Summary

1. The metabolism of an intravenous pulse dose of double-isotope-labelled cholecalciferol has been studied in control subjects with widely differing states of vitamin D nutrition and in patients with primary disorders of parathyroid function.

2. The formation of labelled 1,25-dihydroxycholecalciferol [1,25-(OH)₂D₃] and labelled 24,25-dihydroxycholecalciferol [24,25-(OH)₂D₃] has been related to the prevailing concentrations in serum of 25-hydroxycholecalciferol [25-(OH)D₃], immunoreactive parathyroid hormone, calcium and orthophosphate (P₄).

3. In control subjects with relative vitamin D deficiency [serum 25-(OH)D₃ < 2.5 nmol/l (10 ng/ml)], serum labelled 1,25-(OH)₂D₃ was related inversely to the serum 25-(OH)D₃ and serum calcium, and directly to serum immunoreactive parathyroid hormone. No formation of 1,25-(OH)₂D₃ was detectable in vitamin D-replete individuals, who appeared to form labelled 24,25-(OH)₂D₃ preferentially.

4. No control subject produced significant amounts of both labelled 1,25-(OH)₂D₃ and labelled 24,25-(OH)₂D₃ simultaneously.

5. All subjects with primary hyperparathyroidism produced significant amounts of labelled 1,25-(OH)₂D₃ and labelled 24,25-(OH)₂D₃ simultaneously; the renal turnover of 25-(OH)D₃ was apparently greater than in nutritionally matched controls. Serum labelled 1,25-(OH)₂D₃ in this disease was not correlated with serum 25-(OH)D₃, immunoreactive parathyroid hormone, calcium or P₄. Production of labelled 24,25-(OH)₂D₃ was inappropriately high for the prevailing nutritional state.

6. The indirectly estimated molar concentration of 1,25-(OH)₂D₃ showed only a fourfold variation in control subjects (45–180 pmol/l), compatible with its having a regulated hormonal function.

7. The data suggest that the production of 1,25-(OH)₂D₃ from a pulse dose of cholecalciferol is normally regulated, directly or indirectly, by the parathyroid hormone.

Key words: cholecalciferol, 1,25-dihydroxycholecalciferol, 24,25-dihydroxycholecalciferol, 25-hydroxycholecalciferol, parathyroid hormone, primary hyperparathyroidism, vitamin D deficiency.

Introduction

The metabolism of vitamin D₃ (cholecalciferol) to its hydroxylated derivatives has been shown to occur in man along pathways similar to those established in experimental animals. 25-Hydroxycholecalciferol [25-(OH)D₃] has been isolated from human plasma (Holick et al., 1972); the active metabolite, 1,25-dihydroxycholecalciferol [1,25-(OH)₂D₃], has been detected in serum (Mawer, Backhouse, Lumb & Stanbury, 1971a), as has 24,25-dihydroxycholecalciferol [24,25-(OH)₂D₃] (Holick et al., 1972; Mawer, 1974). The kinetics of the production and decay of 25-(OH)D₃, and its metabolites, are complex.
in man, after a pulse of isotopically labelled cholecalciferol, have been studied in detail, and it has been shown that the state of vitamin D nutrition is the most important single factor affecting the conversion of a test dose of radioactive cholecalciferol into labelled 25-(OH)D₃ (Mawer, Lumb, Schaefer & Stanbury, 1971b; Mawer, Backhouse, Holman, Lumb & Stanbury, 1972). Study of the formation of 1,25-(OH)₂D₃ in man is much more difficult. Only a very small fraction of an administered dose of labelled cholecalciferol appears subsequently in the blood in the form of 1,25-(OH)₂D₃ (Mawer, Backhouse, Taylor, Lumb & Stanbury, 1973) and this can be measured only when the subject is vitamin D-deficient (Mawer, 1974). The quantitative state of vitamin D nutrition required to prevent the formation of labelled 1,25-(OH)₂D₃ is not known, nor are the mechanisms by which the nutritional state determines this effect. In studies of the formation of 1,25-(OH)₂D₃ in disease, this particular problem has been circumvented by the deliberate selection for study of patients with a state of vitamin D nutrition matching that of control subjects shown to be capable of producing detectable amounts of labelled 1,25-(OH)₂D₃ (Mawer et al., 1973; Mawer, Backhouse, Taylor, Lumb & Stanbury, 1973) and it is considered that these twenty subjects differ significantly only in respect of their 'vitamin D status'; for convenience in description and for comparison with patients with primary disorders of parathyroid function, they are referred to as the 'control' group.

Methods

Subjects studied

Studies with radioactive cholecalciferol were undertaken in twenty individuals with a wide range of nutritional state with respect to vitamin D (group 1, Table 1). These included patients with overt clinical signs of vitamin D deficiency or with reasons for the potential development of vitamin D deficiency (cases 1–9), and volunteers, healthy individuals or patients with indifferent diseases, considered clinically to be replete with the vitamin (cases 10–20). Of the latter, two (cases 11 and 15) had recently been exposed to sunshine on holiday and three (cases 18–20) had previously received 5·2 μmol (2 mg) of cholecalciferol orally for the purpose of the investigation (see Table 1). It is considered that these twenty subjects differ significantly only in respect of their 'vitamin D status'; for convenience in description and for comparison with patients with primary disorders of parathyroid function, they are referred to as the 'control' group.

A second group (group 2, Table 2) comprised nine patients with surgically proved primary hyperparathyroidism and two with hypoparathyroidism complicating thyroid surgery. Trephine biopsies (2 mm) were obtained from the iliac crest, after tetracycline labelling in vivo, in the patients with primary hyperparathyroidism: all showed binding of the label at the sites of bone formation (Dr J. Ball) and thus, on this basis, none had a primary defect of mineralization.

