Diagnosing

the

Endometrial Biopsy

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INASMUCH AS THE REMARKS I am about to make are based on endometrium obtained by hysterectomy, rather than by biopsy, you may feel that the title is somewhat of a misnomer. However, for purposes of accurately studying and depicting the endometrial cycle, large blocks of carefully selected endomyometrium are preferable to material obtained by endometrial biopsy. This is in spite of the fact that we are necessarily forced to evaluate the endometrium of a patient, suspected of being sterile, by examination of such biopsy material.

The criteria used in interpreting endometrium at the Free Hospital for Women were originally elaborated by Dr. Rock[1] and his collaborator, Marshall Bartlett. The application of these criteria to a good many thousand endometrial specimens during the past seven years is largely responsible for what I am able to tell you today.

The stages subsequently to be described and depicted are based upon a classic menstrual cycle of twenty-seven days elapsed time, with day one and day twenty-eight coinciding, since it is customary to regard menstruation as occurring every twenty-eight days. Actually, such regularity is the exception rather than the rule; the variations in the length of recorded cycles being due to variation in the length of the proliferative rather than the secretory phase. The latter, or progestational phase, tends to be more constant in length and pattern. Upon these and other facts depends the consensus of opinion[2] that ovulation occurs approximately 14 ± 2 days prior to the next expected menstrual period.

In submitting an endometrial biopsy from a patient suf-

fering from sterility one hopes the pathologist will be able to say whether (and preferably when) the patient ovulated and whether there are any pathological conditions present which are incompatible, *per se,* with pregnancy. Parenthetically, I may add, such things as cancer, tuberculosis, leiomyosarcoma, endometritis and proliferative hyperplasia have all been found in the uteri of patients who originally came to the physician because of sterility.

THE PROLIFERATIVE PHASE

This phase of the cycle is variable but in the average patient is about fourteen days in length. However, in cases of irregular or prolonged cycles it may be forty to fifty days in length or even longer. Since there are not such distinctive day-by-day histological criteria in the proliferative as in the secretory phase and since the former phase varies in length we tend to diagnose the stages of proliferation as being early, mid- or late. Low and medium power illustrations* of these representative phases are seen in Figures 1 to 3, respectively.

The early proliferative phase coincides with about the seventh day of a classic cycle. The surface epithelium has repaired but is still thin in places—especially at a distance from the gland mouths. The entire thickness of endometrium naturally has been reduced but to a variable degree. Apparently, in some patients, as much as the upper two-thirds of the endometrium is lost, whereas in others only the upper third is sloughed off. Upon this variation will depend the respective amounts of glandular regeneration and involution which

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* All plates are composed of a low and medium power photomicrograph of each of the endometria depicted. The field represented within the higher-powered picture is, in each instance, a part of the lower-powered picture.

