their bibliographies, for these will consolidate for the reader references, which he might otherwise search long to find. Abstracts will serve an obvious purpose. From time to time Fertility & Sterility will publish reports of committees appointed by the American Society for the Study of Sterility to review current practices and policies in the treatment of infertile couples, as for example, Artificial Insemination. The journal will publish notices of meetings bearing upon its subject, correspondence, pertinent general news, and it may, if there is a demand, develop a section devoted to questions and answers.

The immediate purpose of Fertility & Sterility is to assist clinicians in the treatment of infertile couples. In itself; this is a worthy end. But there is a further objective. We are today reminded often enough that the world is beginning to contain too many human beings. If war and famine and pestilence are conquered then indeed there may be too many. Before that time comes it will be the duty of medical men, and particularly of those physicians interested in fertility, to discover not merely how more, but how fewer people, can be bred. We must learn what factors produce idiots, imbeciles, morons, and mongols; what genetic attributes if any produce epileptics, psychotics, and criminals; what influences lead to the yet unexplained congenital abnormalities; and we must see to it that unfortunate defectives are not conceived to suffer in and to deplete human society. To improve the quality of man, not to increase the number of human beings, should be the ultimate goal of all investigations of fertility and infertility. This journal is founded to further that design.

PENSOLE TOMPKINS

Dating the Endometrial Biopsy

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The original observation of histologic changes in the endometrium by Hitchcock and Adler, amplified by Schroeder, Novak, O'Leary, and Hartman, and correlated with coincident changes in the ovary by Frankel, and by R. Meyer, established the sequence of preovulatory (proliferative) and postovulatory (secretory) morphologic phases. Further work in the monkey by Corner, Allen, Hartman, and Hinaw, and in man by Kaufman, and by Clauberg, disclosed the nature of the hormonal control of the cycle. Development of the biopsy method greatly aided correlation of the histology of the endometrium with the stage of the cycle (Herrell and Broders; Sturgis and Meigs; Campbell, Lendrum, and Sevringhaus; Kott and Parker). The increasing need for a more quantitative interpretation, especially in infertility studies, led Rock and Bartlett to study the endometrial picture as it changes with each day of the cycle. The original criteria have been modified somewhat in the past ten years (Hertig) during which time approximately 8,000 endometrial biopsies have been examined in this laboratory. It is the purpose of this paper briefly to review these criteria, analyze their accuracy, and evaluate the usefulness of dating the endometrial biopsy. Not all variations that occur in the endometrium are useful for dating. For example, the tortuosity of the endometrial glands and the collaring of the

We are indebted to Dr. C. H. Duparc for the use of his private cases; to Dr. P. V. Latou for the use of his endometrial dating chart prepared in 1947 from the criteria used in the pathology laboratory of the Free Hospital for Women, and to Dr. J. R. Mitchell for use of his survey of 856 sterile cases.

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spiral arterioles increase so gradually that quantitative differences from day to day cannot be detected. We propose to consider, therefore, only those few components that we feel change rapidly, constantly, and characteristically enough under hormonal influence to indicate how long such action has been effective. Figure 1 summarizes the criteria most useful in endometrial dating.

PROLIFERATIVE PHASE

This phase is variable in length and day-by-day changes are not sufficiently distinct to permit recognition of sub-phases other than perhaps early, middle, and late stages. Furthermore, biopsies are rarely taken during the pre-ovulatory phase since the proof of ovulation is one objective of the procedure in fertility studies. A brief description, therefore, will suffice to review the broad morphologic progression of the proliferative phase. Early proliferation (Fig. 2), which in the classic twenty-eight-day cycle lasts from about the fourth through the seventh days, can be identified by the thinness of the regenerating surface epithelium, especially between the mounds of the glands. Most of the straight, short, narrow glands are of proliferative type, as shown by epithelial mitoses. Some, having weathered the recent menstrual storm, still show involutinal changes such as in the cuboidal, ragged, inactive epithelium typical of "secretory exhaustion." The stroma is compact and undergoes some mitotic activity, its cells being stellate or spindle-shaped with anastomosing processes. The nuclei are relatively large because of scanty cytoplasm.

