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ANDROGEN METABOLISM BY HEPATIC AND RENAL TISSUES OF THE FETAL RHESUS MONKEY

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Summary—Liver and kidney from fetal monkeys (day 125 of gestation) were fractionated into low speed pellets, microsomal and cytosolic fractions. Liver cytosols converted as much testosterone (T) to 5β-androstane-3α,17β-diol (5β-diol) at 0°C as at 4°–45°C without exogenous cofactors. The principal product formed from 5α-dihydrotestosterone (5α-DHT) was 5α-diol. A 1000-fold molar excess of radioinert 5β- or 5α-DHT inhibited 5β-diol formation from [^{3}H]T by cytosols and increased 5β-DHT formation. Similarly, using 5α-DHT as substrate, 5α-diol formation was inhibited. Microsomal and low speed pellets with added cofactors formed products which recrystallized with either etiocholanolone or androsterone from [^{3}H]T or [^{3}H]DHT, respectively. Little product was formed without cofactor.

Whole liver homogenates produced 5β-reduced products from [^{3}H]T in the presence of an NADPH generating system whereas kidney homogenates produced 5α-reduced products.

These data provide new information on the capacity of fetal monkey liver and kidney to metabolize androgens. The 3α-reductases are cytosolic. The 5α- and 5β-reductases are mostly in the low speed pellet but are sufficiently represented in cytosols to mediate diol formation. The 17-hydroxysteroid dehydrogenases are in the microsomal fraction. Our results suggest that 5α-DHT is the active androgen in fetal liver since testosterone is metabolized to 5β-DHT and 5β-diol which are inactive androgens.

INTRODUCTION

In nonhuman primates, as in other species, androgens secreted by the fetal testes exert irreversible, permanent effects on target tissues that render them sexually differentiated [1]. The liver of the fetal monkey contains high affinity androgen receptors [2]; therefore, we presume that the liver is a target organ for androgen action in fetal life similar to the brain and the reproductive tract. Although the capacity of the fetal monkey liver to metabolize androgens remains virtually untested, low amounts of 16α-hydroxylation of DHEA† have been reported in adult monkey liver [3].

Data obtained from rodents indicate that the liver plays a major role in androgen metabolism [4] and the pattern of metabolism may be imprinted during a "critical period" in the fetal and/or neonatal periods [4, 5]. In a previous publication [2], we demonstrated that T and DHT are metabolized differently by cytosols of fetal nonhuman primate liver. Specifically, T is metabolized to 5β-reduced compounds. This metabolism of androgen occurs at low temperatures and influences binding specificity studies of the androgen receptor. The present investigation extends these observations further by demonstrating effects of temperature, cell fractionation and cofactors on androgen metabolism by fetal monkey liver.

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†For the sake of brevity, abbreviations or trivial names have been used throughout this manuscript for the following steroid hormones: *Androsterone* (3α-Hydroxy-5α-androstan-17-one); *DHEA* (Dehydroepiandrosterone, 3β-Hydroxy-5-androsten-17-one); *5α-diol* (Dihydroandrosterone, 5α-Androstan-3α-17β-diol); *5α-DHT* (5α-Dihydrotestosterone, 17β-Hydroxy-5α-androstan-3-one); *5β-DHT* (5β-Dihydrotestosterone, 17β-Hydroxy-5β-androstan-3-one); *Etiocholanolone* (3α-Hydroxy-5β-androstan-17-one); *5β-diol* (Etiocholan-3α, 17β-diol, 5β-Androstan-3α-17β-diol); *P* (Progesterone, 4-Pregnen-3-20-dione); *T* (Testosterone, 17β-Hydroxy-4-androsten-3-one).

MATERIALS AND METHODS

Rhesus monkey (*Macaca mulatta*) fetuses were obtained from time-bred females housed with fertile males for 48 h beginning on day 10 following menstruation. The first day of menstruation was considered day 0. The day of gestation was computed from the time of fertilization which was estimated to occur 24 h after pairing of the animals. On days 125–127 of gestation (term being approximately 166 days in our colony) the pregnant females were anesthetized with ketamine hydrochloride (5 mg/kg body wt) and taken to surgery. Details of the surgical procedure, general anesthesia and post-operative care have been published previously [6]. The fetuses (4 males and 3 females) were delivered with the umbilical cord intact. A sample of blood was taken from the saphenous vein of the mother via an indwelling catheter and from the umbilical artery of the fetus using a 3 ml syringe attached to a 22 gauge needle that was bent at a 45° angle. The umbilical cord was tied, the fetus removed and quickly decapitated. Five of the fetuses (3 males and 2 females) were injected with 500 μg DHT 2 h before delivery for use in another research protocol. No apparent differences between treated and untreated animals were observed; therefore, the data were combined. Liver and kidney were placed in cold TEMGD buffer (10 mM Tris, 10 mM EDTA, 25 mM molybdate, 10% glycerol and 10 mM dithiothreitol, pH 7.4) for transportation to the laboratory.

