

Faecal tritium excretion after intravenous administration of ^3H -25-hydroxyvitamin D_3 in control subjects and in patients with malabsorption

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SUMMARY Faecal tritium excretion after intravenous ^3H -25-hydroxyvitamin D_3 administration was measured in three control subjects and in six patients with small intestinal resection or bypass. The mean daily faecal tritium excretion over four to six days ranged from 0.8-1.6% of the injected dose in the controls (mean 1.2) and 0.9-6.8% in the patients (mean 3.7). There was a significant positive correlation between stool volume and the mean daily faecal tritium excretion. No correlation was found between the faecal tritium excretion and the plasma 25-hydroxyvitamin D concentration. Between 2.5 and 19.0% of faecal radioactivity eluted as ^3H -25-hydroxyvitamin D_3 on silicic acid chromatography. We conclude that faecal loss of endogenous 25-hydroxyvitamin D may be increased after small intestinal resection or bypass. Although the amount lost by this route is relatively small, it may contribute to the development of vitamin D deficiency in patients with malabsorption when endogenous vitamin D_3 synthesis is also reduced.

Low plasma 25-hydroxyvitamin D (25OHD) concentrations, indicating vitamin D deficiency, are common in patients with small intestinal disease, resection or bypass¹⁻⁵ and histological evidence of osteomalacia may also occur.¹⁻⁴ The pathogenesis of vitamin D deficiency and osteomalacia in these patients is not fully understood. As endogenous vitamin D_3 synthesis is normally the major source of vitamin D in man,^{6,7} a poor dietary intake or malabsorption of dietary vitamin D cannot explain vitamin D deficiency provided that exposure to ultraviolet irradiation is adequate.

Evidence that 25OHD may undergo enterohepatic circulation in man⁸ has led to the suggestion that malabsorption of the metabolite from an enterohepatic circulation could account for loss of both endogenously and exogenously derived vitamin D in patients with small intestinal disease. Arnaud *et al.*⁹ reported that the plasma half-life of intravenously administered ^3H -25OHD₃ was reduced in four patients with coeliac dis-

ease when compared with normal controls and that faecal tritium excretion was accelerated in the patients with malabsorption. We have studied faecal tritium excretion after intravenous ^3H -25OHD₃ in three control subjects and in six patients with intestinal resection or bypass. In addition, we have performed chromatographic studies to establish whether unchanged ^3H -25OHD₃ is present in the faecal radioactivity.

Methods

PATIENTS

Three control subjects and six patients with small intestinal resection or bypass were studied. Clinical details are shown in Table 1. The control subjects had no symptoms or past history of gastrointestinal disease. In two of the controls (subjects 1 and 2) radiological examination of the small intestine and jejunal biopsy were normal. Plasma creatinine and liver function tests were normal in all nine subjects. No patient was receiving anticonvulsant therapy or cholestyramine. Patients 7 and 8 were taking oral prednisone, 5 and 7.5 mg daily respectively, for active Crohn's disease of the small intestine. None of the patients was receiving any vitamin D therapy. The study was approved by

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Table 1 Clinical details of nine subjects

Patient no.	Age (yr) Sex	Diagnosis	Length of remaining intestine (cm)	Colonic resection	Time since operation (yr)
1	22F	Sciatica	Normal	None	—
2	38F	Nodular prurigo	Normal	None	—
3	55F	Post-viral meningitis	Normal	None	—
4	27F	JIB	36*	None	3
5	41F	JIB	36*	None	4
6	47F	JIB	36*	None	2
7	43F	Crohn's	140	R hemi-colon	2
8	27M	Crohn's	152	None	2
9	58F	Radiation enteritis	140	Sigmoid	5

* In continuity
JIB: Jejunioileal bypass (end-to-side anastomosis of 18 cm jejunum to 18 cm ileum).

the Hospital Ethical Committee and informed consent was obtained from all subjects. The study was carried out between October 1979 and April 1980.

