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Autosomal-recessive vitamin D dependency
calcium
dihydrotachysterol

1α-hydroxyvitamin D_3
vitamin D dependency

Response to Crystalline 1α-Hydroxyvitamin D_3 in Vitamin D Dependency

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Extract

The therapeutic response to chemically synthesized 1α-hydroxycholecalciferol (1α-OH-D_3) was studied in three patients with autosomal recessive vitamin D dependency (ARVDD). The daily maintenance dose for vitamin D_2, to prevent signs of vitamin D deficiency in these patients, was 40–54.5 μg/kg, or about 100 times normal (Table 1). Withdrawal of maintenance therapy with vitamin D_2 resulted in the ultimate reappearance of the vitamin D depletion syndrome in *patients 1* and *2* (Figs. 1 and 2). The third patient presented with the deficiency syndrome despite adequate vitamin D nutrition and was recognized to have ARVDD.

Treatment with 1α-OH-D_3 by mouth in all three patients at dose levels of 1–3 μg/24 hr (80–100 ng/kg) corrected hypocalcemia and suppressed parathyroid hormone-dependent renal loss of amino acids (Figs. 1, 2, and 4). Rickets healed in 7-9 weeks on 1α-OH-D_3 alone (Fig. 3) The therapeutic response was rapid. It was usually seen first in the rise of serum calcium (Figs. 5 and 6). Withdrawal of 1α-OH-D_3 was followed first by a fall of serum phosphorus, then by a fall in serum calcium; the latter occurred within about 2 weeks of withdrawal.

Because the synthesis of 1α-OH-D_3 is simpler than for 1α,25-dihydroxycholecalciferol and because the former is an effective therapeutic analog of vitamin D hormone, we believe these studies in ARVDD reveal 1α-OH-D_3 to be the agent of choice for treatment of this and analogous diseases.

Speculation

Vitamin D dependency or pseudodeficiency rickets is believed to be an inborn error of vitamin D hormone biosynthesis. The putative abnormal enzyme is 25-hydroxycholecalciferol 1-hydroxylase in the recessively inherited trait. Consequently, this experiment of nature offers a special opportunity to examine the requirement in human subjects, for 1α-hydroxyvitamin D_3 metabolites.

Patients with ARVDD (14, 18, 36) develop signs of severe postnatal vitamin D deficiency, despite a nutritional intake of vitamin D_2 or vitamin D_3 (40) that would prevent rickets in normal subjects; hence the term pseudodeficiency preferred by some investigators (34). Persistent hypocalcemia appearing soon after birth is accompanied by an excess of circulating parathyroid hormone (1) which in turn is associated with hyperphosphaturia and hyperaminoaciduria (1, 37). Elevated serum alkaline phosphatase activity, severe rachitic bone lesions, and enamel hypoplasia affecting teeth that form postnatally complete the clinical syndrome (1, 11, 14, 18, 34, 36). Maintenance treatment with vitamin D_2 only at levels about 100 times the normal requirement fully reverses the manifestations of deficiency hence the term vitamin D dependency (18, 36).

The origin of the disturbed physiology in ARVDD lies in a selective disturbance of calcium absorption by intestine (18). A defect either in the biosynthesis of the active hormone form of vitamin D or in the ability of target organ(s) to respond to vitamin D hormone has been proposed (36) to explain the dependency on vitamin D_2, D_3, or 25-OH-D_3 of patients with ARVDD. ARVDD patients respond to microgram doses of chemically synthesized 1α,25-dihydroxycholecalciferol (1α,25-$(OH)_2$-D_3) (12). This finding has been corroborated by several investigators (3). It is surmised that a missing form of vitamin D hormone had been supplied and that ARVDD is likely to be an inborn error of vitamin D hormone biosynthesis, probably at the 1-hydroxylation step (12); this hypothesis is in keeping with the autosomal recessive inheritance of the disease.

Treatment of ARVDD with a "surrogate" vitamin D hormone, such as 1α-hydroxycholecalciferol (1α-OH-D_3) (22, 24), would be advantageous. Crystalline 1α-OH-D_3 is more easily synthesized than 1α,25-$(OH)_2$-D_3, and the former can be effectively administered by mouth. Moreover, ARVDD is an experiment of nature which provides an admirable opportunity to observe the effect of 1α-OH-D_3 in man. The present report reveals the therapeutic value of crystalline 1α-OH-D_3 in ARVDD and its preference over a similar synthetic analog of vitamin D, namely dihydrotachysterol (DHT_2).

METHODS AND MATERIALS

PATIENTS

Three patients, between the ages of 17 months and 9.5 years, were investigated. A case study for *patient 1* has been previously reported in detail (1); she is the second-born of dizygotic twins and exhibited intrauterine growth retardation at birth. *Patient 2* was diagnosed at 18 months of age and has been followed by us since 1965. *Patient 3* presented at 17 months of age during the course of the present investigation at which time the diagnosis of vitamin D dependency was made. She was enrolled in the study before maintenance treatment was prescribed.

Each patient has shown the characterictic clinical and biochemical features of ARVDD in the absence of adequate treatment with vitamin D. *Patient 1* inherited the trait in autosomal recessive fashion (1). Inheritance of the trait in *patients 2* and *3* who are isolated probands cannot be proven through heterozygote detection by available clinical methods(1).

PROTOCOLS FOR ASSESSMENT OF 1α-OH-D_3 EFFICACY(41)

Maintenance Requirements for Vitamin D_2. The requirements for vitamin D_2 in *patients 1* and *2*, to maintain normal serum calcium levels and prevent rickets have been observed over many years and range between 0.6 mg and 1.25 mg/24 hr (42) (41 and 55 μg/kg), and are about 100 times normal. The vitamin D_2 requirement for *patient 3* has yet to be finalized but will be of similar order.

