Evaluation of a Role for 1,25-Dihydroxyvitamin D_3 in the Pathogenesis and Treatment of X-linked Hypophosphatemic Rickets and Osteomalacia

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ABSTRACT Although a defect in renal transport of phosphate seems well established as the primary abnormality underlying the pathogenesis of X-linked hypophosphatemic rickets and osteomalacia, several observations indicate that renal phosphate wasting and hypophosphatemia cannot solely account for the spectrum of abnormalities characteristic of this disease. Thus, in the present study, we investigated the potential role of abnormal vitamin D metabolism in the pathogenesis of this disorder and the effect of 1,25-dihydroxyvitamin D_3 therapy on both the biochemical abnormalities characteristic of this disease and the osteomalacia. Four untreated patients, ages 14–30 yr, had normocalcemia (9.22±0.06 mg/dl); hypophosphatemia (2.25±0.11 mg/dl); a decreased renal tubular maximum for the reabsorption of phosphate per liter of glomerular filtrate (2.12±0.09 mg/dl); normal serum immunoreactive parathyroid hormone concentration; negative phosphate balance; and bone biopsy evidence of osteomalacia. The serum 25-hydroxyvitamin D_3 concentration was 33.9±7.2 ng/ml and, despite hypophosphatemia, the serum level of 1,25-dihydroxyvitamin D_3 was not increased, but was normal at 30.3±2.8 pg/ml. These data suggested that abnormal homeostasis of vitamin D metabolism might be a second defect central to the phenotypic expression of X-linked hypophosphatemic rickets/osteomalacia. This hypothesis was supported by evaluation of the long-term response to pharmacological amounts of 1,25-dihydroxyvitamin D_3 therapy in three subjects. The treatment regimen resulted in elevation of the serum 1,25-dihydroxyvitamin D levels to values in the supraphysiological range. Moreover, the serum phosphate and renal tubular maximum for the reabsorption of phosphate per liter of glomerular filtrate increased towards normal whereas the phosphate balance became markedly positive. Most importantly, however, repeat bone biopsies revealed that therapy had positively affected the osteomalacic component of the disease, resulting in normalization of the mineralization front activity. Indeed, a central role for 1,25-dihydroxyvitamin D_3 in the mineralization of the osteomalacic bone is suggested by the linear relationship between the serum level of this active vitamin D metabolite and the mineralization front activity. We, therefore, suggest that a relative deficiency of 1,25-dihydroxyvitamin D_3 is a factor in the pathogenesis of X-linked hypophosphatemic rickets and osteomalacia and may modulate the phenotypic expression of this disease.

INTRODUCTION

X-linked hypophosphatemic rickets and osteomalacia (XLH)[1] is a familial syndrome characterized by inadequate mineralization of cartilage and/or bone, conse-

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[1] *Abbreviations used in this paper*: $1,25(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; 25(OH)D, 25-dihydroxyvitamin D; PTH, parathyroid hormone; TmP/GFR, renal tubular maximum for the reabsorption of P_i normalized to glomerular filtration rate; XLH, X-linked hypophosphatemic rickets/osteomalacia.

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quent skeletal deformities, and growth retardation. The hallmarks of this disease include a reduced serum concentration of inorganic phosphate (P_i) (1–3) and resistance to therapy with vitamin D (4–6). Current evidence suggests that the primary defect governing the genesis of XLH is an abnormality of P_i transport in the renal tubule (7). This abnormal function results in P_i wasting and consequent hypophosphatemia, upon which all other components of the disorder apparently depend.

Despite the established primacy of renal P_i wasting in the pathogenesis of XLH, treatment aimed only at overcoming the resulting hypophosphatemia fails to normalize all of the manifestations of the disease (8, 9), indicating that an additional factor(s), or an alternate physiological abnormality, may contribute to the pathogenesis of this disorder. Traditionally, an alteration of vitamin D-dependent calcium homeostasis has been suspected as such a potential abnormality (10). However, the presence and/or nature of this suspected defect remains undetermined. Nevertheless, the recent elucidation of the metabolic pathways for vitamin D activation (11, 12) has focused interest on the possibility that an abnormality in this process may be operative in XLH.

Therefore, in the present investigation, we studied whether compromised availability of vitamin D or its active metabolite, $1,25(OH)_2D_3$, is a factor in the pathogenesis of XLH. In these studies we performed detailed examination of vitamin D metabolism in untreated subjects with XLH and, in addition, evaluated the effects of long-term $1,25(OH)_2D_3$ therapy on the biochemical and bone histomorphological abnormalities characteristic of this disease.

METHODS

Patient population. Four patients, a female aged 15 yr and three males, 14, 21, and 30 yr of age (cases 1–4, respectively) were selected for study. The adolescent subjects were probands from kindreds with an X-linked dominant transmission of the rachitic-osteomalacic disorder and the adult males were brothers in a third kindred in which XLH had been likewise documented.

At the initiation of the study the chronologically aged 15- and 14-yr-old subjects had a height age of 12 and 11 yr and a bone age (13) of 17 and 16 yr, respectively. In addition, premature fusion of the epiphyseal centers in the distal femur and proximal tibia had occurred in both subjects and consequently there was no evidence of active rickets. However, characteristic postrachitic skeletal deformities were present and there was radiographic evidence of active osteomalacia. At the completion of the 1-yr study, height age in these subjects was unchanged whereas bone age was 17 yr in both subjects. The adult subjects (cases 3 and 4) likewise presented with growth retardation (height 155 and 163 cm) and postrachitic deformities. In addition, active osteomalacia was similarly evidenced radiographically by pseudo-fractures, coarsened trabeculation, and rarified areas in the long bones.

Therapy with pharmacological amounts of vitamin D and/or vitamin D and oral phosphates had been previously administered to each subject. Initiation of treatment was at age 5–8 yr of age and adherence sporadic therafter. However, no form of therapy with oral Pi or vitamin D and its metabolites had been administered to the patients for 4–9 yr before evaluation.

Complete metabolic studies and bone biopsies were performed in all four subjects in the untreated state on the Duke University Medical Center Clinical Research Unit. Subsequently, therapy with $1,25(OH)_2D_3$ alone was initiated in cases 1–3. Daily treatment with 0.25 μg orally was begun and gradually modified by 0.25-μg increments to 2.25–3.00 μg/d (0.036–0.062 μg/kg per d), given in a split dose regimen at 9:00 a.m. and 9:00 p.m. Serial measurements of the serum calcium concentration and 24-h urinary calcium excretion were employed to monitor potential complications of therapy and 3.0 μg of $1,25(OH)_2D_3$ per d arbitrarily established as the maximum amount of drug used. Ultimately, $1,25(OH)_2D_3$ was continued at established maintenance levels for 12 mo. Complete metabolic studies were repeated at the 6-mo interval and bone biopsies after both 6 and 12 mo of therapy. In case 2 the dosage of $1,25(OH)_2D_3$ was increased from 2.50 to 2.75 μg/d after 6 mo of therapy. All studies were performed with the informed consent of the patient, or the patient and his parents, and were approved by the Duke University Human Investigations Committee. The $1,25(OH)_2D_3$ for oral administration was supplied by the Chemical Research Department, Hoffmann-La Roche Inc. (Nutley, N. J.).

