Vitamin D from skin: contribution to vitamin D status compared with oral vitamin D in normal and anticonvulsant-treated subjects


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Summary

1. The plasma 25-hydroxycholecalciferol [25-(OH)D₃] response to measured u.v. irradiation applied thrice weekly for 10 weeks was investigated in normal and in anticonvulsant-treated subjects.

2. Levels of plasma 25-(OH)D₃ achieved after u.v. irradiation were similar in both normal and anticonvulsant-treated subjects, suggesting that hepatic microsomal enzyme induction does not lead to low plasma 25-(OH)D₃ concentrations.

3. Cholecalciferol was present in plasma of normal subjects in very low concentrations (<5.0 nmol/l) and did not increase until plasma 25-(OH)D₃ levels exceeded 62.5 nmol/l.

4. Cholecalciferol occurred in significant concentrations in plasma during whole body u.v. irradiation or during oral dosage of 62.5 nmol (1000 i.u) or more daily.

5. Plasma 25-(OH)D₃ concentrations reached a steady state after 5–6 weeks of u.v. irradiation or of oral intake within the usual intake range.

6. Cholecalciferol synthesis in skin calculated from the steady-state equation was 0.0015 ± 0.0008 nmol/mJ.

7. Cholecalciferol synthesis in skin was also calculated from the oral dosage required to yield the same plasma 25-(OH)D₃ concentration as u.v. irradiation and was 0.0024 ± 0.0018 nmol/mJ.

8. Rates of cholecalciferol synthesis calculated from these data suggest that many of the population of England receive insufficient u.v. irradiation to maintain vitamin D status throughout the year.

Key words: anticonvulsant drugs, cholecalciferol, skin, ultraviolet irradiation, vitamin D.

Abbreviations: 25-(OH)D₃, 25-hydroxycholecalciferol; 25-(OH)D₂₆, 25-hydroxyergocalciferol; D₃, cholecalciferol.

Introduction

Cutaneous synthesis of cholecalciferol in response to u.v. irradiation is considered to be important in maintaining vitamin D status in man [1, 2]. Little is known about the influence of environmental u.v. energy, or of the effect of age, anatomical site or drugs associated with derangement of vitamin D metabolism on the capacity of human skin to synthesize cholecalciferol in vivo. It is important to consider the skin response in vivo since isolated skin may respond differently [3]. Acquisition of a skin biopsy after u.v. irradiation may be unacceptable to the patient, especially if it is taken from an area such as the face, and the information must therefore be obtained indirectly by measuring the main circulating metabolite of cholecalciferol, 25-(OH)D₃ [4, 5].

Although the importance of sunlight in maintaining vitamin D status is recognized, the relative contribution arising from diet or u.v. energy is controversial [6, 7], since many subjects have little exposure to u.v. irradiation [8] or do
not go out of doors [9]. It would thus be of value to establish the amount of cholecalciferol that may be synthesized in skin by a known quantity of u.v. energy.

We have compared the pattern and magnitude of the plasma 25-(OH)D₃ response both to standardized u.v. irradiation and to oral dosage with cholecalciferol. Cutaneous cholecalciferol synthesis has been calculated as mass of cholecalciferol per millijoule of u.v. energy. The influence of anticonvulsant drugs on the plasma 25-(OH)D₃ response to u.v. irradiation has also been examined.

Subjects and methods

Subjects

Subjects investigated were white skinned and had no clinical or biochemical features of osteomalacia (Table 1). Subjects in groups 1–5, who were selected from patients in the Ida Darwin Hospital, Cambridge, rarely went outside. Since there was a considerable variation in weight amongst the subjects, care was taken to match the two groups receiving u.v. irradiation (groups 1 and 2) for weight. The subjects in group 6, who were older than the subjects in groups 1–5, were selected from patients in the Hinchingbrooke Hospital, Huntingdon, and over the period of observation they did not go outside.

Subjects in whom the relationship between plasma concentrations of 25-(OH)D₃ and cholecalciferol were investigated came from the above groups, from volunteer subjects receiving no therapy, from subjects receiving whole body u.v. irradiation for experimental or therapeutic purposes and from two male subjects taking ergocalciferol (1·25 mg/day) for iatrogenic hypoparathyroidism. Supplementary vitamin D or u.v. irradiation had been administered for at least 9 weeks in all subjects receiving these treatments.

