mc Donald T.W., Annegers J.F., O'Fallon W.M., Dockerty M.B., Malkasian G.D. and Kurland L.T. Exogenous estrogen and endometrial carcinoma; Case control and incidence study. Am. J. Obstet. Gynecol 1977, 127 572-580.

Mc Guire W.L., Carbone P.O. and Vollmer E.P. "Estrogen Receptors in Human Breast Cancer". New-York, Raven Press, 1975.

Mc Guire W.L. "Hormones Receptors and Breast Cancer". New York, Raven Press, 1978.

Meissner W.A., Sommers S.C. and Sherman G. Endometrial hyperplasia, endometrial carcinoma, and endometriosis produced experimentally by estrogen. Cancer 1957, 10:500-509.

Mercer W.D., Lippman M.E., Walh T.M., Carlson C.A., Wahl D.A., Lezotte D. and Teague P.O. The use of immunocytochemical techniques for the detection of steroid hormones in breast cancer cells. Cancer 1980, 46:2859-2868.

Merriam J.C., Easterday C.L., Mc Kay D.G. and Hertig A.T. Experimental production of endometrial carcinoma in the rabbit. Obstet. Gynecol. 1960, 16:253-262.

Mester J. and Baulieu E.E. Dynamics of estrogen receptor distribution between the cytosol and nuclear fractions of immature rat uterus after estradiol administration. Biochem. J. 1975, 146:617-623.

Milgrom E., Thi L., Atger M. and Baulieu E.E. Mechanisms regulating the concentration of progesterone receptors in the uterus. J. Biol. Chem. 1973, 248: 6366-6374.

Molinari A.M., Medici N., Armetta I., Nigro V., Moncharmont B. and Puca G.A. Particulate nature of the unoccupied uterine estrogen receptor. Biochem. Biophys. Res. Commun. 1985, 128:634-642.

Morrow B., Leav I., Delellis R.A. and Raam S. Use of polyestradiol phosphate and anti 17β-estradiol antibodies for the localization of estrogen receptors in target tissues. Cancer 1980, 46:2872-2879.

Mortel R., Levy C., Wolff J.P., Nicolas J.C., Robel P. and Baulieu E.E. Female sex steroid receptors in postmenopausal endometrial carcinoma and biochemical response to an antiestrogen. Cancer Res. 1981, 41:1140-1147.

Mortel R., Zaino R. and Satyaswaroop P.G. Heterogeneity and progesterone receptor distribution in endometrial adenocarcinoma. Cancer 1984, 53:113-116.

Nenci I., Beccati M.D., Piffamelli A. and Lanza G. Detection and dynamic localization of estradiol receptor complexes in intact target cells by immunofluorescence technique. J. Steroid. Biochem. 1976, 7:505-511.

Norris H.J., Tavassoli F.A. and Kurman R.J. Endometrial hyperplasia and carcinoma. Diagnostic considerations. Am. J. Surg. Path. 1983, 7:839-847.

Novak E. and Rutledge F. Atypical endometrial hyperplasia simulating adenocarcinoma. Am. J. Obstet. Gynecol. 1948, 55:46-63.

O'Malley B.W. and Schrader W.T. The receptors of steroid hormones. Sci. Am., 1976, 234,32-43.

Oud P.S., Reubsaet-Veldhuizen J.A.M., Henderik J.B.J., Pahlplatz M.M.M., Hermkens H.G., Tas J., James J. and Vooijs G.P. DNA and nuclear protein measurement in isolated nuclei of human endometrium. Cytometry 1986, 7:318-324.

Panko W B., Mattioli C.A. and Wheeler T.M. Lack of correlation of a histochemical method for estrogen receptor analysis with biochemical assay results. Cancer 1982, 49:2148-2152.

Perrot-Applanat M., Logeat F., Groyer-Ricard M.T and Milgrom E. Immunocytochemical study of mammalian progesterone receptor using monoclonal antibodies. Endocrinology 1985, 116:1473-1484.

Perrot-Applanat M , Groyer-Ricard M.T., Lorenzo F., Jolivet A., Thu Vu Hai M., Pallud C., Spyratos F. and Milgrom E. Immunocytochemical study with monoclonal antibodies to progesterone receptor in human breast tumors. Cancer Res. 1987, 47:2652-2661.

Pertschuk L.P. Detection of estrogen binding in human mammary carcinoma by immunofluorescence. a new technique utilizing the binding hormone in a polymerized state. Res. Commun. Chem. Pathol. Pharmacol. 1976, 14:771-774.

Pertschuk L.P., Tobin E.H., Brigati D.J., Kim D.S., Bloom N.D., Gaetjens E., Berman P.J., Carter A.C. and Degenshein G.A., Immunofluorescent detection of estrogen receptors in breast cancer .Comparison with dextran- coated charcoal and sucrose gradient assays. Cancer 1978,

41:907-911.

Pertschuk L.P., Gaetjens E., Carter A.C., Brigati D.J., Kim D.S and Fealey T.E. An improved histochemical method for detection of estrogen receptors in mammary cancer Comparison with biochemical assay. Am J. Clin. Pathol. 1979, 71:504-508.

Pertschuk L.P., Tobin E.H., Brigati D.J. and Gaetjens E. Morphologic assay of steroid hormone receptors in human neoplasia. Pathol Annu. 1980, 15(part 2) 143-180.

Pertschuk L.P., Eisenberg K.B., Carter A.C. and Feldman J.G. Immunohistologic localization of estrogen receptors in breast cancer with monoclonal antibodies. Cancer 1985, 55·1513-1518.

Pertschuk L.P., Beddoe A.M., Gorelic L.S. and Shain S A , Immunocytochemical assay of estrogen receptors in endometrial carcinoma with monoclonal antibodies. Comparison with biochemical assay. Cancer 1986, 57:1000-1004.

Pollow K., Lubbert H., Boquoi E., Kreuzer G. and Pollow B. Characterization and comparison of receptors for 17β-estradiol and progesterone in human proliferative endometrium and endometrial carcinoma. Endocrinology 1975, 96:319-328.

Press M.F., Nousek-Goebl N., King W.J., Herbst A.L and Greene G L Immunohistochemical assessment of estrogen receptor distribution in the

human endometrium throughout the menstrual cycle. Lab. Invest. 1984. 51:495-503.

Press M.F., Nousek-Goebl N.A. and Greene G.L. Immunoelectron microscopic localization of estrogen receptor with monoclonal estrophilin antibodies J. Histochem. Cytochem. 1985, 33:915-924.

Prodi G., Nicoletti G., De Giovanni C. Multiple steroid hormone receptors in normal and abnormal human endometrium. J. Cancer Res. Clin, Oncol. 1980, 98:173-183.

Pryse Davies J., Ryder T.A. and Mackenzie M.L. In vivo production of the nucleolar channel system in postmenopausal endometrium. Cell Tissue Res. 1979, 203·493-498.

Raam S , Richardson G.S. and Gradley F. Translocation of cytoplasmic estrogen receptors to the nucleus: immunohistochemical demonstration utilizing rat .t antibodies to estrogen receptors of mammary carcinomas. Breast Cancer Res. Treat. 1983, 3:179-199.

Rao B.R., Fry C.G., Hung S., Kuhnel R. and Dandliker W.B. A fluorescent probe for rapid detection of estrogen receptors. Cancer 1980, 46:2902-2906.

Richardson G.S. Hormonal biology of endometrial cancer, (G.S. Richardson and D.T. Mac Laughlin eds). International Union against Cancer Series. Geneva. 1978, 42:3-9.

Roberts D.K., Lavia L.A., Freedman R.S., Horbelc D.V. and Busby-Walker N. Nuclear and nucleolar areas: a quantitative assessment of endometrial neoplasia. Obstet. Gynecol. 1986, 68:705-708.

Rodriguez J., Sen K.K., Seski J.C., Menon M., Johnson T R. and Menon K.M.J. Progesterone binding by human endometrial tissue during the proliferative and secretory phases of the menstrual cycle and by hyperplastic and carcinomatous endometrium. Am. J. Obstet. Gynecol. 1979, 133:660-665.

Rome M., Brown J.B., Mason T., Smith M.A., Lowerty C. and Fortune D. Estrogen excretion and ovarian pathology in post menopausal women with atypical hyperplasia, adenocarcinoma and mixed adenosquamous carcinoma of the endometrium. Brit. J. Obstet. Gynecol 1977, 84:88-97.

Rosenberg S.M., Maslar I.A. and Riddick D.H Decidual production of prolactin in late gestation: further evidence for a decidual source of amniotic fluid prolactin. Am. J. Obstet Gynecol. 1980, 138:681-685.

Sachs H. Wamtach E V. and Wurthner K. DNA content of normal, hyperplastic and malignant endometrium, determined cytophotometrically Arch. Gynecol. 1974, 217:349-365.

Satyaswaroop P.G., Wartell D.J. and Mortel R. Distribution of progesterone receptor, estradiol dehydrogenase and 20α dihydroprogesterone dehydrogenase activities in human endometrial glands

and stroma progestin induction of steroid dehydrogenase activities in vitro is restricted to the glandular epithelium. Endocrinology 1982, 111:743-749

Satyaswaroop P G., Zaino R.J. and Mortel R. Human endometrial adenocarcinoma transplanted into nude mice: growth regulation by estradiol. Science 1983, 219:58-60.

Sarff M. and Gorski J. Control of estrogen binding protein concentration under basal conditions and after estrogen administration. Biochemistry 1971, 10:2557-2563.

Schrader W T., Birnbaumer M.E., Hughes M.R., Weigel N.L., Grody W.W. and O'Malley B.W. Studies on the structure and function of the chicken progesterone receptor Rec. Prog. Horm. Res. N.Y. 1981, 60:583-632.

Shanklin D.R Endometrial carcinoma. Diagnostic criteria, pathogenesis, natural history and associations. Pathol Annu. 1978, 13:233-287.

Sheridan P.J., Buchanan J.M., Anselmo V.C. and Martin P.M. Equilibrium: the intracellular distribution of steroid receptors. Nature 1979, 282:579-582.

Shinada A., Kimura S., Abe S., Nagasakik , Adachi I., Yanaguchik, Suzuki M., Nakajima T. and Miller L.S. Immunocytochemical staining of estrogen receptor in paraffin sections of human breast cancer by use of monoclonal antibody. comparison with that in frozen sections. Proc.

Natl. Acad. Sci. 1985, 4803-4807.

Shintaku P.L. and Said J.W. Detection of estrogen receptors with monoclonal antibodies in routinely processed formalin-fixed paraffin sections of breast carcinoma. Am. J. Clin. Pathol. 1987, 87, 161-169.

Shyamala G. and Ferenczy A. The effect of sodium molybdate on the cytoplasmic estrogen and progesterone receptors in human endometrial tissues. Diagn Gynecol. Obstet. 1981, 3·277-282.

Silverberg E. and Lubera J.A. Cancer Statistics, Ca J Clin 1988. 38:5-22.

Smith D.C., Prentice R., Thompson D.J. and Herrman W L. Association of exogenous estrogen and endometrial carcinoma. N. Engl. J. Med. 1975, 293:1164-1167.

Speert H. The premalignant phase of endometrial carcinoma. Cancer 1959, 5:927-944.

Sternberger L.A. Immunocytochemistry, second edition, Prentice-Hall, New York, John Wiley and Sons Inc., 1979.

Stumpf W.E. Subcellular distribution of ^{3}H estradiol in rat uterus by quantitative autoradiography. A comparison between ^{3}H estradiol and ^{3}H norethynodiel. Endocrinology 1968, 83:629-632.

Stumpf W.E. High resolution autoradiography and its application to in vitro experiments, In· Radioisotopes in medicine in vitro studies. (R.L Hayes, F A Goswitz and B E.P Murphy ed), AEC symposium series No 13 (conf 671111) Oak ridgel Tn. 1968, 633-660.

Stumpf W.E. Nuclear concentration of ^{3}H-estradiol in target tissues: Drymount autoradiography of vagina, oviduct, ovary, testis, mammary tumor, liver and adrenal. Endocrinology 1969, 85:31-37.

Stumpf W.E and Roth L.J. Autoradiography using dry-mounted freeze-dried sections-. In: Autoradiography of diffusible substances (L.H Roth and W E Stumpf ed). New York, Academic Press. 1969, 69-80.

Stumpf W.E. and Sar M. Histochemical approaches for the localization of steroid hormone receptors. Acta Histochem. Cytochem. 1982, 15:560-571.

Sullivan W.P , Beito R.G., Proper J., Krco C J. and Toft D.O. Preparation of monoclonal antibodies to the avian progesterone receptor. Endocrinology 1986, 119:1549-1557.

Swanson P.E., Hagen K.A. and Wick M.R. Avidin-Biotin peroxidase anti peroxidase (ABPAP) complex. An immunocytochemical method with enhanced sensitivity. Am. J. Clin. Pathol. 1987, 88, 162-176.

Takeda A. and Leavitt W. Progestin-induced down regulation of nuclear estrogen receptor in uterine decidual cells: Analysis of receptor synthesis and turnover by the density shift method. Biol. Biophys. Res.

Commun. 1986, 135:98-104.

Tavassoli F. and Kraus F.T. Endometrial lesions in uteri resected for atypical endometrial hyperplasia. Am. J. Clin. Pathol 1978, 70:770-779.

Tchernitchin A., Tseng L., Stumpf W.E and Gurpide E. Radio autoradiographic study of human endometrium superfused with estradiol 17β, estrone, estriol and progesterone. Steroid Biochem. 1973, 4 451-455.

Terenius L., Lindell A. and Persson B.H. Binding of estradiol-17β to human cancer tissue of the female genital tract. Cancer Res. 1971, 31 1895-1898.

Toft D O., Shyamala G. and Gorski J. A receptor molecule for estrogens studies using a cell-free system. Proc. Natl. Acad Sci USA. 1967, 57,1740-1743.

Tseng L., Stolee H. and Gurpide E Quantitative studies on the uptake and metabolism of estrogens and progesterone by human endometrium Endocrinology 1972, 90,390-404.

Tseng L. and Gurpide E. Induction of human endometrial estradiol dehydrogenase by progestins. Endocrinology 1975, 97 825-833

Tseng L., Gusberg S.B. and Gurpide E. Estradiol receptor and 17β dehydrogenase in normal and abnormal human endometrium Ann. N Y. Acad

Sci. 1977, 286,190-198.

Trent J.M. and Davis J R. D-group chromosome abnormalities in endometrial cancer and hyperplasia. Lancet 1979, ii, 361.

Tuohimaa P J., Renoir J.M., Radanyi C., Mester J., Joab I., Buchou T., and Baulieu E.E., Antibodies against highly purified B-subunit of the chick oviduct progesterone receptor Biochem. Biophys. Res. Commun. 1984, 119,433-439.

Varga A. and Henriksen E. Clinical and histopathologic evalution of the effect of 17 alpha-hydroxyprogesterone on endometrial carcinoma. Obstet. Gynecol 1961, 18,658-672.

Vellios F Endometrial hyperplasias, precursors of endometrial carcinoma. In: Pathology Annual (Sommers, S C, ed.). South Carolina, Appleton-Century-Crufts, 1972, 201-229.

Wagner D , Richart R.M. and Turner J.Y. Deoxyribonucleic acid content of presumed precursors of endometrial carcinoma. Cancer 1967, 20:2067-2077

Wei L.L., Sheridan P.L., Krett N.L., Francis M.D., Toft D.O., Edwards D.P and Horwitz K.B Immunologic analysis of human breast cancer progesterone receptors. 2. Structure, phosphorylation and processing. Biochemistry 1987, 26:6262-6272.

Weissman G. Labilization and stabilization of lysosomes. Fed. Proc.

1964, 23:1038-1044.

Welch W.R. and Scully R.E. Precancerous lesions of the endometrium. Hum. Pathol. 1977,8:503-512.

Welshons W.V., Lieberman M.E. and Gorski J. Nuclear localization of unoccupied estrogen receptors: cytochalasin enucleation of GH3 cells. Nature 1984, 307:747-749.

Wentz W.B. Treatment of persistent endometrial hyperplasia with progestagen. Am. J. Obstet. Gynecol. 1966, 96:999-1004.

Wynn R.M. Histology and ultrastructure of the human endometrium. In: Biology of the uterus, (R.M Wynn ed), New York, Plenum Press. 1979, 341-375.

Zaino R.J., Satyaswaroop P.G., and Mortel R., Morphology of human uterine cancer in nude mice. Arch. Pathol. Lab Med 1984, 108 571-578

Ziel H.K., and Finkle W.D. Increased risk of endometrial carcinoma among users of conjugated estrogens. N. Engl. J. Med. 1975, 293.1167-1170.

Chapter II

DISTRIBUTION OF ESTROGEN RECEPTORS IN VARIOUS CELL TYPES OF NORMAL, HYPERPLASTIC AND NEOPLASTIC HUMAN ENDOMETRIAL TISSUES

DISTRIBUTION OF ESTROGEN RECEPTORS IN VARIOUS CELL TYPES OF NORMAL, HYPERPLASTIC AND NEOPLASTIC HUMAN ENDOMETRIAL TISSUES

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INTRODUCTION

The incidence of endometrial carcinoma and its precursors has considerably increased during the past decade and at the present time, endometrial carcinoma is the leading malignancy of the female genital tract in women over 50 years old (30). Despite the growing importance of endometrial carcinoma, the mechanisms underlying its pathogenesis and in particular, its relationship with hyperplasia remains obscure. It is well established that normal endometrial growth is estradiol (E_2) dependent and that progesterone (Pg) can inhibit the E_2-mediated endometrial cell proliferation (11). Thus, consistent with the hypothesis that the presence of steroid hormone receptors is a prerequisite of steroid hormones to mediate their effects in target tissues (18,21), estrogen receptors (ER) and progesterone receptors (PgR) have been identified using biochemical techniques in a variety of human endometrial tissues by our laboratory (29).

Studies on a variety of experimental systems reveal that epithelial growth may be initiated and sustained by the surrounding mesenchymal tissue (6). Therefore, it is conceivable that epithelial-stromal interactions play a role in the induction and growth of epithelial tumors. In the endometrium such a possibility is strenghthened by the observation that ERs are not only present in epithelial, but also in endometrial stromal cells (6).

Recently developed monoclonal antibodies to ER (22) make it possible to immunolocalize receptors and to determine specifically their cytologic localization in target cells. Accordingly, to facilitate our understanding of the pathogenic relationship between

endometrial carcinoma, its precursors and hyperplasia and the possible influence of the surrounding mesenchyme on these abnormal endometrial growths, we have analyzed by immunohistochemistry the ER content and its relative distribution between the stroma and the epithelium in a variety of human endometrial tissues. Preliminary results of this work have been previously published (2).

MATERIALS AND METHODS

Tissue Samples

Thirty normal cyclic, 16 hyperplastic and 21 neoplastic tissues [including four endometrial intraepithelial neoplasia (EIN)] were obtained from women ranging in age from 24 to 58, 25 to 65 and 52 to 87 years old, respectively. The specimens were obtained after informed consent of the patient. Fifteen normal cyclic tissue samples were obtained from hysterectomy specimens performed for treatment of uterine intramural leiomyomata (eight cases), adenomyosis (four cases), invasive squamous carcinoma of the cervix (one case) and chronic pelvic pain (two cases). Patients had no history of exogenous hormone use for at least one year prior to their surgery and had a normal endometrium. Fifteen normal cyclic tissue samples were obtained from biopsies performed in infertile patients. Ten hyperplastic tissue samples were obtained from hysterectomy specimens. In two of 10, pre-hysterectomy biopsies contained a mixture of endometrial hyperplasia and intraepithelial neoplasia. Six hyperplastic tissue samples were obtained from biopsy performed as a diagnostic procedure of dysfunctional uterine bleeding. Twenty-one neoplastic tissue samples were obtained from hysterectomy specimens. Two of the four cases of EIN were associated with hyperplasia.

Hysterectomy specimens immediately after their removal in the operating room were opened along the lateral border in order to obtain undistorted endometrial surfaces. Tissue sections were taken longitudinally from the endometrial mucosa or the grossly abnormal proliferations. All the tissue samples were divided in two pieces. One

for histology was fixed in 10% buffered formalin (hysterectomy specimens) or in Bouin's solution (biopsy specimens) and processed and stained in a routine fashion. The other one was snap frozen in liquid nitrogen and kept at -70°C until it was processed for immunohistochemistry and for biochemical analysis in the cases of carcinoma.

