11984

Use of Colchicine in Detecting Hormonal Effects on Vaginal Epithelium of Menstruating and Castrate Women.*

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The interesting observation that colchicine accumulates dividing cells in metaphase by preventing a completion of mitotic division has been made use of in the study of the action of estrogens,[1] androgens,[2] and gonadotropins[3] in animals. The great increase in the number of mitotic figures obtained with the colchicine technic over the small number seen when the hormones are used alone, has served to throw light on several previously obscure aspects of the action of these hormones.

The use of the colchicine technic in relation to hormonal action has hitherto been confined to animals, presumably because doses comparable to those effective in animals would exceed the limit of safety for this drug in man. However, it seemed likely that this technic could be safely applied to man were the alkaloid brought, in adequate concentrations, in direct contact with accessible structures like the vaginal epithelium and skin, and the total amount kept within recognizably safe limits. This report deals with experiments designed to explore the usefulness of the colchicine technic in revealing the effects of various hormones on the vaginal mucosa of menopausal castrates and menstruating women.

Methods. Colchicine was incorporated into gelatin pessaries for insertion into the vaginal vault. Vaginal biopsies were taken 17-24 hours after insertion of pessaries containing 1-3 mg of the alkaloid. A number of control biopsies were taken just prior to the insertion of the pessary. The subjects were 4 menopausal castrates and 6 menstruating women. Vaginal smears were taken daily to ascertain the effects of the hormones used with the castrate group.

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1 Allen, E., Smith, G. M., and Gardner, W. U., *Am. J. Anatomy*, 1937, **61**, 321.

2 Martins, T., *Compt. rend. Soc. de Biol.*, 1937, **126**, 131; Fleischmann, W., and Kann, S., *Adak. Anz. Akad. d. Wissensch. Wien*, 1937, No. 21; *Biochem. Z.*, 1938, **296**, 373.

3 Allen, E., Smith, G. M., and Gardner, W. U., *Anat. Rec.* (Suppl. 3) 1937, **67**, 3.

and to guide the selection of the appropriate time for biopsy in the menstruating group. The smears were stained by the method recently developed in this laboratory.[4] The biopsies, 69 in number, were fixed either in Carnoy's or Flemming's solution. After the former fixative they were stained by a modified Masson technic, after the latter, by Safranin and Fast Green F.C.F.

Experimental Procedures. (1) *The menopausal castrate group:* Estradiol benzoate was given intramuscularly in ascending doses until a dose was reached which produced a fully cornified follicular smear. This amount of estrogen was then given concomitantly with progesterone in amounts of from 25-50 mg daily for a period of 2-3 weeks (Table I). It has been previously shown[5] that under this regime the cornified vaginal smears induced by estrogens assumed the characteristics of the vaginal secretion of the second half of the menstrual cycle, and that the vaginal epithelium was transformed from the induced mid-menstrual type to that found in premenstruum or, with the larger doses of progesterone, in pregnancy. Vaginal biopsies following the insertion of colchicine pessaries were taken at various stages of treatment.

(2) *The Menstruating Group:* No hormonal therapy was given, but vaginal biopsies after colchicine pessaries were taken when

TABLE I.

A. Menopausal Castrate Group.
Schedule of Hormone Treatment.

Case	Estradiol benzoate estrous unit and maintenance dose R.U. per day	Progesterone, mg (No. days)
1	5,000	25(6), 35(9)
2	4,000	25(8), 35(3), 50(2)
3	10,000	—
4	3,000	25(8), 35(9)

B. Menstruating Women.
Day of Biopsy in Relation to Menstrual Cycle.

Case	Menstrual cycle, days	Day of biopsy
5	28	14, 27
6	26-30	27
7	27-28	11, 17, 24
8	23-26	11, 25
9	23-26	25
10	28	13

[4] Shorr, E., *Science*, 1940, **91**, 321, 579.

[5] Shorr, E., PROC. SOC. EXP. BIOL. AND MED., 1940, **43**, 501.

vaginal smears showed (a) maximal follicular activity and (b) during the last week of the premenstruum.

Results. The findings are presented as qualitative rather than quantitative evidence of the action of colchicine on the vaginal mucosa under these experimental conditions, since some leakage from the vagina could not be prevented, and there were differences in the time after insertion of the pessaries at which the biopsies were taken. No symptoms attributable to colchicine were encountered.

The changes in the vaginal smears under the hormonal regime described, were in agreement with those previously reported from this laboratory,[5] the cornified smear induced by estrogens assuming the appearance characteristic of the premenstrual phase of the normal menstrual cycle[6] when progesterone was added. The structure of the vaginal epithelium was altered by the combined progesterone-estrogen treatment in the manner previously described,[5] the extent of the increased proliferation varying directly with the amount of progesterone used.