Incubation experiments began approximately 2 h after removal of the tissues from the fetus. At this time tissues were weighed and homogenized (1:20; w:v) in TEMGD buffer for 10 s using a Polytron homogenizer (setting 6, Brinkman Instrument Co., Westbury, N.Y.). In some cases the tissues were fractionated into a low speed pellet (800 *g*), a purified cytosolic supernatant following a high speed centrifugation (107,000 *g*; L735 Ultracentrifuge, Beckman Instrument Co., Palo Alto, Calif.) and a pellet of this centrifugation (microsomes). These fractions were frozen in 1 ml aliquots at −85°C (Harris Low Temperature Freezer, Rheem Mfg Co., Asheville, N.C.) until incubations were performed.

In order to determine metabolism of androgens by whole tissue homogenate, liver and kidney (1 g) were obtained from three 125 day fetal rhesus monkeys and homogenized in 5 ml of 0.01 M phosphate buffer (10 mM KH_2PO_4, 10 mM K_2HPO_4, 100 mM KCl, 1 mM EDTA, pH 7.4). The homogenate was diluted further with buffer so that the final dilution was 1:20 (w:v) for liver and 1:10 for kidney. This homogenate was used immediately.

In the initial experiments 45.5 nCi of 1,2,6,7 [^{3}H]T; (specific activity of 99.1 Ci/mmol, NEN Research Products, Boston, Mass.) or 56.8 nCi of 1,2,4,6,7 [^{3}H]DHT; (sp. act. of 148.1 Ci/mmol), were dissolved in 50 μl TEMGD buffer. In experiments that tested the effects of cofactor and whole tissue homogenates on androgen metabolism, radioactive steroid was suspended in phosphate buffer supplemented with a NADPH generating system [1 mM $NADP^+$, 5 mM glucose-6-phosphate and 0.1 units glucose-6-phosphate dehydrogenase (Type XV, Sigma Chemical Co., St Louis, Mo.), pH 7.4]. To this, 100 μl of tissue homogenate (low speed pellet, cytosol or microsomal pellet), were added to make a final radioactive substrate concentration of 3.0 nM. Appropriate blanks were carried with each incubation to control for time of incubation, temperature and the addition of cofactors.

Incubations were terminated by addition of 500 μl of ice-cold distilled water and extracted twice with 7 ml of distilled ether. The ether extractions were combined and dried under a gentle stream of air. The residue was dissolved in 100 μl (×2) of hexane:benzene:methanol (85:15:5; v:v:v) and applied to a 14 × 0.92 cm (i.d.) glass column packed with 2.5 g Sephadex LH-20 (Sigma Chemical Co., St Louis, Mo.). Details of gel filtration chromatography have been published previously [7]. Samples were chromatographed in the above solvent system and the following fractions were collected and the mobility of steroid standards designated: 1(0–12 ml, progesterone), 2(13–17 ml, androsterone and etiocholanolone), 3(18–23 ml, 5α and 5β-DHT), 4(24–25 ml), 5(26–33.5 ml, T), 6(33.6–37.5 ml), 7(37.6–46.5 ml, 5α- and 5β-diols), 8(46.6–66.5 ml), 9(66.6–80.5 ml). Fractions were collected in 10 ml test tubes, dried under a stream of air, dissolved in 1 ml of ethanol from which a 100 μl aliquot was counted in a Packard Tricarb liquid scintillation spectrometer, Model 640, (United Technologies Packard, Downers Grove, Ill.). Radioactive products from fractions 2 and 3 were subjected to a second chromatography on thin-layer plates [Kodak Chromogram Sheet, 13181 silica gel (Eastman Kodak Co., Rochester, N.Y.)] in

the solvent system, benzene:ethyl acetate (6:4; v:v). Areas with the mobility of 5α- and 5β-DHT, androsterone or etiocholanolone were eluted from the thin-layer strip with 5 ml of ethanol. The product with the mobility of etiocholanolone was acetylated as reported previously [8] and chromatographed again on thin-layer plates as mentioned above. After chromatography, the steroids were subjected to reverse isotope dilution [8]. We have previously shown that metabolites eluting in fraction 7 from the gel filtration column are 5α-diol when 5α-DHT is the substrate and 5β-diol when T is the substrate [2].

The metabolism of [^{3}H]T and [^{3}H]5α-DHT was studied in cytosols in which 3 μM (1000-fold molar excess) of either radioinert 5α- or 5β-DHT was added to the incubation tubes. The samples were incubated for 1 h and chromatographed on Sephadex LH-20. Fractions 3 and 7 were rechromatographed on thin-layer in the solvent system, benzene:ethyl acetate (6:4; v:v). The product with the mobility of 5β-DHT was subjected to reverse isotope dilution.

