THE METABOLISM OF ISOTOPICALLY LABELLED VITAMIN D₃ IN MAN: THE INFLUENCE OF THE STATE OF VITAMIN D NUTRITION

E. BARBARA MAWER, G. A. LUMB, K. SCHAEFER* AND S. W. STANBURY

Division of Metabolism, University Department of Medicine, The Royal Infirmary, Manchester

(Received 20 May 1970)

SUMMARY

1. The metabolism of radioactive vitamin D₃ has been studied in individuals low or deficient in vitamin D (group I) and in vitamin D treated subjects (group II).
2. In group I there was a smaller serum pool of vitamin D, turning over more rapidly than in group II. The principal metabolite, peak IV, appeared more rapidly in the serum of group I; the level of radioactivity attained in this and in the more polar metabolites, peak V and VI, was also higher than in group II. Peak VI was the major radioactive component in serum after 100 days.
3. Vitamin D treatment of individuals in group I converted the pattern of metabolism of radioactive vitamin D₃ to that characteristic of group II. This effect was observed in healthy individuals and in patients with vitamin D deficiency or with chronic renal failure.
4. The metabolic disposal of vitamin D entering the body appears to be determined by the state of vitamin D nutrition in the individual. Reported changes of vitamin D metabolism in diseases such as renal failure could be determined by the nutritional state of the patients studied rather than by the primary disease.

The metabolism in man of a test dose of isotopically labelled vitamin D has been studied by several groups of workers during the last few years. Administration of the radioactive vitamin was either by mouth (Thompson, Lewis & Booth, 1966; Avioli, Birge, Lee & Slatopolsky, 1968) or by intravenous injection (Avioli, Lee, McDonald, Lund & Deluca, 1967a; Mawer & Stanbury, 1968). The methods have involved serial sampling of serum after administration of the test dose with subsequent measurement of the radioactivity due to vitamin D and its metabolites. Separation of metabolites has been achieved on silicic acid columns; more components have been resolved as techniques have been refined (Haussler, Myrtle & Norman, 1968; Mawer & Backhouse, 1969; Ponchon & DeLuca, 1969).

* Present address, Medizinische Klinik und Poliklinik der Freien Universität, Berlin.
Correspondence: Professor S. W. Stanbury, Division of Metabolism, University Department of Medicine, The Royal Infirmary, Manchester M13 9WL.
Differences in the metabolism of such a labelled dose of vitamin D have been claimed in studies of patients suffering from various pathological conditions (Avioli, Williams, Lund & DeLuca, 1967b; Avioli et al, 1968; DeLuca, Lund, Rosenbloom & Lobeck, 1967). The present investigation was initiated as an extension of our studies of the apparent vitamin D resistance in patients with chronic renal failure (Stanbury & Lumb, 1962, 1967; Stanbury, Lumb & Mawer, 1969; Lumb, Mawer & Stanbury, 1970). In examining healthy people it soon became apparent that an important factor affecting the disposal of the test dose of radioactive cholecalciferol in an individual was his state of vitamin D nutrition. The same factor also appeared to operate in patients with chronic renal failure. In some of the studies reported by Avioli et al. (1967a, 1968), the subjects received 800 IU/day of vitamin D for the 14 days prior to the administration of the radioactive tracer. It was implied that this procedure would induce comparable states of vitamin D nutrition in the patients studied and in the healthy individuals used as controls. This assumption was not tested by biological assay of the vitamin D activity in the serum of the groups compared. Earlier studies from our laboratory had demonstrated the prolonged persistence in human serum of radioactive vitamin D_3_ and its metabolites (Mawer, Lumb & Stanbury, 1969) and, similarly, of assayable vitamin D activity in subjects who had received a course of treatment with the vitamin (Lumb et al., 1970). It thus seems likely that the previous intake of vitamin D by an individual would remain relevant over a correspondingly long period.

In the study reported here the metabolism of labelled vitamin D_3_ was observed for 10 days following intravenous injection in two groups of subjects selected on the basis of differences in their state of vitamin D nutrition. The members of the first group had received no supplementary vitamin D; they were either patients with clinical evidence of vitamin D deficiency or healthy individuals with a habitually low intake of the vitamin. Their estimated dietary intake and biologically assayed serum vitamin D activity (0-0.4 IU/ml) are shown in Table 1. The second group of subjects had received known doses of vitamin D and their levels of serum activity were correspondingly raised (0.7 to 5.9 IU/ml; Table 1). Three subjects were studied before and after treatment with vitamin D.