The rapid-proliferative phase (Fig. 3), lasting from about the eighth through the tenth days of the classic cycle, is transitional and is recognizable by columnar surface epithelium, longer, curving glands, and a variable amount of stromal edema, which tends to regress. Mitoses are numerous in the "naked nucleus" type of stroma. The late proliferative stage (Fig. 4) lasts from about the eleventh through the fourteenth days, and is characterized by a somewhat undulant surface with tortuous glands showing active growth, and pseudotratification of the epithelium. There is a moderately dense, actively growing stroma.

SECRETORY PHASE

In contrast to the proliferative stage, progressive changes occur in the endometrium of the secretory phase at a rate that we believe justifies recognition of daily periods in most cases. Largely for practical purposes, to these

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**Figure 1.** This chart has been slightly modified from an original by P. F. Laidlaw. The curves represent approximately estimated quantitative changes in each of eight factors we consider most helpful in dating endometrium.
periods we have assigned "dates," so that each numerical designation represents a certain morphologic picture. Since there is some variation in the length of the secretory phase, our dates are somewhat arbitrary and represent sequential phases characterized by the histologic appearance peculiar to each, rather than actual days of the cycle. The accuracy of correlation between these numbers and observed menstruation, and of presumed ovulation will be given later. We have applied dates as of a classic twenty-eight-day cycle, assuming that ovulation occurs on the fourteenth day and menstruation on the twenty-eighth.

We now believe that no appreciable change in the endometrium occurs in the thirty-six- to forty-eight-hour period following ovulation. Thus, we do not date endometrium as of the fourteenth or fifteenth day because we are unable to identify this period. On the sixteenth day, considered as the second postovulatory day (Fig. 5), subnuclear vacuolation of the gland epithelium becomes prominent. One may not safely diagnose ovulation on the basis of a few small, scattered vacuoles, however, since they may occasionally occur in anovulatory cycles, as seen in Figure 6. This patient "menstruated" two days following this biopsy in a cycle presumed to be anovulatory on the basis of a monophasic basal body temperature record. Moreover, Hsiao and Greep have shown that in castrated monkeys subnuclear vacuoles appear after estrogen therapy alone. Once on guard, an observer will not confuse these rare, small, and irregularly distributed basal vacuoles with the larger, more regular vacuolation characterizing early progesterone effect.

At first the vacuoles push some nuclei into an exaggerated pseudostratification (Fig. 5); then, as all the nuclei are pulled centrally they line up and lose the pseudostratified configuration. Increase in the diameter and tortuosity of the glands accompanies this alignment of the nuclei. On the seventeenth, or third postovulatory day (Fig. 7), the picture is one of a more or less orderly row of nuclei with homogeneous cytoplasm above them, and large vacuoles below.

On the eighteenth day (Fig. 8) the vacuoles have decreased in size, apparently because their contents slip by the nucleus into the cytoplasm near the gland lumen, and thence into the lumen. The nuclei approach the base of the cell, improving their linear arrangement. On the nineteenth day few vacuoles remain. Superficially this phase may resemble the early vacuolation of the sixteen-day phase, but the presence here of intraluminal secretion and absence of pseudostratification and of mitoses serve to distinguish it.
Figure 3. Mid-proliferative endometrium. A: Glands, slightly tortuous; surface epithelium, tall columnar. Stromal edema is not always as marked as in this section. (X 150) B: The glands show numerous mitoses with pseudostratification becoming marked. Note "naked nucleus" type of stromal cell with fine anastomosing processes. (X 400)

Figure 4. Late proliferative endometrium. A: Glands, tortuous; stroma, usually quite dense. (X 150) B: Epithelial nuclei are pseudostatified and are oval in shape. (X 400)
Figure 5. Sixteen-day endometrium (second postovulatory day). A: Clumps, terraces, stratum, dense; cells, consisting of nearly naked nuclei. (X 150). B: Gland nuclei, very numerous; pseud stratification of nuclei exaggerated by subnuclear vacuoles. (X 400).

