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Action of Gonadotropic Hormones in Amenorrhea as Evaluated by Vaginal Smears.*†

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We reported that the vaginal smear could be used in women to evaluate the action of estrogens, adequate amounts of which transformed the menopausal smear to the follicular type seen during the normal menstrual cycle at the height of follicular activity.[1] Full replacement therapy could, by its use, be insured not only in the menopause but in amenorrhea as well. Its value for the biological assay of estrogens in the human was pointed out. Subsequent studies from this laboratory demonstrated the applicability of the smear to the analysis of the action of androgens in women.[2] They were shown to be capable of suppressing menstruation, transforming the normal smear to an atrophic type seen in the menopause and primary amenorrhea. It was also found that androgens, given to menopausal women receiving full replacement therapy with estrogens, caused a regression of the induced follicular smear to the original menopausal level.[3] The smear could thus be used to control the therapeutic use of androgens in women wherever indicated.

The present study deals with the application of the smear method to a functional classification of amenorrhea and to the evaluation of another group of hormones, the gonadotropic principles, which are being employed in this condition in an effort to induce ovarian activity.

The status of our present knowledge of the amenorrheas and their therapy is quite unsatisfactory. This is due in part to the lag in chemical progress in the field of the gonadotropic principles, as compared with the estrogens, as well as to our ignorance of the

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1 Papanicolaou, G. N., and Shorr, E., *Am. J. Obst. and Gyn.*, 1936, **31**, 806.

2 Papanicolaou, G. N., Ripley, H., and Shorr, E., PROC. SOC. EXP. BIOL. AND MED., 1938, **37**, 689; *Endocrinology*, 1939, **24**, 339.

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

exact mechanisms underlying the production of the amenorrheas.

A more satisfactory classification of this condition than now exists should help clarify this problem. It is customary to divide the amenorrheas into two groups. If menstruation has never occurred, they are classified as "primary." If amenorrhea has interrupted a period of cyclic menstrual activity, it is considered as the "secondary" type. Examination of the vaginal smears of approximately 40 such patients makes it clear that such a classification is too static, and might well be supplemented by a functional one, based on the degree of associated ovarian under-function as well as the presence or absence of cyclic ovarian activity. On the basis of the smear picture, the amenorrheas can be subdivided into 3 groups. The first is characterized by the constant presence of the atrophic smear which we have interpreted as indicative of virtual absence of ovarian activity.[1] This group includes most "primary" and many "secondary" amenorrheas. The second group exhibits smears which are quite constant for each case and are indicative of some degree of subnormal and uniform ovarian activity. Into this group fall most of the "secondary" amenorrheas. The third and smallest group shows irregular cyclic smear changes, imitating those seen during the normal menstrual cycle, and reflecting periodic ovarian activity, which is however insufficient to produce overt menstruation. This picture is seen chiefly in "secondary" amenorrheas and in adolescents with delayed puberty.

With such a variety of types, the present confusion as to the effectiveness of available gonadotropic agents might be anticipated. Our own experience as to the unpredictability of the response to this group of hormones agrees with that of other workers. However, sufficient positive results have been obtained to indicate the potential value of the gonadotropic hormones and the usefulness of the smear method in their evaluation.

The changes induced in the smears in the amenorrheas, by these hormones, range from a brief congestive response, marked chiefly by an increased secretion of mucus, with little or no change in the cellular picture, through intermediate stages to the development of a full set of changes comparable to those seen in the course of a normal menstrual cycle. For the sake of brevity, the smear changes induced in one such case by a gonadotropic extract will be described in detail as exemplifying a fairly complete type of ovarian response to these hormones.

Case History: Age 19, single, in good health. Catamenia at 14. For first 6 months irregular, at 2-5 week intervals, then regular,

at 4 week intervals, lasting 2-4 days, and somewhat scant until 18 years when they ceased. Duration of amenorrhea was 15 months, except for 4 months during which mixed hormonal therapy including thyroid, estrone, and pregnancy urine extract resulted in three bleedings, the last 4 months prior to admission to Endocrine Clinic.

