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Evidence for Maternal and Fetal Differences in Vitamin D Metabolism (40337)

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Pregnancy induces striking changes in mineral homeostasis including the translocation of calcium and phosphorus from the mother to the fetus and elevations in the maternal blood of parathyroid hormone (1) and 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) (2). These hormonal changes enhance the intestinal absorption of calcium and phosphorus in the mother and the net movement of these two ions from the maternal bone mineral to the blood (3). The effects of these physiological alterations on the feto-placental unit are unknown at present. Furthermore, our knowledge of the relationship between mother and fetus regarding the metabolism of vitamin D and the potential interdependence in terms of the regulation of these metabolic processes is fragmentary. During the third trimester of pregnancy, maternal blood levels of 25-hydroxyvitamin D_3 ($25OHD_3$) have been shown to decrease (4). A maternal to fetal gradient for the blood levels of $25OHD_3$, the D-metabolite produced by the liver, has been described (5). Metabolites more polar than $25OHD_3$ were identified in fetal rat homogenates after the administration of [^{3}H]$25OHD_3$ to pregnant rats (6). In a study of similar design, differences in the maternal and fetal distribution of metabolites more polar than $25OHD_3$ were observed but the exact identity of these metabolites was not determined (7). Our studies were designed to examine the distribution and metabolism of [^{3}H]$25OHD_3$ in selected tissues from the D-deficient pregnant rat and its fetuses during vitamin D supplementation.

Materials and Methods. Female Sprague–Dawley rats were obtained at 2 to 3 months of age and fed a synthetic, vitamin D free diet (8). Vitamin D deficiency was documented by the analysis of plasma $25OHD_3$ levels by competitive binding assay (9). After 6 weeks on this diet, the plasma levels of $25OHD_3$ were not detectable. These rats were bred with normal males after at least 8 weeks of the diet. The presence of spermatozoa in vaginal aspirates was used to identify the first day of pregnancy. On the 19th and 20th days of pregnancy, 0.125 μg of 25OH-([^{3}H]26,27)-D_3 (Amersham/Searle, sp act 11 Ci/mmole) dissolved in 0.2 ml of ethanol was injected intravenously. On the 21st day the pregnant rats were anesthetized with ether and bled by cardiac puncture. The uterus and fetuses were exposed via a midline abdominal incision. Each fetus was removed and fetal blood was obtained by cardiac puncture. Fetal kidneys and small intestine were removed by microdissection. Plasma was separated from red blood cells by centrifugation and other tissues were minced, washed in Tris buffer (0.1 *M*, pH 7.4, 4°), and frozen pending homogenization. Maternal kidneys were removed, cleaned of extraneous tissue, and handled as described. Maternal small intestine was removed, cleaned with cold buffer, and opened and mucosal scrapings were obtained. Wet weight of all tissues was obtained prior to freezing and subsequent homogenization. In some animals, maternal kidneys were removed surgically under ether anesthesia prior to the first injection of [^{3}H]$25OHD_3$. The kidneys are currently known to be the sole organs containing the enzymes which convert $25OHD_3$ to its two dihydroxylated metabolites, $1.25(OH)_2D_3$ and $24,25(OH)_2D_3$ (10). Maternal nephrectomy was performed to determine if the reduction in maternal metabolites was accompanied by a parallel reduction in the fetal metabolites.

Tissues were homogenized in Tris buffer and extracted with methanol:chloroform (2:1) for 1 hr. Chloroform fractions were dried under N_2 and chromatographed on Sephadex LH-20 columns (2 × 30 cm) with chloroform:hexane (65:35) elution solvent. Radioactivity recovered from the LH-20 columns averaged 96% of the total extracted counts. Peak areas of radioactivity in the eluates were pooled, dried, and chromatographed on a

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Spherisorb column (Laboratory Data Control, 5-μm microsilica) using a high-pressure liquid chromatography (HPLC) system (Laboratory Data Control, Riviera Beach, Florida) for further separation and confirmation of peak identity by cochromatography with synthetic standards ($25OHD_3$, $24R,25(OH)_2D_3$, and $1\alpha,25(OH)_2D_3$).