Studies with radioactive cholecalciferol were undertaken in two other groups of subjects, at a time before we were able to trace the formation of labelled 1,25-(OH)₂D₃. The state of vitamin D nutrition in these individuals was assessed by the biologically assayed anti-ricketic activity of serum and not by the serum 25-(OH)D₃. Group 3 comprised sixteen otherwise healthy 'control' subjects, with as wide a range of vitamin D nutrition as in
<table>
<thead>
<tr>
<th>Subjects</th>
<th>Diagnosis</th>
<th>No.</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Serum calcium [mg/dl (mmol/l)]</th>
<th>Serum phosphorus [mg/dl (mmol/l)]</th>
<th>Serum alkaline phosphatase (K.A. units/dl)</th>
<th>Serum iPTH (ng/ml)</th>
<th>Serum 25-(OH)D₃ [ng/ml (nmol/l)]</th>
<th>Serum radioactive metabolites (% of dose/I)</th>
<th>Vitamin D metabolism and parathyroid function</th>
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<td>1</td>
<td>50</td>
<td>F</td>
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<td>5 (13)</td>
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<td>1·8 (0·58)</td>
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<td>3·4 (1·09)</td>
<td>18</td>
<td>0·7 5</td>
<td>6 (15)</td>
<td>7·0 0·05 0·07</td>
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<td>68</td>
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<td>71</td>
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<td>0·9 7</td>
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<td>63</td>
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<td>1·8 1·7</td>
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<td>F</td>
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<td>31</td>
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<td>4 (10)</td>
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<td>57</td>
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<td>0 0</td>
<td>3 (8)</td>
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<td>10</td>
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<td>3·0 (0·96)</td>
<td>13</td>
<td>0·2 1</td>
<td>10 (25)</td>
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<td>37</td>
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<td>3·3 (1·06)</td>
<td>—</td>
<td>0 0</td>
<td>30 (75)</td>
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<td>0 0</td>
<td>7 (18)</td>
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<td>13</td>
<td>57</td>
<td>F</td>
<td>9·3 (2·33)</td>
<td>3·0 (0·96)</td>
<td>13</td>
<td>0·1 0·7</td>
<td>7 (18)</td>
<td>7·4 0·02 0·04</td>
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<td>14</td>
<td>61</td>
<td>F</td>
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<td>3·9 (1·25)</td>
<td>11</td>
<td>0·2 0·1</td>
<td>28 (70)</td>
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<td>15</td>
<td>34</td>
<td>M</td>
<td>10·0 (2·50)</td>
<td>3·4 (1·10)</td>
<td>—</td>
<td>0 0</td>
<td>38 (95)</td>
<td>4·7 0·10 0·04</td>
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<td>3·0 (0·96)</td>
<td>—</td>
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<td>9 (23)</td>
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<td>—</td>
<td>0·7 0·2</td>
<td>8 (20)</td>
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<td>F</td>
<td>9·6 (2·40)</td>
<td>3·6 (1·16)</td>
<td>—</td>
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<td>31 (78)</td>
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<td>33</td>
<td>M</td>
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<td>3·1 (1·00)</td>
<td>—</td>
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<td>40 (100)</td>
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<td>43</td>
<td>M</td>
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<td>3·3 (1·06)</td>
<td>—</td>
<td>0·2 0·3</td>
<td>27 (68)</td>
<td>0·6 0·07 0·07</td>
<td>Vitamin D metabolism and parathyroid function</td>
</tr>
</tbody>
</table>

(1) Bone biopsy showed evidence of osteomalacia with varying degrees of osteitis fibrosa. Other cases designated as osteomalacic had overt clinical and radiographic features of the disease.

(2) Cases 18–20 received 5·2 μmol (2 mg) of cholecalciferol orally, 1 week before administration of the dose of radioactive cholecalciferol.
Table 2. Details of patients with primary disorders of parathyroid function

O.F., osteitis fibrosa; resorption+, evidence of increased bone resorption without osteitis fibrosa. For other abbreviations see Table 1. In case 23, the biopsy findings led to the diagnosis of myelomatosis after removal of a parathyroid adenoma. Diagnosis of primary hyperparathyroidism was confirmed by surgical removal of a single parathyroid adenoma in all patients but case 22, who was not submitted to operation.

<table>
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<tr>
<th>Subjects</th>
<th>Bone biopsy</th>
<th>Creatinine clearance (ml/min)</th>
<th>Serum calcium [mg/dl (mmol/l)]</th>
<th>Serum phosphorus [mg/dl (mmol/l)]</th>
<th>Serum alkaline phosphatase (K.A. units/dl)</th>
<th>Serum iPTH (ng/ml)</th>
<th>Serum 25-(OH)D3 [ng/ml (nmol/l)]</th>
<th>Serum 1,25-(OH)2D3 (OH)D3 [ng/ml (nmol/l)]</th>
<th>Serum 25-(OH)D3 (OH)D3 (OH)D3 (OH)D3 (% of dose/l)</th>
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<tr>
<td>No.</td>
<td>Age (years)</td>
<td>Sex</td>
<td>Normal</td>
<td>55</td>
<td>13.9 (3.47)</td>
<td>1.5 (0.48)</td>
<td>16</td>
<td>0.8</td>
<td>0.5</td>
</tr>
<tr>
<td>22</td>
<td>73</td>
<td>F</td>
<td>Normal</td>
<td>60</td>
<td>12.5 (3.12)</td>
<td>2.5 (0.75)</td>
<td>15</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>23</td>
<td>78</td>
<td>F</td>
<td>O.F.; myeloma</td>
<td>45</td>
<td>12.5 (3.12)</td>
<td>2.3 (0.74)</td>
<td>25</td>
<td>1.1</td>
<td>1.4</td>
</tr>
<tr>
<td>24</td>
<td>49</td>
<td>F</td>
<td>Resorption+</td>
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<td>12.4 (3.10)</td>
<td>2.0 (0.64)</td>
<td>12</td>
<td>0.4</td>
<td>0.9</td>
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<tr>
<td>25</td>
<td>77</td>
<td>F</td>
<td>O.F.± osteoporosis</td>
<td>40</td>
<td>11.0 (2.75)</td>
<td>2.1 (0.67)</td>
<td>39</td>
<td>5.0</td>
<td>8.0</td>
</tr>
<tr>
<td>26</td>
<td>57</td>
<td>F</td>
<td>O.F.± osteoporosis</td>
<td>38</td>
<td>14.0 (3.50)</td>
<td>2.3 (0.64)</td>
<td>16</td>
<td>3.4</td>
<td>1.7</td>
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<tr>
<td>27</td>
<td>41</td>
<td>F</td>
<td>Normal</td>
<td>90</td>
<td>11.9 (2.97)</td>
<td>1.7 (0.54)</td>
<td>13</td>
<td>1.1</td>
<td>1.0</td>
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<tr>
<td>28(i)</td>
<td>47</td>
<td>M</td>
<td>Normal</td>
<td>112</td>
<td>11.8 (2.95)</td>
<td>2.0 (0.64)</td>
<td>18</td>
<td>0.4</td>
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<tr>
<td>29(i)</td>
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<td>Normal</td>
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<td>2.2 (0.70)</td>
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<td>1.4</td>
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<td>Primary hyperparathyroidism; after resection of adenoma</td>
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<td>28(ii) 47 M</td>
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<td>2.4 (0.77)</td>
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<tr>
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<td>F</td>
<td>—</td>
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<td>32</td>
<td>70</td>
<td>F</td>
<td>—</td>
<td>6.0 (1.50)</td>
<td>4.0 (1.29)</td>
<td>—</td>
<td>8 (20)</td>
<td>7.3</td>
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Vitamin D metabolism and parathyroid function

Serum total anti-ricteretic activity

FIG. I. Relationships between the biologically assayed anti-ricteretic activity in serum and the fraction of serum radioactivity in the form of cholecalciferol, 24 h after injection of labelled cholecalciferol. ●, Group 3; ○, Group 4. Abscissa values are given in the equivalents of cholecalciferol (ng/ml).

group 1. Group 4 consisted of thirteen patients with chronic renal failure and a similar range of nutritional state. Previous studies indicate that the formation of 25-(OH)D₃ from a pulse dose of cholecalciferol is apparently unimpaired in renal failure (Mawer et al., 1971a, b). Data from groups 3 and 4 appear only in Fig. 1; they were not used in any of the statistical analyses.

