

Diagnosis of Pregnancy by Cytologic Criteria in Catheterized Urine.*

GEORGE N. PAPANICOLAOU.

From the Departments of Anatomy and Obstetrics and Gynecology, Cornell University Medical College, New York, N.Y.

Pregnancy causes marked proliferation and growth in the epithelium of the vagina and the cervix.¹ This results in characteristic cytologic changes, which permit the diagnosis of pregnancy through the microscopic examination of fluid aspirated from the vagina. The use of the vaginal smear in diagnosing pregnancy was advocated by me in 1925.²

The extreme growth of the epithelium of the vagina and the cervix during gestation is accompanied by a considerable increase in the secretion of glycogen. Glycogenic cells become thus very prominent in the vaginal smear of pregnancy. The most typical of these cells are the so-called "navicular" cells,³ which have a definite diagnostic value. Their differentiation from corresponding cells of the normal cycle is possible through certain criteria such as enlargement and elongation of their nuclei and thickening of their surface membranes.

The vaginal smear method of diagnosing pregnancy, though morphologically sound, did not prove to be of great practical value as a routine diagnostic procedure. The identification of the "navicular cells of pregnancy" requires great experience and is not always dependable. It is often difficult to differentiate these cells from other forms of navicular cells found during the normal menstrual cycle or in certain types of secondary amenorrhea. Furthermore, other factors, such as intercourse or bacterial and parasitic infections may distort the vaginal smear picture, thus impairing its diagnostic value.

In the course of studies of smears prepared from urine sediment for the purpose of detecting cancer cells,^{4,5,6} I have been impressed by the greater cytologic uniformity of such smears in comparison with vaginal smears. The normal exfoliation in the urinary tract is much scantier than in the female genital tract and the variety in the types of desquamated cells is much more limited. The appearance of any significant cytologic changes can thus be noted much more readily in the "urine sediment" than in the vaginal smear.

The effect of the estrogenic hormone upon the cellular make-up of the urine sediment smear may be cited as an example. This effect is noticeable in the urine of women as well as of men. I first observed it in the case of a man receiving estrogenic therapy for a carcinoma of the prostate.⁵ It consists in the appearance of squamous acidophilic cells with a pyknotic nucleus and cytoplasmic granules greatly resembling the cells found in the vaginal smear either normally during the follicular phase or after estrogenic therapy. The recognition and identification of these cells is indeed easier and less equivocal in the "urine" than in the "vaginal" smear. This is because the vaginal smear contains almost invariably other non-specific squamous acidophilic small-nucleated cells which complicate the cytologic picture. The urine sediment smear may thus prove to be more accurate in assaying estrogens than the vaginal smear. The urine of women should be obtained by catheterization in order to prevent the admixture of vaginal cells.

More recently, my attention was directed

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¹ Stieve, H., *Z. f. mikrosk.-anat. Forsch.*, 1925, **3**, 3.

² Papanicolaou, G. N., *Proc. Soc. Exp. Biol. and Med.*, 1925, **22**, 436.

³ Papanicolaou, G. N., *Am. J. Anat.*, 1933, **52**, 3 (Supplement), 519.

⁴ Papanicolaou, G. N., and Marshall, V. F., *Science*, 1945, **101**, 2629, 519.

⁵ Papanicolaou, G. N., *J. Am. Med. Assn.*, 1946, **131**, 372.

⁶ Papanicolaou, G. N., *J. Urol.*, 1947, **57**, 375.

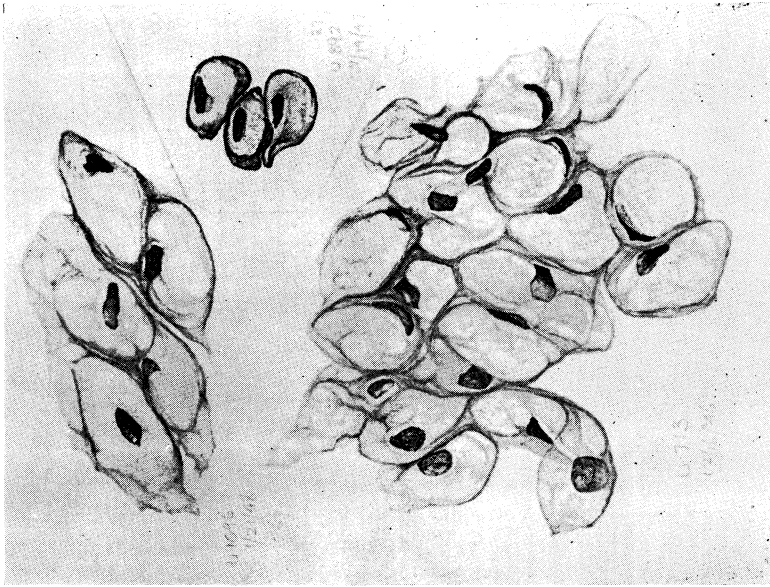


FIG. 1.

