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Bone histology in incipient and advanced renal failure

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Bone histology in incipient and advanced renal disease. Quantitative bone histology (micromorphometry of undecalcified sections, analysis under polarized light; fluorescence microscopy with tetracycline double labelling) as well as serum and urinary chemistry (creatinine clearance, parathyroid hormone, ionized Ca, bone phosphatase, pH), were studied in 50 patients with incipient to advanced (glomerular filtration rate, 80 to 6 ml/min × 1.73 m²) renal insufficiency. In incipient renal failure, indirect evidence of parathyroid hormone excess was found in the skeleton (empty osteoclastic lacunae, woven osteoid). Osteoclastic surface resorption was abnormally high when GFR fell below 50 ml/min × 1.73 m² and endosteal fibrosis appeared below GFR of 30 ml/min ×1.73 m². With the tetracycline double-labelling technique, a mineralization defect was demonstrable in many but not all patients.

Histologie de l'os au cours de l'insuffisance rénale débutante et de l'insuffisance rénale majeure. Chez 50 malades atteints d'insuffisance rénale à des degrés divers (débit de filtration glomérulaire de 80 à 6 ml/min · 1,73m²) ont été réalisés l'étude histologique quantitative de l'os (micromorphométrie des coupes non déclacifiées, analyse en lumière polarisée, microscopie en fluorescence après double marquage par la tétracycline) ainsi que les déterminations de la clearance de la créatinine, du calcium ionisé, de l'hormone parathyroïdienne, des phosphatases osseuses et du pH. Au cours de l'insuffisance rénale débutante on trouve des signes indirects d'une augmentation de l'hormone parathyroïdienne à l'examen de l'os (lacunes ostéoclastiques vides, osteoïde lamellaire). La surface de résorption ostéoclastique est anormalement grande le débit de filtration glomérulaire est inférieur à 50 ml/min · 1,73 m² et il apparaît une fibrose dans les lacunes de résorption quand ce débit est inférieur à 30 ml/min × 1,73 m². Un défaut de minéralisation est mis en évidence par le double marquage par la tétracycline chez beaucoup de malades mais non chez la totalité d'entre eux.

Whereas the skeletal lesions of the terminal stage of renal insufficiency are well known, skeletal histology in early stages of renal insufficiency has not been investigated to any large extent [1-4]. This is somewhat surprising, since cellular mechanisms leading to abnormal bone histology can more easily be unravelled in a skeleton with incipient lesions rather than in a skeleton with far-advanced lesions.

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Therefore, we investigated in a cross-sectional type of study 50 patients with chronic renal disease at various levels of glomerular filtration rate (GFR). Evidence of fibroosteoclasis was found even in very early renal failure; with the tetracycline double-labeling technique, defective mineralization was demonstrable only in a fraction of the patients studied.

Methods

Patients. Fifty patients (19 male, 31 female) with chronic renal disease (duration: one month to 7 yr) who had presented themselves for diagnostic evaluation in the outpatient department were examined (age: 43.2 ± 11.3; range, 20 to 61 yr). The underlying renal disease was glomerulonephritis (19/50), renal malformation and or pyelonephritis (21/50), polycystic disease (6/50), analgesic abuse, malignant hypertension, Kimmelstiel-Wilson and unknown etiology (one case each).

None of the patients had received treatment such as vitamin D or analogous sterols or orally administered phosphate binders or calcium supplements.

For the histological study, 22 victims of traffic accidents without known skeletal or renal disease were chosen as controls (12 male; 10 female; age: 42.5 ± 15.2; range, 24 to 65 yr).

Bone histology. All patients were subjected to tetracycline double-labelling: they were given tetracycline hydrochloride, 500 mg/24 hr, from day 18 to 16 and 4 to 0 prior to biopsy. Biopsy specimens were taken from the iliac crest (4 cm behind the spina iliaca anterior superior) with an electric drill [5]. The specimens were fixed in alcohol, embedded in methylmethacrylate, cut (3 μ) and stained with Masson-Goldner as described previously [6, 7]. Micromorphometric measurements were performed by the method of Merz and Schenk [8]; areas were measured

by point counting and perimeter lengths by counting intersections with semicircular sampling lines.

