

## STUDY OF ESTROGEN ACTIVITY IN 1300 MENOPAUSAL WOMEN\*

by

USHA B. SARAIYA,\*\* M.D., D.G.O., F.I.A.C., F.I.C.S.

JAYSHREE V. JOSHI,\*\*\* M.D., D.G.O., D.F.P.

BERNADETTE CARVALHO,† B.Sc.

and

WINIFRED FERNANDES,†† M.D., F.C.P.S., F.R.C.O.G.

We became aware of estrogen activity in menopausal women in 1963 when a study of postmenopausal endometrium was presented by Thakar and Fernandes. Out of 104 hysterectomy specimens studied, at least 50 per cent of the cases showed some degree of estrogen activity in the endometrium. Although this was an incidental finding while studying results of operation for prolapse, it served as a beginning and a basis for further studies on estrogen activity in this age group.

Later from 1969-1974, a cytohormonal study was undertaken in 1200 menopausal women. The present communication, deals with our results under the following headings:

1. Postmenopausal endometrium—104 cases.

\*Study carried out at Cytology Clinic of Association of Medical Women in India at Cama Hospital, Bombay 1, India; assisted partly by I.C.M.R. (New Delhi).

\*\*Hon. Asst. Prof. in Obstetrics & Gynaecology at Grant Medical College, Bombay 8, India and Hon. Cytologist, Cytology Clinic Cama and Albess Hospital, Bombay 1.

\*\*\*Research Officer in Cytology, Cytology Clinic Cama Hospital.

†Cytotechnologist, Cytology Clinic Cama Hospital.

††Hon. Prof. in Obstetrics & Gynaecology Grant Medical College, Bombay 8.

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2. Routine Cytology in 1200 women.
3. Women with postmenopausal bleeding—120 cases.
4. Women after hysterectomy and oophorectomy—159 cases.
5. Estrogen estimations in urine and correlation with cytology—125 cases.

### 1. Postmenopausal Endometrium

From 1961-63, endometrium from 104 uteri removed for genital prolapse were studied and classified according to Parks *et al* (1958). Cases with bleeding and/or hormone therapy were excluded. We had 9.6 per cent atrophic, 17.3 per cent inactive cystic glands, 2.8 per cent hyperplasia, 66.3 per cent proliferative, 2.8 per cent secretory and 0.96 per cent polyps. Table I confirms that our results compare well with those of others in the field. Gianaroli (1947), Speert (1949), Davies and Williams (1953) had independently studied hysterectomy specimens and concluded that functional activity of the ovaries seems to be continued in old age. Our patients belong to a younger age group, 34 being under the age of 50 years. This may in part explain the high incidence of proliferative endometrium, indicating presence of estrogen activity. Ovarian function may continue for 1-2 years after the menopause.

TABLE I  
Cytohormonal Status Given by Different Authors

	Atrophic	Inactive Cystic Glands	Hyperplasia	Proliferative	Secretory	Polyp.
Gianaroli 50 cases 1950	15%	15%				
Speert 60 cases 1949		72%	1.5%			17%
Foix 100 Curettings 1952	6%	80%		10%	3%	
Davies 40 cases 1953	40%	30%	25%	5%		
McBride 1955	60%	20%	5%			
		15%				
Novak & Richardson 137 cases 1941	45.2%	24%	20%	10%		
Parks 337 cases 1958	49.6%	48.9%	.9%	.3%		
Present Series 104 cases 1964						
Cama & Albless Hospital	9.6%	17.3%	2.9%	66.3%	2.9%	.9%

Distribution of cases according to the duration of menopause is given below. This is a more reliable criterion than the age. Most of our patients are not aware of their correct age.

	No. of cases
Under 5 years	34
6-10 years	21
11-15 years	24
16-20 years	16
Over 20 years	9
<b>Total</b>	<b>104</b>

Since this was a retrospective study we were not able to do further endocrine studies on these women.

Fig. 1 shows endometrium with inactive cystic glands.

2. Routine Cytology in 1200 Women

The object of collecting this data was to know the cytohormonal status of post-menopausal Indian women with special reference to age, duration of menopause and clinical findings.

Cyto-analysis in 1200 women is as follows:

Normal	297 cases	24.7%
Inflammatory	719 cases	59.9%
Dysplasia	50 cases	4.1%
Suspicious	26 cases	2.1%
Carcinoma	64 cases	5.3%
Unsatisfactory	44 cases	3.6%

The cytohormonal states given by different author is given in Table I.

There were only 297 normal smears in which cytohormonal evaluation could be done. The incidence of senile vaginitis was high (nearly 60%).

We have followed the 3 basic cytologic patterns of the menopause as described by Koss (1968) early menopause, crowded menopause and advanced or atrophic menopause.

*Early Menopause.* There is slight deficiency of estrogens. The smears are essentially those of the childbearing age except for a diminution of the number of superficial squamous cells. The intermediate cells show uniform nuclear enlargement. The K I should not exceed 30 and M I 0/70/30.