Photograph of drawings of cells of the navicular type found in smears prepared from the urine of pregnant women. $\times 400$.

toward the cytologic changes occurring in the urine of pregnant women. For this study catheterized urine was used, since voided urine is always contaminated with cervical, vaginal, and vulval cells. The pure catheterized urine contains normally a relatively small number of exfoliated cells, most of which are of the transitional type. The variety of cell types is limited. Leucocytes are, as a rule, scanty, except in cases of bacterial or parasitic infections of the urinary tract, which are much less frequent than those of the vagina and the cervix. Histiocytes are also relatively rare.

In the pregnancy urine, there is an apparent increase in the total number of desquamated cells. The cells appear singly or in clusters. Some of these acquire a characteristic form closely resembling that of the navicular cells found in the vaginal smear of pregnancy (Fig. 1). Here again, under the effect of the hormonal factors of pregnancy, the cytology of the urine sediment smear acquires a great similarity with that of the vaginal smear. Many of the cells show pronounced vacuolation and a displacement of the nucleus toward the periphery or one of the poles of the cell. The presence of glycogen within

the vacuoles may be demonstrated by special staining. There is, thus, evidence of an increased production of glycogen during gestation in the epithelium of the bladder, parallel to that of the epithelium of the cervix and the vagina. Leucocytes are, as a rule, inconspicuous. In general, the urine sediment smear shows a simpler and more uniform cytology than the vaginal smear and is much less contaminated with bacteria, leucocytes, and histiocytes.

Thus far, approximately 350 specimens of catheterized urine of women have been examined. Of these, 38 were from pregnant women. Cytologic changes characteristic of pregnancy were found in all specimens of this latter group. The earliest specimen examined was of a gestation of 14 weeks and the latest of seven months. A study of earlier as well as of more advanced cases is now in progress. Also, of abnormal conditions resulting from pregnancy, such as abortions, ectopics, hydatidiform moles, chorio-epitheliomas, etc.

The technic used in these studies is similar to the one used in cancer diagnosis. Catheterized urine, varying in quantity from 25 to 100 cc is mixed immediately with equal parts of 95% ethyl or with isopropyl alcohol and

centrifuged without much delay for 30 minutes at medium speed. The sediment is spread evenly on a number of slides which have been thinly coated with Mayer's albumen. The smears thus prepared are allowed to stand only for a short time until they begin to show dryness around the edge and then they are immersed in a solution of equal parts of 95% alcohol and ether.

After a fixation of at least 15 minutes the slides are carried through 80%, 70%, and 50% alcohols to distilled water.

The staining is as follows:

Harris Hematoxylin (which has been prepared without acetic acid, and diluted with an equal amount of distilled water) for 6 minutes.

Rinse in distilled water.

† EA65 is the same as EA36 with the difference that the Light Green is one-half strength (0.25% instead of 0.5%). It has the advantage of giving a lighter and more transparent staining which is very desirable in cancer diagnosis. The differentiation between acidophilic and basophilic cells is better with the EA36 (or EA50). This differentiation is more important in vaginal, endocervical, and endometrial smears. Therefore, EA36 (or EA50) is preferable for vaginal, endocervical, and endometrial smears, whereas EA65 is better adapted to other types of smears. Either one of these two stains can be used for all types of smears.

Dip in 0.5% HCl (aqueous solution) 6 times.

Place in running tap water for 6 minutes.

Rinse in distilled water, 50%, 70%, 80%, and 95% alcohols.

Stain in OG6 for 1½ minutes.

Rinse in 95% alcohol, 2 changes.

Stain in EA65† (procedure No. 267) for 1½ minutes or in EA36⁷ (procedure No. 268).

Rinse in 95% alcohol (3 changes); absolute alcohol and xylol, equal amounts; xylol; mount with a coverslip.

Be sure that smears are never allowed to dry at any time.

Summary. Pregnancy causes distinct morphologic changes in the epithelium of the urinary tract which are reflected in the cytology of the "urine sediment smear." There is an appearance of certain characteristic cell forms such as cells of the "navicular" type corresponding to those described in the vaginal smear of pregnancy. The urine sediment smear provides, thus, a simple, rapid and dependable method for diagnosing pregnancy. It appears to be superior to the vaginal smear in that its cytology shows greater uniformity and is more distinctive. Its practical value as a routine diagnostic procedure and its applicability in the early diagnosis of pregnancy are still to be determined.

⁷ Papanicolaou, G. N., *Science*, 1942, **95**, 438.

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Streptomycin as an Essential Nutrilite.

GEOFFREY RAKE.

From the Squibb Institute for Medical Research, New Brunswick, N.J.

In consideration of the mode of action of streptomycin one of the possibilities explored has been that of its functioning as a metabolite antagonist. Little evidence of this has been forthcoming, however, and it was therefore with interest that one read the report of Miller and Bohnhoff¹ on their so-called B variant meningococcus. This variant (mutant) resembled the A variant (mutant)

in that both were resistant to, or developed in high concentrations of, streptomycin. The B mutant differed from the A in that it required streptomycin for multiplication either *in vitro*, or *in vivo* in the mouse, while the A mutant did not. The B mutant never

¹ Miller, C. P., and Bohnhoff, M., *Science*, 1947, **105**, 620.