The following indexes were calculated: *(1) volumetric density of bone* (Vv) indicating the fraction of spongiosal volume occupied by bone matrix (%); *(2) volumetric density of osteoid* (Vo) indicating the fraction of spongiosal volume occupied by unmineralized (lamellar or woven) osteoid (%); *(3) surface density* (SD), i.e., endosteal (= trabecular) surface per unit volume of spongy bone, indicating the size of the interface between bone tissue and marrow cavity (mm^2/cm^3); *(4) specific surface* (S/V), the ratio of trabecular surface to trabecular volume (mm^2/mm^3) (this ratio falls as trabecular diameter rises); *(5) osteoid surface density* (OS) indicating the size of unmineralized osteoid seams on the trabecular surface per unit volume of spongy bone (mm^2/mm^3); *(6) osteoid surface fraction* (OSF) indicating the fraction trabecular surface covered by unmineralized osteoid seams (%); *(7) mean thickness of osteoid seams* (S̄), calculated as the ratio of absolute osteoid volume and absolute osteoid surface; *(8) osteoclastic resorption surface* (HO) indicating the fraction of mineralized (= nonosteoid) trabecular surface covered by osteoclasts; *(9) lacunae* (L) indicating the fraction of trabecular surface covered by inactive resorption cavities (not filled with osteoclasts); and *(10) endosteal fibrosis surface fraction* (EOFS) indicating the fraction of trabecular surface covered with layers of collagen fibers.

In addition, all sections were analyzed under polarized light; the fraction of osteoid seams exhibiting nonlamellar warp-and-woof pattern (woven osteoid) was counted (Fig. 1, a and b; 2, a and b). Unstained sections (3 μ) were analyzed by fluorescence microscopy [7]. "Mineralizing osteoid seams" were defined as seams showing two distinct tetracycline bands (Fig. 3); seams with homogenous fluorescence ("diffuse staining") (Fig. 4) or with one band (slow mineralization with fusion of bands?) were counted as nonmineralizing seams. Since woven osteoid usually, though not invariably, fails to mineralize with a distinct mineralization front, osteoid recognizable as woven osteoid was omitted when mineralizing osteoid seams were counted. The fraction of seams showing a tetracycline double band (irrespective of its extension) was counted. The concepts underlying this technique have been discussed in more detail previously [7].

The normal range for mineralizing seams was defined by studying biopsy specimens of ten non-hyperparathyroid renal stone formers labelled with tetracycline. (Mineralization in this group appears not to be grossly abnormal, although normalcy of mineralization remains to be proven.)

Serum and urine chemistry. In fasting serum, Ca and P concentrations and alkaline phosphatase activity were measured by autoanalyzer. None of the patients had elevated serum transaminase values. Ionized serum Ca was measured with a flow-through electrode (Orion, model 99-20) with a digital voltmeter (model 701). Blood samples were obtained in Vacutainers under anaerobic conditions and measured according to the method of Lindgärde [9]. Commercial standards (Orion Co.) were used. The normal range was 2.30 ± 0.085 mEq/liter for female and 2.39 ± 0.085 mEq/liter for male individuals.

Urinary hydroxyproline was measured according to the method of Firschein and Schill [10].

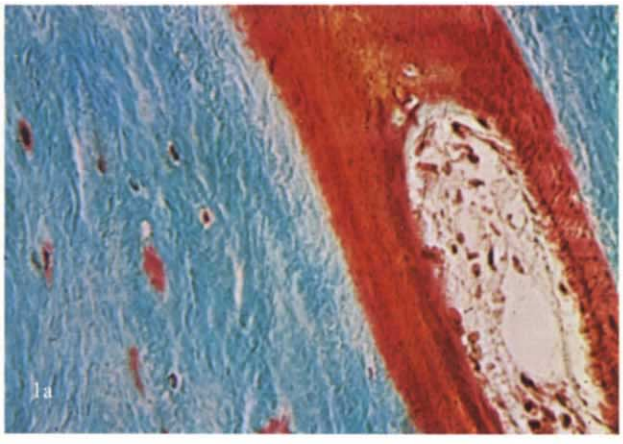
Bone phosphatase in serum was measured after electrophoretic separation on cellulose acetate (Siede G, et al: unpublished data). Parathyroid hormone (PTH) was measured with a guinea pig antibody against bovine PTH (normal range <220 pEq of bovine PTH/ml) by the method of Lequin, Hackeng and Schopman [11].