Results and discussion. Figure 1 depicts the LH-20 chromatograms of the maternal tissue extracts. Peaks I, II, and III cochromatographed on HPLC with $25OHD_3$, $24R,25(OH)_2D_3$, and $1\alpha,25(OH)_2D_3$, respectively. The amounts of dihydroxylated metabolites formed from [^{3}H]$25OHD_3$ are shown in Table I. The amount of each metabolite was calculated from the recovered radioactivity of the tissue extracts and the specific activity of the [^{3}H]$25OHD_3$ given the assumption that the injected $25OHD_3$ was the only source of vitamin D in these D-deficient animals. Based on these calculations, maternal plasma contained 145 pg/ml of $1,25(OH)_2D_3$ and 34 pg/ml of $24,25(OH)_2D_3$ while the maternal kidneys contained 75 pg/g wet wt of $1,25(OH)_2D_3$ and 12 pg/g wet wt of $24,25(OH)_2D_3$. Mucosa from the maternal small intestine contained 125 pg/g wet wt of $1,25(OH)_2D_3$ and no detectable $24,25(OH)_2D_3$. Fetal tissues contained different amounts and proportions of the dihydroxylated metabolites of vitamin D compared to maternal tissues. Figure 2 shows the two dominant peaks in the fetal tissues which cochromatographed on HPLC with $25OHD_3$ and $24R,25(OH)_2D_3$, respectively. Fetal plasma contained 40 pg/ml of $1,25(OH)_2D_3$ and 109 pg/ml of $24,25(OH)_2D_3$. Fetal kidneys and small intestine had no detectable $1,25(OH)_2D_3$ but contained 58 pg/g wet wt and 49 pg/g wet wt of $24,25(OH)_2D_3$, respectively. These findings are in sharp contrast to the distribution of the metabolites in the maternal tissues (Figs. 1 and 2). Fetal plasma

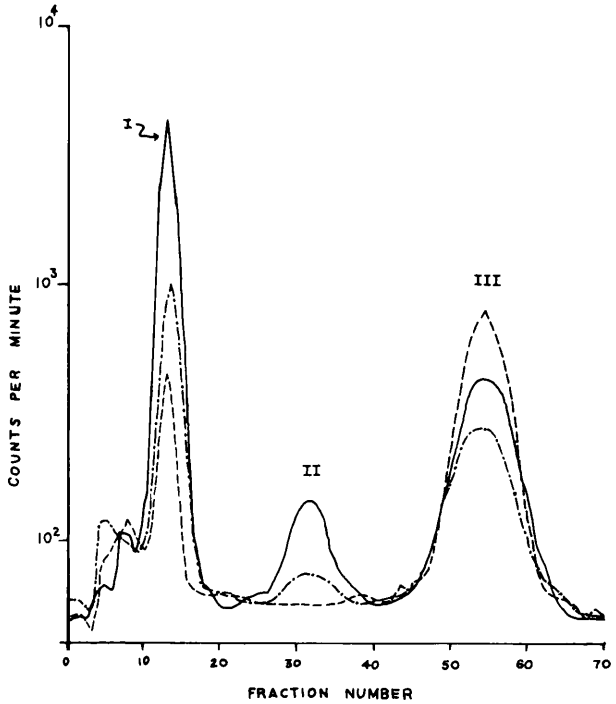

FIG. 1. Sephadex LH-20 chromatograms of maternal tissue extracts from pregnant, D-deficient rats. Maternal blood (——), kidneys (—·—), and small intestinal mucosal scrapings (– – –) from pregnant D-deficient rats treated with [^{3}H]$25OHD_3$ were extracted. Dried extracts were chromatographed on LH-20 columns with chloroform:hexane solvent system. Fractions 5 ml in volume were collected. Aliquots were taken for radioactive counting on a Beckman LS-230 liquid scintillation counter (50% efficiency) in a toluene base cocktail. Remaining fraction volumes were reserved for analysis by HPLC. Radioactivity in peaks I, II, and III comigrated with synthetic $25OHD_3$, $24R,25(OH)_2D_3$, and $1\alpha,25(OH)_2D_3$, respectively, when analyzed by HPLC.

