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Immunohistochemical Study of Distribution of Estrogen Receptors in Corpus and Cervix Uteri*

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Summary. An immunohistochemical assay based on monoclonal antiestrophilin antibodies has been used to localize estrogen receptor (ER) in frozen sections of normal human endometrial, myometrial and cervical tissues from menstruating, hormonally treated, pregnant and postmenopausal women. Specific staining was confined to the cellular nuclei. In proliferative phase endometrium, postmenopausal emdometrium, and endometrium from patients treated with hormone ERs were easily detected in most glandular and stromal cells. After ovulation and in early pregnancy a quick and distinct decrease of ER expression was noted. This was especially the case with the more superficial layers of endometrium (endometrium functionalis), the majority of whose cells had either weak localization of ER or none at all. In the endometrium basalis, however, the reduction of ER localization turned out to be more moderate. More then half of the epithelial and stromal cells displayed nuclear staining, partly strong. The myometrium of the corpus uteri showed a similar ER localization and dependence on hormonal stage when compared with the endometrium functionalis. The endocervical mucosa displayed a high degree of ER expression in the proliferative phase, in postmenopausal women and in women who had been treated with hormones. Unlike the endometrium and myometrium, the endocervical glands underwent minimal changes in nuclear ER content during the menstrual cycle. Although the endocervical stroma showed cyclic alterations in ER levels, their reduction after ovulation was less marked than in the corresponding endometria. In cervical squamous epithelium ER localization was predominantly confined to the basal layers. In the course of cellular maturation, specific nuclear staining vanished. In the proliferative phase, after the menopause and in early pregnancy, the basal, parabasal and intermediate cells were specifically stained. In the postovulatory phase,

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However, nuclear staining was confined to the basal and parabasal cells. Hormonally treated squamous epithelia almost completely lacked nuclear ER localization.

Key words: Estrogen receptors – Uterus

Introduction

Estrogens elicit their biological responses in target tissues by means of high affinity specific binding proteins, called estrogen receptors (estrophilin, ER) [9, 15]. Although, biochemical assays have been used for years to measure estrogen receptor levels, they have not taken into account tissue heterogenity or the histological appearance of the sample. With the availability of specific monoclonal antibodies prepared against human breast cancer ER [11] specific and also very sensitive methods for immunocytochemical assay of ER [2, 12] have become feasible. This permits the identification of the receptor itself and establish its anatomical location.

Such immunohistochemical investigations using monoclonal antibodies have been carried out on human and animal endometria at different phases of the hormonal cycle [22, 29]. However little is known on the distribution of ER in the human cervix.

The aim of the present study, using a monoclonal antibody to ER, has been to examine the distribution of ER in the uterine cervix as compared with the uterine corpus and to clarify the dynamics of ER localization under various hormonal conditions.

Material and Methods

Samples of fundal endometrium, fundal myometrium, endocervix, and ectocervix were obtained from 30 hysterectomy specimens. Details of hormone therapy, menstrual history and the date of the last menstrual period were recorded. Nine of the women had been post-menopausal for 10 to 15 years, two of them had local cervical estrogen applications over a period of 10 days. Eighteen patients with normal ovulatory menstrual cycles were operated on and one woman had her hysterectomy during the 9th week of pregnancy. Two premenopausal women had had preoperative estrogens and gestagens for 10 days (Table 1). The specimens were placed on ice, serial cryostat sections (4–6 μm) were cut immediately and mounted on glass slides.

The first section of each sample was stained with hematoxylin and eosin for conventional histological examination and endometrial dating [24]. Only specimens with a histological appearance appropriate to age and hormonal status were included. The histological appearance of the endometrium was classified as early proliferative (days 3 to 9), late proliferative (days 10 to 14), early secretory (days 15 to 17), mid-secretory (days 18 to 22), and as late secretory (days 23 to 28).

