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ESTROGEN METABOLISM AS MEASURED IN BLOOD AND URINE IN FEMALE RHESUS MONKEYS

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Summary—In order to measure the interconversions of estrone (E1) and estradiol (E2) and their conversion to the 16α-hydroxylated estrogens, 16α-hydroxy estrone (16α-OHE1) and estriol (E3), we infused 11 female rhesus monkeys with [^{3}H]E2 and [^{14}C]E1 and measured radioactivity in the blood as E1, E2 and 16α-OHE1 ($n = 9$) and in the urine as the glucuronides of E1, E2, 16α-OHE1, and E3 ($n = 11$).

The mean conversion of E1 to E2 as measured in blood (percent of infused E1 measured in blood as E2, $[\rho]^{1,2}_{BB}$) was $29.2 \pm 1.6\%$ and as measured in the urine of the same animals, $[\rho]^{1,2}_{BM}$, was $77.4 \pm 5.9\%$. The mean conversion of E2 to E1, $[\rho]^{2,1}_{BB}$ was $21.5 \pm 1.0\%$ and as measured in urine, $[\rho]^{2,1}_{BM}$ was $67.7 \pm 4.6\%$. Thus for both estrone and estradiol only 30–35% of the interconversions occurred in pools which were in equilibrium with the blood pool of these estrogens. The remaining 65–70% occurred in a pool, probably liver, in which glucuronidation occurred immediately after conversion.

The conversion ratios (the ratio of the concentration in the blood of radioactivity as 16α-OHE1 to its precursor, $CR^{Prec,16\alpha\text{-}OHE1}$) was 0.036 ± 0.008 for $CR^{E1,16\alpha\text{-}OHE1}$ and 0.0039 ± 0.0010 for $CR^{E2,16\alpha\text{-}OHE1}$.

The percentages of administered E1 excreted in the urine as the glucuronides of E1, E2, 16α-OHE1 and E3 were 20.1 ± 1.5, 1.6 ± 0.2, 0.96 ± 0.20 and 0.76 ± 0.07 respectively. The percentages of administered E2 excreted in the urine as E1, E2, 16α-OHE1 and E3 were 14.4 ± 1.0, 2.2 ± 0.3, 0.57 ± 0.05 and 0.68 ± 0.11 respectively.

Thus there are minor differences in the patterns of excreted metabolites of E1 and E2. Furthermore, 16α-OHE1 and E3 are not major metabolites of E1 or E2 in the female rhesus monkey.

INTRODUCTION

In the human it has been shown that the calculated interconversions of the androgens, androstenedione (A) and testosterone (T) are greater when measured from urinary metabolites than from the blood pool of the hormones [1, 2]. This is because the pool in which androgen metabolites are formed is not in equilibrium with the blood pool of the unmetabolized androgens.

While it has been suggested that such a result would also hold true for the estrogens estrone (E1) and estradiol (E2) [3], this has not been rigorously tested with measurements of the hormones in blood and measurements of the hormone metabolites in the urine in the same individuals.

In addition, while conjugates of estriol (E3) have been noted to be present in the urine of rhesus monkeys (*Macaca mulatta*) [4, 5], the contributions of estrone and estradiol to the excretion of the estriol conjugates have not been measured nor has the precursor of estriol, 16α-hydroxy estrone (16α-OHE1) been measured in monkey urine or blood. Since the activity of the 16α-hydroxylase enzyme has been reported to be increased in breast and endometrial carcinoma [6, 7], we wished to determine whether the female rhesus might be a useful model system to study the activity of this enzyme and its control.

The present study was designed to compare the interconversions of estrone and estradiol as measured in the blood pool of the unconjugated estrogens with measurements made from the conjugated estrogens in the urine, and to measure the conversions of estrone and estradiol to the 16α-hydroxylated metabolites, 16α-OHE1, and E3.