Figure 6. Proliferative endometrium mimicking sixteen days. A: Biopsy from a clinically non-evaluating patient. Only this area of slide showed subnuclear vacuolation. (X 150) B: Diagnosis of secretory endometrium should not depend on few, small, irregular vacuoles as are shown in these glands. Compare with Fig. 5. (X 400)
FIGURE 7. Seventeen-day endometrium (third postovulatory day). A: Gland nuclei are pushed to the center of the epithelial cells with cytoplasm above, and vacuoles below, them. (X 150) B: Gland mitoses, rare; pseud stratification, decreasing. (X 400)

FIGURE 8. Eighteen-day endometrium (fourth postovulatory day). A: Gland nuclei are returning to the base of the cells. Note wisps of secretory material appearing in the lumina. (X 150) B: Some vacuoles are pushed past the nucleus on their way to empty contents into the lumen. Mitosis and pseud stratification of nuclei absent. (X 400)
Acidophilic intraluminal secretory material begins to appear in small amounts as early as the third and fourth postovulatory day, but visible secretion reaches its peak on the twentieth day (Fig. 9). During subsequent days the secretion becomes inspissated, taking a darker stain and assuming a central position in the lumen.

The behavior of the glandular epithelium has been the key to dating the first half of the secretory phase. Though further notable changes take place in the epithelium, dating of the second half depends largely on stromal characteristics. Tissue edema, though variable in the proliferative phase, is characteristically marked in the mid-secretory stage, becoming evident rather abruptly on the twenty-first, and reaching its peak on the twenty-second day (Fig. 10). The stromal cells at this stage look small, dense, nearly "naked" nuclei with only filamentous cytoplasm. In interpreting the edema of biopsy specimens one must be on guard not to mistake intercellular fluid caused by operative trauma, improper tissue fixation, and faulty histologic technique.

On the twenty-third day (Fig. 11) the spiral arterioles, previously somewhat difficult to distinguish in the edematous stroma, become much more prominent. This is due to enlargement of nuclei and increase of cytoplasm within the perivascular stromal cells. Mitoses may be present. These findings constitute the earliest visible predecidual change. * By the twenty-fourth day (Fig. 12) definite collections of predecidual cells may be identified around the arterioles; and stromal proliferation occurs, as evidenced by mitosis. Predecidua begins to differentiate under the surface epithelium about the twenty-fifth day (Fig. 13). Soon islands coalesce and by the twenty-seventh day (Fig. 14) predecidua usually appears as a solid sheet of well-developed decidua-like cells.

A few scattered lymphocytes may be found in proliferative and early secretory stroma, but the differentiation of predecidua is accompanied by a sharp increase in lymphocytic infiltration. Polymorphonuclear leukocytic invasion begins on the twenty-sixth, and becomes characteristic by the twenty-seventh day (Fig. 14B). Microscopic areas of focal necrosis and hemorrhage occur a few hours before onset of overt menstruation.

In the absence of pregnancy, gland secretion diminishes to a variable extent, and involution of the gland epithelium begins about the twenty-sixth day.

* We consider the term "predecidua," preferable when referring to this change in endometrial stroma, to the term "predecidua" as used in Figure 1 by Latour.
Figure 10. Twenty-two-day endometrium (eighth postovulatory day). A: Stromal edema at its height. The walls of the spiral arterioles are not prominent. (X 150) B: The stromal cells still appear as small, dense "naked nuclei" widely separated by extracellular fluid. Glandular secretion still active but subsiding. (X 400)

Figure 11. Twenty-three-day endometrium (ninth postovulatory day). A: Spiral arterioles become prominent due to condensation of their surrounding stroma. (X 150) B: Both the nuclei and the cytoplasm of the peritrophoblastic stromal cells are enlarging, thus in the earliest predecidual reaction. (X 400)
Figure 12. Twenty-four-day endometrium (tenth postovulatory day). A: Spiral arteriolae and surrounding pseudodecidua still more prominent, and edema subsiding. (X 150) B: Thickening of the perivascular pseudodecidua cuff. Stromal mitosis evident. (X 400)

Figure 13. Twenty-five-day endometrium (eleventh postovulatory day). A: Pseudodecidua begins to differentiate under the surface epithelium. Stromata of the stratum spongiosum is still edematous save for areas near a spiral arteriole. (X 150) B: Round cell infiltration accompanies pseudodecidual differentiation. Note swelling of stromal cell to become pseudodecidual in type. (X 400)
fourth day. The glands are dilated and tortuous, and the epithelium is thrown into folds causing the characteristic saw-toothed effect, but the previously all columnar epithelium is now low, the nuclei are shrunken, and the cytoplasmic edges are ragged and indistinct. Though often striking, the picture of secretion culminating in "secretory exhaustion" is too variable to aid in dating. Some early menstrual endometria show active secretion in occasional glands. Likewise, interesting but variable changes such as constriction and dilation of the spiral arteries, and dilation of the venous sinuses, may be noted after the seventh postovulatory day.