Methods

25-hydroxy-(23,24 (n)-³H)vitamin D₃, 110 Ci/mmol (4.07 TBq/mmol) was obtained from the Radiochemical Centre, Amersham, Bucks, UK, 10 μCi (0.37 MBq) in 250 μl toluene/ethanol 1:1 vol/vol was dried under nitrogen and redissolved in 2 ml absolute alcohol. Eighteen millilitres of distilled water were added to make up the injection. Fifty microlitres of the solution were withdrawn immediately before the injection and their radioactivity estimated. The mean recovery of radioactivity after preparation of the injection was 74.9%, losses mainly occurring during the filtration procedure. The solution was injected intravenously over a period of three minutes. All faecal specimens were then collected in 24 hour periods for four to six days. Specimens from at least four days were collected from each subject.

ESTIMATION OF FAECAL RADIOACTIVITY

Twenty-four hour faecal samples were homogenised, adding water when necessary, to give final homogenate volumes of between 115 and 3044 ml. Ten millilitre aliquots of the 24 hour faecal homogenates were evaporated to dryness in a graphite bath at 200-250°C for two hours and the dried faeces ground to a fine powder. Approximately 100 mg of the dried aliquot was placed in Visking tubing and combusted in an oxygen flask.¹⁰ Combustion of all samples was performed in duplicate. The mean recovery of radioactivity after homogen-

isation, drying, and combustion was 75.5% ± 1.5% (mean ± SD), the recovery being determined by addition of a known amount of radioactivity to unlabelled faeces in four duplicate experiments.

DETERMINATION OF EFFECT OF DILUTION OF FAECAL SAMPLES ON MEASURED RADIOACTIVITY

An unlabelled 24 hour faecal sample was homogenised with water to give a volume of 500 ml. ³H-250HD₃ (99754 dpm) was added to the homogenate and dilutions of 1 in 2, 1 in 4, and 1 in 8 were made, corresponding with total faecal homogenate volumes of 1000, 2000, and 4000 ml. Approximately 5 mg of dried weight from each dilution was combusted as previously described; samples being combusted in duplicate. The percentage recovery from the dilutions was calculated after correction for procedural losses.

EXTRACTION AND CHROMATOGRAPHY OF FAECES

Five grams of freeze dried faeces were extracted with chloroform/methanol 2:1 vol/vol by a method based on that of Bligh and Dyer.¹¹ The chloroform extract was dried under nitrogen and reconstituted in 15 ml chloroform. This was passed through a Type HA Millipore filter (0.45 μm), the filter subsequently being washed through three times with 6 ml chloroform. The filtrate was then dried under nitrogen, redissolved in 2 ml n-hexane, and applied to a 30 × 1 cm silicic acid column (SIL-B-200, 200 mesh, Sigma London Chemical Co, Poole, Dorset, UK).¹² The recovery of the procedure was estimated by addition of a known amount of radioactivity to unlabelled freeze-dried faeces. The mean ± SD recovery of ³H-250HD₃ after extraction, filtration, and chromatography of the faeces in five separate experiments was 44.5 ± 3.4%.

MEASUREMENT OF RADIOACTIVITY

Radioactivity was determined by liquid scintillation counting using Nuclear Enterprises 260 scintillation fluid (Micellar Scintillator, Sighthills, Edinburgh, UK) and an LKB 1215 Rack Beta Scintillation Counter (efficiency 62%). Faecal samples were bleached with 0.5 ml 30% hydrogen peroxide when necessary. Correction for quenching was performed by the channels ratio method.

PLASMA 250HD CONCENTRATION

Plasma 250HD concentrations were measured by a competitive protein-binding assay¹³ after extraction and chromatography using normal human serum as binding protein.

FAECAL FAT EXCRETION

Faecal fat excretion over the period of the study was estimated by the method of van de Kamer *et al.*¹⁴

CALCULATION OF RESULTS

The faecal tritium excretion was corrected for losses occurring during the preparation of the injection and combustion of the faecal samples as determined in the recovery experiments. The results were expressed as the mean daily tritium excretion as a percentage of the dose administered. The percentage of radioactivity eluting as ^3H -250HD₃ was expressed as a percentage of the total tritium content in the 24 hour stool specimen. Correction was made for losses occurring during extraction, filtration, and chromatography as determined in the recovery experiments.

