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25-Hydroxyvitamin D_3: Evidence of an Enterohepatic Circulation in Man (38853)

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25-Hydroxyvitamin D_3 (25-OH-D_3) is a metabolite of vitamin D_3 that has greater biologic potency than D_3 (1) and is the predominant form of vitamin D in plasma (2), where it circulates bound to specific carrier proteins (3). The physiologic role of 25-OH-D_3 is not entirely understood, but it is a precursor of more polar and biologically active forms of vitamin D in deficient animals (4, 5). Physiologic quantities may not be directly involved in biologic effects of vitamin D but pharmacologic doses of 25-OH-D_3 have direct effects on calcium transport in tissue culture (6), induce calcium-binding proteins *in vitro* (7), and mobilize calcium from bone in the anephric animal (8).

In the course of studies on the distribution and excretion of small doses (5 μg) of intravenously administered [26,27-^{3}H]-25-hydroxyvitamin D_3 (25-OH-D_3), we discovered that, in addition to a role in the 25-hydroxylation of vitamin D_3 (9), the liver participates in the secretion of this metabolite into the intestine. Subsequent reabsorption of intestinal radioactivity suggests that 25-OH-D_3 undergoes an enterohepatic circulation similar to that of other constituents of bile, such as bile acids (10).

Methods and Materials. In order to quantify the intestinal and plasma distribution of intravenously administered 25-OH-D_3 simultaneously, normal adults (two men and one woman) were prepared for study by an overnight fast and by intubation a few hours before the study. A triple-lumen nasoduodenal tube was positioned so that the first aperture was located proximal to the ampulla of Vater, the second was distal to it at the ligament of Treitz, and the third was located 30 cm distal to the second (11). The lumen ending most proximally was perfused at a rate of 2 ml/min with polyethylene glycol (PEG), 5 g/liter, a nonabsorbable marker used for quantification of duodenal contents. From the second lumen, duodenal aspirates were collected every hour, for 30–36 hr, into a chilled beaker, and the volume was measured; 10-ml samples were taken and the remainder was reinfused through the third lumen.

Meals were given in liquid form while the tube was in place (11). Oral administration of a fecal marker (chromium sesquioxide, 0.5 g three times daily) was started just prior to the intravenous bolus injection of 8–10 μCi of [26,27-^{3}H]-25-OH-D_3[1] (New England Nuclear, 1.2 Ci/mmole) dissolved in 1 ml of propylene glycol. Serial blood specimens and all the urine and feces excreted for 12 days after injection were collected for analysis.

Total ^{3}H in homogenized stool samples (1–2 g) and duodenal aspirates (0.3–0.5 ml) was determined by liquid scintillation counting after combustion in an oxidizer; values were corrected for volume and for recovery of isotope added to the samples. The volume and bile acid content of duodenal contents were determined by previously validated methods (12). Because of variable delays in passage of intestinal contents from duodenum to colon, fecal data were corrected, on the basis of the chromium sesquioxide marker, to correspond to the appropriate blood and duodenal samples (13). For example, complete fecal collections for 12 days

[1] Purity of the administered [26,27-^{3}H]-25-OH-D_3 was established by cochromatography of it with a crystalline standard (kindly provided by Dr. J. Babcock, the Upjohn Company); there was a single symmetric radioactive peak and the uv spectrum of this peak was identical to the uv spectrum of the standard alone.

in each of the three subjects actually represented 9.3, 9.9, and 7.6 days after administration of tracer.

To measure disappearance of labeled 25-OH-D_3 from blood, serum was extracted with methanol–chloroform, 2:1 (vol/vol) (14); the extract was chromatographed on 1 × 30- or 1 × 60-cm columns of Sephadex LH-20 with chloroform–hexane, 1:1 (vol/vol), as solvent system (15). Fractions containing the 25-OH-D_3 peak were freed of solvent, taken up in scintillation solution, and counted. Count rates were corrected for recovery of isotope added to control serum and multiplied by an assumed plasma volume of 39.6 ml/kg (16). The radioactivity was almost entirely extractable and could be recovered in the 25-OH-D_3 chromatographic peak. The fraction of extractable radioactivity that chromatographed as 25-OH-D_3 remained constant throughout the period of study, indicating little circulation of metabolites that may have been formed from tracer.

Results. Within 5 min after injection of labeled 25-OH-D_3, 75%–86% of the administered ^3H could be accounted for in the plasma. The curve of plasma [^3H]-25-OH-D_3 vs time for 12 days is shown in Fig. 1. During the first 6 hr, there was a rapid loss of 45% of the administered dose from plasma at the same time as 17.1% of the dose appeared in the duodenum. Thereafter, radioactivity in plasma decreased more slowly, to 26.7% of the dose at 24 hr, while the cumulative intestinal radioactivity doubled, to 35.5% of the dose.