Biochemical studies and radioimmunoassays. Serum calcium (normal 8.7–10.3 mg/dl) was measured by atomic absorption spectrophotometry and serum P_i (for age specific normals, see Table I) by the colorimetric method of Dryer et al. (14). Serum creatinine (normal 0.7–1.2 mg/dl) and alkaline phosphatase were determined on the Multichannel Technicon Autoanalyzer (Technicon Instruments Corp., Tarrytown, N. Y.). Urine specimens were stored at −20°C before analysis of calcium (by atomic absorption spectrophotometry), P_i (15), and creatinine (16). Fecal fat excretion was determined by the method of Van de Kamer et al. (17) on 72-h fecal collections marked by carmine red dye and collected during ingestion of 70 g fat/d.

Serum parathyroid hormone (PTH) concentration was measured by four separate radioimmunoassays. Carboxy-terminal-specific assays were purchased from the Mayo Medical Laboratory (Rochester, Minn.) and The Upjohn Laboratory (Kalamazoo, Mich.); normals were <40 μl eq/ml (18) and <150–375 pg eq/ml (19), respectively. An amino-terminal-specific assay (normal < 0.125 ng/ml) employing a guinea pig antibody raised against the synthetic (1–34) amino-terminal peptide of human PTH was performed in the Duke University Laboratories (20, 21) and Dr. Leonard Deftos (University of California, La Jolla, Calif.) measured PTH (normal < 300 pg/ml) by a predominantly carboxy-terminal immunoassay employing previously published methods (22).

Competitive binding protein assays of Vitamin D metabolites. Serum 25-hydroxyvitamin D (25(OH)D) concentration was measured by a modification of the methods of Haddad and Chyu (20, 23). In each subject, in the base-line and treated state, the reported value is the mean±SEM of triplicate determinations made on three separate samples. The seasonally adjusted normal range (mean±2 SD) for this measurement in 60 normal subjects, aged 13–55 yr, is 15–80 ng/ml.

Serum concentration of $1,25(OH)_2D$ was quantitated according to previously published methods (24–26). The reported value in each subject, in the base-line and treated state, is the mean±SEM of triplicate measurements made on two separate samples. When patients were treated with $1,25(OH)_2D$, samples for this determination were obtained

at 11:00 a.m. This time of sampling was selected after preliminary investigations of divided dose $1,25(OH)_2D_3$ therapy (9:00 a.m., 9:00 p.m.) indicated that circulating levels of this metabolite, measured on single samples obtained at either 11:00 a.m., 3:00, 5:00, or 7:00 p.m., closely approximated (±8.9%) the mean serum concentration maintained throughout the day (26). Previous observations that serum $1,25-(OH)_2D$ concentration varies with chronological age (27, 28) and presumably growth (28) necessitated that we establish normal values for this measurement, appropriate for both the adolescent and adult subjects whom we studied. In 79 adults, aged 18–70 yr, the normal serum $1,25(OH)_2D$ concentration (mean±2 SD) is 20–45 pg/ml. In contrast, the normal range in 20 normal adolescents, aged 13–17 yr, is 23–70 pg/ml, levels comparable to values reported by Chesney et al. (27) in a group of similarly aged subjects. However, in the eight adolescents with a bone age > 16 yr the normal range is similar to that in adults, 23–48 pg/ml.

Balance studies and assessment of renal phosphate handling. Calcium and phosphorus balance studies were performed in the base-line and treated state in two 5-d periods according to previously published methods (20). Gastrointestinal absorption of the minerals represents the net difference between measured dietary intake and fecal excretion and mineral balance the difference between absorption and urinary excretion. The results of balance studies performed in 12 normal adults, aged 20–40 yr, were used as the age and/or growth rate matched control data for these studies.

The renal tubular maximum for the reabsorption of P_i normalized to glomerular filtration rate (TmP/GFR) was calculated by the method of Bijvoet (29). For these studies serial 2-h urine collections on selected mornings were preceded by a water load of 20 ml/kg body wt. The urine P_i (15) and creatinine (16) were determined in each specimen, serum determinations of P_i (14) and creatinine at the midpoint of each collection, and the TmP/GFR estimated (for age-specific normals, see Table I) by appropriate calculations and use of a published nomogram (30). Unpublished observations in our laboratory have confirmed that the estimated value accurately approximates the value of TmP/GFR determined during P_i infusion. The values reported are the mean±SEM of six determinations.

Bone studies. Transcortical bone biopsies were obtained from the anterior iliac crest under local anesthesia. Tetracycline (250 mg orally every 6 h) was administered to each subject over the 96- to 48-h period preceding the biopsy. Bone specimens were fixed in ethanol and embedded in methyl methacrylate. Sections were made with a Buehler Isomet sectioning machine (Buehler Ltd., Evanston, Ill.), ground to a thickness of 40±2 μm and then mounted either in the unstained state or after staining with toluidene blue and basic fuchsin.

Histomorphometric analysis of the trabecular bone in the 40-μm sections was accomplished by analysis of 50 microscopic fields in a single plane of focus employing a Merz integrated reticle (31). The results of such studies are no different than those obtained by analysis of 5-μm thick Goldner stained sections.[2] Identification of mineralized bone-osteoid granular interface by light microscopy and tetracycline label by fluorescent microscopy was employed to quantitate mineralization front activity.

The following histological parameters were quantitated: (*a*) Mineralization front activity (percent), the percentage of osteoid covered trabecular bone surface exhibiting a fluorescent tetracycline label and a bone-osteoid granular interface; (*b*) Mean osteoid seam width (micrometers), the mean width of 50 randomly selected osteoid seams which were measured using a linear reticle calibrated with a stage micrometer; (*c*) Osteoid surface (percent), the percentage of trabecular bone surface covered by osteoid; and (*d*) Active resorption (percent), the percentage of trabecular bone surface on which Howship's lacunae containing multinucleated osteoclasts are present. Normal values for these measurements were determined in bone biopsies from 10 normal subjects ages 15–35 yr.

Statistical analyses. Statistical evaluation of the data was performed with Student's *t* test for unpaired observations (32) with appropriate modification, when necessary, to accomodate comparisons between an unequal number of data points in the groups compared. Where necessary (Fig. 3) data was fit to straight lines by the method of least squares (33).

