has been shown that the toxicity of urine for fibroblasts is not destroyed by heat. Urine heated to 60°C and 100°C causes the same degree of inhibition of growth as unheated urine. It was also shown that the toxic substance in urine is non-dialysable. The addition to cultures of 1 cc or 0.5 cc of the residue after dialysis (corresponding more or less to the same volume of urine) results in a markel inhibition of growth (Fig. 2A and B), though somewhat less than is seen with whole urine.

The method described here of demonstrating the toxicity of urine by its effect on the growth rate of fibroblast colonies is thus exceedingly sensitive and enables us to measure the toxicity of urine with a great degree of exactitude. This method may therefore help in the investigation of the nature of the toxic substance present in urine as well as the toxicity of urine in various pathological conditions.

Summary. A method is described of demonstrating the toxicity of urine by its effect on the growth rate of cell colonies *in vitro*. Normal human urine in a dilution 1:5 completely inhibits the growth of fibroblasts; urine in a dilution of 1:50 still perceptibly inhibits their growth. The cells growing in media containing urine even in small concentrations exhibit signs of degeneration. Heat does not destroy this property of urine. The toxic substance is nondialysable.

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Effect of Concomitant Administration of Estrogens and Progesterone on Vaginal Smear in Man.*

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Of the changes in the vaginal secretion during the menstrual cycle[1] those occurring in the first half concurrent with the growth and ripening of the ovarian follicle are most clearly defined and best understood. The demonstration in this laboratory[2] that they are similar to the smear changes induced in menopause and amenorrhea

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by the administration of estrogens, indicates that during the first half of the cycle the vaginal epithelium and secretion are largely, if not entirely, under the control of the estrogenic hormones. The peak effect of the estrogens as seen in the fully expressed "follicular picture" induced after menopause can, therefore, serve as an index of the extent of spontaneous follicular activity during the normal cycle, and affords a simple histological measure of ovarian function. It can also be employed as a guide for replacement therapy with estrogens[2] and as a measure of ovarian stimulation by gonadotropic agents.[3]

The changes in vaginal smear during the second half of the cycle from the time of ovulation up to the next menstruation are less clear cut and more difficult to evaluate.[1] The lack of uniformity possibly arises from the more complicated hormonal pattern of this phase of the cycle. The estrogenic titer falls from its mid-menstrual peak and, except for a brief moderate rise about a week premenstrually, gradually sinks to the low levels characteristic of the premenstruum and the menstrual phase. The progestational hormone now appears for the first time, is elaborated for a period of about 10 days and then disappears one to 3 days before the flow.[4] If, as is likely, the structure of the vaginal epithelium is a resultant of the interaction of both groups of hormones, any variation in the tempo or the extent of their production could be expected to vary the cytology of the vaginal secretion.

The experiments reported in this paper are part of a study designed to analyze the influence of each of these hormonal factors on the structure of the vaginal epithelium and secretion during the second half of the cycle and to ascertain whether the vaginal smear can yield specific cytological evidence of the influence of progestin. Experiments with mice[5] and monkeys[6] have shown an antagonistic action of estrogens and progesterone on the vaginal epithelium. In the mouse, progesterone, like testosterone, prevented cornification when given simultaneously with estrone or estradiol. In the monkey, progestin (a chemically impure preparation of the corpus luteum hor-

1 Papanicolaou, G. N., *Am. J. Anat.*, 1933, **52**, No. 3, Supplement, May 15.

2 Papanicolaou, G. N., and Shorr, E., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 528; *Idem., J. Obs. and Gyn.*, 1936, **31**, 806.

3 Shorr, E., and Papanicolaou, G. N., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 629.

4 Venning, E. H., and Brown, J. S. L., *Endocrinology*, 1937, **21**, 711.

5 Robson, J. M., *J. Physiol.*, 1937, **90**, 15.

6 Hisaw, F. L., Greep, R. O., and Fevold, H. L., *Am. J. Anat.*, 1937, **61**, 487.

mone) caused a sloughing of the vaginal epithelium previously built up by estrone despite continued estrone administration.

Methods: The subjects were 9 women, 3 in spontaneous menopause and 6 with a menopausal syndrome following removal of the uterus, the ovaries, or both uterus and ovaries. The same general procedure was followed as in a previous study on the peripheral neutralization of estrogens by androgens.[7] A follicular type of vaginal smear was induced by estradiol benzoate, estradiol dipropionate, or estrone. Once this stage was reached the same dose of estrogen was continued along with progesterone, the dose of which was varied from time to time. In 2 cases pregneninolone was given by mouth. Vaginal smears were obtained daily and vaginal biopsies at appropriate times.

Results: As a basis for comparison with the changes induced by this combined therapy a brief, though necessarily incomplete, description is given of the most characteristic cytological changes noted by Papanicolaou[1] during the post-ovulatory and premenstrual phases. (1) There is usually a progressive decrease in the number of cornified cells with small pyknotic nuclei, and they may entirely disappear. (2) The discrete arrangement of the cells at ovulation is replaced by clumps of variable density. (3) The majority of the cells are of the intermediate undifferentiated squamous type, irregularly folded or with curled edges. They assume a variety of shapes including the characteristic navicular, with large, round or oval nuclei usually predominant. (4) Leukocytes usually increase in number. There is a rich bacterial growth often associated with cytolysis; and the smears have a "dirty" appearance. (5) There is an increase in mucus from time to time. (6) Cells from the deeper layers of the vaginal epithelium are occasionally seen.

