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Factors in human vitamin D nutrition and in the production and cure of classical rickets

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Vitamin D reaches the body from two totally dissimilar sources, first by cutaneous synthesis under the action of ultraviolet light and secondly in the diet. The relative contribution of these sources to human vitamin D nutrition is uncertain; this paper will examine some of the factors which determine their efficacy and the ultimate production of rickets. Our studies have also provided evidence for an action of vitamin D on renal tubular phosphate reabsorption in healing rickets.

The natural vitamin D, cholecalciferol, is produced in skin by the action of ultraviolet light on the physiological precursor 7-dehydrocholesterol, which in man is concentrated in the Malpighian layer of the epidermis (Wheatley & Reinertson, 1958). Diets contain cholecalciferol (and probably a significant amount of 25-hydroxycholecalciferol (25-HCC)) and in addition may be fortified, especially in the USA and Canada, with the yeast vitamin product, ergocalciferol. With the exception of oily fish, natural foods are surprisingly deficient in vitamin D, considering the usual connotations of the word 'vitamin'. We rely heavily on eggs as a natural source of vitamin D but they too are fortified with vitamin D, or rather the food of the battery hen is.

Vitamin D from dietary and cutaneous sources is metabolized in liver to form 25-hydroxy-vitamin D (25-OHD), the major circulating form of the vitamin (DeLuca, 1974*a*, *b*). We have used a precise radio-competitive protein-binding assay for 25-OHD (Haddad & Chyu, 1971) which measures together the equally effective 25-hydroxy derivatives of both ergocalciferol and cholecalciferol. Isotopic tracer studies with radioactive cholecalciferol and comparisons between measured 25-OHD levels and bioassayable anti-rachitic activity indicate that, under physiological conditions, vitamin D circulates almost exclusively as its hydroxylated derivatives (Stamp, 1973). Plasma levels of 25-OHD are therefore a precise measurement of nutritional status.

During a study of possible sex and age differences in plasma 25-OHD, we noted apparent inconsistencies as the months progressed, with new control populations giving different results from those already established. Eventual comparison of several populations within single assays led us to demonstrate a marked seasonal variation in plasma 25-OHD regardless of age or sex (Stamp & Round, 1974): this is shown in Table 1.

Table 1. *Plasma 25-hydroxy-vitamin D (25-OHD) levels of 198 healthy white British subjects of both sexes sampled over a period of 22·5 months and grouped according to age and season of the year*

Age range (years)	Sex	No.	Season	Plasma 25-OHD (μg/l) Mean	Plasma 25-OHD (μg/l) Range (95% confidence limits)	Vitamin D intake* (μg/d) Mean	Vitamin D intake* (μg/d) Range (95% confidence limits)
12–17	Male	59	Late September–early October 1971	22·6[a]	13·4–37·9	3·67 (n=38)	1·08–12·38
7–10	Both	26	October–early November 1972	21·3[a]	10·1–45·3		
18–37	Both	27	October–early November 1972	21·3[a]	10·8–41·8	3·75 (n=19)	1·18–12·10
75–86	Both	18	Late November 1972–late January 1973	12·4[b]	5·1–29·8		
14–17	Female	15	Late January 1973	12·9[b]	7·2–23·2		
18–37	Both	19	Late March 1973	12·9[b]	6·6–25·2		
14–17	Female	14	Early July 1973	17·0[c]	7·6–38·4		
19–36	Both	20	Early August 1973	20·4[a]	9·7–42·8		
	Total	198					

Mean values with a different superscript letter are significantly different; significance of differences: [a-b] $P<0·001$, [b-c] $P<0·05$, [a-c] $P<0·01$.

*As cholecalciferol equivalents.

When dietary vitamin D intake was assessed, five out of thirty-eight boys showed an intake of less than 1·8 μg cholecalciferol/d. All had entirely normal plasma biochemistry (Round, 1973). In a small series of Caucasian patients with nutritional osteomalacia reported by Dent & Smith (1969), vitamin D intakes were all less than 1·8 μg/d. Thus, estimated vitamin D intake may bear little relationship to nutritional status and, even in the denigrated British climate, summer sunshine may contribute the major part of our vitamin D provided we achieve average exposure. The situation is similar in the USA, and Table 2 (from Haddad & Stamp, 1974) shows mean values, this time in the same subjects, sampled in late January and again in late July; there was actually a tendency towards an increase in dietary vitamin D during winter.

