grams of mass-reared insects. In the Sterile Screwworm Release Program, factory conditions may be altered to improve flight capacity under natural conditions. Alternatively, new strains may be synthesized at appropriate times to meet changing environmental conditions and to maintain high quality of factory-released flies in the field. Knowledge of the existence of geographic races and clines that reflect local adaptations are also important factors to consider in designing future sampling programs for establishing new production lines.

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17 February 1976; revised 5 May 1976

Side Chain Metabolism of 25-Hydroxy-[26,27-^{14}C]vitamin D_3 and 1,25-Dihydroxy-[26,27-^{14}C]vitamin D_3 in vivo

Abstract. *Radioactive CO_2 was detected in expired air after the administration of 25-hydroxy-[26,27-^{14}C]vitamin D_3 to vitamin D–deficient hypocalcemic rats; $^{14}CO_2$ was also detected after the administration of 1,25-dihydroxy-[26,27-^{14}C]vitamin D_3 to rats raised on the same diet. Nephrectomy totally abolished $^{14}CO_2$ formation after administration of 25-hydroxy-[26,27-^{14}C]vitamin D_3, but not after the administration of 1,25-dihydroxy-[26,27-^{14}C]vitamin D_3. The production of $^{14}CO_2$ commenced within 4 hours after injection of 1,25-dihydroxy-[26,27-^{14}C]vitamin D_3, suggesting a possible relevance of this reaction to the function of 1,25-dihydroxyvitamin D_3. These results at least demonstrate a new metabolic pathway of vitamin D_3 metabolism involving the oxidation of a portion of the side chain of 1,25-dihydroxyvitamin D_3 to CO_2.*

It is now clear that, physiologically, 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$] or a further metabolite is the metabolically active form of vitamin D_3 (*1*). Although alternate metabolites derived from 25-hydroxyvitamin D_3 (25-OH-D_3) are known (*2*), little is known concerning the further metabolism of 1,25-$(OH)_2D_3$. At 6 and 12 hours after the administration of 1,25-$(OH)_2$-[26,27-^{3}H]D_3 to rats and chicks, no polar metabolites were detected in the lipid extracts of intestine and bone (*3*, *4*). Since at that time both organs had responded to 1,25-$(OH)_2D_3$, it appeared likely that 1,25-$(OH)_2D_3$ is not further metabolized before it functions. However, radioactivity was detected in water-soluble material (*3*, *5*) and, furthermore, the overall recovery of the ^{3}H in the animals was considerably less than 100 percent, suggesting loss of the label as ^3H_2O. To examine this question fully, 25-OH-[26,27-^{14}C]D_3 was synthesized chemically by methods described for 25-OH-[26,27-^{3}H]D_3 (*6*). The 25-OH-[26,27-^{14}C]D_3 was converted to 1,25-$(OH)_2$-[26,27-^{14}C]D_3 as described (*7*). Both ^{14}C-labeled compounds are fully biologically active, giving antirachitic activity of 180 ± 20 unit/μg and 410 ± 30 unit/μg for the 25-OH-[26,27-^{14}C]D_3 and 1,25-$(OH)_2$-[26,27-^{14}C]D_3, respectively, when tested as described by Tanaka *et al.* (*8*). Furthermore, the 1,25-$(OH)_2$-[26,27-^{14}C]D_3 cochromatographs exactly with synthetic 1,25-$(OH)_2D_3$ (*9*), on high-pressure liquid chromatography, which separates all known metabolites of vitamin D_3 (*10*) (Fig. 1). The possible metabolism of these compounds to $^{14}CO_2$ was then examined in search of a pathway resulting in loss of the ^{3}H and ^{14}C from the 26 and 27 positions.