Evaluation of Response to 1α-OH-D_3. The effect of 1α-OH-D_3 was studied *de novo* in *patient 3*. In *patients 1* and *2* it was necessary to discontinue maintenance therapy with vitamin D_2 in order to determine their requirement for 1α-OH-D_3. They were observed carefully by home visiting until evidence of modest hypocalcemia and reappearance of the parathyroid hormone-dependent mainifestations was apparent. Linear growth rate and bone mineralization were also monitored. The response to 1α-OH-D_3 was then observed in hospital while the patients received normal diets. The 1α-OH-D_3 was given by mouth at 9 AM in doses of 1–3 μg/24 hr. The response was evaluated by serial, daily measurements of total calcium, inorganic phosphorus, and alkaline phosphatase activity in serum. Urinary amino acids were monitored at less frequent intervals. Bone mineralization was evaluated by periodic x-ray examination. It was not possible to perform external balance studies for calcium and phosphorus. *Patients 1* and *3* received one course each of 1α-OH-D_3; *patient 2* was given the substance on three successive occasions at doses of 1 μg, 2 μg, and 3 μg daily, respectively, during the evolution of vitamin D depletion.

Therapeutic Agents. Crystalline 1α-OH-D_3 was given by mouth as a solution containing 2 μg/ml dissolved in propylene glycol. The stock solution was stored at −20° in the dark under nitrogen. The substance was chemically synthesized in the Department of Biochemistry, College of Agriculture and Life Sciences, University of Wisconsin, Madison. 1α,25-$(OH)_2$-D_3 and 25-OH-D_3 were obtained from the same source and stored in similar fashion.

Dihydrotachysterol was obtained in liquid form (Hytakerol) from Winthrop Laboratories, Aurora, Ontario. *Patient 2* was given 5 μg/24 hr by mouth for the first 15 weeks after maintenance therapy with vitamin D_2 was terminated.

Laboratory Methods. Blood was drawn from the antecubital vein at a constant time of day in each patient and allowed to clot. Serum was collected and total calcium and inorganic phosphorus were determined by the methods of Gitelman (15) and Kraml (27), respectively, adapted for the Technicon AutoAnalyzer. Serum total alkaline phosphatase activity in *patients 1* and *2* was determined by a modification of the method of Bessey *et al.* (16); the King-Armstrong method (25) was used for *patient 3*. Urine amino acids were determined by partition chromatography in two dimensions by the method of Dent (10) and measured semiquantitatively as described previously (13, 16). Serum 25-hydroxy-vitamin D was measured by the competitive protein-binding assay of Haddad and Chyu (17).

ANALYSIS OF DATA

Time courses for the biochemical responses were plotted in conventional fashion. The cumulative sum (cusum) method of analysis (23) was then used to study the clinical response to 1α-OH-D_3. The cusum values were derived by subtracting a selected baseline value from the serial daily values for which the pretreatment baseline is the mean of the last three serial determinations before administration of 1α-OH-D_3; the baseline for the withdrawal response is the mean of the values obtained on the last 3 days of treatment with 1α-OH-D_3. The Δ values (observed value minus baseline) during the particular study were then summed cumulatively and plotted as a time course according to their sign (upward for positive values, downward for negative). The cusum method permits significant trends in response to a therapeutic agent to be distinguished amidst the customary analytic noise in clinical studies (23).

RESULTS

RESPONSE TO WITHDRAWAL OF VITAMIN D_2 MAINTENANCE THERAPY

Time Courses. Patients 1 and *2* had been treated with vitamin D_2 for a number of years (Table 1). The time courses for reappearance of the ARVDD phenotype are shown in Figures 1 and 2. The first

Table 1. *Relationships between maintenance requirements for vitamin D_2, concentration of serum 25-OH-vitamin D, and requirement for 1α-OH-D_3, in patients with vitamin D dependency*

Patient	Sex	Age, yr	Maintenance dose of vitamin D_2[1] mg/24 hr	Maintenance dose of vitamin D_2[1] μg/kg	Serum 25-OH-D conc,[2] ng/ml	Effective dose of 1α-OH-D_3,[3] μg/24 hr	Elapsed time for initial response, days
1[4]	F	4 11/12	0.66	41.0	66.8	2	<2
2	F	9 6/12	1.25	54.5	217.7	1—3	<2
3	F	1 5/12	0.75	50.0	(153)[5]	1	1

[1] Established during previous 12 months of observations. Stated dose of vitamin D_2 maintained serum calcium in normal range, suppressed hyperparathyroidism and the parathyroid hormone-dependent tubulopathy, and prevented rickets.

[2] Measured by displacement binding assay (17) on serum samples obtained when phenotype of hereditary vitamin D dependency fully expressed after withdrawal of maintenance vitamin D_2 therapy 6–8 months earlier and before administration of 1α-OH-D_3. Upper range for healthy, seasonal, and age-matched children in Montreal is 29 ± ng/ml (mean ± SD).

[3] 1α-OH-D_3 was given by mouth at 8 AM in propylene glycol vehicle.

[4] Case of patient has been reported previously (1).

[5] Value is probably high because patient received 5,000 IU vitamin D_2/24 hr for 5 days before diagnosis. Serum 25-OH-vitamin D level was 50 ng/ml, 59 days later after total correction of phenotype with 1α-OH-D_3 therapy alone.