There is a sizeable public health problem of rickets and osteomalacia among the immigrant Asian community in Britain. Fig. 1 (from Gupta, Round & Stamp, 1974) shows the seasonal variation in plasma 25-OHD among a group of Asians sampled in early spring and again at the end of summer. Values for Caucasian subjects were derived from the results of Stamp & Round (1974). Spring values among the Asians were associated with significant hypocalcaemia, but when sampled again later in the season, all but one subject were cured of their hypocalcaemia.

The suggested causes of rickets and osteomalacia in Asians include dietary vitamin D deficiency, racial pigmentation with melanin, which is popularly supposed to interfere with ultraviolet transmission through the epidermis, genetic

Table 2. *Seasonal variations in serum 25-hydroxy-vitamin D (25-OHD) levels in thirteen Caucasian students* †

(Mean values and standard deviations)

	Vitamin D intake‡ (μg/week)		Time spent outdoors (h/week)		Serum 25-OHD (μg/l)	
Season	Mean	SD	Mean	SD	Mean	SD
Summer	31·6	8·6	16·1	6·5	29·1	7·8
Winter	35·9	11·7	10·3*	6·5	20·3**	6·1

Values significantly different from those for summer: *$P<0.05$, **$P<0.005$.
†From Haddad & Stamp (1974).
‡As cholecalciferol equivalents.

factors, social customs by which subjects avoid sunshine exposure and finally dietary consumption of large quantities of phytate, for example in chupatties, which may bind calcium in the gut as insoluble calcium phytate and thereby lessen Ca absorption. We studied most of these factors in a 14-year-old, dark-skinned

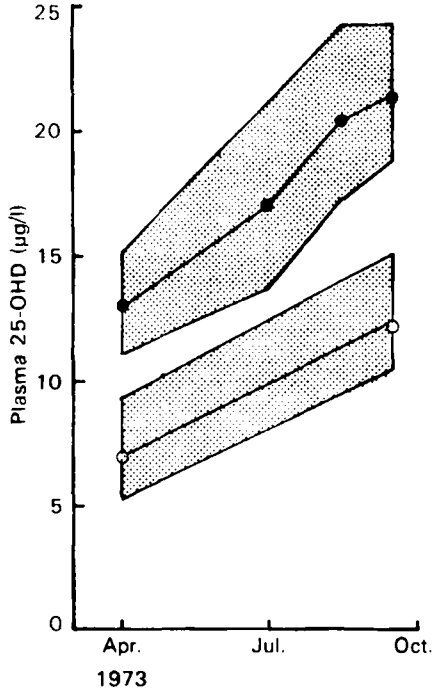

Fig. 1. Plasma 25-hydroxy-vitamin D (25-OHD) levels at different times of the year in healthy Asian (○———○) and white (●———●) populations in the UK; ▣, 95% confidence limits (mean±2×SE). From Gupta, Round & Stamp (1974).

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Asian boy, with results shown in Fig. 2 (Dent, Round, Rowe & Stamp, 1973). Radiology had shown that his rickets was healing well and the healing phase was confirmed by a positive Ca balance of about 300 mg/d. Nevertheless, he was severely hypocalcaemic, with a plasma Ca of 1·55 mmol/l. He was given a diet deficient in vitamin D throughout. Substitution of a low-phytate diet made no