The next sections underwent a immunocytochemical process for the detection of estrogen receptors using the ER-ICA monoclonal kit (Abbott GmbH, Wiesbaden, FRG). Using a sandwich technique [33], amplification and detection of specific sites were achieved by attachment of a rat-peroxidase-antiperoxidase complex through a bridging antibody (goat antirat IgG) to the specific monoclonal rat antibody against human ER, bound to estrogen receptors present in tissue sections. The complex-bound peroxidase was visualized using a substrate containing diaminobenzidine (DAB) and hydrogene peroxide, which forms an insoluble brown deposit on the areas containing the enzyme. In control sections the primary antibody was replaced by normal rat IgG. After several

Table 1. Estrogen receptor specific staining of endometrium and myometrium according to hormonal status of endometrium. By multiplying the percentage of cells of a given type by their degree of staining (+ weak, 2+ strong, 3+ very intense) we got scores of 1 to 74 for +, 75 to 150 for ++ and more than 150 for +++. We scored +++ for very intense/strong specific staining in more than half of the cells, ++ when more than half of the cells were specifically stained, and + when at least one cell but less than half of the cells of a given type were specifically stained [29]

Hormonal stage	Endometrium				Myometrium
	Basalis		Functionalis		
	Glands	Stroma	Glands	Stroma	
Normal biphasic menstrual cycle					
Early proliferative $n = 6$	+++	+++/++	+++/++	++/+++	++/+++
Late proliferative $n = 5$	+++	+++/++	+++/++	++/+++	+++
Early secretory $n = 3$	+++	+++/++	+++	++	++/+
Midsecretory $n = 1$	++	++	+	+	+
Late secretory $n = 3$	++/+	++/+	+	+	+
Postmenopause					
Without hormonal treatment $n = 7$	+++/++	+++/++	++/+++	++/+++	++/+++
Local estrogen treatment $n = 2$	+++	++	+++	++	++
Hormonal treatment $n = 2$	+++	+++/++	+++	++	++
Early pregnancy $n = 1$	+	+	−	−	−

washings with phosphate-buffered saline (PBS), tissue sections were mounted with Eukitt. The intensity of specific staining was scored on a four-rate scale namely − negative, + weak, 2+ strong, 3+ very intense staining. The percentage of specifically stained cells was also counted.

Results

Estrogens receptors were found in the nuclei of uterine myometrial, stromal and epithelial cells. Occasionally, weak cytoplasmic staining was observed. How-

