Estriol: A Review

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ESTRIOL was first isolated in 1930 from human pregnancy urine independently by Doisy and associates (1) and by Marrian (2). It was converted into estrone, the first female sex hormone isolated, by dehydration *in vacuo* with potassium bisulfate. Within 5 years, through the efforts of Doisy, Marrian, Butenandt, Haworth, Bernal, Adam, Cook and others, its formula was determined to be $\Delta 1,3,5,10$ estratriene,$3,16\alpha,17\beta$ triol (fig. 1). Estriol is extracted by an aqueous solution of 0.1 N sodium hydroxide from ethanol or benzene extracts of human urine, due to its somewhat greater solubility in water (although still practically insoluble) and slightly greater acidity as compared with the other female hormones. The former property is probably responsible for the fact that estriol is of value for oral therapy, whereas estrone and estradiol are not. Estriol may be synthesized from the more readily obtained estrone by a process developed independently by Huffman and by Butenandt (3). This review summarizes the occurrence of estriol, its physiological and pharmacological properties and therapeutic uses, with particular reference to recent investigations showing that it is a potent female hormone with qualitative as well as quantitative differences from other estrogens.

Estriol was isolated from human placentas in 1931 by Browne (4). The minimum concentrations of the three 'free' estrogens in the full-term placenta in micrograms per kilogram of wet tissue were determined as 51.3 for estrone, 170 for estradiol and 315 for estriol, by Diczfalusy and Lindkvist (5). Somewhat higher values for the free estrogens, but lower values for each in its conjugated and protein-bound forms, were obtained by Mitchell and Davies (6). Estriol is also found in human bile (7), in meconium (8) (where it is apparently the principle estrogen) and, probably, in the corpus luteum (9) as determined by a histochemical color reaction. The amounts of the estrogens in micrograms per liter of human semen are 60 for estrone, 10 for estradiol 17β, and 30 for estriol (10). Except for its reported isolation from the female pussy willow (11) and placentas of the chimpanzee (12), estriol has been isolated only from human sources and is believed characteristic of the human species. However, this substance, or one closely resembling it in physical properties and/or biological actions, may be found in the guinea pig, rabbit, monkey, dog and rat after administration of estradiol or other estrogens (13).

Excretion of Estrogens. Quantitative data on the excretion of estriol, estradiol 17β, and estrone under various conditions are summarized in table 1; most of the values were obtained by the recently developed method of J. B. Brown (14). Excretion of estrogens increases twice during the menstrual cycle, first at the time of ovulation and then during the luteal phase (18). The quantity of each estrogen in the urine increases and decreases in parallel, and the quantity of estradiol is almost always less than that of estrone, the ratio remaining approximately constant at 1 to 2. There is no such constant ratio between the quantities of estriol and estrone; the amount of estrone may be equal to or greater than the amount of estriol. Estrogen

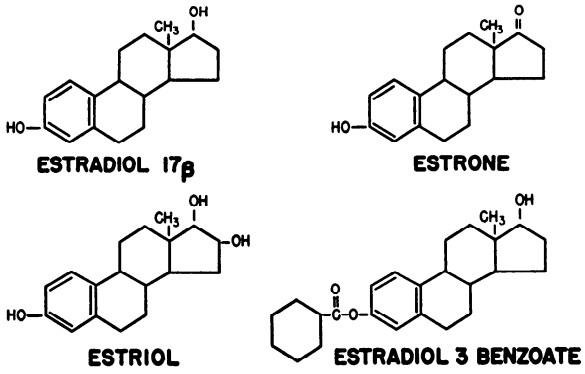

FIG. 1. Structure of estrogens.

excretion is least during the first week of the menstrual cycle; at about the seventh day it begins to increase, reaching a well-defined maximum, 'the ovulation peak,' at about the thirteenth day, at which time the rise in basal body temperature also occurs. Then estrogen excretion suddenly decreases; estrone and estradiol levels remain parallel, while the changes in estriol tend to lag behind about 24 hours. This lag phenomenon is obscured by 24-hour urine collections, but is clearly seen at the time of ovulation and immediately thereafter. Estrogen excretion then increases to the second, the 'luteal,' maximum on about the twenty-first day, then, just prior to onset of menstruation, it again decreases. During the luteal phase the estriol lag is not apparent. Minimum excretion of estrogens often occurs several days after the onset of menstruation. This pattern of excretion is constant, although the amounts in different subjects vary widely.

During pregnancy there is a rapid increase in the urinary excretion of all estrogens, the quantities of estrone and estradiol excreted just prior to parturition being 100-fold those during the luteal phase; these two estrogens are usually excreted in a constant ratio of 3 to 1 (19). Estriol in urine increases 1,000-fold and is, as first shown by Cohen (24) in 1935, by far the most abundant estrogen during pregnancy. Urinary estrogen levels increase rapidly about 7 weeks after the last menstrual period. This suggests the onset of placental synthesis at that time. There is apparently no correlation between estrogen excretion and the onset of labor. Estrogen excretion decreases rapidly postpartum, following sudden removal of the placenta. Estriol is eliminated more slowly than estrone and estradiol 17β, and this is the case also following injection of the estrogens.

During lactation urinary excretion of all three estrogens remains low. After lactation there is a slow increase to the regular rise and fall during the menses. When menstrual bleeding first begins postpartum the excretion of estrone and estradiol is about 7 μg of each per 24 hours, which provides some indication of the minimal levels required for endometrial growth.

Bilateral oophorectomy decreased the estrogen excretion in premenopausal women, but had no effect after the menopause (20). Bilateral adrenalectomy performed after bilateral oophorectomy did not abolish estrogen excretion, perhaps due to incomplete adrenalectomy or the presence of accessory tissue. Thus, failure of a tumor (of breast origin) to respond to these operations is not necessarily due to estrogen independence. The urinary excretion of estrogens from ovariectomized

TABLE 1. URINARY EXCRETION OF ESTROGENS (μg/24 hr.)