Data were analyzed by one-way analysis of variance. *Post hoc* comparisons were performed by the method of Newman–Keuls [9].

RESULTS

Effect of incubation time and temperature

The effects of time of incubation on the metabolism of T and DHT by liver cytosols are shown in Fig. 1. Testosterone was converted to a metabolite with the mobility of 5β-DHT which reached maximum production of 1 h (Panel A). Both T and 5α-DHT produced significant amounts of 5β- and 5α-diol, respectively, at 37°C (Panels B and C). The quantities of 5α-diol produced from [^{3}H]5α-DHT declined rapidly throughout the incubation period indicating that 5α-diol was being metabolized further. In order to identify these metabolites, further studies were conducted using liver fractions incubated for 1 h.

Effects of temperature on the metabolism of T and 5α-DHT by cytosols obtained from fetal liver and kidney are shown in Fig. 2. Testosterone was converted by the liver to a metabolite with the mobility of 5β-diol (fraction 7) on Sephadex LH-20 columns (Fig. 2A). We have previously shown this metabolite to be 5β-diol [2]. Similar amounts (≈800 fmol·mg protein^{-1}) of 5β-diol (fraction 7) were produced by liver cytosols at all temperatures studied except

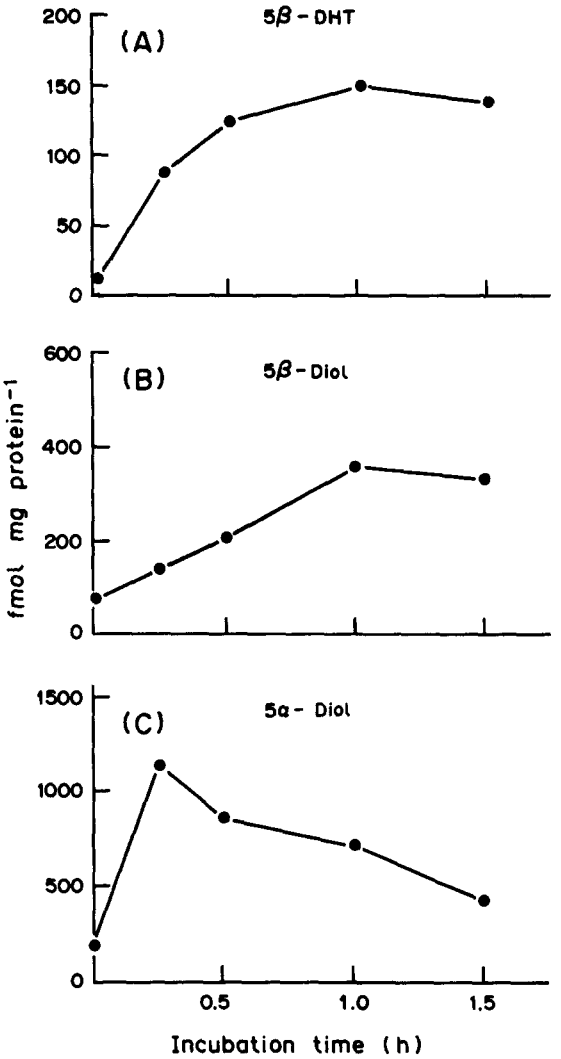

Fig. 1.(A,B) Effects of time of incubation at 37°C on the metabolism of [^{3}H]T and (C) [^{3}H] 5α-DHT by cytosols of fetal monkey liver *in vitro*. Metabolites were separated on Sephadex LH-20 columns. Evidence for 5α- and 5β-diol production was obtained by recrystallization the radiolabeled metabolites with known crystalline androgens [2]. See Methods for complete details.

at 45°C at which temperature small amounts (≈200 fmol·mg protein^{-1}) were produced (Fig. 2A). Kidney cytosols did not metabolize T at any temperature tested. The data presented above and elsewhere in the text were computed as activities per mg protein whereas the same data in the figures are presented as fmol produced per h.

[^{3}H]5α-dihydrotestosterone, however, was converted to a metabolite with the mobility of 5α-diol (fraction 7) on Sephadex LH-20 columns (Fig. 2B) and recrystallized to constant specific activity with radioinert 5α-diol [2]. Incubation temperature did not seem to affect 5α-diol production by liver cytosols in these

