ESTRIOL PRODUCTION AND METABOLISM IN NORMAL WOMEN

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Summary—Studies on estriol metabolism and production were carried out in normal reproductive-aged and post-menopausal women using pulse injections and constant infusions of radiolabeled estriol, estrone and estradiol. The circulating levels were measured by radioimmunoassay.

Following an intravenous pulse of [^{3}H]estriol, the disappearance of radioactivity as estriol could be described as a function which was sum of two exponentials with $t_{1/2}$'s of 3.6 and 64 min. The initial volume of distribution was 20 l. Using the constant infusion technique the metabolic clearance rate (MCR) of estriol was 2100 l/day in the follicular phase of the cycle and similar in the luteal phase. In postmenopausal women the MCR was 1890 l/day. The circulating levels of estriol were 7 and 11 pg/ml in the follicular and luteal phases respectively and 6 pg/ml in post-menopausal women. The production rates of estriol were 14 and 23 μg/day in the follicular and luteal phases of the cycle and 11 μg/day in post-menopausal women. In many of the women infused with radiolabeled estrone or estradiol, no radioactivity could be identified in the blood as estriol The maximal conversion of estrone and estradiol was < 0.4%. Estriol circulates at low but relatively steady levels in the blood. In some women estriol appears to be secreted by the ovary, especially in the luteal phase.

INTRODUCTION

Estriol, one of the three classic estrogens along with estradiol and estrone, arises as a result of 16α-hydroxylation of estrone and subsequent reduction of 16α-hydroxyestrone at the 17 position [1]. While it was once thought to have certain properties suggestive of an estradiol antagonist [2, 3] it is now described as a 'short-acting' estrogen [4], its properties being dependent in part on its mode of presentation to the target-tissue. Thus it has been shown to be an estradiol agonist when present under steady state conditions [4].

Sandberg et al.[5] noted that estriol was rapidly excreted in the urine after an IV dose of radiolabeled estriol. There did appear to be some enterohepatic circulation, and estriol -3-glucuronide was found to be a metabolite formed exclusively in the intestine and then later excreted in the urine [6, 7, 8]. However, the degree of enterohepatic circulation appeared to be somewhat less than that for the other estrogens.

Most of the early studies of estriol metabolism were done to elucidate its urinary excretion patterns and its production in pregnancy. The results of these studies indicated that estriol was excreted primarily as the 16-glucuronide [8], and in pregnancy estriol was formed primarily in the feto-placental unit with its immediate precursor being 16α-hydroxydehydro-epiandrosterone [9].

However, there may not be a good correlation between urinary excretion of conjugates and the circulating blood pool of biologically active steroids [10]. Therefore, we felt that an approach involving studies of estriol in the blood compartment might shed light on its role as an active estrogen. We wished to characterize the disappearance of estriol after its introduction into the blood compartment, to determine its rates of clearance and production and its sources.

METHODS

To determine the volume of distribution and half times of disappearance of estriol from the plasma compartment, normal women were given intravenous pulse injections of [^{3}H]estriol. Blood samples were drawn at increasing time intervals, from 3–270 min, after the pulse and the ^{3}H as estriol was extracted and purified by multiple chromatographic and derivative formation steps. The ^{3}H as estriol was measured in a liquid scintillation spectrometer and the volume of distribution, and half-times of disappearance calculated by standard techniques [11]. To determine the metabolic clearance rate of estriol, [^{3}H]estriol was given as a pulse followed by a constant infusion of [^{3}H]estriol for sufficient time to achieve the isotopic steady state. Blood samples were then obtained, extracted and the [^{3}H]estriol purified by chromatographic and derivative formation, with appropriate correction for losses during the procedures. The MCR was calculated as usual [12].

To determine the endogenous concentration blood samples were obtained from women at various times during the day and during the menstrual cycle. The plasma extracts were analyzed for estriol by RIA using a highly specific antibody and a celite column purification step of the plasma extracts [13].

