TRIAL WITH LOCAL INJECTION OF ESTRADIOL BENZOATE

(Aqueous Suspension)

A Preliminary Report of its Effect on Hypoplasis of Uterus and Crystallization Phenomenon of Cervical Mucus

by

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The uterus reaches an adult size, on an average about one to two years before the menarche. The period of uterine growth preceeds any sign of cyclic ovarian function and it coincides with the other external evidences of puberty as part of that complex. That all of these changes are under the control of estrogen is clear, and it bespeaks of the onset of ovarian function; although conditions are not yet right for menstruation.

The uterus and vagina respond to various estrogens in the following order of decreasing potency,

Estradiol, Estradiol benzoate, Estrone, Equilin, Equilemin, Estrone benzoate, and Estrol.

The effects of estrogenic hormones upon the non-distended uterus are well-known. One may generalise that estrogen stimulates the growth of uterus in immature animals and prevents or relieves the atrophy of the uterus which follows ovariectomy.

The principal features of estrogen-stimulation of the uterus are a generalised hyperemia, accumulation of fluid throughout the tissues as a whole, infiltration of the tissues with eosinophiles and macrophage cells, presecretory changes and mitosis in glands and epithelium of this tissue. Mitosis is almost inappreciable in the myometrium under the influence of estrogen unless the uterus is distended with fluid. It also acts on cervical mucus converting thick, opalescent and scanty mucus to glairy, clear and abundant.

When estrogen is given for a prolonged period, uterine enlargement occurs in the beginning for a time but, later, hormone ceases to be effective — metaplasia sets in with decreasing vascularity of the whole uterus and diminishing motility of its muscle. The reason for this

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* Estradiol monobenzoate prepared by Messrs. Unichem Loboratories. This research was supported by grant from Unichem Laboratories and was conducted under the guidance of Dr. J. N. Karande, M.S., F.C.P.S., at B. Y. L. Nair Charitable Hospital.

change of metaplasia is not known.

Growth Potentialities of Immature Uterus

The somatic substrate is receptive to the ovarian hormone, therefore it is the lack of the external hormonal factor during prepubertal life that accounts for the failure of the uterus to grow to a size beyond that of the early post-natal period.

The reactivity of the uterus of immature animals to estrogen varies with age. The growth response is much greater to a constant dose among animals near pubescence than earlier.

The conclusion that uteri respond to estrogenic hormonal stimulation holds a certain significance for adult women with infantile uteri, but with normal menstrual cycles. In such cases, the administration of large quantities of estradiol benzoate may be without effect. Clearly, therefore, there are instances in which the uterus itself is somatically incapable of being activated by the primary hormonal stimulant of uterine growth. At present, we are wholly in the dark concerning the mechanisms of this lack of responsiveness of hypoplasic uteri in adults in the presence of menstrual cycles.

Contrary to such reports, Sara Field Richards obtained fairly satisfactory response in selected cases of hypoplasia of uterus with local intracervical injection of estradiol monobenzoate in aqueous suspension.

Impressed by her results, we decided to try out local intracervical injection of estradiol benzoate in aqueous suspension in cases of hypoplasis of uterus. In her series, only selected cases (i.e. those with regular menstrual cycle, with secretory endometrium, etc. etc.) were taken up, whereas our series included all cases of hypoplasis of uterus irrespective of their menstrual status. Our study was mainly directed towards its effects on uterine enlargement and cervical mucus.

Messrs. Unichem Laboratories were very kind to prepare an aqueous emulsion of estradiol monobenzoate for the purpose of this study.

In the past one year, 54 cases of clinically diagnosed hypoplasis of uterus have been referred from the O.P.D. of which 29 attended regularly. Only the cases, with uterine length of less than 8 cm., were considered hypoplasic.

Each patient was examined twice a week through one or more menstrual cycles. At each examination, uterine length was measured and samples of cervical mucus were studied. Endometrial biopsy was taken once a month, generally during the premenstrual phase. Aqueous suspension of estradiol benzoate was injected intracervically once a month at the commencement of menstrual cycle.

Technique

1. *Collection of Cervical Mucus*

An unlubricated Sims' speculum is introduced into the vagina. Cervix is exposed and cleaned. The cervical mucus is collected from the lower portion of cervical canal by means of a uterine packing forceps. Mucus is grasped between its blades

and is transferred to a glass slide by opening the forceps with the blades in contact with the slide surface. Both the slide and forceps are dried well before use as the crystallization pattern depends upon the electrolyte content of the mucus (the idea being not to permit any dilution of mucus). All care is taken not to traumatise the cervix as the presence of blood may inhibit crystallization. The mucus is allowed to dry at room temperature and examined under the microscope with 100 x. Crystallization with its typical features does not occur when the air is saturated with steam. Therefore, many a time, the mucus was dried by heat and immediately examined under the microscope.

