Vitamin D activity of plasma in idiopathic hypercalcaemia

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Idiopathic hypercalcaemia is a condition noted in young children, from a few months to a few years old, and is so called because of the absence of any obvious cause for the typical elevated levels of serum calcium. The signs and symptoms of the disease have been described by Lightwood (1952*a,b*), Payne (1952), Fanconi, Girardet, Schlesinger, Butler & Black (1952), Lightwood & Stapleton (1953) and Creery (1953). The condition is characterized by hypercalcuria, hypercalcaemia, azotaemia and renal dysfunction. There is also an increased absorption of Ca from the intestine, elevation of serum citrate level, increased renal phosphate reabsorption and growth retardation; all these effects are closely similar to those noted after administration of excess vitamin D (Fellers, 1959), except that in idiopathic hypercalcaemia there is usually no evidence that abnormally large amounts of vitamin D have been administered.

Lightwood & Stapleton (1953) observed that the disease could occur in one or other of two forms, the benign or mild, and the severe. The signs listed above are noticeable in both types of patient, but those with the severe form typically display several further features, physical and mental retardation, osteosclerosis and cardiac systolic murmurs, and they frequently demonstrate a typical facies known as 'elfin' face; the severe condition may be chronic and so further developed than the benign or mild form, which may be a more acute and transient manifestation of the disease.

The toxic manifestations, the renal and cardiac damage, the osteosclerosis and the mental and physical retardation are probably the result of the elevated serum Ca levels, because the disease can be controlled by measures that control serum Ca, e.g. cortisone administration, induction of a negative Ca balance by administration of a diet low in Ca or low in Ca and rich in phytic acid, or administration of ethylene-diaminetetra-acetate (EDTA) (Creery & Neill, 1954; Ferguson & McGowan, 1954; Bonham-Carter, Dent, Fowler & Harper, 1955; Stapleton, McDonald & Lightwood, 1956; Morgan, Mitchell, Stowers & Thomson, 1956).

Investigators have frequently remarked on the close correspondence between the condition noted in vitamin D intoxication and idiopathic hypercalcaemia (e.g. Lowe, Henderson, Park & McGreal, 1954; Creery, 1953; Creery & Neill, 1954; Schlesinger, Butler & Black, 1956; Lightwood & Stapleton, 1953; Bonham-Carter *et al.* 1955; Fellers, 1959; Morgan *et al.* 1956; Fellers & Schwartz, 1958*a*; Rhaney & Mitchell, 1956) and it has indeed been suggested that this condition may be in fact vitamin D intoxication in children especially susceptible to the toxic action of this vitamin (Morgan *et al.* 1956).

The observation that idiopathic hypercalcaemia is much rarer in the USA, Canada and Sweden than in the UK has also been put forward as evidence that vitamin D may be implicated (Morgan *et al.* 1956), because, in 1956, the vitamin D

content of infant milk foods was lower in those countries (about 40 i.u./100 ml in the USA) than in the UK (about 130 i.u./100 ml). Since 1957 the vitamin D content of infant dried milks in the UK has been reduced (now about 40 i.u./100 ml), and the vitamin D content of infant cereal foods has also been similarly decreased (from about 600–1000 i.u./oz to about 200 i.u./oz) thus markedly decreasing the average vitamin D intake of children in this country. It is not yet possible to state whether these changes have been associated with a significant reduction in the incidence of idiopathic hypercalcaemia, which, over the past few years, has amounted to about seventy to eighty cases yearly.

Idiopathic hypercalcaemia could easily be explained if there had been an excessive intake of vitamin D (and no evidence for it could be discovered in most instances in spite of detailed inquiries). An abnormal sensitivity to the vitamin, an abnormally high absorption and retention, a decrease in breakdown of the vitamin or its elimination from the body or an abnormality in Ca metabolism might also each offer an explanation for this disease.