In recapitulation, dating the endometrium during the first week of luteal activity depends primarily upon recognition of changes occurring in gland epithelium; namely, mitosis, pseudostratification, basal vacuolation, and secretion. During the second week stromal changes, namely, edema, predecidual reaction, mitosis and leukocytic infiltration are the key criteria. These changes are graphically illustrated in Figure 1.

Some clinicians, fearing to abort a possible pregnancy, submit biopsies from sterility patients taken during menstruation. Since menstruation causes loss of morphologic detail necessary for dating, we report simply early, mid- or late menstrual endometrium or "proliferative endometrium consistent with involutionary bleeding." We do attempt broadly to evaluate the quality and amount of predecidual in early menstrual endometrium.

Difficulties encountered in dating often depend on the tissue submitted and on its preparation. Tissue from the fundus of the uterus gives more reliable information than that from the lower uterine segment. Likewise, biopsies including the stratum compactum and spongiosum are datable, whereas the stratum basalis is not. A single long, firm sweep of the curette affords the best specimen. Immediate fixation (Bouin's solution is preferred in this laboratory), careful dehydration, embedding, sectioning, and staining are essential. Vertical orientation of the specimen when sectioning is desirable. The illustrations used in this paper are photomicrographs of routinely prepared biopsy specimens from sterility patients taken from the laboratory file.

We make no attempt to date specimens of abnormal endometria, such as of polyps, of hyperplastic tissue, and of chronic endometritis. The presence of plasma cells serves to distinguish endometritis from physiologic leukocytic invasion. Estrogen therapy, given in doses small enough to permit ovulation, does not seem to alter datable details. For reasons developed below we now
date the entire endometrium on the morphology of the most advanced portion or feature rather than on the average picture.

**ANALYSIS OF ACCURACY**

Using their original criteria, Rock and Bartlett correlated the dating of 200 endometrial biopsies in the secretory phase with the day on which menstruation actually occurred. Of these, 31 patients (16 per cent) menstruated on the day predicted, 53 (17 per cent) menstruated later, and 116 (68 per cent) menstruated earlier than expected. The error ranged from 13 days early to 4 days late (e.g., biopsy was reported as “twenty-six days after endometrium” and the patient menstruated 6 days later rather than 2 days later as predicted; error—4 days late). The mean of error from error in interpreting the biopsy, 97 patients (45 per cent) were found to menstruate at the time predicted.

In the present study in which the criteria above outlined were used for dating, 300 cases are reviewed. Most of the biopsies were taken during routine sterility studies, about half from the clinic and half from the private practice of two staff members. Since a recent survey in this hospital showed that 8% of biopsies in fertility patients 43 per cent were normal, we feel justified in considering this group of 300 fertility biopsies representative of normally menstruating women. The 300 cases were taken consecutively from the files of the pathology department over a three-year period, and were selected only for absence of organic endometrial disease and for availability of accurate menstrual histories.

The dating was done in this series by approximately a dozen different observers. Of the 300 patients, 42 (14 per cent) menstruated on the day predicted, 36 (12 per cent) menstruated later, and 222 (74 per cent) menstruated earlier than expected. The error ranged from 12 days early to 8 days late, the mean being 1.81 days early (standard deviation, 2.33). Again, allowing a “plus or minus” 1 day error in interpreting, 112 patients (37 per cent) were found to menstruate at the time predicted.