SOURCE	ESTRONE	ESTRADIOL	ESTRIOL	TOTAL	REF.
30 males, 25–35 yr	6 (0–11)†	2 (0–7)	6 (1–12)	14 (9–25)	(15)
9 postmenopausal women*	3 (1–3)	1 (0–4)	3 (1–9)	6 (3–11)	(16)
15 amenorrheic females*	3 (1–5)	1 (0–1)	3 (1–9)	7 (2–11)	(15)
1 female, 35 yr., 9th day of cycle	7.2	3.8	27	38	(17)
1 female, 32 yr., early menopause	1.5	1.0	3.2	5.7	(17)
8 patients, 10 cycles, onset of menstruation	5 (4–7)	2 (0–3)	6 (0–15)	13 (4–25)	(18)
8 patients, 10 cycles, ovulation peak	20 (11–31)	9 (4–14)	27 (15–34)	56 (30–79)	(18)
8 patients, 10 cycles, luteal maximum	14 (10–23)	7 (4–10)	22 (8–72)	43 (22–105)	(18)
4 pregnant women, 1 wk. antepartum	1,400 (930–1600)	520 (380–630)	29,000 (22,000–35,000)	30,800 (23,200–37,200)	(19)
14 women, 1–66 mon. postovariect.	2.2 (0–18.67)	1.2 (0–12.1)	38 (0–16.4)	7.2	(20)
3 patients, 5–16 months postadrenalect. after previous ovariect.	0.8 (0–5.2)	1.3 (0–6.1)	0.8 (0–5.2)	3.0 (0–10.5)	(20)
Normal man‡	8.5	3.4	5.1	17	(21)
Castrated man‡	5	1	4	10	(21)
4 males, chronic liver disease, testicular atrophy, alcoholism§	4.5 (2–7)	0	17.5 (13–27)	22 (15–34)	(15)
Woman, carcinoma of uterus	to 896	0	to 504	to 1400	(23)
Male, benign adrenal tumor, pre-op.	86	15	57	158	(26)
Male, benign adrenal tumor, post-op.	9	10	13	32	(26)

* Values are for conjugated plus free forms; less than 1 μg/24 hr. excreted in free form. † Range of values given in parentheses. ‡ Assuming daily urine volume of 2 l. § Selected cases; (15) gives value for 12 patients with chronic liver disease.

women was increased by injection of ACTH, and postoperatively, perhaps due to liberation of ACTH by the stress of the operation. Urinary estrogen excretion continued after hypophysectomy in 7 of 11 cases; in some for more than a year postoperatively (25). This is most likely due to incomplete removal of the gland or to the presence of accessory pituitary tissue; e.g., in the pharynx.

Increased urinary excretion of all three estrogens occurs in patients with adrenal cortical tumors (22, 26) and carcinoma of the uterus (23); the amount of estriol excreted per day has exceeded 500 μg, and of total estrogens, 1,000 μg. Similar data on other types of adrenal and ovarian tumors will be of interest. Urinary estrogen excretion by males appears to be decreased only about 20 per cent after castration (21).

Total urinary excretion of estrogens was increased in 11 of 17 patients with liver disease when the urine was separated chemically into fractions, each of which was assayed by the Allen-Doisy method (27). This may be partly due to decreased biliary excretion. Excretion of estrogen increases with the severity of the liver disease as measured by sulfobromophthalein sodium retention and total bilirubin. Both conjugated and unconjugated estriols were increased more frequently and to a greater extent than estrone or estradiol. Excretion of unconjugated estriol was significantly increased in patients with spider nevi.

Using Brown's chemical method for the separation and determination of estrogens, Cameron (15) found the combined urinary excretion of estriol, estradiol and

estrone to be above normal limits in only 2 of 12 patients with chronic liver disease. One excreted greater than normal amounts of all three estrogens; the other excreted more estriol only. In addition, 4 men and 1 woman excreted more estriol than normal, although their total estrogen excretion was still within normal limits. While there was no association between increased estrogen excretion and gynecomastia, ascites, jaundice, spider nevi, and palmar erythema, there was a suggestive association of increased estriol excretion, testicular atrophy, and a history of alcoholism.

Estrogens occur in urine mostly in the form of glucuronides and sulfates. The most abundant estrogen of pregnancy urine, estriol glucuronide, increases rapidly in amount during pregnancy, but just prior to parturition it decreases almost precipitously (24). At the same time there is a rapid increase in the concentration of free estriol, so that estrogens are then present principally in the free, unconjugated state. While this change may be involved in the initiation of labor, further studies are needed to establish this. Bacterial splitting of estriol glucuronide and subsequent development of the color reaction with phenolsulfonic acid has been proposed by Patterson (28) as a biochemical test for the diagnosis of early pregnancy. His results agreed well with those obtained by the Friedman test.

As a test of placental function during the progress of pregnancy, Zondek and Goldberg (29) recommended the determination of urinary estriol and claimed this test reliable after the nineteenth week of gestation. In advanced pregnancy the estriol test is superior to the chorionic gonadotropin test because excretion of the gonadotropin decreases after the fourth month and the excretion continues several days after expulsion or damage of the placenta, whereas elimination of estriol lasts only 24–48 hours. An estriol excretion below 1,000 μg in 24 hours indicates irreversible placental dysfunction and secondary death of the fetus. Only in rare cases (1 of 75 or more) of primary fetal death with a surviving placenta can the child be dead in spite of a normal estriol excretion. In toxemias of pregnancy with a high chorionic gonadotropin excretion, the estriol test is of special importance. In these cases, in spite of fetal death, the urinary gonadotropin may still be high, but the estriol excretion is always low.