2. *Measurement of Uterine Length*

Anterior lip of the cervix is held with a tenaculum forceps. A Meakers' type of hysterometer is introduced through the cervical canal into the uterine cavity till it reaches the top of the cavity. Length in centimeters is read directly on the hysterometer. The measurement is repeated twice or thrice to avoid any error.

3. *Injection of Estradiol Benzoate Intracervically*

10 mgm. of estradiol benzoate in aqueous suspension (1 c.c.) is collected in a 10 c.c. Luerlock syringe to which is attached No. 18 gauge spinal puncture needle. Cervix is held with a tenaculum and the suspension is deposited in the posterior or lateral lip, 1/2″ to the side of the cervical canal and 3/4″ to 1″ deep in the substance of the cervix. In some cases of tough (less vascular) cervices, it was very difficult to introduce whole or even part of the suspension. The problem was, however, overcome after one or two treatments as the resulting hyperemia made the deposit easier. In some rare cases where even part of the suspension could not be injected, preliminary oral estrogen eased the subsequent intracervical injections.

4. *Collection of Endometrial Biopsy*

Endometrial biopsy is taken 2 or 3 days prior to the expected date of the period. After having taken the cervical mucus for examination and measuring the uterine length, the vagina and cervix are disinfected with dettol. Endometrial biopsy curette is passed up the cervical canal upto the fundus of the uterus and, while withdrawing, its serrated surface is swept over the endometrium of anterior uterine wall. A portion of the latter usually comes out in a small strip. In cases with very poor endometrium more than one sweep of the curette were tried. The endometrial tissue is transferred to Bouin's fluid. In some cases, the internal os was very tight during premenstrual period and will not admit the biopsy curette (not even Meaker's hysterometer). These cases were subjected to dilation and curettage and the biopsy obtained.

It has been known that in normal women the uterus shows a small degree of cyclic growth which may be related to the cyclic variation of ovarian estrogen or to the cyclic changes in uterine vascularity or both. The great increase in the size

during pregnancy is mainly due to the direct mechanical stimulation in addition to the effect of enormously increased estrogen output by placenta.

While trying to study the effects of intracervical injection of estradiol monobenzoate we also studied the normal physiological variations in the size of the uterus and cervical mucus in these cases throughout the menstrual cycles. Our observations and inferences are described in the following paragraphs.

Results

1. *Uterine Growth*

In the majority of our cases, we measured the length of the uterus throughout the menstrual cycle even before starting the treatment. In all the cases with regular menstrual cycles there was associated cyclic variation in uterine length. The variation in length ranged from 0.2 cm. to 1.2 cm. depending upon the type of the menstrual cycle (and hence the ovarian function), e.g. there was no variation in length in cases of primary amenorrhoea. The average variation before treatment with intracervical injection was 0.41 cm. and the variation in length after treatment was from 0.4 cm. to 1.7 cm. with an average of 0.85 cm.

On an average 3 injections have been given to each patient, some having had only one injection while others having as many as 5 injections. The average increase in length of uterus after intracervical injection of estradiol monobenzoate was 0.84 cm., the minimum being 0.3 cm. and maximum 1.6 cm.

The maximum length of uterus during a particular cycle was found to be closely related to the type of the menstrual cycle (i.e. anovulatory or ovulatory). Out of our 29 cases, endometrial biopsy, done 2 to 3 days prior to menstruation, showed secretory phase in 12 cases (Table I). In these 12 cases, the maximum length of uterus was present during premenstrual phase in 10 of them and during intermenstrual phase in only 2 cases. The endometrial biopsy showed proliferative phase in 14 cases (biopsy taken similarly 2-3 days prior to period). In all these 14 cases, the maximum enlargement of uterus was found to be during intermenstrual period. In 3 cases of primary amenorrhoea the uterus did not show any cyclic variation and biopsy showed no endometrium. The length of the uteri increased by as much as 1.5 cms. within 4 days of intracervical injection of estradiol benzoate, but gradually within about 20 days the effect started wearing off till just before the next menstruation (withdrawal bleeding) the size was little more than that at the time of injection. In one case with infrequent periods, where endometrial biopsy showed scanty endometrium, there was no uterine enlargement whatsoever following the injection. Three patients showed ovulatory as well as anovulatory cycles during the period of study and the type of uterine enlargement can be seen from Table I.