Normal endometria was dated according to previously described histologic criteria (25). Normal endometria represented 4 early, 6 mid, 4 late proliferative, seven early secretory (17th day or POD 3), four mid secretory (23rd day or POD 9 and 25th day or POD 11), two late secretory (26th day or POD 12 and 28th day or POD 14) and three menstrual (Table 1) Hyperplastic endometria contained architectural alterations of endometrial glands ranging from minimal (or simple) to complex (adenomatous). These included lesions traditionally named anovulatory, persistent proliferative endometrium, cystic glandular hyperplasia, simple hyperplasia and adenomatous hyperplasia. By definition, the glandular lining epithelium was devoid of cytologic atypia. On the other hand, lesions in which the lining epithelium displayed significant nuclear atypia including nuclear rounding, pleomorphism, loss of nuclear organization and macronucleoli were classified as endometrial intraepithelial neoplasia (EIN) Most of those lesions contained glands with architectural alterations with complex intra- and extraluminal epithelial buddings. Traditionally these lesions were referred to as atypical adenomatous hyperplasia, adenomatous hyperplasia with cytologic atypia, CIS and dysplasia Endometrial carcinoma with invasion either of the endometrial stroma

and/or the myometrium were graded according to the Gynecologic Oncology Group grading system. They were subdivided into grade 1 (well-differentiated with 0-5% of undifferentiated cells), grade 2 (moderately differentiated with 5-50% of undifferentiated cells), and grade 3 (poorly differentiated with more than 50% of undifferentiated cells) carcinoma

Immunohistochemical Analysis of Estrogen Receptor

Frozen tissue blocks were cut with a cryostat at 4 μm and thaw-mounted on glass slides. The initial section was stained with hematoxylin-eosin for tissue diagnosis. Assignment of histologic diagnosis, particularly tumor type and grade, was confirmed by review of formalin- or Bouin-fixed paraffin-embedded, hematoxylin-eosin stained sections taken from the same specimens.

For analysis of ER, ER-ICA kits were provided by Abbott Laboratories which contains the specific antihuman ER antibody H 222. This antibody is derived from a male Lewis rat immunized with estradiol-receptor complex from MCF 7 human breast cancer cells. The peroxidase-antiperoxidase method for immunohistochemical localization of ER was performed according to instructions specified by Abbott Laboratories except incubation with the ER antibody was done overnight at 4°C instead of 30 minutes at room temperature.

Positive staining with monoclonal ER antibody was defined as golden brown granular staining. Staining was absent in controls. For evaluation of ER content a score corresponding to the sum of both, a) the percentage of positive cells (1=0-25%, 2=26-50%, 3=51-75%, 4=76-100%) and b) the staining intensity (1 to 3) was established. The

intensity of specific staining was characterized as absent (0), weak but detectable above control (1), strong (2) and very strong (3) Evaluations were recorded for each observed tissue component, i e epithelium, stroma, and, if available, myometrium. The entire tissue section was scanned. Three randomly chosen low power microscopic fields (X40) in each tissue section were chosen and the intensity and the percentage of positive cells of each cellular component were evaluated at a magnification of 400X. The mean value for the assayed fields was expressed as the percentage of positive cells and the intensity values for the tissue. The maximum score was 7, but a score greater or equal to 2 was the value of a "positive" immunohistochemical assay.

Biochemical Analysis of Estrogen Receptors

Only neoplastic tissues were assayed. Tissue extracts were prepared by homogenisation of tissues in a phosphate glycerol buffer (5 mM sodium phosphate, 12 mM monothioglycerol, 10% glycerol, pH 7 4) containing 20 mM sodium molybdate and were centrifuged at 100,000 xg for one hour. The ER contents were measured using the dextran-coated charcoal (DCC) assay as described by us previously (29). The data were expressed as fmol per mg of tissue protein and were considered positive when greater than or equal to 10 fmol/mg protein. Biochemical assay values and immunohistochemical assay values were coded separately in a blind fashion in the neoplastic tissues.

Statistical analysis was made by means of students' modified T tests.

RESULTS

Immunohistochemical Analysis of ER in Normal Cyclic Endometrial Tissue

The immunolocalization of ER in normal, cyclic endometrium is shown in Figure 1 In all cases, ER were identified specifically in the nuclei of epithelial and stromal cells. As shown in Table 1, the vast majority of both the epithelial and stromal component of the 14 cyclic proliferative endometria had a high score regardless of whether the tissues corresponded to early, mid or late proliferative phases; the average score for the epithelium and the stroma was 5.78 ± 1.05 and 6 1 ± 1.41, respectively (mean ± SD). In the early secretory phase [17th day or postovulatory day 3 (POD 3)] more than 50% of the nuclei of epithelial cells contained a very strong staining, whereas the mid secretory phase endometria (23rd or POD 9 -25th day or POD 11) contained only 25% of positive epithelial cells with a weak or strong staining The epithelial component of the late secretory phase (26th or POD 12 -28th day or POD 14) and menstrual endometria was devoid of receptors. The stromal cells lost their receptors faster than their epithelial counterparts having 25% of positive cells with a weak staining by the 17th day of the cycle and remained poorly stained until the end of the menstrual cycle except in one case (specimen 22). During the menstrual phase, the stromal cells had a variable score. They were unstained in one case (specimen 29) and weakly stained in two cases (specimens 28 and 30) Both the epithelial and stromal component of the endometrial basal layer stained strongly positive, irrespective of cycle days (Table 1). Where the myometrium was available for study, the nuclei of normal smooth muscle cells stained

strongly in all 5 cases, whereas those in myometrial blood vessels failed to stain. The serosal (mesothelial) uterine surface epithelium also lacked staining reaction (data not shown).

Immunohistochemical Analysis of ER in Hyperplastic and Neoplastic Endometrial Tissues

The 16 hyperplastic endometria without cytologic atypia contained a high score both in the epithelium (5 6 ± 0.95; x̄ ± SD) and stroma (5.68 ± 0.94; x̄ ± SD) (Figure 2; Table 2). The four cases of endometrial intraepithelial neoplasia (hyperplasia with cytologic atypia) had low levels of receptors in their epithelial component with less than 25% of cells being ER positive (2 x̄) However, the score of stromal cells remained relatively high (4.75 ± 2 21, x̄ ± SD) and in two cases (specimens 1 and 3) more than 75% of the stromal cells were ER positive (Figure 3, Table 3).

The ER content in the 17 invasive carcinomas are shown in Table 4. The average score for grade 1 carcinomas was 3.2 ± 2 94 (x̄ ± SD) in the epithelial component and 1.2 ± 1.78 (x̄ ± SD) in the stromal component. In 3 of 5 grade 1 carcinomas, the epithelial component had a high ER score (Figure 4A), whereas the remaining two failed to stain in either the epithelial or stromal component They were ordinary carcinomas and occurred in a 74 and an 81 year old patient, respectively All grade 2 carcinomas contained an ER positive epithelial component (Table 4). However, neither the epithelial (4 ± 1; x̄ ± SD) nor the stromal component (1.4 ± 1 16, x̄ SD) was significantly different from grade 1 carcinomas The average ER score of the epithelial component in grade 3 carcinomas (0.8 ± 1.09; x̄ ± SD)

was significantly lower ($p < 0.001$) than in their grade 2 counterparts. Only 2 of the 5 cases were ER positive. These patients were 59 and 60 years old, respectively. The stromal ER content in grade 3 carcinomas remained high (3.4 ± 2.96; $\bar{x} \pm SD$) and was not significantly different from that found in grade 1 or 2 carcinomas (Figure 4B).

Biochemical Analysis for ER Content in Endometrial Carcinoma

Table 5 contains comparisons of ER content in carcinomas made by immunohistochemistry and DCC assay. There was a good correlation in the positivity of cases except in two specimens (3,17) where the epithelial ER content was negative by immunohistochemistry but the total ER content positive by biochemistry due to the stromal or the myometrial ER content.

DISCUSSION

Immunohistochemical analysis of estrogen receptors in various cell types of normal, hyperplastic and neoplastic human endometrial tissues revealed the localization of ER exclusively in the nucleus. These observations are in agreement with similar observations made in previous immunohistochemical studies (3,26,27) and support the hypothesis that ER is a nuclear protein (22,32). Our studies also confirm previous observations that ERs are present in both the stroma and the epithelium of human endometrial tissues in both the upper functionalis and particularly the lower basalis layers (3,6,26,27) The rich ER content of the basal glands including those in the premenstrual phase of the cycle is in agreement with previous electron microscopic and *in vitro* and *in vivo* histoautoradiographic studies on human and experimental endometria suggesting the basal glands to be the origin for the postmenstrual as well as the post-traumatic regenerative endometrial epithelium (9,10,12).

Unlike the present one, previous studies did not specifically examine the modulation of ER in various cell types as it pertains to the normal menstrual cycle and various pathogenic states of the endometrium. Accordingly, the ER content is high in the epithelium of the proliferative endometrium known to contain high levels of PgR (20,29) and decreases with the onset of the secretory phase known to have decreased levels of PgR (4) and is absent in menstrual endometria. The loss of ER as a function of the menstrual cycle is also apparent in the stroma and in fact appears to occur more rapidly than in the epithelium. For example, in the early secretory phase

(17th day or POD 3), while the ER content in the epithelium remains as high as that in the pre-ovulatory proliferative epithelium, the loss of ER in the stroma is very dramatic. Since the onset of secretory phase is known to be accompanied by high plasma levels of progesterone, our data suggest that the loss of both the stromal and epithelial ER may be mediated through progesterone. Such a possibility is strengthened by previous observations that progesterone can down-regulate ER in the uterus (19,31). At present we do not know the precise significance of the presence of ER in the stroma and its modulation through the menstrual cycle. However, it is possible that at least in the proliferative phase of the menstrual cycle the stromal ER is functional for during this period similar to the endometrial epithelium the stroma also undergoes extensive proliferation in response to high plasma level of E_2 (28).

An analysis of the ER content in the various cell types of hyperplasia (without cytologic atypia) reveals it to be similar to normal proliferative endometrium (Table 6). This is consistent with their similar sensitivity to E_2 as revealed by the PgR content in these tissues (20,29). In contrast, the low epithelial ER content in endometrial intraepithelial neoplasia (hyperplasia with cytologic atypia) is similar to the epithelial ER content of carcinoma (Table 6). Based on previous characterization of hyperplasia and EIN by electron microscopy (13), quantitative microscopy (1,5), *in vitro* DNA histoautoradiography (14), it has been proposed that endometrial hyperplasia promoted by E_2 represents an overgrowth of normal proliferative endometrium, while EIN may represent the immediate

precursor of invasive carcinoma (15,16,23). Our present data on the ER content and its relative distribution between the epithelium and the stroma support this hypothesis. Furthermore, our data suggest that endometrial hyperplasia and EIN may in fact represent two separate and distinct pathologic conditions For example, in the two cases where hyperplasia and EIN were associated in the same endometrium (Case 1 hyperplasia, specimen 9, Table 2 and case 1. EIN specimen 2, Table 3; Case 2: hyperplasia, specimen 15, Table 2 and case 2: EIN, specimen 4, Table 3), the distribution of ER was different in each of the coexistent lesion. The findings suggest that coexistent hyperplasia and EIN in the same endometrium are made of different cell populations. Whether EIN represents a neoplastic dedifferentiation of hyperplasia or these two lesions are pathogenically unrelated is to be determined.

In contrast to normal endometrium, endometrial hyperplasia and EIN, in invasive carcinoma there was a considerable heterogeneity in the profile of ER in both the epithelium and the stroma. This suggests that EIN and carcinoma may not necessarily be successive steps on a transformation pathway. Overall the epithelial content of ER in carcinoma appeared to be positively correlated with the degree of tumor differentiation. This confirms similar observations made previously using biochemical assays for ER (7,8,17,24). The stromal ER content of carcinoma failed to be correlated with the degree of tumor differentiation However, the presence of ER in the stroma emphasizes the need to analyze ER by immunohistochemical method so that the epithelial sensitivity to E_2 may be correctly assessed. Indeed, the

stromal and myometrial contribution to biochemical ER analysis, particularly when the epithelial ER is poor or absent (specimens 3, 13 and 17, Table 5) may be very significant and lead to an erroneous interpretation on the supposed epithelial ER content of these cases.

TABLE 1. IMMUNOHISTOCHEMICAL SCORE OF ER IN NORMAL CYCLIC ENDOMETRIUM

HISTOLOGIC DATING	SPECIMEN No.	EPITHELIUM (a)	EPITHELIUM (b)	EPITHELIUM (a+b)	STROMA (a)	STROMA (b)	STROMA (a+b)
Early Proliferative	1	3	3	6	4	3	7
	2	3	2	5	1	2	3
	3	4	2	6	4	3	7
	4	4	3	7	3	3	6
	(Basalis)	4	3	7	4	3	7
Mid Proliferative	5	4	2	6	4	3	7
	6	3	2	5	2	2	4
	7	4	3	7	3	2	5
	8	4	3	7	2	2	4
	9	2	2	4	4	3	7
	10	3	2	5	4	3	7
	(Basalis)	4	3	7	4	3	7
Late Proliferative	11	4	3	7	4	3	7
	12	4	2	6	4	3	7
	13	2	2	4	4	3	7
	14	3	3	6	3	3	6
17th day	15	4	3	7	1	1	2
	(Basalis)	3	3	6	1	1	2
	16	3	2	5	1	1	2
	17	4	3	7			0
	18	4	3	7	1	1	2
	(Basalis)	4	3	7	2	1	3
	19	4	3	7	1	1	2
	20	4	3	7			0
	21	3	3	6			0
23rd day	22	2	2	4	3	3	6
	23	1	1	2	1	1	2
25th day	24	1	1	2			0
	25	1	1	2	2	1	3
26th day	26			0	1	1	2
28th day	27			0			0
	(Basalis)	3	3	6	2	2	4
Menstrual	28			0	1	1	2
	(Basalis)	3	3	6	2	2	4
	29			0			0
	30			0	3	1	4

a= % of positive cells, (1=0-25%, 2=26-50%, 3=51-75%, 4=76-100%)
b= staining intensity, 1 (weak) to 3 (very strong)

TABLE 2. IMMUNOHISTOCHEMICAL SCORE OF ER IN ENDOMETRIAL HYPERPLASIA

SPECIMEN No.	EPITHELIUM (a)	(b)	(a+b)	STROMA (a)	(b)	(a+b)
1	4	3	7	4	3	7
2	4	3	7	3	2	5
3	3	2	5	3	2	5
4	3	2	5	4	3	7
5	3	3	6	3	3	6
6	3	2	5	3	3	6
7	3	2	5	3	2	5
8	2	2	4	3	3	6
9	3	2	5	2	3	5
10	4	3	7	3	2	5
11	3	3	6	4	3	7
12	3	3	6	3	3	6
13	2	3	5	2	3	5
14	4	3	7	4	3	7
15	3	2	5	2	2	4
16	3	2	5	3	2	5

a= % of positive cells, (1=0-25%, 2=26-50%, 3=51-75%, 4=76-100%)
b= staining intensity, 1 (weak) to 3 (very strong)

TABLE 3. IMMUNOHISTOCHEMICAL SCORE OF ER IN ENDOMETRIAL INTRAEPITHELIAL NEOPLASIA

SPECIMEN No.	EPITHELIUM (a)	(b)	(a+b)	STROMA (a)	(b)	(a+b)
1	1	1	2	4	3	7
2	1	1	2	1	1	2
3	1	1	2	4	2	6
4	1	1	2	2	2	4

a= % of positive cells, (1=0-25%, 2=26-50%, 3=51-75%, 4=76-100%)
b= staining intensity, 1 (weak) to 3 (very strong)

TABLE 4. IMMUNOHISTOCHEMICAL SCORE OF ER IN ENDOMETRIAL CARCINOMA BY HISTOLOGIC GRADE

HISTOLOGIC GRADE	SPECIMEN No.	EPITHELIUM (a)	EPITHELIUM (b)	EPITHELIUM (a+b)	STROMA (a)	STROMA (b)	STROMA (a+b)	MYOMETRIUM (a)	MYOMETRIUM (b)	MYOMETRIUM (a+b)
1 well differentiated	1	3	3	6	1	1	2			N/A
	2			0			0	2	2	4
	3			0			0	4	3	7
	4	2	3	5			0	1	1	2
	5	3	2	5	2	2	4	2	2	4
2 - moderately differentiated	6	2	3	5	2	3	5			N/A
	7	2	2	4	2	2	4	3	3	6
	8	1	3	4	1	1	2	3	3	6
	9	2	2	4	1	1	2			N/A
	10	2	2	4	1	1	2			N/A
	11	1	1	2	1	1	2			N/A
	12	2	3	5	2	3	5	3	3	6
3 - poorly differentiated	13	1	1	2	4	3	7	3	3	6
	14			0	3	3	6			N/A
	15	1	1	2	1	1	2	1	1	2
	16			0			0			0
	17			0	1	1	2	2	3	5

a= % of positive cells, (1-0-25%, 2-26-50%, 3-51-75%, 4-76-100%)
b= staining intensity, 1 (weak) to 3 (very strong)
N/A= not available

TABLE 5. IMMUNOHISTOCHEMICAL SCORE AND BIOCHEMICAL ANALYSIS OF ER IN ENDOMETRIAL CARCINOMA BY HISTOLOGIC GRADE

HISTOLOGIC GRADE	SPECIMEN No.	EPITHELIUM (a+b)	STROMA (a+b)	MYOMETRIUM (a+b)	Fmol/mg protein
1 - well differentiated	1	6	2	N/A	117
	2	0	0	4	4.1
	3	0	0	7	114
	4	5	0	2	456
	5	5	4	4	N/A
2 - moderately differentiated	6	5	5	N/A	43.8
	7	4	4	6	180
	8	4	2	6	318
	9	4	2	N/A	79.2
	10	4	2	N/A	546
	11	2	2	N/A	N/A
	12	5	5	6	64.3
3 - poorly differentiated	13	2	7	6	333
	14	0	6	N/A	N/A
	15	2	2	2	50
	16	0	0	0	2
	17	0	2	5	122

N/A= not available
Fmol= femtomol

TABLE 6. MEAN ER CONTENT IN PROLIFERATIVE, HYPERPLASTIC AND NEOPLASTIC ENDOMETRIUM

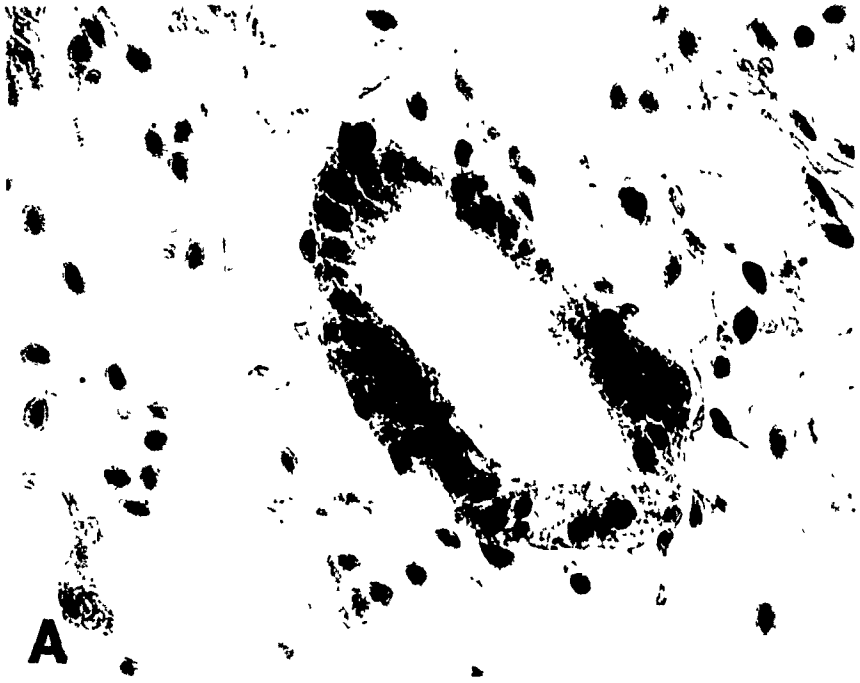
HISTOLOGIC GROUP	EPITHELIUM $\bar{x}\pm SD$	P	STROMA $\bar{x}\pm SD$	P
Proliferative endometrium (n=14)	5.78±1.05		6±1.41	
		NS		NS
Hyperplasia (n=16)	5.62±0.95		5.68±0.94	
		p<0.001		NS
Endometrial intraepithelial neoplasia (n=4)	2.0		4.75±2.21	
		NS		NS
Invasive carcinoma (n=17)	2.82±2.18		2.64±2.17	

SD: standard error deviation
Number of cases in brackets

Figure 1. Immunohistochemical localization of ER in normal cyclic endometrium.

A) A strong nuclear staining is observed in both the epithelial and stromal cells in the mid proliferative phase (x 450. No counterstain).

B) In the early secretory phase (17 day) the nuclei of epithelial cells remain strongly stained compared to the few weakly stained stromal cells (x450. No counterstain).