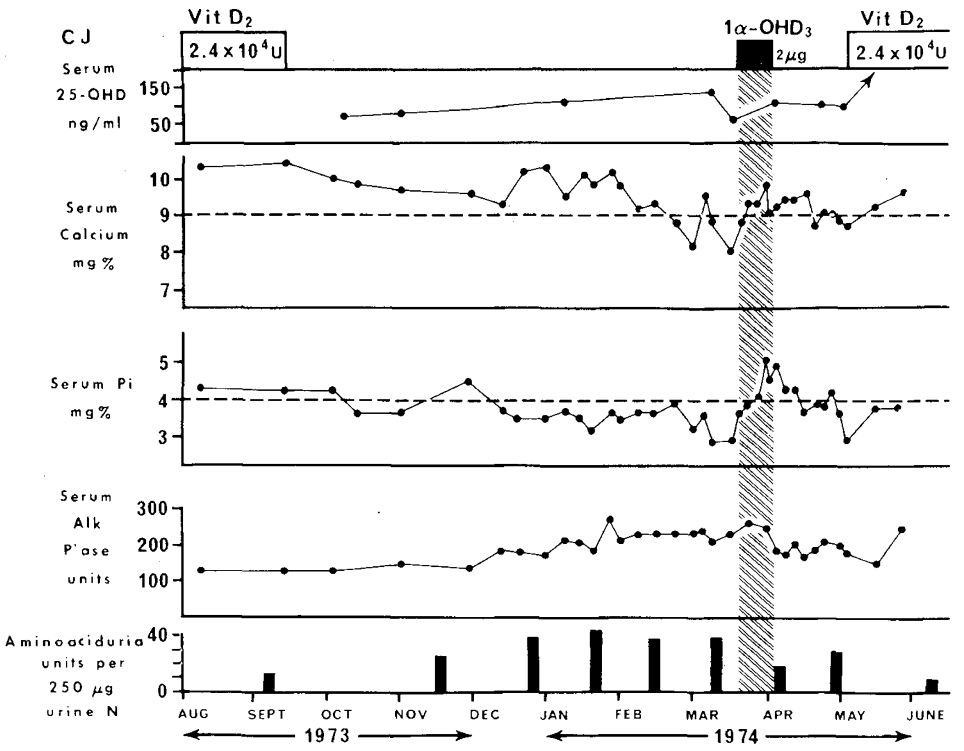

Fig. 1. Time course of the response of *patient 1* with autosomal recessive vitamin D dependency to withdrawal of the maintenance dose of vitamin D_2, (about 40 μg/kg daily, or 100 times normal) and to treatment with crystalline 1α-hydroxyvitamin D_3 (*1α-OHD₃*). The 25-hydroxyvitamin D (*25-OHD*) activity in serum was well above normal for age (29 ± 7 ng/ml) when there were clinical manifestations of vitamin D deficiency syndrome. Mild rachitic lesions in the long bones were apparent which healed during treatment with 1α-hydroxyvitamin D_3 after treatment. *Alk P'ase:* alkaline phosphatase.

manifestations were hypophosphatemia and hyperaminoaciduria, which, in ARVDD, reflect the renal tubular response to elevated levels of parathyroid hormone (1). Normal levels of serum calcium with hypophosphatemia and hyperaminoaciduria occur in the second stage of the vitamin D depletion syndrome in man (1, 13). The elapsed time to reach this stage in our patients was 12–18 weeks. Persistent hypocalcemia then appeared while the hypophosphatemia persisted (Figs. 1 and 2). The resulting calcium × phosphorus product in serum (calcium in milligrams per deciliter × inorganic phosphorus in milligrams per deciliter) was below 40. This constellation of findings corresponds to the third stage of the vitamin D depletion syndrome (1, 13).

Patient 3, who had received no maintenance therapy since birth, presented with hypocalcemia and hypophosphatemia.

Bone Response. Serum alkaline phosphatase activity rose in *patients 1* and *2* beginning about 8 weeks after vitamin D_2 treatment was stopped. Over 80% of the augmented activity was heat labile, indicating bone as its major source (33). Alkaline phosphatase activity in serum plateaued at a level two- to threefold above that present during maintenance therapy with vitamin D_2. Radiographic examinations of long bones and skull revealed mild progressive rachitic changes emerging after withdrawal of antirachitic treatment. The process was most severe in *patient 2* (Fig. 3*A*).

Patient 3, who had not previously received maintenance therapy, presented with florid rickets (Fig. 3*C*) and elevated plasma alkaline phosphatase activity.

Linear Growth. Withdrawal of maintenance vitamin D_2 therapy was followed by slowing of linear growth in *patient 2;* the actual rate was 10% of that expected from her normal percentile during the period of observation. *Patient 1* is a dizygotic twin and has had linear growth failure since birth; she experienced no additional growth failure upon withdrawal of maintenance therapy. *Patient 3* presented below the 3rd percentile.

Serum 25-Hydroxyvitamin D Concentration. Patients 1 and *2* had elevated concentrations of 25-hydroxyvitamin D in serum upon withdrawal of maintenance doses of vitamin D_2 (Figs. 1 and 2). When unequivocal manifestations of the vitamin D depletion

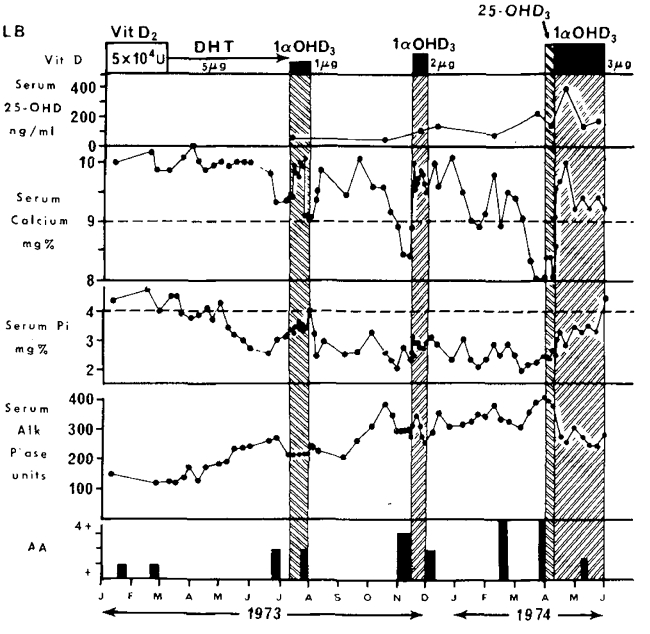

Fig. 2. Time course for the response of *patient 2* with autosomal recessive vitamin D dependency to withdrawal of the maintenance dose of vitamin D_2 (*Vit D_2*) (from 54 μg to 5 μg/kg daily) and to treatment with crystalline 1α-hydroxyvitamin D_3 (*1αOHD₃*), the latter on three occasions. The serum 25-hydroxyvitamin D (*25-OHD*) level was elevated when the vitamin D deficiency syndrome was evolving. Rachitic lesions in bone developed and then regressed during treatment with the 1α metabolite. *25-OHD₃*: 25-hydroxyvitamin D_3; *Alk P'ase:* alkaline phosphatase. *DHT:* dihydrotachysterol.

syndrome had emerged 6-8 months later, the serum concentration of 25-hydroxyvitamin D was still elevated in both patients (Table 1).