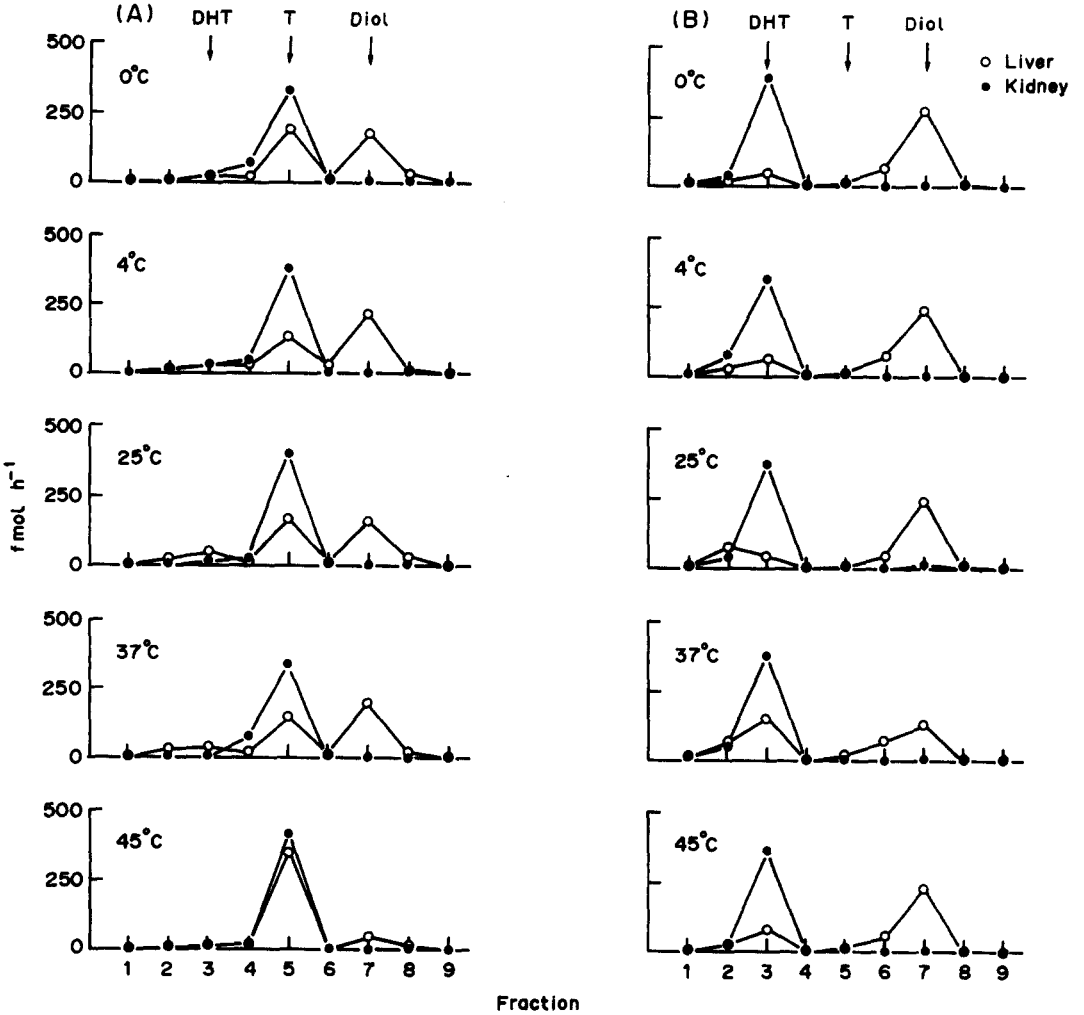

Fig. 2. (A) Effects of temperature on metabolism of [^{3}H]T and (B) [^{3}H]5α-DHT by cytosols (106,000 g supernatant) of female fetal monkey liver (0.229 mg protein per tube) and kidney (0.419 mg protein per tube) at 125 days of gestation. Incubations were performed in TEMGD buffer for 1 h. See Methods for complete details.

experiments. Kidney cytosols did not appear to metabolize 5α-DHT after 1 h, whereas 5α-diol formation was observed in a previous study with a longer incubation time [2]. The above data were replicated on three different occasions.

Effects of cell fractionation and cofactors

We compared the metabolism of T and 5α-DHT by different cell fractions with and without cofactors (Fig. 3). Elution profiles of metabolites produced by previously frozen cell fractions of fetal monkey liver, incubated with [^{3}H]T at 37°C and separated on Sephadex LH-20, are shown in Fig. 3A. Four groups were compared: blanks containing phosphate buffer and a NADPH generating system, liver cytosols from tissue homogenized in phosphate buffer and a NADPH generating system added, liver cytosols with phosphate buffer but without a NADPH generating system and cytosols incubated in TEMGD buffer alone. No metabolism of substrate was observed by the blanks in any of the cell fractions studied (data not shown). As seen in previous experiments, using TEMGD buffer, cytosols converted T to a metabolite with the mobility of 5β-diol. Low speed pellets and microsomes showed little activity with this buffer. The addition of a NADPH generating system to the incubation mixture stimulated the production of radioactive compounds that eluted from the Sephadex columns with either 5α- or 5β-DHT (fraction 3) in all three preparations. Greater amounts of this material (792 fmol·mg protein^{-1}) were produced by the low speed pellet than by either cytosols (214 fmol·mg protein^{-1}) or microsomes (142 fmol·mg protein^{-1}; Fig. 3A). The radioactivity from all three tissue fractions that eluted