Our previous work had shown that the curve of decay of vitamin D_3_ in human serum was biphasic (Mawer et al., 1969). In the present study the characteristics of the two phases of decay of vitamin D, and of the decay of the principal radioactive metabolite of the vitamin, were determined for each subject. The differences in these parameters between the two groups indicate that the state of vitamin D nutrition and the previous intake of the vitamin markedly affect the metabolism of a test dose.

**METHODS**

**Subjects and investigative procedure**

Nine volunteer subjects, details of whom are given in Table 1, participated in the main part of the study. The experiment was explained in detail and the subjects gave informed consent to the procedure. Three of the untreated group were healthy individuals who had low serum vitamin D activity and the habitually low dietary intake of the vitamin characteristic of the British (Lumb et al., 1970). The remaining three were suffering from vitamin D deficiency osteomalacia in association with intestinal malabsorption. Except for D.K., with obstructive jaundice due to chronic biliary cirrhosis, the dietary intake of vitamin D in these patients was also very low. In the treated group two subjects were clinically normal and had taken vitamin
Subsequent to the test D.K. received a total oral dose of 8 x 10^5 IU of vitamin D_3 prior to her death. Blood and tissues obtained at autopsy contained barely detectable amounts of vitamin D activity (to be published). It is evident that her vitamin D deficiency was due to intestinal malabsorption of the vitamin and not to failure of its hepatic hydroxylation (see Table 2), as implied by Ponchon & DeLuca (1969).

D_3 for the purpose of the investigation; the remainder had received vitamin D as treatment for osteomalacia (Table 1). When two radioisotopic studies were performed in the same person, at least 1 month elapsed before the second observation The total therapeutic dose of vitamin D_3 received by each subject in the treated group, prior to injection of radioactive cholecalciferol, is shown in Table 1. Five patients continued to receive their daily therapeutic dose of vitamin D_3 throughout the period of study. The respective oral dose, in IU/day, was: O.C.,
Other 'treated' subjects received only the total dose shown in Table 1. The patient H.B. had previously received massive amounts of vitamin D₂ as treatment for acquired hypophosphataemic osteomalacia, associated with renal phosphaturia and glycosuria; at the time of study, she had taken no vitamin D for 25 months. Four days after administration of radioactive cholecalciferol she was submitted to a mid-thigh amputation for fibrosarcoma. A preliminary account of the distribution of the test dose and its metabolites in the tissues of the amputated leg has been reported (Mawer & Schaefer, 1969); a more comprehensive account and clinical description will be published elsewhere (Stanbury, Ball, Lumb & Mawer, in preparation). The patient T.W. earlier had pseudohyperaldosteronism due to habitual consumption of liquorice; he was found to have haemochromatosis, possibly caused by the high iron content of the liquorice ingested (Holmes, Marrott, Young & Prentice, 1970). Since conventional tests of liver function were all normal, he was regarded as 'normal' for the purpose of this investigation and his therapeutic venesections were used to obtain large amounts of vitamin D metabolites from the plasma.

The tracer dose was dispersed in 'Intralipid' and injected intravenously as described previously (Mawer et al., 1969); at least 5 min was taken for the administration to avoid production of venous pain. The dose of [4-¹⁴C]cholecalciferol, 19.2 mCi/mmmole (Philips-Duphar, Petten, Holland), or [1,2-³H₂]cholecalciferol, 32.0 mCi/mmmole (New England Nuclear Corporation, Boston), given to each subject is shown in Table 1.

Serum samples were collected at 0.5, 1, 2, 4, 7 and 10 days and at longer intervals in some cases. This investigation is not concerned with the changes that occur in serum during the first 5–8 h after the injection (Avioli et al., 1967a) and which are apparently related to uptake of the tracer by the liver and its subsequent release (Ponchon & DeLuca, 1969).