Estrogen Metabolism. Cohen and Bates (30) have shown by means of a specific bacterial phenosulfatase that the sulfate linkage in conjugated estrogens must be with the phenolic OH at C_3, since this enzyme does not hydrolyze alcoholic sulfates. In seven urine samples from pregnant women, the amount of estrogen conjugated as sulfates varied from 5 to 89 per cent for the estriol fraction and 8 to 100 per cent for the estrone fraction.

Estriol is generally believed to be the end product of estrogen metabolism, according to the equation estradiol $\leftrightarrows$ estrone $\rightarrow$ estriol. Injection of either of the first two estrogens, either free or esterified, results in increased excretion of estriol (13). Radioactive estrone and estradiol 17β are converted to radioactive estriol, the ratio of estriol to estradiol excreted increasing with time (31). Ovarian tissue converts radioactive testosterone to radioactive estrone, estradiol and estriol (32).

Estradiol benzoate in doses of 5–15 mg increases the excretion of estrone and estriol in subjects with good liver function, and of estradiol in subjects with poor liver function (33). Lorenzini and collaborators (34) also found that the ratio of the estrone plus estradiol divided by estriol concentration was about 0.4 in an ovariectomized woman; it increased to 0.7 after injection of estradiol benzoate and progesterone, and then returned to the original value. He pointed out that this low ratio may occur after the menopause or ovariectomy or during pregnancy, or even in the

male; in all these cases the estrogens are of extraovarian origin. The conversion of estrone to estriol is believed to be facilitated by progesterone (35), although this has been questioned, and, since administration of estriol does not result in increased excretion of estrone or estradiol (36), is considered irreversible.

Marrian (37) has suggested that oxidation of estrone may occur either to 16α hydroxy estrone, which then is reduced to estriol, or to 16β hydroxy estrone, which forms 16 epiestriol. The latter compound and 16 hydroxy estrone have recently been isolated from urine.

Evidence indicating that estriol is the main estrogen in human blood plasma, where it occurs in an esterified form, possibly as the glucuronide, two-thirds of which is bound to lipoproteins (Cohn's Fraction III-0), has been presented by Roberts and Szego (38, 39). Further studies are needed of the nature of the circulating estrogen. The biological activity of estriol and estradiol is not affected by incubation with rabbit serum and red cells; the ability of estrone to stimulate uterine hypertrophy increases on incubation with human or rabbit red cell serum, so that estrone does not exist in the blood for any length of time (40).

Estriol remains in the tissues longer than estrone; 15 per cent of a dose of estriol can be found in the tissues of animals 12 hours after injection, as compared with only 2–10 per cent of a dose of estrone (41).

Physiological Properties

Vaginal Effects. Estriol, like other estrogens, produces thickening, stratification and cornified epithelial cells in the vagina of ovariectomized and immature animals, and this property has been used in the Allen-Doisy vaginal smear test to compare its potency with that of other estrogens. Emmens (42) summarized in 1950 the ratios of the potencies of estriol and estrone found by eight different investigators working with the Allen-Doisy test in ovariectomized rats. The ratios varied from 1 to 1, to as much as 1 to 250, the apparent relative potency of estriol seeming to improve with the number of injections, and also in oil. Emmens concluded that because of "such wide discrepancies according to the author quoted and technique used it is clear that no useful purpose is served by attempting to assay accurately the potency of estrogenic material of unknown (or even known) constitution in terms of, say, international standard estrone." Pincus (43) also points out wide variations in relative potencies of estrogens by this vaginal smear method when technique, solvent, number of injections and species are changed.

A 200-fold difference in the apparent potency of estriol, depending on vehicle and test animal, is shown in table 2 (data of Zondek and Sulman, ref. 44). The similarity of effect in spayed or intact female infantile rats or mice towards estriol, an estrogenic effect being obtained in mice with 1 μg and in rats with 10 to 15 μg in oily solution, indicates that the ovaries are not important in converting estriol to estrone or other active estrogen. Similar differences were observed by Sondern and Sealey (45) who found that when given orally, estrone, estradiol and estriol were $\frac{1}{65}$, $\frac{1}{20}$ and $\frac{1}{10}$ as effective as stilbestrol in spayed adult mice, and $\frac{1}{80}$, $\frac{1}{65}$ and $\frac{1}{30}$ as effective in spayed adult rats. When given subcutaneously to infantile rats (46), the corresponding activities were $\frac{1}{16}$, $\frac{1}{4}$ and $\frac{1}{40}$ that of stilbestrol, as compared with $\frac{1}{16}$, $\frac{1}{8}$ and $\frac{1}{3}$ when given orally. Imhoffen and collaborators (47) had also found differences in the relative effectiveness; subcutaneously the effective doses (in micrograms) for estrone, estradiol and estriol were 0.8, 0.1 and 10 as compared with corresponding values for the oral route of 60, 50 and 10.

TABLE 2. ESTROGENIC EFFECTS OF ESTRIOL IN SPAYED AND INTACT FEMALE MICE AND RATS (49)

		WT., GM	VEHICLE*	ESTROGENIC ACTION
A. Mice				
	Adult castrate	20–25	H_2O	10
	Infantile intact	12–15	H_2O	10
	Adult castrate	20–25	oil	1
	Infantile intact	12–15	oil	1
B. Rats				
	Adult castrate	150–160	H_2O	200
	Infantile castrate	30–35	H_2O	100
	Infantile intact	30–35	H_2O	100
	Adult castrate	150–160	oil	15
	Infantile castrate	30–35	oil	10
	Infantile intact	30–35	oil	10

* H_2O vehicle contains 0.01 N NaOH + 10% ethyl alcohol; oil is olive oil.

Because of the relative ease of the vaginal smear methods, such procedures are frequently used to compare 'quantitatively' different estrogens; however, such comparisons are valid only under the conditions used and do not necessarily reflect relative potencies under other conditions.

