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THE SITE OF 1α,25-DIHYDROXYVITAMIN D_3 PRODUCTION IN PREGNANCY

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Summary: Metabolism of 25-hydroxyvitamin D_3 (25-OH-D_3) in pregnancy was investigated in vitro in New Zealand White rabbits fed a rabbit chow. Kidney homogenates from pregnant mothers and fetuses were separately incubated with [^{3}H]-25-OH-D_3. The homogenates from fetuses produced significant amounts of [^{3}H]-1α,25-dihydroxyvitamin D_3 [1α,25-$(OH)_2$-D_3] from its precursor, while those from mothers predominantly produced [^{3}H]-24,25-dihydroxyvitamin D_3 [24,25-$(OH)_2$-D_3]. The identity of the radioactive metabolites produced from [^{3}H]-25-OH-D_3 was established by periodate cleavage and comigration with synthetic 1α,25-$(OH)_2$-D_3 or 24,25-$(OH)_2$-D_3 on high pressure liquid chromatography. These results clearly indicate that the fetal kidney is at least one of the sites of 1α,25-$(OH)_2$-D_3 synthesis in pregnancy.

INTRODUCTION

The most biologically active metabolite of vitamin D_3, 1α,25-dihydroxyvitamin D_3 [1α,25-$(OH)_2$-D_3], has been thought to be synthesized exclusively in the kidney (1). The concept was derived from the experimental results that nephrectomy completely abolishes the formation of [^{3}H]-1α,25-$(OH)_2$-D_3 from [^{3}H]-25-hydroxyvitamin D_3 (25-OH-D_3)(1-3), and that the circulating levels of 1α,25-$(OH)_2$-D are not detectable in anephric animals or patients (4,5).

Very recently, Weisman et al (6) and Kenney Gray et al (7) independently demonstrated that nephrectomy of pregnant, vitamin D-deficient rats reduced but did not abolish the in vivo conversion of [^{3}H]-25-OH-D_3 to [^{3}H]-1α,25-$(OH)_2$-D_3. The former group (6) reported that the feto-placental unit is the most likely site of 1α,25-$(OH)_2$-D_3 production in the anephric pregnant ani-

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Abbreviations used: 25-OH-D_3, 25-hydroxyvitamin D_3; 1α,25-$(OH)_2$-D_3, 1α,25-dihydroxyvitamin D_3; 24,25-$(OH)_2$-D_3, 24,25-dihydroxyvitamin D_3.

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mals. The latter group (7) could not find any detectable amounts of [^{3}H]-1α,25-(OH)$_2$-D$_3$ in the fetal kidneys after administration of [^{3}H]-25-OH-D$_3$ to pregnant, vitamin D-deficient animals, so they concluded that the site of 1α-hydroxylation after nephrectomy of pregnant rats was extra-renal, probably maternal and/or fetoplacental, in origin. We now report that fetal kidney homogenates from rabbits fed a laboratory chow produce in vitro 1α,25-(OH)$_2$-D$_3$ from its precursor. These results indicate that the fetal kidney is at least one of the sites of 1α,25-(OH)$_2$-D$_3$ synthesis in pregnant animals.

MATERIALS AND METHODS

Animals: Pregnant rabbits (New Zealand White strain) were obtained from a local distributor. They were fed a rabbit chow (Oriental Co. Ltd., Tokyo) containing 0.85% calcium, 0.66% phosphorus, and 1.0 U vitamin D$_3$/g diet ad libitum throughout the pregnancy. From the 26th day of gestation to the birth, kidneys were removed from pregnant mothers and fetuses under anesthesia with pentobarbital (25 mg/kg). The kidneys were rinsed and homogenized in 4 volumes of 0.25 M sucrose containing 15 mM Tris-HCl (pH 7.4), 2 mM $MgCl_2$, and 5 mM sodium succinate.

Incubation and Chromatography: The homogenates (1.5 ml) from individual mothers and from 2-4 fetuses (pooled) were separately incubated for 30 min at 37°C with 3.8 nmol of [26,27-^{3}H]-25-OH-D$_3$ (Radiochemical Centre, Amersham), as described previously (8,9). Extraction and chromatography of the extracts (Sephadex LH-20 and high pressure liquid chromatography, HPLC, Waters Model 204 equipped with a Zorbax-Sil column) were also performed, as previously described (8,9).