To determine the possible precursors of circulating estriol, [^{3}H]androgen and [^{3}H]estrogen precursors were administered to normal women by pulse injections followed by constant infusions until the isotopic steady state had been reached. Blood samples were
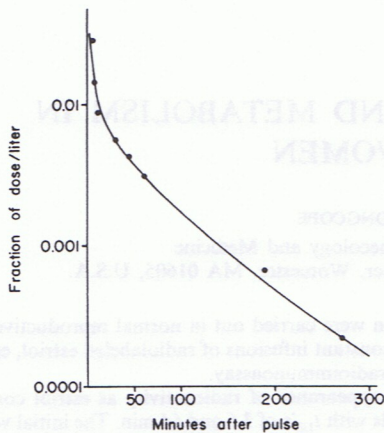
Fig. 1. Semi-log plot of concentration of [³H]estriol expressed as fraction of dose per liter vs time in min after pulse injection.

obtained and extracted and purified as before and the radioactivity in the estriol fraction determined.

RESULTS

Following the administration of iv pulses of [³H]estriol to normal women the radioactivity as estriol disappeared (Fig. 1) in a fashion which could be described as the sum of 2 exponentials. The initial slope has a mean ± SE $t_{1/2}$ of 3.6 ± 0.9 min and the second has a $t_{1/2}$ of 64 ± 11 min. The mean value for the initial volume of distribution was 20 l. MCRs (Fig. 2) calculated from constant infusions were determined in 13 women in the follicular phase (days 5–7) of the cycle and the mean value was 2100 ± 100 l/day. In the luteal phase (days 20–22) of the cycle the mean value in 13 women was 2100 ± 115 l/day. In 4 normal postmenopausal women the mean MCR was 1890 ± 95 l per day, and this value was significantly less than the mean MCR in the premenopausal women.

In 18 normal women (Fig. 3) in the follicular phase of the cycle the mean concentration of estriol was 8 ± 1 pg/ml. In 15 women in the luteal phase the concentration was 11 ± 1 pg/ml. In 8 post-menopausal women ages 50–75 years the concentration was 6 ± 1 pg/ml. In studies in which blood samples were obtained over 10–24 h in 3 normal women there was no trend in the estriol concentrations suggesting that estriol was circulating at a relatively constant level and had no major diurnal variation. In studies in 6 normal young women in which blood samples were drawn throughout the menstrual cycle there were small peaks in the concentration of estriol. These peaks appeared in a somewhat random fashion but were higher in the luteal phase. Measurements of estradiol on the same samples showed that the estriol peaks matched those of estradiol in most instances.

The blood production rates of estriol are shown in

Fig. 4. The mean values in the follicular and luteal phases are 14 ± 2 and 23 ± 2 μg/day respectively. In post-menopausal women the mean value is 11 ± 4 μg/day.

Studies of the conversion of precursor to estriol were carried out in 21 women for estrone, and 24 for estradiol. In many of these studies because of the amounts of radioactivity infused as precursor, volumes of blood drawn and losses during the purification procedures, radioactivity as estriol was not different from background. The mean values noted in Fig. 5 are, therefore, maximal values, and it is probable that the actual values are below these. In any case the conversion of estrone and estradiol to estriol are <0.40% and indicate that there is little contribution of these precursors to the blood pool of circulating estriol.

In no study did we find ¹⁴C as estrone or estradiol indicating that no back conversion of estriol to those estrogens could be demonstrated.

DISCUSSION

Our data on the concentrations of estriol indicate that it is present at low but relatively steady concentrations throughout the day and throughout the menstrual cycle. However, it does appear to be present in somewhat higher concentrations in the luteal phase and there may be occasional spikes in the concentration. Using somewhat similar RIA techniques with different antibodies others have reported that estriol values were considerably higher than the values we found [14, 15, 16] and there has been one report that estriol could not be found in the circulating blood during the follicular phase of the cycle [17]. The levels of estriol that do exist are far lower than those for the other estrogens, estradiol and estrone. However, since estradiol is bound with high affinity to the SHBG only a portion of the circulating concentration is apparently available for entry into the cells [18]. For estriol which is bound at a far lower affinity most of it would be available for biologic activity [18].
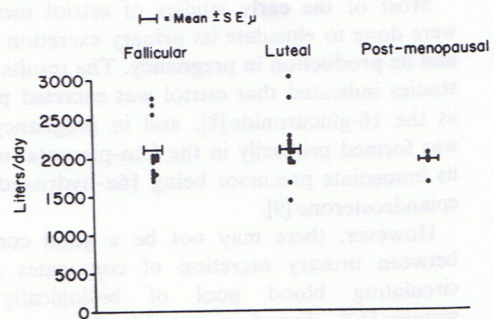