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FIGURE 1. Early proliferative endometrium, corresponding to about the seventh day of a classic menstrual cycle. Note the imperfectly epithelialized surface, the straight tubular glands of pseudostratified epithelium and the somewhat edematous "naked-nucleus" type of stroma. Upper × 75 and lower × 150.
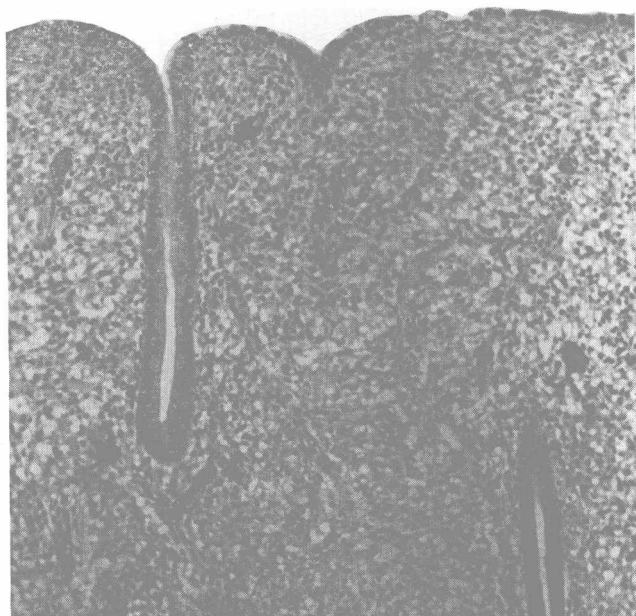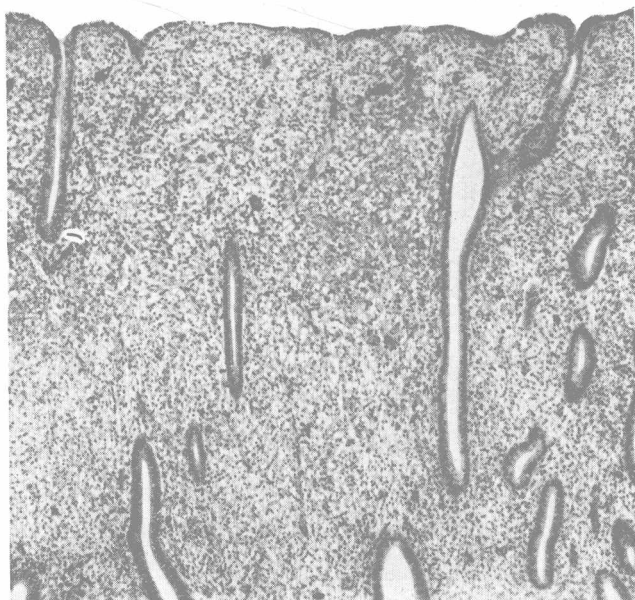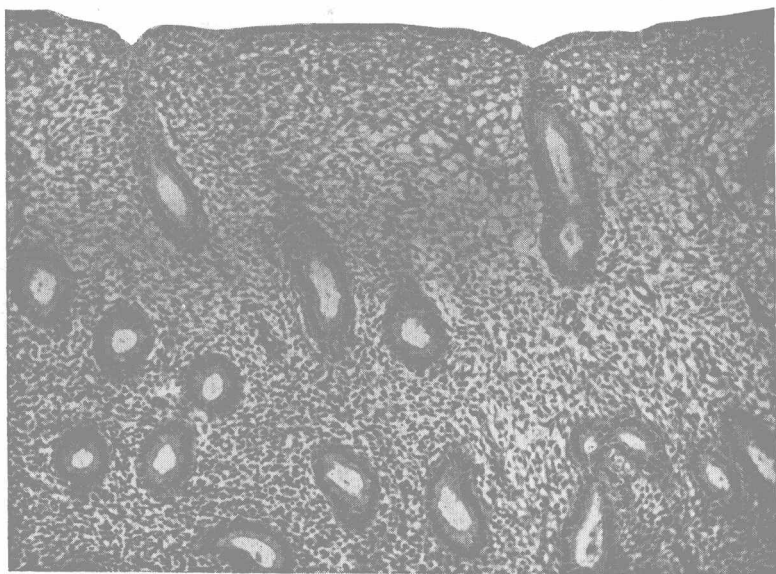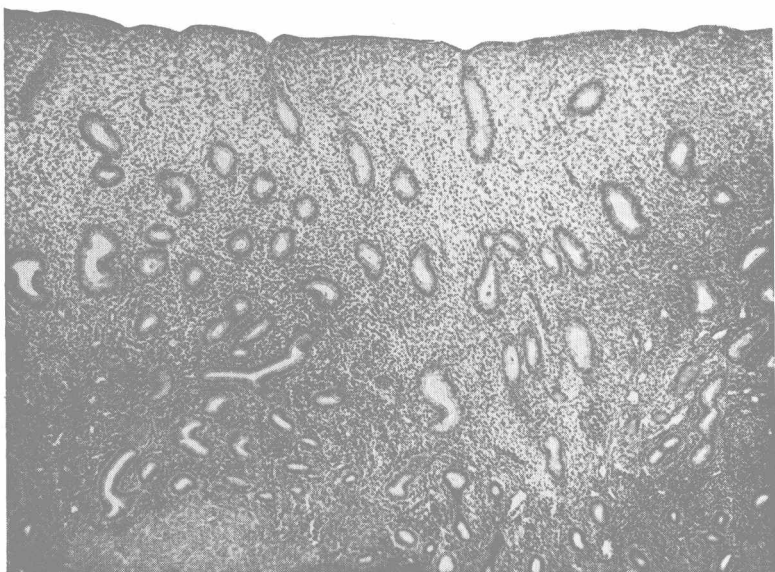
will follow menstruation. Thus, if only the upper third of the mucosa is lost, the middle third needs only to involute rather than to regenerate completely. Because such diametrically opposed phases as proliferation and involution of a secretorily-exhausted gland may occur in the same field, or even the same gland, it is often difficult to interpret biopsies taken late in menstruation, or early in the repair period even though they be normal.