Identification of the metabolites of 25-OH-D$_3$: The metabolites of [^{3}H]-25-OH-D$_3$ was identified by co-chromatography with authentic 1α,25-(OH)$_2$-D$_3$ or 24R,25-dihydroxyvitamin D$_3$ [24R,25-(OH)$_2$-D$_3$] (gifts from Dr. M. R. Uskokovič, Hoffmann-LaRoche Inc., New Jersey). The metabolites of [^{3}H]-25-OH-D$_3$ were also identified by periodate cleavage. The radioactive metabolites on HPLC were dissolved in 2 ml of methanol and treated with 1 ml of 5% $NaIO_4$ at room temperature overnight. The reaction mixture was extracted with chloroform, and the raioactivities of the extracts were determined.

RESULTS

Figure 1 illustrates Sephadex LH-20 chromatographic profiles of extracts of kidney homogenates prepared from a maternal rabbit (on the 28th day of gestation) (A) and her fetuses (B). The homogenates from the mother metabolized in vitro [^{3}H]-25-OH-D$_3$ primarily to a polar peak which appeared in the fractions numbered 18 to 29. The fetal kidneys, on the other hand, in vitro converted [^{3}H]-25-OH-D$_3$ exclusively to a more polar peak which ap-

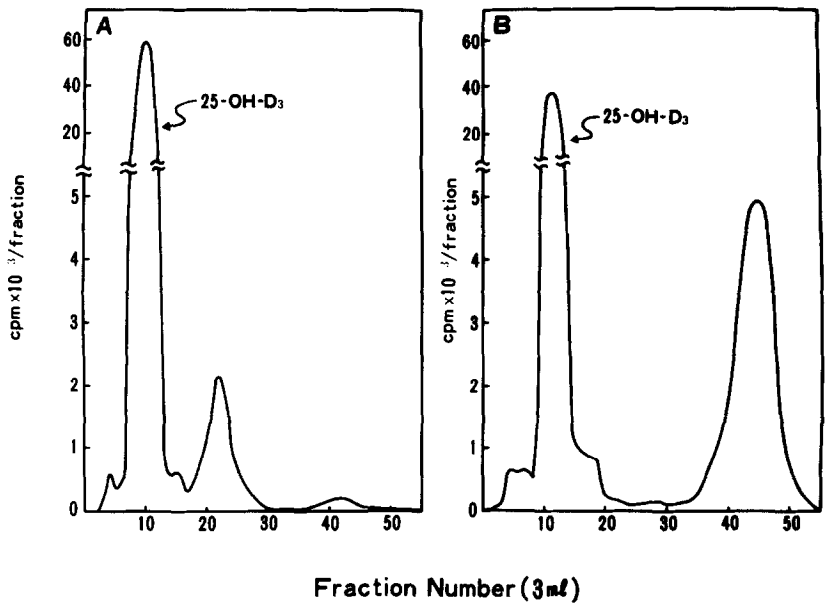

Fig 1. Sephadex LH-20 chromatographic profiles of extracts of kidney homogenates from a pregnant rabbit (on the 28th day of gestation) (A) and her fetuses (B). Kidney homogenates prepared from 3 fetuses were incubated, extracted, and applied to Sephadex LH-20 column. The columns were eluted with a solvent of 65% chloroform - 35% n-hexane. Three ml fractions were collected.

peared in the fractions numbered 35 to 54 on the Sephadex column. No radioactivity appeared in the fractions (tubes 18-29) in the fetuses.

When the polar peak synthesized by the maternal kidney was applied to HPLC column, it was separated into 3 peaks: two unknown peaks referred to as peaks X and Y, and a peak which comigrated to exactly the same position as authentic 24R,25-$(OH)_2$-D_3 (Fig. 2A). When the radioactive fraction which coincided with authentic 24R,25-$(OH)_2$-D_3 on HPLC was treated with 5% $NaIO_4$ overnight, the chloroform extracts lost radioactivity almost completely (Table 1). The maternal kidney produced *in vitro* only small amounts of the metabolite suspected to be 1α,25-$(OH)_2$-D_3 (Fig. 1A).

When the radioactive fraction (tubes 35-54) on the Sephadex column produced by the fetal kidneys was applied to the HPLC column, as much as 89% of the radioactivity comigrated to exactly the same position as authentic 1α,25-$(OH)_2$-D_3 (Fig. 2B). The radioactivity suspected to be 1α,25-$(OH)_2$-D_3 was completely insensitive to the periodate cleavage (Table 1).