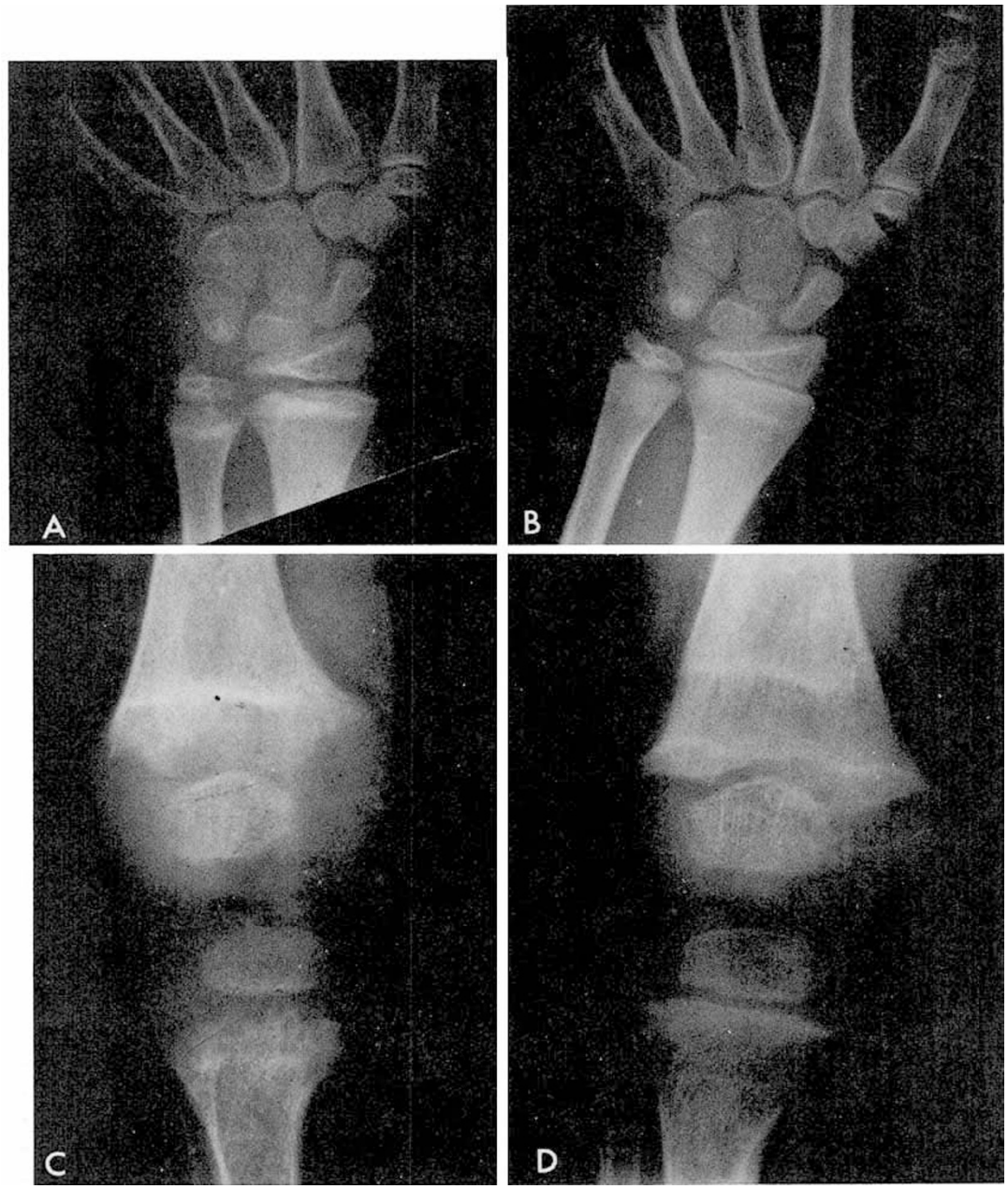

Fig. 3. Radiographs of wrists of *patient 2* (*A*, *B*) and of knees of *patient 3* (*C*, *D*) showing healing of rickets in 7 weeks and 9 weeks, respectively, during administration of 1α-hydroxyvitamin D_3 (2–3 μg/24 hr by mouth).

Patient 3 had an elevated initial concentration of 25-OH-vitamin D in serum (Table 1). Unfortunately, vitamin D_2 had been given before diagnosis (375 μg/24 hr for 5 weeks). Two months later, after treatment with 1α-OH-D_3 alone, the serum 25-OH-D level in this patient was still elevated (50 ng/ml) (Fig. 4).

RESPONSE TO CRYSTALLINE 1α-OH-D_3

Total Calcium and Inorganic Phosphorus in Serum. 1α-OH-D_3 was administered by mouth to each patient in small doses (3 μg or less daily) (Table 1). A prompt response followed; time courses are shown in Figures 1, 2, and 4.

Cusum plots (Figures 5 and 6) reveal that the serum calcium rises within 48 hr of 1α-OH-D_3 treatment, whereas serum inorganic phosphorus rises slightly later. The calcium $\times$ phosphorus product in serum subsequently increases to a value above 30.