There is likewise a variation in the amount of endometrial stroma which may be lost at menstruation. Since only the upper third of the stroma tends to undergo a pre-decidual metamorphosis and is usually lost at menstruation, there remains only the essentially basic type of endometrial stroma, even though it may be much or little in amount. Hence, involution does not tend to complicate the stromal picture as it does the glandular one.

The glands in the early proliferative phase are straight or at most only slightly tortuous. The nuclei within the tall columnar epithelium are arranged in pseudostratified fashion, i.e., the nuclei are at different levels although there is only one layer of cells. Mitoses are frequent.

The stroma is likewise growing or regenerating rapidly and mitoses within the small stellate cells are fairly numerous. Stromal cells, in general, except for their predecidual phase, are of this type, i.e., stellate with relatively large nuclei and little cytoplasm. Their processes anastomose with those of other cells and form a spongy meshwork within the interstices of which is a variable amount of edema fluid. There may be a few phagocytic cells present following the previous menstruation.

FIGURE 2. Mid-proliferative endometrium, corresponding to about the tenth day of the average menstrual cycle. This particular endometrium is thinner than that shown in preceding plate even though the former is more mature than the latter. Note in the upper picture that the upper half of the endometrium (newly-regenerated) is edematous with the relatively straighter glands than in the basal (residual) portion. Upper × 75 and lower × 150.

A word should be said about lymphocytes within the endometrium. Focal accumulations of such cells within the middle and basal thirds are within normal limits. Plasma cells, however, always mean chronic inflammation. Phagocytic cells normally infiltrate the stroma only preceding, during and just after menstruation. Otherwise, their presence indicates acute inflammation of the endometrium.

It should be emphasized that the basal third of the endometrium, by and large, takes little part in the cyclical variation of this tissue. It apparently acts as a germinal bed, so to speak, from which successive crops of endometrium are grown. It isn't until late in the secretory phase that there is any suggestion of secretion within this portion of the endometrium. The relative lack of such cyclical change in the basal layer is best seen in Figures 8 and 12, representing the twenty-second and twenty-sixth days of the cycle.

The mid-proliferative phase, coinciding with about the tenth day of an average cycle, differs from the preceding phase only in degree. The surface epithelium is now a more regular layer and the glands are relatively more tortuous. The glandular epithelium itself, however, is still actively growing and is of pseudostratified type. The stroma still continues to grow in amount but is unchanged as to cell type. The total thickness of the endometrium may vary somewhat from portion to portion of the uterus and quite markedly from patient to patient. Thus Figure 2, of mid-proliferative endometrium, shows the latter to be appreciably thinner than that shown in Figure 1, even though the thinner endometrium is the more advanced of the two.

The late proliferative phase (Figure 3) is characterized by a somewhat undulating surface covering a closely-packed but still "naked-nucleus" type of stroma. The variable edema