Fig. 1. a, *Undecalcified section of iliac crest spongiosa in a 40-year-old woman receiving maintenance hemodialysis (methylmetacrylate embedding, Masson-Goldner stain, ×304).* Note the presence of a broad osteoid seam as seen under nonpolarized light. b, *Same section under polarized light.* Note the presence of parallel lamellae in the osteoid seam. The regular spatial order of the lamellae reflects the supramolecular order of collagen fibers.

Fig. 2. a, *Undecalcified section of iliac crest spongiosa in a 21-year-old man receiving maintenance hemodialysis (methylmetacrylate embedding, ×195).* Under nonpolarized light, a broad unmineralized osteoid seam is seen in the right upper corner. In the center below, a broad zone is seen which also consists of nonmineralized osteoid. The osteoid of these two structures, staining bright red with the Masson-Goldner stain, cannot be differentiated under nonpolarized light. B, *Same section under polarized light.* In the osteoid seam in the upper right corner, well-delineated lamellae ("lamellar osteoid") can be recognized, whereas the osteoid in the center below exhibits a disordered "warp-and-woof" pattern (woven osteoid).

Fig. 3. *Undecalcified section of iliac crest spongiosa in a 23-year-old woman receiving maintenance hemodialysis (methylmetacrylate embedding, unstained section,* in vivo *tetracycline labelling, ×389).* Two distinct bands with yellow fluorescence are visible in the osteoid seam. The lower band corresponds to the first labelling period (2 days), the intermediate unstained band corresponds to the interval without labelling (12 days) and the broad seam on top corresponds to the second labelling period (4 days).

Fig. 4. *Undecalcified section of iliac crest spongiosa in a 40-year-old woman receiving maintenance hemodialysis (methylmetacrylate embedding, unstained section,* in vivo *tetracycline labelling, ×195).* The osteoid seams visible in this section show only "diffuse staining". This indicates that tetracycline is not actively incorporated into the mineralization front. Consequently, the two-band pattern (see Fig. 3) is not visible.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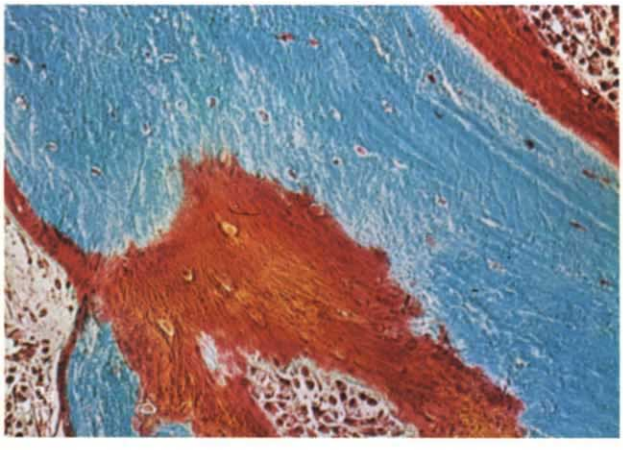
1b