A

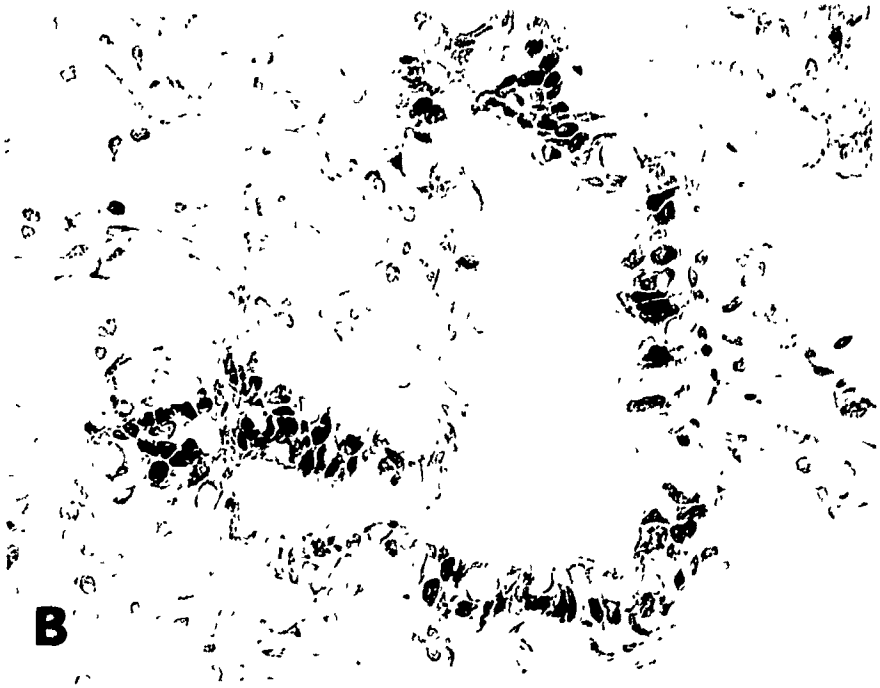
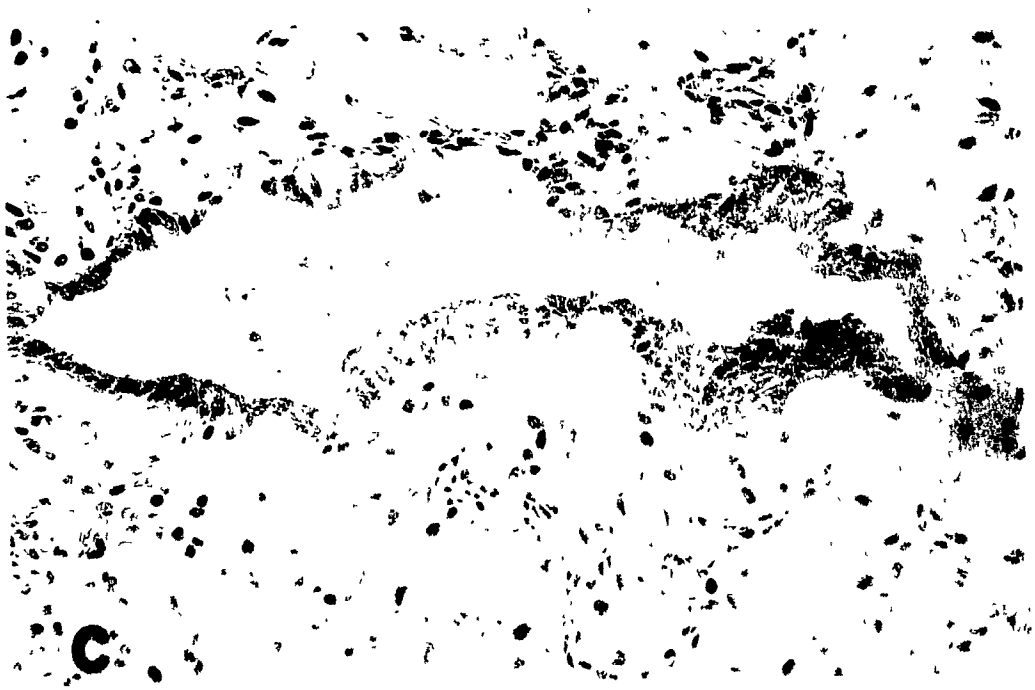

B

Figure 1. Immunohistochemical localization of ER in normal cyclic endometrium.
C) In the mid-secretory phase (25 day) nuclear staining is negligeable in both the epithelial and stromal cells (x450. No counterstain).
D) In the menstrual phase no nuclear staining is observed (x250. No counterstain).


C


D

Figure 2. Immunohistochemical localization of ER in hyperplasia. Both the epithelial and stromal cells have strong nuclear staining (x450. No counterstain).

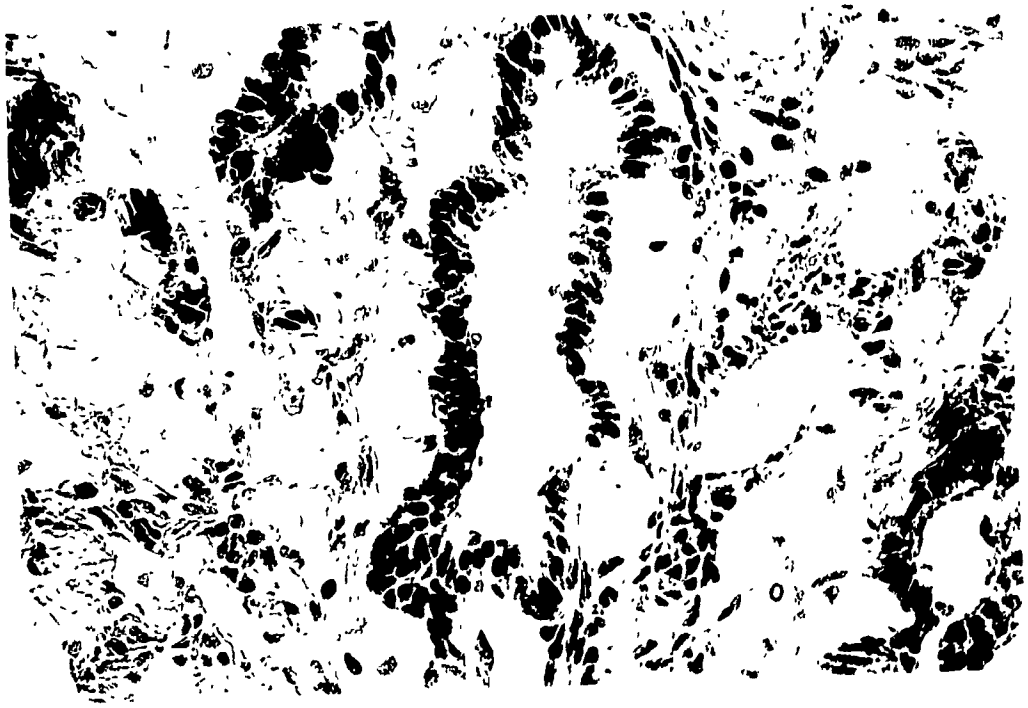


Figure 3. Immunohistochemical localization of ER in endometrial intraepithelial neoplasia. The stromal nuclei stain strongly (*arrows*) while the epithelial cells contain weak or no staining reaction (x 450. No counterstain).

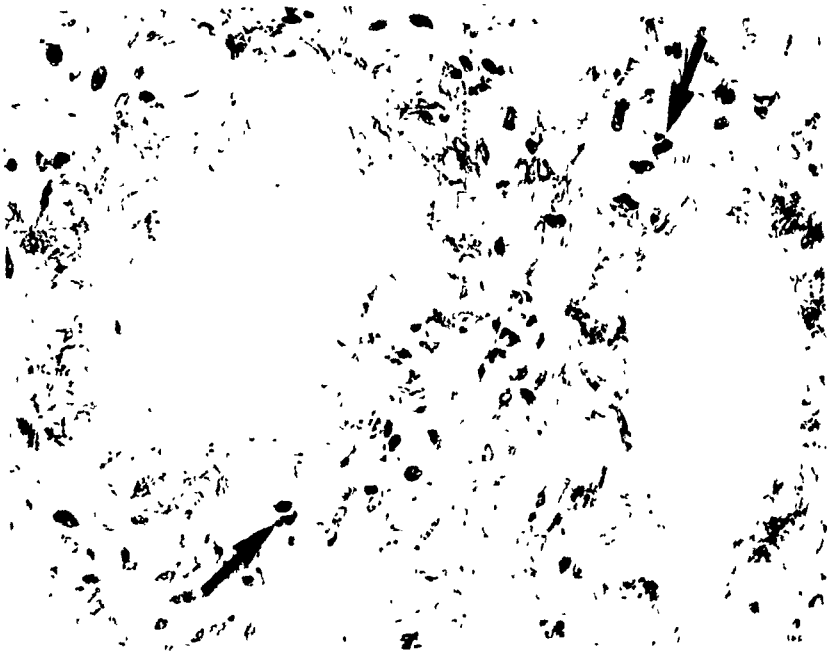
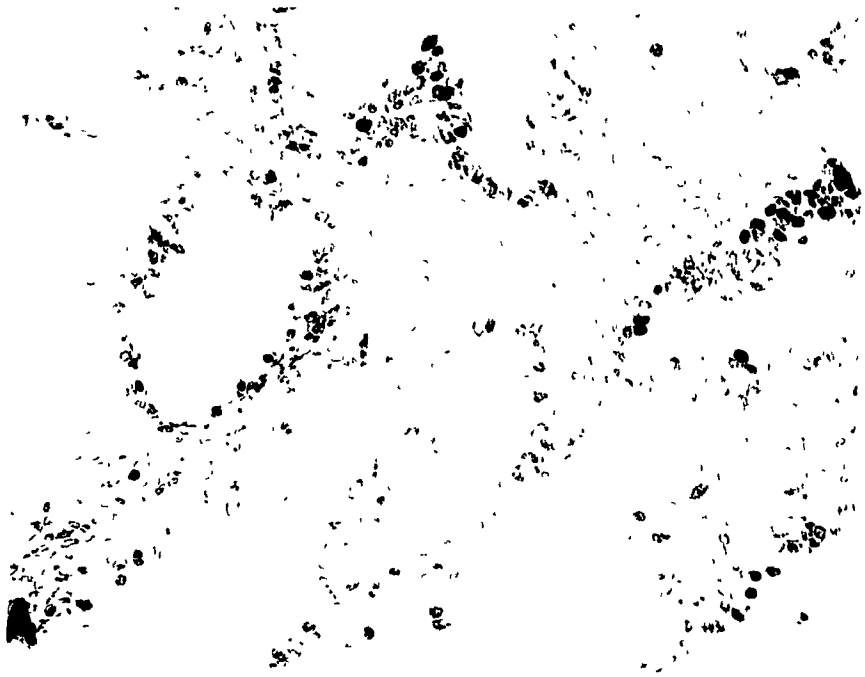


Figure 4. Immunohistochemical localization of ER in carcinoma.
A) Well differentiated carcinoma. Both the epithelial and stromal cells have a heterogenous nuclear staining pattern (x250. No counterstain).
B) Poorly differentiated carcinoma. Immunostaining is confined exclusively to the nuclei of stromal cells (*arrows*) (x450. No counterstain).



B

REFERENCES

1. Aausems EWMA, Vanderkamp JK, Baak JPA: Nuclear morphometry in the determination of the prognosis of marked atypical endometrial hyperplasia. Intl J Gynecol Pathol 4:180, 1985
2. Bergeron C, Ferenczy A, Shyamala G: Distribution of estrogen receptors (ERS) in various cell types of normal, hyperplastic and neoplastic human endometrial tissues. Proceedings of the 78th Annual Meeting of the American Association of Cancer Research, Atlanta, Georgia. Abstract #969 (1987)
3. Budwit-Movotomy DA, McCarthy KS, Cose EB, Soper JT, Mutch DG, Creasman WT, Flowers JL, McCarthy KS: Immunohistochemical analyses of estrogen receptor in endometrial adenocarcinoma using a monoclonal antibody. Cancer Res 46:5419, 1986
4. Clark JH, Schrader WT, O'Malley BW: Mechanisms of steroid hormone action. In Textbook of Endocrinology, edited by Wilson JD, Foster DW, p 33. Philadelphia, WB Saunders Co, 1985
5. Colgan TJ, Norris HJ, Foster W, Kurman RJ, Fox CH: Predicting the outcome of endometrial hyperplasia by quantitative analysis of nuclear features using a linear discriminant function. Intl J Gynecol Pathol 1:347, 1983
6. Cunha GR, Chung LWK, Shannon JM, Taguchi O, Fujii H: Hormone-induced morphogenesis and growth: Role of mesenchymal-epithelial interactions. Recent Prog Hormone Res 39:559, 1983
7. Ehrlich LE, Young RM, Cleary RE: Cytoplasmic progesterone and estrogen receptors in normal, hyperplastic and carcinomatous

endometria: Therapeutic implications. Am J Obstet Gynecol 141:539, 1981

8. Evans LH, Martin JD, Hamel R: Estrogen receptor concentration in normal and pathological human uterine tissues. J Clin Endocrinol Metabol 38:23, 1974

9. Ferenczy A: Studies on the cytodynamics of human endometrial regeneration. II. Transmission electron microscopy and histochemistry. Am J Obstet Gynecol 124:582, 1976

10. Ferenczy A: Studies on the cytodynamics of experimental endometrial regeneration in the rabbit. Historadioautography and ultrastructure. Am J Obstet Gynecol 128:536, 1977

11. Ferenczy A, Bertrand G, Gelfand MM: Proliferation kinetics of human endometrium during the normal menstrual cycle. Am J Obstet Gynecol 133:859, 1979

12. Ferenczy A, Bertrand G, Gelfand MM: Studies on the cytodynamics of human endometrial regeneration. III. *In vitro* short-term incubation historadioautography. Am J Obstet Gynecol 134:297, 1979

13. Ferenczy A: Cytodynamics of endometrial hyperplasia and neoplasia. I. Histology and ultrastructure. In Progress in Surgical Pathology, edited by Fenoglio CM, Wolff M, p 95. New York, Masson Publishing USA, 1982

14. Ferenczy A: Cytodynamics of endometrial hyperplasia and neoplasia. Part II: *In vitro* DNA histoautoradiography. Human Pathol 14:77, 1983

15. Ferenczy A, Gelfand MM: Hyperplasia vs neoplasia: two tracts for the endometrium. Contemp Ob/Gyn 28:79, 1986

16. Fox H, Buckley CH: The endometrial hyperplasias and their relationship to endometrial neoplasia. Histopathol 6:493, 1982

17. Geisinger KR, Marshall RB, Kute TE, Homesley HD: Correlation of female sex steroid hormone receptors with histologic and ultrastructural differentiation in adenocarcinoma of the endometrium. Cancer 58:1506, 1986

18. Gorski J, Toft D, Shyamala G, Smith D, Notides A: Hormone receptors: Studies on interaction of estrogen with the uterus. Recent Prog Hormone Res 29:45, 1968

19. Hsuch AJW, Peck EJ, Clark JH: Progesterone antagonism of the oestrogen receptor and oestrogen-induced uterine growth. Nature 254:337, 1975

20. Janne O, Kauppila A, Kontula K, Syrjala P, Vihko R: Female sex steroid receptors in normal, hyperplastic and carcinomatous endometrium. The relationship to serum steroid hormones and gonadotropins and changes during medroxyprogesterone acetate administration. Intl J Cancer 24:545, 1979

21. Jensen EV, Greene GL, Closs LE, De Sombre ER, Nadji M: Receptors reconsidered: A 20 year perspective. Recent Prog Hormone Res 38:1, 1982

22. King WJ, Greene GL: Monoclonal antibodies localize estrogen receptors in the nuclei of target cells. Nature 307:745, 1984

23. Kurman RJ, Kaminski PT, Norris HJ: The behavior of endometrial hyperplasia: A long term study of "untreated" hyperplasia in 170 patients. Cancer 56:403, 1985

24. McCarthy KS Jr, Barton TK, Fetter BF, Creasman WT, McCarthy KS Sr:

Correlation of estrogen and progesterone receptors with histologic differentiation in endometrial adenocarcinoma. Am J Pathol 96:171, 1979

25. Noyes RW, Hertig AT, Rock J: Dating the endometrial biopsy. Fertil Steril 1:3, 1950

26. Press MF, Nousek-Goebl N, King WJ, Herbst AL, Greene GL: Immunohistochemical assessment of estrogen receptor distribution in the human endometrium throughout the menstrual cycle. Lab Invest 51:495, 1984

27. Press MF, Nousek-Goebl NA, Bui M, Greene GL: Estrogen receptor localization in the female genital tract. Am J Pathol 123:280, 1986.

28. Ross GT, Vandewicke RL, Frantz RG: The ovaries and the breasts. In Text of Endocrinology, edited by Williams RA, p 374. Saunders WB Co, 1981

29. Shyamala G, Ferenczy A: The effect of sodium molybdate on the cytoplasmic estrogen and progesterone receptors in human endometrial tissues. Diagn Gynecol Obstet 3:277, 1981

30. Silverberg E, Lubera J: Cancer statistics. Ca J Clin 37:1, 1987

31. Takeda A, Leavitt W: Progestin-induced down regulation of nuclear estrogen receptor in uterine decidual cells: Analysis of receptor synthesis and turnover by the density shift method. Biol Biophys Res Commun 135:98, 1986

32. Welshons WV, Lieberman ME, Gorski J: Nuclear localization of unoccupied estrogen receptors: cytochalasin enucleation of GH3 cells. Nature 307:747, 1984

Chapter III

IMMUNOCYTOCHEMICAL STUDY OF PROGESTERONE RECEPTORS IN THE HUMAN ENDOMETRIUM DURING THE MENSTRUAL CYCLE

IMMUNOCYTOCHEMICAL STUDY OF PROGESTERONE RECEPTORS IN THE HUMAN ENDOMETRIUM DURING THE MENSTRUAL CYCLE

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INTRODUCTION

In the normal cyclic endometrium estrogens (E_2) induce epithelial and stromal proliferation during the preovulatory phase while progesterone induces epithelial secretory differentiation and stromal decidualization during the post ovulatory phase of estrogen-primed proliferative endometrium (25). These hormonal responses are believed to be regulated by the binding of E_2 and progesterone to their respective receptors (2).

Studies on several experimental systems demonstrate that epithelial growth of the reproductive tract may be initiated and sustained by the surrounding mesenchymal tissue (4). It is known that estrogen receptors (ER) are present in both the stroma and the epithelium of the human endometrium and that the relative distribution of the ER between these cell types varies as a function of the menstrual cycle (1,24). However, the functional significance of ER modulation in the stroma and the epithelium as a function of the menstrual cycle remains unclear. It is well established that in target tissues, the synthesis of progesterone receptors (PgR) is regulated by E_2 and can thus serve as a marker for functional ER (14,17,18). Accordingly the objective of this study was to examine the relative distribution of PgR between the stroma and the epithelium of the human endometrium and its modulation as a function of the menstrual cycle. To achieve our goal, we have analyzed the PgR by an immunocytochemical method using a specific mouse monoclonal antibody against avian PgR (αPR6) known to have cross-reactivity in the human tissue (6,31).

MATERIALS AND METHODS

Tissue Samples

Normal cyclic endometria were obtained from 26 women ranging in age from 23

to 53 years old (Table 1). The specimens were obtained after informed consent of the patient. 18 samples were obtained from hysterectomy specimens performed for treatment of subserosal or intramural leiomyomata (9 cases), adenomyosis (4 cases), invasive squamous carcinoma of the cervix (2 cases) and chronic pelvic pain (3 cases). 8 samples were obtained from biopsies performed in infertile patients.

In all patients the endometrium was of normal morphology and correlated well with the clinical cycle days, irrespective of age of the patients and associated conditions. Patients with intramural leiomyomata and adenomyosis has microscopic disease, only the subserosal leiomyomata were felt clinically. In all these patients the endometrium was free histologically of compression "atrophy" or excessive stromal edema; such endometrial alterations may be found in association with submucosal or extensive

intramural leiomyomata or adenomyosis. The normal cyclic ovulatory nature of the endometrium was ascertained, furthermore, by history of normal cycles of 28 days ± 2 days duration for at least two years prior to endometrial sampling for immunohistochemical assays and by monthly basal body temperature curves during the 6 months preceding the study. To rule out the remote possibility of anovulatory cycles particularly in patients aged 40 years and older, an endometrial biopsy was obtained in each patient during the first or second day of

the menstrual period one to two months prior to the study. In all instances, normal histologic alterations consistent with post-ovulatory menstrual breakdown was found. Finally, no patients used exogenous sex-steroid hormone preparations during the last 2 years prior to the study.

