

CYTOLOGICAL AND CYTOCHEMICAL STUDIES OF HUMAN VAGINA IN HIRSUTISM**

by

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One of us (Shah 1955) had earlier reported the frequent occurrence of smears showing varying grades of cytological atrophy, namely the presence of basal and parabasal cells side by side with superficial cornified cells in hirsute women of active reproductive age (Fig. 1). The present study reports further work to unravel the mechanism involved for this intriguing finding and demonstrates how cytological and cytochemical studies help in detecting the presence of biologically active androgen acting singly or in combination with oestrogen in clinical condition.

Material and Method. The clinical material comprised of 37 hirsute patients, ranging from 15 to 35 years of age. All these patients were found to be abnormally hairy by the criteria of hair rating, laid down by Shah (1957). In each patient, serial vaginal smears to quantitate the oestrogenic effect were taken throughout a single menstrual cycle. Vaginal smears were also repeatedly taken in two more cases of congenital adrenal virilism, before and after administra-

tion of cortisone. In addition to this, vaginal smears in one more adult case having total ovarian insufficiency were studied. Oestrogen and androgen in varying proportions were then given simultaneously to her to bring about comparable cytological changes seen in the smears of hirsute women.

For the cytological changes, the smears were stained according to the technique of Papanicolaou and classified according to the cell morphology as described previously (Shah 1952). The smears were evaluated by the differential cell count technique recording the percentage of only three types of cells: (i) cornified, (ii) pre-cornified and intermediate, and (iii) parabasal and basal cells; the percentage counts shown in this communication are based on the study of 300 cells in each smear.

The cytochemical observations concerned demonstration of glycogen in basal and parabasal cells only. For this, smears were stained by periodic acid-schiff technique of McManus. The percentage counts however were made on 100 basal and/or parabasal cells and only those cells, showing moderate to marked reaction for glycogen, were taken into account.

Observations and Discussion. According to De Allende, presence of 40% or more cornified cells in a vaginal smear suggested good oestrogenic effect. Such counts are commonly

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seen at the peak of maximum oestrogen activity in apparently healthy cyclic women and, therefore, a smear showing percentage of 40 or more of cornified cells is classified as "eutrophic".

In order to have correct assessment of the level of circulating oestrogen in a given individual, we feel that along with the percentage of cornified cells the general pattern of the smear with all the cytological details should be considered. This is more so when the smear depicts low percentage of cornified cells. Considering all the cytological details, we have in the past classified smears of oestrogen deficiency into three categories (Shah 1952). The changes described under "Atrophic Smear" suggested marked oestrogen deficiency, under "Hypotrophic" moderate oestrogen deficiency, and the one under "Hypotrophic with marked Cytolysis" was seen in presence of minimal oestrogen deficiency. This assessment of the level of circulating oestrogen from the changes in the vaginal smear should be related to the clinical data in each case. For example, the total or partial oestrogen deficiency smears in a situation like hirsutism where the vaginal epithelium in addition to oestrogen is alleged to be under the influence of circulating androgen may not give the true picture of the oestrogen production (Shah and Dave 1960). The "atrophic" smear therefore could result because of the poor production of oestrogen or could be due to the production of biologically potent androgen neutralising the normal amount of oestrogen at the vaginal cell level.

Table I shows the vaginal smear patterns in 37 hirsute patients.

Table I
VAGINAL CYTOLOGY IN HIRSUTISM
OF UNKNOWN ETIOLOGY (37)

VAGINAL SMEAR	NO. OF CASES	MAXIMUM CORNIFICATION	BASAL CELLS PRESENT
ATROPHIC	1	2%	1
HYPOTROPHIC	19	2-39%	15
HYPOTROPHIC WITH MARKED CYTOLYSIS	3	NOT DONE	ABSENT
EUTROPHIC	14	41-88%	10
TOTAL	37	-	26

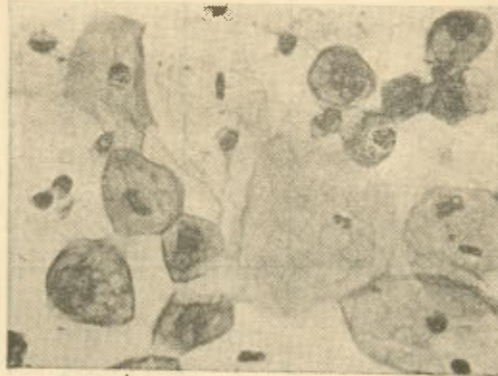


Fig. 1.

