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Do tissues other than the kidney produce 1,25-dihydroxyvitamin D_3 *in vivo*? A reexamination

(vitamin D/metabolism/renal failure)

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ABSTRACT **Recent experiments have shown that 1,25-dihydroxyvitamin D_3-like material is produced in cultured nonrenal cells and may be present in the sera of anephric patients. We reexamined the question of whether 1,25-dihydroxyvitamin D_3 can be synthesized extrarenally in the rat *in vivo*. To intact, sham-operated, ureter-ligated, or acutely nephrectomized vitamin D-deficient rats raised on a diet normal in calcium and phosphorus, we gave a physiologic dose of high-specific-activity 25-hydroxy-[^{3}H]vitamin D_3 (3.6–3.8 μCi; ≈25 pmol per rat). Twenty-four hours later we examined their tissues and plasma for the presence of radiolabeled 1,25-dihydroxyvitamin D_3. Large amounts of radioactivity that behaved chromatographically as identical with authentic 1,25-dihydroxyvitamin D_3 were present in the plasma, bone, and intestine of the intact, sham-operated, or ureter-ligated rats. However, no radioactivity eluting in a manner similar to 1,25-dihydroxyvitamin D_3 was found in plasma, bone, or intestine of acutely nephrectomized rats. We conclude that, in the acutely nephrectomized living rat, 1,25-dihydroxyvitamin D_3 is not present in plasma, bone, or intestine in quantities detectable by the sensitive techniques we have used. No conversion of 25-hydroxyvitamin D_3 to 1,25-dihydroxyvitamin D_3 was observed during a 24-hr period after nephrectomy of vitamin D-deprived rats. This fact casts doubt upon the significance of the *in vitro* production of 1,25-dihydroxyvitamin D_3 by nonrenal cells as an *in vivo* phenomenon.**

Vitamin D_3 is metabolized to its most biologically active form, 1,25-dihydroxyvitamin D_3 [1,25(OH)$_2$D$_3$], via an intermediate, 25-hydroxyvitamin D_3 [25(OH)D$_3$] (1). Fraser and Kodicek (2), and subsequently Gray *et al.* (3), found that radiolabeled 1,25(OH)$_2$D$_3$ was absent from the tissues and plasma of acutely nephrectomized rats that had received low-specific-activity radiolabeled vitamin D_3 or 25(OH)D$_3$ immediately after nephrectomy. In human anephric subjects given radiolabeled vitamin D_3, Mawer *et al.* (4) failed to detect radioactive 1,25(OH)$_2$D$_3$. Gray *et al.* (5) administered 25(OH)[^{3}H]D$_3$ to 10 anephric subjects; in none of the patients studied was radiolabeled 1,25(OH)$_2$D$_3$ detected. In these human studies, radiolabeled 1,25(OH)$_2$D$_3$ was clearly detectable in normal subjects (4, 5). Lambert *et al.* (6) found recently that a substance was present in the plasma of anephric subjects that displaced 1,25(OH)$_2$[^{3}H]D$_3$ from its receptor; additionally, samples processed in a similar manner displaced ^{45}Ca from labeled fetal bone in a bioassay system for 1,25(OH)$_2$D$_3$. The authors concluded that 1,25(OH)$_2$D$_3$ was present in the plasma of anephric subjects. However, other investigators, using radioreceptor, radioimmune, and biological assays for 1,25(OH)$_2$D$_3$, have been unable to detect 1,25(OH)$_2$D$_3$ in anephric persons (7–12).

In certain situations, however, 1,25(OH)$_2$D$_3$ may be synthesized extrarenally. For example, a report by Barbour *et al.* (13) showed that an anephric subject with sarcoidosis had detectable plasma 1,25(OH)$_2$D$_3$-like material at a time when he was hypercalcemic (13); hypercalcemia abated after prednisone treatment that presumably diminished the size of the sarcoid granulomas in the patient. It is likely that sarcoid granulomatous tissue produced the 1,25(OH)$_2$D$_3$. Pregnant nephrectomized rats may synthesize 1,25(OH)$_2$D$_3$ *in vivo* (14, 15). The site of production in pregnant animals could be the placenta because 25(OH)D$_3$ is converted to 1,25(OH)$_2$D$_3$ by placental tissue of rat and human origin *in vitro* (16). Whether the placenta is the sole site of 1,25(OH)$_2$D$_3$ production in the nephrectomized pregnant animal is not known because fetal kidneys could also serve as the source of it. Chicken calvarial cells and human bone cells metabolize 25(OH)[^{3}H]D$_3$ to a material that behaves chromatographically like 1,25(OH)$_2$[^{3}H]D$_3$ (17, 18). The nature of the material was not verified in the original publications, although a recent report suggests that the material is 1,25(OH)$_2$D$_3$ on the basis of mass spectroscopic analysis (†). Two other groups of investigators (19, ‡) have reported that macrophages and cultured intestinal cells can make 1,25(OH)$_2$D$_3$-like material *in vitro*.