**Extraction and chromatography**

Chloroform–methanol extracts of serum were prepared and chromatographed as already described (Mawer & Backhouse, 1969). Because of the large number of fractions produced by the published method, the silicic acid chromatography was modified to limit the number of samples for scintillation counting. Instead of collection as 10 ml fractions, the total volume of each eluant solvent mixture (see Fig. 1) was collected in an Erlenmeyer flask; a further 50 ml wash of the same solvent mixture was then collected separately before proceeding to the next step in the elution. If the radioactive counts recovered in the 50 ml wash were above the background level the chromatograph was rejected. This happened only rarely and was thought to be due to variation in the degree of activation of the silicic acid. The batch fractions were designated A to F and the wash volumes as C⁺, D⁺, etc. (Fig. 1). This batchwise collection of radioactive peaks was checked by running duplicate samples of some extracts in the conventional manner and collecting 10 ml fractions. No difference was observed in the distribution of counts between the peaks, whether collected batchwise or as 10 ml fractions.

**Measurements of radioactivity**

The radioactivity as ¹⁴C or ³H was measured in a Packard Tri-Carb 4000, automatic liquid scintillation counter. The solvent was evaporated from each column fraction and the residue dissolved in 10 ml of scintillator solution (100 mg 1,4-bis(5-phenyloxazol-2-yl) benzene and 4.0 g 2,5-diphenyloxazole per 1 toluene).
Counting efficiency was determined by the channels ratio method (Bruno & Christian, 1961) when appropriate and by the use of n-[1,2-3H₂]- or [14C]hexadecane as internal standard for pigmented samples.

The results were corrected for dose and for body weight and were expressed as

\[
\text{disintegrations min}^{-1} \text{I of serum}^{-1} \quad \frac{\text{disintegrations in dose min}^{-1} \text{kg body weight}^{-1}}{}
\]

This ponderous expression is abbreviated subsequently to 'corrected dpm/l'.

**Biological assay**

The vitamin D activity of serum was assayed by a 10-day prophylactic line test procedure, as described in detail elsewhere (Lumb et al., 1970).

When the assay value in Table 1 is recorded as apparently zero, this implies that the degree of ricketic healing produced by the dose of serum used (0·2–0·3 ml/day for each rat) was either less than that produced by the lowest standard (0·05 IU/day for each rat) or no better than that.

**FIG. 1.** The chromatographic profile of chloroform extracts of serum obtained from different subjects between 4 and 133 days after administering the test dose of cholecalciferol. Details in the text. The volumes of serum extracted were 2·5, 5·0 and 20 ml in subjects O.C., T.W. and B.M. respectively.
which resulted from administration of the arachis oil used as solvent for the standards. In M.C.
and D.K. assay of the chloroform extract of larger volumes of material proved that only trivial
amounts of vitamin D activity were present.
The estimate of potency and its 95% fiducial limits (Table 3) were calculated by a double
analysis of variance, devised by Mr H. Gresley-Grey and based on the methods described by
Finney (1964).

RESULTS

Silicic acid chromatography

The chromatographic profiles of chloroform extracts of serum, obtained from day 4 to day
133 after injection in different treated subjects, are shown in Fig. 1. A clear separation into the
different peaks is evident. Peaks I and II run in the positions of cholecalciferyl esters and pre-

![Graph](image)

Fig. 2. The decay curve of vitamin D$_3$ in the serum. The data plotted are the mean experimental
values from all twelve subjects in the untreated and treated groups. The regression line B represents
the second phase of decay; its extrapolation back to zero is represented by line B'. The values
on B' corresponding with days 0.5-2 were subtracted from the observed points. The line A is the
regression through the values thus derived by difference; it represents the first phase of decay. The
gradients of regression A and B give a measure of the half-lives of the two phases. $T_1 = \log 2/
gradient$. For the combined data $T_1A = 0.596$ days, $T_1B = 3.680$ days.

cholecalciferol respectively. Peak III is unchanged cholecalciferol. In the batchwise elution
system fraction A contains peaks I–III. Peak IV co-chromatographs with 25-OH-chole-
calciferol, which has been shown to be the major metabolite in the pig and to have at least the
biological potency of cholecalciferol (Blunt, Tanaka & DeLuca, 1968). Peaks V, VI and VII
remain unidentified and have little or no biological activity in our assay system. Peak V has
similar chromatographic mobility to peak P of the Cambridge group (Lawson, Wilson & Kodicek, 1969) and to peak 4b of Haussler et al. (1968).

Peak VI, which is the major metabolite persisting after about 100 days, appears to be labile on alkaline hydrolysis. The radioactivity thus lost is no longer extractable by chloroform.

**Time course of vitamin D decay and the appearance of peaks IV to VI (pooled data)**

The corrected dpm/l values for peak III decayed as a double exponential function of time. The decay curves were resolved into two exponential components (Goodman & Noble, 1968).