The nature of the investigation was fully explained to all subjects who consented voluntarily to take part.

Experimental design

An initial blood sample was obtained for the measurement in serum of immunoreactive parathyroid hormone, total 25-hydroxycholecalciferols and total anti-ricteretic activity. Each subject then received an intravenous injection (Mawer, Lumb & Stanbury, 1969) of 26 nmol (10 μg) of [1,2-³H,4-¹⁴C]cholecalciferol of known ³H/¹⁴C ratio. [1,2-³H]Cholecalciferol (492 mCi/mmol) and [4-¹⁴C]cholecalciferol (323 mCi/mmol) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Venous blood samples were obtained 24 h and 48 h after the injection. In a proportion of subjects, additional blood samples were obtained at intervals up to 6–10 days.

Extraction and chromatography of cholecalciferol metabolites

The concentrations of labelled cholecalciferol and its metabolites were measured by counting the radioactivity in chromatographically separated fractions of serum extracts. Lipid extracts of serum were prepared as described previously (Mawer & Backhouse, 1969).

Cholecalciferol and its hydroxylated metabolites were separated by column chromatography on Sephadex LH20 (Pharmacia, Uppsala). The two-stage method, which is a modification of that of Holick & DeLuca (1971), has been described elsewhere (Taylor, Mawer & Reeve, 1973). The first column was eluted with chloroform–hexane (13:7, v/v) and the first 90 ml of eluate was collected, concentrated under nitrogen and re-chromatographed on the second column. This was eluted with chloroform–hexane (1:1, v/v) to separate cholecalciferol and 25-(OH)D₃: the dihydroxylated metabolites of cholecalciferol were eluted from the first column by larger volumes of chloroform–hexane (13:7, v/v).

A peak of radioactivity corresponding to 24,25-(OH)₂D₃ was eluted at 130–140 ml; a second peak at about 250 ml contained 1,25-(OH)₂D₃, together with other material believed to be 25,26-(OH)₂D₃ (Haussler & Rasmussen, 1972). The proportion of 1,25-(OH)₂D₃ in this latter peak was calculated from measurement of the ³H/¹⁴C ratio (Mawer et al., 1971a). During the renal synthesis of 1,25-(OH)₂D₃, tritium is selectively lost from the 1α position, resulting in a decrease in the ³H/¹⁴C ratio (Lawson, Wilson & Kodicek, 1969a, b).

Separated chromatographic fractions were dissolved in scintillator (Mawer et al., 1971b) and monitored for ³H and ¹⁴C radioactivity in a Nuclear-Chicago automatic liquid-scintillation counter. A dual-isotope computer program enabled both isotopes to be counted without cross-contamination.

Radioimmunoassay of parathyroid hormone

Determinations were made on separated serum by a method modified from Arnaud, Tsao & Littledike (1971). Two different antisera (367 and 211/32), prepared in guinea-pigs against the bovine hormone, were always used in triplicate assays.

Purified bovine hormone (biological activity in the thyro-parathyroidectomized rat, 2800–3000
units/mg) was prepared from defatted powdered bovine gland (Wilson Laboratories, Chicago) by phenolic extraction (Aurbach, 1959) and subsequent gel filtration (Rasmussen & Craig, 1962; Aurbach & Potts, 1964). Labelled hormone was prepared from this material by the method of Hunter & Greenwood (1962) and purified according to Schopman, Hackeng & Lequin (1970). The product had a specific radioactivity of 1·0-1·5 mCi/10 µg of hormone. The purified bovine hormone was initially used as standard. Subsequently, the pooled media from cultures of human parathyroid adenomata were assayed at numerous dilutions in human hypoparathyroid serum, given a bovine equivalent value and used for preparing standards in subsequent assays.

Reagents in the assay consisted of: 0·2 ml of unknown serum or dilution of test serum in hypoparathyroid serum; 0·1 ml of antiserum solution; 0·6 ml of Herbert's buffer. Antiserum solution consisted of 1/15 000 antiserum and 1/100 normal guinea-pig serum in Herbert's buffer with Trasylol 100 i.u./ml (Bayer Pharmaceuticals). Control solution was the same with antiserum omitted. After vortex mixing the samples were incubated for 4 days at 4°C: 0·1 ml of labelled hormone (3000 d.p.m.) was added and the samples were remixed and incubated for a further 4 days at 4°C. The free and bound fractions were separated by Dextran-coated charcoal (Herbert, Lau, Gottlieb & Bleicher, 1965).

Both antiserum 367 and 211/32 detect immunoreactive hormone in 85% of normal individuals, with a range of concentration of 0-0-8 ng/ml (bovine equivalents). In a direct comparison of sera from twenty-six normal individuals, the mean values were 0-42 ng/ml (sp 0·31) and 0-35 ng/ml (sp 0·3) with antiserum 367 and 211/32 respectively. Preliminary observations with this assay used to assess static and kinetic secretory states in secondary hyperparathyroidism have been published (Lumb & Stanbury, 1974).

Estimation of total anti-rickettic activity in serum

Total anti-rickettic activity in serum was assayed by the line test as previously described (Lumb, Mawer & Stanbury, 1971). The measured biological activity may include contributions from ergocalciferol, cholecalciferol and both 25-hydroxylated derivatives. The concentration of 1,25-(OH)₂D₃ in serum (q.v.) is too low to make a significant contribution.

Protein-binding assay for 25-hydroxycalciferols

The concentration of total 25-hydroxycalciferols in serum was determined by competitive protein-binding assay, a method modified from Haddad & Chyu (1971) being used. The method does not distinguish between 25-hydroxyergocalciferol and 25-hydroxycholecalciferol; for convenience and simplicity, results of assays are expressed as 25-(OH)D₃.

The lipids were extracted from 2 ml of serum by homogenization with 15 ml of diethyl ether–ethanol (9:1, v/v). The two phases were allowed to separate at room temperature, the ether layer was removed and the remaining lower phase washed twice with 10 ml of ether. The total ethereal extracts were combined and evaporated to dryness under nitrogen. The residue, dissolved in 1·5 ml of chloroform–hexane (1:1, v/v), was applied to a column (50 cm x 0·5 cm) packed with 3·5 g of Sephadex LH20 equilibrated with the same solvent mixture, which was also used for elution. The first 11 ml was discarded; the next 10 ml, which contained the 25-(OH)D₃, was collected and evaporated to dryness in a stream of nitrogen. Recovery (about 85%) from the extraction and chromatographic procedures was estimated by incorporating an internal standard (700 d.p.m.) of 25-[26,27-³H]hydroxycholecalciferol (6·9 Ci/mmol; The Radiochemical Centre). The binding protein was prepared from the kidneys of vitamin D-deficient rats (Haddad & Chyu, 1971).