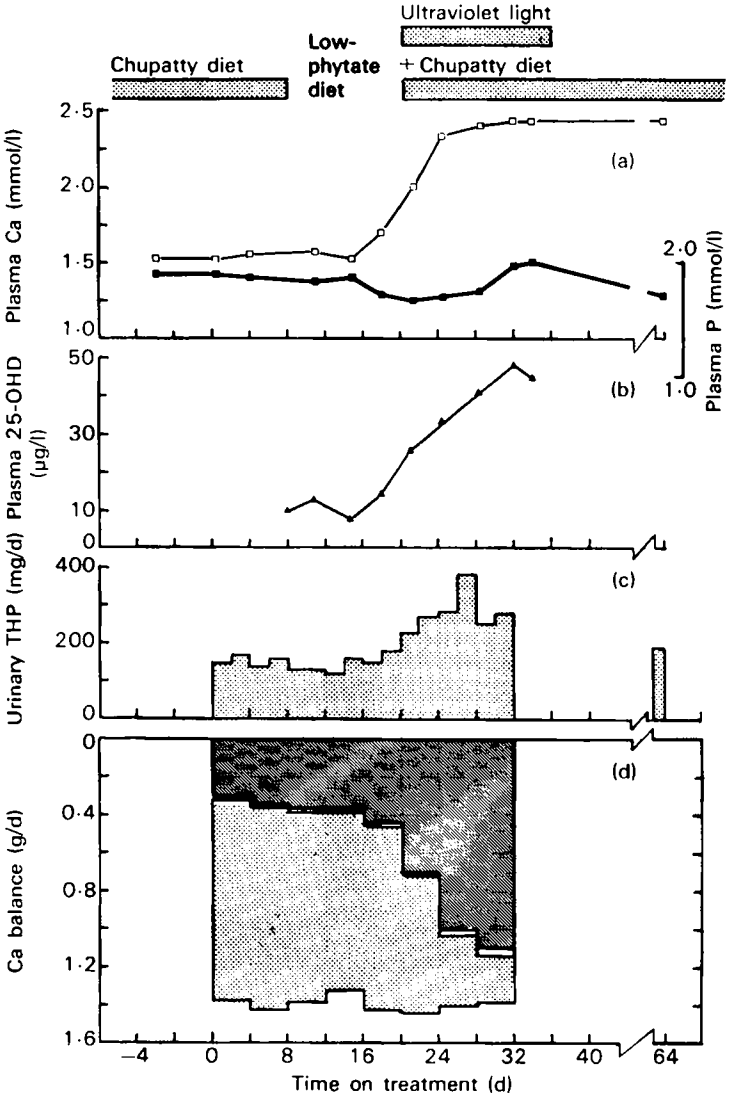

Fig. 2. Metabolic studies during investigation and treatment of a patient with nutritional rickets on a virtually vitamin D-free diet (from Dent, Round, Rowe & Stamp, 1973). Results show negligible change with alterations in dietary phytate (consumption of chupatties), and rapid healing with ultraviolet light. (a) Plasma calcium (□——□) and phosphorus (■——■) levels; (b) plasma 25-hydroxy-vitamin D (25-OHD) level; (c) urinary total hydroxyproline (THP) excretion;. (d) Ca balance: ▦, faecal Ca; □, urinary Ca; ▨, Ca balance.

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detectable difference to his plasma biochemistry or to his Ca balance. He returned to a high-phytate diet and was treated with artificial ultraviolet light. Rapid healing followed with a marked fall in faecal Ca, notwithstanding his high-phytate diet. His urinary total hydroxyproline excretion increased and plasma 25-OHD levels rose steadily to the high normal range. These findings indicated that neither skin pigmentation nor high-phytate diets interfere with the treatment of rickets and osteomalacia by ultraviolet light, and suggest that they may not be important as a cause.

The actual site of vitamin D synthesis within skin has long been uncertain, and indirect evidence has suggested that synthesis may occur at least partly on the skin surface. In rats, the precursor 7-dehydrocholesterol is maximally concentrated in the sebaceous glands and dead keratin fraction of skin (Gaylor & Sault, 1964), suggesting that it is first secreted, then irradiated and vitamin D is then either absorbed or ingested by the process of grooming. Vitamin D can cure rickets when applied to unbroken animal skin (Helmer & Jansen, 1937*a*) and anti-rachitic material has been extracted from human skin washings (Helmer & Jansen, 1937*b*). In order to study this further, the skin of one subject was swabbed with dilute ethanol after vigorous exercise. The swabs were extracted with diethyl ether and the extract was assayed for 25-OHD. A value equivalent to 10 ng 25-OHD was obtained.

In order to determine whether significant surface synthesis of vitamin D occurred during ultraviolet irradiation, a 14-year-old Asian girl with nutritional rickets was studied on full metabolic balance (T. C. B. Stamp & C. E. Dent, unpublished results). Results are shown in Fig. 3. Her severe hypocalcaemia with radiographic changes characteristic of healing rickets and positive Ca balance closely resembled the clinical features of the patient described earlier. Her diet throughout was low in vitamin D (less than 0·5 μg/d) and high in phytate. She bathed thoroughly with soap and water immediately before irradiation, in order to remove any vitamin D precursors from the skin surface, and she bathed again immediately after irradiation in order to remove any vitamin D formed on the skin surface during her brief treatment. Healing was nevertheless rapid and showed a rapid rise in plasma Ca, positive Ca balance and normal plasma 25-OHD. If studies in fur-bearing species or in birds confirm that surface secretion of 7-dehydrocholesterol contributes to their vitamin D nutrition, then in man's evolution his site of vitamin D production has conceivably evolved from skin surface to within the epidermis.