Vaginal smear of a hirsute patient showing good number of basal and parabasal cells side by side with superficial cornified cells. 600 x

Twenty-three of these 37 patients had smears suggestive of some degree of oestrogen deficiency. Since correct idea of cornification cannot be obtained in presence of marked cytolysis of the vaginal epithelial cells, the differential count was not done in 3 cases showing the vaginal smear pattern "Hypotrophic with marked cytolysis". Ten of the remaining 14 cases showing good oestrogenic effect had considerable number of basal cells in their smears. This finding indeed was intriguing. To explain this finding, we subjected these patients to some more investigations. Of the

total 26 cases showing basal and parabasal cells in their vaginal smears, in only 12 cases further investigations were possible.

Table II
URINARY 17-KETOSTEROIDS IN
HIRSUTE PATIENTS (12)

VAGINAL SMEAR	NO. OF CASES	mgm/24hrs
ATROPHIC	1	5.92
HYPOTROPHIC	2	13.95
	3	6.90
	4	9.10
	5	5.20
	6	9.40
	7	10.20
	8	8.40
EUTROPHIC	9	7.75
	10	7.80
	11	10.90
	12	5.85

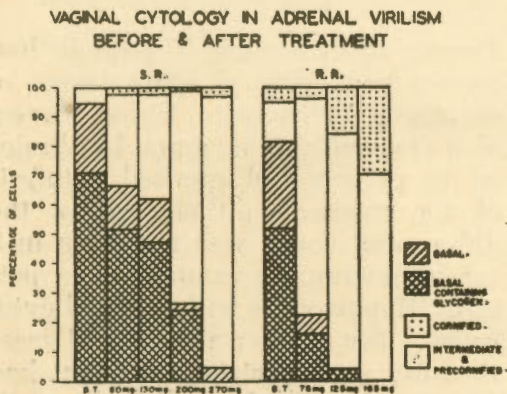


Table II reveals the finding of urinary 17-ketosteroids (the metabolites of circulating androgen) in these

12 cases. In our laboratory the normal excretion range of urinary 17-ketosteroids in women of active reproductive age varies from 4-13 mgm. per 24-hours with the mean value of 8.060 ± 0.484 . It is evident from Table II that none of these cases had elevated level of 17-ketosteroids. Moreover, the mean value of 17-ketosteroids in hirsute women (8.448 ± 0.669) when compared with that seen in our group of normal women was statistically not significant. There was also no correlation between the level of 17-ketosteroids and the pattern of the vaginal smear seen. For example, the finding of 5.9 mgm. of 17-ketosteroids in a case of marked hirsutism having atrophic vaginal smear was rather interesting. The low level of 17-ketosteroids, however, cannot exclude the possibility of the presence of small amounts of biologically potent androgens and such amounts certainly would not make significant changes in the value of 17-ketosteroids in urine. To determine whether the presence of basal and parabasal cells in the vaginal smear of these cases could occur as a result of negation of oestrogen by the circulating androgen, we looked for a clinical situation like congenital adrenal virilism where both oestrogen and androgen are known to be present in large amounts (Migeon and Gardner 1952). Fig. 2 reveals the vaginal smears in two cases of congenital adrenal virilism. Both these cases showed as high a percentage as 95 and 82 of basal and parabasal cells in their smear before treatment and 2-3 fold increase in the urinary values of 17-ketosteroids. Paradoxically, most of these basal and parabasal cells con-

tained glycogen (Fig. 3). It is an observed fact that in physiological states of marked oestrogen deficiency, basal and parabasal cells do not contain glycogen (Cruickshank 1934). Under cortisone therapy, in these cases there was marked reduction in the production of androgens from the adrenal cortex as measured by the estimation of the urinary 17-ketosteroids. Consequently, there was also marked reduction in the basal cells and reduction in the basal cells containing glycogen (Fig. 2).

Interestingly enough, the basal cells in the vaginal smears of our hirsute patients also contained glycogen (Table III). It is evident from Table III that although glycogen was present in the basal cells of all the 12 cases, cervical erosion, however, was present in only one patient. To confirm whether the deposition of glycogen in these cells in hirsute cases was indicative of the presence of endogenously circulating androgen or not, a case of total ovarian insufficiency was administered oestrogen and androgen in various proportions to

Table III
GLYCOGEN IN BASAL AND
PARABASAL CELLS

VAGINAL SMEAR	GLYCOGEN PRESENT	EROSION
ATROPHIC (1)	1	NIL
HYPOTROPHIC (7)	7	NIL
HYPO. M. CYTOL.	-	-
EUTROPHIC (4)	4	1
TOTAL (12)	12	1

bring about cytological changes comparable to those seen in our cases of hirsutism.