The dried 25-(OH)D₃ fraction from the column was dissolved in 1 ml of ethanol and 50 µl samples, equivalent to 0·1 ml of serum, were pipetted into tubes for assay by the method of Haddad & Chyu (1971).

The limit of detection in this assay was 0·625 pmol per assay tube. With sera assaying at less than 7-5 nmol/l the determination was repeated with the equivalent of 0·2 ml of serum. All assays were performed in triplicate. Variation within assay was 10% and between assays 8%.

The normal range established in a group of seventeen healthy laboratory personnel was 22-5-150 nmol/l (mean 72 nmol/l; sp 38) in samples obtained between July and November; 15-70 nmol/l (mean 40-5 nmol/l; sp 15) in sera collected in April and May. In patients with osteomalacia
due to vitamin D deficiency, the corresponding values were 5·25–15·25 (mean 11·0 nmol/l; sd 7·0; n = 8) for June to November; 4·0–21·0 (mean 9·95 nmol/l; sd 4·7; n = 12) for December to May. Other apparently healthy individuals, receiving less solar exposure than the laboratory staff, had concentrations of serum 25-(OH)D$_3$ between 15 and 22·5 nmol/l (e.g. see Table 1). It is to be emphasized that the presence of osteomalacia cannot be equated with particular absolute levels of serum 25-(OH)D$_3$ (Stanbury, Torkington, Lumb, Adams, de Silva & Taylor, 1975).

**Derivation of molar concentrations of cholecalciferol metabolites in the serum**

The specific radioactivity of 25-(OH)D$_3$ in the serum, 24 h after injection of labelled cholecalciferol, was calculated as:

$$\text{radioactivity in 25-(OH)D}_3 \text{ chromatographic fraction}$$

$$\left[\text{assayed mass 25-(OH)D}_3\right] + \left[\text{calculated mass 25-(OH)D}_3 \text{ derived from dose}\right]$$

The basic assumption made is that this corresponds approximately to the specific radioactivity of the labelled dihydroxylated derivatives measured in the serum at 48 h. It is further assumed that the size of the extracellular pool of 25-(OH)D$_3$ is not changing significantly in this period; this is justified by previous observations (Mawer et al., 1972). On this basis, the molar concentrations of 1,25-(OH)$_2$D$_3$ and 24,25-(OH)$_2$D$_3$ were calculated from their respective radioactivities (see Table 4).

**Statistical methods**

Standard procedures were as described by Snedecor (1946) and Bailey (1959) and non-parametric methods by Siegel (1956). Standard deviation is designated throughout as so: the symbol ± is used only with SEM values.

**Results**

Clinical and biochemical data, together with measurements of vitamin D metabolites, are shown in Table 1 for control subjects and in Table 2 for patients with primary disorders of parathyroid function.

For comparative and statistical purposes, data are given for serum concentrations of labelled 25-(OH)D$_3$ at 24 h and of labelled 1,25-(OH)$_2$D$_3$ and 24,25-(OH)$_2$D$_3$ at 48 h after injection of the radioactive cholecalciferol. These are the times at which maximum concentrations of the respective metabolites are normally observed in man (Mawer et al., 1973).

**Effects of vitamin D status on the serum concentration of radioactive 25-(OH)D$_3$**

The percentage of serum radioactivity in the form of unchanged cholecalciferol, 24 h after injection of the labelled pulse, was directly related to the assayed total anti-ricketic activity in the serum (groups 3 and 4; Fig. 1). All subjects with assayed activity exceeding the equivalent of 78 pmol of cholecalciferol/ml of serum had received supplementary vitamin D and previous observations (Mawer et al., 1971) indicate that much of this biological

**FIG. 2. Relationship between the assayed serum concentration of 25-(OH)D$_3$ and the fraction of serum radioactivity in the form of cholecalciferol, 24 h after injection of labelled cholecalciferol.**

- Control subjects of Group 1. Patients: primary hyperparathyroidism before (○) and after (▲) parathyroidectomy; □, hypoparathyroidism. Arrows denote results from two additional cases of primary hyperparathyroidism not included in Table 2. The same symbols are used in subsequent diagrams. 25 nmol/l 25-(OH)D$_3$ = 10 ng/ml.
activity was probably attributable to unmetabolized cholecalciferol. There was, however, a similar direct correlation between the fraction of serum radioactivity present as cholecalciferol and the serum concentration of non-radioactive 25-(OH)D₃ measured by binding assay (group 1: Fig. 2).

The regression of the serum concentration of labelled 25-(OH)D₃ on the concentration of non-radioactive 25-(OH)D₃ in the twenty control subjects is shown in Fig. 3. The data from the patients with primary hyperparathyroidism lie within the 95% confidence limits of this regression. In subsequent description, the serum concentration of non-radioactive 25-(OH)D₃ (serum 25-(OH)D₃) is used as the index of vitamin D status.

**Effects of vitamin D status on the formation of labelled dihydroxylated derivatives**

The relationship between the serum 25-(OH)D₃ and the serum concentration of radioactive 1,25-(OH)₂D₃ at 48 h is shown in Fig. 4. No labelled 1,25-(OH)₂D₃ was detectable in control subjects with serum 25-(OH)D₃ > 25 nmol/l. In fourteen subjects with serum 25-(OH)D₃ ≤ 25 nmol/l, the serum concentration of labelled 1,25-(OH)₂D₃ was
Vitamin D metabolism and parathyroid function

Figure 5. Relationship between serum 25-(OH)D₃ and serum 24,25-(OH)₂D₃ (as in Fig. 4). The regression is calculated for the twelve control subjects with serum 25-(OH)D₃ ≥ 17.5 nmol/l. For symbols see Fig. 2.

Inversely related to the serum 25-(OH)D₃ (r = -0.68; P < 0.01; Fig. 4).

In the primary hyperparathyroidism group, all patients produced labelled 1,25-(OH)₂D₃ but there was no correlation between serum 25-(OH)D₃ and serum radioactive 1,25-(OH)₂D₃ (r = 0.1). Neither patient with hypoparathyroidism produced labelled 1,25-(OH)₂D₃.

Among the fourteen control subjects with serum 25-(OH)D₃ < 25 nmol/l, labelled 24,25-(OH)₂D₃ was detectable in the serum at 48 h in only two individuals, with serum 25-(OH)D₃ of 17.5 and 22.5 nmol/l respectively; above these values, the serum 25-(OH)D₃ and serum 24,25-(OH)₂D₃ were directly correlated (Fig. 5), but the relationship was not significant. However, in some vitamin D-deficient subjects, labelled 24,25-(OH)₂D₃ became detectable in serum samples obtained 6–10 days after injection of the tracer; this occurred only after the concentration of labelled 1,25-(OH)₂D₃ had started to diminish.