MATERIALS AND METHODS

All experiments were performed in adult female rhesus (*Macaca mulatta*) monkeys which were housed in individual cages and fed monkey chow (Purina Co., St Louis, Mo.) *ad libitum*. As part of a study on the biology of the primate uterus, the monkeys were ovariectomized at least 2 months before the initiation of artificial menstrual cycles using capsules made from silastic tubing

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(Dow-Corning, Midland, Mich.), filled with estrogen or progesterone as described previously [8, 9]. Infusions of radiolabeled estrone and estradiol were carried out on days 9, 14 or 23 of the induced cycle [9], and the protocol was approved by the Institutional Animal Care and Use Committee. Some of the data from these infusions have been reported, i.e. the metabolic clearance rates of estrone and estradiol, their interconversions measured in the blood pool [9] and their metabolism in the uterus [10]. For the infusions and the analyses, solvents and reagents were prepared as described previously [9, 11, 12]. [6,7-^{3}H]estradiol (SA 54 Ci/mmol) and [4-^{14}C]estrone (SA 54 mCi/mmol) were obtained from Amersham Corp. (Arlington Heights, Ill.) and purified before use [3]. E1, E2, E3 and 16α-OHE1 were obtained from Steraloids (Wilton, N.H.). E1, E2 and E3 were crystallized from methanol before use, but 16α-OHE1 was used without further purification.

A detailed description of the methods has been reported previously [9]. Briefly, on day 9, 14 or 23 of an artificial cycle, monkeys were tranquilized with ketamine and maintained on halothane/nitrous oxide anesthesia. The animals were given a priming dose intravenously of 15 μCi [^{3}H]estradiol/1 μCi [^{14}C]estrone in 10 ml 8% ethanolic saline and then infused over the next $3\frac{1}{2}$ h with 30 μCi [^{3}H]estradiol/2 μCi [^{3}H]estrone in 20 ml 8% ethanolic saline via a catheter in the cubital vein. Blood samples, 2 ml each, were obtained from the femoral artery at $2\frac{1}{2}$ and $3\frac{1}{2}$ h and stored frozen until analyzed. All urine was collected for 96 h.

The blood samples were processed and the radioactivity as estradiol and estrone measured as previously described [3, 11].

As measured from the blood pool, the overall interconversions of the estrogens, i.e. E1 conversion to E2 and E2 conversion to E1, were the percent of precursor infused into and measured as product in the blood, $[\rho]_{BB}^{Prec,Prod} = 100 \times [(^3H/^{14}C)^{Prod}\ (^3H/^{14}C)$ infused], where measurements were made in arterial blood [13]. Comparisons between group means were made using the Student–Newman–Keul's test [14].

The extraction, and purification of the estrogens from the urine was done essentially as described [12, 15–17]. Briefly, a 25% aliquot of the pooled 96 h urine was extracted once with an equal volume and twice with $\frac{1}{2}$ vol of ethylacetate. The organic phase was backwashed twice with a 10% volume of water. The washes were added to the residual urine. The ethylacetate extract was evaporated but not worked up further, and represented the unconjugated estrogens which amounted to less than 1% of the urinary radioactivity.

The residual urine was made 0.005 M in sodium acetate and brought to pH 5 with 50% H_2SO_4, and β-glucuronidase (500 Fishman U/ml of urine) was added, and the urine was incubated at 37°C for 48 h. Then the carriers E1, E2, 16α-OHE1, and E3 (400 μg of each in ethanol) were added and the urine extracted once with an equal volume and twice with $\frac{1}{2}$ vol of ethylacetate. The organic phase was backwashed twice with a 10% vol of water. The washes were added to the residual urine, and the organic phase further washed with 5% $NaHCO_3$ and water until neutral.

The solvent was removed under vacuum and the residue chromatographed on Silica Gel HF_{254} (Brinkman Instruments, Westbury, N.Y.) in the system benzene–ethylacetate–acetic acid (80:20:2, by vol). Following development 3 zones were separately eluted; those containing (1) E1, (2) 16α-OHE1 and E2, and (3) E3. The individual steroids were then purified by multiple chromatographic and derivatization steps [12, 15–17]. The estrogens recovered from this analysis represented the glucuronide conjugates.