RESULTS

Vitamin D metabolism in XLH. A history of normal dietary intake in affected subjects and documented normal fecal fat excretion (2.6±0.3 g/24 h; normal < 5) provided no evidence for vitamin D deficiency. Normal liver and renal function tests (creatinine clearance 120±5.1 ml/min [Table I]) and lack of drug exposure (e.g., phenobarbital and dilantin) indicated no a priori cause for altered vitamin D metabolism. Thus, we measured the serum levels of 25(OH)D and $1,25(OH)_2D$ to assess whether the XLH mutation is associated with decreased availability of either of these metabolites. The serum 25(OH)D concentration averaged 33.9±7.2 ng/ml and was within the normal range in each subject (Table I), indicating normal vitamin D stores and providing evidence for normal vitamin D–25-hydroxylase activity. However, despite the presence of hypophosphatemia, which is a known stimulus of 25-hydroxyvitamin D-1α-hydroxylase activity, the serum $1,25-(OH)_2D$ concentration averaged 30.3±2.8 pg/ml and was marginally below or within the appropriate age-matched normal range in each adolescent and adult subject (Table I), not increased as expected. These findings indicated that a relative deficiency of $1,25-(OH)_2D_3$ might contribute to the pathogenesis of XLH and, consequently, we initiated therapy with this active vitamin D metabolite.

Effects of $1,25(OH_2D_3$ therapy on the serum concentration of vitamin D metabolites. Therapy with $1,25(OH)_2D_3$ (2.25-3.00 μg/d) was maintained for 6 mo, after which we remeasured the serum concentration of the vitamin D metabolites. The circulating level of 25(OH)D averaged 38.8±7.7 ng/ml, a value not significantly different from that maintained in the base-line period (Table I). In contrast, drug treatment resulted in a significant elevation ($P < 0.001$) of the serum, $1,25(OH)_2D_3$ concentration to levels averaging 51.3 ±1.2 pg/ml. Indeed, in each of the three treated subjects the increase in the serum level of $1,25(OH)_2D$ was

[2] Felsenfeld, A., R. Gutman, and J. M. Harrelson. Unpublished observations.

[3] Lyles, K. W., and M. K. Drezner. Unpublished observations.

TABLE I
Metabolic Data in Four Patients with XLH

Case	Age	$1,25(OH)_2D_3$ Treatment	Serum					Renal	
			Calcium	P_i	Alkaline Phosphatase	25(OH)D	$1,25(OH)_2D$	Cr_{Cl}	TmP/GFR
		μg/d	*mg/dl*	*mg/dl*	*IU*	*ng/ml*	*pg/ml*	*ml/min*	*mg/dl*
1	15	0	9.04±0.06*(18)‡	2.11±0.05(18)	275±7.8 (5)	48.5±3.6(3)	22±1.5(2)	105±7.3 (6)	2.05±0.05(6)
		3.00	9.20±0.07 (18)	2.73±0.06(18)	164±13.4(5)	47.6±4.2(3)	53±3.9(2)	121±10.6(6)	2.31±0.04(6)
			NS	$P < 0.001$	$P < 0.001$	NS	$P < 0.05$	NS	$P < 0.01$
2	14	0	9.21±0.09 (16)	2.12±0.05(16)	246±13.0(6)	25.2±2.4(3)	32±3.0(2)	125±9.8 (7)	2.07±0.06(6)
		2.50	9.69±0.07 (17)	2.87±0.09(17)	185±2.4 (5)	23.4±1.9(3)	52±2.5(2)	119±7.1 (6)	2.51±0.04(6)
			$P < 0.01$	$P < 0.001$	$P < 0.01$	NS	$P < 0.05$	NS	$P < 0.001$
3	21	0	9.33±0.08 (15)	2.18±0.06(14)	245±18.0(7)	43.2±3.7(3)	34±1.9(2)	123±6.7 (5)	1.97±0.07(6)
		2.25	8.92±0.10 (15)	2.71±0.08(15)	84±8.3 (5)	45.4±2.8(3)	49±1.5(2)	119±3.4 (5)	2.48±0.03(6)
			$P < 0.01$	$P < 0.001$	$P < 0.001$	NS	$P < 0.05$	NS	$P < 0.001$
4	30	0	9.29±0.11 (18)	2.59±0.07(18)	187±5.4 (6)	17.6±2.1(3)	33±2.6(2)	127±4.9 (5)	2.37±0.03(6)
Mean		0	9.22±0.06	2.25±0.11	238±18.4	33.9±7.2	30.3±2.8	120.0±5.1	2.12±0.09
		2.25–3.00	9.27±0.23	2.77±0.05	144±30.8	38.8±7.7	51.3±1.2	119.0±0.7	2.43±0.06
			NS	$P < 0.01$	$P < 0.05$	NS	$P < 0.001$	NS	$P < 0.05$
Normal			8.7–10.3	§	§	15–80	‖	100–120	§

* Mean±SEM.

‡ Number of determinations.

§ The normal values for serum P_i, serum alkaline phosphatase and TmP/GFR are age specific. In cases 1 and 2 the normal values for these measurements are 2.9–5.5 mg/dl, <170 IU, and 2.70–5.6 mg/dl, respectively. In cases 3 and 4 these normal values are 2.5–4.5 mg/dl; <110 IU, and 2.55–4.50 mg/dl.

‖ The serum $1,25(OH)_2D$ level varies with age and growth rate. In adults the normal range is 20–45 pg/ml. In contrast in adolescents (13–17), normal values range from 23 to 70 pg/ml. However, when bone age of adolescents is >16 yr and growth rate slowed the normal range is 23–48 pg/ml.

significant ($P < 0.05$), resulting in maintenance of a supraphysiological circulating concentration of this metabolite when values were compared to appropriate age matched and, where necessary, bone age-matched controls (Table I).

Effects of $1,25(OH)_2D_3$ therapy on P_i metabolism. Each of the subjects with XLH presented initially with a characteristically decreased serum P_i and evidence of renal P_i wasting marked by a subnormal TmP/GFR (Table I). In response to therapy with $1,25(OH)_2D_3$ the serum P_i was significantly elevated in each treated subject ($P < 0.001$) and approached, or was within, the age-corrected normal range. Similarly, $1,25(OH)_2D_3$ therapy resulted in a significant increase ($P < 0.01$) of the TmP/GFR (Table I). However, the TmP/GFR remained subnormal in each subject.

An additional abnormality of P_i metabolism manifest at presentation was a negative P_i balance, which ranged from −3 to −60 mg/d (Table II). This abnormality resulted from apparent net gastrointestinal malabsorption of P_i (Table II) and renal P_i wasting (TmP/GFR, 2.12±0.09 mg/dl) (Table I). Treatment with $1,25(OH)_2D_3$ resulted in a remarkably positive P_i balance, ranging from +144 to +264 mg/d in the treated subjects (Table II). This alteration in P_i balance was largely caused by a significant improvement in the net gastrointestinal absorption of P_i in each subject (Table II) and renal P_i wasting (TmP/GFR, 2.43±0.06) (Table I). Despite the improvement in renal P_i wasting, however, 24-h urine P_i increased significantly in response to therapy (Table II).

