Specific estrogen and progesterone binding in human endometrium was studied histochemically using fluorochrome-labeled steroids (estradiol-17β-BSA-FITC and progesterone-BSA-TMRITC), endometrial samples from 36 women being investigated. The binding pattern was similar with both reagents. The relationship between the bindings to glands and to stroma, however, varied with the menstrual phase of the tissue. The specific fluorescence was more intense in the epithelial structures in the proliferative phase. In the secretory phase, the fluorescence from stromal cells was as intense as, or more intense than, that from the glands. The localization of the fluorophores in the glandular epithelial cells also varied by menstrual phase. In the proliferative phase, the fluorescence was most intense in the basal part or the whole cytoplasm of the glandular epithelial cells, while in the secretory phase the fluorescence was most intense in the apical and sometimes also in the basal part of the epithelial cells. (Key words: Estrogen; Progesterone; Endometrium; Histochemistry of steroid binding; Menstrual phases) Am J Clin Pathol 1985; 83: 444-449

Endometrial specimens from 36 women (aged 40 ± 8 (SD) years) were obtained by curettage (33 specimens) or hysterectomy (3 specimens). Eighteen women were operated on because of bleeding irregularities, 16 because of endometriosis, and 2 because of cancer in the uterine cervix. Only samples with a normal histologic appearance were included. No women had been on hormonal medication in recent months.

The date for the last bleeding was noted, and venous blood samples were drawn on the morning of the operation day, or on the following morning, and the serum concentrations of  estradiol-17β, progesterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH) were assayed radioimmunologically.37

The tissue samples immediately were rinsed in isotonic saline at +4 °C to remove blood and mucous and then divided into three parts. One part, about 1–3 mm³ in size, was frozen in liquid propane, cooled by liquid nitrogen within 1 minute, and then stored at −70 °C until required for the histochemical procedure. The second part immediately was placed in a dry plastic bag, stored at −70 °C, and used for biochemical steroid receptor assays within one month. The third part of the tissue sample was fixed in 10% neutral formalin, embed-
ded in paraffin, sectioned, and stained with hematoxylin and eosin for histologic dating according to Noyes and colleagues.24 The cycle day, corresponding to the dominating phase of the tissue specimens, was recorded. A histologic pattern corresponding to cycle days 3–9 in a 28-day cycle was classified as early proliferative, days 10–15 as late proliferative, days 16–19 as early secretory, days 20–22 as midsecretory, days 23–27 as late secretory, and days 28–2 as premenstrual and menstrual endometrium.

Both estradiol-17β-6-carboxymethyl oxime-bovine serum albumin–fluorescein isothiocyanate (E2-BSA-FITC), and progesterone-11α hemisuccinate–bovine serum albumin–tetramethylrhodamine isothiocyanate (progesterone-BSA–TMRITC) FLUORO-CEP® (Estrogen) and FLUORO-CEP® (Progesterone) (Zeus Scientific, Inc., Raritan, NJ) as described by Lee15 were used for the histochemical localization of specific steroid-binding sites. The specificity of the steroid binding of the conjugate has been studied extensively3 and has been found to be acceptable. The specimens were handled in a strictly standardized way as described elsewhere.3 The frozen unfixed specimens were cut in a cryostat to 6–8-μm-thick sections. Two or three consecutive frozen sections were mounted on each gelatinized glass slide, which was immediately placed in a freezer at −70 °C. When the cutting procedure was complete, the glass slides were placed at +4 °C, and the sections were air-dried for 1 hour. After rehydration for a few seconds with 2% BSA in phosphate-buffered saline (PBS), pH 7.4, excess buffer was wiped off and the sections were covered with 0.1 mL of the FLUORO-CEP reagent. One duplicate set of slides for ERe and one duplicate for PRc always were prepared for localization of the estrogen and progesterone binding sites. After incubation for 2 hours in a moist chamber placed on a slow-motion cradle at room temperature, the slides were immersed twice in PBS, pH 7.4, for 30 minutes in each bath. On a fifth slide, consecutive sections were fixed in 10% neutral formalin for at least 15 minutes and stained with hematoxylin and eosin for histologic identification. Only sections containing more than ten well-preserved glands were included.

The sections were examined independently within 24 hours after the incubation by two of us (A.B. and O.L.) according to a standardized schedule. The proportion of fluorescent glandular epithelial cells (none, <10%, >10% but <70%, >70%) and the distribution of the fluorochromes within the glandular epithelial cells (basally, apically, the whole cytoplasm) and the stroma was estimated. A Leitz Orthoplan® fluorescence microscope was used, equipped with a Ploem® illuminator and a HBO2000 mercury lamp. An 460-485-nm excitation filter and a K530 barrier filter were used for FITC and a 535–550-nm excitation filter and a K580 barrier filter were used for TMRITC.

It was possible to estimate the relative proportion of fluorescent epithelial cells because the tissue emitted a weak unspecific fluorescence that permitted the identification of all epithelial structures. This was not possible, however, in the case of the stromal cells, so grading of specific stromal fluorescence was omitted.

The histochemical examination was performed before the results of the biochemical receptor assays or serum hormone concentrations were known.