Hysterectomy specimens immediately after their removal in the operating room were opened along the lateral border in order to obtain undisturbed endometrial surfaces. Tissue sections were taken longitudinally from the endometrial mucosa. All the tissue samples were divided in two pieces. One for histology was fixed in 10% buffered formalin (hysterectomy specimens) or in Bouin's solution (biopsy specimens) and processed and stained in a routine fashion. The other one was snap frozen in liquid nitrogen and kept at -70°C until processing for immunohistochemistry. Endometrial dating was made according to previously described morphologic criteria (19) on the paraffin-embedded sections stained with hematoxylin and eosin. It represented 10 proliferative (cycle days 5 to 14; including three 5-7; four 8-10 and three 11-14), 6 early secretory (17th day or postovulatory day 3 (POD 3)), 4 mid secretory (24th or POD 10 and 25th day or POD 11), 3 late secretory (26th or POD 12 and 27th day or POD 13) and 3 menstrual endometria.

Antibodies

Details about avian PgR purification, immunization of mice, cell fusion, hybridoma cloning and screening procedures used in the preparation of various monoclonal antibodies to PgR have been described elsewhere (31). αPR6 was selected for these studies due to

its cross-reactivity with human progesterone receptor (6,31,33). The normal horse serum, biotinylated immunoglobin against the mouse and the avidin-biotin peroxidase complex were obtained from Vector Laboratories, Burlingame, Ca. The non-immune mouse immunoglobulin was obtained from Dako Laboratories.

Immunohistochemical technique

In preliminary experiments picric acid paraformaldehyde fixation (30) during 15 min was chosen since immunoreactivity of PgR in rabbit tissues and human breast tumors was shown to be well preserved in this condition of fixation in other studies (21,22). Formalin-fixed paraffin embedded sections were tested but did not show any staining using otherwise the identical immunohistochemical method. Accordingly the following protocol was adopted. Frozen tissue blocks were cut with a cryostat at -20°C at 4 μm and thaw mounted on gelatin-coated glass slides. The initial section was stained with hematoxylin-eosin for histological dating. Sections were then fixed immediately without drying in picric acid paraformaldehyde for 15 min at -10 to -20°C. Slides were transferred to PBS for 30 min at 4°C. Sections were treated with a 0.5% solution of hydrogen peroxide in PBS for 10 min, put in PBS for 10 min and incubated for 10 min in normal horse serum to reduce the non-specific binding of primary antibody. The sections were incubated with monoclonal mouse anti-PgR antibody (αPR6) for 1 hr in a humid chamber, biotinylated antimouse IgG for 20 min at room temperature and avidin-biotin peroxidase complexes for 30 min at room temperature. Each incubation was followed by 10 min washings in PBS. Sections were incubated for 10 min with the DAB solution (PBS

containing 0.5 mg DAB/ml and 0.01% H_2O_2) at room temperature. Sections were then dehydrated and mounted for examination by light microscopy without counterstaining.

Various dilutions for the αPR6 were tested (1-20 μg IgG/ml) and the one which gave maximum specific staining intensity was 20 μg IgG/ml. Dilutions for the other antibodies and normal serum were performed according to the instructions specified by Vector Laboratories.

Negative controls consisted of adjacent sections treated with non-immune mouse immunoglobulins at the same dilution as αPR6 or with antibody (αPR6) presaturated with purified PgR. The PgR used for presaturation studies was purified from oviduct cytosol by affinity chromatography using antibody αPR22 (31) covalently linked to protein A-Sepharose (27). For presaturation antibody and antigen were mixed (10 μg receptor/μg IgG) and incubated 2 hr at room temperature. Mixtures were then used on sections instead of antibody (αPR6) alone. Additional controls included the omission of the primary antibody.

Positive staining with monoclonal PR antibody was defined as golden brown granular staining and was absent in the control adjacent section. For evaluation of PR content a score corresponding to the sum of both a) the percentage of positive cells (1=0-25%, 2=26-50%, 3=51-75%, 4=76-100%) and b) the staining intensity was established. The intensity of specific staining was characterized as absent (0), weak but detectable above control (1), strong (2) and very strong (3). Evaluations were recorded for each observed tissue component, i.e. epithelium, stroma and if available myometrium. The entire tissue section was scanned. Because of liquid nitrogen-related ice crystal

fixation artefacts only those areas which were devoid or were relatively free of such alterations were chosen for evaluation of PR content. The intensity and the percentage of positive cells of each cellular component were evaluated at a magnification of 400X. The mean value for 3 assayed fields was expressed as the percentage of positive cells and the intensity values for the tissue. The maximum score was 7, but a score greater or equal to 2 was the value of a "positive" immunohistochemical assay.

Immunoblot Analyses of PgR

Tissues were homogenized in a Tris-EDTA buffer (10 mM Tris pH 7.4, 1.5 mM EDTA, 1 mM dithiothreitol, 77 μg/ml aprotinin, 100 μg/ml bacitracin, 0.1 mM leupeptin and 1 μg/ml pepstatin), centrifuged for one hour at 105,000 x g at 4°C and the supernatant (cytosolic extract) was used for immunoblot analyses. Proteins in the cytosolic extract were denatured in sample buffer (0.135 M Tris pH. 6.8, 1% SDS, 0.005% bromophenolblue, 10% glycerol and 5% β-mercapto-ethanol) at 93°C for 5 min prior to electrophoresis on discontinuous polyacrylamide gels containing 7.5% acrylamide/0.075% Bis in the resolving gel and 3% acrylamide/0.08% Bis in the stacking gel using Laemmli buffer (16). After electrophoresis, proteins were blotted onto nitrocellulose membranes, washed and incubated with 10 μg of αPR6/ml in western buffer (0.5% BSA, 0.5% Tween 20 in phosphate buffered saline, pH 7.2). Subsequently the antibody bound to PgR was visualized using a commercially available kit (Vector Stain) containing biotinylated anti-mouse IgG antibody and avidin/biotinylated peroxidase and using 4-chloronaphthol (11).

RESULTS

Specificity of αPR6 against human endometrial PgR

In previous studies (31), immunoprecipitation analyses of human uterine cytosolic extracts were performed to document that among the various mouse monoclonal antibodies directed against avian PgR, only αPR6 had cross-reactivity. There is extensive documentation that both avian and human PgR isolated from tissue soluble extracts can exist in two different molecular forms commonly referred to as A and B (32). It is also known that αPR6 reacts with only the 110,000 molecular weight form (Form B) of PgR in the oviduct (31) and in human breast cancer cells (6,33). Therefore to further verify the nature of interaction of αPR6 with human endometrial PgR, we performed immunoblot analyses on the human endometrial cytosol. As shown in Figure 1, when the blots were incubated with αPR6 and processed as described in Materials and Methods, a doublet corresponding approximately to 120,000 dalton became visible which was absent in the control blot processed identically except for incubation with αPR6. The molecular weight of the human endometrial PgR reported here is in agreement with that reported for the molecular weight of the B form of the human mammary PgR (32); it is known that the B form of the human PgR can be resolved on SDS gels into a doublet (6,32) or a triplet (3) with a molecular weight somewhat larger than that of the B form of the avian PgR (6,28,32).

Unlike αPR6, αPR 11, 13 and 22 did not exhibit any cross-reactivity (Data not shown); these various mouse monoclonal antibodies to avian PgR have been shown not to interact with soluble PgR isolated from

human uterine tissue when analyzed by immunoprecipitation assays (31).

Immunocytochemical localization of PgR

The immunocytochemical analysis of human endometrium using αPR6 is shown in Figure 2. In this experiment early secretory phase endometrium was used. As shown in Figure 2A immunoreactive material was identified specifically in the nuclei of epithelial and stromal cells. No nuclear staining was observed when αPR6 preincubated with highly purified receptor was substituted for the monoclonal αPR6 (Figure 2B). Nuclear staining was also not observed when instead of αPR6 non-immunized mouse immunoglobulin was used (Data not shown).

a. Proliferative endometrium: In the functionalis of the proliferative phase the surface and the glandular epithelial component was positive in all the cases but at different levels (Table 1). The early proliferative phase (cycle days 5 to 7) contained less than 25% of positive cells. The number of positive cells increased during the mid proliferative phase (cycle days 8 to 10) and was the highest during the late proliferative phase (cycle days 11 to 14) (Figure 3). The latter contained up to 75% positive epithelial cells with a strong staining. In general the stromal PgR content during the proliferative phase was lower than in the epithelial component (Table 1). It had gradually increased, however, and was higher in the late proliferative phase but overall was still lower than in the epithelial component. Indeed less than 50% of stromal cells were positive with a strong intensity.

The PgR levels, both in the stroma and the epithelium of the basalis (Figure 4) also increased during the proliferative phase

(Table 1) and, except for one case (specimen 8, Table 1) in which the stromal component was negative, they were higher than those in the functionalis.

b. Secretory endometrium: In early secretory phase (day 17 or POD 3 of the menstrual cycle) endometria in both the epithelial and the stromal components contained high scores (Figure 2A) similar to those of the late proliferative endometrium. The intensity of the staining was the same in both cell components but the number of positive cells was higher in the epithelial than stromal component (Table 1). In the mid secretory phase (day 24 or POD 10 and 25 or POD 11 of the cycle) the staining was heterogenous but in general was lower than in the early secretory phase especially in the epithelial component (Figure 5). As shown in Table 1, in one case the epithelial cells failed to stain (specimen 18), while they were positive in the remaining 3 cases (specimens 17,19,20). Furthermore, the surface layer epithelium contained more positive cells than the glandular epithelium. The stromal cells were positive in all 4 cases and contained a higher score than their epithelial counterparts. In particular, the predecidual stromal cells located beneath the surface layer epithelium (on day 25 of the cycle) had strong positivity (Figure 5). In the late secretory phase (day 26 or POD 12 and 27 or POD 13 of the cycle) and in the menstrual phase, PgR content was absent from both the epithelial and stromal component except in one case (specimen 21 Table 1).

The basalis had high levels of PgR both in the epithelium and the stroma during the early and mid-secretory phases. In the late

secretory phase and in the menstrual phase the basalis paralelled the functionalis and was equally devoid of PgR.

c. Myometrium: Only a limited number of myometrium was available for study, but in all 6 cases the nuclei of normal smooth muscle cells stained strongly

irrespective of the cycle day (Figure 6). The muscle wall of myometrial blood vessels did not have any PgR. The serosal (mesothelial) uterine surface epithelium and the endothelial cells of endometrial and myometrial vessels also failed to stain for PgR.

DISCUSSION

Immunohistochemical analysis of PgRs in various cell types of 26 normal cyclic endometria revealed the localization of PgR exclusively in the nucleus. These findings confirm previous observations using biochemical techniques in which both the free and the steroid-bound forms of PgR were localized mainly in the nuclei (34). Our studies are also in agreement with immunohistochemical studies done by others in several target tissues for progesterone including human tissues (3,5,10,12,13,21-23).

Our study reveals that, as with the ER (1,24), PgR is also present in both the stroma and the epithelium of normal human endometrial tissues in both the upper functionalis and the lower basalis layer. Furthermore, the relative distribution of the PgR between the stroma and the epithelium also varies with the menstrual cycle, as is the case with ER.

A combined analysis of the data on the distribution of both the ER and PgR in the stroma and epithelium offered us some important insight regarding the estrogenic sensitivity of the normal cyclic endometrium as follows. The levels of ER were high in both the stroma and the epithelium of the proliferative endometrium and remained relatively constant during the proliferative phase (1,24). The PgR (as shown in Table 1) increased gradually from the early to the late proliferative phase. The highest levels of PgR

observed in the epithelium from the mid and the late proliferative endometria correlated with the high plasma and tissue levels of E_2 during this phase of the cycle (26). The PgR content in the stroma of

the proliferative endometrium was not as high as in the epithelium as also noted by Clarke et al (3). These data therefore suggested that the stroma did not respond to E_2 in the same way as the epithelium.

The PgR level remained very high in the early secretory phase (17th day) in both the epithelium and the stroma. This corresponds to the rising plasma and tissue levels of progesterone (26) and important epithelial morphological modifications such as the accumulation of intracytoplasmic glycogen, the appearance of giant mitochondria, and later glycoprotein synthesis (7). Thus it is likely that the morphological changes in the endometrium accompanying the early secretory phase are mediated by progesterone through interaction with the receptors. Furthermore, this high epithelial PgR level in the early secretory phase suggests that the ERs which are still present in the epithelial component during this phase of the cycle are functional. However, the high level of PgRs in the stroma in the early secretory phase did not correlate strictly with the stromal ER content which began to decrease immediately after ovulation (1,24). This suggests that PgR in the stroma may be in part constitutively synthesized and therefore need not reflect the stromal E_2 sensitivity. These findings are similar to our previous observations in murine mammary glands whereby in contrast to epithelial PgR the stromal PgR was found not to increase with E_2 stimulation (29).

The PgR level in the epithelium decreased during the mid secretory (24th - 25th day) phase and was totally absent in the late secretory phase (Table 1).

This correlates with the decrease in ER in the epithelial component

and also with the low levels of plasma and tissue estradiol during this period (26). However, in most of the cases the stroma contained relatively high PgR level (specimens 17,18,19,20,21 Table 1). This is in contrast to ERs which are very few in the stroma during the mid and late secretory phases (1,24). This suggests again that stromal PgR may be synthesized independently of ER and E_2 (29). Predecidual cells were particularly rich in PgR. This supports the role of progesterone in the decidualization process (15). To further our knowledge of decidual cells and their relationship to progesterone stimulation, their PgR content during different periods of gestation is currently being analyzed in our laboratory.

The basalis layer had high PgR levels during the proliferative, early and mid secretory phases in both the epithelium and the stroma. During the late secretory and menstrual phases, PgR was lost in the basalis layer, despite the presence of ERs as shown by us previously (1). The reappearance of PgR in the basalis following the menstrual period during the early proliferative phase seemed to be E_2-induced. This view is supported by the fact that ERs were present at this time in the basalis (1,24) and the endometrium began to be responsive to E_2 stimulation in cycle days 5 to 7 (7-9).

The fact that neither the endometrial nor the myometrial vessels contained ER (1,24) and PgR suggests that vasoconstriction believed to be responsible for menstrual breakdown of the endometrium is not directly induced by sex-steroids but rather mediated by vasoconstrictor proteins or lytic enzymes (9,20).

In summary, monoclonal antiprogesterone receptor antibody was used

to localize PgR in frozen sections of the human endometrium. PgRs were observed in the nuclei of epithelial and stromal cells in both the functionalis and basalis layers and varied with the menstrual cycle. The epithelial content was a good marker of the epithelial E_2 sensitivity while the stromal content in the secretory phase appeared in part to be constitutively synthesized. However, since the number of patients included in these studies were small with a wide age range and had also been selected on the basis of a variety of pathological symptoms, these results must be viewed as preliminary in nature.

TABLE 1. IMMUNOHISTOCHEMICAL SCORE OF PgR IN NORMAL CYCLIC ENDOMETRIUM

HISTOLOGIC DATING	SPECIMEN No.	AGE	EPITHELIUM (a)	EPITHELIUM (b)	EPITHELIUM (a+b)	STROMA (a)	STROMA (b)	STROMA (a+b)
Early	1	29	1	2	3			0
Proliferative	2	47	1	2	3	1	1	2
	3	47	1	1	2	1	1	2
	(Basalis)		1	3	4	1	1	2
Mid	4	49	2	2	4	2	1	3
Proliferative	(Basalis)		3	3	6	2	2	4
	5	40	2	2	4	2	1	3
	6	45	2	3	5	2	2	4
	(basalis)		4	3	7	3	2	5
	7	43	1	2	3	2	1	3
Late	8	44	3	3	6	2	1	3
Proliferative	(Basalis)		3	3	6			0
	9	40	3	3	6	1	3	4
	10	53	4	3	7	2	2	4
17th day	11	44	3	2	5	3	2	5
	12	30	4	3	7	2	3	5
	(Basalis)		4	3	7	3	3	6
	13	30	4	3	7	1	2	3
	14	38	2	3	5	1	2	3
	15	45	3	3	6	3	3	6
	16	40	3	2	5	1	2	3
24th day-25th day	17	44	1	1	2	3	2	5
	18	23			0	1	1	2
	19	32	2	2	4	2	3	5
	(Basalis)		2	3	5	2	3	5
	20	32	2	2	4	3	3	6
	(Basalis)		2	2	4	2	3	5
26th day	21	43	1	1	2	3	2	5
	22	40			0			0
	(Basalis)				0			0
27th day	23	42			0			0
	(Basalis)				0			0
Menstrual	24	30			0			0
	25	36			0			0
	26	43			0			0

a= % of positive cells, (1=0-25%, 2=26-50%, 3=51-75%, 4=76-100%)
b= staining intensity, 1 (weak) to 3 (very strong)

Figure 1. Immunoblotting analysis of cytosol from a proliferative endometrium using αPR6. Cytosol from proliferative endometrium was analysed by sodium dodecyl sulfate gels and blotted onto nitrocellulose as described in text. *Lanes* were cut off the nitrocellulose sheet, incubated with (*lane 1*) or without (*lane 2*) antibody and stained with Vectastain. The position of molecular weight standards is indicated in the figure.

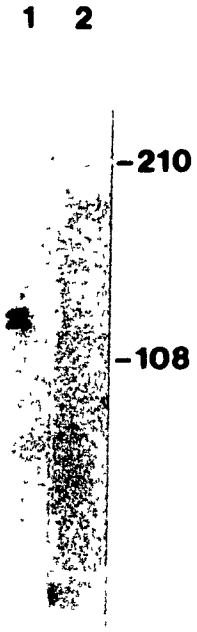
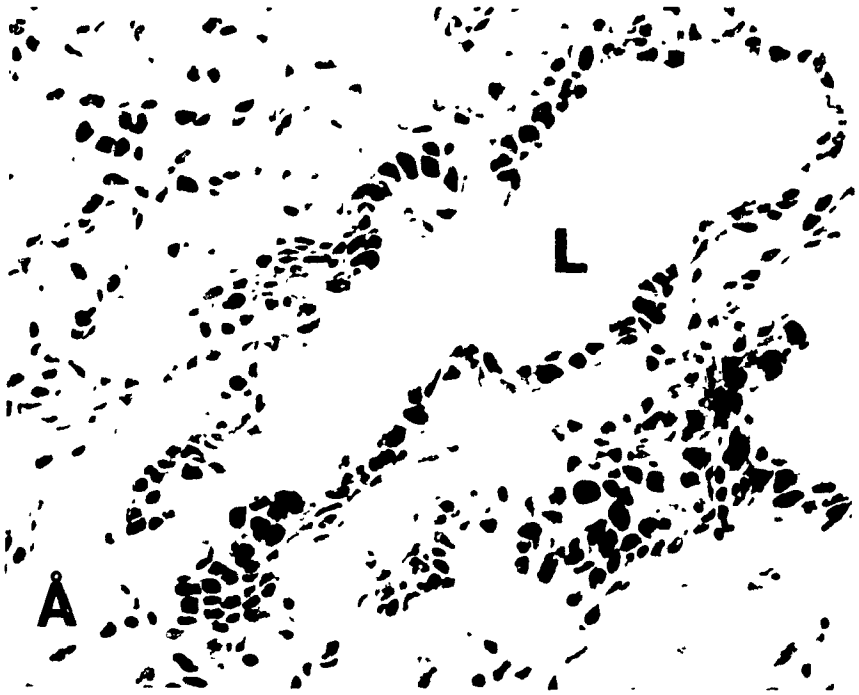

1 2
-210
-108

Figure 2. Immunocytochemical detection of PgR in human endometrium. In the two experiments, early secretory phase endometrium was used.
A) αPR6 was used as is without any prior treatment. The nuclei of epithelial cells and stromal fibroblasts are strongly stained. *L*: glandular lumen (x350, No counterstain).
B) αPR6 pre-incubated with highly purified receptor was used. None of the tissue components contain nuclear staining (x350, No counterstain).


L
A

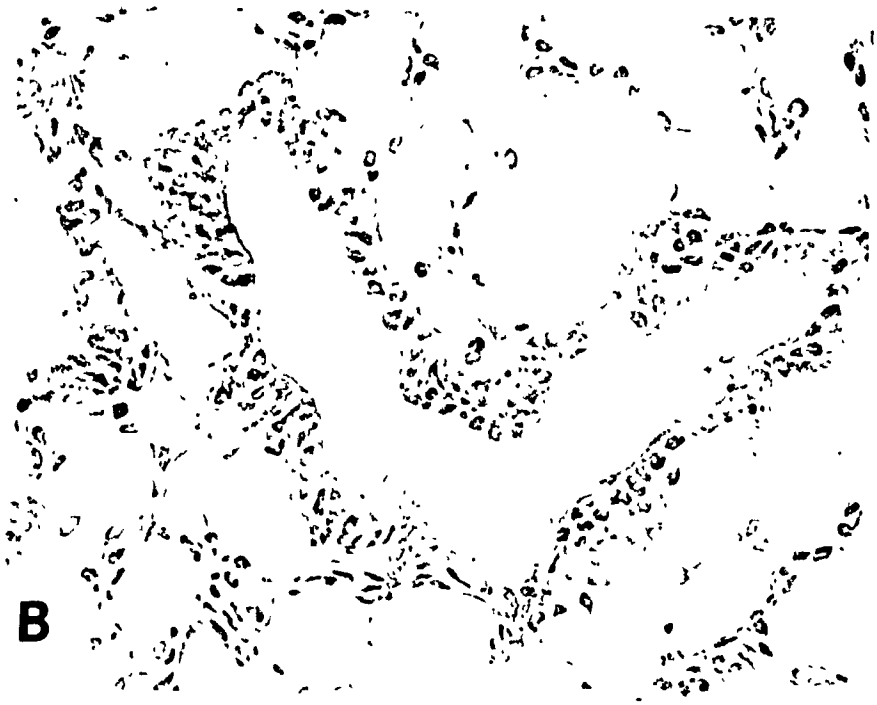

B

Figure 3. Immunocytochemical localization of PgR in late-proliferative phase endometrium. There is strong nuclear staining in most of the epithelial and stromal cells (*arrows*) (x450, No counterstain).