Effects of $1,25(OH)_2D_3$ therapy on calcium metabolism. In the base-line period the serum calcium concentration averaged 9.22±0.06 mg/dl, a value well within normal limits. After treatment with $1,25(OH)_2D_3$ the mean serum calcium remained normal, 9.27 ±0.23 mg/dl, and was not significantly different from the base-line level. However, in case 2, a significant increase, and in case 3, a significant decrease in the serum calcium did occur and persisted (Table I).

Further analysis of calcium homeostasis revealed that the patients maintained a marginally positive calcium balance, 83±30 mg/d, in the base-line period (Table III). This positive balance was maintained, despite a modest decrease in net gastrointestinal malabsorption of calcium, by virtue of renal conservation of calcium (Table III). Treatment with $1,25(OH)_2D_3$ resulted in markedly positive calcium balance of 438±30 mg/d (Table III). This calcium retention occurred in response to a significant increase in net gastrointestinal absorption of calcium in each treated subject and despite an increase in urinary calcium excretion (Table III). The urinary calcium excretion, however, remained within the appropriate normal range and was not significantly different from that in the untreated state.

Effects of $1,25(OH)_2D_3$ therapy on the serum PTH concentration. Because many of the cardinal mani-

TABLE II
Phosphate Balance Studies in Subjects with XLH

	Age	$1,25(OH)_2D_3$ treatment	Dietary intake	Fecal excretion	Gastrointestinal absorption	Urinary excretion	Balance
	yr	*μg/d*	*mg/d*	*mg/d*	*mg/d*	*mg/d*	*mg/d*
Controls (12)*	20–40	0	1,256±42‡ (145)§	387±41 (142)	869±55 (190)	792±43 (149)	77±24 (83)
Case 1	15	0	1,125±15	550±70	575±55	635±30	−60±25
		3.00	1,125±42	128±32	997±74	733±22	264±52
			NS	$P < 0.02$	$P < 0.02$	$P < 0.05$	$P < 0.05$
Case 2	14	0	1,200±40	721±23	479±63	535±40	−56±23
		2.50	1,236±27	168±25	1,068±52	885±35	183±17
			NS	$P < 0.001$	$P < 0.01$	$P < 0.001$	$P < 0.02$
Case 3	21	0	1,210±32	542±37	668±69	671±50	−3±10
		2.25	1,225±41	202±18	1,023±23	876±56	147±33
			NS	$P < 0.02$	$P < 0.02$	$P < 0.05$	$P < 0.05$
Case 4	30	0	1,154±42	639±50	515±65	575±43	−60±22
Mean		0	1,172±20	613±42‖	559±41¶	604±30	−45±14‖
Case 1–4	—	2.25–3.00	1,195±35	116±21**	1,029±21	831±49	198±35**
			NS	$P < 0.001$	$P < 0.001$	$P < 0.01$	$P < 0.001$

The data represent the results of two 5-d balance studies in each subject in the base-line state and, where possible, on treatment with $1,25(OH)_2D_3$. Dietary intake, fecal excretion, gastrointestinal absorption, and urinary excretion and balance are expressed as a mean±SEM of the respective values in each balance period. The control data were obtained in 12 normal subjects, aged 20–40 yr, by performing two 5-d balance studies. (Although phosphate balance varies with age and is significantly greater in rapidly growing adolescents [by virtue of increased gastrointestinal absorption], the completed linear growth of the adolescent subjects in the present study makes subjects aged 20–40 yr appropriate growth rate-matched controls.) The data in these controls are expressed as the mean±SEM of the results in the 12 subjects. In addition the SD of these determinations is provided.

* Number of subjects studied.

‡ Mean±SEM.

§ SD.

‖ Significant difference from controls at $P < 0.02$.

¶ Significant difference from controls at $P < 0.01$.

** Significant difference from controls at $P < 0.05$.

festations of XLH, and the changes in biochemical abnormalities which we observed in response to $1,25(OH)_2D_3$ therapy, might be related to abnormalities or alterations in the serum PTH concentration, we measured this variable in the untreated and treated periods. The serum PTH concentration, measured by four separate radioimmunoassays, with varying antigenic specificity, was normal in each subject in the base-line period, suggesting that altered parathyroid function was not central to the genesis of the syndrome. Nevertheless, $1,25(OH)_2D_3$ treatment did result in a decrease in the circulating level of PTH, as indicated by the uniformly lowered posttreatment values measured by each assay (Table IV). Thus the effects of $1,25(OH)_2D_3$ on the biochemical abnormalities of the syndrome may be modulated, in part, by suppression of PTH secretion.

Effects of $1,25(OH)_2D_3$ on bone histomorphology. To evaluate the effects of $1,25(OH)_2D_3$ therapy on the osteomalacic bone lesions, we obtained bone biopsies in the base-line period and after 6 and 12 mo of therapy. Initial biopsies were marked by decreased mineralization front activity (17.7–29.2%; normal 65.5±7.8); osteoid seams of increased width (24.5–50.9 μm; normal 12.5±4.7); and the presence of increased amounts of osteoid-covered trabecular bone surface (50.2–81.4%; normal 13.5±5.5). These abnormalities are indicative of osteomalacia (Fig. 1; Table V). Moreover, typical of the bone abnormality in XLH, osteocytic osteolysis was uniformly present (Fig. 1). In contrast, after 6 and 12 mo of therapy an apparent resolution of the mineralization defect had occurred. Bone biopsies from each treated subject were marked by a significant increase in tetracycline-labeled mineralization front activity (Fig. 2) and an apparent decrease in the osteocytic osteolysis. This apparent bone healing was accompanied by a significant decline in the serum alkaline phosphatase activity in each treated patient (Table I). Quantitative histomorphological analysis of the bone biopsies confirmed

TABLE III
Calcium Balance Studies in Subjects with XLH

	Age	$1,25(OH)_2D_3$ treatment	Dietary intake	Fecal excretion	Gastrointestinal absorption	Urinary excretion	Balance
	yr	*μg/d*	*mg/d*	*mg/d*	*mg/d*	*mg/d*	*mg/d*
Controls (12)*	20–40	0	935±45‡ (156)§	605±22 (76)	330±33 (114)	205±41 (142)	125±30 (104)
Case 1	15	0	947±42	662±28	285±70	127±13	158±52
		3.00	909±26	194±23	715±49	223±28	492±21
			NS	$P < 0.001$	$P < 0.02$	$P < 0.05$	$P < 0.01$
Case 2	14	0	876±20	787±39	89±19	71±6.8	18±25
		2.50	928±33	360±45	568±12	178±14	390±26
			NS	$P < 0.01$	$P < 0.001$	$P < 0.01$	$P < 0.01$
Case 3	21	0	894±27	745±19	149±33	89±16	60±17
		2.25	890±17	225±21	665±30	233±12	432±50
			NS	$P < 0.001$	$P < 0.01$	$P < 0.01$	$P < 0.01$
Case 4	30	0	898±26	675±24	223±35	127±19	96±31
Mean	—	0	904±15	717±21‖	187±43¶	104±14	83±30
Cases 1–4		2.25–3.00	909±11	260±51**	649±42**	211±17	438±30**
			NS	$P < 0.001$	$P < 0.001$	NS	$P < 0.001$