Direct local application of the estrogen to the vagina produces cornification with much smaller doses than with systemic administration. Muhlböck (48) showed that application of estrogens to the uteri of mice gave sensitive and consistent results. He found the median effective doses of estrone, estradiol and estriol to be 0.00025 μg, 0.0005 μg, and 0.00075 μg. By instilling estrogen solution into the vagina five times, Sulman (49) could detect 0.001 μg estrone, 0.00005 μg estriol and 0.000005 μg estradiol, an increase in sensitivity as compared to subcutaneous injection of 1,000, 20,000 and 2,000 times respectively. Two to four injections of an estrogen solution in 1 per cent egg albumen gave optimal results for estrone, estradiol 17β, and diethylstilbestrol, but not for estriol (50). Under rigid arbitrary conditions, Emmens (51) has obtained a ratio of their effectiveness in promoting vaginal cornification for estriol to estrone of 0.46 as compared with that for estradiol to estrone of 1.52.

Despite the wide variation in both absolute and relative results with small variations in technique, the vaginal smear method has been used not only to compare estrogens but also to draw conclusions regarding their metabolism. Huffman and Grossman (52) found by this method with immature and castrate rats that estriol was the most active of the three known α-glycols obtained by the reduction of 16-ketoestrone. Emmens (53) determined the minimum effective dose to produce vaginal cornification in rats for many estrogens when given systemically and locally. For estriol this minimum dose was 2.0/μg subcutaneously, and 0.001 μg intravaginally, giving a ratio of 2,000. The corresponding doses for estrone were 0.075 μg and 0.00029 μg, giving a ratio of 260, and for estradiol 0.025 μg and 0.0005 μg, corresponding to a ratio of 50. Substances which were active both subcutaneously and intravaginally were true estrogens; those much less active when given intravaginally were called pro-estrogens, since they apparently were converted into active estrogens in the body. The high ratio of 2,000 for estriol is exceptional, probably due to its solubility in body fluids. Estriol is the only substance investigated which appears to increase in potency when more than two subcutaneous injections in oil are given. With four injections the minimum effective dose is 0.14 μg and the ratio of effective-

ness of subcutaneous to intravaginal administration is 140. Thus estrone appears to be only two to three times more active than estriol when four injections are given, but 70 times more active if only two doses are given.

On the other hand, ovariectomized rats may acquire marked tolerance to estriol on prolonged treatment, so that doses 50 or more times the initial dose have to be used to produce the vaginal reaction, although the sensitivity to estrone does not change during that period (54). Curtis, Miller and Witt therefore concluded that the rat is not satisfactory for the assay of estriol. That large doses of estrin or estriol given to pregnant rats did not cause cornification of the vagina was noted by Levin, Katzman and Doisy (55).

The doses of various estrogens which produced vaginal cornification in 50 per cent of adult spayed rats when given subcutaneously, orally and by injection into the portal and femoral veins were studied by Nielsen, Pedersen-Bjergaard and Tonnesen (56). Oral and subcutaneous administration to spayed rats with liver damage was also studied. They concluded that their results indicated that stilbestrol and dienestrol are not inactivated in the liver when given by mouth, while estrone, estradiol and hexestrol are. Estriol appeared to be partially inactivated by the liver whether given orally or parenterally. Estriol was the only estrogen whose activity, both orally and parenterally, was lower in normal animals than in those whose liver was damaged. Given by the oral route it was 100 times less, and parenterally, it was 36 times less.

Comparative data on the relative efficiency of estriol and other estrogens on the vaginal smear in postmenopausal or ovariectomized women are not available. Brown and Bradbury (57) found that 1 mg of estriol daily had no effect on vaginal smears of postmenopausal women, whereas both diethylstilbestrol and estrone did at this dose level. Using the extent of iodine vapor staining of the glycogen in the vaginal smear as a criterion of estrogen activity, Mack (58) also concluded that estriol was less effective than estrone on an equal weight basis in menopausal women.

Mixtures of estriol with estradiol 17β, and estrone, produce additive effects on the vaginal smear of spayed rats, in contrast to their effect on uterine growth where interference occurs (59).

Estriol, like other estrogens, causes establishment and opening of the vaginal orifice. Curtis and Doisy (60) defined a unit of activity as the minimum quantity causing establishment of the vaginal orifice within 10 days in 3 of 5 animals receiving 6 doses of hormones in 3 days, beginning when the animal was 18 days old. This unit was equal to 0.16 μg of estriol or 1.08 μg, of estrone. The latter was as effective orally as subcutaneously, whereas 0.25–0.3 μg of estriol was needed orally. Lloyd, Rogers and Williams (61) report the lowest effective dose in immature female rats to be 2.5×10^{-4} mg, of estrone and 2.0×10^{-4} mg of estriol, but cast doubt on the reproducibility of results obtained by this method.

Uterine Effects. Small doses of estriol given subcutaneously stimulate uterine growth in the immature rat, the increase in weight with increase in dosage being comparable to that obtained with estradiol and estrone in the lower dose range until the uterine weight has reached 50–60 mg, about three times the initial weight (62). However, doses up to 16 times the amount producing a 50-mg uterus (about 1 μg) produce no further increase in uterine weight, and actually 40.0 μg of estriol daily produces less uterine growth than does 20 μg (63). By contrast, estradiol 17β and estrone produce progressively increasing uterine weights up to 300 mg. Moreover, doses of estradiol and estrone producing uterine weights above 50–60 mg cause an accompanying accumulation of intrauterine fluid, proportional to doses, which pro-

duces a uterus weighing about 300 mg before fluid is expressed. Gross accumulation of intrauterine fluid did not occur as a result of estriol injection (62). This difference between estriol and estrone was first shown by Dorfman, Gallagher and Koch (64), who used it to distinguish between the two. On the ascending portion of curves showing uterine weight vs. dose, estriol is comparable to estradiol and estrone. Estriol is relatively more effective in inducing opening of the introitus, the ratios for estradiol:estriol:estrone being 1:25:20, as compared with 1:6:20 for uterine weight (62).

