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Vitamin D Metabolism: The Role of Kidney Tissue

Abstract. *The appearance of a polar metabolite of 25-hydroxycholecalciferol has been studied in the intestinal mucosas of nephrectomized rats and rats which have been made uremic by ureter ligation. In confirmation of previous work by Fraser and Kodicek, it was found that nephrectomy prevents the appearance of this metabolite in the intestine. On the other hand, equivalent amounts of the metabolite were found in uremic rats and in sham-operated controls, showing that the production of this metabolite is independent of the uremic state of the animals. In addition, it was shown that the metabolite produced in vitro by kidney homogenates is identical to that found in vivo in the intestine.*

Impaired renal function has for several years been known to be associated with alterations in vitamin D and calcium metabolism. Kessner and Epstein (*1*) showed the active transport of calcium by everted duodenal sacs to be reduced in the chronically uremic rat, and Avioli *et al.* (*2*) have in addition demonstrated decreased intestinal calcium binding protein activity in such animals. These situations are not reversed by physiological amounts of vitamin D, though they may be partially reversed by 25-hydroxycholecalciferol (25-HCC), the "circulating" or "hormonal" form of vitamin D (*3*, *4*). There is, however, no evidence that the hepatic synthesis of 25-HCC from vitamin D is impaired in uremia.

Recently, Fraser and Kodicek (*5*) have shifted attention from the role of renal function to the role of renal tissue in vitamin D metabolism by their report that the conversion of 25-HCC to a more polar metabolite takes place exclusively in the kidney. This metabolite (designated "peak V" in this laboratory) has been shown to act even more rapidly than 25-HCC in the stimulation of calcium absorption and may very well be the active form of the vitamin in the intestine (*6*). The evidence for the role of kidney tissue in the production of this metabolite is based principally on the observation that the normal appearance of this metabolite in the intestinal mucosa after administration of vitamin D is not seen in nephrectomized rats (*5*). Furthermore, Fraser and Kodicek reported kidney to be the only tissue capable of producing this metabolite in vitro (*5*).

The present study was undertaken with two objectives in mind. The first was to determine whether the absence of the peak V metabolite in the intestine of nephrectomized rats is due to toxic effects of the uremia produced by nephrectomy or whether kidney tissue is required for the further metabolism of 25-HCC. The second objective was to determine whether the peak V produced in vitro by kidney homogenates is identical to that observed in the intestine after in vivo administration of 25-HCC or vitamin D_3.

Male weanling Holtzman rats were fed a vitamin D–deficient diet for 5 to 6 weeks prior to being used for experiments (*7*). The [26,27-^{3}H]-25-HCC used in these experiments was prepared in this laboratory and had a specific activity of 1.3 c/mmole (*8*). Groups of three rats were either bilaterally nephrectomized or sham-operated and were injected intrajugularly with 125 ng of ^{3}H-25-HCC in 95 percent ethanol immediately after surgery. A third group of rats was ureter-ligated 6 hours before being injected with 125 ng of ^{3}H-25-HCC, the 6-hour delay being to ensure a degree of uremia similar to that of the nephrectomized animals at the time of killing. The rats in all groups were killed 12 hours after the injection of ^{3}H-25-HCC, having also been fasted 16 hours prior to killing. The intestinal mucosas from the rats in each group were pooled, homogenized in 0.9 percent saline, and extracted with $CHCl_3$-methanol as previously described (*4*). Identification of ^{3}H metabolites was

