SIDE CHAIN OXIDATION OF 25-HYDROXY-[26,27-^{14}C]VITAMIN D_3 AND 1,25-DIHYDROXY-[26,27-^{14}C]VITAMIN D_3 IN VIVO BY CHICKENS[+]

by

Rajiv Kumar and H. F. DeLuca

Department of Biochemistry

College of Agricultural and Life Sciences

University of Wisconsin-Madison

Madison, Wisconsin 53706

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Radio carbon dioxide was detected in expired air of vitamin D-deficient chicks administered either 25-hydroxy-[26,27-^{14}C]vitamin D_3 or 1,25-dihydroxy-[26,27-^{14}C]vitamin D_3 intravenously. At 48 hours the amounts were 5.13 $\pm$ 1.02% and 11.8 $\pm$ 3.10% of the administered dose, respectively. This phenomenon, similar to the one observed in rats, represents side chain oxidation and elimination of either one or both of the 26 and 27 carbons from the vitamin D molecule. The observation that the rate of $^{14}CO_2$ evolution is maximal 4 hours after a dose of 1,25-dihydroxy-[26,27-^{14}C]vitamin D_3 suggests that this pathway may be of importance to the function of 1,25-dihydroxyvitamin D_3.

The physiologic action of vitamin D_3 is preceded by its conversion to more polar metabolites in the liver and kidney (1-7). The active form of vitamin D_3 in intestine and bone appears to be 1,25-dihydroxyvitamin D_3 (1,25-$(OH)_2D_3$)[1] (6). Six and 12 hours after the administration of 1,25-$(OH)_2$-[26,27-^{14}C]D_3 to the rat, 1,25-$(OH)_2D_3$ is the major metabolite present in these tissues (8, 9). However, a substantial amount of radioactivity was present in the aqueous phase suggesting unknown metabolism. To investigate this matter further 25-hydroxy-[26,27-^{14}C]vitamin D_3 (25-OH-D_3) was synthesized using established methods (10). 25-OH-D_3 was then converted to 1,25-$(OH)_2D_3$ enzymatically using chick kidney homogenates (8).

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[1]Abbreviations: 1,25-$(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; 25-OH-D_3, 25-hydroxyvitamin D_3.

Table 1. Accumulative $^{14}CO_2$ Production After the Injection of 25-OH-[26,27-^{14}C]D_3 and 1,25-$(OH)_2$-[26,27-^{14}C]D_3 into Vitamin D-Deficient Chickens

25-OH-D_3		1,25-$(OH)_2D_3$	
Time (hours)	% Dose	Time (hours)	% Dose
12	2.55 ± 0.50	12	6.62 ± 2.85
24	4.22 ± 0.91	24	10.56 ± 3.12
48	5.13 ± 1.02	48	11.18 ± 3.10

Rachitic chicks were given either 650 pmoles 25-OH-[26,27-^{14}C]D_3 or 325 pmoles 1,25-$(OH)_2$-[26,27-^{14}C]D_3 intravenously in 0.05 ml ethanol. There were 3-4 chicks in each group and the data are expressed as % of dose ± S.D.

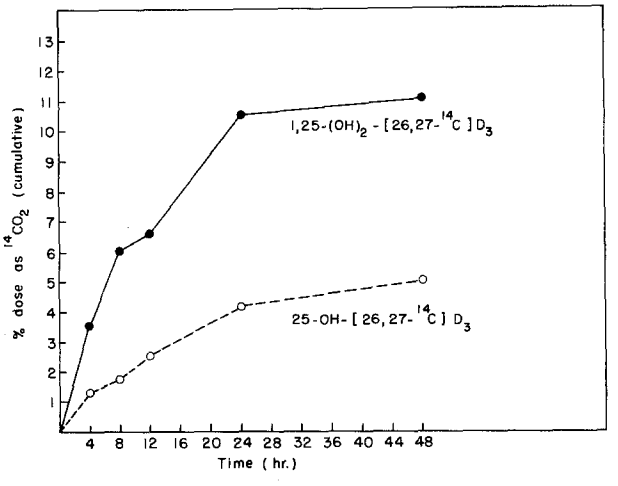

Figure 1. $^{14}CO_2$ appearance in the expired air of rachitic chicks given either 650 pmoles 25-OH-[26,27-^{14}C]D_3 or 325 pmoles 1,25-$(OH)_2$-[26,27-^{14}C]D_3 intravenously.

White leghorn chickens (Northern Hatcheries, Beaver Dam, Wis.) were raised on a vitamin D-deficient diet (11) for a period of 3 weeks. Two groups of 4 chicks were used. Animals in the first group were injected intravenously with 650 pmoles of 25-OH-[26,27-^{14}C]D_3 and the second group with 325 pmoles of 1,25-$(OH)_2$-[26,27-^{14}C]D_3 each dissolved in 0.05 ml ethanol. The animals were immediately placed in metabolism cages and all the CO_2 ($^{12}CO_2$ and $^{14}CO_2$) expired was trapped in ethanolamine-methyl cellusolve (1:2) solution (12). This solution was changed periodically at 4, 8, 12, 24, and 48 hours. Aliquots of this were taken and counted in a liquid scintillation counter (12).

$^{14}CO_2$ was detected in expired air in both groups of chickens. The amounts formed are shown in Table 1 and Figure 1. It is evident that the rate of formation of $^{14}CO_2$ is maximal in the first 4-8 hours after the administration of 1,25-$(OH)_2$-[26,27-^{14}C]D_3.

This phenomenon is similar to the one observed in rats (13, 14). In rats nephrectomy prevented $^{14}CO_2$ formation from 1,25-$(OH)_2$-[26,27-^{14}C]D_3 and conditions suppressing 25-OH-D_3-1-hydroxylase in the kidney (e.g. vitamin D_3 repletion) also suppress $^{14}CO_2$ evolution.

$^{14}CO_2$ in both groups of animals is formed from 25-OH-[26,27-^{14}C]D_3 and 1,25-$(OH)_2$-[26,27-^{14}C]D_3 as a result of loss of either one or both carbons at positions 26 and 27. The fact that $^{14}CO_2$ formation is maximal at a time when 1,25-$(OH)_2D_3$ function is most marked suggests that the pathway may be of importance in 1,25-$(OH)_2D_3$ action. It is also possible that this represents a degradative pathway after 1,25-$(OH)_2D_3$ has stimulated calcium and phosphorus transport in the intestine and calcium mobilization from bone.

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