The chief point of interest lay in the increased number of mitoses which accumulated as a result of the action of colchicine. Under these experimental conditions, vaginal biopsies taken without colchicine show only an occasional mitotic figure in spite of the obvious proliferation. After colchicine, except when leakage or expulsion of the pessary was reported by the patient, a significant increase in the number of mitotic figures occurred. In the *menopausal group*, an increase in the number of arrested mitotic figures was seen following estradiol benzoate alone and in combination with progesterone. In the *menstruating group,* mitotic figures indicative of active proliferation were seen during both the follicular and premenstrual phases.

The mitotic figures were largely confined to the lower layers of the basalis adjacent to the stratum germinativum. Their distribution was patchy, indicating that proliferation was not taking place uniformly throughout the whole epithelium. Except for a rare figure in anaphase, the arrested mitoses were in pro- or metaphase; and there was a high percentage of atypical mitotic figures. Among the latter could be distinguished cells with densely packed chromatin which were considered to represent an atypical prophase in process of degeneration. Cells in atypical metaphase, with the chromosomes grouped to one side of the spindle, were frequent. Many of the cells in arrested division were much larger than the resting cells,

[6] Papanicolaou, G. N., *Am. J. Anat.* (Suppl.), 1933, **52**, 519.

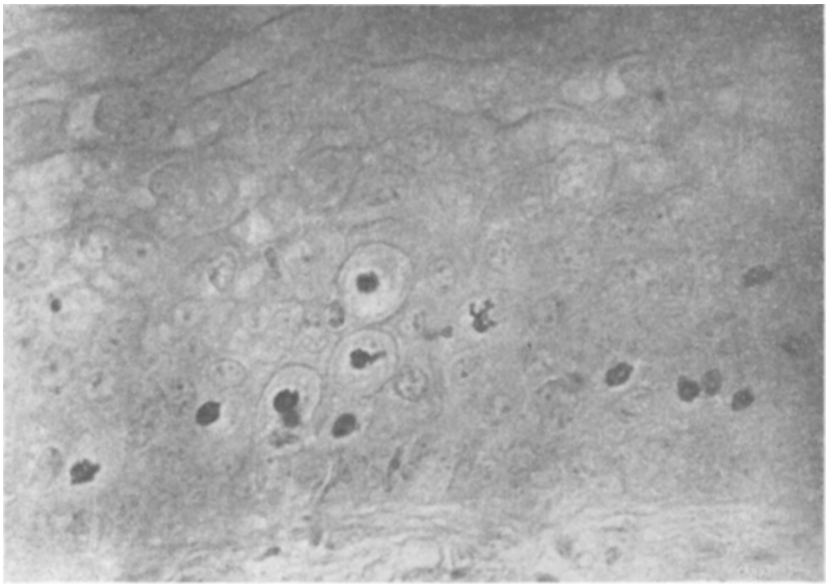

FIG. 1. (Case 7—normal cycle.)
Vaginal epithelium during follicular phase. (11th day.)

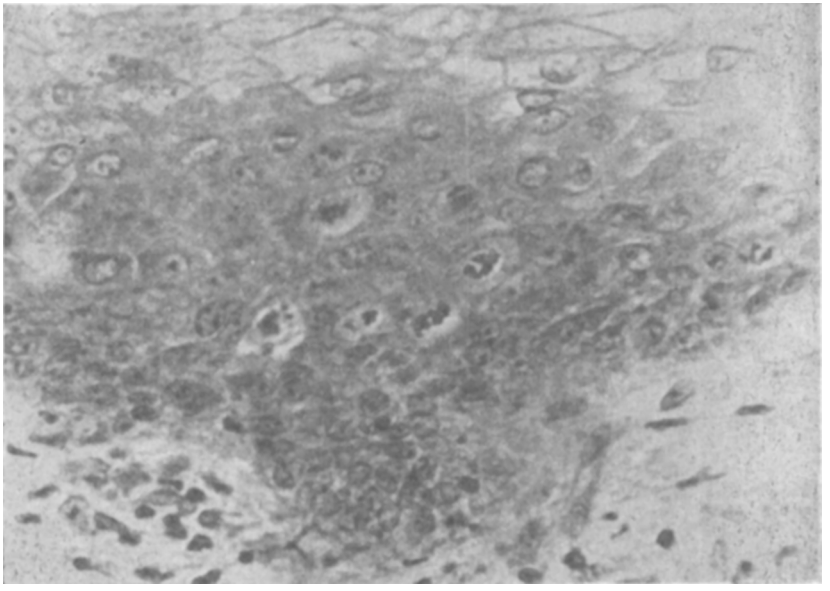

FIG. 2. (Case 7—normal cycle.)
Vaginal epithelium during premenstrual phase. (24th day.)