Fig. 2. Metabolic clearance rates of estriol in liters/day in reproductive-aged women studied during follicular and luteal phases of the cycle and in post-menopausal women.
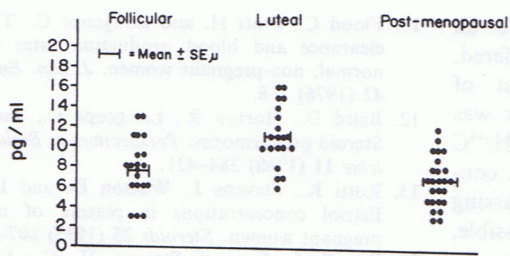
Fig. 3. Concentration of estriol in pg/ml in reproductive-aged women studied during follicular and luteal phases of the cycle and in post-menopausal women.

The initial volume of distribution and the MCR indicate that the binding of estriol to the SHBG is of little physiologic importance. At least the values for V^1 and for MCR are similar to those steroids which are not bound to SHBG [12]. The fact that we could not demonstrate a slowly turning over pool of estriol in plasma, as we could for estrone and estradiol [19] suggests that there is little re-entry into the plasma pool of estriol which has been conjugated previously. This is unlike the situation for estrone and estradiol where the estrone sulfate pool turns over slowly and can contribute to the concentration of both estrogens [19]. The lack of a slowly turning over pool of estriol probably explains the relatively rapid excretion of radioactivity into the urine which has been reported by others [5, 20] following the pulse administration of estriol in plasma.

The finding that the MCRs of estriol are similar in the follicular and luteal phases of the cycle suggests that the rate of metabolism is not altered with changes in estrogen concentrations during the cycle; a finding also noted for estrone and estradiol [21]. We did find a small but significant decrease in the MCR of estriol in postmenopausal women. However, it should be noted that the numbers of subjects studied was small and probably accounts for this difference. In a larger series of patients we could find no such alteration in the MCRs between young and older women for other steroids [22].
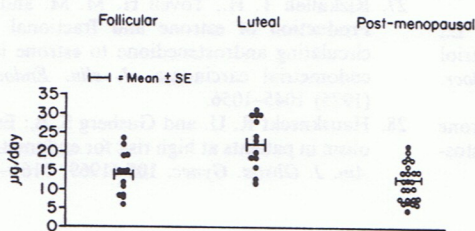
The blood production rates of estriol in both the follicular and luteal phases of the cycle were lower than those of estrone or estradiol. Estriol production was slightly greater in the luteal compared to the follicular phase of the cycle. Others have also noted that the production rate, as determined by isotopic measurements of urinary metabolites, was also greater in the luteal as compared to the follicular phase of the cycle [23]. The production rate of estriol in postmenopausal women is also lower than that in reproductive-aged women.

The production rates that we estimated were considerably lower than those derived form urinary isotopic measurements. This suggests that there may be formation of estriol in a pool(s) not in equilibrium with the blood compartment of estriol a finding similar to that for testosterone in women [10].

The results of precursor conversion to estriol were somewhat unexpected as we had anticipated finding measurable conversion of estrone to estriol in all women. However, for neither of the precursors administered: estrone or estradiol were we able to find evidence of greater than 0.4% peripheral conversion: using these maximal figures we could only explain a portion of the production rate of estriol on the basis of precursor conversion in peripheral tissue. This raises the question as to whether there is a small secretion of estriol from the ovary or the adrenal of reproductive aged women or precursors other than estrone and estradiol. Wotiz et al. noted the conversion of 4-[^{14}C]testosterone to estriol by human ovarian tissues [24]. Smith and Zuckerman reported [25] that in one of four corpus luteum examined there was evidence of synthesis of estriol from precursors. However, this was noted to be small in amount. Thus some of the estriol at least in the luteal phase could be explained by direct secretion of estriol.