Papers alleging such production of 1,25(OH)$_2$D$_3$ *in vitro* generally lack rigorous proof of the material's structure, and those studies failing to detect 1,25(OH)$_2$D$_3$ in anephric individuals suffer from potential problems such as use of low-specific-activity ^{3}H-labeled 25(OH)D$_3$ or vitamin D_3 and use of pharmacologic amounts of precursor. Because this issue is important to the understanding of calcium metabolism, we have reexamined the ability of anephric rats to synthesize 1,25(OH)$_2$D$_3$ *in vivo* by using high-specific-activity labeled 25(OH)D$_3$ in physiologic amounts as the precursor to 1,25(OH)$_2$D$_3$.

MATERIALS AND METHODS

General. The methods used for HPLC, determination of ultraviolet absorbance, and measurement of radioactivity have been reported (20). Counting efficiency for ^{3}H was ≈50%.

25(OH)[26,27-^{3}H]D$_3$ (148 or 158 Ci/mmol; 1 Ci = 3.7×10^{10} Bq) and 1,25(OH)$_2$[26,27-^{3}H]D$_3$ (160 Ci/mmol) were purchased from Amersham and New England Nuclear, respectively. All radiochemicals were purified on a 1 × 60 cm Sephadex LH-20 column with a chloroform/*n*-hexane, 65:35 (vol/vol), solvent system. In both cases, the 25(OH)[^{3}H]D$_3$ and 1,25(OH)$_2$[^{3}H]D$_3$ were the only peaks eluting from these col-

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Abbreviations: 25(OH)D$_3$, 25-hydroxyvitamin D_3; 24*R*,25(OH)$_2$D$_3$, (24*R*)-24,25-dihydroxyvitamin D_3; 1,25(OH)$_2$D$_3$, 1,25-dihydroxyvitamin D_3; 25*S*,26(OH)$_2$D$_3$, (25*S*)-25,26-dihydroxyvitamin D_3.

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† Howard, G. A., Turner, R. T., Puzas, J. E., Knapp, D. K. & Nichols, F., Fifth Workshop on Vitamin D, Williamsburg, VA, Feb. 18, 1982, p. 3 (abstracts).

‡ Roswell, R. H. & Young, M. J., Fourth Annual Scientific Meeting of the American Society of Bone Mineral Research, San Francisco, June 15, 1982, p. 58 (abstracts).

umns. Radiochemical purity of each product was established by co-HPLC with authentic 25(OH)D_3 and 1,25$(OH)_2D_3$; the radiolabeled material was >99% pure. Nonradiolabeled 25(OH)D_3 was obtained from Upjohn; (24*R*)-24,25-dihydroxyvitamin D_3 [24*R*,25$(OH)_2D_3$], (25*S*)-25,26-dihydroxyvitamin D_3 [25*S*,26$(OH)_2D_3$], and 1,25$(OH)_2D_3$ were obtained from Hoffmann–LaRoche.

Animals. Male albino weanling rats (50–60 g) were purchased from Holtzman (Madison, WI). We maintained them in individual hanging wire cages in a room devoid of UV light and fed them ad lib a vitamin D-deficient diet containing 0.47% calcium and 0.3% phosphorus (21). After 7 weeks on this diet, seven bilaterally nephrectomized rats and six intact control rats were given 25(OH)[^{3}H]D_3 as described below. In a second experiment, another group of similar rats was maintained on an identical diet for 7 weeks. Six rats were bilaterally nephrectomized, seven rats underwent bilateral ureteral ligation, and seven rats were subjected to a sham operation. These rats received 25(OH)[^{3}H]D_3 as described below. Additionally, plasma was obtained from 10 vitamin D-deplete rats raised for 7 weeks on the diet described above and used for measurement of 25(OH)D_3 and 1,25$(OH)_2D_3$ levels by described methods (8, 22, 23). Another group of eight rats received 10 international units (250 ng) of vitamin D_3 per day orally for 7 weeks. They served as a positive control group for the measurement of plasma 25(OH)D_3 and 1,25$(OH)_2D_3$. Plasma creatinine and blood urea nitrogen values were measured by described techniques (24, 25).