The uterus of the immature mouse shows a response to increasing doses of estrogen similar to that observed in immature rats (65). The relative activity of the estrogens obtained by the 'uterine mouse method,' based upon an arbitrary 6-mg increase in uterine weight taking estrone as 100, are estriol, 40, and estradiol, 300. Although the shape of the curve showing uterine weight as a function of dose differs for estriol and estrone, within a narrow range of concentrations which is different for each estrogen, the log dose-response relationships are parallel, and, in this range of doses, the potency ratio of estrone to estriol is 20 (66). However, the differences in the dose response curve for estriol and other estrogens makes possible a wide variation in apparent relative potencies, depending on the particular point at which comparisons are made.

Estriol is much more active than the other two natural estrogens in promoting the imbibition of uterine fluid following a single intraperitoneal injection (67). In saline, estriol is five times more active than estrone. The activity of estrone and estradiol seems to be independent of the vehicle, whereas estriol is two to three times more effective in saline than in oil. This greater potency of estriol in producing early evidences of uterine stimulation may be related to its greater solubility in aqueous media relative to estrone or estradiol, thus making the former more readily available for physiological action as well as renal excretion. Rapid excretion may explain the lesser effectiveness of estriol by criteria of estrogenic action requiring prolonged stimulation, as the vaginal smear changes. Initial imbibition of fluid is followed, even after one injection, at least for estradiol, by increased protein synthesis (63), although no significant changes in respiration were observed in the Barcroft-Warburg respirometer (68). Significantly higher amounts of radioactive carbon-14 are found in the uterus of mice who received 1 μg of estrogens labeled with this element 20 hours prior to analysis (69). The order of effectiveness was estradiol 17β $\rightarrow$ estrone $\rightarrow$ estriol.

Estriol, even in small doses (0.1 μg) which by themselves produce little or no increase in weight of the uterus of castrated rats, decreased the growth-promoting action of estradiol and estrone when the hormones were given simultaneously (63). The maximal response to a daily dose of single or mixed estrogens is attained at 72 hours, and the uterine weight thereafter remains unchanged for at least 15 days of continuing treatment. Similarly, the simultaneous injection of estriol together with estrone decreases the response to the same dose of estrone in rats; both weight and nitrogen content of the uteri are less (70). However, a maximally effective dose of estriol did not inhibit the uterine growth-promoting action of estrone in the ovariectomized mouse (71). Estrone had a marked myometrial stimulating action which estriol did not seem to have. Estriol stimulated the stroma of the uterus more than the myometrium. In the presence of estriol, maximum stimulation of the myometrium by estrone did not occur, resulting in a quantitative depression in the total weight response. Estriol does not decrease growth or cornification of vaginal epithelium due to estrone or estradiol.

Estriol is the prototype of a group of 'impeded estrogens,' which Huggins (70) has defined as compounds producing dose response curves with shallow slopes when assayed by uterine growth. Many (70) but not all (72) of these contain either a ketone group at position 6 or a hydroxyl at position 16. Probably a continuous variation of slopes will be found when enough estrogens are studied.

Whereas progesterone inhibits the growth-promoting action of estrone on the uterus of hypophysectomized rats, its effects on estriol-induced growth are biphasic (73). Progesterone depresses uterine growth produced by large quantities of estriol, but enhances the growth-promoting action of small quantities.

The effectiveness of estrogens in increasing the reduced diphosphopyridine nucleotide (DPNH) oxidase activity in the uterus of ovariectomized rats increases in the order estriol ← estrone ← estradiol 17β below 1 μg estrogen per day, and in the order estriol ← estradiol 17β ← estrone above this dose (74). The uterine lactic dehydrogenase (LDH) activity in these uteri was increased most by estriol, to an immediate extent by estradiol 17β, and least by estrone. Estriol seems to inhibit strongly the effect of estradiol on DPNH oxidase, whereas preliminary data indicate no influence or an augmentation of the effect on LDH. Similar specificity is shown by a DPN linked isocitric dehydrogenase in human placenta which is activated *in vitro* by estradiol 17β, estrone, or equilenin in amounts as small as 0.01 μg, whereas estradiol 17α, estriol, or stilbestrol have little or no such effect (75).

Physiological amounts of estrogens increase the β-glucuronidase activity in the uterus and sometimes in the vagina of ovariectomized mice, but not in the liver, kidney, blood cells or spleen (76). The relative effectiveness was estradiol → estrone → estriol at low concentrations, whereas above 10 μg it was estrone → estriol → estradiol. Higher concentrations of estrone (1.7 mg/kg) (77) but not of estriol (5 mg/kg) or estradiol (6 mg/kg) (78) did increase β-glucuronidase activity in the liver.

The effect of estriol, estradiol and estrone on uterine weight has been used to compare their activities during liver regeneration of partially hepatectomized immature female rats (79). Estriol was least dependent on the presence of an intact liver for full estrogenic effectiveness. The effectiveness of estradiol and estrone appeared to be equally reduced by hepatectomy but a slightly greater degree of liver regeneration was necessary for estrone to evoke its maximum effect on the uterus. Similar results were obtained with animals whose livers were damaged by ethionine or aminopterin.

The actions of estrogen on the uterus are apparently the results of a direct influence and occur equally well in the absence of a pituitary and ovary. This was shown early by Hill and Parkes (80), who gave daily subcutaneous doses of 0.5–2 mg of estriol to hypophysectomized and ovariectomized ferrets for 5–15 days. The weight of the uterus and development of the endometrium were the same as those obtained in normal ferrets.

