The Metabolism of Estradiol-17β-4-^{14}C in the Newborn Rhesus Monkey (35515)

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(Introduced by W. F. Cantrell)

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The excretion of estrogens in pregnant monkeys has been studied by several investigators (1–6). The major urinary estrogens identified were estrone, estradiol-17β, and estriol, although estriol was not found consistently (*2, 3*). During the last month of pregnancy there is a moderate increase in the excretion of these estrogens (3–5). In addition, estrone excretion increases markedly approximately 1 week prior to parturition, continuing to rise until the day before birth (5). At this time there is a precipitous decline and within 72–96 hr after birth only small quantities can be measured in the urine. This decrease in urinary estrogens after birth suggests that the fetus may contribute, in part, to the changing pattern seen during pregnancy. Little is known concerning the role of the monkey fetus but it is recognized that the human fetus is very active in the metabolism of estrogens during pregnancy (7, 8).

This report deals with the metabolism of estradiol-17β-4-^{14}C in the newborn rhesus monkey. At this stage of the animal's development the enzyme activity involved in estrogen metabolism should be great and should reflect the potential of the fetus at the time of parturition.

Materials and Methods. Three rhesus monkeys (*Macaca mulatta*), two male and one female ranging in age from 6 to 22 days, were used in this study. After the area of the femoral triangle had been anesthetized with procaine, 1 μCi of estradiol-17β-4-^{14}C (sp act = 58.8 mCi/mole) was injected via the femoral vein. The isotope had been previously repurified and dissolved in 0.5 ml of 50% ethanol in 0.9% saline. Three hr after the injection, the animals were killed.

Urine and bile were aspirated from the urinary bladder and gallbladder, respectively. All samples were extracted with an equal volume of ether (5$\times$). After all traces of ether had been removed, the aqueous phase was diluted with acetate buffer, pH 4.3. All samples were hydrolyzed by incubation for 48 hr at 37° with an extract from *Helix pomatia* (Industrie Biologique Francaise S.A., Gennevilliers, France). A total of 800 units of β-glucuronidase activity/ml was added in four equal portions. After hydrolysis, the samples were reextracted with equal volume of ether (5$\times$). At various steps in the procedure, aliquots were taken for liquid scintillation counting to correct for losses.

Since most of the recovered radioactivity was present in the ether-soluble fraction after hydrolysis, only this fraction was analyzed in detail.

All samples were initially chromatographed on paper in hexane, benzene (1:1)/formamide to the front. This permitted the separation of estrone (R_f 0.32) from estradiol-17β and other polar metabolites. All radioactivity remaining near the origin of the chromatogram was eluted and chromatographed in chloroform/formamide to the front. In this system, estradiol-17β (R_f 0.70 to 0.75) was separated from the α-ketols and estriol. To check for the presence of estradiol-17α, the estradiol-17β like peak was eluted and chromatographed in hexane, benzene (1:1)/formamide for 12 hr. The α-ketols, 16α-hydroxyestrone and 16-ketoestradiol-17β, estriol, 17-epiestriol, and 16-epiestriol were separated when chromatographed in chloroform/formamide for 6 hr. During all of these steps, authentic standards were run

TABLE I. Percentage Distribution of Metabolites of Estradiol-17β-4-^{14}C in Urine of Newborn Rhesus Monkeys.

	Male	Male	Female
"Free" fraction	3	4	10
"Free" AH[a]	92	90	80
Aqueous fraction	5	6	10
Metabolites[b]			
Estrone	56	55	51
Estradiol-17β	9	9	7
Estradiol-17α	1	—	—
16-Ketoestradiol-17β	3	2	3
16α-Hydroxyestrone	2	2	1
Estriol	7	6	8
"X"	8	7	4
"Pre-X"	3	3	2
Unidentified	3	6	4

[a] AH = after hydrolysis.

[b] Metabolites are those present in the "Free" AH fraction.

simultaneously on adjacent strips to check R_f values. Once the various metabolites had been separated, identification was accomplished. To aid in the identification several derivatives were made. Acetylation was performed using acetic anhydride–pyridine (1:2) for 15 hr. The presence of reducible ketone groups was done using potassium borohydride. For methylating phenolic groups, the method of Brown (9) was used. After these derivatives were made, the metabolites were rechromatographed in different systems with authentic standards. The samples were also recrystallized to constant specific activity to complete the identification.

Results. Estradiol-17β-4-^{14}C was rapidly and extensively metabolized by the newborn monkey. In addition to the conversion of estradiol to other estrogens, conjugation was quite extensive. This was indicated by the large fraction that became ether soluble after enzyme hydrolysis (Tables I and II). More than 80% of the radioactivity recovered in urine or bile was present in this fraction.