These two cases provide additional evidence on the action of vitamin D in healing rickets. Recent work has suggested that the only action of vitamin D relevant to healing of rickets is its stimulation of intestinal Ca transport to provide adequate Ca and phosphorus concentrations in plasma for normal calcification to occur in bone matrix and cartilage (DeLuca, 1971). The only direct effect of vitamin D metabolites in bone is the stimulation of bone Ca mobilization; bone resorption is greatly enhanced at minute hormone concentrations of 10^{-10} mol/l (Raisz, Trummel, Holick & DeLuca, 1972; Reynolds, 1974). Nevertheless, present

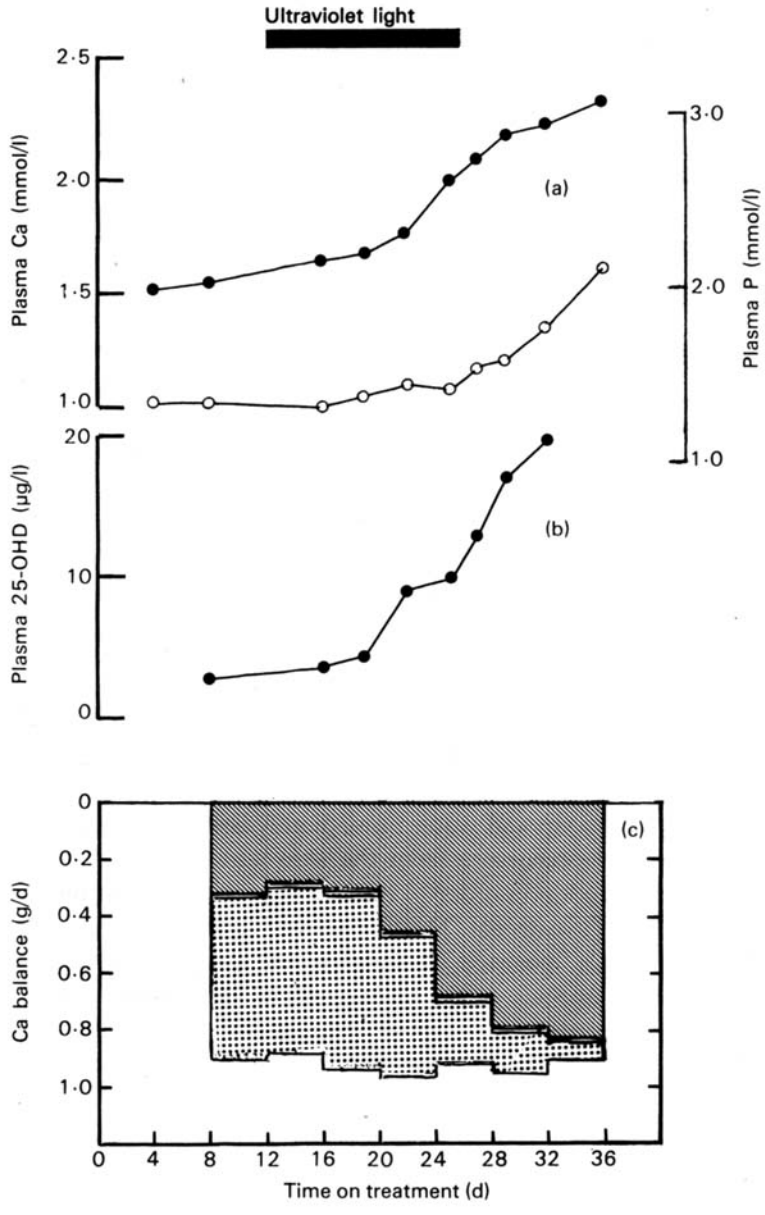

Fig. 3. Metabolic balance studies in a 14-year-old Asian girl with nutritional rickets. During the experiment, she received ultraviolet irradiation once/d and bathed thoroughly before and after irradiation. (a) Plasma calcium (●——●) and phosphorus (○——○) levels; (b) plasma 25-hydroxy-vitamin D (25-OHD) level; (c) Ca balance: ▦, faecal Ca; □, urinary Ca; ▧, Ca balance.