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FIGURE 3. Late proliferative endometrium, corresponding to about the fourteenth day of an average cycle. Note the undulating surface, well covered by an intact epithelial layer. The glands are extremely tortuous but still actively growing. The stroma has lost its edema. Upper × 75 and lower × 150.
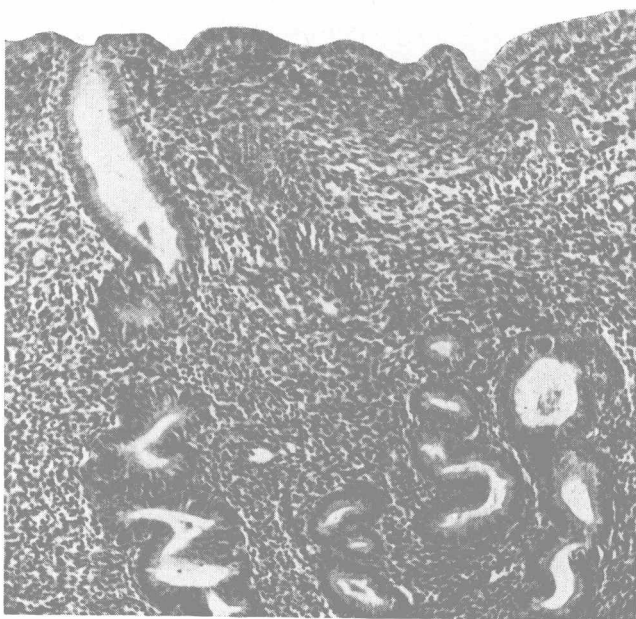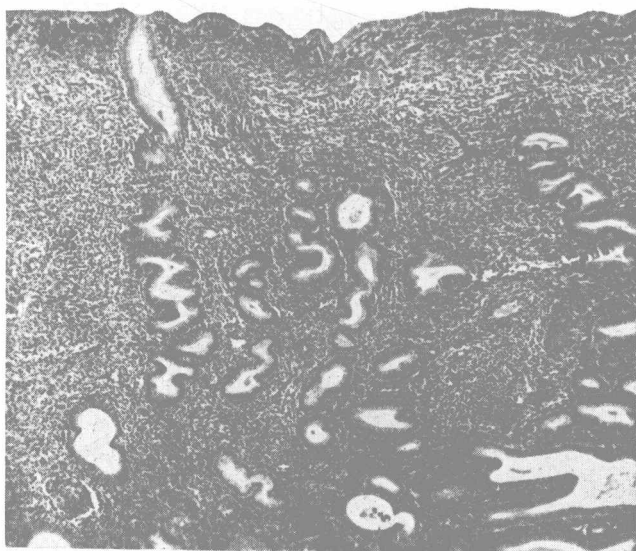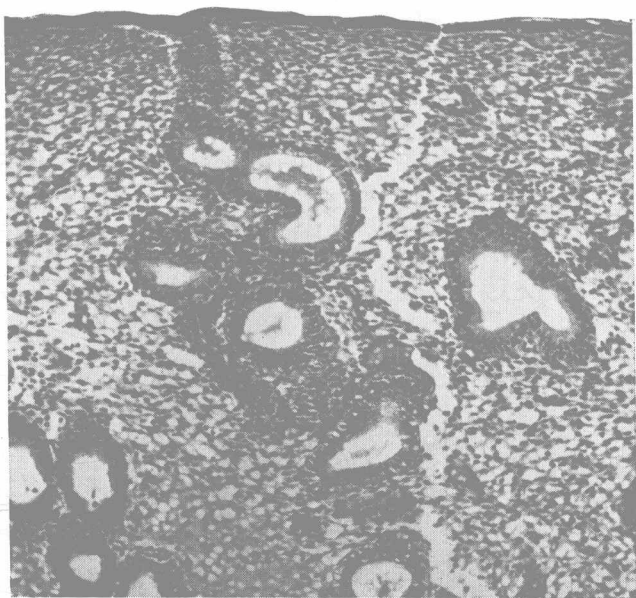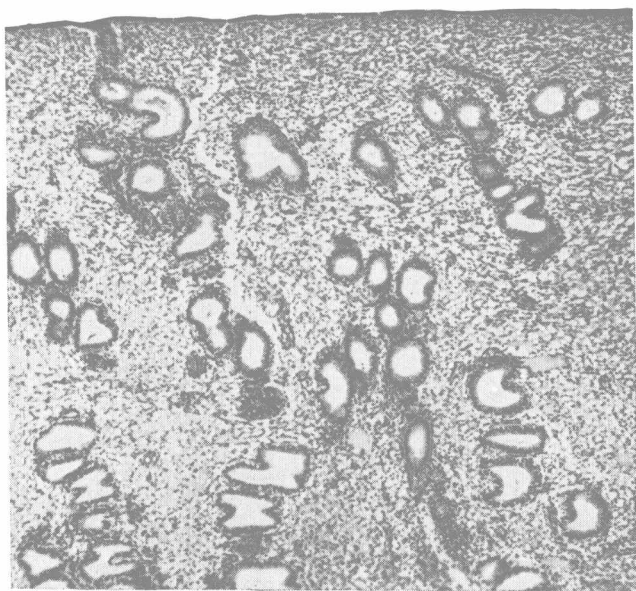
of the earlier phases tends to disappear just before ovulation. Thus, the thickness of the endometrium is about the same as before but the cellular concentration is greater. The glands are continuing to grow in length but because the stroma doesn't easily accommodate their growing bulk they become increasingly tortuous. Secretion during the proliferative phase is minimal and appears to consist only of small amounts of thin mucinous fluid. The latter contains no glycogen as does the secretion of the progestational phase.