When radiolabeled androstenedione and estrone have been administered to women and measurements made of isotope ratios in urinary estrogen conjugates, the ratio for estriol is similar to that of estrone [26].
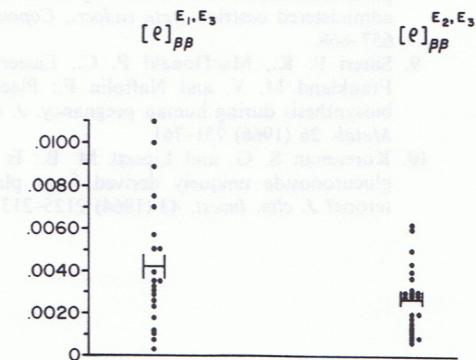

Fig. 4. Blood production rates of estriol in μd/day in reproductive-aged women studied during follicular and luteal phases of the cycle and in post menopausal women. Closed circles (●) indicate production rates calculated from individual's own MCR and plasma concentrations. Open circles (○) indicate production rates calculated from individual's own plasma concentration but group mean MCR.

Fig. 5. Fraction of estrone and estradiol infused which was measured as estriol ($[\rho]_{\beta\beta}^{E_1,E_3}$, $[\rho]_{\beta\beta}^{E_2,E_3}$) in reproductive-aged women studied in follicular phase.

This is evidence that estrone is a major source for all the estriol. In a few studies, these ratios have differed, indicating a source of estriol, independent of estrone [27, 28]. In these studies the precursor was androstenedione and this difference between $^3H/^{14}C$ in estrone compared to estriol indicates direct conversion of androstenedione to estriol without passing through the blood pool of estrone. It is possible, therefore, that there may be other precursors to circulating estriol which we did not infuse.

In summary, therefore, estriol appears to be present at low concentrations in blood and is metabolized at a rate commensurate with minimal if any high affinity binding. We cannot rule out the fact that in some women minimal estriol secretion by the ovary may occur.