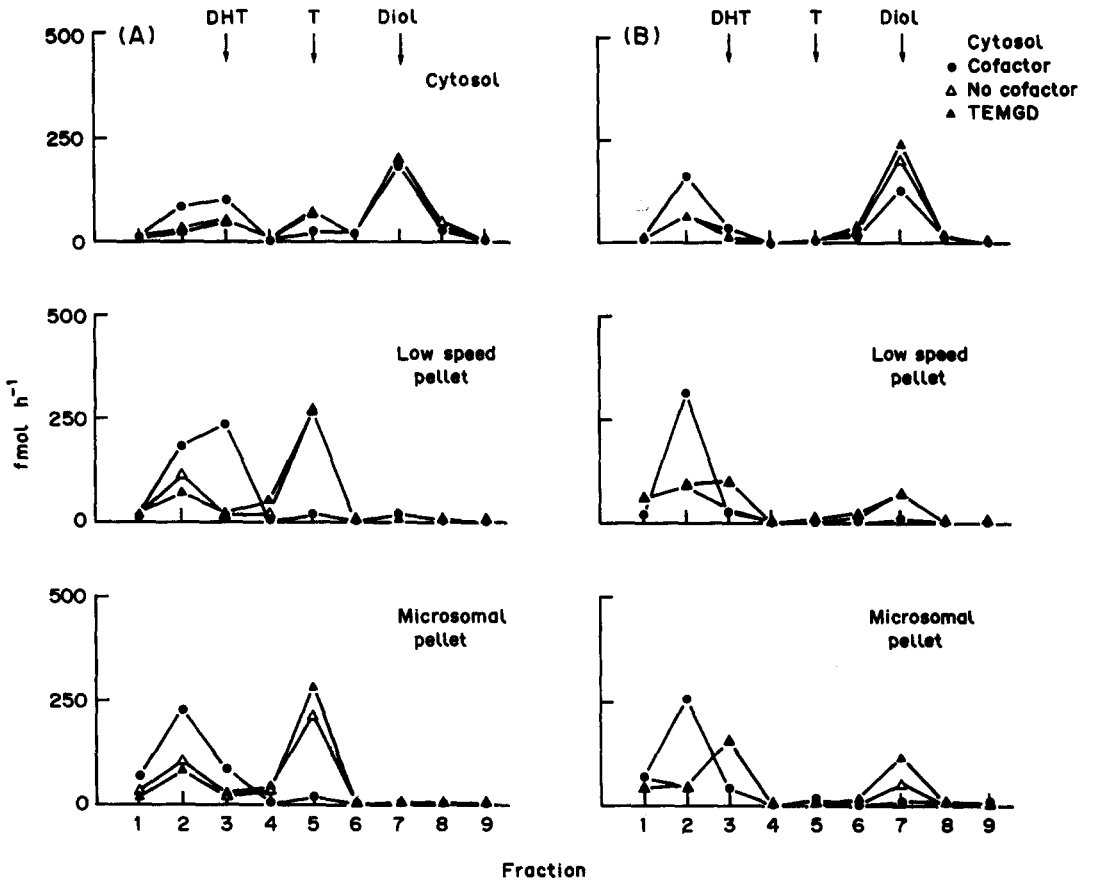

Fig. 3. (A) Metabolism of [^{3}H]T and (B) [^{3}H] 5α-DHT by various cell fractions of fetal monkey liver (female) at 125 days of gestation. Experiments were performed at 37°C for 1 h in TEMGD buffer. Incubation tubes for cytosols, low speed and microsomal pellets contained 0.475, 0.297 and 0.579 mg of protein, respectively. Recrystallization data for Fraction 2 in panels (A) and (B) are presented in Table 1. See Methods for complete details.

in fraction 3 from the Sephadex LH-20 column was chromatographed on thin-layer. The greatest amount of radioactivity was obtained from incubations of the low speed pellet. In this fraction (49.6%) eluted with 5β-DHT and only 6.5% with 5α-DHT. The material with the mobility of 5α- or 5β-DHT was acetylated, rechromatographed on thin-layer and subjected to reverse isotope dilution with authentic 5β-DHT acetate. This material did not recrystallize to constant specific activity from any of the fractions tested; therefore, appeared to be a different metabolite than 5β- or 5α-DHT. In addition to the above, adding a NADPH generating system to the incubation mixture resulted in an increase in metabolites that eluted in fraction 2 from the gel filtration column especially in the low speed pellet and the microsomes. These metabolites had the mobility of etiocholanolone and androsterone on thin-layer chromatography before and after acetylation and recrystallized to constant specific activity (Table 1). In the low speed pellet both etiocholanolone and androsterone were identified as products of T metabolism. In microsomes only etiocholanolone was found in significant amounts (Table 1).