THE OVULATION PHASE

No appreciable change in endometrium occurs in the twenty-four to thirty-six hours following ovulation. Thus we never date endometrium as of the fifteenth day, not because this phase does not exist, but merely because we are not able to recognize it. The endometrium continues to proliferate under the stimulus of the ruptured follicle which is metamorphosing into a corpus luteum. It will be recalled that there is only a slight fall in the estrogenic level at the time of ovulation. It is not until some time during the 16th day that distinctive changes appear in the glands which signalize corpus luteum activity and, therefore, presumably ovulation. It is clear, then, that if one wishes to determine the fact of ovulation it would be unwise to biopsy the patient prior to her 16th day, at the earliest, because the morphologic criteria of ovulation have not yet appeared.

THE SECRETORY OR PROGESTATIONAL PHASE

The sixteenth day of the average cycle is characterized by some increase in tortuosity of the glands, the presence of mitotic figures which began prior to secretion and the ap-

FIGURE 4. Early secretory endometrium, corresponding to about the sixteenth day of an average cycle. The patient has ovulated about thirty-six hours previously. Note the tortuous glands whose epithelium shows an irregular degree of basal vacuolization. The stromal edema is variable at this phase but present in this particular specimen. Upper × 75 and lower × 150.

pearance of glycogen-laden vacuoles in the base of the glandular epithelial cells (Figure 4). Not all such cells contain vacuoles so that the epithelium presents a moth-eaten appearance. One gains the impression that vacuolization occurs first in the cells whose nuclei are not in the basal position. This feature, i.e., irregular basal vacuolization of cells plus the presence of a few mitotic figures is diagnostic of early secretion. It can be superficially confused with the eighteenth to nineteenth day (Figure 6) since at that time there is also basal vacuolization. It must be emphasized at this point, however, that the latter stage represents the residual remains of epithelial secretion and that some of the latter is now in the lumen of the gland. The nuclei are now being or have been pushed back to a basal position so that both the nuclei and the basal vacuoles are fairly regular. Furthermore, there are no mitotic figures at this stage (eighteenth to nineteenth day), apparently because no new mitotic activity begins after the onset of secretion.

The seventeenth day, not illustrated, is characterized by the most marked vacuolization of the cell. Almost two-thirds of the basal portion of such glandular epithelium will contain glycogen-laden fluid. Some of the glands in Figure 6 still show this phase, whereas most of them have progressed beyond it. No two glands, or indeed any two parts of the same gland, are in exactly the same phase of secretion. Edema of the stroma which may still show a few mitoses is variable but often present, as in the examples shown.

It should be mentioned here that the fertilized ovum reaches the uterine cavity on about the eighteenth day of the cycle. One might expect such a fact because of the appearance of secretion at this time within the glandular lumina and hence the uterine cavity. Such expectation is justified because of the recovery of a segmenting ovum from the uterine cavity of a specimen removed by Dr. Rock on the eighteenth day of the cycle.[3] The egg is probably not normal, since some of its blastomeres are multinucleated, as shown in Figure 5.