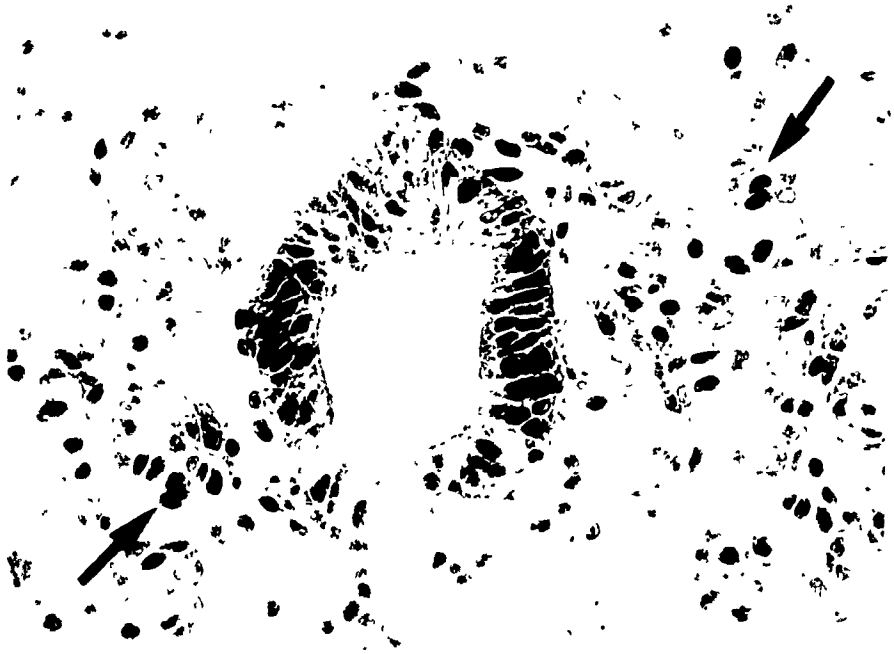


Figure 4. Immunocytochemical localization of PgR in the basalis of mid-proliferative phase endometrium. There is strong nuclear staining in both the epithelial cells and stromal fibroblasts. *M*: myometrium (x250, No counterstain).


M

Figure 5. Immunocytochemical localization of PgR in mid-secretory phase (post ovulatory day 11), endometrium. Nuclear staining is weak and scattered in epithelial cells, whereas most stromal cells display strong nuclear staining reaction (*arrows*) *L*: glandular lumen, *S*: stroma. (x250, No counterstain). Inset: Predecidual cells with cytoplasmic retraction (due to fixation artefact) contain strong nuclear staining (x250, No counterstain).

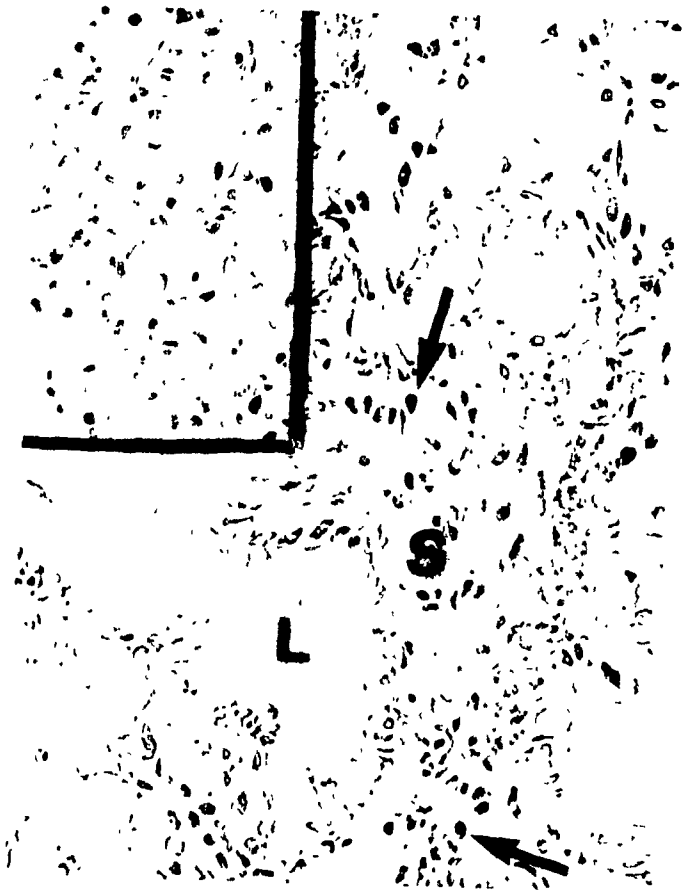

S
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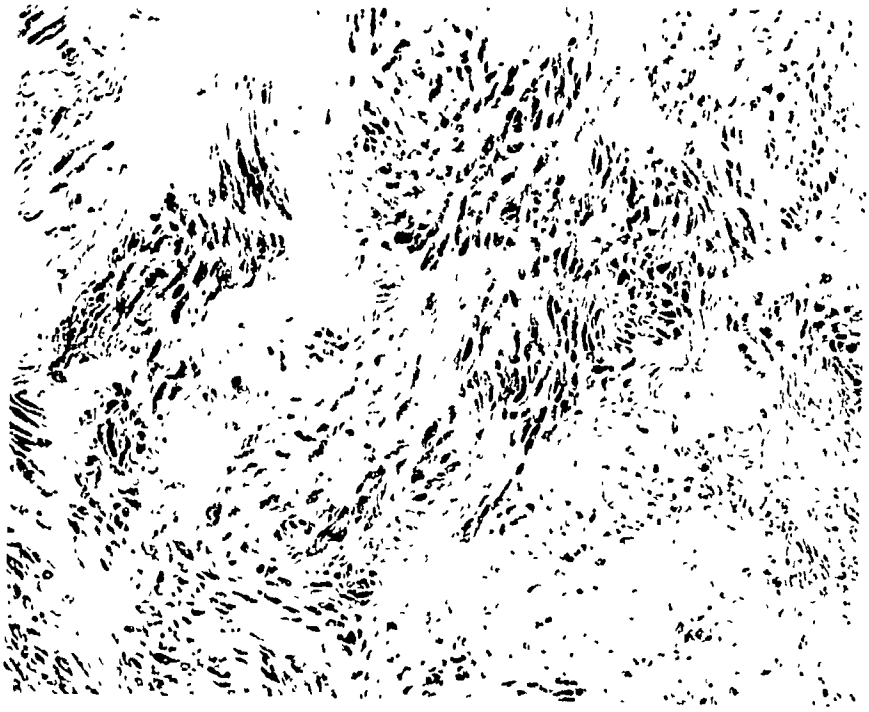
Figure 6. Immunocytochemical localization of PgR in myometrium. Smooth muscle cells have strong nuclear staining throughout the menstrual cycle (x250, No counterstain).



REFERENCES

1. Bergeron C, Ferenczy A, Shyamala G Distribution of estrogen receptors (ERs) in various cell types of normal, hyperplastic and neoplastic human endometrial tissues. Lab Invest 58:338, 1988.
2. Clark JH, Peck EJ Jr Female sex steroid receptors and function. Monog Endocrinol 14:4, 1979.
3. Clarke CL, Zaino RJ, Feil PD, Miller JV, Steck ME, Ohlsson-Wilhelm BM, Satyaswaroop PG Monoclonal antibodies to human progesterone receptor: Characterization by biochemical and immunohistochemical techniques. Endocrinology 121:1123, 1987.
4. Cunha GR, Chung LWK, Shannon JM, Taguchi O, Fujii N Hormone induced morphogenesis and growth. Role of mesenchymal-epithelial interactions. Recent Prog Hormone Res 39:559, 1983.
5. Ennis BW, Stumpf WE, Gasc JM, Baulieu EE Nuclear localization of progesterone receptor before and after exposure to progestin at low and high temperatures: autoradiographic and immunohistochemical studies of chick oviduct. Endocrinology 119:2066, 1986.
6. Estes PA, Suba EJ, Lawler-Heavner J, Elashry-Stowers D, Wei LL, Toft DO, Sullivan WP, Horwitz KB, Edwards DP Immunologic analysis of human breast cancer progesterone receptors. I. Immunoaffinity purification of transformed receptors and production of monoclonal antibodies. Biochemistry 26:6250, 1987.
7. Ferenczy A, Bertrand G, Gelfand MM Proliferation kinetics of human endometrium during the normal menstrual cycle. Am J Obstet Gynecol 133:859, 1979.

8. Ferenczy A, Bertrand G, Gelfand MM Studies on the cytodynamics of human endometrial regeneration. III In vitro short-term incubation historadioautography. Am J Obstet Gynecol 134:297, 1979.

9. Ferenczy A Regeneration of the human endometrium. In: Progress in Surgical Pathology, edited by Fenoglio CM and Wolff M, p 157, New York, Masson Publishing USA, 1980.

10. Gasc JM, Renoir JM, Radanyi C, Joab I, Tuohimaa P, Baulieu EE Progesterone receptor in the chick oviduct: an immunohistochemical study with antibodies to distinct receptor components. J Cell Biol 99:1193, 1984.

11. Hawkes R, Niday E, Gordon J A dot-immunobinding assay for monoclonal and other antibodies. Anal Biochem 119:142, 1982.

12. Isola J, Korte J-M, Tuohimaa P Immunocytochemical localization of progesterone receptor in the chick ovary. Endocrinology 121:1034, 1987.

13. Isola J, Pelto-Huikko M, Tuohimaa P Immunoelectron microscopic localization of progesterone receptor in chick oviduct. J Steroid Biochem 26:19, 1987.

14. Janne O, Kontula K, Luukkainen T, Vihko R Estrogen induced progesterone receptor in human uterus. J Steroid Biochem 6:501, 1975.

15. Kearns M, Lala PK Life history of decidual cells: A review. Am J Reprod Immunol 3:78, 1983.

16. Laemmli UK Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680, 1970.

17. Leavitt WW, Chen TJ, Allen TC Regulation of progesterone receptor

formation by estrogen action. Ann N Y Acad Sci 286:210, 1977.

18. Milgrom E, Thi L, Atger M, Baulieu EE Mechanisms regulating the concentration of progesterone receptors in the uterus. J Biol Chem 248:6366, 1973.

19. Noyes RW, Hertig AT, Rock J Dating the endometrial biopsy. Fertil Steril 1:3, 1950.

20. Orcel L, Smadja A, Roland J, Minh HN Nouvelle hypothèse sur le mécanisme intime de la menstruation. Rev franç Gynécol 68:477, 1973.

21. Perrot-Applanat M, Logeat F, Groyer-Ricard MT, Milgrom E Immunocytochemical study of mammalian progesterone receptor using monoclonal antibodies. Endocrinology 116:1473, 1985.

22. Perrot-Applanat M, Groyer-Ricard MT, Lorenzo F, Jolivet A, Thu Vu Hai M, Pallud C, Spyratos F, Milgrom E Immunocytochemical study with monoclonal antibodies to progesterone receptor in human breast tumors. Cancer Res 47:2652, 1987.

23. Perrot-Applanat M, Groyer-Picard MT, Logeat F, Milgrom E Ultrastructural localization of the progesterone receptor by an immunogold method: effect of hormone adminsitration. J Cell Biol 102:1191, 1986.

24. Press MF, Nousek-Goebl N, King WJ, Herbst AL, Greene GL Immunohistochemical assessment of estrogen receptor distribution in the human endometrium throughout the menstrual cycle. Lab Invest 51:495, 1984.

25. Ross GT, VandeWicke RL, Frantz AG The ovaries and the breasts. In: Textbook of Endocrinology, edited by Williams RH, p 374, Saunders

WB Company, 1981.

26. Schmidt-Gollwitzer M, Genz T, Schmidt-Gollwitzer K, Pollow B, Pollow K Correlation between oestradiol and progesterone receptor levels, 17β-hydroxysteroid dehydrogenase activity and endometrial tissue levels of oestradiol, oestrone, and progesterone in women. In: Endometrial Cancer, edited by Brush MG, King RJB, Taylor RW, p 227, London: Bailliere Tindall, 1978.

27. Schneider C, Newman RA, Sutherland DR, Asser U, Greaves MF A one-step purification of membrane proteins using a high efficiency immunomatrix. J Biol Chem 257:10766, 1982.

28. Schneider W, Toft DO, Sullivan WP, Shyamala G Interaction of murine progesterone receptors with specific monoclonal antibodies to the avian progesterone receptor. J Steroid Biochem 29:297-306, 1988.

29. Shyamala G, Ferenczy A Mammary fat pad may be a potential site for initiation of estrogen action in normal mouse mammary glands. Endocrinology 115:1078, 1984.

30. Stefanini M, DeMontino C, Zambori C Fixation of ejaculated spermatozoa for electron microscopy. Nature 216:173, 1967.

31. Sullivan WP, Beito TG, Proper J, Krco CJ, Toft DO Preparation of monoclonal antibodies to the avian progesterone receptor. Endocrinology 119:1549, 1986.

32. Wei LL, Horwitz KB The structure of progesterone receptors. Steroids 46:677, 1986.

33. Wei LL, Sheridan PL, Krett NL, Francis MD, Toft DO, Edwards DP, Horwitz KB Immunologic analysis of human breast cancer

progesterone receptors. 2. Structure, phosphorylation, and processing. Biochemistry 26:6262, 1987.

34. Welshons WV, Krummel BM, Gorski J Nuclear localization of unoccupied receptors for glucocorticoids, estrogens and progesterone in GH_3 cells. Endocrinology 117:2140, 1985.

Chapter IV

IMMUNOCYTOCHEMICAL STUDY OF PROGESTERONE RECEPTORS IN HYPERPLASTIC AND NEOPLASTIC ENDOMETRIAL TISSUES

IMMUNOCYTOCHEMICAL STUDY OF PROGESTERONE RECEPTORS IN HYPERPLASTIC AND NEOPLASTIC ENDOMETRIAL TISSUES

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and GOPALAN SHYAMALA

INTRODUCTION

Endometrial carcinoma is the most frequent malignancy of the female genital tract (1). However, the mechanisms underlying its pathogenesis and, in particular, its relationship with hyperplasia remains obscure. Since endometrial hyperplasias and well-differentiated carcinomas are frequently found in women with a high plasma estrogen (E_2) level and an absence of biologically effective progesterone (P), these lesions may be the result of unopposed E_2 action (2,3). The precise role of E_2 in endometrial carcinogenesis, however, is not clear (4).

The presence of estrogen receptors (ER) and progesterone receptors (PgR) are a prerequisite for the expression of E_2 and P action, respectively (5). PgR synthesis is mainly regulated by E_2 and therefore its presence also reflects the E_2-sensitivity of a target tissue (6-8). Using hormone binding assays, PgRs were found to be elevated in hyperplasias and well-differentiated carcinomas (9-13). These methods, however, used homogenates of tissue and did not take into account the cellular heterogeneity in receptor content, or the presence of receptors in the adjacent benign epithelium or in the supportive stroma of the lesional epithelium (14,15). Therefore, as a step towards understanding E_2 action in hyperplastic and neoplastic human endometria, we have examined the PgR content and its relative distribution between the epithelium and the stroma in these tissues by an immunocytochemical method, using a specific mouse monoclonal antibody directed against avian PgR (αPR6) (16).

MATERIALS AND METHODS

Tissue Samples. Thirteen hyperplastic and twenty three neoplastic tissues (including five endometrial intraepithelial neoplasia) (EIN) were obtained from 33 women ranging in age from 40 to 68 and 52 to 87 years old respectively. With the exception of three patients with endometrial hyperplasia and intraepithelial neoplasia, only one type of tissue was derived from each patient. Three hyperplastic tissue samples were obtained from biopsy performed as a diagnostic procedure of dysfunctional uterine bleeding. Ten hyperplastic and twenty neoplastic tissue samples were obtained from hysterectomy specimens. Three of the 10 hyperplastic cases contained a mixture of endometrial hyperplasia and intraepithelial neoplasia.

Hysterectomy specimens, obtained immediately after their removal in the operating room, were opened along the lateral border in order to obtain undistorted endometrial surfaces. Tissue sections were taken longitudinally from the endometrial mucosa or the grossly abnormal proliferations. All the tissue samples were divided in two pieces. One for histology was fixed in 10% buffered formalin (hysterectomy specimens) or in Bouin's solution (biopsy specimens) and processed and stained in a routine fashion. The other one was snap frozen in liquid nitrogen and kept at -70°C until it was processed for immunohistochemistry.

Hyperplastic endometria contained architectural alterations of endometrial glands ranging from minimal (or simple) to complex (adenomatous). These included lesions traditionally named anovulatory, persistent proliferative endometrium, cystic glandular hyperplasia,

simple hyperplasia and adenomatous hyperplasia (2). By definition, the glandular lining epithelium was devoid of cytologic atypia. Hyperplastic lesions in which the lining epithelium displayed significant nuclear atypia including nuclear rounding, pleomorphism, loss of nuclear organization and macronucleoli were classified as endometrial intraepithelial neoplasia (EIN). Most of these lesions contained glands with architectural alterations with complex intra- and extraluminal epithelial buddings. Traditionally these lesions were referred to as atypical adenomatous hyperplasia, adenomatous hyperplasia with cytologic atypia, carcinoma *in situ* and dysplasia (2). Endometrial carcinoma with invasion either of the endometrial stroma and/or the myometrium were graded according to the Gynecologic Oncology Group grading system. They were subdivided into grade 1 (well-differentiated with 0-5% of undifferentiated cells), grade 2 (moderately-differentiated with 5-50% of undifferentiated cells), and grade 3 (poorly-differentiated with more than 50% of undifferentiated cells) carcinoma.

Antibodies. Details about avian PgR purification, immunization of mice, cell fusion, hybridoma cloning and screening procedures have been described elsewhere (16). αPR6 was selected for these studies due to its known cross-reactivity with human progesterone receptor (16-19). The normal horse serum, biotinylated immunoglobin against the mouse and the avidin-biotin peroxidase complex were obtained from Vector Laboratories, Burlingame, Ca. The non-immune mouse immunoglobulin was obtained from Dako Laboratories.

Immunohistochemical technique. Frozen tissue blocks were cut with

a cryostat at 4 μm and thaw-mounted on gelatin-coated glass slides. The initial section was stained with hematoxylin-eosin for tissue diagnosis. Assignment of histologic diagnosis, particularly tumor type and grade, was confirmed by review of formalin- or Bouin-fixed paraffin-embedded, hematoxylin-eosin stained sections taken from the same specimens. Sections were then fixed immediately without drying in picric acid paraformaldehyde (20) for 15 min at -10 to -20°C. Indeed, preliminary experiments in normal human cyclic endometria (19) and human breast tumors (21) showed that immunoreactivity of PgR was well preserved in this condition of fixation. Slides were transferred to PBS for 30 min at 4°C. Sections were treated with a 0.5% solution of hydrogen peroxide in PBS for 10 min, put in PBS for 10 min and incubated for 10 min in normal horse serum to reduce the non-specific binding of primary antibody. The sections were incubated with monoclonal mouse anti-PgR antibody (αPR6) for 1 hr in a humid chamber, biotinylated antimouse IgG for 20 min at room temperature and avidin-biotin peroxidase complexes for 30 min at room temperature. Each incubation was followed by 10 min washings in PBS. Sections were incubated for 10 min with the DAB solutions (PBS containing 0.5 mg DAB/ml and 0.01% H_2O_2) at room temperature. Sections were then dehydrated and mounted for examination by light microscopy without counterstaining.

A limiting dilution for the αPR6 was tested from 1 to 30 μg IgG/ml. The immunostaining was optimal at 20 μg IgG/ml and was therefore chosen for these experiments was 20 μg IgG/ml. Dilutions for the other antibodies and normal serum were performed according to instructions

specified by Vector Laboratories.

Negative controls consisted of adjacent sections treated with non-immune mouse immunoglobulins at the same dilution as αPR6 or with antibody (αPR6) presaturated with purified PgR. The PgR used for presaturation studies was purified from oviduct cytosol by affinity chromatography using antibody αPR22 (16) covalently linked to protein A-Sepharose (22). For presaturation antibody and antigen were mixed (10 μg receptor/μg IgG) and incubated 2 hr at room temperature. Mixtures were then used on sections instead of antibody (αPR6) alone. Additional controls included the omission of the primary antibody.