In Fig. 3B are shown the profiles of metabolites of 5α-DHT produced by various cell fractions with and without the addition of a NADPH generating system. The addition of NADPH resulted in an increase in the formation of a radioactive metabolite which eluted in fraction 2 from the column. This occurred to the greatest extent in the low speed pellet (1051 fmol·mg protein^{-1}·h^{-1} vs 340 and 376 fmol·mg protein^{-1}·h^{-1} in cytosols and microsomes, respectively). When this metabolite was submitted to reverse isotope dilution, it recrystallized with androsterone (Table 1). The addition of NADPH did not seem to affect the formation of 5α-diol (Fig. 3B, fraction 7).

Table 1. Metabolites of [^{3}H]testosterone (T) and [^{3}H] 5α-dihydrotestosterone formed *in vitro* by fractions of fetal monkey liver[a] and identified by reverse isotope dilution

Steroids	Substrate[b]	Fraction[c]	Specific activity (dpm/mg) 0[d]	1	2	3	4
5β-dihydrotestosterone (cytosol, Fig. 4, 5β-DHT trap)	T	CX	590	500*[e]	460**	440***	530*
		ML		—	650	470	580
Etiocholanolone (low speed pellet, Fig. 3, Fraction 2, Panel A)	T	CX	710	660*	580*	690*	—
		ML		1310	760	660	—
Androsterone (low speed pellet, Fig. 3, Fraction 2, Panel A)	T	CX	1850	1630†	1720††	1730*	—
		ML		1970	—	—	—
Etiocholanolone (microsomes, Fig. 3, Fraction 2, Panel A)	T	CX	290	260*	300*	270*	—
		ML		501	310	260	—
Androsterone (microsomes, Fig. 3, Fraction 2, Panel B)	DHT	CX	1880	1770*	1700**	1630***	—
		ML		2460	2650	1520	—

[a]Hepatic tissue from 125 day monkey fetuses were fractionated as described in the Methods Section to obtain a cytosolic fraction, a low speed pellet and a microsomal fraction.
[b]The various fractions were incubated at 37°C for 1 h with trace amounts of [^{3}H]T or [^{3}H]5α-DHT designated above as either T or DHT substrate. See Methods Section for methods used in isolation of metabolites and derivative formation. 10 or 20 mg of authentic steroid was used for recrystallization.
[c]Crystals (CX); Mother liquors (ML).
[d]Numbers 0–4 represent crystallization number with 0 representing the initial specific activity.
[e]Specific activity superscripts indicate solvents used for recrystallization: *hexane, **hexane/acetone, ***hexane/benzene, †ethanol, ††ethyl acetate.

Effects of radioinert androgens

In Fig. 4 we tested the effects of the addition of radioinert 5α- and 5β-DHT to the incubation medium on the metabolism of [^{3}H]T by liver cytosols. These additions produced a significant inhibition of radiolabeled metabolites with the mobility of the diols on the gel filtration column (labeled 5β- or 5α-DHT trap in Fig. 4) compared to control values ($P < 0.05$). At the same time significantly greater amounts of a radiolabeled metabolite with the mobility of DHT was formed ($P < 0.05$). When the latter area was rechromatographed on thin-layer (Fig. 4, Inset), the major part of the radioactivity traveled with the 5β-DHT standard and was significantly elevated over control values ($P < 0.05$). This material was eluted from the thin-layer strips, submitted to reverse isotope dilution and recrystallized to constant specific activity (Table 1). These results indicate that 5β-DHT is the intermediate in the formation of 5β-diol from T in liver cytosols. Similar results were obtained when 5α-DHT was used as the substrate (data not shown). The addition of unlabeled 5α- and 5β-DHT inhibited 5α-diol formation. These data indicate that the 3α-reductase can use either 5α- or 5β-DHT as a substrate for diol production and that sufficient 5β-reductase is present in the cytosol to provide intermediate for diol formation.

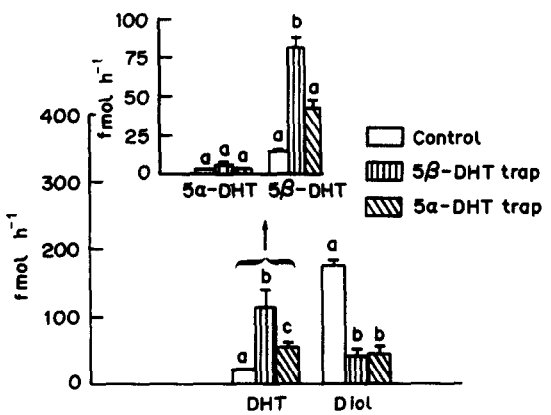

Fig. 4. Effects of the addition of radioinert 5α- and 5β-DHT (3 μM, 1000-fold molar excess) to the incubation mixture on DHT and diol formation (abscissa on lower graph) by fetal monkey liver cytosols (0.415 mg protein per tube). Metabolites of [^{3}H]T were chromatographed on Sephadex LH-20 (lower graph) and eluted from the column in fraction 3 (DHT mobility) or fraction 7 (5α- or 5β-diol). The DHT fraction was chromatographed further on thin-layer chromatography and the areas with the mobility of 5α- and 5β-DHT quantified (inset). Recrystallization data for 5β-DHT trap (inset) after acetylation is presented in Table 1. See Methods section for other details.