Several of the metabolites of estradiol-17β-4-^{14}C were isolated and identified. The percentage of distribution of these metabolites in urine and bile are summarized in Tables I and II, respectively. Quantitatively, estrone was the major metabolite; more than 50% of the radioactivity recovered being identified as this metabolite. Estriol, 16α-hydroxyestrone, and 16-ketoestradiol-17β were also identified and thus indicated that 16α-hydroxylation did occur. However, the amounts of these metabolites were small and identification was possible only after samples from all animals had been pooled. Estradiol-17α was also identified but it likewise was of minor importance. Two other metabolites were isolated but not identified; both being more polar than estradiol-17β. In a chloroform/formamide system, the most polar of the two (Pre-X) had an R_f of 0.20 to 0.24 in several runs; the second (X) had an R_f of 0.28 to 0.35. Metabolite Pre-X had a mobility similar to that of 17-epiestriol and 16-epiestriol. However, it does not appear to be either of these estrogens. Metabolite X likewise did not appear to be either of these estriol isomers; and the possibility that it was either 6-ketoestradiol-17β or 15α-hydroxyestrone was ruled out.

This radioactivity recovered from urine and bile accounted for 15–30% of the injected material. In addition, the liver contained an appreciable fraction (20–26%). Estrone was also the major metabolite identified in this tissue being approximately one-third of the radioactivity present.

TABLE II. Percentage Distribution of Metabolites of Estradiol-17β-4-^{14}C in Bile of Newborn Rhesus Monkeys.

	Male	Male	Female
"Free" fraction	2	2	3
"Free" AH[a]	92	93	80
Aqueous fraction	6	5	17
Metabolites[b]			
Estrone	58	60	46
Estradiol-17β	6	9	11
Estradiol-17α	—	<1	<1
16-Ketoestradiol-17β	3	2	1
16α-Hydroxyestrone	—	2	2
Estriol	1	1	1
"X"	17	11	13
"Pre-X"	5	4	4
Unidentified	2	4	2

[a] AH = after hydrolysis.

[b] Metabolites are those present in the "Free" AH fraction.

Discussion. Previous studies in the rhesus monkey have demonstrated that estrone, estradiol-17β, and estriol were excreted in increasing amounts during pregnancy (3–5). Generally, the concentration of estrone exceeds that of estriol and estradiol-17β combined. These investigations have shown that the pattern of estrogen excretion during pregnancy in the monkey differs markedly from that in man. In the latter, estriol is the major urinary estrogen excreted. The major reason for this difference may be the relative abilities of the fetus to 16-oxygenate steroids. In man it is recognized that the high excretion of estriol is dependent upon a viable fetus which can 16-oxygenate estrogens (7, 8) and neutral steroids (10). This ability to 16-oxygenate estrogens is quite extensive at the time of parturition as demonstrated in the newborn and in infants 2 to 3 months of age (11, 12). In the rhesus monkey, however, it appears that the fetus has only limited 16α-hydroxylase activity. The results of the present study readily demonstrate this as only a small fraction of the recovered metabolites were identified as 16-oxygenated estrogens. Similarly, it was recently demonstrated that the liver of the monkey fetus or newborn has a poor capacity to 16α-hydroxylate the androgen dehydroepiandrosterone (DHA) (13). Thus, these findings are consistent and could explain the small excretion of 16-oxygenated estrogens by pregnant rhesus monkeys. The marked ability of the monkey fetus to metabolize estradiol-17β to estrone could be a major reason for the rise in estrone excretion seen during pregnancy.

The identity of the two metabolites Pre-X and X remains obscure. One of these may be the same metabolite reported in previous studies which has a polarity similar to estradiol-17β (3, 5).

The pattern of estrogen metabolism seen in this study suggests that the monkey fetus, like the human, contributes to the type of estrogen metabolites excreted during pregnancy. As the fetus is exposed to estrogens, especially estradiol-17β, it can rapidly metabolize them to estrone and other estrogens that have little biological activity. In this way it could protect itself from the physiological actions of active hormones.

Summary. Estradiol-17β-4-^{14}C was rapidly and extensively metabolized by the newborn rhesus monkey. Estrone was the major metabolite identified in both urine and bile, accounting for more than 50% of the recovered radioactivity. The newborn monkey has the capacity, although limited, to 16-oxygenate estrogens. Estriol, 16α-hydroxyestrone, and 16-ketoestradiol-17β were identified; but these comprised only a small portion of the metabolites.

The authors thank Mrs. Renee Ellis for her technical assistance. This work was supported by U.S. Public Health Service Gr. AM-11201 and NIH Gr. FR-00163 to the Oregon Regional Primate Research Center. Publication No. 502 Oregon Regional Primate Research Center.

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Received Dec. 10, 1970. P.S.E.B.M., 1971, Vol. 137.