Positive staining with monoclonal PgR antibody was defined as golden brown granular staining and was absent in the control adjacent section. For

evaluation of PgR content a score corresponding to the sum of both a) the percentage of positive cells (1=0-25%, 2=26-50%, 3=51-75%, 4=76-100%), and b) the staining intensity (1 to 3) was established. The intensity of specific staining was characterized as absent (0), weak but detectable above control (1), strong (2) and very strong (3). Evaluations were recorded for each observed tissue component , i.e. epithelium, stroma and if available myometrium. The entire tissue section was scanned. Three randomly chosen low power microscopic fields (X40) in each tissue section were chosen and the intensity and the percentage of positive cells of each cellular component were visually evaluated at a magnification of 400X. The mean value for the assayed field was expressed as the percentage of positive cells and the intensity values for the tissue. The maximum score was 7, but a

score greater or equal to 2 was the value of a "positive" immunohistochemical assay.

Statistical analysis was made by means of students' modified T tests.

RESULTS

Immunohistochemical analysis of PgR in hyperplastic and intraepithelial neoplastic tissues. The immunolocalization of PgR in the nuclei of hyperplastic endometria is shown in Figure 1. In the epithelial cell component, the staining was strong with 75% of the cells being positive (Fig. 1A), which was abolished with the use of antibody preincubated with purified receptor (Fig. 1B). Overall the percentage of positive cells varied from 25% to more than 75% with the mean score for 13 hyperplastic endometria being 5.46 ± 1.26; $\bar{x}$ ± SD (Table 1). The stromal cell nuclei were also positive (Fig. 1) in all the cases but overall had a lower score (4.23 ± 1.36; $\bar{x}$ ± SD) than the epithelial component. Indeed, except in 3 cases (specimens 4,6,8) less than 50% of stromal cells had a positive staining (Table 1).

In contrast to hyperplastic endometria, the PgR content in the epithelial component of endometrial intraepithelial neoplasia (EIN) was low (Fig. 2), the mean score for the five cases of EIN in the epithelial component was 2.2 ± 0.44; $\bar{x}$ ± SD (Table 2). However, the PgR content in the stromal cell nuclei remained relatively high (Fig. 2); the mean score was 3.8 ± 2.04; $\bar{x}$ ± SD. In some cases (specimens 1 and 3) more than 75% of the stromal cells contained PgR (Table 2).

Immunohistochemical analysis of PgR content in invasive carcinoma. Histological patterns of the 18 endometrial carcinomas included 11 endometrial carcinomas, four adenocarcinomas with benign squamous differentiation (adenoacanthoma), two adenocarcinomas with clear cell features and one adeno-squamous carcinoma. There was no difference in the PgR distribution among these histological variants, and the

squamous component of tumors failed to stain. The immunolocalization of PgR in well differentiated and poorly differentiated carcinomas is shown in Fig. 3A and 3B, respectively. As with normal and hyperplastic endometria, the staining was observed also in the nuclei. The average score for grade 1 carcinomas was 3 ± 2.75 ($\bar{x}$ ± SD) in the epithelial component and 4 ± 1.14 ($\bar{x}$ ± SD) in the stromal component (Table 3). Two cases failed to stain in the epithelial component even though histologically they did not differ from the usual endometrial carcinomas; these occurred in a 74 and an 81 year old patient. All grade 2 carcinomas contained PgR both in their epithelial and stromal components. The average score for the epithelial (3.57 ± 0.78; $\bar{x}$ ± SD) or the stromal component (4.85 ± 0.69; $\bar{x}$ SD) was not significantly different from that found in grade 1 carcinomas (Table 3). The average PgR score of the epithelial component in grade 3 carcinomas (0.4 ± 0.89; $\bar{x}$ ± SD) was significantly lower ($P<0.001$) than in their grade 1 and 2 counterparts. Only one of 5 cases contained PgR in the epithelial component. The stromal PgR content remained high (3 6 ± 2.70; $\bar{x}$ ± SD) and was not significantly different from that found in grade 1 or 2 carcinomas (Table 3). This lack of correlation between the score of the malignant epithelial component and the score of the stromal component is shown in Fig. 4 ($r = 0.32$, $p>0.1$).

The myometrium was available in a limited number of cases. The PgR content in normal smooth muscle cell nuclei was generally high irrespective of the tumor grade (Data not shown).

DISCUSSION

Immunocytochemical localization of PgR in hyperplastic and neoplastic human endometria was found only in the nuclei but not in the cytoplasm of epithelial and stromal cells. Similar nuclear localization of PgR has also been observed in other immunohistochemical studies done on normal cyclic human endometria (19,23), and other target tissues for progesterone (21,24-27). This nuclear localization of human PgR is similar to that of ER, in the normal cyclic endometrium (28,29) and hyperplastic and neoplastic endometria (29,30).

The PgR content was highest in the epithelium of hyperplasia without cytologic atypia. We have previously demonstrated that these tissues contain high levels of ER in the epithelium (29). Thus the combined analyses of ER and PgR suggest the presence of functional ER in these tissues and support previous suggestions that the epithelium of hyperplasia may be sensitive to E_2. In contrast, the PgR level was low in the epithelium of endometrial intraepithelial neoplasia (hyperplasia with cytologic atypia) and is consistent with the low epithelial ER levels found in these tissues (29). Previous studies using electron microscopy (31), morphometry (32,33) and *in vitro* DNA histoautoradiography (34) have suggested that hyperplasia and EIN are made of different types of epithelial cells. This suggestion is further supported by the data obtained in our study in the three cases where hyperplasia and EIN were localized in the same endometrium (case 1 hyperplasia specimen 5 Table 1 and case 1 EIN specimen 2, Table 2; case 2 hyperplasia specimen 11 Table 1 and case 2 EIN specimen 4 Table

2; case 3 hyperplasia specimen 13 Table 1 and case 3 EIN specimen 5 Table 2); in these specimens the epithelial content of PgR was characteristic of the respective lesions and hence different within the same endometrium. Overall, these data suggest that the epithelium of endometrial hyperplasia may be relatively more sensitive to progesterone as compared to the epithelium of EIN. This suggestion is supported by recent clinical data showing a better response to exogenous progestagen therapy by hyperplasia as compared to EIN (35).

The carcinomas showed a considerable heterogeneity in the PgR content of both the epithelium and the stroma. Similar heterogeneity has been reported by immunohistochemical methods for ER in endometrial carcinoma (29,30) and in breast carcinoma for PgR (21). Overall, the epithelial content of PgR appeared to be correlated with the degree of tumor differentiation as also found with previous biochemical assays for PgR (9-13). In contrast, there was a lack of correlation between the malignant epithelial content and the stromal content of PgR, irrespective of the tumor grade (Table 3, Fig. 4). Progesterone therapy has been used in advanced and recurrent endometrial carcinoma and it has been recently shown that only 60% of cases positive for PgR by biochemical assays will respond to treatment (36). Immunohistochemical analyses of PgR may therefore provide a good complement to quantitative biochemical analyses in the evaluation of endometrial adenocarcinomas and help to select patients better able to respond to exogenous progestin therapy.

The high PgR level in the stroma in the two types of hyperplastic lesions and in neoplastic lesions is consistent with the high ER

levels found in the stroma of these tissues (29). It remains to be seen whether these stromal PgR are the reflection of functional ER or are consitutively synthesized in these tissues.

TABLE 1. IMMUNOHISTOCHEMICAL SCORE OF PgR IN ENDOMETRIAL HYPER"LASIA

SPECIMEN No.	EPITHELIUM (a)	(b)	(a+b)	STROMA (a)	(b)	(a+b)
1	4	3	7	2	2	4
2	3	3	6	2	1	3
3	4	3	7	2	3	5
4	3	2	5	3	3	6
5	2	2	4	2	2	4
6	4	3	7	3	3	6
7	2	2	4	2	2	5
8	2	3	5	3	3	6
9	4	3	7	1	1	2
10	2	3	5	2	2	4
11	2	2	4	1	1	2
12	3	3	6	2	2	4
13	2	2	4	2	2	4

a= % of positive cells, (1=0-25%, 2=26-50%, 3=51-75%, 4=76-100%)
b= staining intensity, 1 (weak) to 3 (very strong)

TABLE 2. IMMUNOHISTOCHEMICAL SCORE OF PgR IN ENDOMETRIAL INTRAEPITHELIAL NEOPLASIA

SPECIMEN No.	EPITHELIUM (a)	(b)	(a+b)	STROMA (a)	(b)	(a+b)
1	1	1	2	3	3	6
2	1	2	3	1	2	3
3	1	1	2	3	3	6
4	1	1	2	1	1	2
5	1	1	2	1	1	2

a= % of positive cells, (1=0-25%, 2=26-50%, 3=51-75%, 4=76-100%)
b= staining intensity, 1 (weak) to 3 (very strong)

TABLE 3. IMMUNOHISTOCHEMICAL SCORE OF PgR IN ENDOMETRIAL CARCINOMA BY HISTOLOGIC GRADE

HISTOLOGIC GRADE	SPECIMEN No.	EPITHELIUM (a)	(b)	(a+b)	STROMA (a)	(b)	(a+b)	MYOMETRIUM (a)	(b)	(a+b)
1 - well differentiated	1	2	1	3	2	2	4			N/A
	2			0	1	1	2	2	2	4
	3			0	2	1	3	2	2	4
	4	2	3	5	2	3	5	3	3	6
	5	2	1	3	2	1	3			N/A
	6	3	4	7	2	1	3	2	2	4
2 - moderately differentiated	7	2	1	3	2	2	4	3	3	6
	8	1	1	2	3	2	5	3	3	6
	9	3	1	4	2	2	5			N/A
	10	2	2	4	2	2	4			N/A
	11	1	3	4	3	3	5			N/A
	12	2	2	4	2	3	5	3	3	6
	13	3	2	4	3	3	6	3	3	6
3 - poorly differentiated	14	1	1	2	2	3	5			N/A
	15			0	4	3	7			N/A
	16			0	1	1	2	1	1	2
	17			0			0			0
	18			0	2	2	4	3	3	6

a= % of positive cells, (1-0-25%, 2-26-50%, 3-51-75%, 4-76-100%)
b= staining intensity, 1 (weak) to 3 (very strong)
N/A= not available

Figure 1. Immunochemical localization of PgR in endometrial hyperplasia.
A) αPR6 was used as is, without any prior treatment. Strong nuclear staining is observed in the epithelial cell lining of a complex voluminous gland. Some stromal cells nuclei (*arrow*) stain strongly as well (x250; No counterstain)
B) αPR6 preincubated with purified receptor was used. None of the tissue components contain nuclear staining (x200; No counterstain).


A

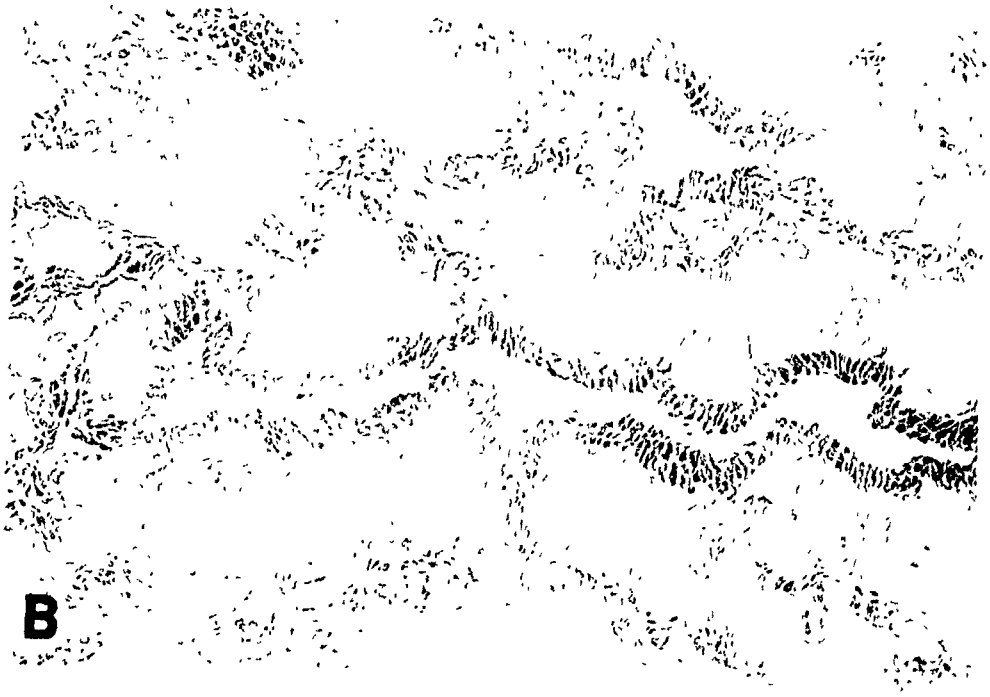

B

Figure 2. Immunohistochemical localization of PgR in endometrial intraepithelial neoplasia. The gland cells growing in a cribriform pattern are devoid of receptors. In contrast the stromal cells contain strong nuclear staining reaction L= glandular lumen, S = stroma (x250; no counterstain)

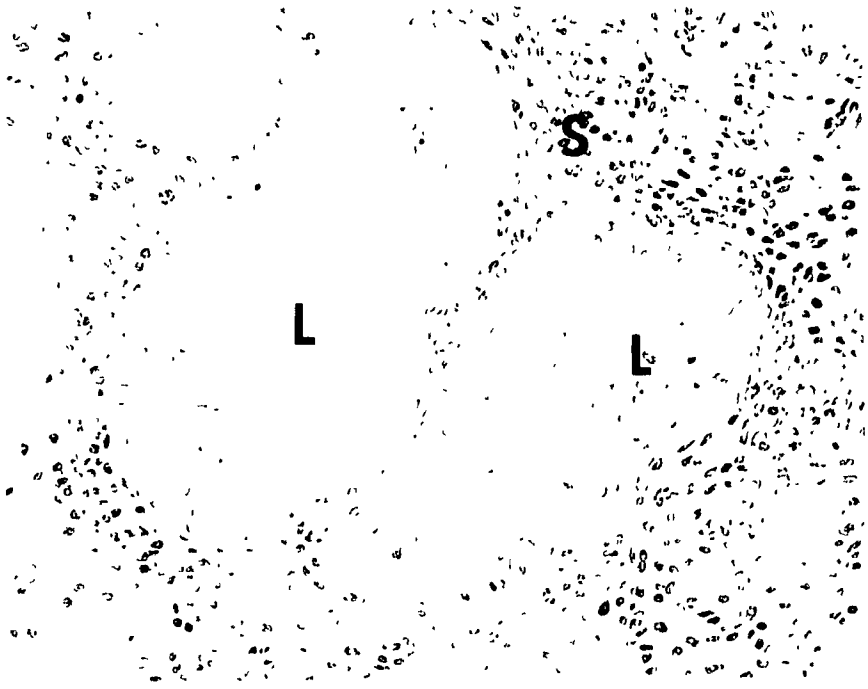

S
L
L

Figure 3 Immunohistochemical localization of PgR in endometrial carcinoma.

A) Well-differentiated adenocarcinoma cells demonstrate intense nuclear staining (x250, no counterstain)

B) Immunostaining is confined exclusively to the nuclei of stromal cells (*arrow*) surrounding poorly-differentiated epithelial proliferations (x450; no counterstain)


A

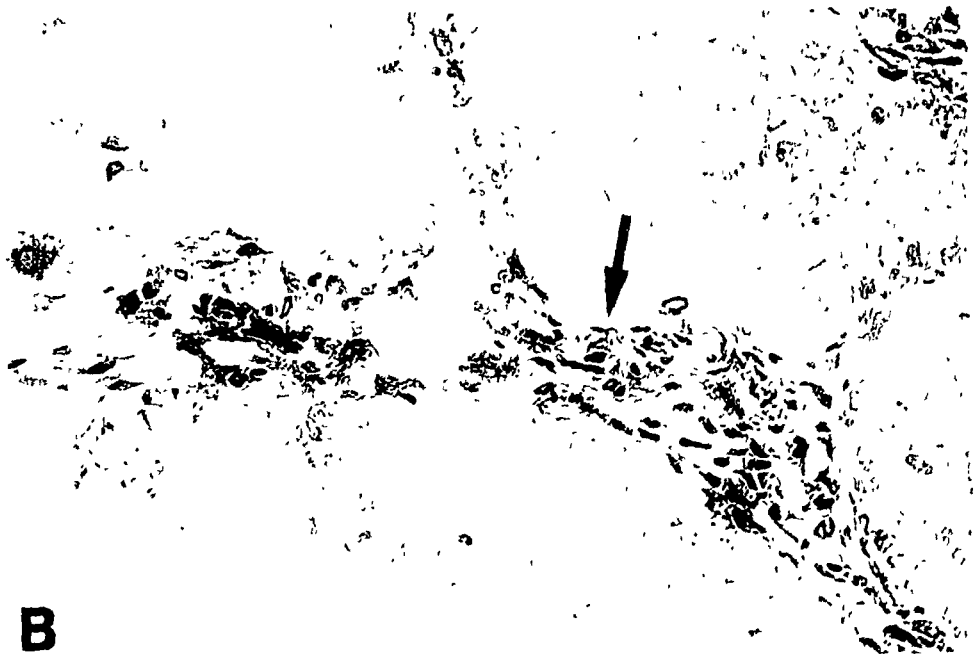
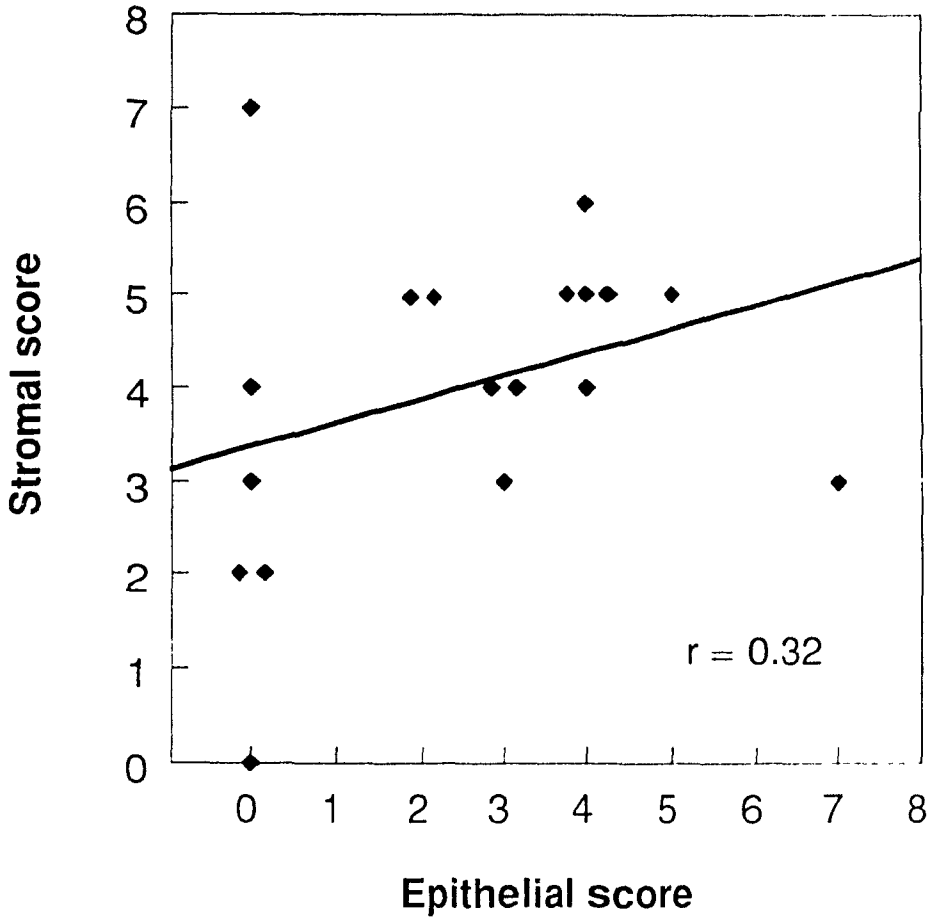

B

Figure 4. There is a lack of correlation of immunochemical scores between the malignant epithelial and the stromal component (n= 18, r = 0.32, y = 3 379 + 0 248 x).


Stromal score
Epithelial score
r = 0.32
0
1
2
3
4
5
6
7
8

REFERENCES

1. Silverberg, E., and Lubera, J. Cancer statistics. Ca. J. Clin., *37:* 2-19, 1987.

2. Ferenczy, A., Gelfand, M.M., and Tzipris, A. The cytodynamics of endometrial hyperplasia and carcinoma. A review. Ann. Pathol., *3:* 189-202, 1983.