Estriol, estrone and estradiol appear to be about equally effective in their motility-inducing property *in vivo* on the uterus (81). The dual action of progesterone on the uterine mucosa is to stimulate glandular growth on the one hand and to favor decidua formation (ovum growth) on the other. Estrone inhibits both effects, while estradiol and estriol inhibit the former and not the latter. Estradiol 17β is by far the most active inhibitor of decidual growth in rats induced by a standard dose of progesterone (82). In decreasing order of effectiveness are estradiol benzoate, diethylstilbestrol, estrone, estriol and equilenin, the latter two being 200–300 and 300–400 times less active than estradiol.

Growth-Stimulating Effects. Their finding (83) that estriol was present in significant quantities in the blood of women whenever changes in the uterine cervix occurred, e.g., just before parturition, in glandular cystic hyperplasia of the endometrium and before menstruation, prompted Puck and Hubner (84) to investigate carefully the action of his hormone. Estriol and estradiol in daily doses of 50–625 μg given for 3–19 days to rabbits and guinea pigs produce proliferation of the endometrium and myometrium, the strongest effect being on the latter. Both hormones cause large increases in the water content of the uterus. Estriol produces widening of the cervical canal, the longitudinal folds becoming deeper and more ramified and their roots in the connective tissue thinner. Mucopolysaccharides become abundant, pushing the nuclei to the base of the cells, and the water content increases. The cervix of guinea pigs that have received 625 μg of estriol is exactly the same as that seen in the pregnant animal during the last trimester. Similar results are obtained with ovariectomized rabbits. The effects of estradiol on the uterine cervix are much less marked.

Estriol produces an 'extraordinary' increase in the size of the vagina of guinea pigs and rabbits, the diameter being several times greater than in the control animals. Formation of mucopolysaccharides in the guinea pig's vagina is more pronounced with estriol than with estradiol, and approaches that seen in pregnancy. The vaginal epithelium of rabbits receiving estriol is changed from cuboidal to high cylindrical cells which contain mucopolysaccharides that cause a flattening and basal position of the enlarged nuclei. Widening of the symphysis pubis of female guinea pigs, as measured by the density of cell nuclei, occurs to about the same extent after 625 μg of estriol and estradiol. X-ray measurements show that this amount of estriol produces greater separation of the symphysis pubis than is obtained during pregnancy.

In a further study (85), Hubner and Puck compare the actions of 20 μg estradiol, 20 μg estriol, 10 μg estriol and 20 ml of a total ovarian extract (which contained 7–20 μg estriol) on the uterus and vagina of adult guinea pigs ovariectomized 2 months previously. Estradiol stimulates growth of all layers of the body of the uterus more strongly than does estriol, producing in many places a cystic hyperplasia. The ovarian extract has little effect. Both estriol and estradiol cause proliferation of the epithelium of the uterine cervix, the former seeming to stimulate mucus formation more than the latter even in one-half the dose, although layer formation may be less definite. Again, the total ovarian extract produces little effect. Estradiol causes noticeable thickening of the vaginal epithelium, but in only one of four animals is much mucopolysaccharide found. On the other hand, estriol, even in half the dose, greatly increases mucus formation in the vaginal epithelium, but this is not much affected by the total ovarian extract. Strong stimulation of the lower vaginal tract by estriol suggests this is the specific function of this hormone during pregnancy.

The actions of estriol in large and small doses on the uterus, uterine cervix and vagina were studied in nine women who had been given the hormone in divided doses orally or intramuscularly for 5 or 6 days prior to biopsy of the vagina and cervix and in most cases hysterectomy, or laparotomy with biopsy of the corpus uteri (86). The large dose of estriol, 5 mg daily for 6 days, produces little or no proliferation of the functionalis layer of the endometrium, whereas even small quantities, 10 μg daily for 5 days, increases the number of cervical glands, stimulates their secretion and induces proliferation of the vaginal epithelium. The basalis layer of the endometrium shows some proliferation. The epithelium of vagina, uterus and cervix proliferate, the vaginal epithelium being nearly normal. Erosions show

signs of healing on estriol therapy. No endometrial bleeding is detected even at the high doses. Mesenchymal proliferation and loosening accompanying increased water content occur in all areas of the genital tract; the metabolism of the connective tissue seems to be increased. The musculature is not greatly affected by estriol. Secretory activity of the cervical epithelia, and the maltase-soluble glycogen and maltase-resistant polysaccharide found in vaginal epithelium are considerably increased by estriol. Puck and Hubner conclude that estradiol is only appropriate for endometrial proliferation and that estriol produces the sex hormonal changes in the cervix uteri and vagina. They believe that increased quantities of estriol prior to parturition are responsible for the loosening and widening of the cervix uteri, the vagina and the pelvic zone preparatory to birth.

Contrary to the recent results of Puck, Korte and Hubner (86), Soule (87) found in 1942 that estradiol, estriol and stilbestrol were equally effective in producing estrogen withdrawal bleeding. However, Schiller (88) injected three adult oophorectomized rhesus monkeys subcutaneously daily for 10 days with 75 rat units of crystalline estriol in 1 cc sesame oil, and produced reddening of the sex skin and atypical cornification of the vaginal cells, but no menstrual-like bleeding occurred following withdrawal of the estriol. The same animals, after return to their original castrate condition, were injected in the same way with the same dosage of estriol of human origin. Responses were the same, but in addition they exhibited menstrual-like flow 10 and 7 days after the last injection. Castrate monkeys show no menstrual bleeding after 650 I.U. of estriol (1 I.U. estimated to equal 0.666 μg) plus 100 I.U. of estradiol; when this treatment is stopped and they are continued immediately on 750 I.U. of estradiol, over half of them menstruate (89).

Ovarian weights of immature rats after subcutaneous daily injection for 4 days of 0.5 mg of estrogens in 0.2 cc sesame oil, beginning immediately after hypophysectomy, is 10.3 for estriol, 16.5 for estrone, and 16.2 for estradiol benzoate, as compared with 8.34 for the uninjected animal (90).