Thus the period of active secretion into the lumen of the gland from the eighteenth through the twentieth days of
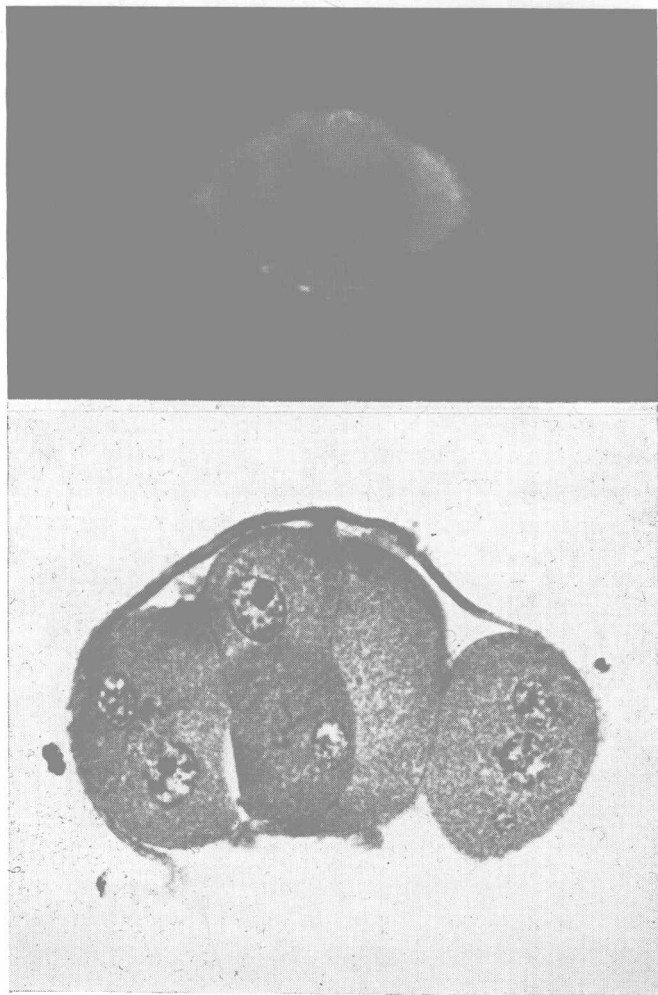

FIGURE 5. A segmenting human ovum of about four days, fertilization age, probably not normal because of the multinucleated blastomeres. This specimen was obtained from the uterine cavity of a patient whose endometrium was in the eighteenth day of its cycle. Upper view shows the whole specimen photographed by reflected light × 250. Lower view is a cross section showing five large blastomeres, some of which contain more than one nucleus. The zona pellucide (shell) is broken below but intact above. × 600.
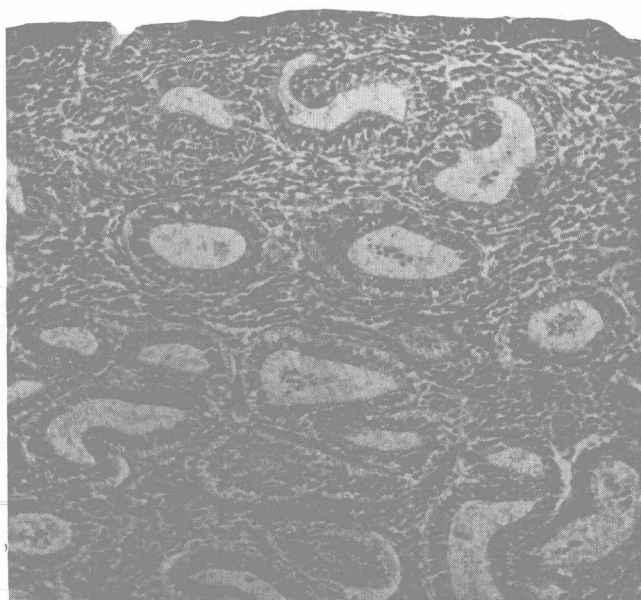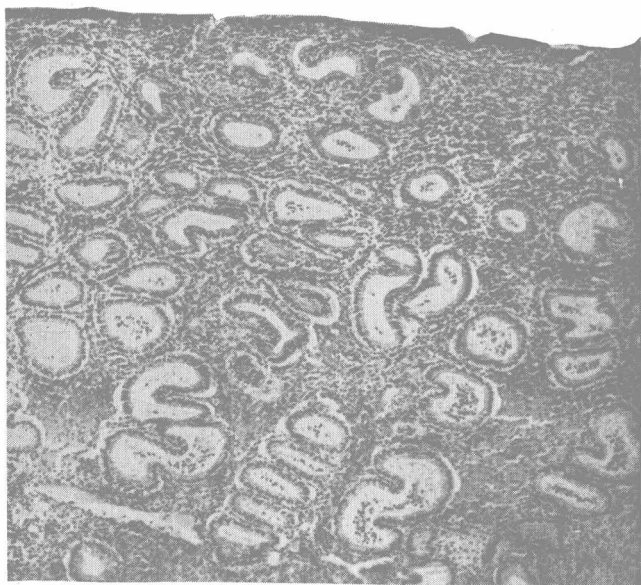
the cycle coincides with the period when the ovum is free in the uterine cavity and must, perforce, derive its nourishment from the uterine secretion.