All patients with primary hyperparathyroidism had detectable amounts of labelled 24,25-(OH)₂D₃ in the serum at 48 h (Fig. 5), and the concentration of this metabolite was directly but insignificantly correlated with the serum 25-(OH)D₃ as in the control subjects (r = 0.52; P > 0.1; n = 9).

Thus in control subjects labelled 24,25-(OH)₂D₃

Table 3. Metabolism of labelled cholecalciferol by patients with primary hyperparathyroidism and by a control group matched for vitamin D nutritional state

The nine patients with primary hyperparathyroidism were matched with an equal number of control subjects. Significance of differences between the two groups was assessed by the two-tailed Mann-Whitney U-test (Siegel, 1956).

<table>
<thead>
<tr>
<th>% of injected dose/I of serum (mean value and so)</th>
<th>24 h after injection</th>
<th>48 h after injection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group</td>
<td>Patients</td>
<td>P</td>
</tr>
<tr>
<td>Cholecalciferol</td>
<td>1.35 (0.68)</td>
<td>1.54 (0.56)</td>
</tr>
<tr>
<td>25-(OH)D₃</td>
<td>6.28 (1.45)</td>
<td>6.96 (1.60)</td>
</tr>
<tr>
<td>24,25-(OH)₂D₃</td>
<td>0.01</td>
<td>0.05 (0.02)</td>
</tr>
<tr>
<td>1,25-(OH)₂D₃</td>
<td>0.02 (0.02)</td>
<td>0.03 (0.02)</td>
</tr>
</tbody>
</table>

(1) Only two or three subjects in the control group produced measurable amounts of 24,25-(OH)₂D₃ and therefore no so value is given.
was the predominant dihydroxylated metabolite produced when body vitamin D was adequate (serum 25-(OH)D₃ > 25 nmol/l) and labelled 1,25-(OH)₂D₃ was produced in states of relative vitamin D deficiency. Formation of these two metabolites appeared to be on a reciprocal basis in that significant amounts of both were not found in any one subject (Table 1). In the primary hyperparathyroidism group this relationship was not evident and all patients produced significant amounts of both 1,25-(OH)₂D₃ and 24,25-(OH)₂D₃ (Table 2; Figs. 4 and 5). The range of vitamin D status in that group (Table 2) was much narrower than in the twenty controls (Table 1). In Table 3, data from the primary hyperparathyroidism group are compared with selected controls, who were matched for vitamin D status on the basis of the 25-(OH)D₃ assay. The groups were indistinguishable in their conversion of cholecalciferol into 25-(OH)D₃, and the concentrations of 1,25-(OH)₂D₃ were not significantly different; the concentration of 24,25-(OH)₂D₃ was much higher in the primary hyperparathyroidism group \( (P < 0.02, \text{Table 3}) \). The sum of the concentrations of these two labelled metabolites in the last-named group was approximately 2.7 times that in the matched controls (Table 3). The mean creatinine clearance \( (\text{Ccr}) \) of 63 ml/min (SD 25.5) in the group implies that renal function, and thus probably also renal mass, was reduced in several of these patients. This could imply an even greater production of labelled dihydroxylated metabolites, per unit mass of renal tissue, in the hyperparathyroidism group than in their control subjects. There was, however, no significant correlation between \( \text{Ccr} \) and the serum labelled 1,25-(OH)₂D₃ in the hyperparathyroidism group \( (r = 0.22) \).

The metabolism of labelled cholecalciferol was observed in three patients after removal of a parathyroid adenoma (cases 28, 29 and 30, Table 2). The formation of labelled 25-(OH)D₃ (Fig. 3), 1,25-(OH)₂D₃ and 24,25-(OH)₂D₃ (Table 2) was indistinguishable from that of control subjects with comparable vitamin D status.

**Relation of vitamin D metabolites in the serum to the serum concentrations of calcium and phosphorus: control subjects**

There were significant direct relationships between the serum 25-(OH)D₃ and the serum calcium, phosphorus and [Ca] \( \times [P] \) product (Fig. 6).

The relationships between the serum calcium and the concentrations of labelled dihydroxylated derivatives at 48 h are shown in Fig. 7. Serum 1,25-(OH)₂D₃ was inversely related to the serum calcium \( (P < 0.001) \). No labelled 24,25-(OH)₂D₃ was detect

![Fig. 6. Relationships between serum 25-(OH)D₃ and (a) serum calcium, (b) serum phosphorus and (c) serum [Ca] \( \times [P] \) product, in the twenty control subjects. The regression equations are as follows: (a) \( y = 1.944 + 0.204 \) (± 0.03) log \( x \); \( r = 0.814 \); \( P < 0.001 \). (b) \( y = 0.493 + 0.320 \) (± 0.084) log \( x \); \( r = 0.671 \); \( P < 0.01 \). (c) \( y = 0.792 + 0.982 \) (± 0.189) log \( x \); \( r = 0.763 \); \( P < 0.001 \).](image-url)
Vitamin D metabolism and parathyroid function

Interrelationships of the variables correlating with the formation of labelled 1,25-(OH)₂D₃: control subjects

In the thirteen subjects who produced measurable amounts of labelled 1,25-(OH)₂D₃, its serum concentration (Y, 10⁻² × % of dose/l) was related to the serum calcium (X₁, mmol/l) and the serum 25-(OH)D₃ (X₂, nmol/l) by the partial regression equation:

\[ y = 34.2 - 12.28 (±4.24)x_1 - 0.156 (±0.068)x_2 \]

Relation of serum immunoreactive parathyroid hormone to the formation of 1,25-(OH)₂D₃ in control subjects

In the fifteen control subjects for whom measurements of immunoreactive parathyroid hormone were available (Table 2) the serum concentrations of the hormone and of labelled 1,25-(OH)₂D₃ were closely correlated (P<0.001, Fig. 8).

Interrelationships of the variables correlating with the formation of labelled 1,25-(OH)₂D₃: control subjects

In the thirteen subjects who produced measurable amounts of labelled 1,25-(OH)₂D₃, its serum concentration (Y, 10⁻² × % of dose/l) was related to the serum calcium (X₁, mmol/l) and the serum 25-(OH)D₃ (X₂, nmol/l) by the partial regression equation:

\[ y = 34.2 - 12.28 (±4.24)x_1 - 0.156 (±0.068)x_2 \]

in twelve control subjects with serum P₁ > 0.97 mmol/l there was a positive but insignificant correlation (r = 0.32; P > 0.1) between serum P₁ and serum 24,25-(OH)₂D₃.

No labelled 1,25-(OH)₂D₃ was detectable when the serum [Ca] × [P] exceeded a value of 2.34 mmol²/l² (29 mg²/dl²), and no 24,25-(OH)₂D₃ below a value of 2.26 mmol²/l² (28 mg²/dl²). Above the limiting value, the serum 24,25-(OH)₂D₃ and serum [Ca] × [P] were positively correlated (r = 0.48; P > 0.1; n = 10).