TABLE I. PICOGRAMS OF METABOLITES FORMED FROM [^{3}H]-250HD$_3$ IN MATERNAL AND FETAL TISSUE EXTRACTS.[a]

	24,25(OH)$_2$D$_3$	1,25(OH)$_2$D$_3$	24,25/1,25
Maternal			
Plasma (pg/ml)	34	145	0.23
Kidneys (pg/g)	12	75	0.16
Intestinal mucosa (pg/g)	N.D.[b]	125	—
Fetal[c]			
Plasma (pg/ml)	109	40	2.8
Kidneys (pg/g)	58	N.D.	—
Intestine (pg/g)	49	N.D.	—

[a] Data were calculated as picograms of metabolite based on the specific activity of the injected isotope and an assumed 1:1 conversion of 250HD$_3$ to metabolites. Metabolite amounts were expressed either per milliliter (plasma) or per gram of wet tissue weight. Data shown are average values from three experiments.

[b] Not detectable.

[c] Tissues from all fetuses in each pregnant rat were pooled and results represent pooled organ content.

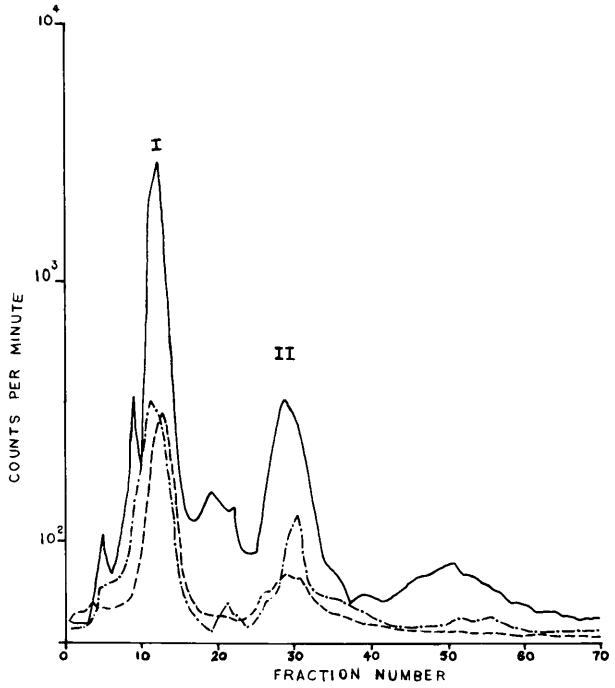

FIG. 2. Sephadex LH-20 chromatograms of fetal tissue extracts from pregnant, D-deficient mothers. Blood (——), kidneys (—·—), and intestine (– – –) from fetuses of D-deficient rats treated with [^{3}H]25OHD$_3$ were extracted. Dried extracts were chromatographed on LH-20 columns with chloroform:hexane solvent system. Fractions 5 ml in volume were collected. Aliquots were taken for radioactive counting in a Beckman LS-230 liquid scintillation counter (50% efficiency) in a toluene base cocktail. Remaining fraction volumes were reserved for analysis by HPLC. Radioactivity in peaks I and II comigrated with synthetic 25OHD$_3$ and 24*R*,25(OH)$_2$D$_3$, respectively, when analyzed by HPLC.

contained 320% more 24,25(OH)$_2$D$_3$ than the corresponding maternal plasma. In contrast, maternal plasma contained 360% more 1,25(OH)$_2$D$_3$ than the corresponding fetal plasma. When expressed as a ratio of 24,25(OH)$_2$D$_3$:1,25(OH)$_2$D$_3$, the ratio in maternal plasma was 0.23 and the ratio in fetal plasma was 2.8, a 12-fold difference between the mother and the fetus (Table I).