STATISTICAL ANALYSIS

Statistical analysis was performed using the Spearman rank correlation coefficient.

Results

FAECAL TRITIUM EXCRETION (Table 2)

The mean daily faecal tritium excretion expressed as a percentage of the intravenous dose ranged from 0.8-1.6 in the three control subjects (mean 1.2%) and from 0.9-6.8 in the patients (mean 3.7%). The mean daily faecal tritium excretion was higher than the control values in four of the patients with malabsorption, although in one of these (patient 8) the difference was small. In the remaining two patients with malabsorption the values were similar to those found in the control group. There was a significant positive correlation between the mean daily faecal tritium excretion and stool volume in the nine subjects studied ($r = 0.75$, $P < 0.05$) (Fig 1). There was also a positive correlation between the mean daily faecal tritium excretion and faecal fat excretion in the six patients with malabsorption ($r = 0.77$) but this was not statistically significant. No correlation was found between the mean daily faecal tritium excretion and the plasma 250HD concentration.

Table 2 Faecal data and plasma 250HD concentrations in nine subjects

Patient no.	Mean stool volume g/23 h	Faecal fat excretion (mmol/24 h*)	Mean faecal ^3H excretion (%/dose/day)	Plasma 250HD (nmol/l)†
1	92	NE	1.3	40.5
2	108	NE	0.8	11.0
3	44	NE	1.6	16.2
4	1408	724	6.1	<2.5
5	972	52	4.4	66.0
6	298	192	1.5	13.5
7	150	36	0.9	4.2
8	180	50	2.5	17.0
9	2525	328	6.8	37.5

NE: not estimated

*Normal < 17 mmol/24 hours.

†Normal 12-100 nmol/l.

The maximal faecal tritium excretion occurred on day 2 or day 3 in eight of the nine subjects. The cumulative faecal tritium excretion is shown in Fig 2. At four days after injection of ^3H -250HD₃ 2.41-8.5% of the tritium had been excreted in the controls (mean 5.5) and 3.03-27.2% in the patients (mean 14.5%).

The recovery of radioactivity from increasing dilutions of faecal homogenate is shown in Table 3. There was a small decrease in recovery with increasing volume of faecal homogenate, but even when the aliquot for combustion was taken from a volume of 4000 ml of faecal homogenate 84% of the added radioactivity was recovered.

FAECAL EXTRACTION AND CHROMATOGRAPHY

Faecal extraction and chromatography were carried out in seven subjects (one control and six patients), the analysis being performed on one 24 hour stool sample in each case, from day 1 (two subjects), day 2 (three subjects), or day 3 (two subjects). After extraction the percentage of radioactivity in the chloroform layer ranged from 26-79% (mean 47%). In the control subject (patient 3) 8.9% of the faecal radioactivity eluted as ^3H -250HD₃; in the six patients with malabsorption values ranged from 2.5-19.0% (mean 9.7%).

PLASMA 250HD CONCENTRATIONS (Table 2)

The plasma 250HD concentration was just below the lower limit of normal in one control subject and was greatly reduced in two of the patients with malabsorption. In the remaining six subjects the plasma 250HD concentration was within the normal range.

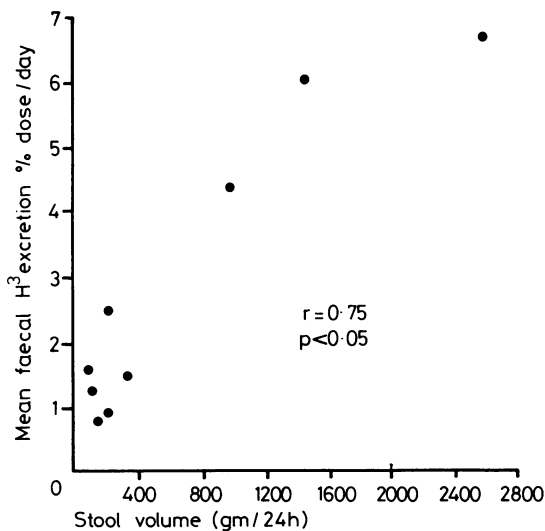


Fig 1 Relationship between daily faecal tritium excretion and stool volume in the nine subjects studied.