In the control subjects with serum P₁ < 0.97 mmol/l (9.2 mg/100 ml) there was no significant correlation between these two variables. No labelled 24,25-(OH)₂D₃ was detectable in subjects with serum P₁ < 0.97 mmol/l:

able when the serum calcium was less than 2.3 mmol/l (9.2 mg/100 ml); above this value the two variables were positively but insignificantly correlated (r = 0.37; P > 0.1; n = 7; Fig 7).

Labelled 1,25-(OH)₂D₃ was formed in association with serum concentrations of P₁ between 0.58 and 1.1 mmol/l (1.8 and 3.4 mg/100 ml) (Table 1), and there was no significant correlation between these two variables. No labelled 24,25-(OH)₂D₃ was detectable in subjects with serum P₁ < 0.97 mmol/l;
Both partial regression coefficients were significantly different from zero \((x_1, P<0.01; x_2, P<0.05)\). This implies that an increase of either independent variable \((x_1, x_2)\) was associated with a decrease of serum labelled \(1,25-(OH)_2D_3\) when the influence of the other was eliminated; or a decrease of either the serum \(25-(OH)D_3\) or serum calcium might independently increase the formation of \(1,25-(OH)_2D_3\). Because assays of immunoreactive parathyroid hormone were not available in all subjects (Table 1), partial regression analysis could not be extended to the third variable \((x_3)\), the serum hormone. The relationship between serum labelled \(1,25-(OH)_2D_3\) and the serum hormone \((y = 2.35 + 3.58 \times 0.57 x_3)\) was, however, of a high order of significance \((P<0.001)\). There are thus no mathematical grounds for attaching primary significance in control of the formation of \(1,25-(OH)_2D_3\) to any of the three variables considered.

**Relations of serum concentrations of labelled 1,25-(OH)_2D_3 and 24,25-(OH)_2D_3 to serum calcium, phosphorus and immunoreactive parathyroid hormone in patients with primary hyperparathyroidism**

In this group of patients there was no significant correlation between the serum concentration of labelled \(1,25-(OH)_2D_3\) and the serum calcium, \(P_i\) or \([Ca_0 \times P_i]\) product. The concentration of labelled \(24,25-(OH)_2D_3\) correlated positively, almost reaching significance, with the serum calcium \((r = 0.61; 0.05<P<0.1; \text{Fig. 7})\) and inversely but insignificantly with the serum \(P_i\) \((r = -0.43; P>0.1)\).

Measurements of serum immunoreactive parathyroid hormone from eight patients with primary hyperparathyroidism showed no correlation with the serum concentration of labelled \(1,25-(OH)_2D_3\) (Fig. 9). There was also no relationship between the serum hormone and the serum concentration of labelled \(24,25-(OH)_2D_3\) in this group of patients.

**Metabolism of cholecalciferol in hypoparathyroidism**

The formation of labelled \(25-(OH)D_3\) was appropriate to the vitamin D status of the two patients studied (Table 2; Figs. 2 and 3). Neither subject produced detectable amounts of labelled \(1,25-(OH)_2D_3\). The serum concentration of labelled \(24,25-(OH)_2D_3\) (Table 2) was inappropriately

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>3H, 14C-labelled</th>
<th>Specific radioactivity</th>
<th>3H, 14C-labelled</th>
<th>3H, 14C-labelled</th>
<th>Calculated concentrations (nmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(25-(OH)_2D_3)</td>
<td>(25-(OH)_2D_3)</td>
<td>(25-(OH)_2D_3)</td>
<td>(25-(OH)_2D_3)</td>
<td>(1,25-(OH)_2D_3) (24,25-(OH)_2D_3)</td>
</tr>
</tbody>
</table>
| 5            | 753             | 17.4                  | 43.2            | 8               | 0
d| 0.13 \(0.19\) | \(0.07\) \(0.10\) |
| 8            | 545             | 13.6                  | 40.1            | 4               | 0
d| 0.07 \(0.10\) | \(0.07\) \(0.10\) |
| 11           | 665             | 79.3                  | 8.4             | 6               | 0
d| 0.21 \(0.25\) | \(0.27\) \(0.31\) |
| 14           | 278             | 71.8                  | 3.9             | 6               | 0
d| 1.50 \(1.54\) | \(1.50\) \(1.54\) |
| 24           | 421             | 25.3                  | 16.8            | 4               | 0
d| 0.11 \(0.14\) | \(0.17\) \(0.23\) |
| 27           | 822             | 13.7                  | 35.6            | 5               | 0
d| \(1.35\) \(1.42\) | \(1.35\) \(1.42\) |

\(1\) Sum of the concentrations of unlabelled \(25-(OH)_2D_3\) measured by protein-binding assay and the \(25-(OH)_2D_3\) derived from the injected pulse of labelled cholecalciferol.

\(2\) A, concentration before injection of tracer dose; B, concentration 48 h after the injection.
high for the prevailing serum calcium but probably appropriate to the serum phosphorus.

Derived molar concentrations of metabolites of cholecalciferol in the serum

The method of derivation of the molar concentrations of 1,25-(OH)₂D₃ and 24,25-(OH)₂D₃ in the serum is demonstrated for representative subjects in Table 4.

The mean increment in the concentration of 25-(OH)D₃ in the serum 24 h after the injected pulse of cholecalciferol, was not significantly different in the primary hyperparathyroidism group (4.35 nmol/l; sd 1.0) and in the twenty control subjects (3.67 nmol/l; sd 1.69).

The range of estimated concentrations of 1,25-(OH)₂D₃ in the serum of the thirteen control subjects producing labelled metabolite was 45-132 pmol/l (mean 90 pmol/l; sd 25); the mean value increased to 122 pmol/l (sd 25) after injection of labelled cholecalciferol. In the nine patients with primary hyperparathyroidism, the corresponding mean values of 148 pmol/l (sd 66) and 177 pmol/l (sd 74) were both significantly higher (P < 0.01). This difference is adequately explained by the different nutritional state in the two groups; the mean serum 25-(OH)D₃ was 21.1 nmol/l (sd 6.0) in the hyperparathyroidism group and 14.5 nmol/l (sd 6.0) in the thirteen control subjects, among whom were several patients with clinical vitamin D deficiency (Table 1).

The estimated mean serum concentration of 24,25-(OH)₂D₃ was 3.4 nmol/l (sd 3.48) in the eight control subjects producing labelled metabolite and 173 pmol/l (sd 99) in the nine patients with primary hyperparathyroidism. This difference was related to the much higher values of serum 25-(OH)D₃ in these eight control subjects (65.6 nmol/l; sd 30.4).