*Crowded Menopause.* There is moderate deficiency of estrogens. This type of smear is characterized by thick crowded clusters of numerous intermediate and large parabasal cells. The cells are well preserved and the nuclei show an uniform enlargement. The cytoplasm frequently contains deposits of glycogen. The K I should not exceed 10 and the M I should be around 10/80/10.

*Atrophic or Advanced Menopause.* The cytologic patterns of the advanced atrophic menopause are influenced by dryness of the genital tract and attendant infection. The predominant cell is of the size and the degree of maturity corresponding to the parabasal cells. Two main

effects of dryness may be observed. One is uniform enlargement of the cell accompanied by a characteristic uniform gray discoloration of the degenerated nucleus (enlargement type)—the second is marked eosinophilia of the cytoplasm accompanied by nuclear pyknosis and Karyorrhesis (the eosinophilicpyknotic type).

These changes together with the lack of properly preserved cells make it very difficult to exclude malignancy.

We had 65 smears of early menopause giving an incidence of 22.0 per cent. Of these 56 were in the first 10 years of menopause, 6 in the next decade and only 3 were after 20 years of menopause. These 3 cases were probably because extra-ovarian sources of estrogen had developed.

We had 77 cases of crowded menopause giving an incidence of 26.0 per cent. Of these, 55 were in the first decade, 20 in the second decade and only 2 after that.

There were 155 atrophic smears giving an incidence of 52.0 per cent. Of these, as many as 104 were in the first 10 years. Here the extra-ovarian sources had not had a chance of becoming established and there was a sudden fall in the circulating estrogen.

Therefore, out of 215 cases in the first decade of menopause, one half show some degree of estrogen activity and the other half show an atrophic pattern. In the second decade, out of 76 cases only 24 show some degree of estrogen activity, whereas 52 showed an atrophic pattern after 20 years of menopause only an occasional smear will show an estrogenic smear.

Fig. 2 indicates in greater detail the incidence of the 3 smear patterns with reference to the duration of menopause.

TABLE III  
Cyto-Histo Correlation—25 cases

Cytology			Histopathology	
Positive	Suspi- cious	Nega- tive	Posi- tive	Nega- tive
9			8	1
	15		8	7
		1	1	

were available in only 25 cases. The rest of the cases were not treated in this Institute. Of the 9 cytologically positive cases, 8 were confirmed positive by histopathology while 1 case showed negative findings. There were 15 cases with suspicious cytological smears of which 8 were found to be histologically positive while 7 were negative. Only 1 case which had a negative smear had an early ovarian carcinoma on hysterectomy.

All cases of postmenopausal bleeding even if negative on investigations require a very close follow-up. We advise cytology every 3 months and insist on an endometrial aspiration smear. It is the opinion of our senior author who has over 25 years of experience in the field that a very high per cent of these women develop an overt carcinoma sooner or later. We have come to the conclusion that hysterectomy would be justifiable in a large number of these women, especially those who are unable to come for a regular check-up. Paloucek, *et al* (1964) studied 257 patients with postmenopausal bleeding and it is their experience also that cancer at all sites develops more frequently in women with postmenopausal bleeding than in those who do not experience this symptom.

Figure 5 shows a cluster of adenocarcinoma cells from a 52 year old patient with postmenopausal bleeding.

Cytology has a definite place in the management of cases with menopausal

bleeding and if used routinely will diagnose a few more cases which may be missed on biopsy.

However, routine cervico-vaginal smears should be supplemented with endocervical and endometrical aspiration smears. Dilatation and curettage should be scheduled after due consideration is given to cytology report. In cases with suspicious clinical lesions and negative cytology biopsy is still justified. Finally we advocate a very close follow-up of all cases who have experienced this symptom and for this cytology is very suitable.

#### 4. Women after Hysterectomy

Vault smears from 159 hysterectomised women were studied. Seventy-one had normal smears, 77 had inflammatory smears, 10 showed abnormal cells and 1 smear was unsatisfactory. Women with diabetes, liver diseases or digitalis therapy were excluded as these are known to induce estrogen-like responses.

There were 24 women in whom both the ovaries had been removed. Out of these 2 showed an early menopause smear—one 6 months and the other 2 years after menopause. Seven showed crowded menopause and 15 had atrophic smears.

In 4 women who had only one ovary removed the smear pattern was early in 2 upto 2 years after hysterectomy and crowded in 2. One woman was under 40 years of age and 3 were over 40.

There were 20 cases in whom ovaries were conserved at hysterectomy. One 45 year old woman had persistent estrogen effect and is kept under observation. Two women had early menopause smears one within 6 months, and the other 5 years after hysterectomy. Ten cases showed crowded menopause smears. Out of these, 7 were over the age of 40 years.

There were 7 cases of atrophic smears but they were all over the age of 45 and so have now reached natural menopause.

Whitelock (1968) also studied 2 groups of women by smears after hysterectomy, one in whom the ovaries were preserved and the other in whom, bilateral oophorectomy was performed. In the first group the estrogen activity of the ovaries was probably equal to that of normal woman, while those without ovaries had nil to very low estrogen activity.