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clinical results show that hypocalcaemia may persist during healing rickets and therefore that calcification within bone may proceed in advance of stimulated intestinal Ca transport. There is thus a further action of vitamin D which requires clarification. Its explanation may involve the renal action of vitamin D on tubular phosphate reabsorption. There is evidence that 25-HCC enhances renal tubular phosphate reabsorption directly (Puschett, Moranz & Kurnick, 1972) and we have previously drawn attention to the supranormal rise which occurs in plasma P during healing rickets (Dent *et al.* 1973) and which is illustrated here. We have moreover confirmed that this may be associated with a rise in maximum renal tubular phosphate reabsorption to unusually high levels (Dent & Stamp, 1970; Stamp & Stacey, 1970) and that ergocalciferol may be much more effective than dihydrotachysterol in this respect (Stamp & Stacey, 1970). Since the hypocalcaemic effect of raised plasma P levels is enhanced in metabolic bone disease (Stamp, 1971), indicating preferential precipitation of Ca and P within bone in these conditions, present results suggest that healing of rickets with vitamin D may therefore be due to the combined action of enhanced intestinal Ca transport and enhanced renal tubular P reabsorption, the P retention producing hypocalcaemia and further enhancing calcification. Such a combined action may reconcile some of the earliest human metabolic balance results (Rominger, 1939) which led to the belief that vitamin D acted primarily on intestinal P absorption.

It is commonly believed that skin pigmentation with melanin retards vitamin D synthesis (Loomis, 1970; Bronowski, 1973). I studied this further by comparing the rise in plasma 25-OHD during standard ultraviolet irradiation in the two Asian patients described above, four white subjects and one black African volunteer. Subjects received a standard course of whole-body irradiation at a distance of 450 mm from the light source using a Theraktin lamp (Rank Medical Equipment, Welwyn Garden City, Herts.) giving a strong emission at 290 nm. Subjects received irradiation for 1 min on the front and 1 min on the back the first day, and irradiation to each surface was increased by 1 min on successive days. Mild erythema occasionally required constant irradiation on successive days. Plasma 25-OHD levels during this therapy are shown in Fig. 4 and changes were similar in every subject. While these changes may represent maximum stimulation of vitamin D synthesis, overcoming a partial pigment barrier, it is clear that even the blackest skin is far from an absolute barrier to vitamin D synthesis. Alone among these subjects, the black volunteer showed no subjective trace of skin tenderness. It is more sensible to conclude that black skin evolved for protection against tropical sunburn than to suggest that white skin evolved to ensure vitamin D nutrition in temperate climates. Avoidance of direct sunshine and more extensive clothing are likely to be at least as important as dietary deficiency in the production of rickets and osteomalacia among the Asian community in Britain.

Intestinal absorption of 25-HCC and hepatic 25-hydroxylation of vitamin D has been studied in man. Haddad & Stamp (1974) found a dose-response relation between plasma 25-OHD and constant daily pharmacological dosage of vitamin D. Over a shorter time-scale, a single large oral dose of cholecalciferol (25 mg) gave

the same peak plasma 25-OHD as a daily dose of 1 mg given for 25 d, despite a 7 d lag with the latter treatment (Haddad & Stamp, 1974). There is nevertheless strong evidence from in vitro studies that hepatic 25-hydroxylation of vitamin D is regulated by feedback control, probably product inhibition (DeLuca, 1974*b*). In