Vitamin D activity can normally be demonstrated in a number of tissues of the body, since these tissues, or suitable extracts thereof, when orally administered to rachitic rats may cure rickets to some degree. Only in a very few instances has the compound responsible for this vitamin D activity been identified, by, for instance, radiochemical methods combined with chromatographic and chemical procedures (Kodicek, 1956; Kodicek & Ashby, 1960; Kodicek, Cruickshank & Ashby, 1960). Thomas, Morgan, Connor, Haddock, Bills & Howard (1959) have shown that the vitamin D activity of serum is non-dialysable, that this activity is located, after electrophoresis, between the α_1- and α_2-globulins and that it is so whether the vitamin D (ergocalciferol) is administered to the patient before examination of the serum or added to the serum sample in vitro. Assay of pooled human serum fractions demonstrated vitamin D activity in the α-globulin-containing fractions (IV–1, IV–4), with traces in fraction IV but none in fractions I, II or III. Fellers (1959) reported that the vitamin D activity of the serum of a child receiving 60 000 i.u./day was distributed about equally between the α-lipoprotein and albumin fraction and the high-density β-lipoprotein fraction. From these observations it may be concluded that the vitamin D activity of serum is probably due to the presence of a compound having vitamin D activity and associated with a macromolecule.

Little is known of the fate of vitamin D when administered in physiological doses because of the extreme difficulty of measuring the minute quantities involved. Cruickshank & Kodicek (1953), Kodicek (1956) and Fellers (1959) followed the fate of ergocalciferol by radiochemical procedures, using ^{14}C-marked ergocalciferol, or by biological methods after administering large doses (40 000 i.u.) to the rat. These workers have shown that, under these conditions, the concentrations in the liver 24 h after dosing were much higher than in the blood and other tissues, but that, within the next few days, the concentrations fell more rapidly in the liver than in the blood. Cruickshank & Kodicek (1953) also showed that only a small proportion of the administered vitamin D could be recovered from the test rats (6% after 1 day and less than 2% after 4 days). This poor recovery was shown, by tests with ^{14}C-

marked ergocalciferol (Kodicek, 1956; Kodicek & Ashby, 1960) to be due partly to poor absorption and partly to degradation of the vitamin within the body. The percentage absorption and degradation were also found to be similar for doses of 40 000 i.u. or 400 i.u./rat, and, at least at 24 h after administration, the vitamin concentrations were higher in the liver than in the other organs of rats given either of these doses. Vollmer (1939) demonstrated vitamin D activity (about 20 i.u./g) in skin and liver samples obtained at post-mortem from a child who had been given, within the $3\frac{1}{2}$ days before death, 1.6×10^6 i.u. vitamin D.

Eaton, Spielman, Loosli, Thomas, Norton & Turk (1947) showed that liver concentrations in the young calf were initially higher than plasma concentrations of the vitamin after administration of 50 000 i.u. vitamin D, but that the plasma concentration fell only slowly during the subsequent 42-day period. Heymann (1937) noted in the rabbit that 1 week after administration of 200 000 United States Pharma-copoeia units of vitamin D the vitamin activity was higher in blood plasma than in the other tissues and that plasma levels remained higher than those in other tissues for at least the next 12 weeks. In a series of tests on dogs and rabbits, Warkany (1936, 1937) showed, like Heymann (1937), that the serum vitamin D activity, once elevated by administration of a single large dose (100 000 United States Pharma-copoeia units of vitamin D), only slowly returned to previous levels (4–6 weeks), even though the initial rise in vitamin D activity was rapid, occurring within the first 1 or 2 days after dosing.

There is little evidence as to the relative concentrations of the vitamin in liver and blood under physiological conditions, but Devaney & Munsell (1935) reported the vitamin D activity of liver from pigs, sheep and cattle as ranging from about 0.1 to 0.5 i.u./g, and Warkany (1937) has shown the vitamin D activity of serum samples from these species to be between about 0.5 and 1.5 i.u./ml. Thus, under physiological conditions the liver reserves may be small compared with the quantity circulating in the blood.

In the pig, Quarterman, Dalgarno & Adam (1963) have shown that, after treatment for 1 month with daily doses of 250 000 i.u. vitamin D_3/day, the vitamin D activities of the liver and blood were both elevated to about the same extent (to 27 and 23 i.u./g respectively), whereas at a much lower dose level (350 i.u./day) the liver had an activity only about half that of the blood (0.21 and 0.52 i.u./g respectively). Within a week of withdrawal of the vitamin D_3 doses, it was noted that the vitamin D activity of the liver in both groups had fallen to about half that of the blood. Blood vitamin D activities steadily fell over the next 6 months, to 0.15 and 0.08 i.u./g in those groups that had been given the high and low doses of vitamin respectively. Liver vitamin activities were found to be about half those of the blood in both groups throughout this period.