In 22 women it was not known whether the ovaries were removed or conserved. There were 2 women with persistent estrogen effect aged 45 and 62 years. Nine women had early smears, 11 showed crowded and atrophic patterns.

In conclusion, out of 159 vault smears, cyto-hormonal evaluation was possible in 71 cases. Forty-three showed some degree of estrogen activity, whereas 28 smears were atrophic. When ovaries are conserved the smears show normo-estrogenic pattern till the age of natural menopause. Some show atrophic pattern and in these operative trauma is responsible for ovarian atrophy. When the ovaries are removed, smears are atrophic. However, persistent estrogen effect is seen quite significantly and indicates an extra-ovarian source of estrogen.

##### 5. Estrogen Estimations in Urine and Correlation with Cytology

Chemical estimation of urinary estrogens has now become a simple laboratory procedure as a result of recent advances in biochemistry. Whereas in the past the clinician had to depend only on cyto-hormonal assay, he can now validate his results by chemical estimation of urinary hormones. This is particularly significant now because estrogen therapy in the menopause has become even more of a

controversial subject. On one hand, women are demanding estrogen to stay youthful and on the other there is a reportedly increased risk of breast and genital cancer following estrogen therapy.

We have been using a simple chemical method after Brown and McLeod (1968) for estimating total estrogens in the pooled 24 hour urine specimens by fluorimetry.

We have found that the mean average value in our series was 7.6/ugms. The mean age was 54 years and the duration of menopause ranged from 1 to 25 years. This value compares favourably with the results of others in the field. Brown *et al* 1955 reported a study of 20 women with mean excretion value of 6.4% ugms. Brown and Beischer (1972) reported a study of 18 women with mean excretion rate of 7/ugms. McBride (1957) has reported a study of 7 cases with mean age 59 and mean excretion value as 5.9/ugms. So far we have not come across any report of a large series of women.

Table IV analyses the urinary estrogen level in relation to years of menopause. It is seen that fairly wide variations occur in the first 10 years of menopause, estrogen levels ranging from 0 to 20 ugms were seen. This goes to prove that minimal ovarian function and steroidogenesis are maintained upto 10 years after menstruation has ceased. After this period however one can expect a fairly rapid fall in estrogen levels. Occasional patient will even then show a high estrogen level and this is the patient who needs to be watched carefully. From our study, 11 patients were selected for a close follow-up. These had levels above 10 ugms persistently.

We were able to do cyto-hormonal estimation in only 47 cases out of 125 cases. Rest of the smears showed the presence of senile vaginitis or abnormal

*Metropolitan and Urban Centres*TABLE IV  
*Estrogen in/ugms and Duration of Menopause in Years*

Yrs. of menopause	5 /ugms	5-9	10-14	15-19	20
1-5 yrs.	16	10	5	7	3
6-10 yrs.	9	11	9	9	
11-15 yrs.	11	8	5	1	
16-20 yrs.	5	2	2	-	1
20 yrs.	5	5	1	1	

cells. Table V correlates the cytohormonal assessment according to Koss (1968) and the estrogen levels.

TABLE V  
*Cytohormonal (Koss) & Estrogen in  $\mu$ gms*

	5	5-9	10-14	15-19	20
Early					
3 Cases		2	1		
Crowded					
20 Cases	9	4	2	5	
Atrophic					
24 Cases	10	7	4	1	2

There were only 3 cases of early menopause and the estrogen levels, as expected, were within normal range. There were 20 cases of crowded menopause, out of which 7 showed levels which were higher than expected. Out of 24 cases of atrophic menopause, 6 showed unexpected high levels. One of these cases was highly diabetic. These cases need to be investigated for the source of this estrogen. It is possible that the vaginal epithelium is refractory to this circulating estrogen.

We find that patients and clinicians accept both methods equally and do not indicate a preference. Brown and Beischer (1972) however state that in their hospital when an unrestricted service for steroid measurement was made available it quickly replaced vaginal cytology. He

feels that by not involving trained medical personnel and operating theatre facilities, hormone assays are cheaper and provide a more extended picture of ovarian function.

*Conclusions*

Just as puberty is a long period of establishment of ovarian function so is the menopause a long period of hormonal withdrawal. The waning ovary is of great concern today to the clinicians because of the increasing number of aged persons.

Spritz (1949) gave evidence that there is protection of the postmenopausal woman from coronary artery disease, arteriosclerosis and osteoporosis by use of exogenous estrogens. Utian (1971) states that as yet there is no proof that estrogen therapy prevents these diseases.

Though most helpful, cyto-hormonal evaluation is only an indirect attempt at estimating estrogen effect and wide variations are seen in normal women and also in surgical castrates. We agree with Liu (1968) that cytology must not be exploited as a screening test on which to base hormone therapy. Clinical indications must be taken into consideration. Replacement therapy should be geared to the individual with symptoms and the response monitored by cytology. However, vaginal cytology as an index of estrogen activity

in the post climacteric woman is very helpful.

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See Figs. on Art Paper I-II