Metabolism of 25(OH)[26,27-^{3}H]D_3 *in Vivo*. In the first experiment, six rats with intact kidneys and seven bilaterally nephrectomized rats were lightly anesthetized with ether and given (intrajugular injection) 25 pmol of 25(OH)[^{3}H]D_3 (3.6 μCi) dissolved in 68 μl of 95% ethanol. In the second experiment, six bilaterally nephrectomized, seven sham-operated, and seven ureter-ligated rats received (intrajugular injection) 25 pmol of 25(OH)[^{3}H]D_3 (3.8 μCi) dissolved in 88 μl of 95% ethanol. We took 0.3 ml of blood prior to administration of the dose in both experiments for measurement of serum calcium.

Twenty-four hours after the injection of 25(OH)[^{3}H]D_3, rats were exsanguinated and plasma was separated from the blood cells. We immediately removed the entire small intestine and the fore- and hindlimbs. We dissected the fore- and hindlimb bones free from adhering muscle and ligaments and weighed them. The bones were then cooled in liquid nitrogen and pulverized. We removed the intestinal contents by flushing with ice-cold saline and then scraped off the mucosa with a glass microscope slide. Radioactivity was measured in homogenates of intestine and in plasma prior to lipid extraction. Plasma, 50% aqueous suspensions of pulverized bone, and intestinal mucosa were extracted with methanol/chloroform, 2:1 (vol/vol), as described (26). The aqueous layer was reextracted with an equal volume of chloroform. We then evaporated the chloroform fractions to dryness under reduced pressure and resuspended them in the appropriate column solvent.

In the first set of experiments, the residues from plasma, intestine, or bone were suspended in 1 ml of chloroform/*n*-hexane, 65:35 (vol/vol), and chromatographed on Sephadex LH-20 columns (1 × 60 cm) developed and eluted with 350 ml of the same solvent. Five-milliliter fractions of the eluate were collected, an aliquot of each fraction was transferred to a 5-ml scintillation vial and dried, and radioactivity was determined (20). After chromatography, the Sephadex LH-20 in the column was washed with methanol. The methanol wash was collected and dried in order to determine the presence of more polar radioactive material.

In the second set of experiments, the residues from the plasma, bone, and intestine were suspended in an appropriate volume of *n*-hexane/isopropanol, 9:1 (vol/vol), and applied directly to a HPLC system as described below.

HPLC of Metabolites. We pooled and dried (under nitrogen) fractions containing radioactivity that eluted from Sephadex LH-20 columns in a manner identical to 1,25$(OH)_2D_3$ or 25*S*,26$(OH)_2D_3$. The radioactive material was redissolved in 0.1 ml of *n*-hexane/isopropanol, 9:1 (vol/vol). We added 90 ng of authentic 1,25$(OH)_2D_3$ to the radioactive samples prior to chromatography on a 0.4 × 30 cm μPorasil column (Waters Associates) in an *n*-hexane/isopropanol, 9:1 (vol/vol), solvent system developed at a flow rate of 2 ml/min and a pressure of 800 psi. In order to determine the elution volume of 25*S*,26$(OH)_2D_3$, 100 ng was chromatographed in the same system. Absorbance of the effluent was monitored continuously at 265 nm.

In the second experiment, 2 μg of each authentic 25(OH)D_3, 24*R*,25$(OH)_2D_3$, 25*S*,26$(OH)_2D_3$, and 1,25$(OH)_2D_3$ was added to the plasma, intestine, and bone lipid extracts. Chromatography was performed on a 0.4 × 30 cm μPorasil column with a *n*-hexane/isopropanol, 9:1 (vol/vol), solvent system run at a flow rate of 2 ml/min. Fractions were collected at 0.5-min intervals. Effluent from the 1,25$(OH)_2D_3$ region was further chromatographed on a reverse-phase 0.4 × 30 cm C_{18} μBondapak column with a methanol/water, 8:2 (vol/vol), solvent system

