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The estrogenic potential of estriol

A clinical and laboratory re-evaluation

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The estrogenic effect of estriol was studied in humans through the observation of vaginal cytology, ferning of the cervical mucus, estrogen withdrawal bleedings, progestin withdrawal bleeding, and endometrial stimulation. The findings indicate that estriol possesses a low order of estrogenicity at 1 mg. a day. The relative efficacy of estriol is less than 10 per cent of stilbestrol. Specific polarity of estrogenic response in the human reproductive tract could not be demonstrated.

REVIVAL OF interest in estriol has been occasioned by recent observations of variations in urinary estriol during compromised human pregnancies. In the past, research interest in estriol had been primarily concerned with specific tissue response to the hormone. These studies produced a number of inconsistencies and contradictions concerning estriol and its estrogenicity. The usual assumption that estriol is biologically inert could not be verified, since there were conditions in which estriol was more estrogenic than estradiol or estrone.

It is quite apparent that the method by which estrogenicity is evaluated is of the greatest importance. However, it is difficult to determine which procedures indicate true estrogenicity with greatest significance. Table I indicates some of the variation in the estrogenicity of estriol relative to the other major estrogens as reported by several investigators under stipulated testing conditions. There is excellent agreement, as expressed by Sealey and Sondern,[1] Evans, Varney, and Koch,[2] and Huffman and Grollman,[3] that estriol is less estrogenic than estradiol. Szego[4] indicates to the contrary that estriol is highly estrogenic when compared to estrone and estradiol. The difference in the observations could not be totally related to the test conditions since, in some instances, the conditions were similar although the findings were at variance.

The effect of the solvent on the estrogenicity of the hormone has also been at issue. As summarized in Table II, Szego, as well as Burn and Elphick,[5] observed an enhancement of the estrogenicity of estriol when the solvent was aqueous. Contrarily, Zondek and Sulman[6] reported that an aqueous solvent for estriol reduced the estrogenicity to a 10 per cent level. It is of some interest to note that in addition to technique variation, the "aqueous solvent" varied in constitution.

Discrepancies in the results of estrogenicity studies concerned with the clinical effectiveness of estriol in humans were also noted. When estrogenicity was determined by vaginal cytology, Table III, Brown and Bradbury[7] were unable to demonstrate a positive effect with 1 mg. of estriol daily for 10 days. Mack,[8] however, with the same

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Table I

Investigator and method	*Estrogenicity relative to estradiol (100%)*		
	Estradiol	*Estrone*	*Estriol*
Sealey and Sondern[1]			
immature rat, uterine weight and vaginal cytology (oil)	100	25	10
Szego[4]			
immature and castrated rats, uterine weight (oil)	100	18	256
Huffman and Grollman[3]			
immature rat, opening of vagina (aqueous)	100	10	33
Evans, Varney, and Koch[2]			
immature mouse, uterine weight (oil)	100	33	12

Table II. Estrogenicity of estriol in lipoid and "aqueous" solutions

Investigator and method	*Oil (%)*	*Water (%)*	*Composition*
Zondek and Sulman[6]			
immature mouse, castrated adult mouse, and rat, uterine weight	100	10	0.01N NaOH 10% ethanol
Szego[4]			
immature rat, uterine weight	100	300	Saline
Burn and Elphick[5]			
castrated rat, vaginal cytology	100	940	Water ethanol

dosage schedule demonstrated a positive cytologic effect similar in order to that achieved with estrone, estradiol, and stilbestrol. Puck[9] observed a positive vaginal cytologic effect with 0.1 mg. estriol daily for 5 days.

In determining estrogenicity of estriol by withdrawal bleeding in women, Table IV,

Table III. Estrogenicity of oral estriol in humans as determined by vaginal cytology

Investigator	*Dose*	*Response*
Brown and Bradbury[7]	1.0 mg./day for 10 days	No effect
Mack[8]	1.0 mg./day for 10 days	Positive
Puck[9]	0.1 mg./day for 5 days	Positive

Table IV. Estrogenicity of estriol in humans as determined by withdrawal bleeding

Investigator	*Total dose (mg.)*	*Bleeding*	*No. of patients*
Soule[10]	50	yes	1
Puck[9]	30	no	5

Soule[10] noted this phenomenon in one patient. He compared the effectiveness of estriol as being approximately that of estradiol and stilbestrol. Puck observed that estriol given at 5 mg. a day for 6 days would not induce withdrawal bleeding. In addition to this, there was no evidence of endometrial stimulation by microscopic examination, although there was stimulation of the cervical and vaginal mucosa at this and lower dose levels.

