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Osteopathy in Maintenance Hemodialysis

Micromorphometric and Microradiographic Studies with Correlations to Serum Parathyroid Hormone and Calcitonin Levels *

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Summary. 50 iliac crest biopsy specimens taken from hemodialyzed uremic patients (average duration of dialysis 16.7±12.3 months), 20 specimens from patients in terminal renal failure and 20 specimens from healthy controls were analyzed by micromorphometry of undecalcified microtome sections. In addition undecalcified ground sections were studied by microradiography. None of the biopsy specimens of dialyzed and non-dialyzed uremic patients showed entirely normal bone histology. The osteopathy was characterized in both uremic groups by secondary hyperparathyroidism with increased cellular activity, endosteal fibrosis and appearance of woven osteoid and by osteomalacia. Micromorphometry revealed no differences between uremic patients with and without hemodialysis: volumetric density, the fraction of total bone volume represented by osteoid, the fraction of trabecular surface covered by osteoid and the mean osteoid seam thickness were increased; the fraction of osteoid covered by osteoblasts remained unchanged when compared with normal controls, whereas the fraction of trabecular surface covered by active Howship's lacunae was elevated. The specific surface of trabecular bone was unaltered. Microradiography showed marked periosteocytic osteolysis, abundance of low density zones (particularly in dialyzed patients) and microheterogeneity of bone structure.

There was a good degree of correlation between the increased amounts of osteoid and active Howhsip's lacunae and increased area of osteocytic lacunae and increased serum parathyroid hormone levels. There was no correlation between serum clacitonin levels and micromorphometric parameters. Fibro-osteoclasia and osteomalacia apparently did not progress with continued dialysis.

Zusammenfassung. Unentkalkte Schnitte und Schliffe der Beckenkammspongiosa von 50 Patienten unter Langzeithämodialyse (durchschnittliche Dialysedauer 16.6±12.3 Monate), von 20 Patienten mit terminaler Niereninsuffizienz ohne Hämodialyse und von 20 skeletgesunden Patienten wurden mikromorphometrisch und mikroradiographisch untersucht.

Keine der Beckenkammbiopsien urämischer Patienten mit und ohne Hämodialyse zeigte einen normalen Skeletbefund. Die Skeletveränderungen waren durch einen sekundären Hyperparathyreoidismus mit gesteigerter Zellaktivität, Endostfibrose und Auftreten von atypischem Faserosteoid sowie durch eine Osteomalacie geprägt. Die mikromorphometrische Analyse ergab zwischen urämischen Patienten mit und ohne Hämodialyse keinen Unterschied: Volumetrische Dichte, Osteoidsaumlänge und mittlere Osteoidsaumbreite waren gesteigert; der Anteil der mit Osteoblasten bedeckten osteoiden Säume am Gesamtosteoid war gegenüber skeletgesunden Kontrollen nicht verändert. Die Zahl aktiver Howshipscher Lakunen war beträchtlich vergrößert. Die spezifische Oberfläche der Trabekel blieb unverändert. Die Mikroradiographie zeigte periosteocytäre Osteolyse, Mineralisationsdefekte (vor allem bei Dialysepatienten) und eine starke Mikroheterogenität der Knochenstruktur.

Die Serum-Parat-Hormonspiegel waren bei Dialysepatienten ausnahmslos erhöht. Osteoidmenge, Anzahl der aktiven Howshipschen Lakunen und Größe der Osteocytenlakunen korrelierten mit der Höhe der Serum-Parat-Hormonspiegel. Die Serum-Calcitonin-Spiegel lagen bei

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Dialysepatienten im Normbereich. Zwischen morphometrischen Daten und der Höhe der Serum-Calcitonin-Spiegel ergab sich keine Korrelation.

Dissezierende Fibrosteoklasie (Ostitis fibrosa) und Osteomalacie erfuhren mit zunehmender Dialysedauer keine Veränderung.

Chronic renal failure is associated with a peculiar form of bone disease, the pathogenesis of which has not been completely clarified so far (Kleeman *et al.*, 1969; Schaefer and Opitz, 1970; Ritz *et al.*, 1971). Since the lifes of patients in terminal renal insufficiency can now be prolonged by maintenance hemodialysis and renal homotransplantation the problem of uremic bone disease has attracted considerable interest and remains one of the major unsolved problems of maintenance hemodialysis.

The histological changes that are found in the skeleton of uremic patients have been well described by Follis and Jackson (1943), Ammann (1961) and by Garner and Ball (1966). According to these authors uremic osteopathy is characterized by both osteomalacia and osteitis fibrosa. In our own histomorphometric studies we were able to confirm these findings by quantitative measurements (Krempien *et al.*, 1971).

There have been conflicting reports on the course of uremic osteopathy under maintenance hemodialysis. Both improvement (Curtis *et al.*, 1968; Ritz *et al.*, 1968) and worsening (Pendras and Ericson, 1965) have been described. It was the purpose of the following study, to clarify the natural history of dialysis bone disease and to identify some pathogenetical factors. Quantitative measurements of the histological changes in iliac crest spongiosa of patients in terminal renal failure were compared with those obtained from uremic patients under maintenance hemodialysis of various duration and with those obtained from normal controls. Measurements of parathyroid hormone levels and serum calcitonin levels were performed to find a possible correlation between these hormone levels and micromorphometric data of bone disease. The clinical and roentgenological findings of this study have been reported elsewhere (Ritz *et al.*, 1971).