On three occasions 1α-OH-D_3 was given to patient 2; once at a dose level of 1 μg/24 hr in the normocalcemia, hypophosphatemic state of the vitamin D depletion syndrome; and twice in the hypocalcemic, hypophosphatemic stage, first at a dose level of 2 μg/24 hr and then at 3 μg/24 hr. With each trial of 1α-OH-D_3, the serum calcium rose first, anticipating the rise in serum phosphorus by at least 1 day.

Patient 3, who presented with florid rickets (Fig. 3*C*) experienced an unusual serum response to 1α-OH-D_3 (Fig. 4). Serum calcium rose immediately and reached the normal range within 1 week. However, serum inorganic phosphorus fell initially, rising only after the 4th week of treatment. The initial calcium $\times$ phosphorus product in serum rose to exceed 30 but during the subsequent 3 weeks, the average value was below 30. Movement of mineral into bone may explain these findings, because radiographic evidence of remineralization appeared within 2 weeks of 1α-OH-D_3 therapy.

BONE RESPONSE

Alkaline phosphatase activity in serum fell immediately after initiation of 1α-OH-D_3 treatment, and radiographic examination

of bones showed repair of the rachitic lesions in all three patients. Rickets in *patient 2* was healed with 1α-OH-D_3 treatment alone in 7 weeks (Fig. 3*B*). Florid rickets was healed in *patient 3* in 9 weeks on 1α-OH-D_3 (Fig. 3*D*).

AMINOACIDURIA

During the course of treatment with 1α-OH-D_3, hyperaminoaciduria subsided, in keeping with repair of the hypocalcemia and suppression of endogenous hyperparathyroidism (1, 13, 16, 37). (Figs. 1 and 2).

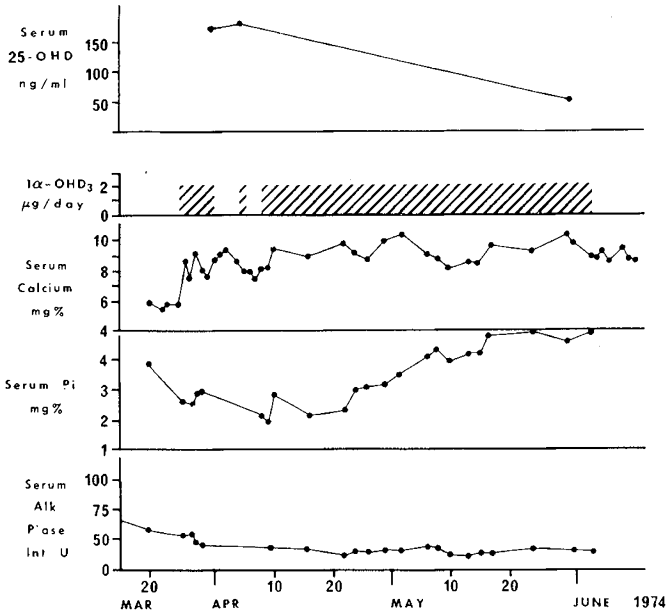

Fig. 4. Response of *patient 3* to crystalline 1α-hydroxyvitamin D_3 (*1α-OHD*$_3$) (2 μg/24 hr by mouth) after initial diagnosis of vitamin D dependency at 17 months of age. The patient presented with hypocalcemia, hypophosphatemia, rickets, and growth failure despite normal vitamin D intake during the first 16 months of life. Vitamin D_2 treatment (375 μg/24 hr for 5 weeks) did not heal the rickets. All manifestations were corrected by 1α-hydroxyvitamin D_3 alone (2 μg/24 hr) in 9 weeks. Alk P'ase: alkaline phosphatase. *25-OHD:* 25-hydroxyvitamin D.

LINEAR GROWTH RATE

Acceleration of the linear growth rate was observed in *patient 3*, to 160% of her normal percentile during the 9-week long third course of 1α-OH-D_3 (3 μg/24 hr) treatment. Accelerated growth continued when maintenance with vitamin D_2 was reinstituted.

RESPONSE TO WITHDRAWAL OF 1α-OH-D_3

The 1α-OH-D_3 therapy was intermitted in *patients 1* and *2*. Serum calcium remained elevated in the first patient for 18 days, and then declined; serum phosphorus fell within the first few days after withdrawal of 1α-OH-D_3 (Figs. 2, 5, and 6). In *patient 2*, *1α*-OH-D_3 was stopped after 2-week courses at dose levels of 1 μg/24 hr and 2 μg/24 hr, respectively. On both occasions, the vitamin D depletion syndrome reappeared.

RESPONSE TO OTHER FORMS OF VITAMIN D

DHT_2. DHT_2 was administered by mouth at low doses (5 μg/24 hr of the equivalent of 15 μg of vitamin D_2 (21)) for 18 weeks to *patient 2* in place of maintenance therapy with vitamin D_2. Appearance of the vitamin D depletion syndrome was not significantly retarded by DHT_2 in comparison with *patient 1* who did not receive DHT_2.

1α,25-(OH)$_2$-D$_3$. Synthetic vitamin D_3 hormone was administered by mouth (3 μg/24 hr for 10 days) to *patient 2*, when the ARVDD phenotype had appeared. A prompt rise in serum calcium was observed (Δ = +1.3 mg/dl); serum inorganic phosphorus subsequently also rose (Δ = +0.5 mg/dl). This finding extends the observations (12) obtained with intravenous 1α-25-OH-D_3 (1 μg/24 hr), inasmuch as the substance is shown to be modestly effective by mouth.

25-OH-D$_3$. *Patient 2* also received 25-OH-D_3 by mouth at dose levels of 40 μg/24 hr; the requirement for the normal person is thought to be on the order of 3 μg/24 hr (4, 12). The patient had no significant response in comparison with the effect of 1α-OH-D_3.