2. *Cervical Mucus*

The functional significance of cervical mucus has been recognised only during the past few decades. Se'guy

TABLE I

The Effect of the Type of Menstrual Cycle on the Period of Maximum Growth of Uterus

Result of endometrial biopsy taken 2 to 4 days prior to the next menstrual period.	The phase of menstrual cycle during which the length of uterus was maximum.	
Secretory	Premenstrual	Intermenstrual
12	10	2
Proliferative		
14	—	14
Scanty or no endometrium		
4	—	1

(In other 3 cases the length increased only after injection of estradiol benzoate and the effect was transitory).

& Vimex described cyclic changes in the cervical mucus and pointed out that during the intermenstruum the mucus becomes abundant, watery, glassy, elastic and clear and its viscosity decreases while its elasticity increases. Papanicolaou demonstrated that when cervical mucus is spread on a slide and allowed to dry, it crystallizes with arborization, a phenomenon which is particularly characteristic at the time of ovulation. B. Zondek refers to this as palm-leaf (PL reaction) reaction, as the crystals look like palm-leaf or fern. The influence of estrogen production upon the secretion of cervical mucus was demonstrated by Se'guy and Simmonet, Momicard, Zondek, etc.

Campos de Paz describes the following degrees of crystallization:

1. Total & typical crystallization (****): In the examination of a dried sample of cervical mucus a crystallization pattern like a carpet made of palms can be observed. No other structures can be seen (Fig. 1).

2. Typical partial crystallization (***): On examining the dried mucus typical crystallization can be seen at some places and atypical or negative crystallization at other places (Fig. 2).

3. Atypical crystallization: Here a pattern similar to an outline of the characteristic palm can be seen. It is impossible to appreciate clear picture. In other areas some amount of cellular content can be seen (Fig. 3).

4. Negative crystallization (-ive): The dried mucus does not show any fernlike structure. It has only a cellular structure (Fig. 4).

In normal women the crystallization phenomenon runs parallel with the amount of cervical mucus and spinbarkeit, whereas the cellular content of cervical mucus is inversely proportional to that of crystallization (Fig. 5).

The crystallization phenomenon is much less marked (negative or atypical) following menstruation. It gradually increases till, at ovulation, it rises to total typical crystallization (****). Thereafter it again decreases and becomes negative just prior to the next menstrual period.

Fig. 1
Total typical crystallisation—x 250.

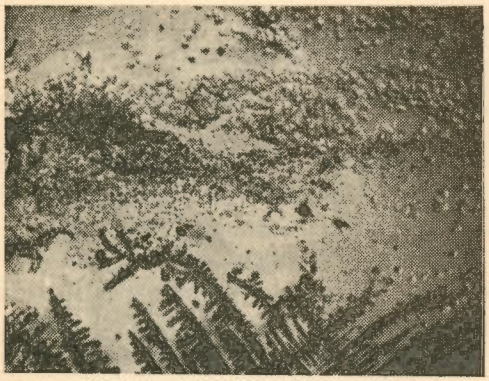

Fig. 2
Partial typical crystallisation—x 70.

Fig. 3
Atypical crystallisation—x 70.

Fig. 4.
Negative crystallisation. It shows dried secretion with cellular pattern—x 70.

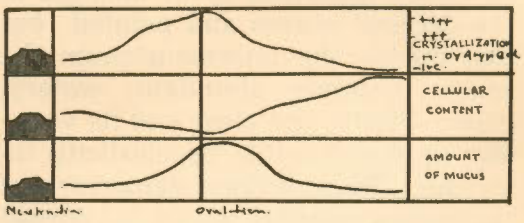

Fig. 5.
Chart showing crystallisation, amount of mucus and cellular content during the menstrual cycle.

It is not an abrupt but a gradual change, the degree of which varies in different cases, depending upon the estrogen secretion.

It has been observed by many workers now, that the crystallization phenomenon depends upon the amount of estrogen and that it is inhibited by progesterone. Thus the crystallization phenomenon may remain longer or persist throughout the cycle in the following conditions:

(i) In cases of failure of progesterone secretion.
(ii) In cases of insufficient progesterone secretion.

(iii) In cases of increased estrogen production.

The crystallization phenomenon may be absent or poor in the conditions as follows:

(i) Absent or poor estrogen secretion.
(ii) Sufficient production of progesterone secretion.
(iii) Dysmucorrhoea—a condition named by Zondek where due to defects in the cervical glands there is no secretion of cervical mucus.