Details of the subjects receiving u.v. energy and cholecalciferol and of the groups into which they were divided appear in Table 1. Subjects in groups 2–5 had been established on anticonvulsant drugs before the experimental period and continued to receive these drugs during the study.

U.v. irradiation

U.v. irradiation was administered to 600 cm² dorsal skin for 3 min/day for 3 days/week over a 10 week period in the subjects comprising groups 1 and 2 beginning 30 January 1978. Subjects in group 6 received u.v. irradiation to 900 cm² dorsal skin for 3 min/day on 3 days/week over 5 weeks in February/March 1979. In all groups the same area of skin was exposed to u.v. irradiation on each occasion.

The u.v. energy source was a Hanovia 7A prescription lamp situated 50 cm from the skin surface in groups 1 and 2, and at various distances in group 6. The energy received by each subject in group 6 was known accurately since the power emitted was found to behave in a manner predicted by the inverse square law of irradiation (Dr J. Haybittle, Medical Physics Department, Addenbrooke's Hospital, Hills Road, Cambridge). The power spectrum of this lamp has previously been described [10]. Since the energy required either to heal rickets or to convert 7-dehydrocholesterol into cholecalciferol

<table>
<thead>
<tr>
<th>Group</th>
<th>Age (years)</th>
<th>Weight (kg)</th>
<th>Treatment</th>
<th>Vitamin D₃ dosage (nmol/day)</th>
<th>U.v. energy (mJ day⁻¹ cm⁻²)</th>
<th>Skin area irradiated (cm²)</th>
<th>Pheno-barbitone (mg day⁻¹ kg⁻¹)</th>
<th>Phenytoin (nmol/l)</th>
<th>Initial plasma 25-(OH)D₃ (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>21.8 ± 4.6</td>
<td>6</td>
<td>6</td>
<td>2</td>
<td>33.5 ± 9.4</td>
<td>U.v.</td>
<td>18.9</td>
<td>Nil</td>
<td>14.7 ± 9.0</td>
</tr>
<tr>
<td>2</td>
<td>20.6 ± 2.8</td>
<td>6</td>
<td>6</td>
<td>2</td>
<td>34.1 ± 13.6</td>
<td>U.v.</td>
<td>18.9</td>
<td>Nil</td>
<td>5.5 ± 6.5</td>
</tr>
<tr>
<td>3</td>
<td>21.2 ± 7.6</td>
<td>7</td>
<td>2</td>
<td>50.2 ± 17.6</td>
<td>Oral</td>
<td>25</td>
<td>18.9</td>
<td>Nil</td>
<td>16.5 ± 4.8</td>
</tr>
<tr>
<td>4</td>
<td>21.4 ± 8.9</td>
<td>7</td>
<td>2</td>
<td>45.9 ± 14.9</td>
<td>Oral</td>
<td>62.5</td>
<td>16 ± 4.4</td>
<td>(n = 5)</td>
<td>(n = 7)</td>
</tr>
<tr>
<td>5</td>
<td>22.5 ± 9.1</td>
<td>6</td>
<td>2</td>
<td>45.6 ± 18.4</td>
<td>Oral</td>
<td>625</td>
<td>2.7 ± 1.5</td>
<td>(n = 5)</td>
<td>(n = 6)</td>
</tr>
<tr>
<td>6</td>
<td>77.2 ± 9.4</td>
<td>2</td>
<td>7</td>
<td>55.5 ± 12.1</td>
<td>U.v.</td>
<td>16.7 (n = 3)</td>
<td>8.4 (n = 3)</td>
<td>Nil</td>
<td>10.6 ± 6.8</td>
</tr>
</tbody>
</table>

Table 1. Details of subjects receiving u.v. irradiation or oral cholecalciferol
is different for each wavelength in the spectrum of this lamp, a factor was calculated from previously published data [11-13] to express the power of each wavelength as a proportion of the most efficient wavelength. The total power emitted from the Hanovia lamp, expressed in terms of the most efficient wavelength, is 140·2 µW/cm² calculated from the data of Knudsen & Benford [12], 245·3 µW/cm² calculated from the figures of Kobayashi & Yasumura [11] and 168·7 µW/cm² from activity spectrum of Bunker & Harris [13]. The total effective energy over 60 s was found by multiplying the power by 60 and dividing by 1000 to obtain the result in mJ/cm²: in turn this yielded 8·4, 14·7 and 10·1 mJ/cm² for the power emissions stated above. The procedure has been described in detail previously [10]. The energy (14·7 mJ/cm²) calculated from the conversion factor (in vitro) of Kobayashi & Yasumura [11] has been used in the calculation of vitamin D synthesis. Thus each subject in groups 1 and 2 irradiated for 9 min/week received an average of 1·29 min/day or 18·9 mJ/cm² over 1 day. The energy received each day appears in Table 1, corrected by use of the conversion factor (in vitro).