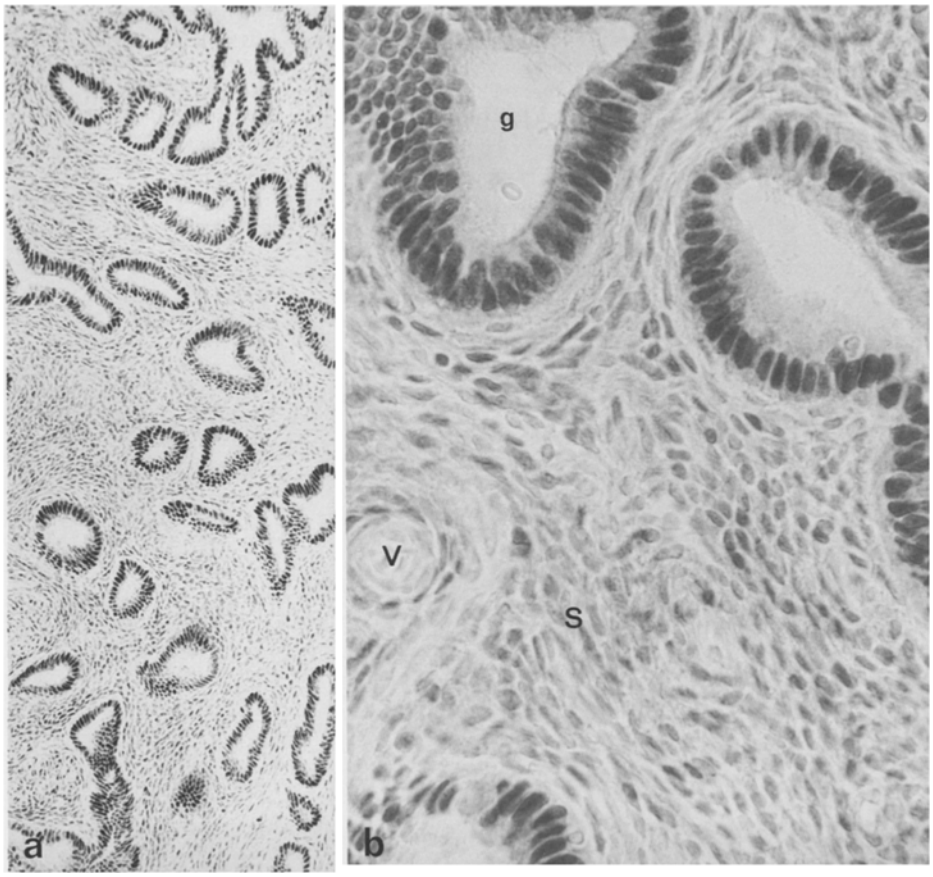

Fig. 1a and b. Proliferate phase endometrium immunostained for ER: strong nuclear staining is present in the majority of endometrial cells. Staining intensity and the number of specifically stained cells are less in stromal than in epithelial cells. Muscular coats and endothelium of vessels are negative for ER (g = gland, s = stroma, v = vessel) (ER-ICA, no counterstain, **a** × 100, **b** × 500)

ever, since it was also present in controls, it was considered insignificant. Specific cytoplasmic staining was not identified in any of the material studied. No ERs were found in the muscular coats or endothelium of uterine vessels (Fig. 1) or in mitotic figures.

Corpus Uteri, Endometrium

In proliferative phase endometria of women having normal biphasic menstrual cycles, the surface and glandular epithelial cells and the stromal cells showed strong or very intense staining in the majority of nuclei (Table 1). The staining intensity of the stromal cells seemed on average to be less than that of the epithelial cells. More stromal than epithelial cells remained unstained (Fig. 1).