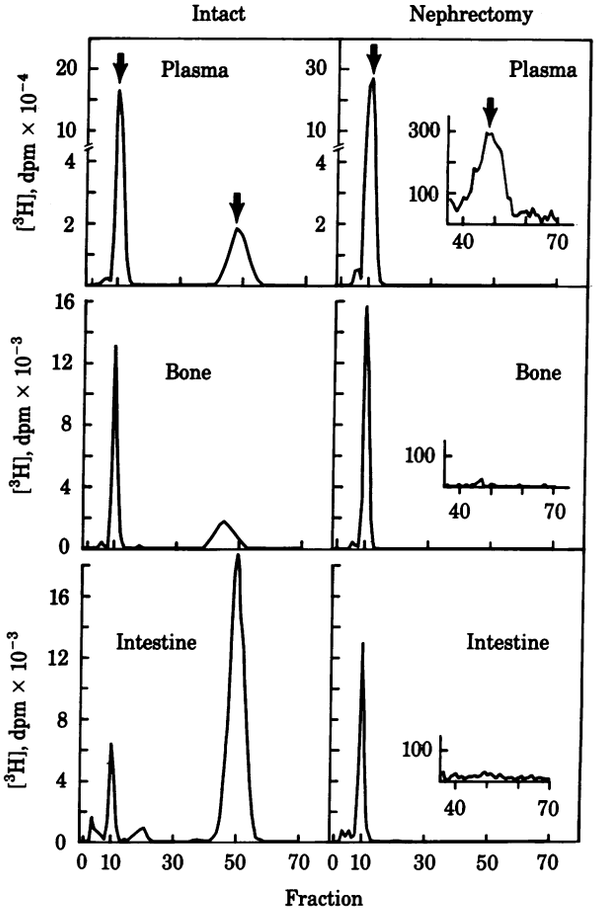

FIG. 1. Representative chromatograms of the lipid extracts of plasma, bone, and intestinal mucosa taken 24 hr after intact and nephrectomized rats had received 25 pmol of 25(OH)[^{3}H]D_3. Chromatography was performed on a Sephadex LH-20 column (1 × 60 cm) developed in and eluted with 350 ml of chloroform/*n*-hexane, 65:35 (vol/vol). After 350 ml, the column was washed with methanol. Arrows show the elution position of authentic 25(OH)[^{3}H]D_3 and 1,25$(OH)_2$[^{3}H]D_3. (*Inserts*) Expanded scales for radioactivity in actual dpm.

run at a flow rate of 2 ml/min. Fractions were collected at 0.5-min intervals.

RESULTS

25(OH)D_3 levels were undetectable in the vitamin D_3-deficient group and the mean ± SEM was 9.2 ± 0.6 ng/ml in the group given D_3. 1,25$(OH)_2D_3$ levels were <5 pg/ml in the vitamin D-deficient group and 213.4 ± 16.5 pg/ml in the vitamin D_3-treated group. Mean (± SEM) serum calcium levels were 4.8 ± 0.2 mg/dl in the vitamin D-deficient sham-operated, ureter-ligated, and nephrectomized animals and 9.9 ± 0.1 mg/dl in the control vitamin D_3-treated group ($P < 0.001$). Plasma creatinine and blood urea nitrogen levels were significantly higher ($P < 0.001$) in the nephrectomized and ureter-ligated rats than in the sham-operated controls (nephrectomized, 4.83 ± 0.04 and 151.6 ± 3.4 mg/dl; ureter-ligated, 4.37 ± 0.08 and 133.4 ± 6.4 mg/dl; sham-operated, 0.47 ± 0.02 and 10.09 mg/dl).

The distributions of radioactivity in the plasma, bone, and intestine of vitamin D-deficient intact and nephrectomized rats were comparable after an intravenous injection of 25 pmol of 25(OH)[^{3}H]D_3. Twenty-four hours after injection, plasma contained the largest percentage of the radioactivity administered per gram of tissue followed by intestine and then bone. From 98% to 104% of the radioactivity in the plasma and 72% to 90% of that in the intestine was chloroform extractable.