REFERENCES

1. Fishman J., Hellman L., Zumoff B. and Cassouto J.: Pathway and stereochemistry of the formation of estriols in man. *Biochemistry* 5 (1966) 1789–1794.
2. Hisaw F. L., Velardo J. T. and Goolsby C. M.: Interaction of estrogens on uterine growth. *J. clin. Endocr. Metab.* 14 (1954) 1134–1143.
3. Wotiz H. H., Shane J. A., Vigersky R. and Brecher P. I.: The regulatory role of oestriol in the proliferative action of oestradiol. In *Prognostic Factors in Breast Cancer* (Edited by A. P. M. Forrest and P. B. Kunbler). Williams and Wilkins, Baltimore (1968) pp. 368–382.
4. Clark J. H., Peck E. J., Jr., Hardin J. W. and Eriksson H.: The biology and pharmacology of estrogen receptor binding: relationship to uterine growth. In *Receptors and Hormone Action* (Edited by B. W. O'Malley and L. Birnbaumer). Academic Press, New York, Vol. II (1978) pp. 1–31.
5. Sandberg A. and Slaunwhite W. R. Jr: Studies on phenolic steroids in human subjects. VII. Metabolic fate of estriol and its glucuronide. *J. clin. Invest.* 44 (1965) 694–702.
6. Goebelsmann U., Sjoberg K., Wiqvist N. and Diczfalusy E.: Oestriol-3-glucosiduronate, a major urinary metabolite of oestriol and oestriol-16(17?)-glucosiduronate. *Acta endocr., Copenh.* 50 (1965) 261–272.
7. Goebelsmann U., Cooke I., Wiqvist N. and Diczfalusy E.: Comparison of the metabolism of oestriol-3-glucosiduronate and oestriol-16-glucosiduronate in pregnant women. *Acta endocr., Copenh.* 52 (1966) 30–42.
8. Stoa K. F. and Levitz M.: Comparison of the conjugated metabolites of intravenously and intraduodenally administered oestriol. *Acta endocr., Copenh.* 57 (1968) 657–668.
9. Siiteri P. K., MacDonald P. C., Easterling W. E., Frankland M. V. and Naftolin F.: Placental estriol biosynthesis during human pregnancy. *J. clin. Endocr. Metab.* 26 (1966) 751–761.
10. Korenman S. G. and Lipsett M. B.: Is testosterone glucuronoside uniquely derived from plasma testosterone? *J. clin. Invest.* 43 (1964) 2125–2131.
11. Flood C., Pratt H. and Longcope C.: The metabolic clearance and blood production rates of estriol in normal, non-pregnant women. *J. clin. Endocr. Metab.* 42 (1976) 1–8.
12. Baird D., Horton R., Longcope C., and Tait J. F.: Steroid prehormones. *Perspectives in Biology and Medicine* 11 (1968) 384–421.
13. Rotti K., Stevens J., Watson D. and Longcope C.: Estriol concentrations in plasma of normal, non-pregnant women. *Steroids* 25 (1975) 807–816.
14. Powell J. E. and Stevens V. C.: Simple radioimmunoassay of five unconjugated ovarian steroids in a single sample of serum or plasma. *Clin. Chem.* 19 (1973) 210–215.
15. Runnebaum B., Klinga K., Von Holst T. and Junkermann H.: Steroids in human myometrium and peripheral blood during the menstrual cycle. *Am. J. Obstet. Gynec.* 131 (1978) 628–631.
16. Wu C-H. and Lundy L. E.: Radioimmunoassay of plasma estrogens. *Steroids* 18 (1971) 91–112.
17. Raju U., Ganguly M., Weiss G., Zarkin A., and Levitz M.: Serum unconjugated estriol in the menstrual cycle and early pregnancy. *Gynec. Invest.* 6 (1975) 356–364.
18. Dunn J. F., Nisula B. C. and Rodbard D.: Transport of steroid hormones: Binding of 21 endogenous steroids to both testosterone-binding globulin and corticosteroid-binding globulin in human plasma. *J. clin. Endocr. Metab.* 53 (1981) 58–68.
19. Longcope C.: The metabolism of estrone sulfate in normal males. *J. clin. Endocr. Metab.* 34 (1974) 113–122.
20. Fishman J., Zumoff B., Hellman L. and Gallagher T. F.: Metabolism of estriol 17α-^3H in man. *Steroids* 11 (1968) 337–346.
21. Longcope C. and Tait J. F.: Validity of metabolic clearance and interconversion rates of estrone and 17β-estradiol in normal adults. *J. clin. Endocr. Metab.* 43 (1971) 481–490.
22. Longcope C., Jaffee W. and Griffing G.: Metabolic clearance rates of androgens and oestrogens in ageing women. *Maturitas* 2 (1980) 283–290.
23. Barlow J. J. and Logan C. M.: Estrogen secretion, biosynthesis and metabolism: Their relationship to the menstrual cycle. *Steroids* 7 (1966) 309–320.
24. Wotiz H. H., Davis J. W., Lemon H. M. and Gut M.: Studies on steroid metabolism: V. Conversion of testosterone-4-^{14}C to estrogens by human ovarian tissue. *J. biol. Chem.* 222 (1956) 487–405.
25. Smith O. W. and Zuckerman N. G.: Estrogen biosynthesis from testosterone-4-^{14}C by post-ovulatory human ovaries: Comparison between the corpus-luteum ovary and its partner. *Steroids* 22 (1973) 379–399.
26. Grodin J. M., Siiteri P. K. and MacDonald P. C.: Source of estrogen production in postmenopausal women. *J clin. Endocr. Metab.* 36 (1973) 207–214.
27. Rizkallah T. H., Tovell H. M. M. and Kelly W. G.: Production of estrone and fractional conversion of circulating androstenedione to estrone in women with endometrial carcinoma. *J. clin. Endocr. Metab.* 40 (1975) 1045–1056.
28. Hauskneckt R. U. and Gusberg S. B.: Estrogen metabolism in patients at high risk for endometrial carcinoma. *Am. J. Obstet. Gynec.* 105 (1969) 1161–1167.