Control Vaginal Smears: Smears taken for a month prior to treatment were of the atrophic type (Figure 1) we have described as occurring in most "primary" amenorrheas and many castrates.[1] We interpret this smear as indicating profound depression of ovarian activity.

Treatment: Pregnancy urine extract (Follutein) was given as follows: beginning 12/6/38 with 20 R.U. subcutaneously, the extract was given daily for 9 days in increasing doses with a final dose of 1,000 R.U. on 12/15/38 (Total = 4,470 R.U.)

Vaginal Smears: On 12/12-13/38 there was a strong mucous reaction, the cellular picture remaining unchanged. On 12/18-19/38 mucus was again seen together with some fibrination and a few erythrocytes. From 12/28 on there occurred a gradual change in the smear picture with the development of a follicular type which persisted from 1/7-13/39 (Figure 2). Slight microscopic bleeding was seen from 1/7-11/39. Early regression was apparent on 1/15/39 and more advanced by 1/17/39 (Figure 3). There was no distinct premenstrual phase. On 1/18-20/39 moderate bleeding appeared (Figure 4). The early appearance of the bleeding and the lack of a typical premenstrual phase were interpreted as indicating the absence of ovulation. During the bleeding phase (1/18-20/39) deep cells reappeared, increased in number, and the smears changed to their original atrophic character. The patient reported a faint show on 1/8-10/39 and on 1/18-20/39 what she considered an entirely normal menstrual flow.

Second Course of Treatment: Ten injections of Follutein in increasing amounts (50-1,000 R.U. totalling 3,760 R.U.) were given from 2/1-14/39.

Vaginal Smears: The initial level was typically atrophic. After an initial mucous reaction the cell type changed gradually to reach a follicular phase on 2/10/39. Regression began 2/14/39 and was slower than before. Bleeding was seen in the smears of 2/22-24/39 which were a little more typical of the normal menstrual smear. Deep cells reappeared in large numbers on 2/25/39 and persisted. The patient reported what she considered a normal menstrual flow on 2/21-24/39.