Fecal radioactivity corresponding to the first 24.7 hr (mean) of study was only 3.03% of the injected dose, indicating reabsorption of at least 85% of the ^3H in the duodenal contents. Fecal excretion of ^3H for the entire period of collection (22.5% in 8.9 days) did not approach the quantity of ^3H in the duodenum in the first 24 hr. We observed parallel increases in bile acids and ^3H in duodenal aspirates (Fig. 1 inset); postprandial increases in both are probably the result of contraction of the gallbladder.

Urinary excretion was small, accounting for 1.47% of the dose in 24 hr, 2.30% in 48 hr, and negligible quantities thereafter.

Discussion. The secretion of vitamin D_3 in bile prior to fecal excretion has been recog-

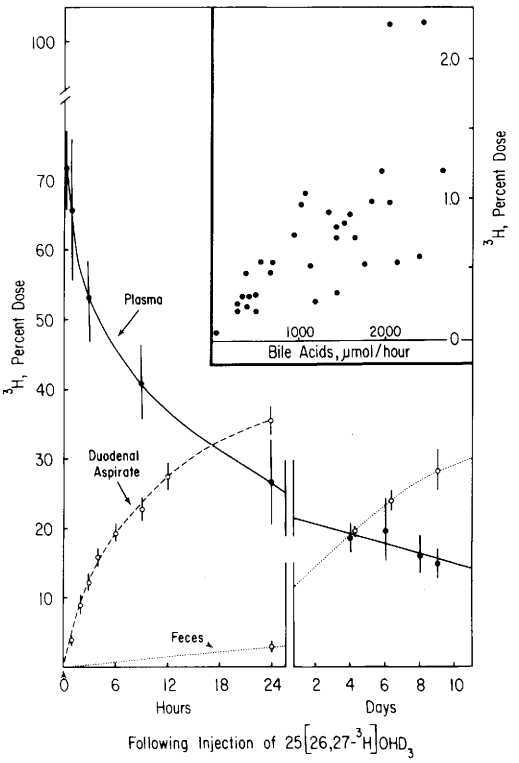

FIG. 1. Each curve is derived from the mean and SE (vertical lines) of values from three normal subjects given an intravenous injection of 8–10 μCi of [26,27-^3H]-25-hydroxyvitamin D_3 (25-OH-D_3). Solid line = plasma disappearance of ^3H in 25-OH-D_3. Broken line = cumulative ^3H in duodenal aspirates. Dotted line = cumulative ^3H in feces (plotted against a time corrected for delay in intestinal transit). Inset, Relationship between ^3H in duodenal aspirates and bile acid secretion rate in the period 14–30 hr after administration of tracer in two subjects; the high correlation coefficient ($r = 0.72$; $p < 0.001$) indicates that the radioactivity is secreted with the bile.

nized in man (17, 18) and animals (19), and the importance of bile for the absorption of orally administered vitamin D is well established (20, 21). In order to compare the behavior of 25-OH-D_3 to that of vitamin D_3, we administered [26,27-^3H]-25-OH-D_3 and [4-^{14}C]-D_3 simultaneously in one subject; compared to ^3H, only one-half as much ^{14}C appeared in the duodenal aspirates in the first 6 hr but twice as much ^{14}C was in the 6-day fecal collection. These data are slightly at variance with the vitamin D_3 data of Avioli *et al.* (18), who measured isotope recovery in T-tube drainage of postcholecys-

tectomy patients, but this may be accounted for by the slight biliary dysfunction in their patients (22).

The early disappearance of [^{14}C]-D_3 from plasma in our subject was identical to that reported by Smith and Goodman (3) in healthy adults and slower than that of the simultaneously administered ^{3}H-labeled 25-OH-D_3. This suggests that the more rapid plasma disappearance of 25-OH-D_3 is not the result of our method of study but is due to more rapid distribution of this metabolite. Although this study was concerned only with the early phases of plasma decay, radioactivity chromatographing with 25-OH-D_3 was detected for as long as 131 days after injection.

Summary. Within 24 hr after intravenous administration of isotopic 25-hydroxyvitamin D_3 to three normal adults for kinetic studies, one-third of the radioactivity was secreted into the lumen of the duodenum, probably with the bile. The subsequent intestinal reabsorption of over 85% of secreted radioactivity suggests that this major metabolite of vitamin D has a hitherto unrecognized enterohepatic circulation.

Our observation of a dynamic hepatic secretion and intestinal reabsorption of radioactivity administered as ^{3}H-labeled 25-hydroxyvitamin D_3 to vitamin D-replete man is indicative of an enterohepatic circulation that may be of physiologic importance. It is conceivable that interruption in the recycling of 25-OH-D_3 may be an important mechanism of acquired deficiency of vitamin D in gastrointestinal disease.

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