In plasma, bone, and intestinal tissues from intact rats a polar metabolite(s) appeared at the elution position of 1,25$(OH)_2D_3$ or 25*S*,26$(OH)_2D_3$ on Sephadex LH-20 chromatography (Fig. 1; Table 1). Plasma, intestine, and bone from intact rats also contained large amounts of radioactive material eluting in a manner identical to 1,25$(OH)_2D_3$ on HPLC (Table 2). On Sephadex LH-20 chromatography, the plasma lipid extracts from nephrectomized animals also contained a small radioactive peak eluting in the position of 1,25$(OH)_2D_3$ or 25*S*,26$(OH)_2D_3$ (Fig. 1; Table 1). However, when this peak was applied to the straight phase HPLC system, it migrated not with authentic 1,25$(OH)_2D_3$ but in a manner similar to authentic 25*S*,26$(OH)_2D_3$ (Table 2). Bone and intestine from nephrectomized vitamin D-deficient rats contained no detectable radioactivity in the elution position of 1,25$(OH)_2D_3$ or 25*S*,26$(OH)_2D_3$ (Fig. 1; Tables 1 and 2). 25(OH)D_3 was the major metabolite appearing in plasma, bone, and intestine of nephrectomized rats 24 hr after injection of radiolabeled 25(OH)D_3, accounting for 88–101% of the radioactivity.

Table 2. HPLC of Sephadex LH-20 elution peaks representing 1,25$(OH)_2D_3$ or 25*S*,26$(OH)_2D_3$

Tissue	dpm applied to column	dpm eluted as 1,25$(OH)_2D_3$	dpm eluted as 25*S*,26$(OH)_2D_3$	Total recovered
Plasma:				
Intact	5,923	4,812	0	5,382
(n = 5)	± 590	± 492		± 490
		(81.4 ± 2.5)		(91.4 ± 3.0)
Nx	838	0	577	789
(n = 5)	± 64		± 36	± 61
			(69.6 ± 3.8)	(94.7 ± 4.0)
Bone:				
Intact	2,852	2,278	0	2,553
(n = 6)	± 148	± 156		± 164
		(79.6 ± 2.6)		(89.4 ± 2.6)
Nx				
(n = 7)	0	0	0	0
Intestine:				
Intact	1,961	1,671	0	1,793
(n = 6)	± 143	± 99		± 101
		(85.6 ± 1.4)		(90.6 ± 1.8)
Nx				
(n = 7)	0	0	0	0

See legend to Table 1.

Table 1. Radioactive vitamin D metabolites in plasma, bone, and intestine after injection of 25(OH)[26,27-^{3}H]D_3 to intact and nephrectomized (Nx) rats

Tissue	dpm applied to column	dpm eluted: Less polar than 25(OH)D_3	25(OH)D_3	Less polar than 1,25$(OH)_2D_3$	1,25$(OH)_2D_3$ or 25*S*,26$(OH)_2D_3$	Column strip	Total recovered
Plasma:							
Intact	512,964	9,742	281,626	2,236	112,600	18,580	424,784
(n = 5)	± 81,720	± 4,569	± 33,089	± 1,308	± 8,297	± 6,372	± 43,432
		(1.6 ± 0.6)	(57.0 ± 3.5)	(0.4 ± 0.2)	(24.6 ± 4.5)	(3.8 ± 1.3)	(87.2 ± 7.2)
Nx	756,247	16,511	666,645	727	2,489	5,598	692,095
(n = 5)	± 43,115	± 2,473	± 75,440	± 300	± 192	± 701	± 78,093
		(2.2 ± 0.3)	(87.7 ± 7.5)	(0.1 ± 0.04)	(0.33 ± 0.01)	(0.8 ± 0.1)	(91.0 ± 7.6)
Bone:							
Intact	30,201	645	18,874	137	12,286	1,344	33,286
(n = 6)	± 3,684	± 118	± 2,085	± 72	± 2,013	± 165	± 3,928
		(2.1 ± 0.2)	(63.8 ± 4.5)	(0.4 ± 0.2)	(40.0 ± 3.0)	(4.5 ± 0.3)	(110.7 ± 4.7)
Nx	34,464	1,232	34,927			1,246	37,272
(n = 7)	± 3,746	± 198	± 3,997	0	0	± 176	± 4,241
		(3.5 ± 0.2)	(101.3 ± 3.1)			(3.7 ± 0.5)	(108.1 ± 3.0)
Intestine:							
Intact	155,196	3,555	14,064	4,564	119,320	11,618	153,146
(n = 6)	± 21,112	± 583	± 1,273	± 562	± 15,255	± 1,686	± 17,581
		(2.3 ± 0.2)	(9.8 ± 1.4)	(3.0 ± 0.2)	(77.5 ± 3.1)	(7.6 ± 1.0)	(100.2 ± 2.4)
Nx	37,929	2,700	33,831	469		6,102	45,140
(n = 7)	± 4,038	± 434	± 4,292	± 146	0	± 1,113	± 5,801
		(6.9 ± 0.4)	(89.5 ± 7.0)	(1.3 ± 0.5)		(15.4 ± 1.4)	(112.9 ± 7.2)