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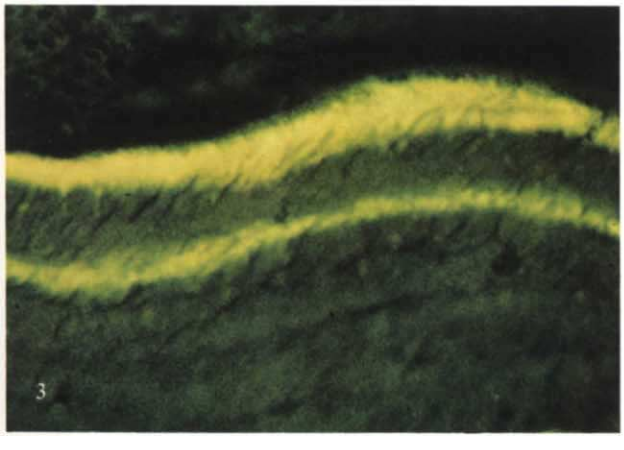
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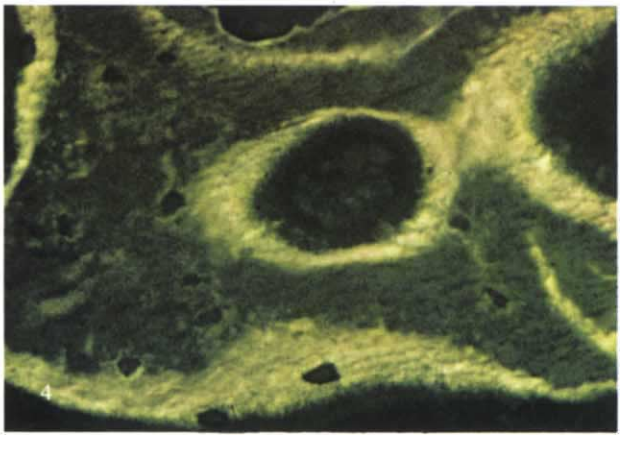
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Urinary Ca and P concentrations were measured after 24-hr urine collection under ambulatory conditions and without dietary restrictions. Completeness of collection was controlled by measuring urine creatinine. Creatinine clearance (urinary creatinine measured according to the method of Popper, Mandel and Mayer [12]) was used as a measure of GFR.

Statistical evaluation. The data were analyzed with a computer (PDP-11 Digital Equipment). Group differences were analyzed with Student's t test (the values conformed to a normal distribution). The significance of correlation was checked by calculating the t values.

Results

Bone histology (Table 1). *1) Osteosclerosis,* i.e., an increase of the fraction of spongiosal volume represented by mineralized bone (Vv), was seen first in individual patients with GFR <60 ml/min × 1.73 m². The 70th percentile of volumetric density was exceeded in more than 50% of the patients at a GFR of 40 ml/min × 1.73 m². At a GFR <20 ml/min × 1.73 m², the volumetric density was beyond the 95th percentile in 4 of 13 patients. The increase of Vv correlated with the accumulation of osteoid (Vo) (r = 0.43).

2) Accumulation of osteoid was demonstrated by measuring the extent of trabecular *surface* covered by *osteoid* seams (OS), the fraction of spongiosal *volume* represented by unmineralized bone matrix (Vo) and by calculating the average seam *thickness* (S). Osteoid increased as renal function deteriorated, i.e., the increase of OS correlated significantly with the fall of GFR (r = −0.6). In individual patients, values of OS beyond the 95th percentile were already seen at a GFR of 70 ml/min × 1.73 m²; at a GFR of 50 ml/min × 1.73 m², OS exceeded the 95th percentile in more than 50% of the patients. At a GFR <20 ml/min × 1.73 m², OS was beyond the 95th percentile in 12 of 13 patients.

Osteoid (OS) was correlated with osteoclastic surface resorption (HO) (r = 0.79) and endosteal fibrosis (EOF) (r = 0.69) pointing to the tight coupling between bone apposition and bone resorption. This correlation is the more surprising, since accumulation of osteoid, as a result of a mineralization defect, should tend to obscure the correlation between resorption indexes and osteoid.

The ratio of trabecular surface and trabecular volume (S/V) which is inversely related to trabecular diameter, correlated with osteoid seam thickness ($\bar{S}$) (r = −0.5) suggesting that trabeculae are coarse as a consequence of their osteoid coating [13]. Osteoid (OS) increased as serum PTH levels rose (r = 0.62). Since both OS and PTH rose as GFR fell, this correlation might merely be fortuitous; however, it might also reflect a rise of osteoid in the skeleton as turnover increases under the influence of PTH.

Osteoid volume (Vo) was loosely but significantly correlated with the degree of metabolic acidosis (r = −0.36).