One of the more interesting chapters in the estriol story is the concept advanced by Puck, in which he postulates a type of polarity attributable to estriol. This, it is suggested, results in a manifestation of estriol estrogenicity in the lower portions of the human reproductive tract (vagina and cervix) but with little or no effect upon the body of the uterus or endometrium.

It was this concept, as well as the conflicting reports concerning the estrogenicity of estriol, that stimulated us to review the problem in our laboratories. The estrogenic effect of estriol in the immature mouse as related to uterine weight with both aqueous and lipoid solvents was to be studied. In addition, the estrogenicity of the hormone in women was to be determined through the

evaluation of the maturation index of the vaginal mucosa, ferning of the cervical mucus, estrogen withdrawal bleeding, progesterone withdrawal bleeding, and microscopic study of the endometrium after estriol administration. The estrogenic effects of estriol in women are the subject of the current presentation.

Methods and material

Estriol, ethinyl estradiol, and stilbestrol were used throughout the study. Each was given orally. The major part of the study was concerned with estriol administration. The other two substances were administered to small control groups of patients to provide a positive estrogenic control in addition to the negative control in the estriol group.

Women to receive estriol were selected from inpatients and outpatients at Baltimore City Hospitals and University of Maryland Hospital. The patients were selected because of evidence of ovarian failure with amenorrhea and atrophic change in the vaginal mucosa. The patients ranged in age from 47 to 94 years with the average of 66 years. Each patient received 1 mg. of estriol daily by mouth for 28 days.

Vaginal smears for cytologic study were obtained through scraping the lateral vaginal wall with a wood spatula. This procedure was followed in order to provide a standard specimen relatively free from uterine contamination and from exfoliated vaginal pool cells. The material obtained was smeared on a glass slide and quickly fixed in 95 per cent alcohol. Subsequently the smears were prepared with Shorr's stain, the slides were then read by two investigators, and, whenever possible, 100 cells were read and differentiated into parabasal, intermediate, and superficial cells. The cervical mucus was studied in 30 patients, 10 of whom received estriol; 10 received 0.1 mg. of ethinyl estradiol daily for 2 weeks and 10 patients received 0.1 mg. of stilbestrol daily for 2 weeks. Crystallization of the cervical mucus was demonstrated by microscopic study.

Three patients received a synthetic progestin, megestrol 5 mg. (daily for 5 days) on completion of the estriol regimen. The purpose of this procedure was to demonstrate the potential of progestin withdrawal bleeding from the estriol-stimulated endometrium.

Two hysterectomy specimens were obtained from patients receiving estriol for 28 days prior to operation. The endometrium was examined for histologic evidence of estrogen stimulation.

Results

Estriol effects on the vaginal epithelium. A significant change in the cell population of the vaginal epithelium was noted after 2 weeks of estriol administration (Table V). The parabasal cells decreased by approximately 50 per cent. There was a concomitant increase in the intermediate and superficial cells. The changes apparent at 2 weeks of administration were not significantly changed at the 4 weeks' medication level.

Comparison with stilbestrol and ethinyl estradiol is shown in Table VI. Stilbestrol at a dosage level of 0.1 mg. daily appears capable of inducing greater estrogen stimulation of the vaginal mucosa than estriol at the 1 mg. level. Ethinyl estradiol at the same dosage level as stilbestrol shows a greater estrogenic effect than stilbestrol or estriol.

Cervical mucus. In 10 patients treated with estriol at 1 mg. a day for 28 days, a positive fern test was noted in 2 patients. The remaining were negative. In similar groups of patients treated with ethinyl estradiol and stilbestrol at 0.1 mg. levels, all patients were found to have positive fern tests as indicated in Table VII.

Estrogen withdrawal bleeding. Three patients of the 60 treated with estriol showed evidence of estrogen withdrawal bleeding following therapy. This occurred within a week of the cessation of therapy and consisted of spotting for 2 to 3 days. As indicated in Table VIII, each of the patients manifesting estrogen withdrawal bleeding was noted to have stimulation of the vaginal mucosa at a level significantly greater than the mean of the study group.