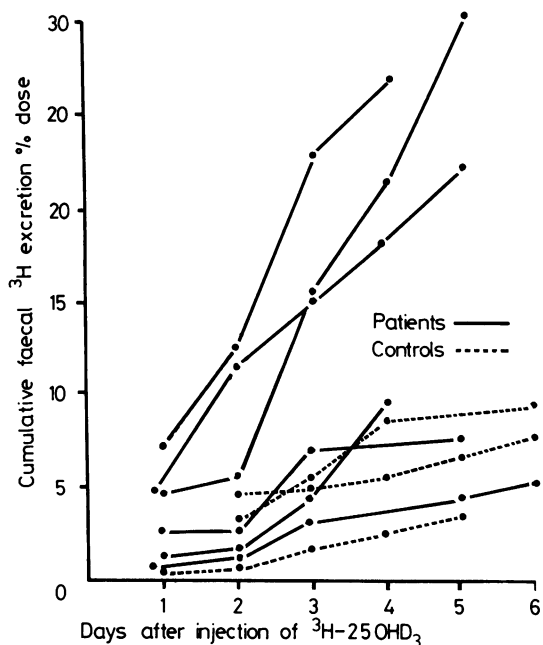


Fig 2 Cumulative faecal tritium excretion expressed as a percentage of the injected dose of ^3H -25-hydroxyvitamin D_3 in the control subjects (.....) and in the patients with malabsorption(——).

Table 3 Recovery of radioactivity from increasing dilutions of faecal homogenate

Vol. faecal homogenate (ml)	DPM/mg dry weight	% Recovery
500	817.5	97.5
1000	772.2	92.0
2000	733.4	87.3
4000	703.1	83.5

Discussion

Our results demonstrate that faecal tritium excretion after intravenous ^3H -250HD₃ may be increased after intestinal bypass or resection and that unchanged ^3H -250HD₃ is present in the faecal radioactivity. These findings support the concept that increased faecal loss from an enterohepatic circulation may contribute to the development of vitamin D deficiency in some patients with intestinal malabsorption.

Previous studies of faecal radioactivity after intravenous ^3H -250HD₃ in normal subjects have demonstrated a mean daily faecal tritium excretion of 2–3%

of the administered dose^{8 9 15 16}; these values are similar to, although slightly higher than, those found in the present study. Gray *et al.*¹⁵ also demonstrated the presence of unchanged ^3H -250HD₃ in the faecal radioactivity, although the amount was not stated. Two previous studies in patients with intestinal disease have produced conflicting results. Mawer¹⁶ reported that faecal tritium excretion after intravenous ^3H -250HD₃ was similar in two ileostomy patients and one normal control subject, whereas Arnaud *et al.*⁹ found that faecal tritium excretion was greater in the first three days after intravenous ^3H -250HD₃ in four patients with coeliac disease when compared with normal subjects. Analysis of faecal radioactivity was not reported by Arnaud *et al.*; Mawer found that less than 1% of the administered dose of ^3H -250HD₃ appeared unchanged in ileostomy fluid. Our results demonstrate that faecal tritium excretion after intravenous ^3H -250HD₃ may be normal or increased in patients with intestinal resection or bypass and, in addition, show that between 2.5 and 19% of faecal radioactivity may be present as unchanged ^3H -250HD₃.