The data represent the results of two 5-d balance studies in each subject in the base-line state and, where possible, on treatment with $1,25(OH)_2D_3$. Dietary intake, fecal excretion, gastrointestinal absorption, and urinary excretion and balance are expressed as mean±SEM of the respective values in each balance period. Control data were obtained in 12 normal subjects, aged 20–40 yr by performing two 5-d balance studies. (Although calcium balance varies with age and is significantly greater in rapidly growing adolescents [by virtue of increased gastrointestinal absorption], the completed linear growth of the adolescent subjects in the present study makes subjects aged 20–40 yr appropriate growth rate matched controls.) The data in these controls are expressed as the mean±SEM of the results in the 12 subjects. In addition the SD of these determinations is provided.

* Number of subjects studied.

‡ Mean±SEM.

§ SD.

‖ Significant difference from controls at $P < 0.02$.

¶ Significant difference from controls at $P < 0.05$.

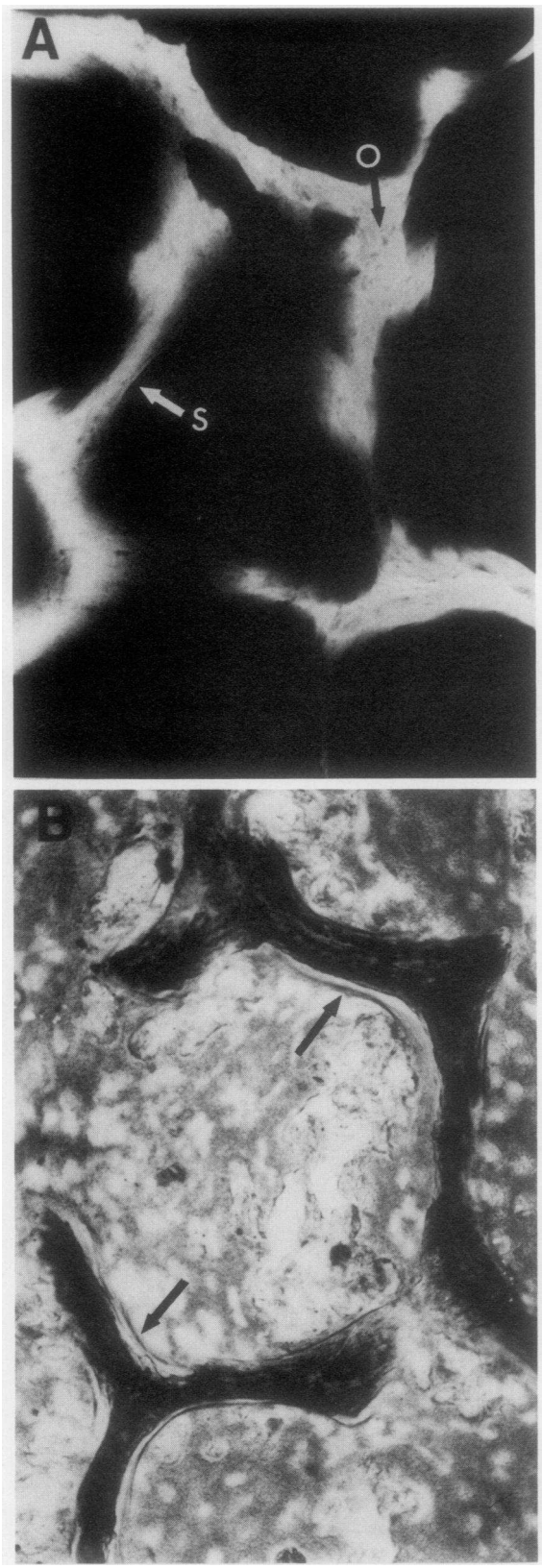
** Significant difference from controls at $P < 0.001$.

that $1,25(OH)_2D_3$ therapy resulted in normalization of the decreased mineralization front activity present in each biopsy in the base-line state (Table V). Further, a central role for the $1,25(OH)_2D_3$ in the induction of mineralization in the osteomalacic bone is suggested by the linear relationship between the serum level of this metabolite and mineralization front activity (Fig. 3). In both the untreated and treated state, mineraliza-

TABLE IV
Concentration of Immunoreactive Serum PTH in Patients with XLH

Case	$1,25(OH)_2D_3$ treatment	Serum PTH			
		-COOH 1	-COOH 2	-COOH 3	$-NH_2$
	μg/d	*μl eq/ml*	*pg eq/ml*	*pg/ml*	*ng/ml*
1	0	23	175	170	0.045
	2.50	14	<150	71	ND*
2	0	23	310	39	0.047
	3.00	19	223	<39	ND
3	0	26	348	—	0.031
	2.25	19	193	—	ND
4	0	35	150	—	0.053
Normal		<40	<150–375	<300	<0.10

* ND, nondetectable.



tion front activity closely correlated with the serum levels of $1,25(OH)_2D$ circulating at the time of biopsy.

Despite the normalization of mineralization front activity, treatment with $1,25(OH)_2D_3$ had variable effects on the wide osteoid seams and the excess osteoid covered trabecular bone surface present on initial biopsies. Although osteoid seam width and the extent of osteoid covered trabecular bone surface progressively diminished over the 12 mo of treatment in case 2, the biopsies from cases 1 and 3 showed no change in the extent of osteoid and variable changes in osteoid seam width (Table V).