Discussion

The present investigation confirms the previous observation in man (Mawer et al., 1973) that the formation of labelled 1,25-(OH)₂D₃ from an administered pulse of radioactive cholecalciferol is detectable only in subjects relatively deficient of vitamin D. Among individual control subjects, no labelled 1,25-(OH)₂D₃ was detected when the serum 25-(OH)D₃ exceeded 25 nmol/l (Table 1); and, by regression analysis, the mean limiting value of serum 25-(OH)D₃ was approximately 37.5 nmol/l (Fig. 4). Below this limiting concentration there was a significant inverse relationship between the serum concentration of non-radioactive 25-(OH)D₃ and of labelled 1,25-(OH)₂D₃. In the fourteen subjects of group 1 (Table 1) with serum 25-(OH)D₃ < 25 nmol/l there was only a small variation in the serum concentration of labelled 25-(OH)D₃ at 24 h (mean 6.91 % dose/l; sd 1.17). There was no correlation between these values and the serum concentrations of labelled 1,25-(OH)₂D₃ at 48 h (r = 0.13); but the latter values were significantly correlated with the specific radioactivity of serum 25-(OH)D₃ at 24 h (r = 0.71; P < 0.01). Thus the principal determinant of the concentration of labelled 1,25-(OH)₂D₃ in the serum was the concentration of serum 25-(OH)D₃ at the time of injection of the tracer. This relationship (Fig. 4) might imply that the formation of labelled 1,25-(OH)₂D₃ was regulated in some way by the prevailing state of vitamin D nutrition; but it could result from dilution of labelled 25-(OH)D₃ into pre-existing body pools of 25-(OH)D₃, the radioactive enrichment of the 1,25-(OH)₂D₃ diminishing with increase of pool size. The lack of a similar relationship between serum 25-(OH)D₃ and labelled 1,25-(OH)₂D₃ in patients of the primary hyperparathyroidism group, with serum 25-(OH)D₃ less than 37 nmol/l (Table 2), makes the latter alternative unlikely. So also do the observations on the formation of radioactive 24,25-(OH)₂D₃. The mean serum concentration of non-radioactive 25-(OH)D₃ (65.5 nmol/l; sd 30.5) in the subjects of group 1 who produced labelled 24,25-(OH)₂D₃ was much greater than the mean value (14.75 nmol/l; sd 5.75) in those producing labelled 1,25-(OH)₂D₃, and this must imply corresponding differences in total body pools of 25-(OH)D₃ (Mawer et al., 1972). The concentration of labelled 24,25-(OH)₂D₃ at 48 h was correlated positively, albeit insignificantly, with both the serum 25-(OH)D₃ (r = 0.3) and the serum labelled 25-(OH)D₃ (r = 0.48). Since both dihydroxylated metabolites are produced from the same precursor substrate, labelled 25-(OH)D₃, it is illogical to postulate that dilution of this radioactive precursor should limit the radioactive enrichment of 1,25-(OH)₂D₃ and at the same time apparently increase the enrichment of 24,25-(OH)₂D₃. The most reasonable interpretation of the data is that, below a limiting concentration of 25–37.5 nmol/l,
the formation of labelled 1,25-(OH)₂D₃ is inversely related to the serum 25-(OH)D₃ (Fig. 4); above that limit, no 1,25-(OH)₂D₃ is formed and labelled 24,25-(OH)₂D₃ is produced in proportion to the body pool of 24-(OH)D₃ (Fig. 5). This interpretation is in general agreement with the results of animal experiments. In the rat, an administered pulse of 25-(OH)D₃ is metabolized to 1,25-(OH)₂D₃ in vitamin D deficiency, and to 24,25-(OH)₂D₃ in animals replete with the vitamin (Galante, Colston, Evans, Byfield, Matthews & MacIntyre, 1973). The effects of pre-existing body pools of vitamin D and its metabolites must inevitably complicate the interpretation of radioisotopic studies (Stanbury, Mawer, Hill, Holman, Jones & Van Den Berg, 1972), but the results obtained in the present investigation suggest that this factor is outweighed by the physiological factors determining the formation of 1,25-(OH)₂D₃.

The observation that the serum concentration of labelled 1,25-(OH)₂D₃ in subjects of group 1 was also significantly correlated with the serum calcium (Fig. 7) and the serum immunoreactive parathyroid hormone (Fig. 8) provides additional, indirect evidence that the regression in Fig. 4 represents a physiological relationship. A reduction of serum calcium and secondary increase of parathyroid secretion are expected consequences of vitamin D deficiency; and the state of vitamin D nutrition in the present study was assessed in the first place in terms of the serum concentration of 25-(OH)D₃ (Table 1). In the thirteen subjects who produced labelled 1,25-(OH)₂D₃ the serum 25-(OH)D₃ was 25 nmol/l or less. On the basis of these values, and the clinical state and nutritional history of these individuals (Table 1), they can reasonably be regarded as having varying degrees of 'vitamin D deficiency'.

The relationship shown in Fig. 4 suggests that the formation of 1,25-(OH)₂D₃ from a single intravenous dose of cholecalciferol is regulated in proportion to the prevailing degree of physiological deficiency, and the state of vitamin D nutrition in the present study was assessed in the first place in terms of the serum concentration of 25-(OH)D₃. In the thirteen subjects who produced labelled 1,25-(OH)₂D₃ the serum 25-(OH)D₃ was 25 nmol/l or less. On the basis of these values, and the clinical state and nutritional history of these individuals (Table 1), they can reasonably be regarded as having varying degrees of 'vitamin D deficiency'.

The data obtained from the subjects of group 1 would be compatible with control of the formation of 1,25-(OH)₂D₃ being mediated through change of serum calcium (Fig. 7) or serum immunoreactive parathyroid hormone (Fig. 8), or by some other function of the state of vitamin D nutrition (Fig. 4); but it was not possible to identify any of these three interrelated variables as the primary determinant of the renal 1α-hydroxylation of 25-(OH)D₃. Experiments in animals have been similarly inconclusive, different investigators attaching primary significance to each of these factors (Boyle, Gray & DeLuca, 1971; Fraser & Kodicek, 1973; Garabedian, Holick, DeLuca & Boyle, 1972; Larkins, MacAuley, Rapoport, Martin, Tulloch, Byfield, Matthews & MacIntyre, 1974; Rasmussen, Wong, Bikel & Goodman, 1972). Primary hyperparathyroidism in man provides the potential for dissociating these normally interrelated factors. Individual patients were matched nutritionally with control subjects capable of producing labelled 1,25-(OH)₂D₃ (Table 3); the serum calcium (mean 3·18 mmol/l; sd 0·28) in each was above the concentration at which formation of this metabolite became undetectable in control subjects (Fig. 7), and the adenomatous basis of the disease implies a reduced or absent reactivity of parathyroid hormone secretion to change in serum calcium (Lumb & Stanbury, 1974). Since these patients produced the same amount of labelled 1,25-(OH)₂D₃ as their control subjects, despite the presence of hypercalcaemia (Fig. 7), it is reasonable to exclude the serum calcium as determinant of its formation. These data conflict with the claim made for the rat that parathyroid hormone inhibits the formation of 1,25-(OH)₂D₃ in the presence of hypercalcaemia (Galante, MacAuley, Colston & MacIntyre, 1972). The lack of correlation between serum concentrations of labelled 1,25-(OH)₂D₃ and of 25-(OH)D₃ in the primary hyperparathyroidism group suggests further that the formation of 1,25-(OH)₂D₃ in primary hyperparathyroidism may be uninfluenced by the state of vitamin D nutrition (see Fig. 4). To establish this point unequivocally, it is necessary to extend observations to patients with serum 25-(OH)D₃ >37·5 nmol/l and such studies are in...
progress. With this reservation, it seems reasonable to attribute the continued formation of 1,25-(OH)₂D₃ in hypercalcaemic primary hyperparathyroidism to the sustained inappropriate secretion of parathyroid hormone. By analogy, the effects of vitamin D deficiency in the subjects of group 1 might also be produced through accompanying changes of parathyroid function.