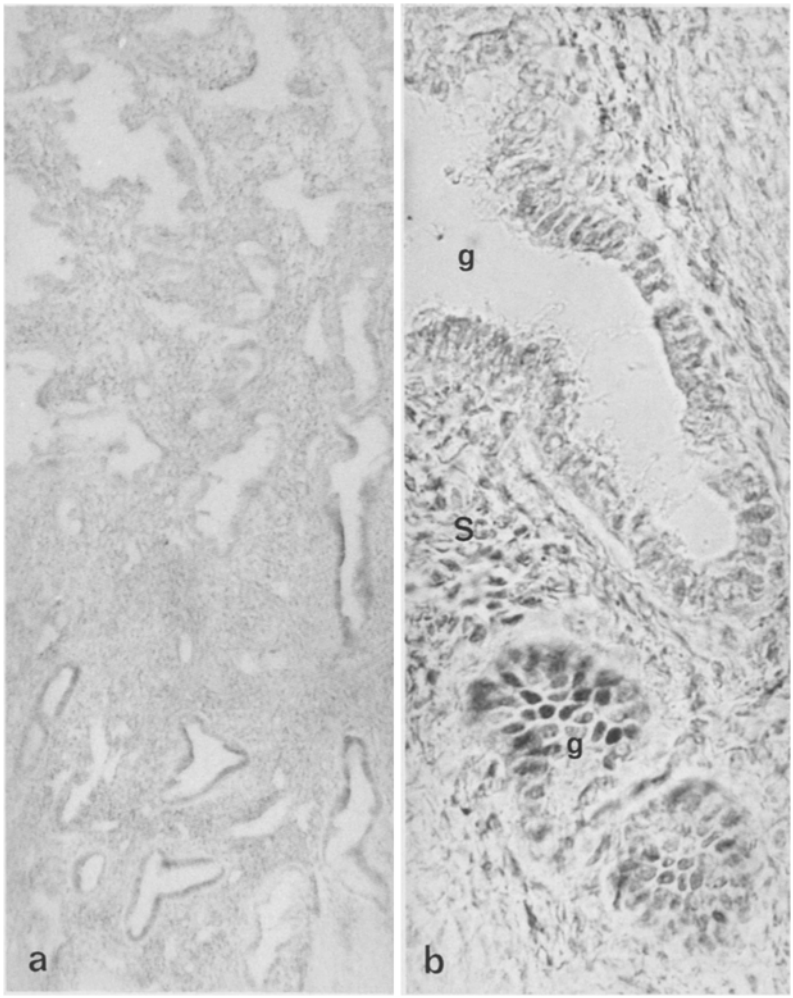

Fig. 2a and b. Late secretory phase endometrium immunostained for ER. **a** Glandular nuclear staining is observed only in the endometrium basalis. Stromal cells are weakly stained for ER (ER-ICA, no counterstain, × 50). **b** Junction between the functional and basal layer. The upper gland in the functional layer is unstained, the lower gland in the basal layer shows nuclear staining (ER-ICA, no counterstain, × 350) (s = stroma, g = gland)

Some of the unstained stromal cells were identified as lymphocytes and granulocytes in sections stained with HE. Consistent changes in staining intensity could not be demonstrated during the proliferative phase.

During the first days after ovulation the pattern and intensity of the peroxidase reaction was similar to that described for proliferative phase endometrium. With secretory transformation, however, a quick and distinct decrease of antigen expression was noted (Table 1). This was especially the case with the upper layers of endometrium (endometrium functionalis), the majority of whose cells in the mid- and late secretory phase have either few or no ERs. The