DISCUSSION

Of the several theories for the pathogenesis of XLH which have evolved, no single explanation has gained universal acceptance and the metabolic basis for this disease remains unknown. Nevertheless, cumulative data from several studies (7, 34, 35) indicate that a defect in transepithelial transport of P_i in kidney (resulting in partial impairment in net P_i reabsorption) and perhaps also in intestine and bone is likely the primary determinant of the XLH phenotype in man. This proposal is supported by several lines of evidence: (*a*) treatment of affected subjects with orthophosphate, in doses sufficient to restore the serum P_i to normal, results in radiographically evident healing of rickets and promotes "catch-up" linear growth in childhood (36, 37); (*b*) subtotal parathyroidectomy does not change renal P_i handling, suggesting that the renal tubular defect is innate (38, 39); and (*c*) animals rendered hypophosphatemic by P_i depletion develop abnormalities in bone mineralization similar to those of XLH (40). Moreover, Tenenhouse et al. (41) have reported that purified renal cortical brush membranes from the murine homologue of XLH, the X-linked hyp-mouse, exhibit a partial loss of the sodium-dependent phosphate transport process. This finding in the hyp-mouse is compatible with a partial loss of renal phosphate transport in XLH. Nevertheless this hypothetical P_i leak and resultant hypophosphatemia do not account for all of the abnormalities of XLH, especially the characteristic decrease in intestinal absorption of calcium and P_i or its normalization after therapy with $1\alpha(OH)D_3$ (42, 43). In addition, the variable presence of the bone lesion and the lack of correlation between the magnitude of the hypophosphatemia and the severity of the rickets or osteomalacia is enigmatic (1). Finally, treatment with P_i alone or in combination with pharmacological amounts

FIGURE 1 (A) Microradiograph showing both hypomineralized "splits" (S) and patchy osteocytic osteolysis (O). (B) Unstained section demonstrating extensive osteoid seams (arrows) with minimal mineralization.

TABLE V
Quantitative Histomorphology of Bone Biopsies

	Case 1			Case 2			Case 3			Case 4	
Time, *mo*	0	6	12	0	6	12	0	6	12	0	Normals (10)*
Mineralization front activity, %‡	17.7	72.5	73.6	23.0	65.5	58.7	29.2	61.9	62.8	26.0	65.5±7.8§
Mean osteoid seam width, μm‖	24.5	18.7	34.1	28.6	20.8	15.2	50.9	25.0	26.4	28.7	12.5±4.7
Osteoid surface, %¶	50.2	57.0	61.5	74.2	64.0	42.5	73.5	64.7	75.3	81.4	13.5±5.5
Active resorption, %**	0.6	0.4	0.3	0.4	0.1	0.1	0.2	0.5	0.3	0.5	0.19±0.22

* Number of normal controls.
‡ Mineralization front activity—the percent of osteoid covered trabecular bone surface over which mineralization is occurring.
§ Mean±1 SD.
‖ Mean osteoid seam width—mean width of osteoid seams determined by measurement of 50 seams using a calibrated reticle.
¶ Osteoid surface—the percentage of trabecular bone surface covered by osteoid.
** Active resorption—the percentage of total trabecular bone surface occupied by Howship's lacunae containing multinucleated osteoclasts.

of vitamin D does not restore bone mineralization to normal (44) refuting the hypothesis that all the component abnormalities are solely dependent upon hypophosphatemia.

The results of the present study indict an abnormality in vitamin D metabolism as an additional factor in the phenotypic expression of XLH. A disordered regulation of vitamin D metabolism is suggested by the serum levels of $1,25(OH)_2D$ in affected subjects in the base-line state (Table I). Recent studies in both man (45) and various animals (46–48) indicate an inverse relationship between serum levels of P_i and production rate or serum concentration of $1,25(OH)_2D$. Therefore, it follows that patients with XLH should manifest both an increased production rate for and an increased circulating level of $1,25(OH)_2D$. However, we found that three affected subjects had only a 'normal' circulating level and one a marginally decreased serum concentration of $1,25(OH)_2D$. These data indicate a functional impairment in the regulation of $1,25(OH)_2D_3$ biosynthesis which may contribute to the clinical expression of XLH.

The therapeutic response to pharmacological amounts of $1,25(OH)_2D_3$ substantiates a role for altered vitamin D homeostasis in the pathogenesis of XLH. Therapy raised the serum $1,25(OH)_2D$ level and partially restored P_i homeostasis; long-term treatment normalized the mineralization defect in bone. The effects of therapy on P_i homeostasis were apparently modulated by the direct action of $1,25(OH)_2D_3$ on a variety of tissues. These included a marked increase in the net gastrointestinal absorption of P_i (Table II), an apparent suppression of PTH secretion (Table IV); and an amelioration of renal P_i wasting, indicated by a significant increase in TmP/GFR (Table I). However, the concomitant increase in serum P_i (Table I) and consequently in the filtered load of P_i, significantly increased the 24-h urine P_i excretion (Table II) obscuring any effects on renal P_i reabsorption. In any case, the TmP/GFR remained below the normal range and the 24-h urine P_i significantly above that in the base-line state, indicating persistent and inappropriate P_i wasting. Thus, $1,25(OH)_2D_3$ treatment did not correct the basic renal defect, but did alter P_i homeostasis to circumvent the abnormality and to achieve a positive P_i balance (Table II) and a significant increase in the serum P_i.

Most importantly, however, $1,25(OH)_2D_3$ therapy repaired the osteomalacic bone lesion. Treatment restored normal mineralization front activity to the endosteal bone surface, reversing a primary abnormality of the XLH osteomalacic disorder. Moreover, the linear correlation between mineralization front activity and the serum level of $1,25(OH)_2D_3$ (Fig. 3) indicates that this metabolite may directly affect deposition of calcium in bone. Although this relationship may be indirect and modulated by other factors, the lack of correlation between serum P_i at the time of bone biopsy and mineralization front activity (data not shown) and the failure of alternate therapeutic means to cause this response (44), despite similar effects on the characteristic biochemical abnormalities of the syndrome, suggest that the action on bone is direct.

Moreover the apparent need to achieve supraphysiological serum levels of $1,25(OH)_2D$ to effect bone healing reaffirms the hypothesis that an abnormality of vitamin D metabolism contributes to the manifestations of XLH. Several lines of evidence confirm that pharmacological amounts of $1,25(OH)_2D_3$ are needed to induce the observed changes in bone