**Calculation of cholecalciferol synthesis**

Effective cholecalciferol synthesis in skin was calculated from the plasma 25-(OH)D₃ response to u.v. irradiation. Use of this measurement may result in a slight underestimate of cholecalciferol synthesis in skin if some cholecalciferol is not converted into 25-(OH)D₃. However, at the concentrations of 25-(OH)D₃ achieved during u.v. irradiation, there is rapid conversion of tracer dosages of cholecalciferol into 25-(OH)D₃ [4, 26], and there is no accumulation of cholecalciferol in plasma (see the Results section). Two methods were used in the calculation of cholecalciferol synthesis. One made use of the steady-state equation [18] and in the second method the oral dosage of cholecalciferol required to achieve the same plasma 25-(OH)D₃ concentration as occurred during u.v. irradiation was calculated.

The steady-state equation [17, 18] used in the first method describes the relationship between dosage of a drug and the plasma concentration achieved at plateau level thus:

\[ C_p = (1.44 \times F \times T_{1/2} \times D)/V_d \]

in which \( C_p \) = plasma concentration (nmol/l), \( F \) = fractional absorption (assumed to be unity), \( T_{1/2} \) = half life determined by \(^{3}^{2}\text{H}25\text{-}(\text{OH})\text{D}_3\) (days), \( V_d \) = volume of distribution (litres) and \( D \) = dosage per unit time (nmol/day). The value for the radioactive half life was used because it is less influenced by exogenous sources of vitamin D than is the actual half life, and it is unrelated to the plasma 25-(OH)D₃ concentration [19]. The half life determined by the tracer method is also closer to the half life of plasma 25-(OH)D₃ after very high dosage of vitamin D, when any extraneous vitamin D will make less impact on the rate of decline of plasma 25-(OH)D₃ from high levels [20]. Moreover the time required for a drug to achieve a concentration of 90% of the plateau level is equivalent to three to four half lives [17, 18]. The radioactive tracer 25-(OH)D₃ half life meets this criterion more closely than does the actual half life, which is longer [19].
volume of distribution was calculated from a single dose of radiolabelled 25-(OH)D₃. Although the volume of distribution calculated from the steady-state method is not identical with that obtained by the single dose injection, the difference between the two is very small [17]. The concentration in the compartment of volume $V_d$ was assumed to equate to that of the plasma compartment since the steady-state period (30 days) was long compared with the time required for equilibration of the $[^3]$H]25-(OH)D₃ (2 days). For the purpose of the steady-state equation $V_d$ was calculated from the term body weight (kg) × 21.6/100. This term represents the fraction of body weight represented by $V_d$ as calculated from a single injection of $[^3]$H]25-(OH)D₃ (see the Results section). Thus with values for $C_p$, $V_d$, $F$ and $T_{1/2}$ known, the steady-state equation may be expressed in terms of $D$, this being the daily input of cholecalciferol from skin with units of nmol/day. Since the total cholecalciferol synthesis per day is derived from a known area of skin, each square centimetre contributes an amount of cholecalciferol equal to total cholecalciferol per day/area of exposure. The u.v. energy applied to each square centimetre of skin during the plateau period of plasma 25-(OH)D₃ being known (see above), cholecalciferol synthesis per mJ of u.v. energy is equal to

$$\text{Total cholecalciferol per day/area of exposure}$$

$$\frac{\text{daily u.v. energy/cm}^2}{\text{nmol/day}}$$

Cholecalciferol synthesis in skin was also estimated from the amount of oral cholecalciferol required to sustain a plasma 25-(OH)D₃ level equivalent to that obtained during u.v. irradiation treatment. Since plasma 25-(OH)D₃ correlates with cholecalciferol intake per kg body weight (see the Results section), a value of cholecalciferol intake/kg could be found for any plasma 25-(OH)D₃ level. Assuming that the metabolism of cholecalciferol does not differ according to the origin of cholecalciferol in the body, and ignoring absorptive losses, any concentration of plasma 25-(OH)D₃ will reflect the same intake of cholecalciferol, be it from the diet or from skin. Thus the relationship of cholecalciferol dosage to plasma 25-(OH)D₃ concentration allows the total amount of cholecalciferol required to sustain a given 25-(OH)D₃ level to be calculated from cholecalciferol intake (nmol/kg) × body weight (kg).