The period during and immediately after implantation of the blastocyst is shown in Figures 7 to 10 and includes the twentieth through the twenty-fourth days of the cycle. From Dr. Rock's and my data[4,5] the ovum probably implants on about the twentieth day although this may be subject to some variation. At any rate, the only two seven-and-one-half-day ova in existence (one shown in Figure 8[4]) are both implanted on twenty-two-day endometrium and appear, on the basis of their development, to have been attached about thirty-six hours.

The implantation and post-implantation phases of the endometrial cycle are characterized by a diminishing secretory activity of glandular epithelium although the glands themselves become more tortuous and contain considerable amounts of inspissated secretion within their lumina. The stroma reaches its peak of physiologic edema by the twenty-second day as is well shown in Figure 8. Perhaps this is because the ovum is implanting at that time. An edematous endometrium, it would seem on purely teleological grounds, might well facilitate the early stages of implantation. With the decline of edema, there begins on the twenty-fourth day, the metamorphosis of stroma about the spiral arterioles. This predecidual reaction is characterized by the reappearance of mitotic figures in the perivascular stroma with swelling of the latter cells, associated with which there is either imbibition or formation of glycogen within their cytoplasm.

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FIGURE 6. Secretory endometrium, corresponding to the eighteenth-nineteenth day of the cycle. Note the active secretion within the glandular lumina (the ovum is free in the uterine cavity at this time). There is still marked basal vacuolization of the glandular epithelium although both the nuclei and vacuoles are now lined up in a fairly orderly fashion. The stroma is resting, although composed of the same type of cells as in the proliferative phase. Upper × 75 and lower × 150.

In general, the peak of secretory activity and stromal edema coincides with the peak of corpus luteum activity as shown by Dr. Brewer.[6] Dr. Rock's[7] and my studies on corpora lutea confirm Dr. Brewer's work, the corpus luteum appearing to reach its peak of secretory activity about the twenty-second day of the cycle.

The twenty-second day of the cycle coincides approximately with the upper limit of what Dr. Hamblen terms "immature progestational endometrium" and would be termed "P minus 6" by him. It must be emphasized at this point that endometria so termed have been removed at the onset of bleeding and not on the apparent date corresponding to the morphology. Such cases bleed at the regular time without altering the cycle length more than three days in either direction. It is most important and must be correlated with an improperly functioning corpus luteum or an endometrium which is incapable of proper progestational response to a normal corpus luteum.

It would be most interesting and valuable to correlate the ovarian and endometrial findings in such cases. Unfortunately, it is not possible to do this with biopsy material from sterility patients unless a laparotomy were done simultaneously. In this event, insufflation probably and properly would precede any sampling of the endometrium—an unwise procedure in view of the possibly resulting endometriosis were menstrual endometrium to land on the pelvic peritoneum. Hence, we must obtain correlative data from the occasional non-sterility patient who happens to be curetted at the onset of flow and who also has one or both ovaries removed.

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FIGURE 7. Secretory endometrium, corresponding to about the twentieth day of an average cycle. Note the markedly tortuous glands containing secretion in their lumina but little in the glandular epithelium. The stroma is beginning to become physiologically edematous. (This edema is constant for the period of early implantation of the ovum but is variable or absent at other times.) Upper × 75 and lower × 150.