**TABLE 2. The decay of plasma vitamin D₃ (peak III): the appearance and decay of peak IV**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Peak III</th>
<th>Peak IV</th>
<th>Peak level (corrected dpm/l)</th>
<th>Peak day (day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T₁ (1st phase)</td>
<td>T₂ (2nd phase)</td>
<td>T₄ (days)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(days)</td>
<td>(days)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Untreated group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M.C.</td>
<td>0.607</td>
<td>5.76</td>
<td>27.1</td>
<td>3.35</td>
</tr>
<tr>
<td>A.J.</td>
<td>0.582</td>
<td>2.72</td>
<td>15.2</td>
<td>4.21</td>
</tr>
<tr>
<td>D.K.</td>
<td>0.276</td>
<td>2.99</td>
<td>12.0</td>
<td>4.00</td>
</tr>
<tr>
<td>B.M.</td>
<td>0.363</td>
<td>7.92</td>
<td>19.9</td>
<td>4.25</td>
</tr>
<tr>
<td>F.McC.</td>
<td>0.285</td>
<td>2.99</td>
<td>10.5</td>
<td>3.76</td>
</tr>
<tr>
<td>A.R.</td>
<td>0.327</td>
<td>4.70</td>
<td>10.6</td>
<td>2.86</td>
</tr>
<tr>
<td>Mean</td>
<td>0.407</td>
<td>4.51</td>
<td>15.9</td>
<td>3.73</td>
</tr>
<tr>
<td>SEM</td>
<td>0.061</td>
<td>0.84</td>
<td>2.7</td>
<td>0.22</td>
</tr>
<tr>
<td><strong>Treated group</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>H.B.</td>
<td>0.825</td>
<td>7.03</td>
<td>27.5</td>
<td>0.42</td>
</tr>
<tr>
<td>O.C.</td>
<td>0.898</td>
<td>3.43</td>
<td>26.2</td>
<td>0.65</td>
</tr>
<tr>
<td>B.M.</td>
<td>0.594</td>
<td>5.42</td>
<td>43.6</td>
<td>1.82</td>
</tr>
<tr>
<td>F.McC.</td>
<td>0.703</td>
<td>3.17</td>
<td>19.5</td>
<td>0.22</td>
</tr>
<tr>
<td>A.R.</td>
<td>0.616</td>
<td>5.12</td>
<td>30.8</td>
<td>0.38</td>
</tr>
<tr>
<td>T.W.</td>
<td>0.641</td>
<td>2.89</td>
<td>36.4</td>
<td>0.40</td>
</tr>
<tr>
<td>Mean</td>
<td>0.713</td>
<td>4.51</td>
<td>30.7</td>
<td>0.65</td>
</tr>
<tr>
<td>SEM</td>
<td>0.050</td>
<td>0.66</td>
<td>3.4</td>
<td>0.24</td>
</tr>
<tr>
<td><strong>P</strong></td>
<td>&lt;0.005*</td>
<td>&gt;0.999*</td>
<td>&lt;0.02*</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

*The significance of the difference between the mean values of the two groups was estimated by Student's t-test, using a two-tailed test with 10 degrees of freedom.
†The significance was estimated by a non-parametric method, the Mann-Whitney U-test.

The method is exemplified in Fig. 2, in which peak III data for all twelve subjects of the treated and untreated groups is combined. A more detailed interpretation of these results involving the use of a 2-compartment model will be the subject of a subsequent publication (Mawer, Mawer & Lucas, in preparation). Values for the half-life (T₄) of the two phases were obtained for each subject by weighted regression analysis and are shown in Table 2. The T₄ of the first phase of vitamin D₃ decay was much shorter in the untreated than in the treated group (untreated 0.41, treated 0.71 days; P<0.005). No statistically significant differences between the two groups was observed in the half-life of the second phase, although the
error in measurement was greater here as the counting rate was very low, particularly in the untreated group.

Isotopically labelled peak IV appeared in serum much more rapidly in the untreated group (maximum concentration on day 0.5–4) than in the treated (maximum concentration on day 4–10). The maximum level of radioactivity was much higher in the untreated group than in the treated (untreated 3.73, treated 0.65 corrected dpm/l; \( P < 0.001 \)). The \( T_1 \) for the decay of peak IV from its maximum concentration was shorter in the untreated group \( (P < 0.02) \). Differences between the two groups for Peaks V and VI were less well defined but higher serum levels of both these more polar peaks were observed in the untreated group.