Metabolites are referred to by their elution positions (Sephadex LH-20 column) relative to authentic 25(OH)D_3 and 1,25$(OH)_2D_3$. n, Number of animals per experiment; Nx, nephrectomized. Results are shown as mean ± SEM. The numbers in parentheses represent percentage of total counts applied.

Table 3. HPLC of lipid extracts from plasma, bone, and intestine after injection of 25(OH)[^{3}H]D_3 to sham-operated, ureter-ligated, and nephrectomized rats

Tissue	Distribution of ^{3}H among metabolites, %			
	25-(OH)D_3	25*S*,26-$(OH)_2D_3$	1,25-$(OH)_2D_3$	Total recovered
Plasma:				
Sham-operated	60.5	0.8	29.0	90.8
(n = 7)	± 1.4	± 0	± 1.2	± 1.3
Ureter-ligated	73.9	0.7	15.3	90.3
(n = 6)	± 1.3	± 0.02	± 0.6	± 1.4
Nx	92.8	0.6	0	93.7
(n = 6)	± 3.9	± 0.04		± 3.8
Bone:				
Sham-operated	53.7	0	38.1	91.9
(n = 6)	± 1.8		± 1.9	±1.3
Ureter-ligated	68.4	0	26.5	95.2
(n = 7)	± 0.8		± 1.7	± 1.4
Nx	93.5	0	0	93.7
(n = 6)	± 2.5			± 2.5
Intestine:				
Sham-operated	9.2	0	76.8	86.2
(n = 7)	± 1.1		± 4.0	± 4.2
Ureter-ligated	17.9	0	50.9	69.2
(n = 7)	± 0.7		± 3.6	± 3.0
Nx	71.4	0	0	72.5
(n = 6)	± 2.3			± 2.1

See legend to Table 1.

The results of the second experiment are shown in Table 3. Radioactivity coeluting with $1,25(OH)_2D_3$ was present in the plasma, bone, and intestine of sham-operated and ureter-ligated rats. None was observed in the plasma, bone, or intestine of nephrectomized rats. Radioactivity coeluting with authentic $1,25(OH)_2D_3$ from the straight phase HPLC of sham-operated or ureter-ligated rats also coeluted with $1,25(OH)_2D_3$ on the reverse-phase system.

DISCUSSION

Our data are consistent with prior reports that $1,25(OH)_2D_3$ is not detected in plasma and intestine of acutely nephrectomized rats (2, 3). In addition, our results show that $1,25(OH)_2D_3$ is not present in the bone of nephrectomized rats. The animals used in our experiments had been maintained on a vitamin D-deficient diet for 7 weeks, resulting in serum calcium levels ≈50% of normal and no detectable $25(OH)D_3$ and $1,25(OH)_2D_3$ in plasma. Under these circumstances, the $25(OH)D_3$ 1α-hydroxylase should be maximally stimulated (1). Furthermore, because we used high-specific-activity 25(OH)[^{3}H]D_3 as the radiolabeled precursor, we were able to give physiologic amounts of $25(OH)D_3$ (≈25 pmol) but large amounts of radioactivity (3.6–3.8 μCi) to each rat. The sensitivity of our technique would have allowed us to detect as little as 100–200 dpm of radioactive $1,25(OH)_2D_3$ in various tissues. This is about 0.1% of the amount of radioactivity actually present in each intact rat plasma or intestinal sample (≈100,000 dpm; see Fig. 1 and Table 1) and about 1% of that present in a bone sample (≈12,000 dpm). Thus, if the nephrectomized rats had circulating plasma or intestinal tissue levels of $1,25(OH)_2D_3$ that were even 0.1% of those present in intact rats, we would have been able to detect them. Similarly, had bone from nephrectomized rats produced $1,25(OH)_2D_3$ in amounts 1% of those of intact rats we would have detected this metabolite.