It is clear from Fig. 4 that before treatment the vaginal smear showed 77% of basal cells. A considerable number of basal cells however revealed presence of glycogen. This could probably be due to sub-clinical vaginitis produced by marked oestro-

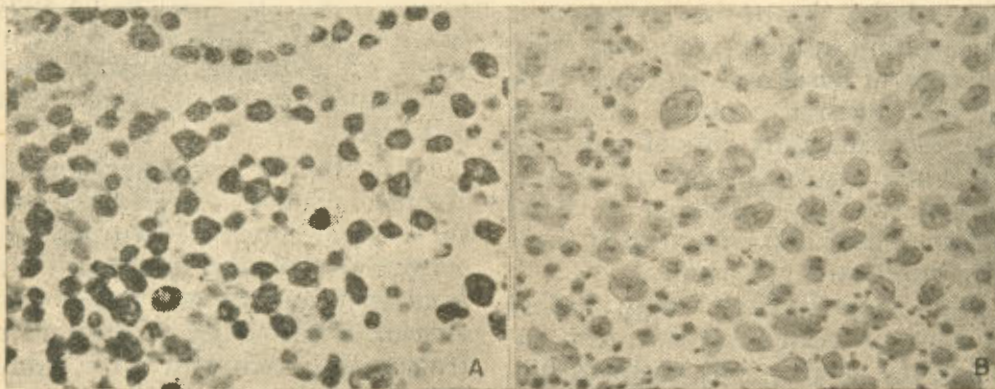


Fig. 3.

Fig. 3—"Atrophic" smear of a case of congenital adrenal virilism. Basal and parabasal cells contained glycogen—(A) before and (B) after distase treatment. Periodic-acid-schiff and Haematoxylin stain. 630 x

EFFECT OF HORMONES ON VAGINAL CYTOLOGY IN OVARIAN INSUFFICIENCY

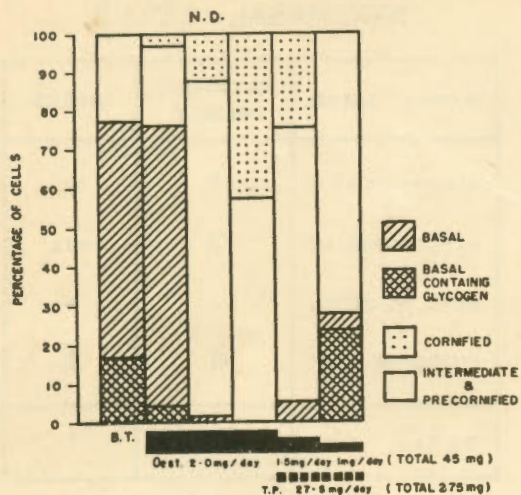


Fig. 4.

gen deficiency. The patient was then put on 2.0 mg. of oestrogen daily till her vaginal smear showed eutrophic changes. At this stage the dosage of oestrogen was gradually reduced to 1.0 mg. and testosterone propionate was simultaneously injected in the dosage of 27.5 mg. per day from the start of 1.5 mg. of oestrogen daily. From this histogram it appears that initially there was reappearance of basal and parabasal cells but they did not contain glycogen. With the same daily dose of androgen and when oestrogen was reduced to 1.0 mg. per day, number of basal cells increased and now most of these cells contained glycogen.

From these findings it appears that the various grades of oestrogen insufficiency seen in our cases of hirsutism are but the end results of the interaction of varied quantitative combinations of androgen and oestrogen. Even if this characteristic

pattern of vaginal smear in presence of circulating androgen fails to pinpoint the source of androgen, viz. ovary or adrenal cortex, it seems worth while to pursue and develop cytochemical methods which would detect the presence of minute quantities of biologically active sex hormones—acting singly or in combination. Such techniques in the study of pathological states would have obvious advantages over the chemical measurement of urinary metabolites of the sex hormones.

Summary

(i) Cytological and cytochemical observations on vaginal smears of 37 hirsute patients ranging from 15 to 35 years of age are presented.

(ii) Twenty-six of the 37 patients showed, in their vaginal smears, mild to severe atrophic changes as judged from the number of basal and parabasal cells. These cells, contrary to the usual findings, contained glycogen. These cytological and cytochemical observations, especially in association with unelevated urinary 17-ketosteroids, appeared to be suggestive but not conclusive of the presence of androgen neutralising the circulating oestrogen at the vaginal cell level.

(iii) Similar cytological and cytochemical changes were observed in the vaginal smear of two patients of congenital adrenal virilism before treatment and of one case of total ovarian insufficiency when both oestrogen and androgen were administered simultaneously in the proportion of 1 : 27.5 mg. per day.

(iv) The implications of these findings are discussed.

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