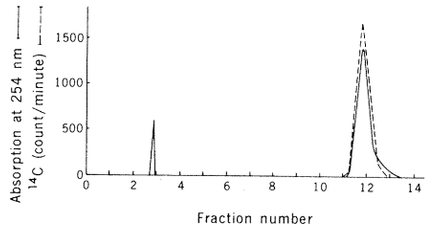

Fig. 1. Cochromatography of 1,25-$(OH)_2$-[26,27-^{14}C]D_3 with crystalline 1,25-$(OH)_2D_3$ (Hoffmann–La Roche Inc.) on a high-pressure liquid chromatograph (Dupont 830) with a fine-grain silicic acid column (*10*) and a solvent of 15 percent isopropanol in Skellysolve B. 1,25-$(OH)_2$-[26,27-^{14}C]D_3 was prepared enzymatically from 25-OH-[26,27-^{14}C]D_3 and mixed with 20 ng of crystalline 1,25-$(OH)_2D_3$; the mixture was injected into the high-pressure liquid chromatograph. The ^{14}C was determined by liquid scintillation with the crystalline 1,25-$(OH)_2D_3$ detected by a monitor at 254 nm.

Male, albino weanling rats (Holtzman) were fed a vitamin D–deficient diet con-

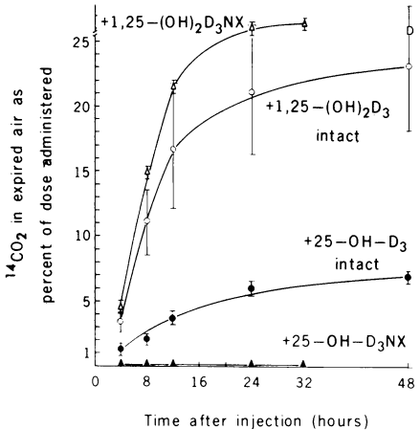

Fig. 2. Cumulative $^{14}CO_2$ production by rats injected with 25-OH-[26,27-^{14}C]D_3 or 1,25-$(OH)_2$-[26,27-^{14}C]D_3. Vitamin D–deficient rats were either left intact or nephrectomized and then injected intrajugularly with 650 pmole of 25-OH-[26,27-^{14}C]D_3 or 325 pmole of 1,25-$(OH)_2$-[26,27-^{14}C]D_3 in 0.05 ml of ethanol; CO_2 was collected at 4, 8, 12, 24, and 48 hours. The $^{14}CO_2$ present at each time point was determined, and the cumulative $^{14}CO_2$ was plotted. The vertical bars represent the standard error of the mean, and there were four rats in each group.

taining 0.02 percent calcium and 0.3 percent phosphorus (*11*). After 3 weeks they were considered vitamin D depleted. Vitamin D deficiency was assessed by a lack of growth and low serum calcium levels (4 to 5 mg/100 ml). At the end of this period the animals were divided into four groups. Groups 1 and 3 received 650 pmole of 25-OH-[26,27-^{14}C]D_3, while groups 2 and 4 received 325 pmole of 1,25-$(OH)_2$-[26,27-^{14}C]D_3 intrajugularly in 0.05 ml of ethanol. Animals in groups 3 and 4 were nephrectomized just prior to the injection of the 25-OH-[26,27-^{14}C]D_3 or 1,25-$(OH)_2$-[26,27-^{14}C]D_3. After injection, all animals were immediately placed in a metabolism apparatus, and all expired CO_2 ($^{12}CO_2$ and $^{14}CO_2$) was trapped in a solution of ethanolamine and methyl Cellusolve (*12*). This solution was changed at 4, 8, 12, 24, and 48 hours in the first two groups and at 4, 8, 12, 24, 28, and 32 hours in groups 3 and 4. Animals in groups 3 and 4 died 36 hours after nephrectomy and, therefore, the experiment was terminated at 32 hours. The $^{14}CO_2$ was determined by liquid scintillation counting (*12*).

The cumulative amounts of $^{14}CO_2$ evolved in the four groups (Fig. 2) indicate that $^{14}CO_2$ is formed as a result of oxidation of at least a portion of the side chain of 25-OH-D_3 and 1,25-$(OH)_2D_3$. Both 25-OH-[26,27-^{14}C]D_3 and 1,25-$(OH)_2$-[26,27-^{14}C]D_3 were metabolized to $^{14}CO_2$; but $^{14}CO_2$ formation was abolished totally in nephrectomized animals given 25-OH-[26,27-^{14}C]D_3, while $^{14}CO_2$ evolution from 1,25-$(OH)_2$-[26,27-^{14}C]D_3 was unchanged by nephrectomy. These data demonstrate that 25-OH-D_3 is almost certainly converted to 1,25-$(OH)_2D_3$ before $^{14}CO_2$ formation from the 26 and 27 carbons occurs. Other extremely unlikely possibilities are that 25-OH-D_3 may be converted solely in the kidney to an unknown compound X, and that 1,25-$(OH)_2D_3$ could be converted to the same intermediate compound at an extra renal site, or alternatively 1,25-$(OH)_2$-[26,27-^{14}C]D_3 forms $^{14}CO_2$ via a pathway totally distinct from that for 25-OH-D_3. These hypotheses are summarized below:

(i) 25-OH-D_3 $\xrightarrow{\text{in kidney}}$ 1,25-$(OH)_2D_3$ $\xrightarrow{?}$ CO_2 + ??

(ii) 25-OH-D_3 $\xrightarrow{\text{only in kidney}}$ compound X $\xrightarrow{?}$ CO_2

1,25-$(OH)_2D_3$ $\xrightarrow{\text{extra renal sites}}$ compound X $\xrightarrow{?}$ CO_2

(iii) 1,25-$(OH)_2D_3$ $\longrightarrow$?Y $\longrightarrow$ CO_2

Although none of these hypotheses can be completely excluded, the first one appears the most likely.

The response of intestinal calcium transport at 4 and 8 hours after a dose of the 1,25-$(OH)_2$-[26,27-^{14}C]D_3 is shown in Fig. 3. In this experiment, groups of six vitamin D–deficient rats prepared as described above were given an intravenous injection of either ethanol vehicle or 130 pmole of 1,25-$(OH)_2$-[26,27-^{14}C]D_3 in ethanol. At either 4 or 8 hours after the dose intestinal calcium transport was determined by the everted sac technique (*13*). The results show a clear response by 4 hours, again illustrating the biological effectiveness of the 1,25-$(OH)_2$-[26,27-^{14}C]D_3 at the time $^{14}CO_2$ was detected. In other experiments, no significant intestinal response was noted at 2 hours after dose with a measurable response at 3 hours, and varying the dose from 650 to 65 pmole of 1,25-$(OH)_2D_3$ did not appreciably alter the time course of intestinal calcium transport response.

It is, therefore, of significance that expired $^{14}CO_2$ begins to appear within 4 hours after administration of 1,25-$(OH)_2$-[26,27-^{14}C]D_3—early enough to be of significance in intestinal calcium absorption. Even if this proves not to be the case, this pathway of 1,25-$(OH)_2D_3$ is quantitatively of major significance, accounting for as much as 30 percent of the

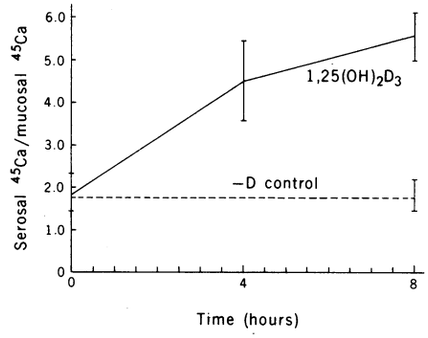

Fig. 3. Intestinal calcium transport response of vitamin D–deficient rats to 1,25-$(OH)_2$-[26,27-^{14}C]D_3. Vitamin D–deficient rats (six in each group) were injected with 0.05 ml of ethanol or 0.05 ml of ethanol containing 130 pmole of 1,25-$(OH)_2$-[26,27-^{14}C]D_3. At 4 or 8 hours after injection, intestinal calcium transport was determined by the everted sac method (*13*).

injected parent compound. Thus, if this pathway represents breakdown or degradation of 1,25-$(OH)_2D_3$, it may have major significance in the control of the tissue levels of 1,25-$(OH)_2D_3$, a potent calcium-mobilizing hormone. Either possibility suggests that this new pathway of vitamin D metabolism may be of major importance to our understanding of vitamin D function.

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14. This work was supported by grant AM-14881 from the Public Health Service, contract E(11-1)-1668 with the Energy Research and Development Administration, and the Harry Steenbock Research Fund of the Wisconsin Alumni Research Foundation.

10 November 1975; revised 28 January 1976