Cholecalciferol synthesis in skin may be expressed as cholecalciferol synthesis (nmol/mJ) × energy (mJ/cm²) × area irradiated (cm²). The cholecalciferol intake being the same these two terms will be equal, and can be rearranged such that daily cholecalciferol synthesis (nmol/mJ)

$$\frac{\text{cholecalciferol intake (nmol/kg) \times body weight (kg)}}{\text{energy (mJ/cm}^2) \times \text{area irradiated (cm}^2)}$$

(2)

**Statistics**

Regression analysis was performed on a Texas TI-51-111 calculator. Other statistical analyses were performed as described in standard texts, and expressed as mean ± S.D.

Ethical approval was obtained for all these experiments from the Ethical committee, Addenbrooke's Hospital, Cambridge. Permission was obtained from all subjects or their guardians.

**Results**

Details of the subjects receiving u.v. irradiation or oral cholecalciferol appear in Table 1. The initial plasma 25-(OH)D₃ concentration was 5.5–16.5 nmol/l, the level being significantly lower in group 2 subjects compared with those in group 1 ($P < 0.05$; Mann–Whitney U-test). The intake of cholecalciferol in subjects in groups 1–6 was 2.6 ± 0.5 nmol/day with no significant difference between the groups.

**Response to u.v. irradiation**

Plasma 25-(OH)D₃ rose in all subjects receiving u.v. irradiation. In groups 1 and 2, plasma 25-(OH)D₃ levels attained a maximum level after 30–40 days: thereafter the levels remained steady until irradiation was discontinued on day 70 (Fig. 1a). In the subjects comprising group 6, plasma 25-(OH)D₃ levels at the end of irradiation were 28.0 ± 14.4 nmol/l in those receiving 16.7 mJ day⁻¹ cm⁻², 17.9 ± 6.3 nmol/l in those exposed to 8.4 mJ day⁻¹ cm⁻² and 12.9 ± 1.0 in those receiving the least u.v. energy. In all the subjects receiving u.v. irradiation the plasma 25-(OH)D₃ concentration after 5 weeks was related to the daily u.v. energy applied by a power function (Table 2; Fig. 2). The incremental plasma 25-(OH)D₃ was linearly related to the daily u.v. energy applied (Table 2; Fig. 3), the relationship being significant for all subjects. One subject, however, had a disproportionate effect on the slope of the regression and thus the slope relating subjects receiving daily u.v. energy up to 500 mJ day⁻¹ kg⁻¹ is also shown (Fig. 3).
FIG. 1. Plasma 25-(OH)D, response (mean ± sd) by subjects in groups 1 and 2 to u.v. irradiation (a), and by subjects in groups 3–5 to oral cholecalciferol (b). (a) ■, Normal subjects; ▲—▲, anticonvulsant-treated subjects. (b) ○, 625 nmol of cholecalciferol/day; □—□, 62.5 nmol/day; △···△, 25 nmol/day.

FIG. 2. Relationship between plasma 25-(OH)D, after 32–35 days of u.v. irradiation and the daily u.v. energy for subjects in group 1 (■), group 2 (▲) and group 6 (□). The equation of the line is (y) in Table 2. U.v. energy is calculated from the product of the daily energy applied per kg body weight (mJ cm⁻² day⁻¹ kg⁻¹) and the area irradiated (cm²).
FIG. 3. Relationship between the increase of plasma 25-(OH)D₃ after 32–35 days of U.V. irradiation and daily U.V. energy for subjects in group 1 (C), group 2 (■) and group 6 (△). The dotted line refers to all subjects, and the continuous line to subjects receiving less than 500 mJ day⁻¹ kg⁻¹ (see the text). The equation of the continuous line is (vi) in Table 2. U.V. energy is derived as in Fig. 2.