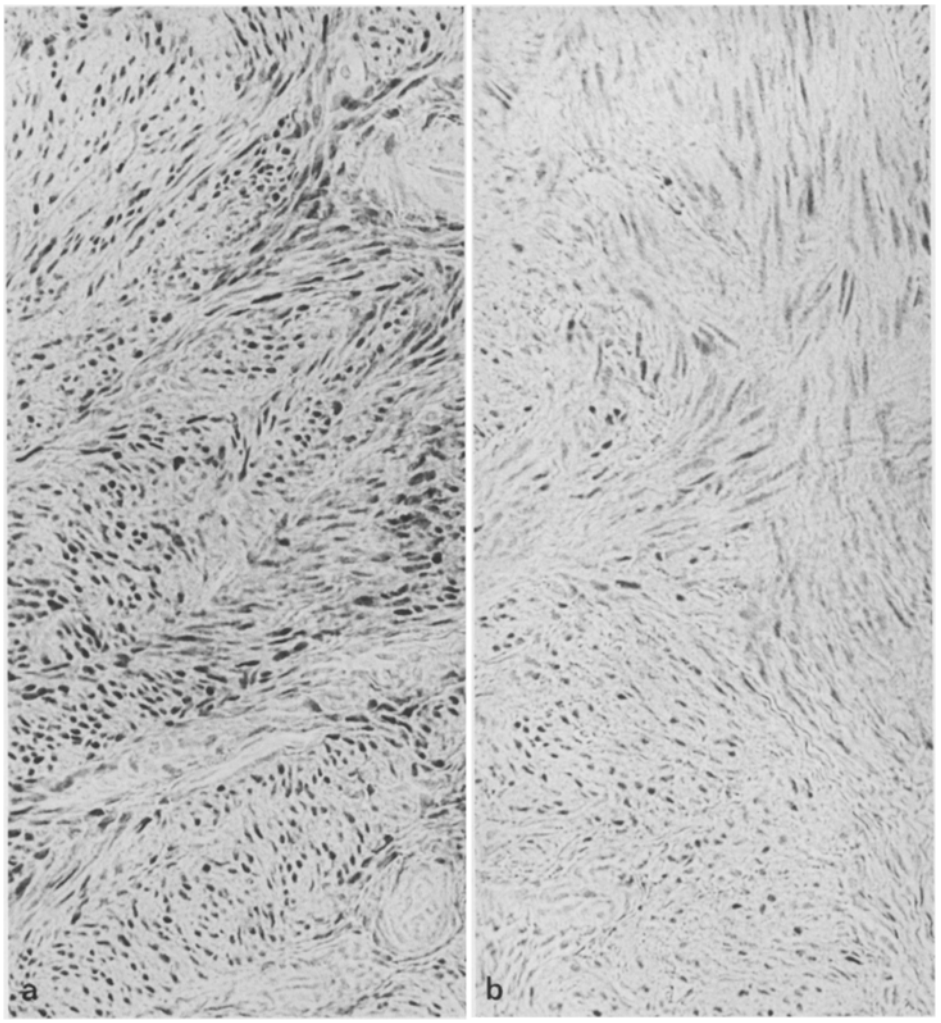

Fig. 3a and b. Follicular phase myometrium immunostained for ER. Most smooth muscle cells of subendometrial myometrium **(a)** show strong nuclear staining. In the subserosal parts of the myometrium **(b)** the staining intensity is lower and a greater number of nuclei appears unstained (ER-ICA, no counterstain, × 250)

stroma showed a higher precentage of cells with specifically stained nuclei than epithelial cells of the adjacent glands. On the other hand, in the endometrium basalis the reduction of ER localization turned out to be more moderate (Fig. 2). More than half of the glandular epithelial cells displayed nuclear staining. The pattern of stromal nuclear staining of the endometrium basalis paralleled that of the epithelial nuclear staining but was generally less intense.

Pattern and intensity of ER expression in the endometria of menstruating women after systemic therapy with estrogens and gestagens were in accordance with ER localization in proliferative phase endometria. A similar distribution of

ER was seen in the endometria of postmenopausal women. In early pergnancy, only some scattered, isolated basal glands and the surrounding stromal cells showed ERs (Table 1).

Myometrium

During the follicular phase the smooth muscle cells of myometrium displayed predominantly strong ER expression in the majority of nuclei. No change of staining pattern was observed during this phase, but a strong decrease of ER localization took place after ovulation (Table 1). More than 50% of the muscular nuclei in mid- and late luteal phases were ER negative, and the remainder were usually weakly stained. The distribution of ER positive and ER negative muscle cells was not diffuse, but arranged in receptor positive and receptor negative muscle bundles. Generally the muscle cells in the outer, subserosal parts of the myometrium seemed to have less ER than those of the subendometrial parts (Fig. 3). The staining intensity was lower and a greater number of nuclei remained unstained.

Nuclear staining in the myometria of postmenopausal women and women after hormonal therapy paralleled that of follicular phase endometria (Table 1).

In the myometrium of the patient in the early stage of pregnancy, ER localization was weak and confined to a few muscle cells (Table 1).

Cervix Uteri, Ectocervix

In cervical squamous epithelium, ER localization was predominantly confined to the basal layers. In the course of cellular maturation, specific nuclear staining vanished. In menstruating women, cyclic alterations of ER content were evident (Table 2). In the follicular phase most of the basal and parabasal and more than half of the intermediate cells demonstrated strong nuclear staining. The superficial layer did not display labelling to any significant degree (Fig. 4a). Clear changes in nuclear staining throughout the proliferative phase did not occur.

In the post-ovulatory phase, nuclear labelling was diminished, the staining intensity and the number of specifically stained cells of the basal and parabasal layers decreased and the loss of ER localization during maturation accelerated (Fig. 4b).

In the postmenopausal squamous epithelia without local estrogen application, no differences in specific nuclear staining were seen when compared with proliferative phase epithelia of menstruating women. In contrast, local estrogen treated postmenopausal squamous epithelia almost completely lacked nuclear ER localization, as did the cervical squamous epithelia of premenopausal, hormonally treated women. In early pregnancy considerable nuclear labelling of squamous epithelial cells was seen (Table 2).