3) Osteoclastic surface resorption (r = −0.6) increased and *endosteal fibrosis* appeared as GFR fell. In only 5 of 18 patients with a GFR>50 ml/min × 1.73 ², osteoclastic surface resorption was beyond the 70th percentile. Patients in whom osteoclastic surface resorption exceeded the 95th percentile were not seen before GFR fell below 50 ml/min × 1.73 m² and endosteal fibrosis did not appear before GFR had fallen below 30 ml/min × 1.73 m². Osteoclastic surface resorption was beyond the 95th percentile in more than 50% of the patients with a GFR of 40 ml/min × 1.73 m²; endosteal fibrosis was present in virtually all (24 of 27) patients with a GFR < 30 ml/min × 1.73 m². Although in early renal failure,

Table 1. Bone histology at various levels of glomerular filtration rate (GFR)[a,b]

GFR *ml/min × 1.73 m²*	Vv %	Vo %	OSF %	$\bar{S}$ μ	SD *mm²/cm³*	S/V *mm²/mm³*	L %	HO %	EOFS %
0-19 (N = 13)	24.7 ± 6.4	2.13 ± 1.71	51 ± 27	9.9 ± 4.2	3871 ± 511	14.6 ± 4.9	23.2 ± 4.4	4.5 ± 2.2	6.6 ± 4.9
20-30 (N = 15)	23.2 ± 6.1	2.54 ± 3.23	49 ± 21	12.0 ± 10.8	3726 ± 644	15.8 ± 3.5	21.8 ± 6.1	4.8 ± 3.6	5.0 ± 6.1
40-59 (N = 12)	21.0 ± 5.1	0.80 ± 0.60	22 ± 13	9.5 ± 4.2	3699 ± 534	16.3 ± 4.2	14.9 ± 5.3	1.8 ± 0.8	0
60-117 (N = 10)	17.0 ± 3.8	0.23 ± 0.23	14 ± 11	6.2 ± 3.3	2963 ± 504	18 ± 4.1	10.8 ± 4.2	1.1 ± 0.8	0
Normal controls (N = 22)	15.6 ± 8.8	0.13 ± 0.24	7.7 ± 12	6.4 ± 11	3124 ± 1394	20.6 ± 6.1	7.4 ± 7.6	0.74 ± 1.8	0

[a]Values are given as $\bar{X}$ ± SD except for normal controls where values are given as $\bar{X}$ ± 2 SD.
[b]Vv = volumetric density of bone; Vo = volumetric density of osteoid; OSF = osteoid surface fraction; $\bar{S}$ = osteoid seam thickness; SD = surface density; S/V = specific surface; L = lacunar surface fraction; HO = osteoclastic resorption surface; EOFS = endosteal fibrosis.

direct evidence of the action of PTH on the skeleton (i.e., increased osteoclast counts) was rather inconstant, *indirect evidence* of excess PTH activity was seen even when osteoclast counts were not yet outside of the normal range. These indirect signs were an increase of empty lacunae (L) on the trabecular surface (as evidence of past osteoclastic activity) and the presence of woven osteoid. Even in early renal insufficiency (GFR > 80 ml/min × 1.73 m²), woven osteoid with disturbed collagen texture was seen filling up former osteoclastic lacunae. The presence of woven osteoid is indicative of precipitous synthesis of bone matrix by osteoblasts that are under the influence of excessive PTH concentrations.

Endosteal fibrosis (EOF) correlated better than any other index with PTH ($r = 0.76$). This is in good agreement with findings of Duursma et al [14] and Mehls et al [15]. The correlation between HO and PTH was much better when osteoclastic resorption was related to nonosteoid surface, i.e., mineralized trabecular surface ($r = 0.63$), than when osteoclastic resorption was related to total trabecular surface ($r = 0.42$). This indicates that under the stimulus of PTH the appearance of osteoclasts is related to the extension of mineralized rather than total trabecular surface. This would support the concept that high local Ca concentrations (which osteoid is unable to provide) are necessary to initiate osteoclastic resorption [16].

The increase of the fraction of nonosteoid trabecular surface covered by osteoclastic resorption (HO) showed some correlation with the fall of ionized serum Ca ($r = -0.44$). Obviously, osteoclastic surface resorption is a PTH-mediated homeostatic effort to bring serum Ca levels back into the normal range.