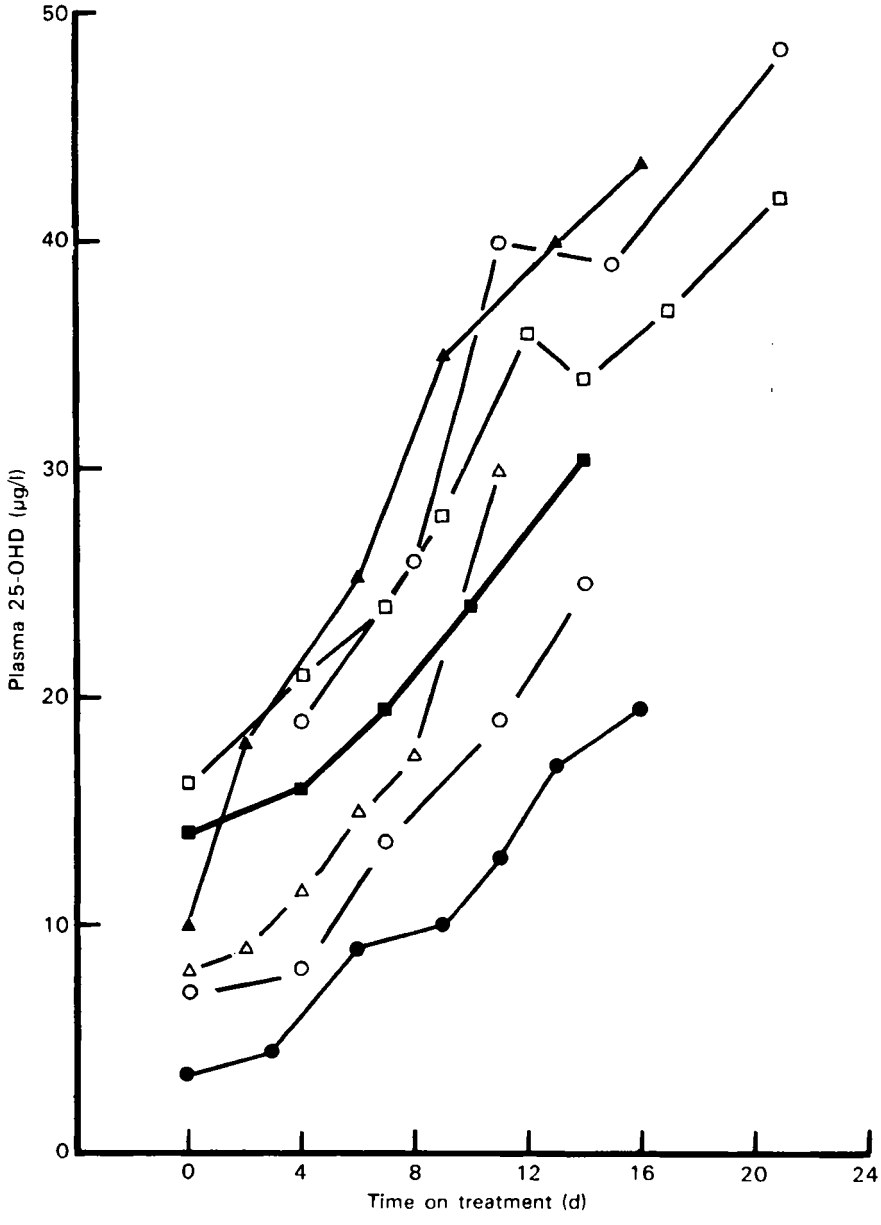

Fig. 4. Plasma 25-hydroxy-vitamin D (25-OHD) levels in volunteer subjects during daily ultraviolet irradiation treatment: ○, □, △, white subjects; ▲, ●, Asian subjects; ■, a black subject. Each line represents the results for one subject.

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man, feedback inhibition would clearly be of low order in view of the dose-response relation found by Haddad & Stamp (1974). Fig. 5 shows the relationship between initial plasma 25-OHD levels and the plasma 25-OHD response to intravenous cholecalciferol. Subjects were arbitrarily divided on the basis of 'high' and 'low' initial levels. Even on high dosage with cholecalciferol (12–15 μg/kg body-weight), subjects with initial plasma 25-OHD levels above 20 μg/l showed no rise in 24 or 48 h, while the rise which occurred in subjects with initially low levels, including those receiving intermediate vitamin D dosage (5–10 μg/kg body-weight) was significant in the first 24 h (J. G. Haddad & T. C. B. Stamp, unpublished results).