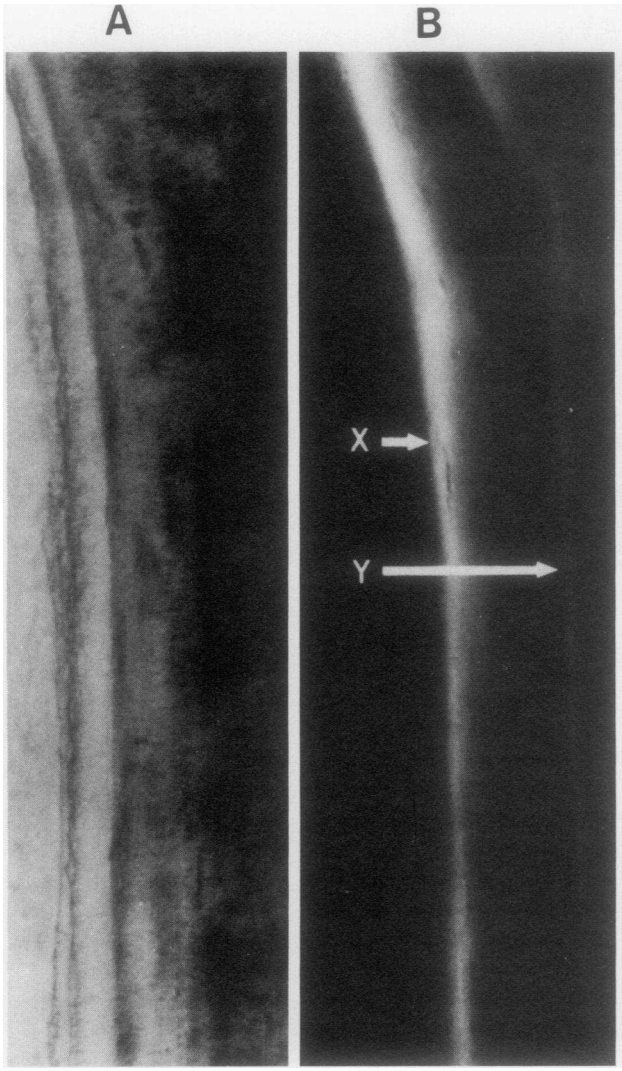


FIGURE 2 (A) Unstained section in posttreatment state showing narrow osteoid seam and a granular interface with underlying bone, indicative of mineralization. (B) Fluorescent photomicrograph of same section showing current active label (X) and prior label of 6 months earlier (Y) with interposed normal lamellar bone.

mineralization. First, Glorieux et al. recently reported (49) the treatment of XLH with near physiological doses of $1,25(OH)_2D_3$ (1 μg/d), but amounts less than those employed in the present study. They found improvement, but not normalization, of mineralization front activity despite concomitant therapy with oral P_i (1.2–3.3 g/d) which increased the serum P_i to values comparable to those achieved in the present study. These data indicate that doses of $1,25(OH)_2D_3$ must be significantly greater than physiological requirements to maintain normal mineralization front activity in XLH. Secondly, previous trials of $1,25(OH)_2D_3$ therapy at doses of 1.0 and 1.3 μg/d and $1\alpha(OH)D_3$ therapy at 1.0 μg/d (50–53) have failed to improve net gastrointestinal absorption of P_i, and/or calcium and P_i balance or have resulted in insignificant changes in tubular reabsorption of P_i and/or TmP/GFR. These observations are similar to our own which we noted during the course of drug titration in the present study (data not shown) and contrast to the response seen (Tables I–III) when we used pharmacological amounts of $1,25(OH)_2D_3$.

Further, in each treated subject in the present study the circulating concentration of $1,25(OH)_2D_3$ increased significantly in response to pharmacological doses of this metabolite, attaining supraphysiological levels (Table I). While the values in the adolescent patients remained within the chronologically age-matched normal range (23–70 pg/ml), they were above values (23–48 pg/ml) maintained in normals matched for bone

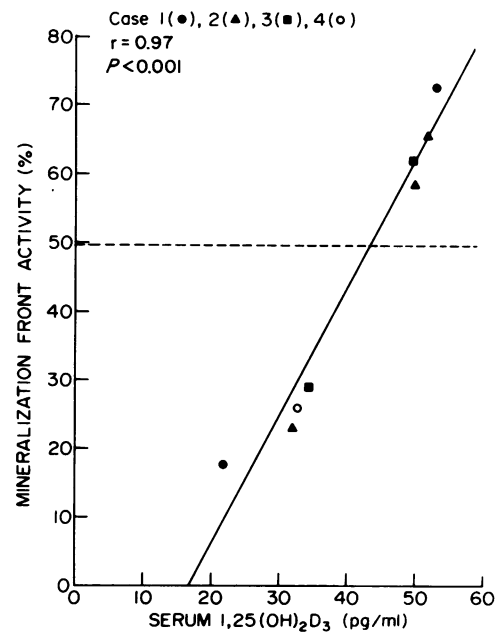


FIGURE 3 Correlation between the mineralization front activity in bone biopsies and the circulating concentration of $1,25(OH)_2D_3$. Bone biopsies were obtained (and mineralization front activity determined) in the four subjects studied in the base-line period, after 6 mo of therapy with $1,25(OH)_2D_3$ in the three treated subjects (while on 3.00, 2.50, and 2.25 μg/d, respectively) and after 12 mo of therapy in cases (while on 2.75 μg/d) 1, 2, and 3. Serum $1,25(OH)_2D_3$ levels were determined at 11 a.m. on the 2 d preceding bone biopsy in each case as described in Methods. Because the 6- and 12-mo biopsies in case 2 were performed on different doses of medication both points are displayed. In contrast, the 6- and 12-mo biopsies on case 1 and 3 were performed on the same dose of medication and are displayed as a single point, because no significant difference in mineralization front activity or $1,25(OH)_2D$ serum levels were present. The dotted line represents the lower level of normal mineralization front activity. It is evident that a significant correlation exists between the measured parameters.

age (and presumably growth rate). In early childhood and adolescence serum levels of $1,25(OH)_2D$ are normally higher than at other periods of life, probably caused by rapid growth in early childhood and teenage years (28). This relationship to growth is substantiated in the present study by the internal disparity of the values seen in normal adolescents when segregated by bone age rather than chronological age. Thus, commensurate with plateau of growth and a bone age of 16 yr or greater, the normal range for serum $1,25(OH)_2D_3$ is lower than that in the more rapidly growing adolescents who have a bone age ranging from 12 to 15 yr. The adolescent subjects with XLH whom we treated in the present study had a bone age of 16 yr or greater and the attenuated growth characteristic of the disease. Therefore, we used both chronological and bone age to select appropriate controls for serum $1,25(OH)_2D_3$ concentration.

Our findings regarding serum concentration of $1,25(OH)_2D$ are consistent with previous observations. Meyer et al. (54) reported that the serum $1,25(OH)_2D$ levels in the X-linked hyp-mouse model are normal and not elevated as expected and as, in fact, are found in phosphate depleted normal mice of the same strain (55).[3] Scriver et al. (55) reported serum levels of $1,25(OH)_2D_3$ in human patients with XLH which were considerably lower (15.6 ± 7.8 pg/ml) than those obtained in the present study. However, the patients studied by Scriver et al. were younger than ours and the majority were already being treated with oral P_i and vitamin D. This therapeutic regimen may lower serum levels of $1,25(OH)_2D_3$ and account for the noted differences (56). In any case, the observations of Scriver et al. substantiate that there is no increase in serum $1,25(OH)_2D_3$. Moreover, previous studies in the X-linked hyp-mouse, provide data which may explain the relative deficiency of $1,25(OH)_2D$. Despite renal P_i wasting, the hyp-mouse has a normal intracellular P_i content in kidney (41). This finding implies an intact component of P_i transport (perhaps influx at the basolateral membrane) which maintains intracellular renal P_i concentrations and masks the intracellular hypophosphatemia precluding the anticipated increase in 25-(OH)D-1α-hydroxylase activity. Such a compensatory mechanism, if isolated to the kidney, may protect the renal cell from mineral disequilibrium while subjecting other tissues particularly bone to the effects of hypophosphatemia in the absence of an increased serum $1,25(OH)_2D$ concentration.