In patients with renal insufficiency, *specific surface* —i.e., the ratio of trabecular surface to trabecular volume (S/V)—was diminished, indicating an increase of trabecular diameter. This finding is of interest in view of the well-known coarse trabecular structure that is commonly seen in skeletal roentgenograms.

4) Woven osteoid (Fig. 5). Woven osteoid, defined by disordered collagen texture and by its characteristic pattern of birefringence, was seen even in some patients with GFR≧80 ml/min × 1.73 m². The fraction of osteoid seams consisting of woven osteoid rose as GFR fell. With falling GFR the extension of woven osteoid over the trabecular surface rose progressively: at a GFR > 40 ml/min × 1.73 m², woven osteoid was only seen filling up circumscribed lacunae, whereas broad apposition fronts were always of the lamellar variety. Broad osteoid seams of woven collagen texture that extended over a considerable fraction of the trabecular surface were not found

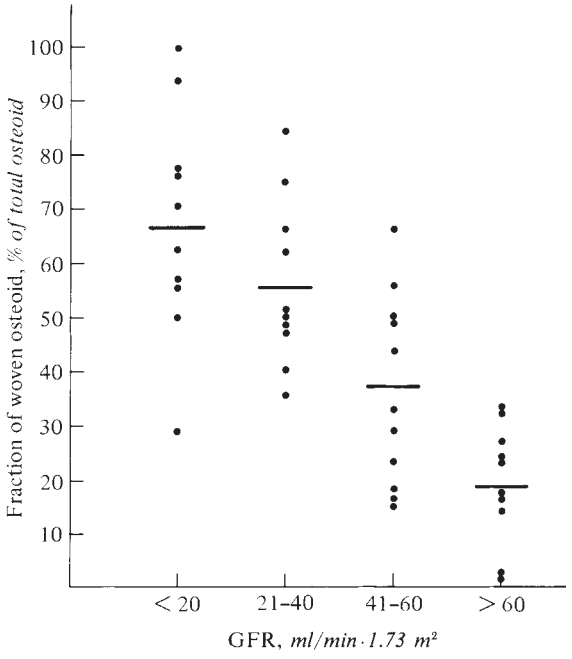

Fig. 5. *Prevalence of woven osteoid at various levels of GFR.*

before GFR had dropped below approximately 30 ml/min × 1.73 m².

5) Mineralization defect (Fig. 6). Although individual patients with GFR over 40 ml/min × 1.73 m² had an increased fraction of nonlabelled osteoid seams, patients with a severe mineralization defect were not seen before GFR had fallen below 40 ml/min × 1.73 m². Yet, it is remarkable that even in advanced renal failure, mineralization may be perfectly normal, when evaluated with the tetracycline double-labelling technique. Due to the limited number of observations, it cannot be decided whether there is a correlation between GFR and incidence of a mineralization defect.

6) Abnormal histology with normal serum PTH concentrations. In 5 of 16 patients with normal serum PTH (<220 pEq of bovine PTH/ml) with GFR < 70 ml/min × 1.73 m², increased numbers of osteoclasts were counted. Although technical or methodological problems with PTH measurements can certainly not be excluded, the finding may also indicate that single PTH measurements are not representative for average serum PTH activity.

7) Correlation between renal pathology and bone histology. There was no recognizable correlation between the nature of renal disease and the severity of histologic lesions. It is our personal impression that at any given level of GFR, bone disease is more pronounced in patients with analgesic abuse; because of the composition of the patient group in this study,