Definite changes were recognizable in the smear within a short time after the institution of combined therapy. In about one-half of the cases they were detectable in 24 hours and in the remainder within 48 hours. The following changes were quite uniformly seen:

1. A leukocytosis of variable extent was present in all of the cases. The degree of leukocytosis varied irregularly, the smear occasionally becoming quite free of them for a day or two. In Case H.S. leukocytes were present in small numbers in only an occasional smear.

2. The typical cornified cells with small pyknotic nuclei rapidly diminished in number and in many instances virtually disappeared. In

7 Shorr, E., Papanicolaou, G. N., and Stimmel, B. F., PROC. SOC. EXP. BIOL. AND MED., 1938, **38**, 759.

most of the smears a small number of large pale flat squamous cells with small pyknotic nuclei persisted. In Case H.S. cornified cells continued to constitute about one-fourth of the epithelial cells but most of them were folded and wrinkled.

3. Characteristic clumping took place early. The cell aggregates were usually quite dense at the end of therapy. From time to time variation in the density of the clumping was seen.

4. Superficial squamous cells of the undifferentiated type dominated the smear picture. In the earlier stages the most characteristic change was the folding of the cell. Later, curling of the edges took place and a variety of shapes and sizes of cells was seen such as the navicular, the straplike, and even the oyster-shaped cell which has been described as characteristic of pregnancy.[1] In the thick smears there was considerable compression of the cells with occasional aggregates such as are also seen in pregnancy. Cells with large nuclei increased in number, but there was a persistence and frequent predominance of cells of various shapes and sizes with small pyknotic nuclei.

5. From time to time increased mucinification was seen. Bacteria appeared frequently in great abundance. In association with this, there occurred cytolysis with large numbers of free nuclei; and the smears often assumed a smudgy appearance.

6. Although a few cells from the deeper layers of the vaginal epithelium were usually seen at the end of combined treatment they were never a prominent feature.

In the 2 cases treated with pregneninolone the changes were of a similar character to those seen with progesterone but were not as marked. Apparently higher doses of this preparation should have been used.

A detailed description of the changes in the vaginal epithelium under this regime will be given in a separate report. It will suffice here to describe the major alterations noted. The biopsies taken during the induced follicular phase showed the typical increase in the height of the epithelium as compared to the untreated state. The intra-epithelial condensation or cornification of Dierks[8] was present and the cells of the functionalis were thick walled and flattened. Following the combined administration of estrogen and progesterone there was a definite increase in the height of the epithelium contributed largely by the functionalis. The intra-epithelial zone of cornification became less prominent and in many instances disappeared. The walls of the cells of the functionalis became thinner,

[8] Dierks, K., *Arch. f. Gynaek.*, 1927, **130**, 46.

TABLE I.
Tabulation of the Dosage of Estrogen and Progesterone Administered Simultaneously to Women in Menopause.

Case	Condition	Estrogen		Progesterone mg (number days)
		Preparation	Estrous unit and maintenance dose R.U. per day	
S.B.	Natural Menopause	Estradiol Benzoate	3,000	10 (4), 20 (6)
M.G.	" "	Estrone	1,500	10 (6), 20 (6)
A.S.	" "	"	3,500	15 (5)
T.K.	Surgical Castrate	Estradiol Benzoate	4,000	10 (6), 20 (6), 35 (6), 100 (6)*
A.B.	" "	" "	3,000	10 (6), 25 (5), 25 (9)
L.C.	" "	" "	7,500	10 (7), 20 (4), 35 (5)
M.L.	" "	" "	10,000	10 (6), 25 (7)
H.S.	Surgical—one ovary present	" Dipropionate	5 mg 3 × week	25 (12)
M.D.	Surgical Castrate	" Benzoate	4,000	50 (7)*, 100 (9)*

*Pregneninolone orally.

the cells wider, presenting in the most extreme cases a chicken-wire appearance. This picture is similar to that described by Dierks, and by Traut, Bloch and Kuder[9] during the proliferative phase; and in the most extreme hyperplasias, resembles the picture seen in pregnancy[9, 10] where the production of both groups of hormones is at a very high level.

Symptomatically, previous relief of the menopausal symptoms induced by estrogen persisted throughout the concomitant administration of progesterone. Occasionally increased fullness and tenderness of the breasts was noticed.

Discussion. It is apparent that the concomitant administration of progesterone and estrogen causes profound alterations in the vaginal smear as compared with the picture seen with estrin alone. Virtually all of the changes seen during the second half of the cycle are reproduced. Since the amount of estrogen was constant throughout, it was not to be expected that the changes would occur in the exact sequence seen during the normal cycle. The inference seems warranted that the changes taking place during the second half of the cycle result not only from the diminution in estrin production but also from the active influence of the progestational hormone in modifying the effect of estrin on the vaginal epithelium. This conclusion must also be true for pregnancy where both groups of hormones are present in such large amounts.

No sloughing of the epithelium as observed in the monkey was seen in this group of women although the proportion of progesterone to estrin was frequently as great as that employed in the animal experiments. The human epithelium appears to be more sensitive to progesterone than that of the mouse in that much less progesterone in proportion to estrin is effective in abolishing cornification.

9 Traut, H. F., Bloch, P. W., and Kuder, A., *Surg. Gynec. and Obst.*, 1936, **63**, 7.
10 Stieve, H., *Ztschr. f. mikros.-anat. Forsch.*, 1925, **3**, 307.