The mean results with their standard errors are plotted on a linear scale in Figs. 3 and 4.

Fig. 3. The decay of labelled peak III (vitamin \( \Delta \_3 \)) from serum and the appearance of peak IV in untreated and treated groups. Each point is the mean from six subjects; the vertical bars represent the standard errors of the mean (SEM).

**Peak III and peak IV curves in individual subjects**

The differences in metabolism of the test dose of labelled vitamin D, which were clearly established for the untreated and treated groups, were also evident in individual subjects studied both before and after treatment with vitamin D (Table 2, subjects B.M., J.McC., A.R.). The vitamin D nutritional state of the individual, assessed from knowledge of the therapeutic dose given and the biologically assayed level of serum vitamin D activity, was always the determining factor in the metabolism of the test dose, irrespective of underlying pathology. Paired curves for peaks III and IV from subjects before and after dosage with unlabelled vitamin D are shown in Figs. 5–7. In Fig. 5 the subject was a healthy volunteer (B.M.); in Fig. 6 the patient (A.R.) had osteomalacia following gastrectomy.
Vitamin D intake and vitamin D₃ metabolism

**Fig. 4.** The appearance of isotopically labelled peaks V (Δ) and VI (●) in serum after injection of labelled vitamin D₃. Each point is the mean from the six subjects in each group; vertical bars represent the SEM.

**Fig. 5.** The curves for radioactive peaks III and IV before and after treatment with vitamin D in a normal individual (Subject B.M.).
FIG. 6. Curves for radioactive peaks III and IV before and after vitamin D therapy in a patient with osteomalacia complicating gastrectomy and inadequate dietary intake. (Subject A.R.: see Table 1).

FIG. 7. The curves for radioactive peaks III and IV in four patients with advanced chronic renal failure. RP. and J.G. (left) were untreated; I.N. and R.H. had received therapeutic doses of vitamin D (see Table 1) but the amount given to I.N. was insufficient to produce a metabolic response.
Comparable data from four patients with chronic renal failure (see Table 1) are presented in Fig. 7. None of these patients was studied in both the treated and untreated state; J.G. and R.P. had received no therapy, R.H. and I.N. had been given vitamin D treatment for azotaemic osteodystrophy in the dosage shown in Table 1. The sequential changes in serum levels of peaks III and IV in the untreated and treated pairs, were essentially the same as those occurring in subjects with normal renal function (cf. Figs. 3, 5, 6).

**Table 3. The distribution of serum vitamin D activity between peaks III and IV**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Clinical state</th>
<th>Potency (95% fiducial limits)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Whole serum (IU/ml)</td>
<td>Peak III (IU/ml serum)</td>
</tr>
<tr>
<td>K.S.</td>
<td>Normal</td>
<td>0.64 (0.29–1.08)</td>
</tr>
<tr>
<td>T.W.</td>
<td>Normal (treated)</td>
<td>2.94 (2.24–4.43)</td>
</tr>
<tr>
<td>B.S</td>
<td>Chronic renal failure (treated)</td>
<td>16.94 (8.06–30.45)</td>
</tr>
<tr>
<td>R.H.*</td>
<td>Chronic renal failure (treated)</td>
<td>5.24* (3.87–5.95)</td>
</tr>
</tbody>
</table>

*In subject R.H., two different blood samples were used for assay of the activity of whole serum and of the separated fractions III and IV. The whole serum was assayed in 1968; peaks III and IV activity was assayed in 1970, using pooled frozen serum from two specimens of blood collected in 1968. The total serum vitamin D activity was thus probably different in the two samples of serum assayed.

In all other subjects, all the assays relate to a single sample of blood.