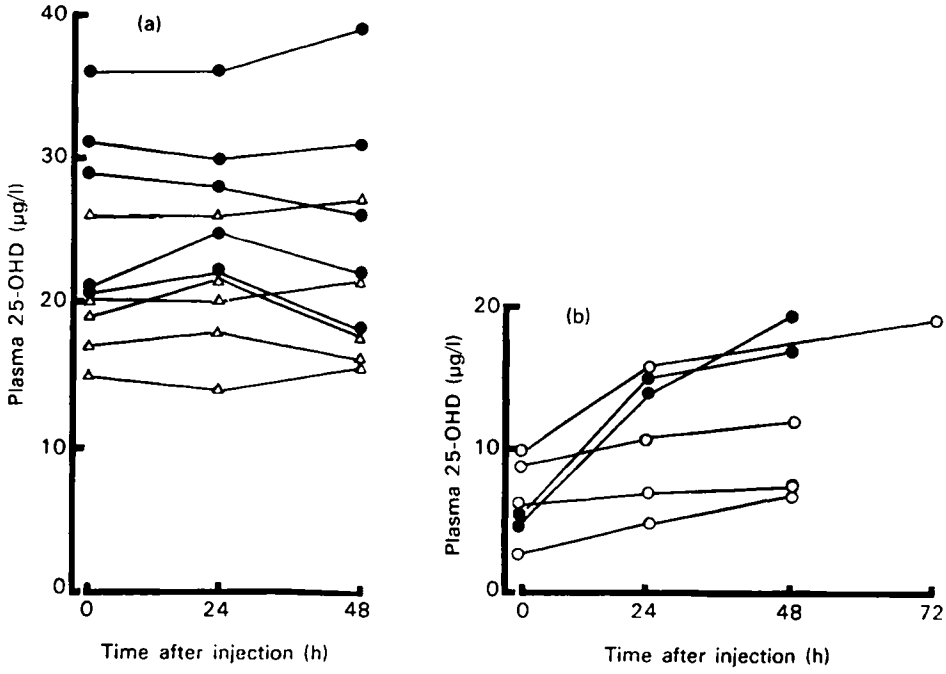

Fig. 5. Plasma 25-hydroxy-vitamin D (25-OHD) levels following intravenous injection of cholecalciferol in normal subjects and in patients with (a) high or (b) low initial levels of plasma 25-OHD. ●———●, Injection of 12–15 μg cholecalciferol/kg body-weight; ○———○, 5–10 μg/kg body-weight; △———△, 1–4 μg/kg body-weight. Mean change in plasma 25-OHD: (a) +0·5 μg/l (not significant); (b) +5·0 μg/l ($P<0·02$).

In order to study intestinal absorption separately from subsequent hepatic 25-hydroxylation, 25-HCC itself was administered orally in a standard dose of 10 μg/kg body-weight to twelve subjects, and postabsorptive 25-OHD levels were measured. The results are shown in Fig. 6 (Stamp, 1974). Further study demonstrated that postabsorptive levels were normal in patients with gross nutritional deficiency and with primary hyperparathyroidism. Normal levels were found in a patient with post-gastrectomy osteomalacia and steatorrhoea; the

dietary history indicated that simple vitamin D deficiency from intolerance of the necessary fatty foods may determine vitamin D deficiency after partial gastrectomy (Morgan, Paterson, Woods, Pulvertaft & Fourman, 1965). The most severe impairment of oral 25-HCC tolerance was seen in patients with obstructive jaundice (Stamp, 1974). We have more recently demonstrated that malabsorption in obstructive jaundice may be improved by simultaneous bile salt administration and that postabsorptive 25-OHD levels may be normal in isolated pancreatic steatorrhoea (A. Reuben, P. Cotton & T. C. B. Stamp, unpublished results).