Metabolism by whole tissue homogenate

In order to determine the pathways for the metabolism of T and DHT in unfractionated tissues, we incubated whole tissue homogenates from three 125 day fetuses (2 males and 1 female) with [^{3}H]T or [^{3}H]5α-DHT for 15 min at 37°C with or without a NADPH generating system. The results are summarized in Fig. 5. When [^{3}H]T was used as a substrate (Panel A), small amounts of 5α- and 5β-DHT were formed by homogenates without cofactors. Little or no 5α- or 5β-diol was produced by these same

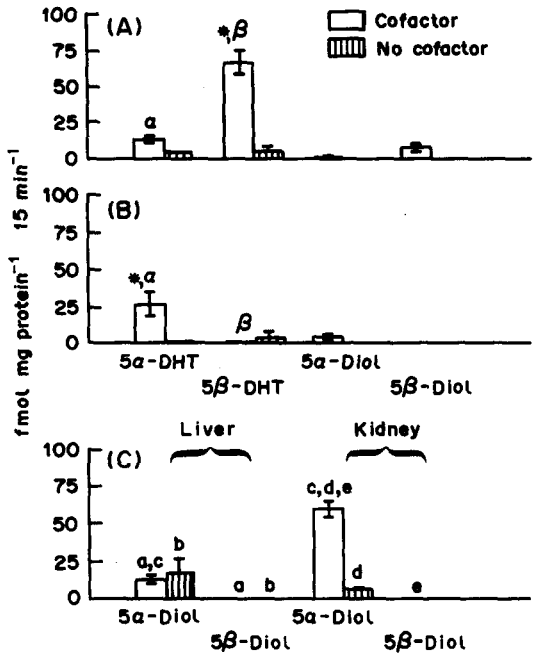

Fig. 5. (A) Metabolism of [^{3}H]T by liver and (B) kidney, and (C) [^{3}H]5α-DHT by liver and kidney homogenates of fetal rhesus monkeys on day 125 of gestation. Data are presented as means (bars, $n = 3$) ± SEM (vertical lines). Asterisks (*) indicate means that differ significantly from all other means within panels A or B ($P < 0.05$). Bars marked with the same Greek letters indicate significant differences between panels A and B ($P < 0.05$). Bars marked with the same letter in panel C differ significantly ($P < 0.05$). Data were analyzed by a two-way analysis of variance followed by a Newman–Keuls test.

incubations. The addition of an NADPH generating system to the incubation mixture did not significantly affect the production of 5α-DHT but stimulated a 5-fold increase in 5β-DHT production ($P < 0.05$) in the incubation tube. In these incubations only small amounts of 5α- and 5β-diol were found. The metabolism of [^{3}H]T by kidney homogenates is presented in Panel B. The kidney, unlike the liver, did not produce large amounts of 5β-DHT with or without cofactors. Furthermore, the amount of 5α-DHT produced by the kidney was significantly greater than the amount produced by the liver.

When [^{3}H]5α-DHT was used as a substrate (Fig. 5C), significantly greater amounts of 5α-diol ($P < 0.05$) were found in the kidney compared to the liver in incubations supplemented with NADPH. The addition of cofactors had no effect on 5α-diol formation in the liver.

DISCUSSION

In rodents the liver and kidney contain enzymes that metabolize androgens by reducing the A ring to 5α- [10] and 5β- [11–13] reduced forms and by forming derivatives hydroxylated at the 3, 17, 16 and other positions on the steroid molecule [4, 14]. In addition, the mammalian liver conjugates androgen to glucuronide in different proportions depending on the structure of the metabolite [15]. The result of these conversions is the production of androgens having reduced or no biological activity. The androgen metabolizing system in the rat liver is imprinted during the neonatal period by the action of T [4, 5, 11, 14, 16, 17]. Microsomal enzymes such as 17α-hydroxy and 3β-hydroxysteroid reductases as well as cytosolic enzymes such as 5β-reductase are induced by androgen imprinting [4]. The 5α-reductases, on the other hand, are suppressed by androgen treatment during the neonatal period [4, 11, 18].