Most of the blood vitamin D activity has been shown to be in the plasma rather than the corpuscles (Hess, Light, Frey & Gross, 1932). The foregoing observations would suggest that, at least when large doses of the vitamin, whether vitamin D_2 or vitamin D_3, are administered, some of it may be preferentially deposited in the liver, though within a relatively short period the liver concentration may fall and

that in the blood may then be higher. At lower, presumably physiological, doses, the blood levels are higher than those of the liver and all other organs examined.

In man the vitamin D activity of blood serum was found, in Cincinnati, to be about 1 i.u./ml (Warkany, 1936; Warkany & Mabon, 1940), whereas in Baltimore (Thomas *et al.* 1959) and in the UK (Cuthbertson, 1963) a similar but slightly higher mean value (about 2 i.u./ml serum or plasma) was reported. The liver vitamin D activity has been determined in two subjects (Cuthbertson, 1963), both aged 10–12 months. The control, not suffering from any disease of Ca metabolism, showed an activity of about 3 i.u./g liver, but the hypercalcaemic patient's liver contained less, about 1 i.u./g liver. Serum vitamin D assays were not carried out on either subject, but it can be seen that the liver activity was not greatly different from what would have been expected in the plasma of these patients.

The serum vitamin D activity may quickly be raised in man by administering the vitamin; with daily doses the rise may continue from a few hours after the first dose to reach a maximum level after several weeks (Thomas *et al.* 1959; Warkany, Guest & Grabill, 1942). As in animals, the serum activity, once elevated, is slow to subside, several months at least being required for return to normal levels (Warkany *et al.* 1942).

Table 1. *Serum vitamin D activity (i.u./ml) of patients given daily doses of ergo-calciferol for periods of more than 1 month*

	Serum vitamin D activity	
	Arthritic	Hypoparathyroid
Daily dose	patients	patients
(i.u.)	(Warkany *et al.* 1942)	(Thomas *et al.* 1959)
50 000	16·5	40, 40
100 000	—	30, 48, 50
150 000	66	—
180 000	33	—
200 000	44	18, 8
240 000	33	—
400 000	130, 90	—
500 000	90, 130	—

Table 1 shows the maximum serum vitamin D activities attained in two groups of patients who had been prescribed known high levels of the vitamin for periods of time never less than 1 month and mostly considerably longer. One group (Warkany *et al.* 1942) suffered from chronic proliferative arthritis and the other (Thomas *et al.* 1959) from hypoparathyroidism. The results in Table 1 clearly show that the serum vitamin D activity cannot be precisely predicted from the dose given. Even the level attained with a constant dosage may vary unpredictably (Warkany *et al.* 1942), but in at least one patient, and perhaps in others, part of this variation may have been due to failure to take the dose prescribed. Thomas *et al.* (1959) have shown that the relation between serum Ca level and serum vitamin D activity is also variable. Thus it appears that there are from one person to another large quantitative differences in response to a given dose of vitamin D.

A number of determinations of vitamin D activity have been made on serum (or plasma) samples from patients with idiopathic hypercalcaemia and are summarized in Table 2 (Lang & Eiardt, 1957; Fellers & Schwartz, 1958a,b; Fellers, 1959; Thomas et al. 1959; Smith, Blizzard & Harrison, 1959; Cuthbertson, 1963).