3. Siiteri, P.K. Extraglandular oestrogen fraction and serum binding of oestradiol: relationship to cancer. J. Endocrinol., *89:* 119-129, 1981

4. Lucas, W.E. Estrogen - A cause of gynecologic cancer? Cancer, *48:* 451-454, 1981.

5. Clark, J.H., and Peck, E.J., Jr. Female sex steroid receptors and function. Monog. Endocrinol., *14:* 4, 1979.

6. Leavitt, W.W., Chen, T.J., and Allen, T.C. Regulation of progesterone receptor formation by estrogen action. Ann. N.Y. Acad Sci., *286:* 210-225, 1977.

7. Milgrom, E., Thi, L., Atger, M., and Baulieu, E.-E. Mechanisms regulating the concentration of progesterone receptors in the uterus J Biol Chem., *248:* 6366-6374, 1973.

8. Janne, O , Kontula, K., Luukkainen, T., and Vihko, R. Estrogen induced progesterone receptor in human uterus. J. Steroid Biochem , *6:* 501-509, 1975.

9. Janne, O., Kauppila, A., Kontula, K., Syrjala, P., and Vihko, R. Female sex steroid receptors in normal, hyperplastic and carcinomatous endometrium. The relationship to serum steroid hormones and gonadotropins and changes during medroxyprogesterone

acetate administration. Int. J Cancer, *24:* 545-554, 1979.

10. McCarthy, K.S. Jr., Barton, T K., Fetter, B.F., Creasman, W T., and McCarthy, K.S Sr. Correlation of estrogen and progesterone receptors with histologic differentiation in endometrial adenocarcinoma. Am. J. Pathol., *96:* 171-184, 1979

11. Ehrlich, C.E., Young, P.C.M., and Cleary, R.E. Cytoplasmic progesterone and estradiol receptors in normal, hyperplastic and carcinomatous endometria: Therapeutic implications. Am. J Obstet Gynecol., *141:* 539-546, 1981.

12. Shyamala, G., and Ferenczy, A. The effect of sodium molybdate on the cytoplasmic estrogen and progesterone receptors in human endometrial tissues. Diagn. Gynecol. Obstet , *3:* 277-282, 1981.

13. Geisinger, K.R., Marshall, R.B , Kute, T E., and Homesley, H.D Correlation of female sex steroid hormone receptors with histologic and ultrastructural differentiation in adenocarcinoma of the endometrium. Cancer, *58:* 1506-1517, 1986

14. Cunha, G.R., Chung, L W.K , Shannon, J.M., Taguchi, O , and Fujii, N. Hormone induced morphogenesis and growth Role of mesenchymal-epithelial interactions. Recent Prog. Hormone Res , *39:* 559-598, 1983.

15. Mortel, R , Zaino, R., and Satyaswaroop, P G. Heterogeneity and progesterone receptor distribution in endometrial adenocarcinoma. Cancer *53:* 113-116, 1984.

16. Sullivan, W.P., Beito, R.G , Proper, J., Krco, C J., and Toft, D.O. Preparation of monoclonal antibodies to the avian progesterone receptor. Endocrinology, *119* 1549-1557, 1986.

17. Estes, P.A., Suba, E.J., Lawler-Heavner, J., Elashry-Stowers, D., Wei, L.L., Toft, D.O , Sullivan, W.P., Horwitz, K.B , and Edwards, D P. Immunologic analysis of human breast cancer progesterone receptors. I. Immunoaffinity purification of transformed receptors and production of monoclonal antibodies. Biochemistry, *26:* 6250-6262, 1987.

18. Wei, L L., Sheridan, P.L., Krett, N.L., Francis, M.D., Toft, D.O., Edwards, D.P., and Horwitz, K.B. Immunologic analysis of human breast cancer progesterone receptors. 2. Structure, phosphorylation, and processing. Biochemistry, *26:* 6262-6272, 1987.

19. Bergeron, C., Ferenczy, A., Toft, D.O., Schneider, W., and Shyamala, G. Immunocytochemical study of progesterone receptors in the human endometrium during the menstrual cycle. Lab. Invest., *59*. 862-869, 1988.

20. Stefanini, M., DeMartino, C., and Zamboni, L. Fixation of ejaculated spermatozoa for electron microscopy. Nature *216:* 173-174, 1967.

21 Perrot-Applanat, M., Groyer-Ricard, M.T., Lorenzo, F., Jolivet, A., Thu Vu Hai, M , Pallud, C., Spyratos, F , and Milgrom, E. Immunocytochemical study with monoclonal antibodies to progesterone receptor in human breast tumors. Cancer Res., *47:* 2652-2661, 1987.

22. Schneider, C, Newman, R.A., Sutherland, D.R., Asser, U., and Greaves, M F. A one-step purification of membrane proteins using a high efficiency immunomatrix. J. Biol. Chem., *257:* 10766-10769,

1982.

23. Clarke, C.L., Zaino, R.J., Feil, P.D., Miller, J.V., Steck, M.E., Ohlsson-Wilhelm, B.M., and Satyaswaroop, P.G. Monoclonal antibodies to human progesterone receptor: Characterization by biochemical and immunohistochemical techniques. Endocrinology *121:* 1123-1132, 1987.

24. Perrot-Applanat, M., Logeat, F., Groyer-Ricard, M.T., and Milgrom, E. Immunocytochemical study of mammalian progesterone receptor using monoclonal antibodies. Endocrinology, *116:* 1473-1484, 1985.

25. Ennis, B.W., Stumpf, W.E., Gasc, J.M., and Baulieu, E.-E. Nuclear localization of progesterone receptor before and after exposure to progestin at low and high temperatures: autoradiographic and immunohistochemical studies of chick oviduct Endocrinology, *119.* 2066-2075, 1986.

26. Isola, J., Korte, J.-M., and Tuohimaa, P Immunocytochemical localization of progesterone receptor in the chick ovary Endocrinology, *121:* 1034-1040, 1987.

27. Isola, J., Pelto-Huikko, M., and Tuohimaa, P. Immunoelectron microscopic localization of progesterone receptor in chick oviduct. J. Steroid Biochem., *26·* 19-23, 1987.

28. Press, M.F., Nousek-Goebl, N , King, W.J., Herbst, A.L., and Greene, G.L. Immunohistochemical assessment of estrogen receptor distribution in the human endometrium throughout the menstrual cycle. Lab Invest , *51·* 495-503, 1984

29. Bergeron, C., Ferenczy, A., and Shyamala, G. Distribution of estrogen receptors (ERs) in various cell types of normal,

hyperplastic and neoplastic human endometrial tissues. Lab. Invest. *58*: 338-345, 1988.

30 Budwit-Movotomy, D.A., McCarthy, K.S., Cose, E.B., Soper, J.T., Mitch, D G., Creasman, W.T., Flowers, J.L., and McCarthy, K. Immunohistochemical analyses of estrogen receptor in endometrial adenocarcinoma using a monoclonal antibody. Cancer Res., *46:* 5419-5425, 1986.

31. Ferenczy, A. Cytodynamics of endometrial hyperplasia and neoplasia. I. Histology and ultrastructure. In: Progress in Surgical Pathology, Fenoglio CM, Wolff M (eds) p 95, New York. Masson Publishing, 1982.

32. Aausems, E.W.M.A., Vanderkamp, J.K., and Baak, J.P.A. Nuclear morphometry in the determination of the prognosis of marked atypical endometrial hyperplasia. Int. J. Gynaecol. Pathol. 4: 180-185, 1985.

33. Colgan, T.J , Norris, H.J., Foster, W., Kurman, R.J., and Fox, C H Predicting the outcome of endometrial hyperplasia by quantitative analysis of nuclear features using a linear discriminant function. Int. J. Gynaecol. Pathol. *1*: 347-352, 1983.

34 Ferenczy, A. Cytodynamics of endometrial hyperplasia and neoplasia. Part II: *In vitro* DNA histoautoradiography. Hum. Pathol. *14*: 77-83, 1983.

35 Ferenczy, A., and Gelfand, M. The biologic significance of cytologic atypia in progestagen treated endometrial hyperplasia. Am. J. Obstet. Gynecol., *160*: 126-131, 1989.

36. Ehrlich, C.E., Young, P C.M , Stehman, F.B., Sutton, G.P., and

Alford, W.M. Steroid receptors and clinical outcome in patients with adenocarcinoma of the endometrium. Am. J. Obstet. Gynecol. *158*: 796-807, 1988.

Chapter V

GENERAL DISCUSSION

Major regulatory factors controlling the growth of the normal uterus are sex-steroid hormones, especially estrogens (E_2) and progesterone. However, the hormonal regulation of endometrial hyperplasia and carcinoma and their mutual relationships are still highly debated (see Chapter I, part III). These, in turn, may jeopardize the appropriate management of uterine carcinoma and its precursors. The presence of ER and PgR is a prerequisite for the action of E_2 and progesterone, respectively. These receptors have been measured mainly using biochemical techniques in both healthy and pathologic endometrium (see Chapter I, part IV). Accordingly, we have analyzed by immunohistochemistry the ER and PgR content and its relative distribution in the stroma and the epithelium in a variety of human endometrial tissues: normal, hyperplastic and neoplastic (see Chapter II, III, and IV).

Immunohistochemical analysis of ER in the normal cyclic human endometrium was first published by Press *et al*, 1984, using H222 monoclonal antibody. The specificity of this antibody for ER has been largely demonstrated (see Chapter I, part V). cDNA for the ER receptor has recently been cloned from MCF7 human breast cancer line (Green *et al*, 1986) and shown to be recognized correctly by the monoclonal antibody H222, developed against MCF7 human breast cancer ER. We have confirmed and extended the observations made by Press *et al*, 1984, in Chapter II using the same antibody. Since then, two other studies (Garcia *et al*,

1988; Scharl *et al*, 1988) have been published on the normal cyclic human endometrium supporting our results; that is a differential loss of ER expression in the various cell populations of cyclic human endometrium. Accordingly, the highest levels of ER in both the glandular and stromal components occur in proliferative phase endometrium, they decrease in the stroma of early secretory phase endometrium but remain high in its glandular component; finally, an important decline and a complete disappearance are observed in both stromal and glandular components of the functionalis during the mid/late secretory and menstrual phase endometrium, respectively.

Recently, several monoclonal antibodies against rabbit, chicken and human PgR have been developed (Logeat *et al*, 1983; Schrader *et al*, 1981; Tuohimaa *et al*, 1984, Sullivan *et al*, 1986; Clarke *et al*, 1987; Greene *et al*, 1988). Monoclonal antibodies to rabbit and avian PgR have been shown to crossreact with human PgR (Logeat *et al*, 1983; Sullivan *et al*, 1986; Wei *et al*, 1987, Estes *et al*, 1987) Using these antibodies, immunohistochemical studies have first localized PgR in target cells of the chick oviduct (Gasc *et al*, 1984; Isola *et al*, 1987a), chick ovary (Isola *et al*, 1987b), rabbit uterus, oviduct, cervix and vagina (Perrot-Applanat *et al*, 1985), rabbit ovary (Korte and Isola, 1988), and monkey ovary (Hild-Petito *et al*, 1988) PgR in the chick oviduct has been shown in two distinct forms designated "A" and "B" with molecular weights of approximately 79 and 108 Kd (Wei and Horwitz, 1986). In the human, neoplastic breast cells have been the most studied by immunohistochemistry for PgR (Perrot-Applanat *et al*, 1987; Greene and Press, 1987; Helin *et al*, 1988; Pertschuk *et al*, 1988; Elashry-Stowers *et al*, 1988; Charpin *et al*,

1988). A high correlation between immunohistochemical methods and biochemical assays has been found Akin to avian PgR, its counterpart in humans, is also found in two forms with molecular weights of approximately 95 and 120 Kd (Wei and Horwitz, 1986) αPR6 recognizes only the B form of PgR in the oviduct (Sullivan *et al*, 1986) and in human breast cancer cells (Wei *et al*, 1987; Estes *et al*, 1987) cDNA for the PgR receptor has recently been cloned from rabbit uterus (Loosfelt *et al*, 1986), chick oviduct (Jeltsch *et al*, 1986, Conneely *et al*, 1986), and human breast cancer cells (Misrahi *et al*, 1987) It indicates the presence of only one gene for these PgRs. It is unclear at this time whether the existence of these two receptor forms is a consequence of proteolysis either in vivo or during experimental conditions from a unique product (Loosfelt *et al*, 1986, Lamb *et al*, 1986) or whether A and B forms are the result of differential splicing events (Carson *et al*, 1987, Gronemeyer *et al*, 1987)

We have studied the distribution of PgR in the normal human cyclic endometrium (Chapter III) using the mouse monoclonal antibody (αPR6) to the avian PgR. Its specificity for human endometrial PgR has been shown by Sullivan *et al*, 1986. There is no evidence that, in human endometrial tissues, the receptor exists exclusively in the "A" form or in a different ratio to the "B" form. The fact that αPR6 recognizes only the "B" form may lead to reduced immunostaining intensity, however this is probably not restricted to any particular type of endometrial tissue Furthermore, recent immunohistochemical studies on the normal cyclic human endometrium with rat antibodies, JZB39 and KD68, recognized both the A and B forms of human PgR (Press *et al*, 1988; Press and Greene, 1988; Lessey *et al*,1988) The distribution of PgR in these studies was similar to our results presented in Chapter III.

Immunoblot analysis with αPR6 on the human endometrial cytosol (Chapter III) confirmed the specificity of αPR6 for the B subunit of human endometrial PgR, demonstrated by Sullivan *et al*, 1985 Furthermore, our competition studies (Chapter III) demonstrated the specificity of the immunocytochemical staining for human endometrial PgR. Specific staining was found only in the nuclei of epithelial and stromal cells of the endometrium when incubated with αPR6, but disappeared when αPR6 was preincubated with highly purified receptor The exclusive nuclear distribution of PgR in the epithelial and stromal cells of the endometrium was also found in nuclei of smooth muscle cells of the myometrium Endometrial PgR concentrations varied in the menstrual cycle PgR content increased between the early and late proliferative phase, remained elevated in the early secretory phase and declined by the mid-secretory phase in the epithelial component It remained high, however, in stromal cells throughout the secretory phase However, future investigations for endometrial tissue fixation may improve distortion due to ligand nitrogen in the secretory endometrium Investigators have reported on the distribution of PgR in the normal cyclic endometrium with mouse monoclonal antibodies to human endometrial PgR (Clarke *et al*, 1987), to rabbit uterine PgR cross-reacting with human PgR (Garcia *et al*, 1988), and rat monoclonal antibodies to human breast cancer cells PgR (Press *et al*, 1988, Lessey *et al*, 1988) The distribution of PgR in these studies was similar to ours described in Chapter III. However, future investigations for endometrial tissue fixation may improve distortion due to ligand nitrogen in the secretory endometrium.

The combined analysis on the distribution of both ER and PgR provides new information on PgR synthesis in the human cyclic endometrium. PgR concentrations have been shown by biochemical assays to be the highest during the late proliferative-early secretory phase, declining thereafter during the remaining of the

menstrual cycle. These studies led to the conclusion that PgR synthesis is mainly induced by E_2 in target cells via the E_2-receptor complex mechanism (see Chapter I, part II; Nardulli *et al*, 1988). Progesterone decreases the synthesis of ER, thus indirectly decreases the synthesis of PgR. However, the existence of an E_2-independent pool of PgR has also been suggested in hamster and rabbit uterus and vagina (Isotalo *et al*, 1981, Allen and Leavitt , 1983; Okulicz, 1986). As a result, PgR synthesis in these tissues may exist independently of either E_2 or progesterone levels. Toppila *et al*, 1986 and Hild Petito *et al*, 1988 have suggested that the same phenomenon may exist in the normal human and monkey ovary, respectively, and Korte and Isola, 1988 failed to find E_2-inducibility of rabbit ovarian PgR Our study in Chapter III confirmed the concept of E2-regulat › of PgR synthesis and only progesterone down-regulation in the epithelial component of the endometrium *The presence of PgR in the epithelium may thus be a good marker of epithelial E_2-sensitivity Conversely, lack of PgR in estrogen primed endometrial epithelium reflects the action of progesterone* PgR in the stroma of the endometrium and the myometrium, however, appears to exist independently of either E_2 or progesterone levels This led to the suggestion that *PgR may be in part constitutively synthetized in the latter* The precise significance of this finding is not clear. The functional response of uterine tissue to E_2 and progesterone seems to be determined in part by the fluctuationing levels of ER and PgR in the epithelium and stroma The declining functional importance of the glandular epithelium during the late secretory phase parallels the depletion of PgR. The stroma which becomes decidualized in the mid-secretory phase would logically require receptors to support its development induced by progesterone. The same is true for the myometrium

during gestation, since progesterone is thought to inhibit its contractibility.

In our study, ER and PgR were not found in endothelial cells of endometrial and myometrial vessels nor in the muscular wall of myometrial blood vessels Press *et al*, 1984, Press *et al*, 1988; Lessey *et al*, 1988, found the same results with rat monoclonal antibodies to human ER and PgR. However, Perrot-Applanat *et al*, 1988 recently showed the presence of ER and PgR in muscle cells of myometrial uterine arteries in rabbits and humans The discrepancy between this immunocytochemical study and ours and others may be due to differences in the monoclonal antibobies used. However, the study of Perrot-Applanat *et al*, 1988, if verified, offers a new concept to explain uterine vascular changes observed during the menstrual cycle and pregnancy Steroid hormones may act directly on arterial muscle cells rather than only through their indirect action on prostaglandins Whether E_2 or progesterone may constrict or dilate the uterine vascular system is yet unknown

Immunohistochemical study of ER and PgR provides a new tool for diagnosing luteal phase defect in women with infertility. In such cases, the glandular epithelium and the stroma are out of phase with respect to cellular maturation In general, maturation is more advanced in the stroma than in the gland cells. The possibility that this asynchronous maturation is receptor-related is entertained but has not been proven so far. However, epithelial PgR decrease and eventual absence of as a marker of gland cell maturation under the action of progesterone may be used together with endometrial histology and steroid plasma levels, for studying infertility during short luteal phase or luteal phase defect and related conditions of infertility.

ER and PgR have been found elevated in endometrial hyperplasia by biochemical assays (see Chapter I, part IV) However, the epithelial and stromal cell components of endometrial hyperplasia have not been evaluated separately in these studies Our immunohistochemical analysis of the cellular distribution of both ER and PgR (Chapter II and IV) in hyperplastic endometria provides insight to understand the complexity of possible steroid hormone effects on these tissues Endometrial hyperplasia with or without cytologic atypia displayed differential expression in their ER and PgR immunoreactivity. In hyperplasia without cytologic atypia, ER and PgR were found in high concentrations in the nuclei of both the epithelial and stromal cells in agreement with the previous biochemical data In endometrial intraepithelial neoplasia (EIN), however, ERs and PgRs were mostly confined to the stromal cell component The levels in this endometrial component reflects probably most of the value measured by biochemical assays

The various biomorphologic characteristics (other than PgR) that distinguish EIN from hyperplasia have been described (see Chapter I, part III) They support the two disease hyperplasia-neoplasia concept (Ferenczy, 1988). The difference in cellular distribution of steroid receptors between hyperplasia and EIN is an additional characteristic which distinguishes these two pathological states Although it is admitted that the presence of ER and PgR in normal target tissues is a prerequisite for the action of E_2 and progesterone, little is known about the hormonal regulation of hyperplastic proliferations in the human endometrium *High levels of ERs and PgRs in hyperplasias suggest their sensitivity to E_2 and their possible response to progesterone*. The low levels of ERs and PgRs

in the epithelial component of EIN suggest *a different*, if any, *hormonal regulation of this lesion and a potential insensitivity to* E_2 *and progesterone*. This hypothesis seems to be supported by a recent clinico-pathologic study on hyperplastic endometrium with and without cytologic atypia (Ferenczy and Gelfand, 1989) Endometrial response to exogenous progestagens, medroxyprogesterone acetate (Provera (R)), was significantly higher in hyperplasia without cytologic atypia (80%) than EIN (25%). Histologically, endometrial response to Provera was ascertained by secretory, "pill-effect", menstrual-like or inactive changes, suggesting that PgR in hyperplastic endometrium are indeed functional, but less or not at all in EIN It is also possible that the difference in steroid receptor distribution in these tissues is a reflection of dedifferentiation and loss of ER and PgR expression in hyperplasia prior to its conversion to neoplasia.In this case, hyperplasia and EIN may be considered as a continuum of disease In contrast to normal endometrium, hyperplasia and EIN, invasive carcinoma displayed heterogenous staining for ER and PgR in both their epithelial and stromal components This may indicate that EIN and carcinoma are not necessarily successive pathologic steps on a transformation pathway. Unfortunately, at present, there are no experimental models to verify our contentions on hormonal regulation of hyperplastic and neoplastic endometria. Even in mice, the best experimental model for studying human endometrium in relation to hormonal influence, it is impossible to ascertain morphologically and distinguish between hyperplastic and intraepithelial neoplastic endometrium

Our observations of the heterogenous distribution of ER and PgR in the epithelial and stromal cell components of endometrial carcinoma is in

agreement with the findings of Mc Carthy *et al*, 1985; Budwit-Novotny *et al*, 1986; Pertschuk *et al*, 1986; Charpin *et al*, 1988, for ER and Zaino *et al*, 1988; Charpin *et al*, 1989; Segreti *et al*, 1989 for PgR Similar heterogeneity has been reported for ER and PgR in breast carcinoma (King *et al*, 1985, Mc Carthy *et al*, 1985, Pertschuk *et al*, 1985; Perrot-Applanat *et al*, 1987, Charpin *et al*, 1988; Elashry-Stowers *et al*, 1988, Helin *et al*, 1988, Pertschuk *et al*, 1988) It may indicate that regulation of steroid receptors during malignant transformation of endometrial tissue is altered and/or different from the normal cyclic counterpart. However, Satyaswaroop *et al*, 1983 and Zaino *et al*, 1984 have shown that receptor-positive human endometrial adenocarcinoma transplanted to nude mice showed growth response and histologic alterations after estradiol or progesterone similar to those observed in normal endometrium Conversely, steroid receptor-negative carcinoma failed to respond to E_2 injection The results indicate that response of endometrial carcinoma to exogenous, suppressive hormone therapy requires the presence of steroid receptors, whereas their absence implies a probable lack of response to hormone therapy.