Third Course of Treatment: This time a gonadotropic extract

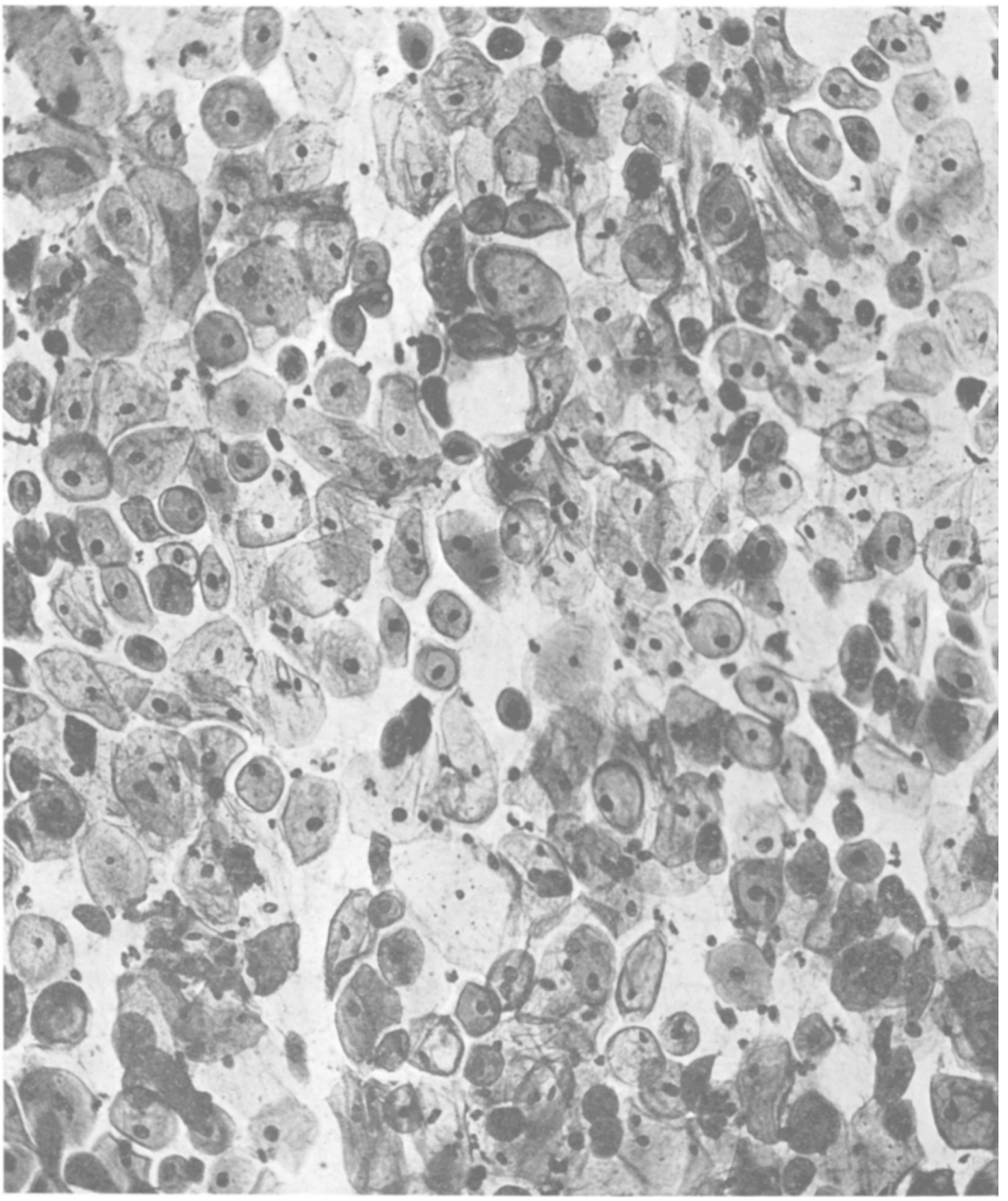

FIG. 1.
Original type of atrophic vaginal smear, showing many deep cells. × 250.

from pregnant mare serum (Gonadogen) was used, 150 units being given intravenously on 3/11/39.