Following the final HPLC, the mass of each estrogen was determined by u.v. spectrometry and the losses through the procedure calculated. The radioactivity in each estrogen was measured in a liquid scintillation spectrometer and corrected for losses through the procedures.

As measured using the glucuronide conjugates in the urine, the interconversions of E1 and E2 were calculated as $[\rho]_{BM}^{Prod,Prec} = 100 \times (^3H/^{14}C)^{Prod}/(^3H/^{14}C)$ infused [18]. The percent of administered dose was calculated as the radioactivity in the product, corrected for losses, divided by the radioactivity administered as precursor.

Comparisons between means were done using paired t-tests.

RESULTS

As shown in Table 1, for both $[\rho]^{1,2}$ and $[\rho]^{2,1}$ the measurements made using urinary metabolites were significantly greater, $P < 0.05$, than the measurements made using the blood pool of unconjugated estrogens. However, there did not appear to be a significant shift in any of the interconversions measured at different times of the cycle.

The mean conversion ratio measured in blood, i.e. the ratio of radioactivity as precursor to product, $CR^{Prec,Prod}$, of estrone to 16α-OHE1 was 0.036 ± 0.007 which was significantly greater, $P < 0.01$, than the mean CR of E2 to 16α-OHE1, 0.0044 ± 0.0007.

The monkeys excreted $46.0 \pm 2.1\%$ of the administered dose of radioactivity in the urine, and

Table 1. Interconversions of estrone and estradiol measured in the blood ($[\rho]_{BB}$) and in the urine ($[\rho]_{BM}$) of female rhesus monkeys

Day of cycle	n	$[\rho]_{BB}^{1,2}$ [a] (%)	$[\rho]_{BM}^{1,2}$ (%)	$[\rho]_{BB}^{2,1}$ (%)	$[\rho]^{2,1>BM}$ (%)
9	3	26.3 ± 1.5[b]	62.1 ± 3.7	22.2 ± 1.3	84.9 ± 3.1
14	3	32.0 ± 2.2	86.0 ± 7.7	20.9 ± 2.3	61.3 ± 5.2
23	3	30.2 ± 3.2	78.2 ± 6.5	21.7 ± 4.4	84.6 ± 4.2

[a] $[\rho]_{BB}^{1,2}$ is the percent of estrone infused into the blood and measured in the blood as estradiol. $[\rho]_{BM}^{1,2}$ is the percent of estrone infused into the blood and converted in the body to estradiol.
[b] Mean ± SEM.

87.9 ± 1.3% of the urinary radioactivity was in the enzyme-hydrolyzed, glucuronide fraction. Because most of the urinary radioactivity was in the glucuronide fraction, we did not study the free or pH 1 hydrolyzable (sulfate) fractions. Extending the time of the urine collection did not alter these percentages.

Following the administration of either E1 (Table 2) or E2, a greater percentage of that dose was excreted as E1 glucuronide than E2 glucuronide and both these percentages were greater than the similar percentages excreted as 16α-OHE1 or E3. Following the administration of E1 (Table 2) a greater percentage of the dose was excreted as E1 glucuronide than E2 glucuronide, and a greater percentage of administered E2 was excreted as E2 glucuronide than E1 glucuronide.

The percentages of 16α-OHE1 and E3 arising from E1 were greater than the percentages arising from E2.

In one of the monkeys measurements were also made of the percentages of administered E1 and E2 excreted as the glucuronides of 2 hydroxyestrone and 2 methoxyestrone and in this monkey the sum of these metabolites was 2.64% of E2 and 3.15% of the administered doses of E1.