TABLE 2. Relationships between oral vitamin D intake or U.V. irradiation and plasma concentrations of 25-(OH)D₃ or 25-(OH)D₃ + D₁

| Oral cholecalciferol | 25-(OH)D₃ (nmol/l) = log 56.7 + 0.31 log D₁ intake* | n = 26; r = 0.79; P < 0.001
| (i) log plasma 25-(OH)D₃ (nmol/l) = log 65.3 + 0.5 log D₁ intake* | n = 25; r = 0.90; P < 0.001
| (ii) log plasma 25-(OH)D₃ + D₁ (nmol/l) = log 65.3 + 0.5 log D₁ intake* | n = 26; r = 0.90; P < 0.001
| (iii) log D₁ intake* = log 0.0003 + 2.1 log plasma 25-(OH)D₃ (nmol/l) | n = 25; r = 0.90; P < 0.001
| (iv) log D intake* = log 0.0012 + 1.62 log plasma 25-(OH)D₃ + D₁ (nmol/l) | n = 25; r = 0.90; P < 0.001
| U.V. irradiation | 25-(OH)D₃ (nmol/l) = log 2.0 + 0.49 log daily U.V. irradiation† | n = 25; r = 0.67; P < 0.001
| (v) log plasma 25-(OH)D₃ (nmol/l) = log 2.0 + 0.49 log daily U.V. irradiation† | n = 23; r = 0.71; P < 0.001
| (vi) Increase plasma 25-(OH)D₃ (nmol/l) = 2 log plasma 25-(OH)D₃ + D₁ (nmol/l) | n = 25; r = 0.67; P < 0.001

* Oral intake of cholecalciferol = nmol of cholecalciferol day⁻¹ kg⁻¹.
† U.V. irradiation = mJ/cm² cm⁻² day⁻¹ kg⁻¹ (of mJ day⁻¹ kg⁻¹).
‡ Relationship used to determine oral equivalent of cutaneously derived cholecalciferol.
§ Regression equation omits two subjects; one initial value was not available and one subject had an exceptionally high degree of exposure (see the text). This equation describes the relationship up to 500 mJ day⁻¹ kg⁻¹.

Plasma 25-(OH)D₃ levels were followed for 6 weeks after cessation of u.v. irradiation in groups 1 and 2. At the end of the period of irradiation plasma 25-(OH)D₃ levels were 44.0 ± 15.5 nmol/l and 44.3 ± 12.3 nmol/l in groups 1 and 2 respectively. Six weeks later the levels were 33.8 ± 16.0 nmol/l and 33.5 ± 15.3 nmol/l in the two groups.

Oral cholecalciferol dosage

Plasma 25-(OH)D₃ levels also reached a plateau during oral administration of cholecalciferol (Fig. 1b). Subjects receiving 62.5 nmol/day had similar plateau levels of 25-(OH)D₃ as subjects receiving 25 nmol/day. Oral cholecalciferol dosage (as nmol of cholecalciferol/kg body weight) correlated with plasma 25-(OH)D₃ concentration (Table 2). Cholecalciferol dosage was also related to total plasma [25-(OH)D₃ + D₁] if this term were used for groups 4 and 5, and 25-(OH)D₃ only in group 3, since there was very little cholecalciferol present in the plasma of group 3 subjects. A power function also best described this relationship (Table 2; Fig. 4), the slope of which was similar to that relating u.v. energy and plasma 25-(OH)D₃ concentration.
Cutaneous vitamin D synthesis

Plasma cholecalciferol

Cholecalciferol was present in plasma at a concentration below 5 nmol/l in subjects whose plasma 25-(OH)D₃ levels were less than 62.5 nmol/l (Fig. 5). The plasma concentration of cholecalciferol exceeded that of 25-(OH)D₃ in six subjects (five females, one male), three of whom were receiving u.v. irradiation and three oral cholecalciferol. Cholecalciferol was present at a concentration below 5 nmol/l in subjects from groups 1-3, and was not measured in the subjects in group 6. Plasma cholecalciferol reached a plateau during oral administration of 62.5 nmol of cholecalciferol/day, the mean level being 14.5 ± 7.5 nmol/l. In subjects receiving 625 nmol daily (group 5), high levels of cholecalciferol were found in plasma (Table 3), but, unlike the levels in group 4, plasma cholecalciferol rose rapidly in group 5 subjects and in four (nos. 2, 4, 5, 6; Table 3) levels gradually fell even though dosage was continuing. Plasma cholecalciferol fell rapidly when oral supplementation was discontinued.

Radioactive 25-(OH)D₃ studies

The disappearance of [³H]25-(OH)D₃ fitted a two-compartment system. The slow half life, measured from 48 to 504 h (2–21 days) was 12.9 ± 3.6 days, and the total volume of distribution was 11.45 ± 3.70 litres or 21.6 ± 5.5% of body weight. The term (21.6/100) × body weight (kg) was used to calculate the value for $V_d$ in the steady-state equation.