DISCUSSION

The original hypothesis (1, 11, 18, 34, 36) that ARBDD is an inborn error in the pathway from prohormone to the hormonal form of vitamin D (26) is supported by considerable, indirect

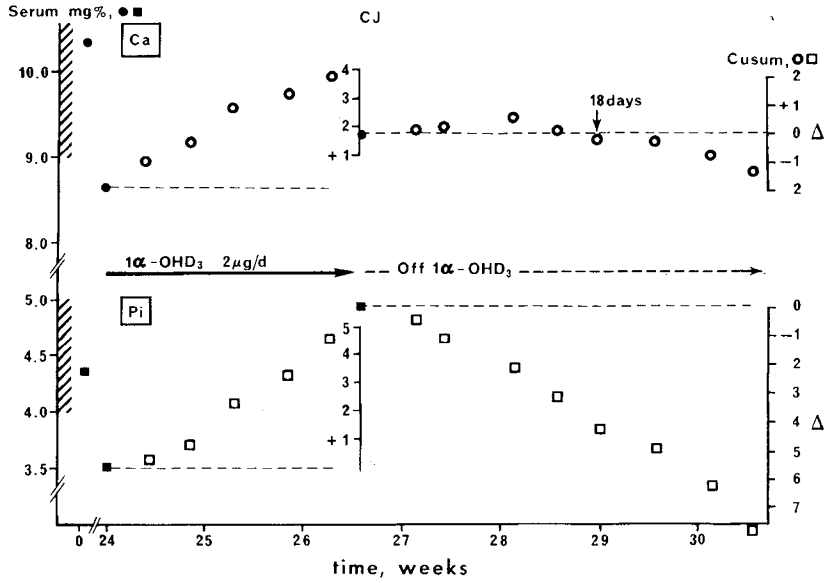

Fig. 5. Cumulative sum (cusum) method of analysis (23) to show response to 1α-hydroxyvitamin D_3 (*1α-OHD*$_3$) in *patient 1*. ○, □: cusum response (Δ) of serum calcium and serum inorganic phosphorus, respectively, during administration of 1α-hydroxyvitamin D_3 and after its withdrawal (plotted against right ordinate). ●, ■: baseline values (plotted against left ordinate) used in calculating the cusum values (Δ). See test for details. Time elapsed between 0 and 24 weeks represents withdrawal of vitamin D_2 treatment.

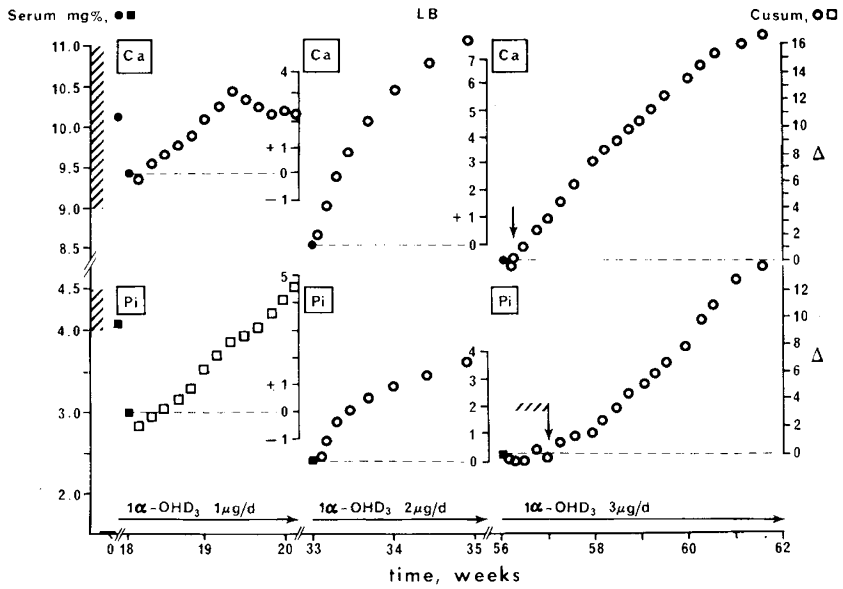

Fig. 6. Cumulative sum (cusum) method of analysis (23) showing response of *patient 2* to 1α-hydroxyvitamin D_3 (*1α-OHD₃*) on three occasions. (See legend to Fig. 5 for explanation of symbols). Elapsed time between 0 and 18 weeks represents withdrawal of vitamin D_2 treatment.

evidence. A selective defect in calcium absorption by the intestine (18), an unequivocal clinical response to small doses of 1α,25-$(OH)_2$-D_3 (12), and the need for about 100 times the estimated normal requirement of vitamin D_2 (or vitamin D_3) (12) and its first hydroxylation product, 25-OH-vitamin (4, 5, 12) place the putative enzymatic defect, in the majority of patients studied so far, at the level of 25-hydroxycholecalciferol 1-hydroxylase in kidney (12).

The possibility that other patients with the vitamin D dependency syndrome will have an attenuated response of target tissues to normal amounts of vitamin D hormone, or will have a block in hormone biosynthesis at another level, or of a different degree of "completeness," is entirely possible. It is the nature of recessive mendelian traits in man to exhibit genetic heterogeneity (9, 28). Differences in the maintenance requirement for 25-OH-D_3 and vitamin D_2 among patients with apparent vitamin D dependency have already been reported (35, 38). A description of the cellular events which determine the vitamin D dependent trait might be obtained in more detail through investigation of a promising animal model in swine (19, 30).

Several investigators (11, 18) have reported that "antirachitic activity" in serum, as determined by various bioassays, is at least normal, or higher than normal, in vitamin D dependency. However, this type of assay often does not discriminate between vitamin D and its more active polar metabolites. In the study of Fraser *et al.* (12), a chromatographic fraction of vitamin D, mainly comprising 25-OH-D_3, was elevated in the serum of patients depleted of maintenance doses of vitamin D_2 or D_3. We found the serum level of 25-OH-vitamin D, as measured by competitive binding assay (17), to be significantly elevated in our patients. However, this finding does not tell us whether the level of 25-OH-D_3 in the cytosol of target cells is elevated. Nonetheless, normal calcium metabolism was not sustained under the observed conditions implying that adequate specific replacement of 1α-hydroxylated vitamin D is necessary in ARVDD. The parathyroid hormone-dependent disturbance of tubular reabsorption affecting amino acids and phosphorus is not prevented by the elevated levels of 25-OH-D_3 in this "calciopenic" form of rickets (37).