Estriol, estradiol, and estrone increased the body weight of male mice castrated at 16.5–19 gm body weight and implanted subcutaneously 1 month later with estrogen pellets at low doses, but inhibited or decreased it at higher doses (91). The kidneys of these rats were not, or were only slightly, increased in size. Their thymus weight was decreased. Seminal vesicle and prostate weights were increased about the same by two different doses of estrogen. The arginase activity of the kidney was increased equally by both dose levels, while that of the liver was not remarkably increased. The estrogens had no effect on the alkaline or acid phosphatases of the liver or kidney.

Stimulation of mitotic activity by estrogens and other hormones has been studied by Bullough (92), who found the average number of mitoses per unit volume of mouse ear epidermis to be 12.5 for estriol, 11.7 for estradiol and 10.0 for estrone, as compared with 7.5 without the hormones. The effect on mitoses of cells in tissue culture decreases in the following order: estradiol, estriol and estrone (93). Chick fibroblastic growth is stimulated by estriol; the effect of this estrogen should be studied in wound healing (94). Although increased incidence of cervical erosion in pregnancy has been attributed to the persistent action of high estrogen levels (95), estriol glucuronide promotes healing of recurrent ulcerative stomatitis and vulvitis (96). Daily doses of 500–15,000 I.U. of estriol have a protective effect against cincophen ulcers in dogs (97). Stimulation by estriol, estrone, and estradiol of phagocytosis of injected carbon by the reticuloendothelial system of mice has been observed (98).

Prolonged administration of from 0.65 to 32.5 μg of estriol and from 1.65 to

6.68 μg of estrone to male rats daily from weaning for 113–242 days results in a body weight averaging 87 per cent that of the controls, and the weights of testes, prostate and Cowper's glands are decreased (99). This amount of estrogen does not affect the fertility of the males tested. Similar administration of estrone and estriol to female rats decreases fertility and interferes with lactation. The effects of various estrogens on the prostate, seminal vesicles and preputial glands of rats castrated when 20–30 days old and given five daily injections are summarized in table 3 (100). Prostate and seminal vesicle weights are increased as compared with controls; effects on the preputial glands are not definite. Five hundred micrograms of estrone or estradiol arrest growth of the capon's comb due to 400 μg of testosterone when smeared on simultaneously, whereas the same amount of estriol does not (101). However, estriol, like estrone and estradiol, arrests development and descent of the testes into the scrotum of mice, produces scrotal hernias in mice, reduces testis size and produces bisexual gonads of chicks when injected into fertile eggs, and produces a female type pelvis when given to mice before they are 30 days old (102).

Administration of 200 μg of estriol three times daily and, later, 500 μg daily to male monkeys produces in 8–10 days swelling and edema of the external genitalia (103). This edematous phase lasts 10 days, and at its height the face is brilliantly flushed. During the next 10–40 days this reaction disappears despite continuation of the estrogen. With continued treatment a second phase begins after 20–25 days, consisting of a pallid or colorless edema entirely different in type and distribution. The skin is thrown up into tensely swollen ridges intersected with irregular grooves, beginning at the groin and knee and extending over the whole trunk and then down the arms and legs. The animal returns to normal in a month after cessation of treatment. Reddening of the sexual skin, scrotal swelling and mammary gland hyperplasia are produced by applying by unction 1,000 I.U. of estriol to a male rhesus monkey (104). Administration of one rabbit unit of progesterone on alternate days prevents scrotal swelling but does not prevent reddening of the sexual skin or development of the breast.

Castrate rats receiving 200 rat units of estriol daily for 6 days show definite hypertrophy of the pituitary, thyroid and adrenals, whereas the same amount of estrone produces pituitary hypertrophy only (105). At double this concentration the results are more marked, but larger doses of estrone and estradiol are effective. Estrone or estriol (7 μg subcutaneously daily for 2–3 weeks) decreases thyroid activity in infantile guinea pigs (106), and these estrogens and estradiol increase the serum calcium level in pigeons (107). The effectiveness of estrogens in stimulating new bone formation in mice given 1500 rat units weekly for 3 weeks decreases in the order estrone → estriol → estradiol 17β, whereas with higher doses over a longer period of time, the order is estradiol 17β → estrone → estriol (108).

TABLE 3. EFFECTS OF VARIOUS ESTROGENS ON ORGAN WEIGHTS OF CASTRATED RAT (100)

HORMONE	DOSE, γ/DAY	PROSTATE	SEMINAL VESICLES *mg/100/gm/B.W.*	PREPUTIAL GLAND
None		22, 17	13, 14	34
Estrone	2	44	25	30
Estrone	10	39	17	48
Estriol	2	28	18	22
Estriol	10	48	19	38
Estradiol	2	25	46	35
Estradiol	6	75	45	45

Estriol, like estrone and estradiol, when injected into rats who subsequently receive intravenous trypan blue, produces an abnormally large concentration of the dye in the uterus and vagina, but this is not due to increased affinity of the tissues for the dye (109). All three estrogens stimulate ciliary movements of the buccopharyngeal mucosa of the frog when applied topically in concentration ranges per 100 cc of saline of 0.07–0.2 mg for estrone, 0.002–0.02 mg for estriol and 0.0005–0.0002 mg for estradiol (110).

Subcutaneous injection of estriol, estradiol and estrone into castrate female guinea pigs for 21–120 days produces abundant and repeated metrorrhagias of long duration and forms extragenital tumors not limited to the abdominal cavity (111). Induction of such tumors by estriol is much less than that due to equal or smaller quantities of estrone or estradiol. Lipschutz and collaborators (112) show that while the fibromatogenic action of estriol in the guinea pig is less than that of estrone or estradiol, the increase in uterine weight produced by each was almost equal. Mello (113) also finds the fibromatogenic action of estradiol and estrone to be about the same as that of seven to eight times the weight of estriol. However estriol, like estrone and estradiol, prevents formation of adrenal cortical tumors in susceptible strains of mice (114). Urinary excretion of estriol is greater in noncancerous females than in cancerous, as is the excretion of total estrogens, whereas estradiol excretion is higher in cancerous than noncancerous males (115).