Table V. Estriol effect on vaginal epithelium maturation index

60 patients — average age 66 years range 47 — 94 years Treatment: 1 mg. estriol by mouth for 28 days			
Pretreatment	2 weeks	4 weeks	posttreatment
51-41-8	23-57-20	18-63-19	31-63-6

Table VI. The maturation index of the vaginal epithelium after 14 days of therapy

	Daily dose (mg.)	*Duration (days)*	*Cytology*	*No. of patients*
Estriol	1.0	14	23-57-20	60
Ethinyl estradiol	0.1	14	1-53-46	10
Stilbestrol	0.1	14	1-73-26	10

Table VII. Ferning of cervical mucus after 14 days of therapy

No. of patients	*Daily dose (mg.)*	*Drug*	*Result*	
			Positive	*Negative*
10	1.0	Estriol	2	8
10	0.1	Ethinyl estradiol	10	0
10	0.1	Stilbestrol	10	0

Table VIII. The maturation index of the 3 patients exhibiting estrogen withdrawal bleeding

Patient	*Age*	*Pretreatment*	*During treatment*
E. S.	75	30-52-18	5-55-40
M. C.	58	6-68-26	2-48-50
L. H.	70	3-92-5	0-81-19

Table IX. Megestrol withdrawal bleeding

		Pretreatment			*Posttreatment*		
Patient	*Age*	*Maturation index*	*Fern*	*Bleeding*	*Maturation index*	*Fern*	*Bleeding*
E. L.	61	100-0-0	No	No	32-56-12	No	No
K. K.	52	0-86-14	No	No	0-86-14	No	No
L. H.	47	88-9-3	No	No	1-74-25	Yes	Yes

Progestin withdrawal bleeding. Three patients were given 5 mg. of megestrol by mouth for 5 days upon the completion of the 28 day course of estriol. As indicated in Table IX, only one patient showed evidence of progestin withdrawal bleeding. This patient showed additional evidence of estrogenicity greater than that observed in the rest of the group in that the fern test was positive and the maturation index showed an exaggerated estrogen response.

Endometrial stimulation. Hysterectomy was accomplished on 2 patients who were in the estriol study group. Minimal estrogen stimulation of the endometrium was noted. The maturation index at the time of hysterectomy in each, respectively, was 0-84-16 and 14-75-11.

Conclusions

When given orally at a dose level of 1.0 mg. daily for 28 days, estriol produced an estrogenic effect in women. There is stimulation of the vaginal mucosa which becomes apparent by the second week of administration and remains constant through the following 2 weeks of administration. Return to pretreatment levels of maturation of the vaginal mucosa is noted on the smears taken 4 weeks after therapy. When compared to the vaginal cytologic effect of stilbestrol and ethinyl estradiol, it is noted that 1 mg. of estriol does not induce as great a degree of estrogenic stimulation of the vaginal mucosa as does 0.1 mg. stilbestrol. Ethinyl estradiol at this dosage level induces an intense estrogenic effect on the vaginal mucosa.

Other evidence of weak estrogenicity of estriol is seen in the occasional development of a positive fern test in the cervical mucus as compared to the 100 per cent development of positive fern tests with smaller amounts of ethinyl estradiol and stilbestrol.

Estrogen withdrawal bleeding with estriol occurred in 3 patients of the 60 study group. Withdrawal bleeding following the administration of a progestational agent occurred in one of three trials.

The studies indicate that estriol is estrogenic in the various modalities tested. The degree of estrogenicity is relatively slight and equivalent to less than 0.1 mg. of stilbestrol.

Estriol and megestrol were supplied by Organon, Inc., and Mead Johnson & Company, respectively.

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Discussion

Dr. Willard M. Allen, St. Louis, Missouri. The studies reported by Dr. Haskins do show that estriol is a weak estrogen which has a specific effect on the cervix and the vaginal epithelium. A histologic effect on the endometrium was not detected. However, an effect must have been produced; estrogen withdrawal bleeding occurred in three trials of 60, and progesterone withdrawal bleeding in one of three trials. The three target tissues tested—vaginal epithelium, cervical glands, and endometrium—respond to estriol in the same way as they respond to other estrogens.

Estriol has been available for clinical use under the trade name of Theelol for at least 35 years. It has not been utilized very much, I suppose, because it is a "weak estrogen." The descriptive adjective "weak" has little real significance. Let me ask a pertinent question: "Which is a better estrogen for clinical use, ethinyl estradiol or Premarin?" The first is a highly potent estrogen, the second is a weak estrogen, yet both in proper dosage are highly effective and useful estrogens.

This careful study was done to see whether or not there was a qualitatively different effect on the three target tissues tested. It would be nice, for example, if estriol had a relatively greater effect on the vaginal epithelium and cervical glands than on the endometrium. If that were the case, oral estriol would be better for the oral treatment of atrophic vaginitis, or perhaps even the menopausal syndrome than estrogens now in use. No such difference was detected—all target tissues responded equally.