**DISCUSSION**

The interpretation of data based solely on measurements of radioactivity is difficult and uncertain. At present there are no chemical methods available for the estimation of cholecalciferol and its 25-hydroxylated derivative at physiological concentrations, so that specific activities cannot be determined. The biological assay measures as ‘vitamin D activity’ the sum total of substances in serum possessing antiricketic activity. This assay provides a reasonably reliable index of the state of vitamin D nutrition in the individual (Lumb et al., 1970) and it was used for the initial separation of the two groups in the present study. But it cannot differentiate between vitamin D₃ and 25-hydroxycholecalciferol, so the result of a particular assay of serum may be due to the presence of either or both of these compounds. In a preliminary study of subjects with normal or increased serum vitamin D activity, the chloroform extract of serum has been fractionated into peaks III and IV which were assayed separately. In these circumstances, the biological activity of the whole serum was fairly evenly distributed between the two peaks (Table 3). It cannot be assumed that the same is true when the body content and serum activity of vitamin D is very low. Judging by the very rapid formation of radioactive
peak IV from the test dose of cholecalciferol in our untreated group (Figs. 3, 5–7) it seems likely that the proportion of biological activity due to unchanged vitamin D is very small in these individuals.

The biological assay thus provides a maximum estimate of the concentration of unlabelled vitamin D in the serum; it will variably overestimate the actual concentration. Despite these limitations, the data obtained (Table 1) establish that the radioactive cholecalciferol was introduced into a smaller serum pool of unlabelled vitamin in the untreated subjects as compared with the treated group. The fractional turnover of this smaller pool was greater than in the larger pool of the treated group (Table 2). Theoretically, turnover may consist of the three components, excretion, metabolism and redistribution. Excretion appears to account for a relatively small proportion of turnover following an intravenous injection of vitamin D₃ in man (Avioli et al., 1967a; Mawer et al., 1969). In the rat the liver is probably the principal site of hydroxylation of vitamin D₃ to peak IV (Ponchon, Kennan & DeLuca, 1969) and the same might be expected to obtain in man. In the untreated group the appearance of radioactive peak IV was much more rapid and the level of radioactivity reached in this fraction was higher than in the treated group. This is compatible with the faster fractional turnover of vitamin D₃ in the former group. The smaller pool of unlabelled vitamin in the untreated group would provide a substrate of higher specific activity for metabolic transformation and thus ensure the formation of peak IV of correspondingly high specific activity. The radioactive peak IV formed would dilute into a total mass of pre-existing, unlabelled peak IV that was probably much smaller in the untreated than in the treated subjects (see Tables 1 and 3).

The decay of peak IV in the serum was also more rapid in the untreated group of subjects (Table 2; Figs. 3, 5–7) and turnover in this fraction might similarly be due to excretion, distribution or further metabolic transformation. There is no evidence that peak IV is normally an excretion product of the vitamin, although small amounts may be recovered in the protein-containing urine of patients with chronic renal failure (Avioli et al., 1968; Mawer, Lumb & Stanbury, unpublished observations). In this laboratory continuing investigations of the bodily distribution of vitamin D and its metabolites in man have detected the presence of peak IV in addition to cholecalciferol in most tissues studied so far (Mawer & Schaefer, 1969). It is also possible that peak IV may be removed from serum by conversion to other compounds such as peaks V and VI. In the chick Lawson et al. (1969) have demonstrated the formation from peak IV of one or possibly two metabolites possessing the same chromatographic mobility as peak V. Peak VI appears to be the most persistent metabolite of vitamin D in man (Fig. 1) but so far there is no evidence for its metabolic derivation from peak IV.

The differences between the mean values of the various parameters measured in the ‘treated’ and ‘untreated’ groups (Table 2; Fig. 3) simply demonstrate the effect of extreme differences in the bodily content of vitamin D. Within the untreated group the turnover of vitamin D₃ was apparently more rapid in the three patients with vitamin D deficiency severe enough to cause osteomalacia than in the three healthy subjects; osteomalacic, mean $T_{1/2}$, first phase 0.296 days, second phase 3.56 days; healthy, mean $T_{1/2}$, first phase 0.517 days, second phase 5.47 days. Since cholecalciferol is apparently biologically inert (DeLuca, 1969), this suggests that the rate of formation of the biologically active hydroxylated derivative (peak IV) may be regulated in some way by the level of vitamin D in the body or at the site of hydroxylation. There was also a comparable difference in the rates of decay of peak IV; osteomalacic, mean $T_{1/2}$, 11 days; healthy, mean $T_{1/2}$, 20.7 days ($P = 0.05$). This could imply that severe vitamin D deficiency
Vitamin D intake and vitamin D₃ metabolism

determines the more rapid formation of some other significant metabolite from peak IV. It is evident that the sequence of events detectable in the serum following an intravenous test dose of cholecalciferol depends critically on the state of vitamin D nutrition in the individual studied. There is the further suggestion that the intermediary metabolism of the vitamin may be controlled by the body content of the vitamin or its individual metabolites. Alternative models are discussed in the computer assisted analysis of Mawer, Mawer & Lucas (in preparation).