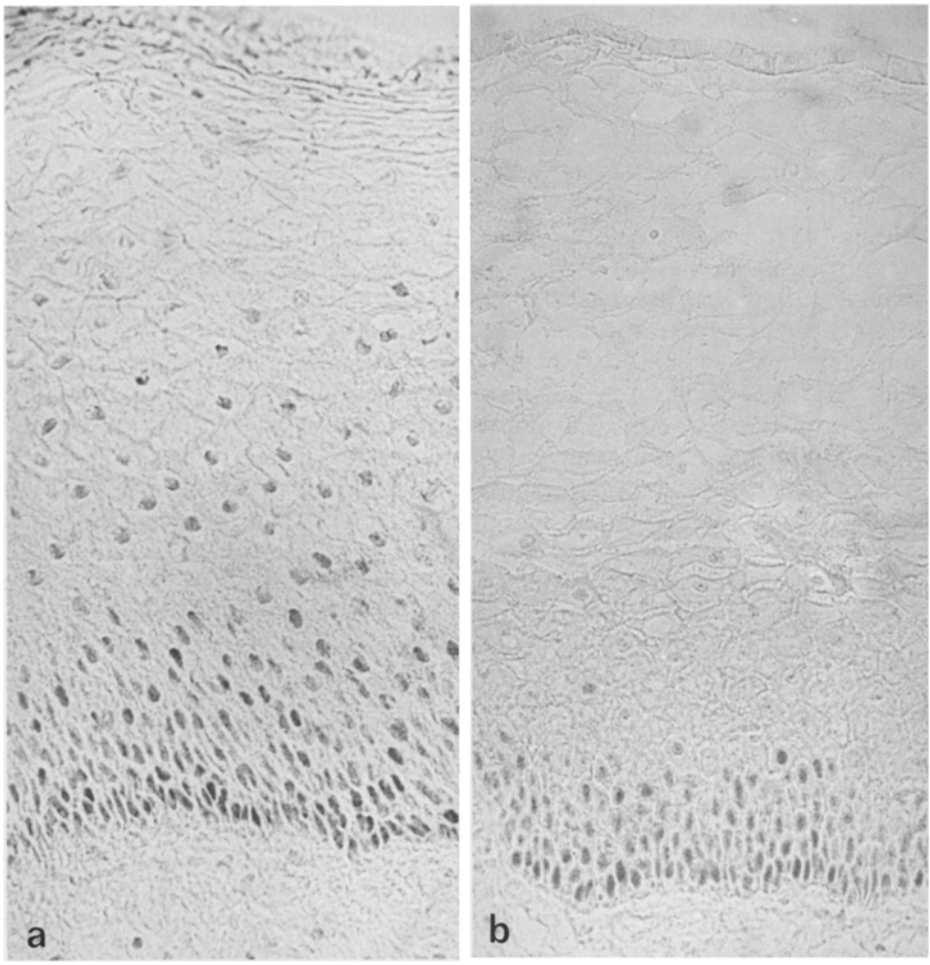

Fig. 4a and b. Squamous epithelium of the cervix uteri immunostained for ER. **a** Follicular phase epithelium. Specific nuclear staining is confined to the basal and intermediate cell layers, the superficial layer displays no ER (ER-ICA, no counterstain, × 300). **b** Luteal phase epithelium. Specific staining is confined to the basal cell layer. The intermediate and superficial layers lack ER (ER-ICA, no counterstain, × 300)

Endocervix

Columnar mucous epithelial cells displayed a high degree of labelling in all samples. The dependence on hormonal alterations in menstruating women turned out to be small (Table 2). The majority of proliferative phase columnar epithelial cells displayed predominantly strong nuclear staining. After ovulation the staining intensity decreased little and there seemed to be a slight increase in the number of unstained cells. More distinct variations throughout the cycle were seen in cervical stromal cells. During the follicular phase, on average 80%

Table 2. Estrogen receptor specific staining of endocervix and ectocervix according to hormonal status