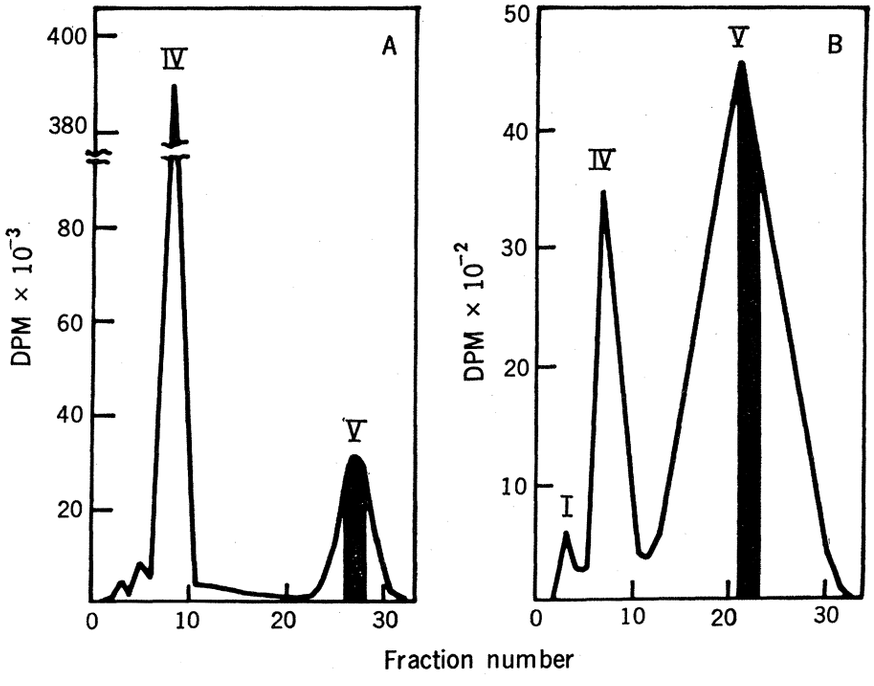

Fig. 1. (A) Sephadex LH-20 chromatography of the combined lipid extracts from six in vitro incubation mixtures. Incubation was for 2 hours at 37°C. A column containing 12 g of Sephadex LH-20 was used and 5.3-ml fractions were collected. The fractions in the shaded area were pooled and taken up in a known volume of solvent. (B) Sephadex LH-20 chromatography of the lipid extract of the mucosal scrapings from six vitamin D–deficient chicks each given 125 ng of [26,27-^{3}H]-25-HCC 12 hours prior to killing. A column containing 10 g of Sephadex LH-20 was used and 5.5-ml fractions were collected. The fractions in the shaded area were pooled and taken up in a known volume of solvent.

accomplished by Sephadex LH-20 chromatography (*9*). A 30 by 2 cm column containing 10 g of Sephadex LH-20 was used, and 5-ml fractions were collected. The samples were applied to the column in less than 1 ml of 65 percent chloroform in Skellysolve B (by volume) and were eluted with 175 ml of the same solvent. The column was stripped with 100 ml of 70 percent chloroform in Skellysolve B. Radioactivity in the chromatographic fractions was determined by liquid scintillation counting using a Packard Tri-Carb spectrometer No. 3375 equipped with external standardization. In vitro conversion of 25-HCC to the peak V metabolite was accomplished by using a 25 percent homogenate of kidney tissue from chicks fed a vitamin D–deficient diet for 4 to 5 weeks. Incubation conditions were the same as those used by Fraser and Kodicek except that 25 ng of [26,27-^{3}H]-25-HCC was used as substrate (*5*). Also, identification of the peak V metabolite was determined by chromatography on Sephadex LH-20 as described above.

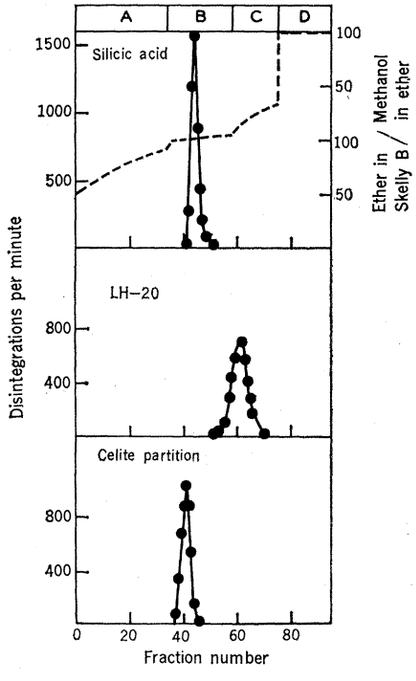

Fig. 2. Cochromatography of combined aliquots of the metabolites isolated in Fig. 1. Silicic acid chromatography was done as described by Suda *et al.*, using a multibore column containing 14 g of silicic acid with 11.5-ml fractions being collected (*11*). Total recovery was 90 percent. Sephadex LH-20 chromatography was done (*12*) using a 60- by 1-cm column containing 20 g of Sephadex LH-20 with 3.2-ml fractions being collected. Total recovery was 100 percent. Celite partition chromatography was done as described by Suda *et al.* with 90 percent methanol, 10 percent water as the stationary phase and 30 percent chloroform, 70 percent Skellysolve B as the mobile phase, and 5.3-ml fractions were collected (*11*). Total recovery was 87 percent.