Cholecalciferol synthesis in skin

Effective cholecalciferol synthesis in skin was calculated for subjects in group 2 (Table 4). The energy available from the Hanovia lamp was calculated as described and the value of 14.7 mJ/cm² derived from the activity spectrum in vitro (see the Methods section) was used to
FIG. 5. Relationship between plasma cholecalciferol and 25-(OH)D₃ levels, in subjects not receiving u.v. irradiation or cholecalciferol supplements (△), subjects receiving u.v. irradiation from an artificial u.v. source (▲), subjects receiving supplementary cholecalciferol (■) and subjects receiving supplementary ergocalciferol (●). The inset is an enlargement of the area between 0 and 62.5 nmol/l on the ordinate and 0 and 125 nmol/l on the abscissa.

TABLE 3. Plasma cholecalciferol levels in group 5 subjects during and after dosage with 625 nmol of cholecalciferol daily

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Plasma cholecalciferol (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>4.5</td>
</tr>
<tr>
<td>2</td>
<td>0.0</td>
</tr>
<tr>
<td>3</td>
<td>0.0</td>
</tr>
<tr>
<td>4</td>
<td>4.3</td>
</tr>
<tr>
<td>5</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>0.0</td>
</tr>
<tr>
<td>7</td>
<td>0.0</td>
</tr>
<tr>
<td>8</td>
<td>0.0</td>
</tr>
</tbody>
</table>

calculate the results depicted in Table 4. Use of the activity spectrum of Bunker & Harris [13] gives a result 45% higher and the spectrum of Knudsen & Benford [12] gives a value 75% higher.

Discussion

The pattern of plasma 25-(OH)D₃ response is similar irrespective of the source of cholecalciferol, and plateau levels are achieved after equivalent periods with low dosage of oral cholecalciferol or of u.v. energy. Thus the initial exposures of u.v. irradiation lead to a rapid increase of plasma 25-(OH)D₃ from a low starting level [10]. By following the plasma 25-(OH)D₃ response to u.v. irradiation over a 10 week period, it was evident that a plateau level was eventually reached.

Unmetabolized cholecalciferol appeared in
Subjects were from group 2 and received 18.9 mJ/cm² over 600 cm² daily. Calculations do not allow for dietary vitamin D as this contributed less than 10% to the total plateau level (see the text).

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Plateau plasma 25-(OH)D₃ (nmol/l)</th>
<th>Cholecalciferol synthesis (nmol/mj)</th>
<th>(b) as % of (a)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Equivalent to oral intake</td>
<td>Calculated from plateau level</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>57.1</td>
<td>0.0048</td>
<td>0.0024</td>
</tr>
<tr>
<td>2</td>
<td>39.0</td>
<td>0.0021</td>
<td>0.0016</td>
</tr>
<tr>
<td>3</td>
<td>45.6</td>
<td>0.0020</td>
<td>0.0013</td>
</tr>
<tr>
<td>4</td>
<td>43.6</td>
<td>0.0018</td>
<td>0.0012</td>
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<tr>
<td>5</td>
<td>32.8</td>
<td>0.0015</td>
<td>0.0014</td>
</tr>
<tr>
<td>6</td>
<td>31.8</td>
<td>0.0010</td>
<td>0.0009</td>
</tr>
<tr>
<td>7</td>
<td>36.5</td>
<td>0.0005</td>
<td>0.0004</td>
</tr>
<tr>
<td>8</td>
<td>53.3</td>
<td>0.0055</td>
<td>0.0031</td>
</tr>
</tbody>
</table>

| Total       | 0.0024 ± 0.0018                    | 0.00154 ± 0.0008                  | 72.5 ± 15.6     |