We have observed our 29 cases of hypoplasis of uterus through 66 menstrual cycles. The result of the comparative study of cervical mucus crystallization, amount of mucus and endometrial biopsy can be seen in Table II.

showed typical total crystallization and 41.3% partial crystallization just prior to period. Amount of cervical mucus ran parallel to that of crystallization phenomenon. According to expectations, provided there was normal estrogen secretion, there should have been total crystallization during premenstruum in all cases of anovulatory cycles, but negative and partial crystallization in 41.3% and 10.3% respectively in the present series suggests deficient estrogen secretion in these cases. Campos de Paz, who studied the cervical mucus in anovulatory cycles, also reports similarly. In his series, 44.5% showed total typical crystallizations, 8.43% atypical crystallization and 46.5% negative crystallization.

In Ovulatory Cycles

40.9% of our cases showed secre-

TABLE II

Comparative Study of Cervical Mucus Crystallization, Amount of Cervical Mucus and Endometrial Biopsy

Result of endo-biopsy performed 2-4 days prior to the onset of menstruation	Result of crystallization test performed at the same time as taking the endometrial biopsy				Amount of mucus secretion at the same time of taking the biopsy		
	****	***	Atypical	Negative	Abundant	Moderate	Scanty
Proliferative phase 29 (44% of total)	14	3	1	10	14	3	12
	49%	10.3%	41.3%				
Secretory phase 27 (40.9% of total)	2	—	5	20	—	2	25
	7.43%		18.5%	74.07%			
Material scanty 3	—	—	1	2	—	1	2
No endometrium 1	—	—	1	—	—	—	1

Out of 66 cycles, 29 showed proliferative phase premenstrually, i.e. 44% of total. Out of these, 48% tory endometrium during premenstrual phase. Amongst these, 74.07% of cases showed negative crystalliza-

tion and in 18.5% it was atypical and 7.43% showed total typical crystallization. Crystallization would be expected to be negative if progesterone secretion happens to be sufficient. But, as seen from the table, progesterone secretion must be deficient in cases with total crystallization and atypical crystallization. There was negative crystallization during premenstruum in 72% of normal ovulatory cycles, and in 26% of cases there was partial crystallization (probably deficient progesterone).

There were 3 cases in which cervical mucus secretion was thick, dirty and very scanty throughout the menstrual cycle. The mucus did not show any crystallization pattern. Probably these were the cases of dysmucorrhoea—as described by Zondek.

Effect of Intracervical Injection of Estradiol Benzoate on Cervical Mucus

The most striking effect was found in those cases of dysmucorrhoea where, within 4 days of injection of estradiol benzoate, the mucus became transparent, increased in amount and showed partial crystallization. The effect lasted for about 20 to 25 days and again the condition reverted to original.

In those cases of anovulatory cycles with partial or atypical crystallization, the effect of injection of estradiol benzoate was beneficial. The mucus increased in quantity, was glairy and showed total crystallization. Here again, the effect was transient.

In cases of ovulatory cycles, the injection increased the mucus in early phase of the cycle but it did not have any effect during later part as the injection was given immediately after the menstruation. Thus with the dosage used and with the mode of administration the injection of estradiol benzoate did not change the picture of premenstrual cervical mucus and that of endometrial biopsy.

Conclusion

(A) *Regarding Uterine Length*

1. In all women with normal menstrual cycles there is cyclic variation in uterine length.
2. In ovulatory cycles the maximum increase in length of the uterus occurred during premenstrual phase of the cycle.
3. In anovulatory cycles the maximum increase in uterine length occurred during intermenstrual phase of the menstrual cycle.

Effect of Intracervical Injection of Estradiol Benzoate on Uterine Length

4. In the majority of cases of hypoplasia of uterus, there is a definite increase in length of the uterus, although small in some cases.
5. The extent of the cyclic variation in length of uterus also increases.
6. The phase of the menstrual cycle during which maximum increase in length occurs depends upon the type of the menstrual cycle (i.e. in ovulatory cycles, the increase is during premenstrual phase, and, in anovulatory, it is during intermenstrual phase).

(B) *Regarding Cervical Mucus*

7. The amount of cervical mucus secreted and the crystallization phenomenon run parallel to each other.

8. The phenomenon of mucus crystallization depends upon the amount of estrogen present.

Effect of Intracervical Injection of Estradiol Benzoate on Cervical Mucus

9. Within 4 days of injection the quantity of cervical mucus secreted increases and that it shows crystallization phenomenon on drying. The effect of injection of 10 mgm. lasts for about 25 days.

10. With the dosage used and the mode of administration employed, the injection of estradiol benzoate does not alter the usual pattern of mucus crystallization that occurs during ovulatory or anovulatory cycles.

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