Clinical Applications

Although estriol was commercially available for several years, few quantitative clinical investigations comparing this with other estrogens have apparently been made. Israel (116) found the estriol glucuronide product available in 1936 to be of practically no value in treating primary dysmenorrhea. Pratt and Thomas (117) in 1937 found 2–5 mg estriol orally, or estriol in oil injected, to be no more effective in relieving menopausal symptoms than placebos. However, Puck (118) has recently reported that synthetic estriol is an effective estrogen for treating many conditions.

Puck's clinical investigations were stimulated by his finding that the estriol content of woman's blood was correlated with the state of the uterine cervix, and that women with rigid cervices complaining of dysmenorrhea had less estriol in their blood than those who had no menstrual complaints. Both the estriol and estradiol in the blood were found to be elevated in patients with glandular cystic hyperplasia of the endometrium, in which condition the cervical canal is increased in width. The characteristic intermenstrual changes in the cervix involving widening of the canal, which Puck suggests facilitate entry of sperm, are accompanied by increased estriol excretion. The increased width of the lower genital canal in the last months of pregnancy also occurs concurrently with a great increase in excretion of estriol. These observations lead to the hypothesis, partially verified by animal and human experiments, that estriol stimulates the cervix, vagina and vulva, while estradiol is effective on the corpus uteri.

Estriol was therefore administered by Puck to patients with dysmenorrhea and sterility involving an insufficiently developed cervix, or with cervicitis and parametritis associated with dyspareunia. Vaginitis and vaginal ulcers healed, apparently because of its action on the vagina and vulva. Since estriol occurred in the blood of menstruating females in higher concentrations than estradiol and estrone, Puck assumed that it influenced the general condition of the female, and this, with the

observation that estriol occurs rarely in the blood during the menopause, led to the use of estriol in treating menopausal complaints. Puck regards estriol as a physiological female hormone that is active in minimum quantities, but which does not cause proliferation of the endometrium even when high doses are given, avoiding danger of hemorrhage when treating women in the menopause. Estriol did not delay or advance the cycle.

Treatment of more than 133 women with estriol was reported by Puck and collaborators (118). Twenty-six women with vaginitis and cervicitis received from 0.01 mg to 0.25 mg daily orally, or 1 mg by injection every third day. There were 25 satisfactory results after 5–10 days therapy. The same dose schedule relieved all of 16 women with ulcers of the vagina due to pessaries. Pruritus of the vulva associated with kraurosis and leukoplakia was satisfactorily relieved in 15 of 16 patients after 5–20 days with a dosage schedule of 1 mg injected every third day, or 0.25 mg orally, once or twice daily. Dysmenorrhea, probably due to hypoplastic uteri, myometritis, endometriosis or possibly vascular disorders, was relieved after two to three cycles in 28 of 33 patients; the regimen during the last half of the cycle was three to five injections of 1 mg estriol, or 0.25 mg orally, once or twice daily.

Sixteen women with menopausal symptoms during estriol treatment either ceased to have hot flushes or noted a marked decrease in their intensity. Sensations of vertigo, headaches and depressions disappeared or became less troublesome. One tablet containing 0.01 mg of estriol daily was usually sufficient; this dosage was administered during a long period of time without noting any action on the endometrium or signs of hemorrhage or neoplasia, as may occur when estrone or estradiol is given. A few very severe cases received 0.25 mg estriol daily. Dyspareunia due to a narrow vagina was relieved by estriol treatment, eliminating the stenosis, possibly because of greater elasticity of the vaginal structures and improvement in the epithelial texture.

These new investigations on estriol have increased its importance as a physiologically important female sex hormone and point to the need for further research.

Summary

Newly developed methods for the quantitative determination of estriol, estrone and estradiol in blood and urine have shown different concentrations of each in a variety of conditions, and have suggested the quantitative importance of estriol. This suggestion has been confirmed by studies of the relative potencies of the various estrogens in different actions which show that estriol itself is a potent estrogen, contrary to the usual conception of its being just a metabolite of the more potent estrone and estradiol. Although ordinarily less effective than estrone and estradiol in promoting vaginal cornification, estriol, under optimum conditions, approaches their effectiveness for this purpose.

Estriol is more potent than estrone or estradiol in causing establishment and opening of the vaginal orifice, in promoting imbibition of uterine fluid, in increasing lactic dehydrogenase activity in the uterus, and in stimulating mitotic activity in the epidermis of the mouse ear. The activity of estriol is of the same order of magnitude as that of estrone and estradiol in other estrogenic actions, such as to promote uterine growth at low concentrations (although less effective at high doses), to increase β-glucuronidase and reduced diphosphopyridine nucleotide oxidase activity in the uterus, to reduce motility of the uterus *in vivo*, and to stimulate ovarian growth, body weight, phagocytosis of carbon by reticuloendothelial cells, ciliary movements

of the buccopharyngeal mucosa of the frog, and new bone formation. The fibromatogenic activity in the guinea pig of estriol is much less than that of estrone or estradiol.

Recent experiments have suggested and partly verified the hypothesis that estriol stimulates the cervix, vagina and vulva more effectively than estrone or estradiol, whereas the latter are much more effective on the corpus uteri. These findings, together with the observation that the synthetic estriol now available did not cause endometrial proliferation and bleeding even when high doses were given, led to its successful use in the treatment of disorders due to estrogen deficiency, such as menopausal complaints, dysmenorrhea and sterility involving an insufficiently developed cervix, dyspareunia due to a narrow vagina, vaginitis, cervicitis, vaginal ulcers, and pruritus of the vulva involving kraurosis and leukoplakia.

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