It was felt that some of this rather high percentage of error was due to individual interpretation. The slides were therefore reviewed by a single observer (R.W.N.). Instead of dating from the average morphologic picture as had been done previously in this series, the date for the most advanced area in the biopsy was chosen. Of the same 300 cases thus reviewed, 65 patients (22 per cent) menstruated on the day predicted, 70 patients (24 per cent) menstruated later, and 168 (55 per cent) menstruated earlier than expected. The error ranged from 7 days early to 10 days late, the mean being 0.70 days early (standard deviation, 2.02). 179 patients (57 per cent) menstruated within “plus or minus” 1 day of the day predicted.

These results led to the suspicion that either the dating criteria were in error or that biopsy in some way caused early menstruation. In order to test the former hypothesis, another reference point, the change in basal body temperature, was used to correlate endometrial dating with ovulation instead of with onset of menstruation. This was felt justifiable since recent experiments have shown that the change in basal body temperature as well as the change in endometrial morphology is due to progesterone. Tompkins and others feel that the mid-cycle temperature change is probably associated with ovulation. Greulich showed ovulation occurring on a rising temperature, but it is the opinion of Farris that ovulation usually takes place a day or two before the change. For the present study the day of the lowest temperature preceding the sustained rise was considered as indicating the day before progesterone dominance.

The records of 40 patients who had taken adequate temperature records and had been biopsied in the secretory phase of the cycle were studied. Of the 40 patients, 19 (48 per cent) ovulated on the day predicted, 10 (25 per cent) ovulated earlier, and 11 (27 per cent) ovulated later than expected. The error ranged from 2 days early to 2 days late, the mean being 0.1 days early (standard deviation, 1.1). Allowing a “plus or minus” 1 day error in interpreting, 31 patients (78 per cent) ovulated as predicted. The higher percentage of accuracy, narrower range and equal distribution of error, and lower standard deviation in this group indicate if the selected temperature level (approximated ovulation) that the dating criteria are not significantly inaccurate. Dating is a better gauge of duration of progesterone effect than it is a prediction of onset of menses. If ovulation and the low end point of the first temperature phase are concomitant, endometrial dating correlates well with the time of ovulation.

Does biopsy alter the length of the secretory phase? In the above series of 40 patients, 25 had recorded temperatures in at least two cycles in addition to that in which the biopsy was taken. In two-thirds of these patients, the secretory phase was definitely shorter in the biopsy cycle than in the control cycle. This was found to be equally true whether the biopsy was
taken near mid-cycle or near menstruation. It is suggested, therefore, that biopsy accelerates the onset of flow, and thus that catamenia before the day anticipated by the dating may not prove its inaccuracy.

Does biopsy alter the length of the menses? In the series of 300 cases data on menses preceding and following biopsy were available in 107 cases. In 55 per cent of these, the menses following biopsy were no longer than the preceding period, and in the remaining 45 per cent the menses were rarely increased over two days. Sometimes biopsy causes spotting which may have been misinterpreted as the beginning of catamenia when the biopsies were done late in the cycle. Nor was menstrual rhythm interfered with as judged by length of the succeeding cycle. In 148 cases where it was recorded, the next cycle was of average length (mean "plus or minus" 2 days) in 86 per cent, longer in 6 per cent, shorter in 8 per cent.

One may suspect on the basis of this small series that biopsy may shorten the secretory phase of the cycle, bringing on menstruation early; but it does not materially interfere with the length of the flow or the succeeding rhythm.

A speculation on the mechanism may be based on the observations of Mackee, who noted blanching of endometrial vessels, thought to be due to hormone withdrawal, one to three days before onset of bleeding. The thought might arise whether trauma to the endometrium of this extent might possibly release prematurely a local factor (such as the mensural toxin of Smith and Smith) earlier than it would be liberated in the normal course of events, with resultant premature menstruation.

The usefulness of dating the endometrium lies in the fact that it gives a rough idea of quantitative progestosterone effect, and also indirectly of the time of ovulation. This progestosterone effect depends on duration of action as well as amount of steroids acting, and possibly on the sensitivity of the endometrial end-organ as well. Selke considers endometrial biopsy a superficial form of hormone assay because of its freedom from a multitude of technical uncertainties. The value of an assay, however, is no greater than the accuracy of its standardization, and this method we think offers a better basis for standardization than any other in general use.

SUMMARY

1. The criteria involved in dating endometrial biopsies are given.
2. The dating of 300 biopsies is correlated with the onset of subsequent menstruation.

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