Comparison between healthy control and diseased subjects, of the rate of turnover of vitamin D₃ or of the pattern of appearance of metabolites in the serum, will thus provide a valid index of the effects of the disease only if all the subjects studied have a comparable nutritional state with respect to vitamin D. In individuals receiving a constant, high daily dose of the vitamin, the biologically assayed antiricketic activity in the serum appears to be a power function of the intake (Lumb et al., 1970) and the assay provides a reasonable estimate of the nutritional state. In other circumstances the results of the serum assay can be deceptive and it may be impossible to assess the body content of the vitamin. Comparable bioassay values were obtained on the sera of subjects H.B. and O.C. (Table 1), although the total dosage received varied by a factor of 10⁴ and H.B. had taken no vitamin D for 2 years. Direct assays made on the tissues of H.B. showed that the concentration of vitamin D activity in muscle, bone, bone marrow, skin and subcutaneous fat was some 8–10 times greater than in the serum (Stanbury, Ball, Lumb & Mawer, unpublished observations). If the total dose administered to the vitamin D deficient subject O.C. had been absorbed and evenly distributed throughout the body, the average tissue concentration could have been no more than ~1 IU/g. The vastly different body pools of ‘vitamin D’ in these two subjects must have influenced the disposition of the test dose of radioactive cholecalciferol. The particular example of H.B. and other evidence (Warkany, Guest & Grabill, 1942; Lumb et al., 1970) demonstrates that the prevailing body pool of vitamin D can be predetermined by the intake of the vitamin in the relatively remote past.

The differences in the half-life of vitamin D₃ and in the rate of appearance of peak IV in the serum between our treated and untreated patients closely resemble those found by Avioli et al. (1968) between patients with chronic renal failure and healthy American control subjects. The latter findings were ascribed to an acquired abnormality of vitamin D metabolism in chronic renal failure; but, since neither dietary histories nor serum bioassay data were provided, it is impossible to determine whether the results reported represent a phenomenon of disease or simply reflect differences in the nutritional state of the groups compared. Similarly, these authors claimed that the peak IV material isolated from the serum of their patients with renal failure had a lower biological activity than that from their control subjects. This comparison of biological activities was based on the specific activity of the injected cholecalciferol, with the implicit assumption that the serum pools of both peak III and peak IV before the injection were similar in the two groups. No direct evidence was available to support this assumption. The present observations indicate that the patient with chronic renal failure apparently can hydroxylate vitamin D in the normal way, and that treatment with vitamin D probably influences the distribution kinetics of the injected tracer in the same manner as in healthy individuals (Fig. 7). Significant biological activity has been demonstrated in the peak IV material isolated from the serum of patients with chronic renal failure treated with vitamin D (Table 3). On the basis of the present study and of other evidence (Lumb et al., 1970), it seems that the apparent vitamin D resistance of chronic renal failure is not caused by a failure of
such patients to produce biologically active peak IV nor by rapid metabolic inactivation of this metabolite. This metabolic abnormality in renal failure would be compatible either with a defect at some subsequent step in the intermediary metabolism of the vitamin or with relative unresponsiveness of the target organ. It has conclusively been demonstrated that this 'vitamin D resistance' can invariably be overcome if the administered dose of vitamin D, and thus probably also the serum level of peak IV (Table 3), is sufficiently increased (Lumb et al., 1970).

ACKNOWLEDGMENTS

This work was supported by a grant from the Medical Research Council. Dr K. Schaefer was Research Fellow of the Free University of Berlin.

We are grateful to Dr Philip Bell of the Dunn Nutritional Laboratory, Cambridge, for a sample of authentic 25-hydroxycholecalciferol, and to Dr G. E. Mawer for stimulating discussions and suggestions.

We are indebted to Mrs Joan Backhouse for her excellent technical assistance, and to the staff of the Wellcome Metabolic Ward for their co-operation in collecting samples. We are grateful to Mrs M. Jones for assessing the levels of vitamin D intake.

Dr W. F. J. Cuthbertson, Mr G. W. Flynn and Mr H. Gresley-Grey, of Glaxo Research Ltd, have provided generous and continued help in establishing the vitamin D assay in our laboratory.

REFERENCES