DISCUSSION

Measurements made from the pool of hormone metabolites will reflect with accuracy the pool of unmetabolized hormone in the blood when the only source of the hormone metabolite is from the pool of unmetabolized hormone in the blood [1, 2, 19]. This has been shown to be true for cortisol but not for the androgens A and T, in women [19]. Thus in the case of the latter, it has been clearly shown that T glucuronide arises from sources other than the blood pool of T [1, 20], and the interconversions of A and T measured from urinary glucuronides will be much greater than the interconversions as measured in the blood pool of unconjugated A and T [1]. Our data indicate that an analogous situation holds for E1 and E2 as well in the female rhesus monkey. The interconversions of E1 and E2 are in the range of 25–30% when measured in the blood pool of these estrogens. However, when measured in the metabolite pool the interconversions are in the range of 70–80%. Thus in the female rhesus monkey there is a source of E1 glucuronide which is not in equilibrium with the blood pool of E1. Most likely this source is E1 formed from E2 in the splanchnic bed and then conjugated to a glucuronide before leaving the liver. Formation and conjugation in this liver pool accounts for about 60–70% of the E1 glucuronide formed in the body.

The conversion of E2 to E1 is similar in that 60–70% appears to take place in a pool not in equilibrium with the blood pool of estradiol. Our data on the interconversions of E1 and E2 as calculated from the respective glucuronides in the urine are similar to those reported by Barlow and Logan[21] and Gurpide *et al.*[22] in normal women.

Edman and MacDonald[23] and ourselves [18] have shown that in obese women there can be a disparity between the aromatization of A as measured in the blood pool of E1 during a $3\frac{1}{2}$ h infusion, and as measured from the urine pool of E1 glucuronide after a 4 day urine collection. In such individuals the disparity is due to the time needed to reach equilibrium in the blood because of the effect of obesity, and not because of other sources of the radiolabeled E1 glucuronide. However, it should be noted that if our findings in the rhesus monkey apply to humans then one cannot determine the aromatization of A to E2 using measurements made in the E2 glucuronide pool unless one injects radiolabeled A and E2.

As we have reported in the past [9, 11] the interconversions of E1 and E2 as measured in the blood pool do not vary during the menstrual cycle in the rhesus monkey, and our current values are in keeping with these earlier reports [9, 10]. Our present data indicate that there is no variation during the cycle in the interconversions measured using the glucuronide pool although our groups were small and a very small variation could have been missed.

Although, only about 50% of the administered radioactivity as estrone or estradiol was recovered in the urine, this amount is similar to that which we and others have reported in women [16, 24, 25]. It is probable that a portion of the remaining radioactivity was excreted in the feces, but we did not investigate that further.

From our urinary data, we found that a greater percentage of E1 glucuronide arose from E1 than from E2, and a greater percentage of E2 glucuronide arose from E2 than E1. Thus, the pattern of these estrogen metabolites differs somewhat depending on the precursor administered. Musey *et al.*[26] reported a difference in the amounts of E1 glucuronide and E2 glucuronide formed after incubations of E1 and E2

Table 2. Percent of administered [^{14}C]estrone or [^{3}H]estradiol excreted in the urine as the glucuronide conjugate of estrone (E1), estradiol (E2), estriol (E3) and estrone 16α-hydroxyestrone (16α-OHE1) without regard to time of the artificial menstrual cycle

Precursor	*n*	% Of administered dose as the glucuronide of E1	E2	E3	16α-OHE1
[^{14}C]Estrone	11	20.1 ± 1.5[a,c]	1.6 ± 0.2[a]	0.76 ± 0.07[a]	0.96 ± 0.20[c]
[^{3}H]Estradiol	11	14.4 ± 1.0[b]	2.2 ± 0.3[b]	0.57 ± 0.05[b]	0.68 ± 0.11[d]

[a,b] Difference between means significant, $P < 0.01$. [c,d] Difference between means significant, $P < 0.05$. [c] Mean ± SEM.

with rhesus monkey liver. The relative relationships that they found were similar to ours although the actual amounts differed because of the difference in technique.

Hopper and Tullner[4] reported that estrone glucuronide was the major estrogen in the urine of female rhesus monkeys, and this finding was borne out by our data.

There have been few studies looking at the extent of 16α-hydroxylation of E1 and E2 in the rhesus monkey [27]. Our data confirm that this occurs, and that there is a blood pool of 16α-hydroxy estrone. This is probably a pool which is relatively small because the CRs of both E1 and E2 to 16α-OHE1 are small. The CR by itself is not a direct indicator of the amount of conversion of product to precursor, but if the MCR of 16α-OHE1 were similar to that of E1 or A in the rhesus monkey then the percent conversion of E1 to 16α-OHE1 would be similar to the CR and hence in the range of 1–2%.