Maternal nephrectomy (Nx) reduced the conversion of [^{3}H]25OHD$_3$ to its dihydroxylated metabolites. The mean plasma level of 1,25(OH)$_2$D$_3$, determined from three separate experiments, in the Nx mother was 36 pg/ml,

75% lower than the concentration in animals with intact kidneys. The observed difference in the maternal plasma level of $1,25(OH)_2D_3$ was highly significant ($p < 0.001$).[1] The mean maternal plasma level of $24,25(OH)_2D_3$ was 19.5 pg/ml, a 43% reduction compared to the plasma level in animals with intact kidneys. The difference was also significant ($p < 0.02$). The levels of $1,25(OH)_2D_3$ and $24,25(OH)_2D_3$ in the plasma of fetuses from these Nx mothers were 39.8 and 114.5 pg/ml, respectively. These fetal plasma levels were not significantly different from the levels observed in the fetuses from mothers with intact kidneys. When the maternal plasma levels of the dihydroxylated metabolites were lowered by Nx, the fetal plasma levels were essentially the same as the levels observed in the fetuses from mothers with intact kidneys.

The results of these studies show that the distribution and metabolism of $[^3H]25OHD_3$ in the mother and fetus were different in blood, kidneys, and the small intestine. At a time when $1,25(OH)_2D_3$ was the dominant metabolite in maternal tissues, $24,25(OH)_2D_3$ was the dominant metabolite in fetal tissues. This difference is emphasized by the lack of detection of $1,25(OH)_2D_3$ in fetal kidneys and small intestine as well as by the 12-fold differences in the $24,25(OH)_2D_3$:$1,25(OH)_2D_3$ ratio between maternal and fetal plasma. As expected, maternal Nx reduced the plasma levels of both dihydroxylated metabolites of vitamin D in the mother but, surprisingly, this reduction in maternal plasma levels was not associated with a parallel reduction in the fetal plasma levels. The maintenance of fetal plasma levels of $24,25(OH)_2D_3$ and $1,25(OH)_2D_3$ after maternal Nx indicates that the feto-placental metabolism of $[^3H]25OHD_3$ is to some degree independent of the maternal metabolism. This concept of independent feto-placental metabolism is a heretofore unsuspected aspect of vitamin D metabolism in pregnancy and fetal development. Despite these results, which demonstrate that $24,25(OH)_2D_3$ is the dominant fetal metabolite, the role of this metabolite in fetal development is unknown at present. Recent reports describing the formation of $24,25(OH)_2D_3$ from $25OHD_3$ in cultured chondrocytes and the stimulation of $^{35}SO_4$ incorporation into these cells by $24,25(OH)_2D_3$ indicate that this metabolite may be involved in the growth and differentiation of the fetal skeleton (11, 12).

Summary. Vitamin D metabolism was studied in pregnant, D-deficient rats and their fetuses. D-depleted, pregnant rats were supplemented with $[^3H]25OHD_3$ on the 19th day of pregnancy. The distribution and metabolism of radiolabeled D metabolites was different in maternal and fetal blood, kidneys, and small intestine. $24,25(OH)_2D_3$ was the predominant dihydroxylated D metabolite in the fetus, whereas $1,25(OH)_2D_3$ was the predominant dihydroxylated D metabolite in the mother. The ratio of $24,25(OH)_2D_3$:$1,25(OH)_2D_3$ was 12-fold greater in fetal plasma than maternal plasma. Maternal nephrectomy reduced the metabolism of $[^3H]25OHD_3$ to $24,25(OH)_2D_3$ (43%) and $1,25(OH)_2D_3$ (75%). However, plasma levels of these two metabolites were unchanged in the fetuses of these animals when compared with levels observed in fetuses from mothers with intact kidneys. These results suggest the possibility of independent control of $25OHD_3$ metabolism by the feto-placental unit and raise questions as to the possible role of $24,25(OH)_2D_3$ in fetal development.

[1] Percentage differences were calculated by comparison of the mean value and p values were determined by the analysis of variance.

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