That uremia *per se* is not responsible for the lack of $1,25(OH)_2D_3$ production is shown by the ureter-ligated group of rats, in which $1,25(OH)_2D_3$ production was observed (Table 3) despite blood urea nitrogen and plasma creatinine values similar to those in nephrectomized animals. Our data confirm and strengthen the observations of Fraser and Kodicek (2) and Gray *et al.* (3) in rats. Our observations are also supported by the fact that anephric man does not produce radiolabeled $1,25(OH)_2D_3$ from an appropriate precursor (4, 5). The absence of circulating $1,25(OH)_2D_3$ in anephric humans as measured by radioreceptor, radioimmunoassay, and biological assays also supports this contention (7–12).

Lambert *et al.* (6) recently detected material in the plasma of anephric patients that resembles $1,25(OH)_2D_3$ in a radioreceptor assay and a bioassay. It is conceivable that some interfering substance was present in the sera from anephric persons that caused displacement of $1,25(OH)_2$[^{3}H]D_3 from the cytosolic intestinal receptor and mobilization of ^{45}Ca from fetal long bones. Certainly, vitamin D-deficient plasma contains materials that displace $1,25(OH)_2$[^{3}H]D_3 from its receptor—hence the need for extensive purification before assay (7–12). It should also be kept in mind that both laboratories involved in the Lambert *et al.* study (6) previously reported plasma $1,25(OH)_2D_3$ levels to be undetectable in anephric subjects (10, 12). If, as one must assume, larger amounts of plasma were extracted than before, then one cannot dismiss the possibility that larger amounts of interfering material were also extracted. The authors did not present results from appropriate control samples—i.e., vitamin D-deficient plasma samples of a volume equal to that extracted in the anephric patients. It is also puzzling that, with the stated sensitivity of the bioassay method, ≈1–2 pg/ml, $1,25(OH)_2D_3$ levels were not detectable earlier (12) in 2- to 5-ml plasma samples from anephric persons [the average value of $1,25(OH)_2D_3$ reported later in the non-vitamin-D-supplemented anephrics was 3.2–7.9 pg/ml] (6). The report by Lambert *et al.* (6) should be interpreted cautiously because of the points raised above and because of contrary evidence from groups using diverse techniques (2–5, 7–12, 27). Despite these criticisms, we must emphasize that our experiments were performed in acutely nephrectomized rats and the possibility of induction of the $25(OH)D_3$ 1α-hydroxylase in the chronic uremic state cannot be dismissed.

Several laboratories have reported that $1,25(OH)_2D_3$ is made in tissues other than the kidney (14–19, †). In the pregnant animal this may well be the placenta or the fetoplacental unit (14–16). The *in vitro* data are supported by the *in vivo* observations that nephrectomized pregnant rats can synthesize a material resembling $1,25(OH)_2D_3$. Chicken and human bone cells reportedly synthesize $1,25(OH)_2D_3$ *in vitro*. The existence of $1,25(OH)_2D_3$ in these cultures has also been confirmed recently by mass spectrometry (†). The cultures were maintained in serum-free media wherein vitamin D-binding protein is absent, a circumstance that does not occur *in vivo*. This might influence the ability of these cells to synthesize $1,25(OH)_2D_3$. Reports showing that intestinal cells synthesize $1,25(OH)_2D_3$ are preliminary; the identity of the putative $1,25(OH)_2D_3$ has not been unambiguously established (‡). A report that macrophages synthesize $1,25(OH)_2D_3$ has now been withdrawn (19, §). Our data show that $1,25(OH)_2D_3$ is not detectable in the plasma, bone, or intestine of anephric rats. Although it is possible that certain cells can synthesize $1,25(OH)_2D_3$ *in vitro*, it is unlikely that this phenomenon is of significance *in vivo*.

We conclude that the acutely nephrectomized rat does not form $1,25(OH)_2D_3$ in amounts detectable by highly sensitive methods. If any $1,25(OH)_2D_3$ is formed outside the kidney of

§ Gray, T. K., Maddux, F. W., Mentz, W. M. & Williams, M. E., Fourth Annual Scientific Meeting of the American Society of Bone Mineral Research, San Francisco, June 14, 1982, p. S52 (abstracts).

acutely nephrectomized rats, it must be in amounts less than 0.1% of normal. Our data may not be applicable to the anephric human because our experiments were performed in the acutely nephrectomized state which may not mimic the chronic anephric state. Our experiments, however, do question the role of *in vitro* production of $1,25(OH)_2D_3$ by nonrenal cells.

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