Similar comments would apply to E2 conversion to 16α-OHE1 so that one can calculate a production rate of 16α-OHE1 of 2–3 μg/day at peak production rates, compared to 90–150 μg/day of E1 and E2 during the artificial menstrual cycle [9].

The $CR^{E1,16\alpha\text{-OHE1}}$ was 10-fold that of $CR^{E2,16\alpha\text{-OHE1}}$, while the circulating E1 concentration is about 50% that of E2 [9]. Therefore, using the approach of Mann and Gurpide[28], we calculate that E1 is a more important source of 16α-OHE1 than is E2.

The relatively small conversions of E1 and E2 to the blood pool of 16α-OHE1 are reflected in the small percentages of administered E1 or E2 which are excreted in the urine as 16α-OHE1 and E3. Although the percent of administered dose is not a direct measurement of 16α-hydroxylase activity it does indicate that such activity is probably low in comparison to the percent of administered dose excreted as E1 and E2 glucuronide. This pattern differs from the pattern we found in women in whom a greater percent of the dose is excreted as E3 than E1, E2, or 16α-OHE1 [15, 16], and the greatest percentage of the dose is excreted as catechol estrogens. The apparent low 16α-hydroxylase activity in the monkey would indicate that it would not be a good model for the study of this reaction.

Wortmann *et al.*[27] infused estrone sulfate through the liver of a rhesus monkey and reported rapid conversion to unconjugated E3 and slower conversion to E3 glucuronide. They also noted conversion to catechol estrogens. We also noted the conversion of E1 and E2 to catechol estrogens and this conversion was somewhat greater than conversion to 16α-OHE1 and E3. However, the percentage of catechol estrogens found was far less than E1 glucuronide.

Thus, in the female rhesus monkey, a major portion of the interconversions of E1 and E2 occur in the liver and the quantitative pattern of estrogen metabolites differs somewhat from that reported in women.

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REFERENCES

1. Horton R. and Tait J. F.: Androstenedione production and interconversion rates measured in peripheral blood and studies on the possible site of its conversion to testosterone. *J. Clin. Invest.* **45** (1966) 301–313.
2. Tait J. F. and Horton R.: The *in vivo* estimation of blood production and interconversion rates of androstenedione and testosterone and the calculation of their secretion rates. In *Steroid Dynamics* (Edited by G. Pincus, T. Nakao and J. F. Tait). Academic Press, New York (1966) pp. 393–427.
3. Longcope C., Layne D. S. and Tait J. F.: Metabolic clearance rates and interconversions of estrone and 17β-estradiol in normal males and females. *J. Clin. Invest.* **47** (1968) 93–106.
4. Hopper B. and Tullner W. W.: Urinary estrone and plasma progesterone levels during the menstrual cycle of the rhesus monkey. *Endocrinology* **86** (1970) 1225–1230.
5. Breckwoldt W., Murawec T. and Touchstone J. C.: Urinary estrogens II. Excretion of estrogens in rhesus monkeys stimulated with human gonadotropins. *Steroids* **17** (1971) 305–316.
6. Fishman J., Schneider J., Hershcopf R. J. and Bradlow H. L.: Increased estrogen-16α-hydroxylase activity in women with breast and endometrial cancer. *J. Steroid Biochem.* **20** (1984) 1077-1081.
7. Bradlow H. L., Hershcopf R. E. and Fishman J. F.: Oestradiol 16α-hydroxylase: a risk marker for breast cancer. *Cancer Surv.* **5** (1986) 573-583.
8. Hodgen G. D.: Surrogate embryo transfer combined with estrogen-progesterone therapy in monkeys. *JAMA* **250** (1983) 2167–2171.
9. Longcope C., Bourget C., Meciak P. A. *et al.*: Estrogen dynamics in the female rhesus monkey. *Biol. Reprod.* **39** (1988) 561–565.
10. Longcope C., Bukowski C., Okulicz W. C., McCracken J. A., Hoberg L. M. and Padykula H. A.: Estrogen interconversions in the induced cycle in female rhesus monkeys. *Biol. Reprod.* **40** (1989) 949–952.
11. Longcope C., Billiar R. B., Takaoka Y., Reddy S. P., Hess D. and Little B.: Tissue metabolism of estrogens in the female rhesus monkey. *Endocrinology* **109** (1981) 392–396.
12. Bourget C., Femino A., Franz C. and Longcope C.: Estrogen and androgen dynamics in the cynomolgus monkey. *Endocrinology* **122** (1988) 202–206.
13. Baird D., Horton R., Longcope C. and Tait J. F.: Steroid prehormones. *Perspect. Biol. Med.* **11** (1968) 384–421.
14. Steel R. G. D. and Torrie J. H.: *Principles and Procedures of Statistics*. McGraw-Hill, New York (1960) pp. 110–111.
15. Longcope C., Femino A., Flood C. and Williams K. I. H.: Metabolic clearance rate and conversion ratios of 3H-2-hydroxyestrone in normal men. *J. Clin. Endocr. Metab.* **54** (1982) 347–380.
16. Longcope C., Gorbach S., Goldin B. M., Woods M., Dwyers J. and Warman J.: The metabolism of estradiol: oral compared to intravenous administration. *J. Steroid Biochem.* **23** (1985) 1065–1070.
17. Longcope C., Femino A. and Johnston J. O.: Inhibition of peripheral aromatization in baboons by an enzyme-activated aromatase inhibitor (MDL-18,962). *Endocrinology* **1223** (1988) 2007–2011.
18. Longcope C.: Methods and results of aromatization studies *in vivo*. *Cancer Res.* **42** (1982) 3307s–3311s.