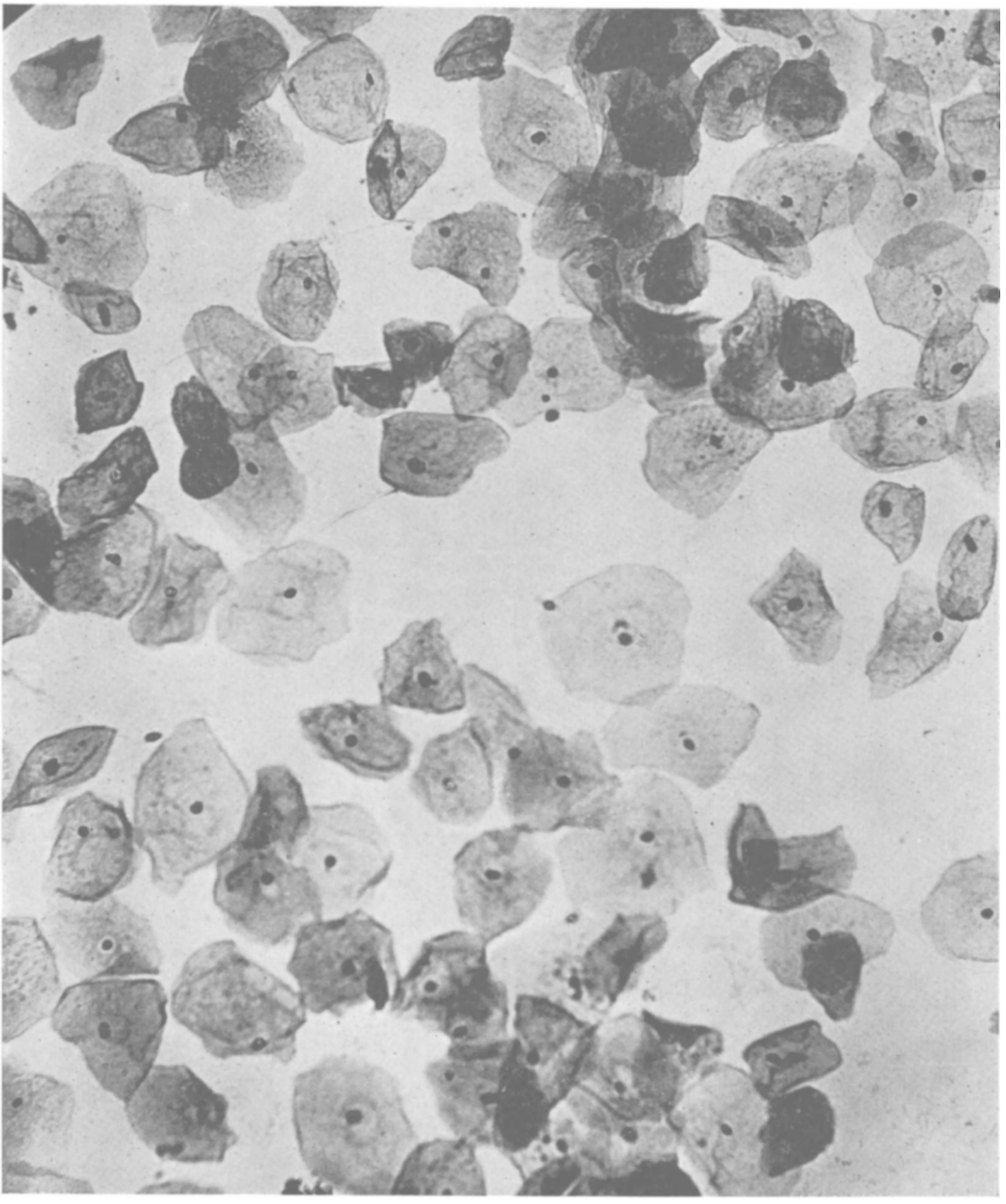

FIG. 2.
Follicular type of vaginal smear, induced by treatment. × 250.

Vaginal Smears: An early or pre-follicular phase was reached on 3/18-19/39, followed by a rapid regression with reappearance