The quantitative ERc assays were performed using isoelectric focusing on polyacrylamide gel,9 and the quantitative PRc assays were performed using a dextran-coated charcoal (DCC) method originally described by Korenman.414 The amount of receptors found was related to the amount of cytosol protein assayed according to Lowry and associates.19

Results

The number of cases with different histologic phase pattern appears in Table 1.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Cycle Days</th>
<th>No. of Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early proliferative</td>
<td>3–9</td>
<td>2</td>
</tr>
<tr>
<td>Late proliferative</td>
<td>10–15</td>
<td>14</td>
</tr>
<tr>
<td>Early secretory</td>
<td>16–19</td>
<td>6</td>
</tr>
<tr>
<td>Midsecretory</td>
<td>20–22</td>
<td>6</td>
</tr>
<tr>
<td>Late secretory</td>
<td>23–27</td>
<td>7</td>
</tr>
<tr>
<td>Premenstrual and menstrual</td>
<td>28–2</td>
<td>1</td>
</tr>
</tbody>
</table>

Table 1. Distribution of Endometrial Samples in Relation to Menstrual Phase

There was a good correspondence between the histologic dating of the endometrium and the peripheral plasma hormone concentrations in all cases. The concentration of FSH was consistently within the normal range for premenopausal women.

The binding pattern was the same irrespective of whether the sample was obtained by curettage or hysterectomy. In most cases the extent and the site of fluorescence were similar with both conjugates.

The relationship between the binding of fluorophores to glands and to stroma varied with the menstrual phase of the tissue. In the proliferative phase the fluorescence was more intense in the epithelial structures than in the stroma (Fig. 1). In the secretory phase the reverse pattern was found, the fluorescence from the stromal cells being as intense as or even more intense than that from the glands (Fig. 2).

The localization of the fluorophores in the glandular epithelial cells varied with menstrual phase. In the proliferative phase, when the glands were straight or


slightly tortuous and narrow, a strong specific fluorescence was observed mainly in the basal parts of the epithelial cells or in their whole cytoplasm (Fig. 3). On the other hand, the tortuous glands found in the secretory phase displayed a weaker specific fluorescence that was located apically and sometimes basally as well, whereas the central part of the cell body was less fluorescent (Fig. 4). Saw-toothed glands in late secretory and menstrual phase were always negative. In very early proliferative phase the epithelium had not yet became fluorescent and the stroma was still fluorescent (Fig. 5). When present, the epithelium covering the endometrium was invariably positive, even in sections where the glandular epithelium was negative, and the binding was not influenced by the menstrual phase. When stromal fluorescence occurred, this appeared to be more intense in the upper part of functionalis than in the deeper layers of the endometrium.

The results of the biochemical assays are presented in Figure 6. In six samples the tissue was too sparse to allow both types of biochemical receptor assays to be done, and only PRc was assayed. The highest values of ERc were found in late proliferative and early secretory phase and those of PRc in the late proliferative phase.
Discussion

The present study showed that the distribution of the specific fluorescence within the cytoplasm of the glandular cells and its distribution between the glandular structures and the stroma varied during the menstrual cycle. In the proliferative phase both conjugates were detected preferably in the epithelial structures, often localized basally or to the whole cytoplasm of the cells, whereas the binding to stromal cells was less noticeable. This is compatible with the morphologic appearance during this phase, when high mitotic activity in the epithelial cells denotes estrogen effect. During this phase the stromal cells are small, with poorly developed organelles and scanty cytoplasm. Stromal cells in mitosis generally abound until in late proliferative phase. Nonetheless, electron microscopy does reveal a slow maturation of these cells, with gradual enlargement of their nuclei and development of cytoplasmic organelles. It may be that the priming of the stromal cells by estrogen has a rapid effect on cell replication but a slower effect on cytoplasmic differentiation. In the secretory phase, the distribution of the specific fluorescence tended to be reversed, in that specific fluorescence often was most pronounced in the stroma instead. During this phase mitotic activity is uncommon in the epithelial cells and the influence of progesterone induces the formation of vacuoles, which are located basally in the early secretory phase but move to the apical region of the cells during the midsecretory phase. However, mitoses occur in the stroma when the predecidual cells, rich in cytoplasm, develop as an effect of the second estrogen surge. This may explain the more intense fluorescence seen in the stromal cells of the secretory endometrium.

The ERc and PRc levels found in this study corresponded to those previously reported. In some studies, histochemical semiquantitative evaluation of steroid binding sites has been compared with steroid receptor concentrations assayed by biochemical methods. Results have been contradictory, however, and in our opinion such comparisons can be considered invalid for a number of reasons. Although specificity studies have shown that the histochemical method has a reasonable degree of ligand specificity, it is still not known whether the two methods demonstrate the same types of steroid binding. Even if this were the case, however, there remain several other objections to the validity of such comparison. The histochemical method visualizes the steroid binding in separate cells that might be disseminated unevenly throughout a tissue sample, while the biochemical method determines the quantity of steroid receptors from a tissue homogenate that also contains varying amounts of nonreceptor-bearing tissue components.

The distribution of the endometrial glands varies in different parts of the uterine cavity and also at different depths of the endometrium, and the steroid receptor concentration has been found to decrease stepwise from the fundal part to the cervix region. Histochemically the extent of steroid binding has been found to differ between different areas of the endometrium, being commonly more extensive in the functionalis. Furthermore, in most tissue sections some glands differ from the majority of glands with respect to the menstrual phase pattern and also with respect to binding distribution of the fluorophores. This corresponds to morphologic variations found in hematoxylin and eosin stained sections. Since samples taken for biochemical assay differ from those used for histochemical examination and may even derive from different parts of the uterine cavity, this is a further important reason why the results of the two methods are not comparable from a quantitative point of view.

This, however, does not exclude the possible relevance of qualitative comparison. Our finding that there exists a cyclic covariation of the ERc and PRc levels, determined biochemically, and of the extent and distribution of the specific steroid binding, demonstrated histochemically, may be of physiologic significance.

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