The percentage distribution of ^{3}H metabolites in the lipid extracts of tissues from vitamin D–deficient rats subjected to various treatments is seen in Table 1. In the case of both intestinal mucosa and blood plasma, the nephrectomized rats failed to show any of the peak V metabolite, while equivalent amounts of this metabolite were seen in both the sham-operated controls and the ureter-ligated rats. On the other hand, the ureter-ligated rats had a blood urea nitrogen value similar to that for the nephrectomized rats, indicating that the state of uremia in these two groups of animals is about the same. From this data, we can conclude that the uremic state of the animals has little effect on the appearance of the peak V metabolite in the intestine and that the presence of kidney tissue is required for 25-HCC metabolism to peak V to occur.

In order to establish the identity of the peak V metabolite produced in vitro by kidney homogenates with the metabolite observed in the intestine in vivo after administration of ^{3}H-25-HCC, it was first necessary to isolate metabolites from these two sources. The in vitro metabolite was isolated by combining the lipid extracts from six of the incubation mixtures described above. The combined lipid extracts were chromatographed on Sephadex LH-20 and the metabolite was isolated as shown in Fig. 1A. The in vivo metabolite was isolated by injection of six vitamin D–deficient chicks with 125 ng each of ^{3}H-25-HCC 12 hours prior to killing, and isolating the metabolite from the lipid extract of the mucosal homogenate by Sephadex LH-20 chromatography as shown in Fig. 1B. The smaller elution volume obtained for this metabolite is due to the fact that this column contained 10 g of Sephadex, while the other contained about 12 g. Also, the large amount of lipid observed in the in vivo sample could have caused some peak broadening. Aliquots of each of the metabolites isolated in Fig. 1 were combined and chromatographed in three different chromatographic systems as shown in Fig. 2. It can be seen that in each case virtually all of the recovered radioactivity can be found in a single, symmetrical peak. These results provide further evidence that the metabolite produced in vitro by kidney homogenates is, in fact, identical to that found in intestinal mucosa after in vivo administration of ^{3}H-25-HCC or vitamin D_3.

Table 1. Effect of nephrectomy and ureter ligation on peak V accumulation in rat tissues. The rats were killed 12 hours after the intravenous administration of 125 ng of [26,27-^{3}H]-25-HCC. Urea nitrogen determinations were done on an aliquot of the pooled plasma from the rats in each group by the method of Marsh *et al.* (*10*) using a Technicon AutoAnalyzer.

Metabolite	Nephrectomized rats	Sham-operated rats	Ureter-ligated rats
*Small intestine mucosa**			
25-HCC ester	7.7	11.9	6.7
25-HCC	92.3	25.7	33.7
Peak V	0	62.4	59.6
*Blood plasma**			
25-HCC	100	89.9	91.6
Peak V	0	10.1	8.4
Blood urea nitrogen†			
	129	21	141

* These values are percentages of the recovered radioactivity, the recoveries usually being about 90 percent. † These values are milligrams per 100 milliliters.

The present results clearly confirm the observation of Fraser and Kodicek (*5*) that kidney tissue is necessary for the conversion of 25-HCC to a more polar metabolite. It has also been shown that the acute elevation of blood urea nitrogen caused by nephrectomy does not by itself impair this conversion in the short term. We have furthermore provided convincing evidence that the metabolite produced in vitro by kidney homogenates is identical to that found in the intestine in vivo after administration of 25-HCC.

These findings and those of Fraser and Kodicek open the possibility that in azotemic chronic renal disease there is a failure to produce the intestinal metabolically active form of vitamin D (peak V), thus resulting in impaired calcium absorption.