Whether the raised serum 25-OH-vitamin D level represents metabolite accumulation in a blocked biosynthetic pathway or merely a persistent increase because of previous maintenance therapy at high dose levels is not clear from our study. Serum 25-OH-D_3 does not accumulate in chronic renal failure (2, 29) or after nephrectomy (31), when there is interference with renal 1-hydroxylase activity, except when large doses of vitamin D are administered for a long time (39).

DHT_2 has a hydroxyl group in position 3 rotated 180° so that the hydroxyl occupies a position analogous to position 1. Nonetheless, DHT_2 appears not to be effective in ARVDD in the low microgram dose range, and our limited observations suggest that DHT_2 is not equivalent to the 1α-hydroxy metabolites of vitamin D in man, corroborating the earlier findings of Harrison and Harrison (20) in the rat.

The response of 1α-OH-D_3 in ARVDD is equivalent to the earlier reported effect of 1α,25-$(OH)_2$-D_3 (12). These observations offer a more direct evaluation of the efficacy of 1α-OH-D_3 than earlier studies with the agent in patients with malabsorption syndromes (7, 32) and renal failure (8). It is not yet certain whether responsiveness to 1α-OH-D_3 is dependent on further 25-hydroxylation, but observations *in vitro* (26) suggest this is the case. Because dependence on vitamin D hormone is not likely to be outgrown in ARVDD, we anticipate a preference for 1α-OH-D_3 over vitamin D_2 during long term treatment. The response to 1α-OH-D_3 in ARVDD requires only a matter of a day or two; by contrast, weeks are required to achieve a stable treatment response with vitamin D_2. We have also shown that the vitamin D depletion syndrome appears within a few days after withdrawal of 1α-OH-D_3 in ARVDD, an advantageous feature for the avoidance of vitamin D toxicity.

The apparent normal maintenance dose for 1α-OH-D_3 is on the order of 80 ng/kg in later childhood and early puberty.

SUMMARY

Vitamin D dependency (pseudodeficiency rickets), a postnatal autosomal recessive condition in which calcium absorption is compromised and with attendant manifestations of secondary hyperparathyroidism, is believed to be the result of deficient 1-hydroxylation of 25-hydroxyvitamin D. Therefore, the therapeutic effect of synthetic crystalline 1α-hydroxyvitamin D_3 was examined in three patients who fully expressed the syndrome, despite serum 25-hydroxyvitamin D levels elevated two- to sevenfold above normal. Whereas pharmacologic doses (40-54.5 μg/kg/24 hr) of vitamin D_2 were required to prevent manifestations of vitamin D deficiency in these patients, the effective maintenance dose of 1α-OH-D_3 was only 80-100 ng/kg/24 hr. The latter substance is effective by mouth within 1 or 2 days and its biologic effect is short-lived (days only). These studies yield a first approximation of the requirement for 1α-OH-D_3 and reveal that it may be the agent of choice for management of this and analogous diseases.