Little information is available describing the capacity of the primate liver to metabolize androgens. Livers from adult male and female rhesus monkeys do not differ in their capacity to metabolize DHEA to derivatives substituted at the C^7 and the C^{16} positions on the steroid molecule [18]. Using the profiles of urinary excretion as an endpoint, sex differences in the ratio of etiocholanolone (a 5β-reduced product) to androsterone (a 5α-reduced product) excreted in human urine have been found [19]. Normal males excrete these two compounds in a ratio of 1:2, whereas this ratio changes to 2:1 in females. In the rhesus monkey, samples of liver cytosols obtained from late gestation fetuses converted greater amounts of T to 5β-diol than to the 5α-derivative. These data agree with urinary studies in women mentioned above [19]. Our data suggest that the liver of male and female monkey fetuses do not differ in their capacity to metabolize androgen in late gestation, however, we have not studied a sufficient number of animals to draw a firm conclusion.

In contrast to liver, kidney cytosols did not metabolize T or 5α-DHT after being incubated for 1 h. After 20 h, however, kidney cytosols converted 5α-DHT to 5α-diol but were unable to metabolize T [2]. Furthermore, kidney homogenates metabolized T to 5α-reduced products *in vitro*, whereas liver produced 5β-reduced metabolites.

A surprising observation in our study was the fact that production of 5α- and 5β-diol by liver cytosols was not temperature sensitive since equal or greater amounts were produced at 0°C compared to other temperatures e.g. 37°C. In addition, greater quantities of diol were formed

in the cytosols compared to the low speed pellet in which only small amounts were formed and in the microsomes which produced little or no product. Thus, the 5β-reductases and the 3α-reductases were concentrated in the cytosols of the fetal monkey liver. One could speculate about the significance of possessing an enzyme system that functions as well at 0°C (on ice) as at 37°C, the presumed normal temperature at which mammalian cells operate. Perhaps, this capacity offers no immediate advantage to the nonhuman primate fetus but represents, instead, a vestigal remnant of liver function.

These data take on added significance because the metabolism of T and DHT takes place under the exact conditions in which the cytosolic androgen receptor is quantified. In tissues such as the liver, radiolabeled ligands used to estimate receptor numbers may be metabolized to inactive products that do not bind to the AR. This point is clearly demonstrated by the data which show that T is converted to 5β-diol that does not bind to the AR, whereas 5α-diol, the metabolic product of 5α-DHT, does bind [2]. Our data from the fetal rhesus monkey are similar but not the same as data obtained from the adult rat [20]. In the latter, T is an effective competitor for the radioactive ligand but 5α-DHT is only partially active because DHT is rapidly metabolized to 5α-diol at 4°C [20]. This latter point was clearly demonstrated when it was shown that 5α-DHT displaces a significant amount of the radioactive ligand bound to the receptor when the binding protein is separated from the metabolic enzymes on heparin: Sepharose columns [20].

The significance of these metabolic conversions in fetal monkey liver for understanding the biological effectiveness of T and 5α-DHT on liver function or, perhaps, on sexual differentiation of the liver is difficult to access at this time. Interpretation of the cytosolic and the whole tissue homogenate data, at face value, would indicate that 5α-DHT but not T, supplied to the liver by the systemic circulation, can exert androgenic actions on the fetal liver. Thus, the metabolism of T to an inactive metabolite is important for hormone action. This observation would not be the first example of specificity of steroid hormone action depending on steroid metabolism by target tissues. Binding of aldosterone to Type I receptors in target organs such as the kidney depends upon metabolism of cortisol, which competes with aldosterone for binding to the Type I receptor whereas its metabolite, cortisone, does not [21]. The hypothesis that metabolism of androgen in the fetal liver is important for hormone action must be qualified by the knowledge that metabolic conversions of androgens can take place in other subcellular fractions in the liver. The potential effectiveness of T as an androgen in the liver would depend on the amount of T that is converted to 5α-DHT. We addressed this issue by testing the capacity of whole tissue homogenates to metabolize T *in vitro*. Our data suggest that T is preferentially converted to 5β-DHT by the liver provided that NADPH is not limiting.

The fact that T and 5α-DHT can be converted by cytosols to 5β- and 5α-diol, respectively, in significant quantities at 0°C indicates that endogenous quantities of NADPH are present in sufficient amounts to provide a hydrogen donor for the 5β- and the 3α-reductases to produce their effects. This possibility is confirmed by the fact that no significant change in the amount of diol occurred in the presence of an exogenous NADPH generating system. In addition, it is well-known that in the liver NADPH is generated via the phosphogluconate pathway which is located in the cytosol [22]. Thus, exogenous NADPH is necessary for metabolism of androgen to occur in the microsomal and low speed pellets.

These data indicate that fetal monkey liver can metabolize T and 5α-DHT even at 0°C. These two androgens are metabolized differently by liver cytosols. The principal product of T being 5β-diol, an inactive androgen, whereas 5α-DHT is converted to 5α-diol which can be androgenic.

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