Evidence that 250HD undergoes enterohepatic circulation requires demonstration both of its biliary secretion and intestinal reabsorption. Although biliary excretion of metabolites of injected ^3H -250HD₃ is well documented in man, there is as yet no evidence that reabsorption of 250HD itself occurs from the intestine; thus biliary excretion might serve to eliminate rather than to conserve the metabolite. Quantitative studies of biliary radioactivity in man after intravenous ^3H -250HD₃ have been reported by two groups. Arnaud *et al.*,⁸ in a study of three normal subjects, found that 35% of the injected radioactivity appeared in bile within the first 24 hours, but did not characterise the radioactivity; Gray *et al.*¹⁵ reported a figure of 12% in one patient with a biliary fistula, most of the radioactivity being in the form of polar metabolites. In contrast, a greater proportion of faecal radioactivity is chloroform soluble,^{15 17} suggesting either that the more polar metabolites are preferentially reabsorbed or that the biliary metabolites are altered within the intestinal lumen—for example, by bacterial metabolism. Reabsorption of water soluble conjugates would support the concept that biliary excretion serves to eliminate the vitamin, as the biological activity of the more polar metabolites is low.¹⁶ However, the intraluminal release of 250HD from biliary conjugates and subsequent reabsorption of the metabolite would provide a mechanism for its conservation which could be damaged by intestinal disease, resection, or bypass.

Faecal tritium excretion varied considerably in the patient group, being raised in three patients and near normal or normal in the remaining three; these differences could not be explained on the basis of remaining functioning small intestinal length. There was a posi-

tive correlation between faecal tritium excretion and both stool volume and faecal fat excretion, although the latter relationship was not statistically significant, but there was no correlation between faecal tritium excretion and the plasma 250HD concentration. Although the large dilution factor and the low concentration of radioactivity in the patients with high stool volumes was a potential source of inaccuracy, recovery experiments performed on similar or greater volumes of faecal homogenates demonstrated that these relatively small amounts of radioactivity could be detected with reasonable accuracy. Thus our results suggest that factors determining stool volume and faecal fat excretion were more important than vitamin D status in determining faecal tritium excretion; these factors may include dietary fat intake¹⁸ and fluid consumption,¹⁹ transit time,²⁰ bacterial contamination of the small intestine,²¹ and the degree of compensatory structural and functional changes in the remaining small intestine.²²⁻²³ In addition, the presence of active disease in patients with Crohn's disease or radiation enteritis might further impair small intestinal function.

The amount of 250HD excreted in the faeces both in normal subjects and in the patients with malabsorption was small in relation to the total body pool and would be unlikely to result in vitamin D depletion unless endogenous vitamin D₃ synthesis was also reduced. Thus patient 9, who had a comparatively high faecal tritium loss, maintained a normal plasma 250HD concentration; however, in patients with less exposure to ultraviolet irradiation, similar faecal losses may gradually lead to vitamin D depletion, as in patient 4. This suggests that the contribution made by increased faecal loss to the development of vitamin D deficiency in patients with malabsorption depends critically upon the rate of endogenous vitamin D₃ synthesis. As the amounts synthesised in the skin in subjects with normal exposure to ultraviolet irradiation are large relative to the faecal losses,²⁴ a substantial reduction in endogenous synthesis over a period of time is probably required before faecal 250HD loss results in vitamin D deficiency.

As biliary radioactivity was not measured in this study the possibility that some of the faecal radioactivity was derived from isotope passing through the intestinal mucosa cannot be excluded. In diseases affecting the intestinal mucosa, such as Crohn's disease or coeliac disease, loss of 250HD by this route might be increased; if 250HD passes through the intestinal wall unchanged, as has been reported for 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) in rats,¹⁶ the contribution made by ³H-250HD₃ to the total faecal radioactivity might be greater than if excretion occurred only by the biliary route. In addition, our results do not exclude the possibility that increased biliary excretion of 250HD or reduced hepatic metabolism might have contributed to

the increased faecal tritium loss in some patients with malabsorption.

Although a tracer dose of ³H-250HD₃ was used in our experiments, its administration as a bolus may be unphysiological and lead to falsely high biliary excretion. Nevertheless, differences between the control and patient group were observed using an identical method of administration. We conclude that increased faecal loss of endogenous 250HD may occur after intestinal bypass or resection. The development of vitamin D deficiency and osteomalacia in these patients is probably multifactorial and the rate of endogenous vitamin D₃ synthesis is likely to be an important determinant. In addition, end-organ resistance of the intestine to normal circulating concentrations of vitamin D metabolites may also contribute to the development of malabsorption osteomalacia.

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