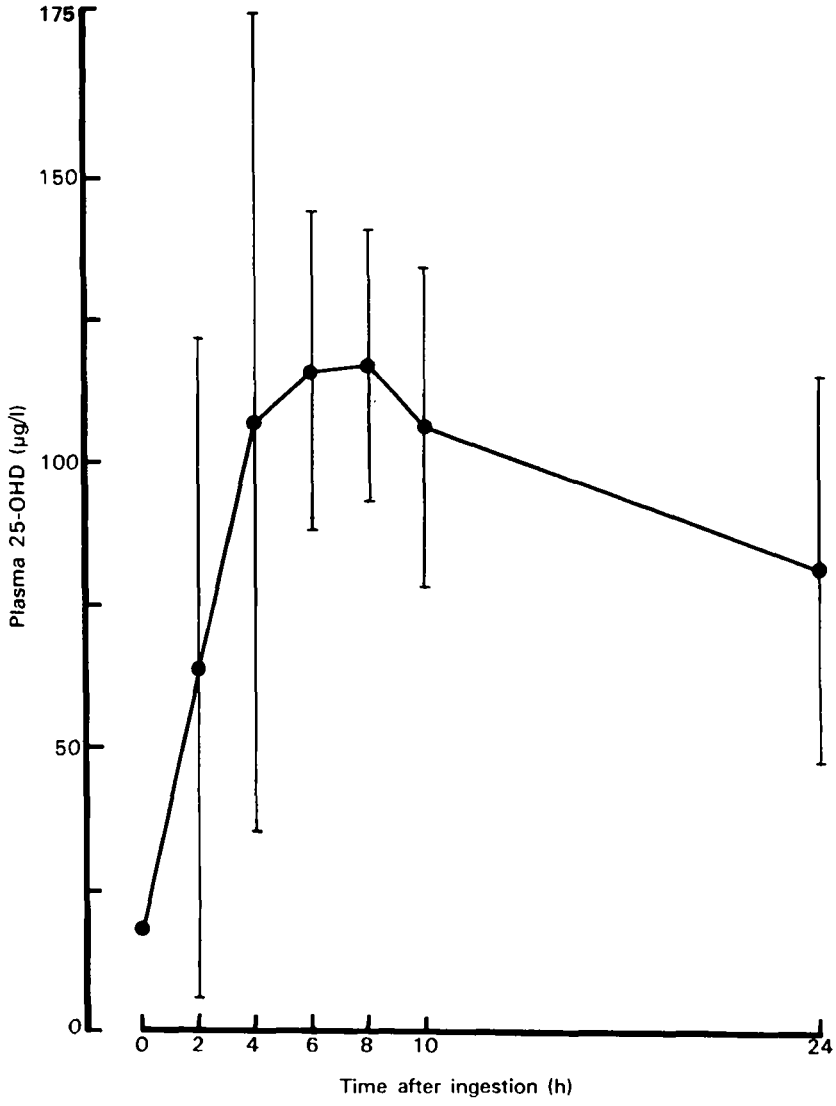

Fig. 6. Mean plasma 25-hydroxy-vitamin D (25-OHD) level in twelve healthy adult subjects following oral administration of 10 μg 25-hydroxycholecalciferol/kg body-weight; vertical bars, limits of 2×SD from the mean. From Stamp (1974).

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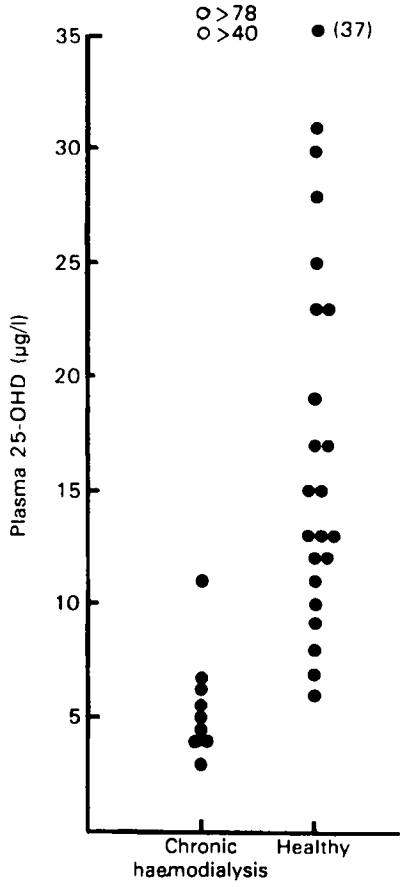

Fig. 7. Plasma 25-hydroxy-vitamin D (25-OHD) levels in eleven patients receiving chronic haemodialysis treatment and in twenty-three healthy subjects sampled during the same month (●); ○, two patients who had received vitamin D supplements and whose values are not included in calculations. From unpublished results of W. R. Cattell, L. R. I. Baker, J. G. Haddad & T. C. B. Stamp.

Vitamin D nutrition may be disturbed in chronic renal failure, and it has been reasonably supposed that low vitamin D intake is an important factor in the production of renal osteodystrophy (Lumb, Mawer & Stanbury, 1971). Fig. 7 shows the very low plasma 25-OHD levels in most patients undergoing regular dialysis treatment for renal failure compared with a healthy population sampled in the same month (W. R. Cattell, L. R. I. Baker, J. G. Haddad & T. C. B. Stamp, unpublished results). Dietary intake in these patients was nevertheless very carefully controlled and they had a converted log mean intake of 3·8 μg cholecalciferol/d (95% confidence limits 2·5–5·7 μg/d). This intake should be optimal in normal subjects, and other factors may clearly determine vitamin D deficiency in patients with chronic renal failure. Problems of vitamin D nutrition

exist in every aspect of human biology, human evolution, physiology, public health and in many aspects of human disease. Scope for research into all these aspects continues.

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