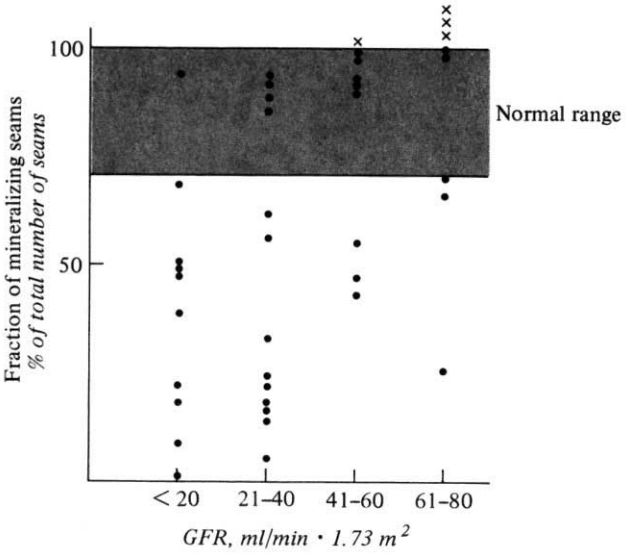

Fig. 6. *Fraction of mineralizing osteoid seams (tetracycline labelling) at various levels of GFR.* (× = no fronts.)

the hypothesis, unfortunately, could not be tested directly.

Serum biochemistry as an indicator of bone histology. 1) Serum calcium. As had been found by Duursma et al [14] and Ritz et al [17], total serum Ca ($r = -0.64$) and ionized serum Ca ($r = -0.69$) fell as the amount of osteoid (Vo) in the skeleton increased. A similar correlation between serum Ca was also found for surface fraction of osteoid (OSF) and mean osteoid seam thickness ($\bar{S}$). Osteoclastic surface resorption increased as ionized serum Ca decreased ($r = -0.44$).

2) Serum alkaline phosphatase. Although serum alkaline phosphatase and specific bone phosphatase were closely correlated ($r = 0.94$), the discrimination between normal and abnormal bone histology was better with the specific bone phosphatase. In patients with abnormal bone histology, total bone alkaline phosphatase was abnormal in 16% of the cases and specific bone phosphatase was abnormal in 77% of the cases. Both phosphatase values correlated equally well with bone histology: the osteoblastic enzyme alkaline phosphatase correlated not only with histologic indicators of bone apposition—i.e., osteoid volume ($r = 0.76$) and osteoid surface ($r = 0.53$)—but also with histologic markers of bone resorption—i.e., with HO ($r = 0.36$)—and endosteal fibrosis ($r = 0.58$). This is presumably due to the fact that in bone remodelling, apposition and resorption are coupled processes.

Serum alkaline phosphatase correlated better with total osteoid surface ($r = 0.53$) than with the surface fraction of active osteoid, i.e., osteoid covered with osteoblasts ($r = 0.3$). This discrepancy may be related to the difficulty of identifying active osteoblasts in the presence of woven osteoid, thus resulting in a large variance for this index (figures therefore not given in Table 1). It is also conceivable that nonendosteal cells (i.e., osteocytes) in woven bone may secrete alkaline phosphatase and thus vitiate the correlation.

3) Urinary Ca excretion. As GFR fell, urinary Ca excretion decreased ($r = 0.44$). As shown by Lichtwitz and Parlier [18], a low urinary Ca concentration was found even with very modest reductions of GFR.

4) Urinary hydroxyproline. Total urinary hydroxyproline, an indicator of bone matrix turnover, did not correlate at all with bone histology.

5) Metabolic acidosis. As found previously by Cochran and Nordin [19], arterial pH correlated slightly but significantly ($r = -0.36$) with osteoid volume (Vo). One need not necessarily accept their [19] conclusion, that metabolic acidosis is detrimental to osteoid mineralization. The correlation may be fortuitous, since in advanced renal failure, patients tend to be in metabolic acidosis and to have more osteoid at the same time; alternatively, the correlation may reflect the well-known [20] tendency of hyperparathyroid subjects to be in metabolic acidosis.

6) Serum PTH concentrations. Fasting serum PTH concentrations were increased (>220 pEq of bovine PTH/ml) in 1 of 7 patients with GFR > 70 ml/min × 1.73 M^2 and in 26 of 42 patients with GFR < 70 ml/min × 1.73 m^2. Serum PTH increased exponentially with decreasing GFR ($r = -0.61$). An inverse relationship between ionized serum Ca and serum PTH was found ($r = -0.54$).

Serum PTH correlated with osteoid volume ($r = 0.45$) and osteoid surface ($r = 0.61$), with osteoclastic resorption ($r = 0.63$) and even better with endosteal fibrosis ($r = 0.76$).