plasma during oral dosage of 62.5 nmol of cholecalciferol or more daily or during whole body u.v. irradiation. Although subjects in group 4 had a plasma 25-(OH)D₃ concentration similar to those in group 3, the total response of 25-(OH)D₃ + D₃ exceeded that of group 3 subjects. Plasma levels of cholecalciferol were higher than 25-(OH)D₃ on several occasions in subjects receiving 625 nmol of cholecalciferol daily, such high dosages probably leading to inhibition of cholecalciferol conversion into 25-(OH)D₃ [21]. In the course of dosing with large quantities of vitamin D, changes in the metabolism of vitamin D may take place, leading to lower plasma levels of vitamin D activity than might otherwise be expected. Not only did plasma cholecalciferol levels start to fall even during the course of cholecalciferol administration (Table 3), but levels fell very rapidly after supplementation was stopped. Moreover in subjects receiving 25 nmol of cholecalciferol daily (group 3), the concentration of 25-(OH)D₃ predicted by the steady state equation assuming 75% absorption [27] was 36 nmol/l compared with the value of 47.0 nmol/l calculated from the relationship of plasma 25-(OH)D₃ + D₃ and oral cholecalciferol dosage (ii; Table 2). In contrast the predicted levels of vitamin D activity in groups 4 and 5, derived from the steady-state equation, are 90-3 and 909-0 nmol/l respectively; these values compare with the levels of 70-0 and 215-0 nmol/l calculated from the actual regression (ii; Table 2). At high dosages of cholecalciferol, vitamin D activity may accumulate in tissues [22, 23], resulting in a different value for Vₐ. Moreover cholecalciferol excretion may be increased.

U.v. irradiation was applied both to normal subjects and to subjects receiving hepatic microsomal enzyme-inducing drugs. Initial plasma 25-(OH)D₃ levels were lower in subjects receiving these drugs, but the resulting plateau levels and the rate of decline on cessation of u.v. irradiation were unaffected. The phenomenon of hepatic microsomal enzyme induction has been proposed to explain the low levels of plasma 25-(OH)D₃ frequently found in association with phenytoin and phenobarbitone administration [24], together with the impaired response to oral vitamin D [25]. The present data show that it is important to continue dosing until a plateau level of 25-(OH)D₃ is reached: otherwise the plasma 25-(OH)D₃ response to vitamin D may appear to be impaired. If the plasma level of 25-(OH)D₃ had been compared after 2 weeks (Fig. 1a), the level of plasma 25-(OH)D₃ would have been lower in anticonvulsant-treated subjects than in the normal subjects. This may explain the differences of plasma 25-(OH)D₃ concentration found in normal and anticonvulsant-treated subjects after oral dosage with vitamin D for 3 weeks [25].

The existence of hepatic microsomal enzyme induction might also be expected to reduce the value of D in the steady-state equation by restricting the amount of cholecalciferol available for conversion into 25-(OH)D₃, thereby leading to lower plasma 25-(OH)D₃ concentrations if Vₐ and T₁/₂ remain unchanged. Tracer studies with 25-(OH)D₃ suggest that T₁/₂ is not affected by anticonvulsant drugs, although Vₐ may be slightly lower [26]. Diminished vitamin D storage is also unlikely since the rate of decline of plasma 25-(OH)D₃ after cessation of u.v. irradiation was similar in groups 1 and 2. The initial low plasma 25-(OH)D₃ levels in group 2 do, however,
indicate that the dosage of anticonvulsant was sufficient to exert an effect on vitamin D metabolism.

In calculating cutaneous vitamin D synthesis from the plasma 25-(OH)D₃ levels, it is assumed that all cholecalciferol is converted into 25-(OH)D₃. Cholecalciferol undergoes rapid 25-hydroxylation at low plasma 25-(OH)D₃ concentrations [4, 16], and there is no evidence that cholecalciferol accumulates whilst plasma 25-(OH)D₃ is below 62.5 nmol/l (Fig. 5): such a level was not achieved by any subject receiving limited area U.V. irradiation. Accumulation in tissues other than plasma is unlikely, since, even during high dosage of cholecalciferol, concentrations in plasma are equivalent to those in other tissues. When dosing is discontinued, some tissues, notably fat, release cholecalciferol more slowly than plasma and may exhibit higher levels [23]. Cholecalciferol could also suffer biliary excretion, rendering itself unavailable for 25-hydroxylation; indeed excretion of radioactivity after intravenous H or ¹⁴C as cholecalciferol was slightly greater in the faeces of a normal subject [28], or in the urine of subjects with biliary obstruction [29] compared with the excretion after administration of [³H]25-(OH)D₃. If significant losses occurred by means of this pathway, plasma 25-(OH)D₃ concentrations during oral administration would be lower than those predicted by the steady-state equation. Although this was true for high intakes of cholecalciferol (see above), it did not occur with oral doses of vitamin D that were associated with plasma 25-(OH)D₃ levels corresponding to those found during irradiation.