The significance of ER and PgR as markers of estrogen sensitivity in the stroma of EIN and some endometrial carcinomas by immunohistochemistry is not clear. Although the presence of PgR in these tissues suggests the presence of functional ERs and estrogen sensitivity, PgR is only one measure of estrogen action The latter is better ascertained by a combination of measurements. For exemple, measuring estrogen-induced mitogenic action of endometrial stroma with synthesis and/or secretion of stromal estradiol-induced growth factors such as fibroblast growth factor (FGF), platelet derived growth factor (PDGF),

ßeta transforming growth factor (ßTGF) and insulin-like growth factor 1 (IGF1) would confirm that ER are functional (Baird *et al* 1985; Neufeld *et al*, 1987; James and Bradshaw, 1984, Sporn *et al*, 1987; Clemmons, 1984; Huff *et al*, 1986) Also, akin to secretory phase endometrium, PgR in the stroma of EIN and carcinoma may not be a marker of estrogen action but rather may be constitutively synthetized. If so, presence of PgR may not necessarily reflect cellular response to exogenous progestogens. On the other hand, presence of stromal progestin-induced proteins such as 51K or pregnancy associated endometrial α-globulin (α1 PEG or IGFBP) (Maudelonde and Rochefort, 1987; Waites *et al*, 1988) together with histologic evidence of stromal cell secretory differentiation (decidualization) after progestogen administration would suggest the functional nature of PgR. A recent clinical study (Ferenczy and Gelfand, 1989) has analyzed the longterm efficacity of exogenous progestogens in 85 patients with endometrial hyperplasia with (20 patients) and without (65 patients) cytologic atypia. While 80% of endometrial hyperplasia without cytologic atypia were converted on a longterm basis (mean 7 years) into secretory endometrium, 75% of the 20 cases with cytologic atypia (EIN) failed to respond to oral medroxyprogesterone acetate (Provera (R) therapy). These lesions remained unchanged both in their epithelial and stromal components and 5 of 20 progressed to carcinoma In this series, decidual transformation of the stroma or secretory maturation of the abnormal epithelium has been observed but has not been correlated with the presence or absence of PgRs. Similarly, an earlier clinico-morphological study on progestogen treated endometrial carcinoma failed to evaluate cellular difference, if any in the stroma of these tumors (Ferenczy and Gelfand, 1982). As a result, it is not known whether or not PgR in such lesions is

either absent or, if present, functional An alternative hypothesis is a *paracrine hormonal regulation of epithelial cells via stromal-epithelial interaction mechanism in EIN and some endometrial carcinomas containing ER- and PgR-positive stromal cells* This paracrine stimulation may be induced via stromal-secreted growth factors which are known to have a potent mitogenic effect on epithelial cells (Yee *et al*, 1989)

In conclusion, while intimate interrelationships between hormones which influence growth and development of secretory function in the normal cyclic endometrium have been well documented, much remains to be done for a better insight into the hormonal influence on theand pathogenesis of endometrial hyperplasia and neoplasia Specifically designed experimental studies of human endometria using steroidobinding-immunohistochemistry and molecular biology may improve our understanding of this challenging target tissue of the human reproductive tract

REFERENCES

Allen T C and Leavitt W W. Regulation of estrogen-dependent and estrogen independent levels of progesterone receptor in hamster vagina and uterus. J. Steroid Biochem 1983, 19·1047-1053.

Baird A., Esch F , Mormede P , Veno N., Ling N., Bohlenl P., Ying S Y., Wehrenberg W B and Guillemin R. Molecular characterization of fibroblast growth factor distribution and biological activities in various tissues. Rec Prog. Horm Res 1985, 42:142-205.

Budwit-Novotny D.A , McCarthy K.S., Cose E.B., Soper J.T., Mutch D.G., Creasman W T , Flowers J.L. and McCarthy K. Immunohistochemical analyses of estrogen receptor in endometrial adenocarcinoma using a monoclonal antibody Cancer Res. 1986, 46:5419-5425.

Carson M.A., Tsai M.J., Conneely O.M., Maxwell B.L., Clark J.H., Dobson A D.W , Elbrecht A., Toft D.O., Schrader W.T. and O'Malley B.W. Structure-function properties of the chicken progesterone receptor. A synthesized from complementary deoxyribonucleic acid. Mol. Endocrinol. 1987, 1:791-801.

Charpin C., Jacquemier J., Andrac L., Vacheret H., Habib M.C., Devictor B , Lavaut M and Toga M. Multimeric analysis (SAMBA 200) of the progesterone receptor immunocytochemical assay in non-malignant and

malignant breast disorders. Am. J. Path. 1988, 132·199-211.

Charpin C., Andrac L., Habib M.C., Vacheret H , Lavaut M N , Xerri L., Toga M. Immunocytochemical assays in human endometrial carcinomas A multiparametric computerized analysis and comparaison with nonmalignant changes. Gynecol. Oncol. 1989, 33: 9-22

Clarke C.L., Zaino R.J., Feil P D., Miller J.V., Steck M.E., Ohlsson-Wilhelm B.M. and Satyaswaroop P.G. Monoclonal antibodies to human progesterone receptor: Characterization by biochemical and immunohistochemical techniques Endocrinology 1987, 121:1123-1132.

Clemmons D.R Multiple hormones stimulate the production of somatomedin by cultured human fibroblasts. J. Clin. Endocrinol. Metab. 1984, 58:850-856.

Conneely O.M., Sullivan W.P., Toft D O., Birnbaumer M., Cook R G., Maxwell B.L., Zarucki-Schultz T., Greene G.L., Schrader W T and O'Malley B W. Molecular cloning of the chicken progesterone receptor. Science 1986, 233:767-770.

Elashry-Stowers D., Zava D.T., Speers W C and Edwards D P Immunocytochemical localization of progesterone receptors in breast cancer with anti-human receptor monoclonal antibodies. Cancer Res 1988, 48:6462-6474.

Estes P.A , Suba E.J., Lawler-Heavner J., Elashry-Stowers D., Wei L.L.,

Toft D.O., Sullivan W.P., Horwitz K.B. and Edwards D.P. Immunologic analysis of human breast cancer progesterone receptors. I. Immunoaffinity purification of transformed receptors and production of monoclonal antibodies. Biochemistry 1987, 26: 6250-6262.

Ferenczy A. and Gelfand M.M. Steroid-induced regression in endometrial cancer. In: Prolonged Arrest of Cancer (Stoll B.A Ed). New York, Wiley J. & Sons, 1982, 369-385.

Ferenczy A. Endometrial hyperplasia and neoplasia: A two disease concept, In: Contemporary issues in Obstetrics and Gynecology vol. 5: Gynecology Oncology (Cohen C. and Kale M.G eds). New York, Churchill Levenghoe Inc. 1988, 197-213.

Ferenczy A. and Gelfand M.M. The biologic significance of cytologic atypia in progestagen treated endometrial hyperplasia. Am. J. Obstet. Gynecol. 1989, 160·126-131.

Garcia E., Bouchard P., De Brux J., Berdah J., Frydman R., Schaison G., Milgrom E. and Perrot-Applanat M. Use of immunocytochemistry of progesterone and estrogen receptors for endometrial dating. J. Clin. Endocrinol. Metab. 1988, 67:80-87.

Gasc J.M., Renoir J.M., Radanyi C., Joab I., Tuohimaa P. and Baulieu E.E. Progesterone receptor in the chick oviduct: an immunohistochemical study with antibodies to distinct receptor components. J. Cell. Biol. 1984, 99:1193-1200.

Green S., Walter P., Kumar V., Krust A., Bormert J.M., Argos P. and Chambon P. Human oestrogen receptor cDNA: sequence, expression and homology to V-erb-A. Nature 1986,320:134-139.

Greene G.L. and Press M.F. Immunochemical evaluation of estrogen receptor and progesterone receptor in breast cancer. In: Immunological approaches to the diagnosis and therapy of breast cancer (Ceriani R.L ed). New York, Plenum Press, 1987, 119-135.

Greene G.L., Harris K., Bova R., Kinders R., Moore B. and Nolan C. Purification of T47D human progesterone receptor and immunochemical characterization with monoclonal antibodies. Mol. Endocrinol. 1988, 2:714-726.

Gronemeyer H., Turcotte B., Quirin-Stricker C., Bocquel M.T., Meyer M.E., Krozowski Z., Jeltsch J.M., Lerouge T., Garnier J.M. and Chambon P. The chicken progesterone receptor: sequence, expression and functional analysis. EMBO J. 1987, 6:3985-3994.

Helin H.J., Helle M.J., Helin M.L. and Isola J.J. Immunocytochemical detection of estrogen and progesterone receptors in 124 human breast cancers. Am. J. Clin. Pathol. 1988, 89.137-142.

Hild-Petito S., Stouffer R.L. and Brenner R.M. Immunocytochemical localization of estradiol and progesterone receptors in the monkey ovary throughout the menstrual cycle. Endocrinology 1988, 123:2896-2905.

Huff K.K., Kaufman D., Gabbay K.H., Martin Spencer E., Lippman M.E. and Dickson R.B: Secretion of an insulin-like growth factor 1 related protein by human breast cancer cells. Cancer Res. 1986, 46:4613-4615.

Isola J., Pelto-Huikko M. and Tuohimaa P. Immunoelectron microscopic localization of progesterone receptor in the chick oviduct. J. Steroid Biochem. 1987, 26:19-23.

Isola J., Korte J.M. and Tuohimaa P. Immunocytochemical localization of progesterone receptor in the chick ovary. Endocrinology 1987, 121:1034-1040.

Isotalo H., Isomaa V. and Jänne O. Replenishment and properties of cytosol progesterone receptor in rabbit uterus after multiple progesterone administration. Endocrinology 1981, 108, 868-873.

James R. and Bradshaw R.A. Polypeptide growth factors. Ann. Rev. Biochem. 1984, 53:259-292.

Jeltsch J.M., Krozowski Z., Quirin-Strickei C., Gronemeyer H., Simpson R.J., Garnier J.M., Krust A., Jacob J. and Chambon P. Cloning of the chicken progesterone receptor. Proc. Natl. Acad. Sci. USA. 1986, 86:5424-5428.

King W.J., De Sombre E.R., Jensen E.V. and Greene G.L. Comparison of immunocytochemical and steroid-binding assays for estrogen receptor in

human breast tumors. Cancer Res. 1985, 45:293-304.

Korte J.M. and Isola J.J. An Immunocytochemical study of the progesterone receptor in rabbit ovary, Mol. Cell. Endocrinol. 1988, 58:93-101.

Lamb D.J., Kima P.E. and Bullock D.W. Evidence for a single steroid-binding protein in the rabbit progesterone receptor. Biochemistry. 1986, 25:6319-6324.

Lessey B.A., Killam D.A., Metzger D.A., Haney A.F., Greene G.L. and Mc Carty K.S. Immunohistochemical analysis of human uterine estrogen and progesterone receptors throughout the menstrual cycle. J. Clin. Endocrinol. Metab. 1988, 67:334-340.

Logeat F., Vu Hai M.T., Fournier A., Legrain P., Buttin G. and Milgrom E. Monoclonal antibodies to rabbit progesterone receptor: cross reaction with other mammalian progesterone receptors. Proc. Natl. Acad. Sci. USA 1983, 30:6456-6459.

Loosfelt H., Atger M., Misrahi M., Guischon-Mantel A., Meriel C., Logeat F., Benarous R. and Milgrom E. Cloning and sequence analysis of rabbit progesterone-receptor complementary DNA. Proc. Natl. Acad. Sci USA 1986,83:9045-9049.

Maudelonde T. and Rochefort H. A51K progestin-regulated protein secreted by human endometrial cells in primary culture. J. Clin. Endocrinol. Metab. 1987, 64:1294-1301.

Mc Carthy K.S. Jr, Miller L.S., Cox E.B., Konrath J. and Mc Carthy K.S. Sr. Estrogen receptor analyses correlation of biochemical and immunohistochemical methods using monoclonal antireceptor antibodies. Arch. Pathol. Lab. Med. 1985, 109:716-721.

Misrahi M., Atger M., d'Auriol L., Loosfelt H., Meriel C., Fridlansky F., Guorcon-Mantel A., Galibert F. and Milgrom E. Complete amino acid sequence of the human progesterone receptor deduced from cloned cDNA. Biochem. Biophys. Res. Commun. 1987, 143:740-748.

Nardulli A.M., Greene G.L., O'Malley B. and Katzenellenbogen B.S. Regulation of progesterone receptor messenger ribonucleic acid and protein levels in MCF7 cells by estradiol: analysis of estrogen's effect on progesterone receptor synthesis and degradation. Endocrinology 1988, 122:935-944.

Neufeld G., Ferrara N., Schweigerer L., Mitchell R. and Gospodarowicz D. Bovine granulosa cells produce basic fibroblast growth factor. Endocrinology 1987, 121:597-603.

Okulicz W.C. Progesterone receptor replenishment during sustained progesterone treatment in the hamster uterus. Endocrinology 1986, 118:2488-2494.

Perrot-Applanat M., Logeat F., Groyer-Ricard M.T. and Milgrom E. Immunocytochemical study of mammalian progesterone receptor using

monoclonal antibodies. Endocrinology 1985, 116:1473-1484.

Perrot-Applanat M., Groyer-Ricard M.T., Lorenzo F., Jolivet A., Thu Vu Hai M., Pallud C., Spyratos F. and Milgrom E. Immunocytochemical study with monoclonal antibodies to progesterone receptor in human breast tumors. Cancer Res. 1987, 47:2652-2661.

Perrot-Applanat M., Groyer-Ricard M.T., Garcia E., Lorenzo F. and Milgrom E. Immunocytochemical demonstration of estrogen and progesterone receptors in muscle cells of uterine arteries in rabbits and humans. Endocrinology 1988, 123:1511-1519.

Pertschuk L.P., Eisenberg K.B., Carter A.C., and Feldman J.G. Immunohistologic localization of estrogen receptors in breast cancer with monoclonal antibodies. Cancer 1985, 55:1513-1518.

Pertschuk L.P., Beddsoe A.M., Gorelic L.S. and Shain S.A. Immunocytochemical assay for estrogen receptors in endometrial carcinoma with monoclonal antibodies. Cancer 1986, 57:1000-1004.

Pertschuk L.P., Feldman J.G., Eisenberg K.B., Carter A.C., Thelmo W.L., Cruz W.P., Thorpe S.M., Chistensen J., Rasmussen B.B., Rose C. and Greene G.L. Immunocytochemical detection of progesterone receptor in breast cancer with monoclonal antibody. Cancer 1988, 62:342-349.

Press M.F., Nousek-Goebl N., King W.J., Herbst A.L. and Greene G.L. Immunohistochemical assessment of estrogen receptor distribution in the

human endometrium throughout the menstrual cycle. Lab. Invest. 1984, 51:495-503.

Press M.F., Udove J.A. and Greene G.L. Progesterone receptor distribution in the human endometrium analysis using monoclonal antibodies to the human progesterone receptor. Am. J. Pathol. 1988, 131:112-124.

Press M.F. and Greene G.L. Localization of progesterone receptor with monoclonal antibodies to the human progestin receptor. Endocrinology 1988, 122:1165-1175.

Satyaswaroop P.G., Zaino R.J. and Mortel R. Human endometrial adenocarcinoma transplanted into nude mice: growth regulation by estradiol. Science 1983, 219:58-60.

Scharl A., Vierbuchen M., Graupner J., Fischer R. and Bolte A. Immunohistochemical study of distribution of estrogen receptors in corpus and cervix uteri. Arch. Gynecol. Obstet. 1988, 241: 221-233.

Schrader W.T., Birnbaumer M.E., Hughes M.R., Weigel N.L , Grody W.W. and O'Malley B.W., Studies on the structure and function of the chicken progesterone receptor. Rec. Prog. Horm. Res. N.Y., 1981, 60:583-632.

Segreti E.M., Novotny D.B., Soper J.T., Mutch D.G., Greasman W.T and Mc Carty K.S. Endometrial cancer: histologic correlates of immunohistochemical localization of progesterone receptor and estrogen receptor. Obstet. Gynecol. 1989, 73:780-785.

Sporn M.B., Roberts A.B., Wakefield L.M. and De Grombrugghe B. Some recent advances in the chemistry and biology of transforming growth factor-ßeta. J. Cell. Biol. 1987, 105:1039-1045.

Sullivan W.P., Beito R.G., Proper J., Krco C.J. and Toft D.O. Preparation of monoclonal antibodies to the avian progesterone receptor. Endocrinology 1986, 119:1549-1557.

Toppila M , Tyler J.P.P., Fay R., Baird P.J., Grandon A.J., Eastman C.J. and Hudson C.N. Steroid receptors in human ovarian malignancy. A review of four years tissue collection. Br. J. Obstet. Gynecol. 1986, 93,986-992.

Tuohimaa P.J., Renoir J.M., Radanyi C., Mester J., Joab I., Buchou T. and Baulieu E.E. Antibodies against highly purified B-subunit of the chick oviduct progesterone receptor. Biochem. Biophys. Res. Commun. 1984, 119,433-439.

Waites G.T., James R.F.L. and Bell S.C. Immunohistological localization of the human endometrial secretory protein pregnancy- associated endometrial ∝1-globulin, an insulin like growth factor binding protein, during the menstrual cycle. J Clin Endocrinol. Metab. 1988, 67:1100-1104.

Wei L.L. and Horwitz K.B. The structure of progesterone receptors. Steroids 1986, 46:677-695.

Wei L.L., Sheridan P.L., Krett N.L., Francis M.D., Toft D.O., Edwards

D.P., and Horwitz K.B. Immunologic analysis of human breast cancer progesterone receptors. 2. Structure, phosphorylation and processing. Biochemistry 1987. 26:6262-6272.

Yee D., Paik S., Lebovic G.S., Marcus R.R., Favoni R.E., Cullen K.J , Lippman M.E. and Rosen N. Analysis of insulin-like growth factor I gene expression in malignancy and evidence for a paracrine role in human breast cancer. Mol. Endocrinol. 1989, 3:509-517.

Zaino R.J., Satyaswaroop P.G. and Mortel R. Morphology of human uterine cancer in nude mice. Arch. Pathol. Lab. Med. 1984, 108:571-578.

Zaino R.J., Clarke C.L., Mortel R. and Satyaswaroop P.G. Heterogeneity of progesterone receptor distribution in human endometrial adenocarcinoma. Cancer Res. 1988, 48:1889-1895.

CONTRIBUTION TO THE KNOWLEDGE

1) On the specificity of the monoclonal antibody αPR6 for human endometrial progesterone receptors and,

2) *On the* systematic immunocytochemical study of estrogen (ER) and progesterone receptors (PgR) in normal cyclic, hyperplastic and neoplastic human endometria. The results indicate:

a) the presence of epithelial PgR may be a specific marker of epithelial E_2 sensitivity during the menstrual cycle and lack of epithelial PgR reflects the action of progesterone. PgR in the stroma may be in part constitutively synthetized.

b) high levels of ER and PgR in hyperplasia without cytologic atypia correspond to high E_2 sensitivity of these tissues and their potential to respond to progesterone.

c) low levels of ER and PgR in the epithelium of endometrial intraepithelial neoplasia and the majority of invasive carcinoma correspond to relative epithelial insensitivity of these abnormal proliferations to E_2 and their limited potential to respond to progesterone and,

d) the presence of ER and PgR in the stroma of endometrial intraepithelial neoplasia and in some invasive carcinoma suggests a possible paracrine hormonal regulation of epithelial cells in these lesions.