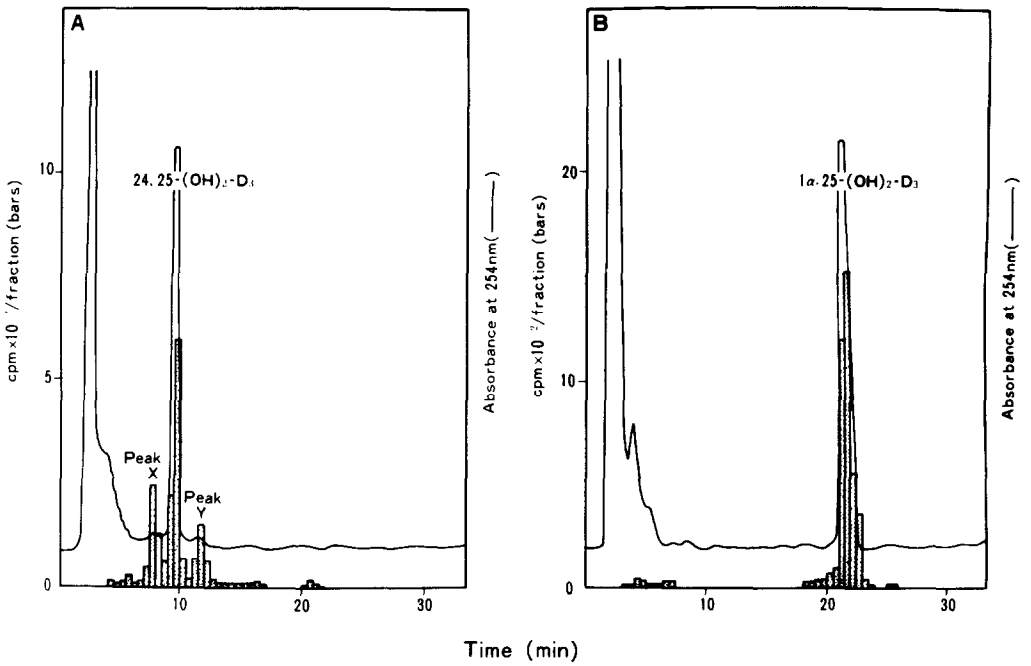

Fig 2. HPLC profiles of the radioactive peaks shown on Fig 1. Panel A and B represent HPLC profiles of the radioactive peak (tubes 18-29) on Fig 1A and that (tubes 35-54) on Fig 1B, respectively. Before applying to HPLC columns, 500 pmol of authentic 24R,25-$(OH)_2$-D_3 (in panel A) or 1α,25-$(OH)_2$-D_3 (in panel B) were added to each sample. The columns were eluted with a solvent of 10% isopropanol in n-hexane (13). The solid line indicates absorbance at 254 nm, and the dotted bar represents radioactivity in each 30 sec fraction.

Table 1 The sensitivity to periodate oxidation of the 24,25-$(OH)_2$-D_3 and the 1α,25-$(OH)_2$-D_3 fractions after Sephadex LH-20 column chromatography and/or HPLC.

	Before $NaIO_4$ treatment	After $NaIO_4$ treatment	Recovery
24,25-$(OH)_2$-D_3 Fraction	dpm	dpm	%
Sephadex LH-20	2391	1086	45
LH-20 + HPLC	2104	65	3
1α,25-$(OH)_2$-D_3 Fraction			
Sephadex LH-20	4636	4676	101
LH-20 + HPLC	2025	2038	100

Table 2 Metabolites of 25-OH-D_3 produced by kidney homogenates from maternal and fetal rabbits.

Pregnant Rabbits (Days after gestation)	(Number of fetuses)	24,25-$(OH)_2$-D_3 Fractions				1α,25-$(OH)_2$-D_3 Fractions	
		Sephadex LH-20	Sephadex LH-20 + HPLC			Sephadex LH-20	LH-20 + HPLC
			Peak X	24,25-$(OH)_2$-D_3	Peak Y		
day 1 (31)	Mother	7.6 %	29.2 %	55.7 %	7.2 %	0.6 %	— %
	Fetus (8)	trace	—	—	—	7.5 ± 2.3	82.1 ± 8.6
2 (28)	Mother	3.2	25.0	56.5	5.7	0.8	
	Fetus (10)	trace	—	—	—	21.4 ± 7.0	89.4 ± 5.0
3 (26)	Mother	3.0	27.7	42.0	8.5	1.1	—
	Fetus (8)	trace	—	—	—	14.0 ± 1.4	88.0 ± 6.2
4 (28)	Mother	1.4	—	—	—	2.2	—
	Fetus (9)	trace	—	—	—	10.0 ± 1.2	86.3 ± 4.3