Table 2.   *Vitamin D activity of plasma from patients with idiopathic hypercalcaemia*

Reference and country of origin of samples	No. of patients and type* of idiopathic hypercalcaemia	Serum (or plasma) vitamin D activity (i.u./ml)	Time taken for vitamin D activity to decline to normal level (months)
Lang & Eiardt (1957); W. Germany	1, severe	4	~8
Fellers & Schwartz (1958a); USA		20–30	>18
Fellers & Schwartz (1958b); USA	3, severe	20–60	>17 <26
Fellers (1959); USA		20	?
Thomas et al.† (1959); UK	2, mild	Normal (1–3)	—
Smith et al. (1959); USA	1, severe	23	>6
Cuthbertson (1963); UK	6, mild 5, severe	Normal (1–3) Normal (1–3)	—

*Mild or severe according to the criteria of Lightwood & Stapleton (1953).
†Patients suffering from mild form of disease (R. G. Mitchell, 1962, personal communication).

The plasma and serum vitamin D activities recorded in samples from two patients with the benign form of the disease (R. G. Mitchell, 1962, personal communication) by Thomas et al. (1959) and by Cuthbertson (1963) on other similar patients, were not significantly different from normal values or from values determined on control patients, i.e. 1–3 i.u./ml. Similarly, in serum from a patient suffering from the severe form of the disease, Lang & Eiardt (1957) found a vitamin D activity of about 4 i.u./ml, i.e. a little above normal, as might perhaps have been expected in this patient, who had received u.v. light treatment and daily doses of 1000–10 000 i.u. vitamin D, apart from the administration of a total of 1 600 000 i.u. vitamin D in three separate doses during the previous 2 years!

The patients with the severe condition, reported by Fellers & Schwartz, 1958a,b) and by Smith et al. (1959), present a different situation, in that the high serum activities (Table 2) found were such as would only be expected to result from the administration of large amounts (more than 20 000 i.u.) of the vitamin daily. The vitamin D intake of these patients, as reported by Fellers & Schwartz (1958a) and by Smith et al. (1959), was in no way abnormal, ranging from about 1000 to 5000 i.u./day and would certainly not be expected to have induced such high serum levels in normal patients. These results differ markedly from those found

by Cuthbertson (1963) (Table 2) in a series of five patients with the severe type of disease, i.e. suffering from mental retardation, systolic murmurs, osteosclerosis and the typical 'elfin' facies. Plasma samples were not obtained from three of these patients until they had been on a diet free from added vitamin D for a period of from 3 weeks to $6\frac{1}{2}$ months; it is thus possible that the vitamin D activity may have fallen during this period. Even so, had these patients resembled those of Fellers & Schwartz (1958*a*) or of Smith *et al.* (1959), the vitamin D activity would still have been elevated even after this lapse of time, for these authors noted elevated serum activity from 6 months to 2 years after stopping the administration of vitamin D. Plasma samples were taken from the other two severely affected patients, in one instance before transfer to a diet free from vitamin D; the sample from the other patient was taken 10 days after removal of all sources of vitamin D from the diet. These two samples were both shown to have vitamin D activities within the normal range and not to differ in this respect from control samples assayed at the same time.

The condition noted in the severely affected patients studied by Cuthbertson (1963) cannot therefore be attributed to unusually large doses of vitamin D (because these infants had received average amounts, from 1000 to 3000 i.u. vitamin D/day) or to unusually high levels of circulating vitamin D (which could have been brought about by abnormally effective absorption, by decreased elimination of vitamin D or by other means); they would seem best to be explained by an unusual sensitivity to doses of the vitamin normally quite harmless or to some other derangement in Ca metabolism. The severely affected patients observed in the USA by Fellers & Schwartz (1958*a*) and by Smith *et al.* (1959) all had exceptionally high serum vitamin D activities, sufficient easily to account for the observed state of these patients, not unlike that to be expected from chronic vitamin D intoxication; it is thus not necessary to postulate any abnormal sensitivity to vitamin D in these patients. The high level of circulating vitamin D is, however, more difficult to explain; it could have arisen from undisclosed or unknowing administration of large doses of vitamin D before or during treatment, though it is unlikely that it would have escaped notice. Even if it had occurred, the serum vitamin D activity would not, to judge from the work of Warkany *et al.* (1942), have been expected to remain elevated for so long after removal of vitamin D from the diet. It may therefore be that certain instances of the severe form of idiopathic hypercalcaemia are brought about by a derangement of vitamin D metabolism that permits high levels of circulating vitamin D activity even when the subjects have received only average amounts from the diet (1000–5000 i.u./day). In patients with the benign form of the disease, all studies on serum and plasma vitamin D activity (Thomas *et al.* 1959; Cuthbertson, 1963) have shown the levels to lie within the normal range, so that the condition may be due to an unusual sensitivity to the vitamin. It is also possible, in both the severe and benign forms of the disease, that a derangement of Ca metabolism is primarily responsible, in that the condition is associated with an unusually efficient absorption of Ca (Creery & Neill, 1954; Bonham-Carter *et al.* 1955; Morgan *et al.* 1956), and that improvement follows reduction of the serum Ca levels, whether it