There was a highly significant proportional relationship between the serum concentration of labelled 1,25-(OH)₂D₃ and serum immunoreactive parathyroid hormone in the subjects of group 1 (Fig. 8), but not in the patients with primary hyperparathyroidism (Fig. 9). This difference may reflect a dose–response relationship or a saturation phenomenon. The serum immunoreactive parathyroid hormone exceeded 1 ng/ml in only two of the fifteen measurements available in subjects of group 1 (mean 0·44 ng/ml; SD 0·53), whereas this value was exceeded in five of the eight patients with primary hyperparathyroidism (Fig. 9; mean 1·74 ng/ml; SD 1·63). It is possible that maximum stimulation of 1α-hydroxylation occurs with the moderate secondary hyperparathyroidism of vitamin D deficiency, and that no further effect is produced by the greater parathyroid activity in the particular group of patients with primary hyperparathyroidism studied.

On the basis of animal experiments, Tanaka & DeLuca (1973) have suggested that parathyroid hormone affects the renal synthesis of 1,25-(OH)₂D₃ indirectly, through its influence on serum P₃, or on the orthophosphate concentration of renal cells. In the present study there was no significant relationship between the serum concentration of labelled 1,25-(OH)₂D₃ and the serum P₃, either in the primary hyperparathyroidism group (r = -0·18) or in the subjects of group 1 (r = -0·14). The mean serum P₃ in the primary hyperparathyroidism group (0·65 mmol/l; SD 0·822) was significantly lower than in the control subjects producing labelled 1,25-(OH)₂D₃ (0·85 mmol/l; SD 0·171; P < 0·01). The reduced glomerular filtration in the patients with primary hyperparathyroidism would tend to limit the reduction of serum P₃ in response to parathyroid hormone, and it is conceivable that there was a greater reduction of renal cellular P₃ in these subjects than is suggested by the concentrations of serum P₃. These patients produced the same mean serum concentration of labelled 1,25-(OH)₂D₃ as their nutritionally matched control subjects (Table 3), despite this evidence of secondary renal damage. It will be important to ascertain if the production of labelled 1,25-(OH)₂D₃, in cases of primary hyperparathyroidism with completely normal renal function is actually greater than in appropriately matched control subjects.

The significance of the derived molar concentrations of 1,25-(OH)₂D₃ in serum (Table 4) depends on the validity of the assumptions made. First, that the specific radioactivity of serum 25-(OH)D₃ does not change materially between 24 and 48 h after injecting the tracer; a limited number of observations indicates that this is the case (unpublished work). Secondly, that serum 1,25-(OH)₂D₃ is turning over sufficiently rapidly for the labelled 25-(OH)D₃ in the serum to be considered the exclusive source of the 1,25-(OH)₂D₃ measured at 48 h. There is no information available on the turnover of 1,25-(OH)₂D₃ in man and this second assumption cannot be validated directly. In the chick, the half-life of injected 1,25-(OH)₂D₃ in serum is 4–5 h (Lawson & Emtage, 1974); in the rat it may be longer than this (Mawer, Backhouse, Hill & Taylor, 1975), and both animal studies indicate that the 1,25-(OH)₂D₃ in the intestinal mucosa accounts for the greater part of the body pool, the amount in circulation being a relatively small fraction of the whole. The molar concentration of 1,25-(OH)₂D₃ in the serum, as estimated with these assumptions (Table 4), showed only a fourfold variation in the vitamin D-deficient subjects of group 1, between the lowest value before (45 pmol/l) and the highest value after (185 pmol/l) injection of the labelled cholecalciferol. Because they produced no labelled 1,25-(OH)₂D₃ (Table 1), it was not possible to estimate the concentration of this metabolite in the vitamin D-replete subjects of this group. With an independent biological assay used for 1,25-(OH)₂D₃ (Hill, Taylor & Mawer, 1974), its mean serum concentration in a separate group of vitamin D-replete subjects was 130 pmol/l (SD 75), and comparable values have been obtained by competitive protein-binding assay (Brumbaugh, Haussler, Bressler & Haussler, 1974). The close agreement between the results obtained by three very different techniques serves indirectly to validate the method of derivation used in the present study. The order of concentration and its small variation are compatible with the concept that 1,25-(OH)₂D₃ is produced in regulated quantity as a hormone, and the present investigation suggests that para-
thyroid hormone functions as a trophic influence controlling its synthesis.

Because most of the subjects in group 1 were selected deliberately for their low vitamin D status, and since labelled 24,25-(OH)2D3 was detected only in those with serum 25-(OH)D3 > 17.5-22.5 nmol/l (Table 1), data on the formation of this metabolite are limited. Derivation of the molar serum concentration of 24,25-(OH)2D3 is also less secure than with 1,25-(OH)2D3, as nothing is known either of its rate of turnover or of the dimensions of its body pool in any animal species. The concentrations calculated for the subjects of group 1 varied between 0-15 and 8.7 nmol/l, which, if valid estimates, would imply a higher range and greater variability than with the hormonal metabolite, 1,25-(OH)2D3. The relationship shown in Fig. 5 suggests that 24,25-(OH)2D3 may be formed preferentially in vitamin D-replete subjects in proportion to the serum 25-(OH)D3. No labelled 24,25-(OH)2D3 was detectable in association with serum calcium <2.33 mmol/l (9.3 mg/100 ml), serum Pi <0.97 mmol/l, which, if valid estimates, would be 0.15 and 8.7 nmol/l, which, if valid estimates, would indicate a net wastage of 25-(OH)D3 by the kidney in this disease is some two to three times greater than in nutritionally matched controls (Table 3). If 24,25-(OH)2D3 has no specific function, this would imply a net wastage of 25-(OH)D3. But the combined renal metabolites represent so small a fraction of the administered dose that it is unlikely that such wastage could actually produce a conditioned deficiency of 25-(OH)D3 or cholecalciferol in this disease.

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References


Vitamin D metabolism and parathyroid function