In any case a relative deficiency of $1,25(OH)_2D_3$ may explain the enigmatic findings which have precluded universal acceptance of a model for the pathogenesis of XLH. Birge and Miller (57) recently reported data which indicates that the calcium and P_i malabsorption, characteristic of XLH, may result from a blunted effect of $1,25(OH)_2D_3$ on gastrointestinal absorption in the presence of inadequate P_i. In their studies, $1,25(OH)_2D_3$ had a diminished effect on radiolabeled calcium and P_i transport across inverted gut sacs from rats in the presence of low medium P_i. Thus, the abnormal gastrointestinal absorption in XLH may reflect an inability of normal levels of $1,25(OH)_2D_3$ to overcome the suppression of absorption induced by hypophosphatemia. Further, it is likely that the effects of therapy on calcium and P_i absorption result from the cumulative effects of both $1,25(OH)_2D_3$ itself and the increased serum P_i. In addition, the absence of hypercalciuria in the presence of hypophosphatemia is no doubt caused by the relative deficiency of $1,25(OH)_2D$ with consequent calcium malabsorption. Finally, the hypothesis that at least two factors underlie XLH, may account in part for the variable severity of bone involvement in affected subjects. For example, if some hypophosphatemic subjects retain a capacity to increase the synthesis of $1,25(OH)_2D_3$, any resulting bone lesion may be remarkably less severe. Indeed, in subjects with hypophosphatemic bone disease, Scriver et al. (55) proposed that a retained capacity to synthesize $1,25(OH)_2D_3$ explains the disparity between the severity of the hypophosphatemia and the severity of the expressed bone disease.

Despite the data supporting our hypothesis that a relative deficiency of $1,25(OH)_2D_3$ is a factor in the genesis of XLH, we cannot exclude a potential role for alternate or additional abnormalities. We did not measure other vitamin D metabolites, including $24,25(OH)_2D$ and $1,24,25(OH)_3D$, thus precluding determination of whether the apparent aberration in vitamin D metabolism is more widespread than appreciated. However, the relatively unknown physiological role of these metabolites makes speculation concerning their importance in the genesis of XLH difficult. Secondly, it is possible that target organ resistance to the effects of $1,25(OH)_2D$ may account for the beneficial responses which we noted in response to pharmacological therapy. However, in those disease states marked by a resistance to $1,25(OH)_2D_3$, affected subjects have hypocalcemia, hypophosphatemia, secondary hyperparathyroidism and, most importantly, a markedly elevated serum level of $1,25(OH)_2D$ (58, 59). Moreover, recent studies (60) in the X-linked hyp-mouse model indicate that transport of $1,25(OH)_2D_3$ into cytoplasm and subsequently to the nucleus of intestinal mucosal cells is normal, eliminating the usual mechanisms of end organ resistance. Thus end organ resistance apparently is not a major factor in genesis of XLH. Finally, we cannot completely exclude the possibility that the changes which we observed in response to $1,25(OH)_2D_3$ therapy may simply represent pharmacological effects of $1,25(OH)_2D_3$ in a group of patients who have preserved the ability to respond to this hormone. However, the coupling of an inappropriately low serum

$1,25(OH)_2D$ level, suggesting inadequate homeostatic regulation, and the beneficial responses to therapy with $1,25(OH)_2D_3$ support the presence of an underlying defect in vitamin D metabolism.

Nevertheless, several observations in the present study are apparently at variance with establishing a relative deficiency of $1,25(OH)_2D$ as central to the pathogenesis of XLH. First, in all of the subjects studied bone age exceeded 16 yr, longitudinal growth had ceased, and there was no evidence of rickets. Thus, we cannot determine whether the rachitic lesions, characteristic of XLH, respond to $1,25(OH)_2D_3$ therapy nor whether treatment promotes catch up growth in affected subjects. However, a requirement for $1,25(OH)_2D_3$ in the healing of the rachitic disease is questionable because there is ample radiographic evidence that the rickets heals in response to a variety of treatment regimens. Moreover, a potential diversity of response is not surprising because there may be differential regulation of the mineralization of epiphyseal and diaphyseal bone. Nevertheless, further studies in young children will be necessary to examine the effects of $1,25(OH)_2$ on the rachitic lesions and statural growth. Secondly, and most importantly, the efficacy of $1,25(OH)_2D_3$ therapy on the osteomalacic lesions may be limited because posttreatment bone biopsies show excess osteoid despite normalized mineralization front activity. The persistence of unmineralized osteoid is most likely secondary to an increased rate of osteoid synthesis or an unappreciated defect in mineralization. Increased osteoid synthesis is a reversible and self-limited cause of excess osteoid associated with disorders of accelerated bone turnover such as hyperparathyroidism and with bone healing (e.g., fractures). At present we cannot determine if the reparative process going on in the treated patients has increased osteoid synthetic rate and hence resulted in a time-limited excess of osteoid. Similarly, our data are insufficient to determine if mineral appositional rate is abnormal. Appositional rate is a second factor, in addition to mineralization front activity, which controls the rate of bone mineralization (61) and is likely abnormal in XLH (62). A persistent defect in this function would result in incomplete healing of the osteomalacia. Thus, at present we cannot predict whether $1,25(OH)_2D_3$ therapy resolves the osteomalacic disorder of XLH. Nevertheless, our data do illustrate that $1,25(OH)_2D_3$ therapy normalizes mineralization front activity which is a primary defect underlying the bone abnormalities in XLH.

Thus, our studies suggest that altered regulation of vitamin D metabolism is a fundamental abnormality in XLH. Whether the resulting 'deficiency' of $1,25(OH)_2D$ is secondary to decreased biosynthesis or increased degradation remains to be determined. However, at the present time we propose that the sequence of metabolic events underlying this disorder includes: (*a*) a genetic lesion in the renal tubule results in Pi wasting and consequent hypophosphatemia; (*b*) the hypophosphatemia fails to elicit the anticipated increase in $1,25(OH)_2D_3$ biosynthesis; and (*c*) relative deficiency of $1,25(OH)_2D_3$ and associated absolute deficiency of P_i result in gastrointestinal malabsorption of calcium and P_i and, most importantly, in the characteristic defect in mineralization front activity in XLH bone. Although additional defects are likely to be revealed with further studies, our findings indicate that therapeutic regimens which include $1,25(OH)_2D_3$ may be beneficial in this refractory disorder.

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