19. Tait J. F. and Burstein S.: *In vivo* studies of steroid dynamics in man. In *The Hormones, Vol. 5* (Edited by G. Pincus, K. V. Thimann and E. B. Astwood). Academic Press, New York (1964) pp. 441–557.
20. Korenman S. G. and Lipsett M. B.: Is testosterone glucuronide uniquely derived from plasma testosterone? *J. Clin. Invest.* **43** (1964) 2125–2131.
21. Barlow J. J. and Logan C. M.: Estrogen secretion, biosynthesis and metabolism: their relationship to the menstrual cycle. *Steroids* **7** (1966) 309–320.
22. Gurpide E., Angers M., Vande Wiele R. and Lieberman S. Determination of secretory rates of estrogens in pregnant and nonpregnant women from the specific activities of urinary metabolites. *J. Clin. Endocr. Metab.* **22** (1962) 935–945.
23. Edman C. D. and MacDonald P. C.: Slow entry into blood of estrone produced in extraglandular site(s) in obesity and endometrial neoplasia. *Gynec. Invest.* **5** (1974) 27.
24. Sandberg A. A. and Slaunwhite W. R. Jr: Studies on phenolic steroids in human subjects. II. The metabolic fate and hepato-biliary-enteric circulation of C^{14}-estrone and C^{14}-estradiol in women. *J. Clin. Invest.* **36** (1957) 1266–1278.
25. Fishman J., Brown J. B., Hellman L., Zumoff B. and Gallagher T. F.: Estrogen metabolism in normal and pregnant women. *J. Biol. Chem.* **237** (1962) 1489–1494.
26. Musey P. I., Collins D. C. and Preedy J. R.: Estrogen metabolism in nonhuman primates I. *In vitro* biosynthesis of estrogen glucosiduronates in rhesus monkey liver. *Steroids* **29** (1977) 93–104.
27. Wortmann W., Johnston D. E., Wortmann B. and Touchstone J. C.: Metabolism of ^{3}H-estrone sulfate perfused *in vivo* through a rhesus monkey liver. *J. Steroid Biochem.* **4** (1973) 271–276.
28. Mann J. and Gurpide E.: Generalized rates of transfer in open systems of pools in the steady state. *J. Clin. Endocr. Metab.* **26** (1966) 1346–1354.