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is brought about by treatment with cortisone, EDTA or phytic acid, or simply by maintenance on a diet low in Ca (Morgan *et al.* 1956). The single observation on liver stores, in a patient suffering from the benign form of the disease (Cuthbertson, 1963) and a control patient, again supports the suggestion that vitamin D metabolism is not deranged. Both the hypercalcaemic patient and the control had been receiving 3000 i.u. vitamin D/day, the control up till the time of the patient's death, though the vitamin had been withdrawn from the diet of the hyper-calcaemic patient about 1 month before death. The fact that rather less vitamin D activity was found in the patient's liver than in that of the control is thus not sur-prising in view of the small amounts of vitamin D found in the livers of animals not given large doses of the vitamin (Devaney & Munsell, 1935) and the known rapid rate of disappearance of vitamin D from the livers of animals given the vitamin (Heymann, 1957; Warkany, 1936, 1937; Quarterman *et al.* 1963). These observations on liver vitamin D activity, as on plasma and serum activities, have failed to demonstrate anything abnormal about vitamin D activities encountered in at least the benign form of idiopathic hypercalcaemia. Rat biological assays, which have been used in all the studies quoted, can throw no light on the suggestion by Thomas *et al.* (1959) that the abnormality may reside in the accumulation within the body, and the failure to eliminate it, of a vitamin D derivative, such as, for instance, dehydrotachysterol, which may have a potent effect on Ca metabolism in man and yet have little or no antirachitic effect in the rat. Further investigation is required to determine whether the disease may be due to an abnormal sensitivity to vitamin D, since these patients show toxic responses to doses closely similar to those commonly needed to ensure normal utilization of Ca, or whether the condition may be due to an error in Ca metabolism and so to a large extent independent of vitamin D intake, at least within the range of vitamin D intakes commonly encountered in this country.

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Nutritional problems in the immigrant population in London

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During the past 10 years the immigrant population of this country has increased enormously. The three main countries of origin have been the West Indies, India and Pakistan. The immigrants largely settled in the big cities where they tended to aggregate in certain areas. In London, Brixton and Camberwell have a very high proportion of West Indian people. It is interesting to note that, although the number of immigrants entering this country has been greatly reduced as a result of the new immigration laws, the birth rate among those already here is very high. In Dulwich and St Giles Hospitals in London the number of West Indian babies born totalled over 1000 last year and is increasing annually. It is obvious that the number of immigrant people in the big cities will continue to increase and their medical problems should therefore be well understood. A few of these problems are genetically determined, such as sickle cell anaemia and thalassaemia, but these diseases are uncommon. Much more important are the problems related to the adaptation which has to take place of these non-indigenous people to their new country.

West Indian children are occasionally seen in our hospital with minor skin changes suggestive of deficiencies of vitamin A and the vitamins of the B complex. These changes are nearly always very mild and disagreement arises as to whether or not they are significant. Of recent years however two nutritional diseases have been described amongst immigrant children, namely iron-deficiency anaemia and rickets. Both these diseases occur with a frequency and severity which leaves no doubt that they are an important aspect of the health of the children of the immigrant communities. Nearly all the affected children were born in this country of parents who had immigrated here. In November 1960 attention was drawn to the high incidence of iron-deficiency anaemia among West Indian children in the Camberwell and Brixton areas of London (Davis, Marten & Sarkany, 1960). They investigated a total of 114 infants whose ages ranged from 5 to 23 months and of whom forty-seven were West Indian and sixty-seven were European. One of their interesting findings was that the weights of these children showed no statistical difference but the haemoglobin levels of the West Indians were significantly lower at all ages than those