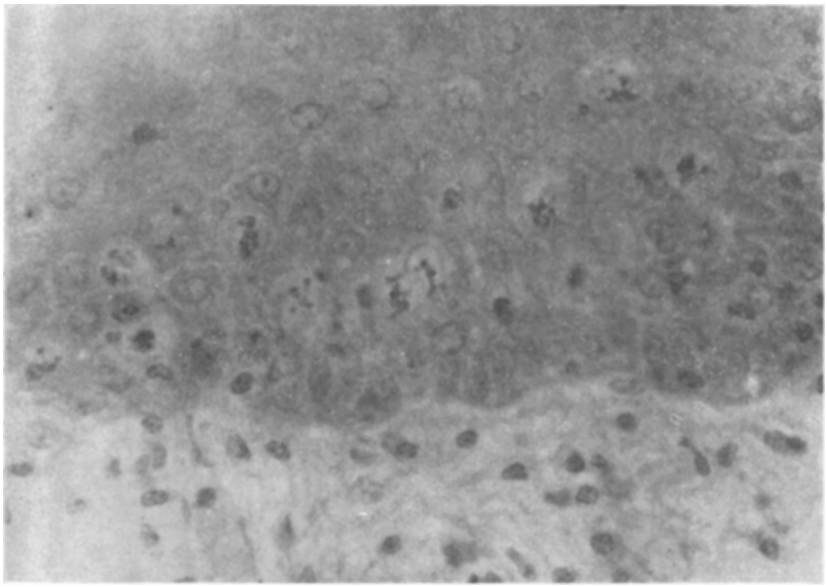

FIG. 3. (Case 2—menopausal castrate.)
Vaginal epithelium during induced follicular phase.

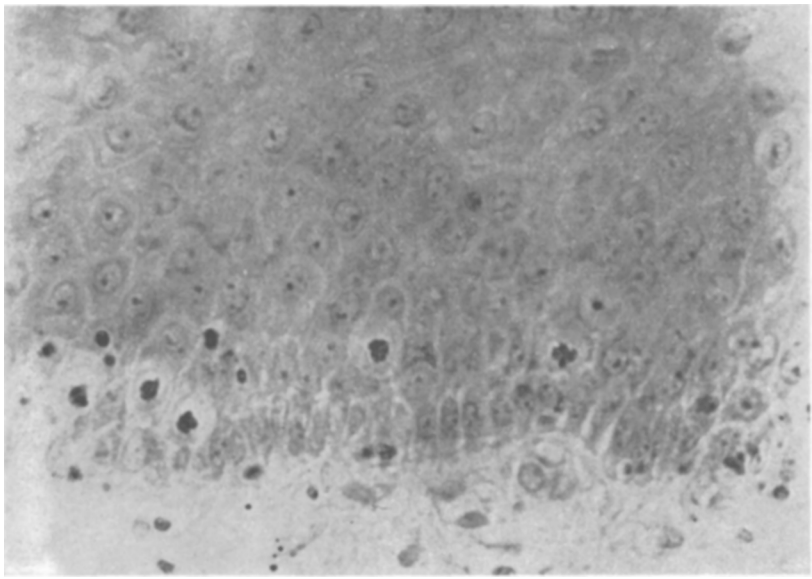

FIG. 4. (Case 2—menopausal castrate.)
Vaginal epithelium during estrogen-progesterone administration.

many were vacuolated, and the frequency with which they were devoid of cytoplasm and chromosomes suggested their fragility.

Discussion. The applicability of the colchicine technic to man makes available an additional method for analyzing hormonal effects on structures such as the vagina and skin which are accessible to topical application of the alkaloid. It may also be possible to introduce colchicine in a suitable medium into the uterine cavity prior to endometrial biopsies. What additional information will be derived from the use of this technic can only be determined by further experiments.

The technic was employed in the present study to aid in the analysis of the hormonal factors responsible for the structure of the vaginal epithelium during the menstrual cycle and thereby responsible for the character of the desquamated vaginal secretion. The results reinforce the conclusions drawn from previous experiments of the same character in which colchicine was not employed.

The effects of estrogens in stimulating the growth of the vaginal mucosa and producing a cornified vaginal secretion[7, 8] are now generally accepted, and the accumulated mitoses, after estrogens in the menopause and during the follicular phase of the normal cycle, reflect this proliferative influence. The conclusions were drawn from our previous study[5] that the estrogen-progesterone combination which controls the second half of the menstrual cycle, has two effects: (a) to modify the estrogen effect on the vaginal epithelial cell so that it fails to develop to full cornification, and (b) to enhance the proliferative effects of estrogens in a manner analogous to its action on endometrial growth. The abundant mitoses seen in premenstruum in normal women, and in castrates when this combination of hormones is given, support the conclusion that active proliferation is taking place.

Conclusions. Colchicine, by topical application in vaginal pessaries, permits the detection of the growth stimulating effects of estrogens, and progesterone in combination with estrogens, on the vaginal epithelium of menopausal castrates. In menstruating women, this technic demonstrates clearly the proliferative effect of these hormones of endogenous origin during both the follicular and premenstrual phases of the cycle.

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[7] Davis, M. E., and Hartman, C. G., *J. A. M. A.*, 1935, **104**, 279.

[8] Papanicolaou, G., and Shorr, E., *Am. J. Obs. and Gyn.*, 1936, **31**, 3.