Since 25-(OH)D₃ is the main circulating metabolite in plasma, values of V_d and T_1/2 relating to this metabolite were used in the steady-state equation. T_1/2, calculated by this method is shorter than the T_1/2 of plasma 25-(OH)D₃, possibly as a result of daily vitamin D intake [20]. The value for the radioactive 25-(OH)D₃ half life is at the lower end of the range 13.3-23.0 days previously reported [30, 31], but compares well with the value of 13.5 days found in normal subjects [26]; P. Siklos & M. Davie, unpublished work). Vitamin D synthesis in human skin (Table 4) is similar to that found in rat skin, 0.0011-0.0027 nmol/mJ being synthesized according to the wavelength used [12]. A higher value for synthesis calculated from the oral equivalent (Table 4) is expected since not all the cholecalciferol ingested will be absorbed [27]. At these rates of synthesis considerable exposure would be required to make any impact on the 2-4 nmol/cm² of 7-dehydrocholesterol present in skin [3]. For the purpose of the calculation, no account was taken of the initial plasma 25-(OH)D₃ level or of the contribution of dietary vitamin D, since both were low in subjects making up group 2. In subjects from group 1, use of the incremental plasma 25-(OH)D₃ relationship with u.v. energy (Fig. 3) suggested that cholecalciferol synthesis was 0.001 ± 0.0003 nmol/mJ. Dietary vitamin D intake was very low in all subjects, and from the steady-state equation, assuming 75% absorption to calculate the expected steady-state level, it was apparent that no more than 10% of the plateau level could be accounted for from this source.

The potential contribution of u.v. energy to vitamin D status may be assessed by using the data in Table 4 relating to cholecalciferol synthesis and published incident u.v. energy data [8, 32]. From the data of Bunker & Harris [13] and Johnson et al. [32] up to 315 nm, the total antirachitic activity was calculated in the same manner as the activity of the Hanovia lamp [10], for latitude 45°N. Calculation of the area under the curve relating the wavelength with (energy at λ × antirachitic activity at λ) showed that 17.5 mJ day⁻¹ cm⁻² are incident in January compared with 712.5 mJ day⁻¹ cm⁻² in July. However 60% of daily u.v. energy falls in the 4 h around midday G.M.T. [33, 34]: thus about 15% of total energy will be available in each hour around midday. Partial cloud cover may reduce energy by 25% and this hour might contain 15 × 75% = 11.25% of daily u.v. energy. The incident u.v. energy over this 1 h period would thus be 1.97 (17.5 × 0.1125) and 80.2 mJ/cm² in January and July respectively. However, direct measurement of u.v. energy in June/July in southern England has shown that gardeners were exposed to 21.8 mJ/cm² per day and indoor workers only to 3.7 mJ/cm² per day. Expected plateau plasma 25-(OH)D₃ levels associated with these incident energy values may be calculated either from the relationship in Fig. 2 or by the steady-state equation, with the values for cholecalciferol synthesis depicted in Table 4. Assuming that the dorsum of the hands and the face are exposed (about 600 cm²) in a 70 kg man, the relationship described in Fig. 2 predicts that daily exposure to 80.2 mJ/cm² would lead to a plateau level of plasma 25-(OH)D₃, of 82.7 nmol/l, and that the gardeners and indoor workers would have 26.0 and 10.9 nmol/l respectively. To obtain values from the steady-state equation, a value for D is calculated from the product of area irradiated × energy applied × cholecalciferol synthesis, using the figure of 0.00154 nmol/mJ for cholecalciferol synthesis (Table 4), and values for T_1/2 and
Note added in proof
That exposure must be longer or the area irradiated greater than has been accounted for in 24.7 nmol/l for the gardeners and 4.2 nmol/l for subjects with exposure of 600 cm², exposure to (OH)D, level of 91.0 nmol/l, compared with that institutionalized subjects lack both U.V. exposure and vitamin D in the diet, since both are 25-(OH)D, levels in groups 1–6 (Table 1) suggest effective at increasing plasma 25-(OH)D, levels ends, and since the half life of 25-(OH)D, extends for additional U.V. exposure, especially at week-

There is also an advantage in using u.v. irradiation in anticonvulsant-treated subjects. Although doubt exists about the effect of oral vitamin D on the plasma 25-(OH)D₃ level [25, 37], it is evident from the present study that u.v. exposure yields identical responses in both normal and anticonvulsant-treated subjects.

Note added in proof
Since this paper was submitted, our attention has been drawn to similar conclusions arising out of work in animals [Takada, K. et al. (1981) Journal of Steroid Biochemistry, 14, 1361–1367].

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