Hormonal stage	Endocervix		Ectocervix		
	Glands	Stroma	Basal	Intermed.	Superfic.
Normal biphasic menstrual cycle					
Early proliferative $n = 6$	+++/++	++/+++	++/+++	++/+	−/+
Late proliferative $n = 5$	+++/++	++/+++	+++/++	++/+	−/+
Early secretory $n = 3$	+++	++/+++	++/+++	++/+	−
Midsecretory $n = 1$	++	++	++	−	−
Late secretory $n = 3$	++/+++	++/+	++	−/+	−
Postmenopause					
Without hormonal treatment $n = 7$	+++/++	+++/++	++/++	++/+	−/+
Local estrogen treatment $n = 2$	+++	+++	+/−	−	−
Hormonal treatment $n = 2$	+++	++	+	−	−
Early pregnancy $n = 1$			+++	++	+

of the stromal nuclei demonstrated ERs. This staining was mostly strong, and sometimes weak. In the post-ovulatory phase, the intensity and percentage of nuclear staining lessened (Table 2).

Nuclear labelling of the postmenopausal endocervix resembled that of proliferative phase samples (Table 2, Fig. 5). Local treatment of the postmenopausal cervix with estrogens did not alter the expression of ER in glandular or in stromal cells. The pattern and intensity of endocervical nuclear specific staining after systemic hormonal therapy resembled that of follicular phase endocervix (Table 2). The cervical tissue from the woman in early pregnancy contained no glands.

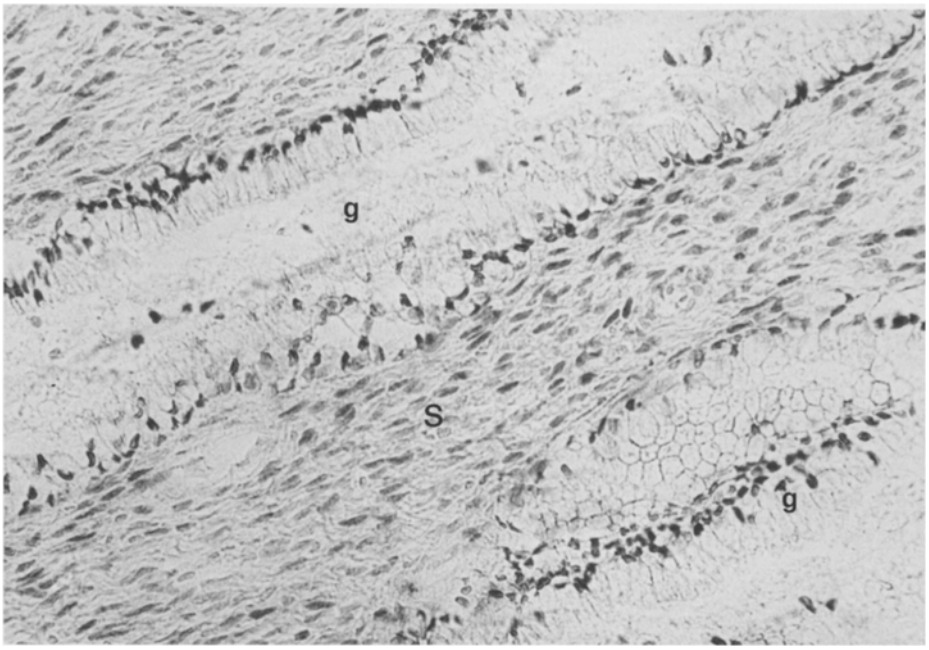

Fig. 5. Immunohistochemical localization of estrogen receptor in postmenopausal endocervix. Columnar mucous epithelial cells appear intensely stained. Most stromal cells exhibit staining (g = gland, s = stroma) (ER-ICA, no counterstain, × 230)

Discussion

The only specific staining obtained by immunoperoxidase method using a monoclonal antibody was nuclear. The absence of specific cytoplasmic staining has already been reported in several studies of normal and neoplastic tissues [4, 13, 16, 18, 28, 29]. This finding coincides with previous results for ^{3H}-estradiol-17β localization [10, 32].

As in previous studies [28, 29], we found that ER detection using monoclonal antibodies was not possible in uterine vessels. Autoradiographic studies also verified their lack of estrogen binding sites [10]. Investigations using conventional receptor assays proved the uterine arteries to have either very low (20 fmol/mg cell protein) or absent receptor levels [19]. Cyclic alterations in microcirculation therefore might be regulated by stromally derived mediators, but not directly by hormones.