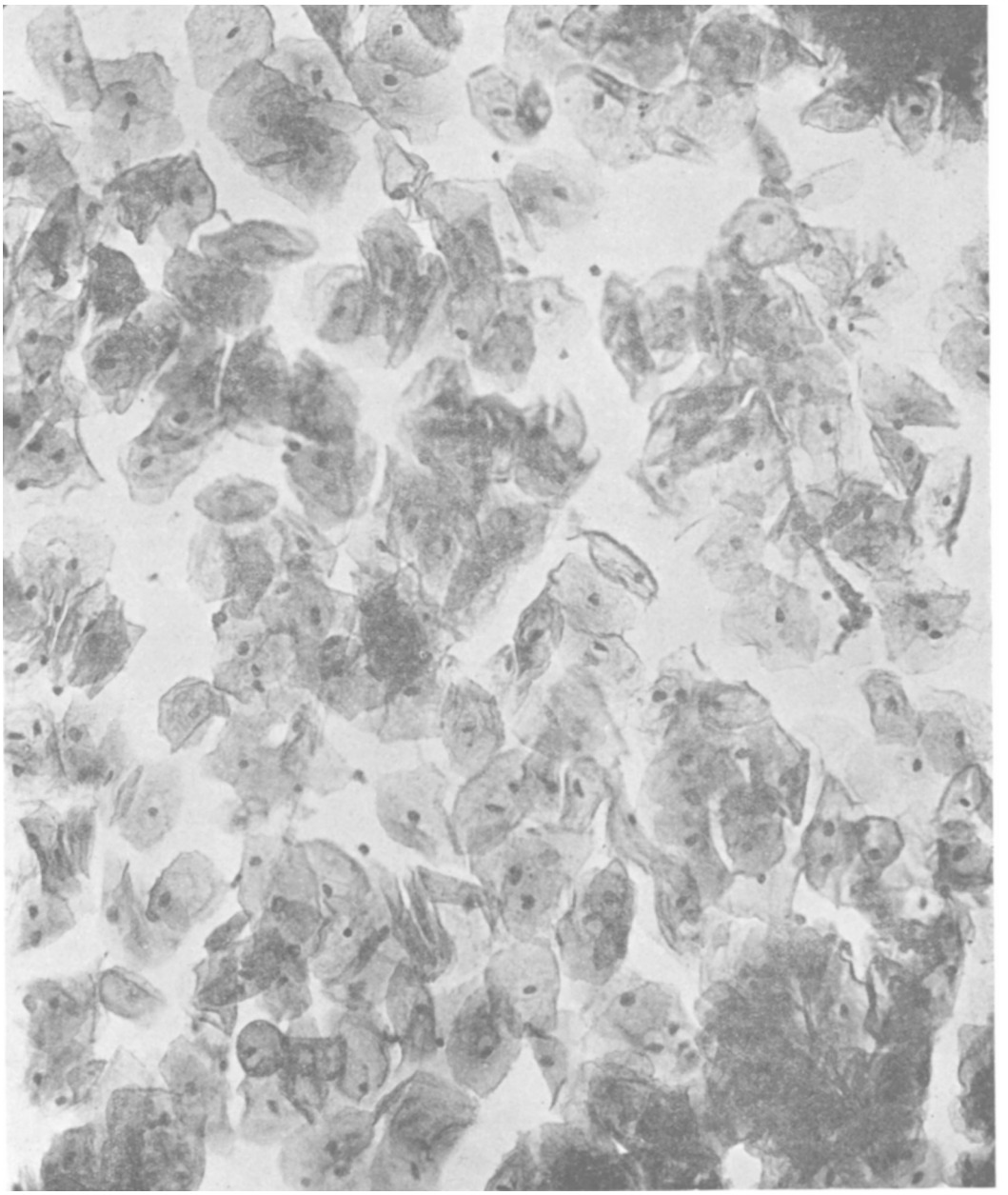

FIG. 3.
Vaginal smear showing regression. × 250.

of deep cells and microscopic bleeding 3/21-22/39. There was no overt bleeding.

FIG. 4.
Vaginal smear showing bleeding. × 250.

Fourth Course of Treatment: Another course of Follutein was iven 4/11-24/39. Initial dose 50 R.U., final dose 1,000 R.U. Total 4,950 R.U.)

Vaginal Smears: Control smears were less atrophic than the original ones. Starting 4/12/39 a gradual change to an early follicular smear occurred which was maximal 4/22/39. The regression proceeded at a slower rate with very few deep cells, and the smears were nearer the normal premenstrual type. Bleeding was seen on 5/3-6/39 with a few deep cells reappearing towards the end of the flow. The patient reported a normal menstrual flow on 5/3-6/39.

Comment: This case we would now classify as a secondary amenorrhea of the stationary or "fixed" atrophic type. This type, in our experience, rarely resumes ovarian activity spontaneously. For this reason we feel that the changes in ovarian activity as reflected in the vaginal smears were actually due to the treatment given. Another reason for this belief is that following each course of treatment the smears reverted to the original atrophic type.

The degree of ovarian stimulation would appear to have been considerable since smear changes of a follicular type were induced, quite comparable to those seen during the normal menstrual cycle. Furthermore, the estrogen elaborated was sufficient to build up an endometrium from which there was a flow equal in amount and duration to the patient's normal flow. Whether true ovulation occurred is questionable and it is quite likely that the first 3 cycles, at least, were anovulatory. The post-follicular smears in these cycles were not of the usual premenstrual character but rather like those seen on estrin withdrawal in amenorrhea or the menopause, where the corpus luteum hormone does not influence the smears and bleeding occurs in 5-6 days after treatment stops. The post-follicular smears of the 4th cycle were more premenstrual in character, the bleeding occurred 12 days after the follicular stage, and the menstrual smears were more normal. This cycle may have been accompanied by ovulation.

Summary: The desirability of a reclassification of the amenorrheas based, in part, on the vaginal smear picture has been suggested. Definite smear changes indicative of ovarian stimulation and resembling the normal cycle have been induced in amenorrhea by means of gonadotropic hormones. The vaginal smear should afford a simple and sensitive method for evaluating the effects of such hormones in the human subject.