Each kidney homogenates prepared from 2-4 fetuses were separately incubated, extracted, and applied to Sephadex LH-20 and HPLC. Data on fetuses are mean ± SE of 3 incubations.
Data on the 24,25-$(OH)_2$-D_3 and the 1α,25-$(OH)_2$-D_3 fractions from Sephadex LH-20 columns are expressed as percentage of the radioactivities recovered in each 1-55 fractions. The 24,25-$(OH)_2$-D_3 and the 1α,25-$(OH)_2$-D_3 fractions were separately applied to HPLC columns. Figures after Sephadex LH-20 + HPLC are expressed as percentage of the sum of the radioactivity recovered in each 1-70 fractions.

Table 2 shows the percentage distribution of the metabolites of [^{3}H]-25-OH-D_3 produced *in vitro* by maternal and fetal kidneys from pregnant rabbits on the 26th - 31st day of gestation. The 24,25-$(OH)_2$-D_3 fraction from the Sephadex columns produced by the maternal kidneys was separated into 3 peaks on HPLC, and the percentage distribution was 25-30% in peak X, 42-57% in 24,25-$(OH)_2$-D_3, and 5-8% in peak Y. Peaks X and Y resembled chromatographically peaks C and E (the metabolites of 24,25-$(OH)_2$-D_3 produced by chick kidney homogenates) recently reported by Takasaki *et al.* (9). The 1α,25-$(OH)_2$-D_3 fraction from the Sephadex columns produced by the fetal kidneys was considered to be mainly 1α,25-$(OH)_2$-D_3, since 82-89% of the radioactivity was eluted in the position to which authentic 1α,25-$(OH)_2$-D_3 comigrated on HPLC. It was characteristic without any exception that all of the fetal kidneys *in vitro* produced only 1α,25-$(OH)_2$-D_3 from its precursor.

DISCUSSION

Recently, attention has been focused on the vitamin D metabolism in pregnancy. Weisman *et al.*(6,10) and Kenney Gray *et al.*(7) independently reported that nephrectomy of pregnant, vitamin D-deficient rats reduced but did not abolish the *in vivo* conversion of 25-OH-D_3 to 1α,25-$(OH)_2$-D_3. Weisman *et al.* (10) reported that homogenates of fetal rat kidneys produced 1α,25-$(OH)_2$-D_3 from 25-OH-D_3 *in vitro*, though its percentage conversion was only 1%, whereas the placenta was unable to perform this transformation *in vitro*. The rat is not a suitable animal to measure *in vitro* renal 1α-hydroxylase activity, because of the presence of the 1α-hydroxylase inhibitor (11). Kenney Gray *et al.*(7) could not find any detectable amounts of [^{3}H]-1α,25-$(OH)_2$-D_3 in the fetal kidneys after administration of [^{3}H]-25-OH-D_3 to pregnant vitamin D-deficient animals. They suggested two sites of 1α-hydroxylation of 25-OH-D_3, one renal and the other extra-renal, either maternal or fetoplacental, in the pregnant, vitamin D-deficient rats.

The present study clearly demonstrates that the fetal kidney is at least one of the sites of 1α,25-$(OH)_2$-D_3 synthesis in pregnant animals. Fetal

kidneys from normal rabbits fed a laboratory chow can produce in vitro significant amounts of 1α,25-$(OH)_2$-D_3 from its precursor. The reason kidney homogenates from rabbit fetuses perform this transformation in vitro even in a vitamin D-supplemented state is not known. It is, however, obvious that the fetal rabbit kidney is useful in measuring in vitro renal 1α-hydroxylase activity in mammals and in studying unique 25-OH-D_3 metabolism in pregnancy.

It is of great interest that the maternal rabbit kidney synthesizes primarily 24,25-$(OH)_2$-D_3, while the fetal kidney produces only 1α,25-$(OH)_2$-D_3 from its precursor. Lester et al.(12) suggested the possibility of independent control of 25-OH-D_3 metabolism in the fetus. Our results confirm their suggestion. According to the calculations of in vivo metabolism by Kenney Gray et al.(7), plasma levels of 1α,25-$(OH)_2$-D_3 are more than 4 times higher than the fetal plasma levels of the metabolite even after nephrectomy. Some portions of the 1α,25-$(OH)_2$-D_3 appearing in maternal plasma after nephrectomy, therefore, may be derived from the fetal kidney. The independent control of 25-OH-D_3 metabolism in the rabbit fetus is of considerable interest, and is currently under investigation in our laboratories.

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