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X-ray Diffraction Patterns of Transfer RNA Consistent with the Presence of Short Parallel Helices in the Molecule

Abstract. *X-ray diffraction data of yeast formylmethionine transfer RNA,* Escherichia coli *phenylalanine transfer RNA, and* Escherichia coli *arginine transfer RNA single crystals are compared with the Fourier transform of a helix. The results are consistent with the presence of short parallel double helical segments in the transfer RNA molecules.*

Transfer ribonucleic acid (tRNA) molecules play a prominent role in protein biosynthesis. They function as adaptor molecules in the translation of the messenger RNA code into the corresponding polypeptide chain. It is of interest to have information on their molecular structure. Several different models of the tertiary structure of tRNA have been proposed (*1*). All these models are based on the cloverleaf structure of Holley (*2*). These models consist of four main double helical stems, amino acid, dihydrouridine, anticodon, and pseudouridine. We now report x-ray diffraction patterns from single crystals of tRNA which are consistent with the presence of short parallel helices in the molecule.

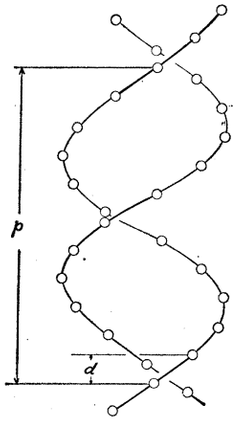

Fig. 1. Elevenfold double helix.

The structural analysis of a macromolecule by single crystal x-ray diffraction can be achieved with the multiple isomorphous derivatives technique. However, although various single crystals of tRNA have been grown and preliminary x-ray diffraction studies have been made (*3*, *4*) deciphering of the phases by the isomorphous derivatives method has not yet been possible. In parallel with our efforts of preparation of the single crystals of heavy atom derivatives, we have attempted to obtain structural information from available diffraction data of the crystals of yeast formylmethionine tRNA ($tRNA^{fMet}$), *Escherichia coli* phenylalanine tRNA ($tRNA^{Phe}$), and *E. coli* arginine tRNA ($tRNA^{Arg}$). X-ray data for these crystals are shown in Table 1.

The two important quantities of a molecular structure that can be compared with the x-ray data are the Fourier transform and the interatomic vector set. We have examined both these quantities and compared them with the x-ray data.

The x-ray diffraction pattern from a crystal is the Fourier transform of all the molecules in the unit cell. Therefore, in general, the diffraction pattern will not directly exhibit the Fourier transform of a single molecule (*5*). However, if the molecule contains a considerable amount of helical structure, the diffraction pattern, may, in favorable cases, be expected to show characteristics of helix diffraction.

The fundamental parameters that define a helix are the pitch p, the number of units per turn N, and the radius r. The distance d between the successive units is then equal to p/N, and the angle of rotation ϕ per unit is equal to $360°/N$ (Fig. 1). The general formula of the Fourier transform of a set of points is

$$F(X,Y,Z) = \sum_j f_j \times \exp\, 2\pi i(Xx_j + Yy_j + Zz_j) \qquad (1)$$

where f_j is the scattering factor of the jth atom whose coordinates are x_j, y_j, and z_j and X, Y, Z are orthogonal coordinates in the reciprocal space. For the infinite helix, Eq. 1 can be written (*6*)

$$F(R,\psi,Z) = \sum_{n=0}^{\infty} J_n(2\pi Rr) \times \exp\, i[n(\psi - \phi + \pi/2) + 2\pi Zz] \qquad (2)$$

where R,ψ,Z are the cylindrical coordinates in the reciprocal space and J_n is the Bessel function of nth order. This function is known to have the following characteristics. (i) The transform has a layer structure, with the distance $1/p$ between the layers; (ii) at the lower layers, the main part of the Bessel function has the same order as the layer number and has a cross-shaped pattern through the origin; (iii) the strong meridional region appears at a distance of about $1/d$; and (iv) for the double helix, the transform is modified by a fringe function corresponding to the relative position of the two strands.

Since the scattering in the low angular region is mainly due to the phosphate group (*7*), the phosphorus coordinates (*8*) of the helix for 11-fold viral RNA with $p = 30$ Å and $N = 11$

Table 1. Crystallographic data for three tRNA crystals.

Species	Crystal system	Space group	Cell constants		Molecules in asymmetric unit (No.)
			$a = b$ (Å)	c (Å)	
Formylmethionine (yeast)	Hexagonal	$P6_222$ ($P6_122$)	115	137	1
Phenylalanine (*E. coli*)	Hexagonal	?	122	198	2(?)
Arginine (*E. coli*)	Trigonal	$P3_121$ ($P3_221$)	99.4	92.9	2