Discussion

Reiss, Canterbury and Kanter [21] were the first to demonstrate elevated serum PTH concentrations in incipient renal failure. The present study supplements their data by showing that even in very early renal failure, evidence of parathyroid overactivity can be demonstrated in the skeleton. The number of osteoclasts becomes abnormal only in advanced renal failure, presumably because their measurement is subject to rather large statistical error [22]. Alternatively, diminished responsiveness of the skeleton to the effect of PTH may be present [23, 24]. In spite of the absence of frankly increased numbers of osteoclasts, the accumulation of empty resorption cavities as well as the appearance of woven osteoid even

in the earliest stages of renal insufficiency point to a stimulatory effect of PTH in the skeleton. Since the presence of woven osteoid is evidence of accelerated deposition of bone, the presence of empty lacunae is unlikely to be the consequence of delayed filling of lacunae by osteoblasts.

Parathyroid hormone activates mesenchymal cells on the trabecular surface and induces remodelling processes of sequential character, bone apposition in each remodelling site being preceded by bone resorption. Consequently, both resorption (HO) and apposition (Vo, OS) indexes are elevated in advancing renal failure secondary to the rise of PTH. Presumably, more intense stimulation by PTH is required for endosteal fibrosis, since it does not appear before GFR is markedly lowered and PTH concentrations are concomitantly elevated. Consequently, endosteal fibrosis is an indicator of more advanced osteitis fibrosa.

Osteoblasts, which are under the influence of PTH, no longer deposit collagen in ordered lamellae with regular spatial orientation of collagen fibers, but in an irregularly disordered "woven" pattern which can be visualized under polarized light. As noted by Binswanger et al [3] in a smaller series, woven osteoid appears quite early in renal failure. Its appearance accounts for several characteristic features of renal osteodystrophy: nontrajectorial structure of bone on a macroscopic level is caused by the loss of spatial orientation of collagen fibers; structural insufficiency (fractures; epiphyseal slipping) is the result of inferior biomechanical qualities of woven bone [25] and undermineralization of the skeleton is primarily the consequence of incomplete mineralization of woven bone.

Defective mineralization (i.e., osteomalacia), once postulated to precede the development of osteitis fibrosa [26], is not consistently demonstrable in renal failure when the *in vivo* tetracycline labelling technique is used. However, this technique does present problems in renal bone disease with high bone turnover. Deposition of mineral in a discrete mineralization front and a defined temporal relationship between bone matrix deposition and bone matrix mineralization are both prerequisites for the tetracycline double-labelling technique [7]. These prerequisites, unequivocally shown in lamellar bone, may no longer hold true in woven bone. Scanning electron microscopy studies have shown that lamellar or woven collagen texture are not mutually exclusive [27]. Rather, in uremic individuals collagen texture may show the complete spectrum of deterioration from the regular spatial order of lamellar bone to the complete structural anarchy of woven bone. Woven osteoid does circumvent the mineralization block of uremia and mineralizes, although incompletely, even in the absence of vitamin D [28]. This might be one explanation for the absence of a demonstrable mineralization defect in a certain number of the patients examined. However, ultrastructural studies are required before a mineralization defect can definitively be excluded in those patients in whom mineralization was found to be normal with the *in vivo* labelling technique.

If mineralization were truly normal in some patients, this would imply that renal insufficiency must be associated with additional factors of unknown nature for osteomalacia to occur.

Acknowledgments

This report is dedicated to Professor W. Rotter (Frankfurt) on the occasion of his 65th birthday. The results of this study were presented in part at the XIth Conference of the European Dialysis and Transplant Association, Tel Aviv (November 2-7, 1974) and at the International Symposium on Vitamin D, Wiesbaden (October 28-31, 1974). The study was carried out with the support of "Deutsche Forschungsgemeinschraft". Dipl. Chem. Mr. G. Krause (Frankfurt/Main) performed the determination of urinary hydroxyproline, and Dr. W. Hackeng (Bergwegziekenhuis, Rotterdam), the determination of serum parathyroid hormone. Ms. H. Tomaskowitz (Frankfurt/Main) provided skillful technical assistance.

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