In 1944, I published a paper in the *Southern Medical Journal* giving the results of the bioassay of several estrogens in 8 young ovariectomized women with an intact uterus. The amount of each estrogen over a 3 week period required to induce estrogen withdrawal bleeding was determined. Ethinyl estradiol given orally proved to be the most active of the estrogens tested. Also ethinyl estradiol was at least 30 times as active as estradiol. The question which now arises is, "How active is estriol?" In Haskins' group estrogen withdrawal bleeding occurred in 3 of 60 trials. I would predict that a dose of 3 to 5 mg. per day would produce withdrawal bleeding in 50 per cent or more of instances.

It seems to me, therefore, that Dr. Haskins' study proves that estriol is an effective estrogen that might be half as active as estradiol. Manifestly, estriol will not be used clinically to treat

estrogen deficiency. However, it just may be that the estriol circulating in the blood of the mother during pregnancy may have a good deal of significance.

Dr. Haskins' paper should give impetus to the study of blood levels of E_1, E_2, and E_3 in the blood during both normal and abnormal pregnancy.

Dr. R. L. Vande Wiele, New York, New York. I would like to make a few comments to the interesting contribution of Dr. Haskins. In analyzing the physiologic significance of his studies, it is necessary to compare the dose of estriol used by Dr. Haskins to the amount of estriol produced in vivo. In the nonpregnant female, 1 mg. of estriol, the dose found by Dr. Haskins to have a significant biologic effect, is an enormous amount of estriol. At the peak of estrogen secretion, the ovary produces in the neighborhood of 200 μg of estradiol per day, only a fraction of which is converted to estriol. Furthermore, it is quite likely that only insignificant amounts of free estriol, the form used by Dr. Haskins, circulate, and that most of it is conjugated before entering into the circulation.

In the pregnant female, however, the situation is very different. Available data indicate that in the third trimester somewhere between 20 and 80 mg. of estriol enters into the maternal circulation. In addition, it has been shown by Dr. Ryan, a member of our Society, that most, if not all, of the estriol enters into the circulation as the free compound. It is quite likely that such high amounts of estriol have significant biologic effects.

Dr. Howard W. Jones, Jr., Baltimore, Maryland. Dr. Allen pointed out that it would be exceedingly helpful to have an estrogen which acted on the vagina and cervix and not the endometrium. Perhaps equally important would be to have some estrogen which had a differential effect on hot flushes and did not effect endometrium.

Therefore, I rise simply to ask Dr. Haskins if he could tell us about the effect of estriol on hot flushes.

Dr. Edward C. Hughes, Syracuse, New York. I presented a paper entitled, "Nutritional Aspects of the Endometrium" at the Annual Meeting of the American Association of Obstetricians and Gynecologists some years ago. I suggested that the dose level to stimulate the function of the endometrium was 0.1 mg. of diethylstilbestrol. I have maintained that idea for a great many years. Lately, I have proved this to myself by testing the amount of estrogen which stimulates the endometrium through organ culture of the endometrium. We find that the size of the dose is most important for the stimulative effects upon the metabolic activity of the endometrium.

For instance, if you add more than 5 μg to the culture media, the uptake of glucose from the fluid is decreased and the metabolism of glucose by the endometrium to glycogen is decreased.

If you use less than a microgram (and we have not decided how far we must go—we are down to a hundredth of a microgram right now) we do find that the uptake of glucose from the culture media does increase. The endometrium does metabolize glycogen in considerable quantities, and the enzymes that are particularly involved in the synthesis, phosphorylase and synthetase, are likewise stimulated.

I think your suggestion, that a small dose of estrogen has a stimulative effect upon the generative tract, is very important. If you wish to depress the action, particularly in the endometrium, a larger dose is appropriate.

Dr. Haskins (Closing). In regard to question concerning 0.1 mg. stilbestrol: we have for a number of years in the treatment of patients with ovarian agenesis administered 0.1 mg. stilbestrol daily without interruption and with 5 days a month of added synthetic progestin. This will produce endometrial stimulation and evoke regular menstrual bleeding.

The symptom of hot flushes was not studied. Indeed, the majority of our patients did not offer this complaint. This finding was undoubtedly relative to the advanced age of the patient population.

The question of the glucuronide or of the conjugation making the estriol inactive, I think, is certainly an excellent point, but one which I am unable to discuss competently.