Like others [4, 29] the amount of immunohistochemically identified ER in the human endometrium showed a great dependence on hormonal status. Maximal ER localization was displayed in postmenopausal and proliferative phase specimens. Increasing gestagens in the post-ovulatory phase or during pregnancy diminished the expression of ER; this is consistent with immunohistochemical findings in gestagen treated monkeys [22]. These findings compared favourably with the receptor content measured by conventional steroid

binding assays in menstruating, postmenopausal, pregnant and hormonally treated women [1, 6, 20, 21, 23, 25, 30]. However, previous methods of investigation failed to detect the remarkable ER content in the endometrium basalis despite the strong reduction of ER in the more superficial layers (endometrium functionalis) in the late secretory phase and early pregnancy. The presence of ER may permit these endometrial cells, thought to be progenitor cells during the next cycle [26], to be distinguished from the endometrium functionalis, which is shed during menstruation or after parturition. Our findings confirmed the results of Press et al. [29] by establishing that glandular nuclear ER localization is more intense than stromal localization, with the exception of the mid- and late phase endometrium functionalis. When analyzing ER content of endometrial stroma and glands separately by biochemical assays, Fleming et al. [8] also detected higher values for epithelium than for stroma, but King et al. [17] did not. The demonstration of ^{3H}-estradiol-17β binding in animal endometrium using autoradiographic or immunofluorescence methods also produced conflicting results: higher [34] versus equal amounts [7] for epithelium than for stroma. In proliferative phase endometria, binding of fluorochrome-labelled estrogens to epithelium exceeded stromal binding, whereas in secretory phase endometria this relation was reversed [3].

As with biochemical measurements [5, 25] the myometrium of the corpus uteri showed a similar dependence on hormonal stage when compared with the endometrium.

Unlike the endometrium and myometrium, the endocervical mucosa showed less dependence on the menstrual cycle. The endocervical glands underwent minimal changes in nuclear ER content. Although the endocervical stroma showed cyclic alterations in ER levels, their reduction after ovulation was less marked than in the corresponding endometria. Conventional biochemical assays have likewise revealed somewhat higher or almost equal ER levels in cervical tissues during the follicular phase compared to the luteal phase [14, 25, 27, 31] with the clearest differences being measured in the endocervical mucosa. Our investigations have shown these alterations to be confined to the stroma. Autoradiographic experiments have however established consistent values of ER binding sites throughout the entire cycle equal in glandular and stromal cells [10].

In the squamous epithelium of the cervix significant localization of ER was limited to the basal and intermediate cell layers in accordance with autoradiographic studies [10] and with investigations on animal uteri using monoclonal antibodies [22]. Throughout cellular maturation ER progressively disappeared. Since the proliferative activity of normal squamous epithelium is located in basal cells, the hormonal dependence of cervical squamous epithelium could be expected to be pronounced, although biochemical analyses of ectocervical tissues in toto yielded rather low ER levels [31]. During the menstrual cycle moderate variations of the ER content were observed. As observed in autoradiographic studies [10] the loss of ER throughout cellular maturation accelerated in the post-ovulatory phase. While the post-ovulatory reduction of ER qualitatively resembled the hormonal dependence of the other uterine tissues, marked differences occurred after hormonal therapy and in early pregnancy.

Both local estrogen treatment and combined systemic estrogen and gestagen application minimized the ER expression in squamous epithelium, whereas other uterine tissues, including the adjacent stroma, showed high ER localization comparable with proliferative phase samples. This is indeed intriguing, since therapy with estrogens was established to increase ER levels in uterine tissues [17, 23]. By contrast, strong nuclear staining was displayed by squamous epithelium in early pregnancy, although very low ER levels were usually noted [20]. As yet we have not been able to explain this result. The number of cases investigated is still low and further studies using the monoclonal antibody against ER are needed to clarify the issue.

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