REFERENCES AND NOTES

1. Arnaud, C., Maijer, R., Reade, T., Scriver, C. R. and Whelan, D. T.: Vitamin D dependency: An inherited postnatal syndrome with secondary hyperparathyroidism. Pediatrics, *46:* 871 (1970).
2. Avioli, L. V., and Haddad, J. G.: Vitamin D: Current concepts. Metabolism, *22:* 507 (1973).
3. Balsan, S., Fanconi, A., and Prader, A.: Personal communications, 1974.
4. Balsan, S., and Garabedian, M.: 25-hydroxycholecalciferol: A comparative study in deficiency rickets and different types of resistant rickets. J. Clin. Invest. *51:* 749 (1972).
5. Balsan, S., Garabedian, M., and LeBouadec, L.: La rachitisme vitamino-résistant pseudocareitial hypocalcémique. Arch. Franç. Pediat. *29:* 287 (1972).
6. Bessey, O. A., Lowry, O. H., and Brock, M. J.: A method for the rapid determination of alkaline phosphatase with five cubic millimeters of serum. J. Biol. Chem., *164:* 321 (1946).
7. Bordier, P., Pechet, M. M., Hesse, R., Marie, P., and Rasmussen, H.: Response of adult patients to treatment with crystalline 1α-hydroxy vitamin D_3 New Engl. J. Med. *291:* 866 (1974).
8. Chalmers, T. M., Davie, M. W., Hunter, J. O., Szaz, N. F., Pelc, B., and Kodicek, E.: 1-alpha-hydroxycholecalciferol as a substitute for the kidney hormone, 1,25,dihydroxycholecalciferol in chronic renal failure. Lancet, *ii:* 696 (1973).
9. Childs, B., and Der Kaloustian, M.: Genetic heterogeneity. New Engl. J. Med., *279:* 1205, 1267 (1968).
10. Dent, C. E.: A study of the behaviour of some sixty amino acids and other ninhydrin reacting substances on phenol-"collidine" filter paper chromatograms with notes as to the occurrence of some of them in biological fluids. Biochem. J., *43:* 169 (1948).
11. Fanconi, A., and Prader, A.: Die hereditäre Pseudomangel rachitis. Helv. Paediat. Acta, *24:* 423 (1969).
12. Fraser, D., Kooh, S. W., Kind, H. P., Holick, M. F., Tanaka, Y., and DeLuca, H. F.: Pathogenesis of hereditary vitamin D dependent rickets. An inborn error of vitamin D metabolism involving defective conversion of 25-hydroxy vitamin D to 1α-hydroxy vitamin D. New Engl. J. Med., *289:* 817 (1973).
13. Fraser, D., Kooh, S. W., and Scriver, C. R.: Hyperparathyroidism as the cause of hyperaminoaciduria and phosphaturia in human vitamin D deficiency. Pediat. Res., *1:* 425 (1967).
14. Fraser, D., and Salter, R. B.: The diagnosis and management of the various types of rickets. Pediat. Clin. N. Amer., *May:* 417 (1958).
15. Gitelman, H. J.: An improved automated procedure for the determination of calcium in biological specimens. Anal. Biochem., *18:* 521 (1967).
16. Grose, J., and Scriver, C. R.: Parathyroid dependent phosphaturia and aminoaciduria in the vitamin D deficient rat. Amer. J. Physiol., *214:* 370 (1968).
17. Haddad, J. G., Jr., and Chyu, K. J.: Competitive protein-binding radioassay for 25-hydroxycholecalciferol. J. Clin. Endocrinol., *33:* 992 (1971).
18. Hamilton, J. R., Harrison, J., Fraser, D., Radde, I., Morecki, R., and Paunier, L. The small intestine in vitamin D dependent rickets. Pediatrics, *45:* 364 (1970).
19. Harmeyer, J., and Plonait, H. Generalisierte Hyperaminoaciduria mit erblicher Rachitis bei Schweinen. Helv. Paediat. Acta, *22:* 216 (1967).
20. Harrison, H. E., and Harrison, H. C.: Dihydrotachysterol: A calcium active steroid not dependent upon kidney metabolism. J. Clin. Invest., *51:* 1919 (1972).
21. Harrison, H. E., Lifshitz, F., and Blizzard, R. M.: Comparison between crystalline dihydrotachysterol and calciferol in patients requiring pharmacologic vitamin D therapy. New Engl. J. Med., *276:* 894 (1967).
22. Haussler, M. R., Zerwelch, J. E., Hesse, R. N., Rizzardo, E., and Pechet, M. M.: Biological activity of 1α-hydroxycholecalciferol, a synthetic analog of the hormonal form of vitamin D_3. Proc. Nat. Acad. Sci. U.S.A., *70:* 2248 (1973).
23. Healy, M. J. R.: The disciplining of medical data. Brit. Med. Bull., *24:* 210 (1968).
24. Holick, M. F., Semmler, E. J., Schnees, H. K., and DeLuca, H. F.: 1α-hydroxy derivative of vitamin D_3: A highly potent analog of 1α,25-dihydroxy vitamin D_3. Science, *180:* 190 (1973).
25. King, E. J.: Microanalysis in Medical Biochemistry, Ed. 3 (J. & A. Churchill, Ltd., London, 1959).
26. Kodicek, E.: The story of vitamin D. From vitamin to hormone. Lancet, *i:* 325 (1974).
27. Kraml, K.: A semi-automated determination of phospholipids. Clin. Chim. Acta, *13:* 442 (1966).
28. McKusick, V. A.: The nosology of genetic disease. Hosp. Pract., *6:* 93 (1971).
29. Mawer, E. B., Backhouse, J., Taylor, C. M., Lunib, G. A., and Stanbury, S. W.: Failure of formation of 1,25-dihydroyxcholecalciferol in chronic renal insufficiency. Lancet, *i:* 626 (1973).
30. Meyer, H., and Plonait, H.: Ueber eine erbliche Kalzuimstoff wechsel storung bein Schwein (erbliche Rachitis). Z. Vet. Med. Reihe A, *15:* 481 (1968).
31. Oettinger, C. W., Merrill, R., Blanton, T., and Briggs, W.: Reduced calcium absorption after nephrectomy in uremic patients. New Engl. J. Med., *291:* 458 (1974).
32. Peacock, M., Gallagher, J. C., and Nordin, B. E. C.: Action of 1α-hydroxyvitamin D_3 on calcium absorption and bone resorption in man. Lancet, *i:* 385 (1974).
33. Posen, S., Neale, F. C., and Clubb, J. S.: Heat inactivation in the study of human alkaline phosphatases. Ann. Int. Med., *62:* 1234 (1965).
34. Prader, Von A., Illig, R., and Heierli, E.: Eine besondere Form der primären vitamin-D resistenten Rachitis mit Hypocalcämie und autosomal-dominatem Erbgang: die hereditäre Pseudo-Mangelrachitis. Helv. Paediat. Acta, *16:* 452 (1961).
35. Rosen, J. F., and Finberg, L.: Vitamin D-dependent rickets: Actions of parathyroid hormone and 25-hydroxycholecalciferol. Pediat. Res., *6:* 552 (1972).
36. Scriver, C. R.: Vitamin D dependency. Pediatrics, *45:* 361 (1970).
37. Scriver, C. R.: Rickets and the pathogenesis of impaired tubular transport of phosphate and other solutes. Amer. J. Med., *57:* 43 (1974).
38. Soriano, J. R., Einhorn, A., Stark, H., and Edelmann, C. M., Jr.: Deficiency-type rickets due to decreased sensitivity to vitamin D. J. Pediat. *68:* 227 (1966).
39. Stanbury, S. W., Mawer, E. B., Lumb, G. A., Hill, L. F., Holman, C. A., Taylor, C. M., and Torkington, P.: Vitamin D metabolism and renal bone disease. pp. In: B. Frame, A. M. Parfitt, and H. Duncan: Clinical Aspects of Metabolic Bone Disease, pp. 562–572 (Excerpta Medica, Amsterdam, 1973).
40. The recommended daily allowance is 400 units or 10 μg crystalline vitamin D_2 or D_3.
41. The protocols for *patients 1* and *2* were approved by a committee of the Department of Pediatrics at the Montreal Children's Hospital. The purpose of the study was explained and consent granted by the parents of the patients. 1α-OH-D_3 was used under appropriate licence from the United States Food and Drug Administration and the Health Protection Branch, Ottawa, Canada.
42. One milligram of vitamin D_2 is equivalent to 40,000 IU.

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