Material and Methods

Iliac crest specimens were obtained from 20 healthy victims of traffic accidents within 5 h after clinical death (bedridden for less than 3 days), from 20 patients in terminal renal failure without hemodialysis (serum creatinine >7 mg-%) and from all ($n=50$) patients in 3 German hemodialysis centers, who were not on anticoagulants (duration of dialysis <1 month—more than 3 years). The ages of the patients ranged from 20 to 55 years. Biopsies were performed with an electric drill ("Myelotomieinstrumentarium" after Burkhardt, 1966) 3 cm behind the spina anterior in a perpendicular direction. The length of the spongiosa cylinder was approximately 2 cm. All specimens were fixed in 70% ethanol, stained in 5% basic fuchsin, dehydrated and defatted and embedded in methylmetacrylate according to Schenk (1965). Undecalcified microtome sections (5 μ) were stained after Masson-Goldner (modification of Schenk *et al.*, 1966) and with von Kossa stain. 4 sections of every iliac crest specimen taken from different depths of the spongiosa cylinder were evaluated independently by two observers (20 areas with 720 counting points, enlargement 1:80, Merz, 1967). The spongiosa near the corticalis was not taken into account. The following parameters were measured (Schenk, 1967; Merz and Schenk, 1970):

1. *volumetric density (Vv)*—fraction of total spongiosa volume (%) represented by bone matrix volume;
2. *osteoid volume (VO)*—fraction of total spongiosa volume (%) represented by osteoid;
3. *osteoid volume (VOb)*—fraction of bone matrix volume (%) represented by osteoid:

4. *osteoid surface (OS)*—fraction of total trabecular surface (%) represented by osteoid;
5. *active osteoid (OSa)*—fraction of surface osteoid (%) covered by osteoblasts;
6. *mean osteoid seam thickness* (μ);
7. *active Howship's lacunae (HO)*—fraction of trabecular surface (%) covered by osteoclasts;
8. *specific surface (S/V)*—relation of bone surface to bone volume (mm/mm^2).

Contact microradiographs of 50–70 μ ground sections were made by a CMR 5 Philips X-ray tube according to Heuck (1966)[1]. In these microradiographs we measured the two mean diameters of 200 elliptical "osteocytic holes", represented by osteocytic lacunae and periosteacytic halos. The area was evaluated in the following way: $\mu^2 = a \times b \times \pi$.

Measurements of serum parathyroid hormone (PTH) and serum calcitonin (CT) were done after Schopman *et al.* (1970)[2]. Details of the protocol of the clinical study have been reported elsewhere (Ritz *et al.*, 1971).

All data are given as mean±standard deviation. The significance of differences between groups was evaluated by the Wilcoxon test.

Results

1. Histological Findings. There was no obvious difference of bone histology between uremic patients with and without maintenance hemodialysis. None of the iliac crest biopsy specimens from both uremic groups showed completely normal bone histology. The most prominent changes observed were an increase of the fraction of the trabecular surface covered by osteoid and an increase of the thickness of osteoid seams (Figs. 1–3). The front of calcification, that was clearly delinated in normal controls, appeared blurred and indistinct in uremic patients. In addition to lamellar osteoid, which appeared birefringent under polarizing light due to a regular orientation of collagen fibers, there were various amounts of woven osteoid which irregularly orientated collagen fibers (Fig. 1a–c). Woven osteoid often showed speckled mineralization. The amount of active osteoid-covered by an almost epitheloid layer of cuboidal or more fusiform osteoblasts—and the amount of inactive resting osteoid seams without an osteoblastic layer were increased (Figs. 2 and 3). A rather typical finding in the uremic skeleton was buried osteoid, covered by mineralized bone (Fig. 4). Marrow fibrosis with replacement of reticular fibers by collagen fibers is rarely marked. Diffuse marrow fibrosis, which is not unusual in primary hyperparathyroidism, is encountered only in the occasional patient with full blown renal osteitis fibrosa (so called tertiary hyperparathyroidism, Fig. 1a–c). Usually there was only a fibrous thickening of the endosteum with a few layers of coarse collagen fibers parallel to the trabecular surface (Fig. 3 and 4). Foci of more marked fibrosis are usually found over osteoblasts and osteoclasts (Fig. 2). The trabecular surface showed shallow erosions by foci of multinucleated osteoclasts (active Howship's lacunae, Fig. 5). These osteoclasts were mostly of modest size and contained only few nuclei. Central trabecular dissections by coni of osteoclasts were less frequently observed. Since osteoid is resistant to osteoclastic destruction osteoid spurs, overlying resorption cavities were often found (Fig. 4).

Osteocytic lacunae were mostly enlarged and often surrounded by broad non-mineralized zones staining like osteoid (Fig. 3). The number of large osteocytes with round or bean shaped nuclei, loose chromatin and broad cytoplasmatic

1 We thank Prof. Dr. F. Heuck, Katharinenhospital Stuttgart, for technical help and advise.
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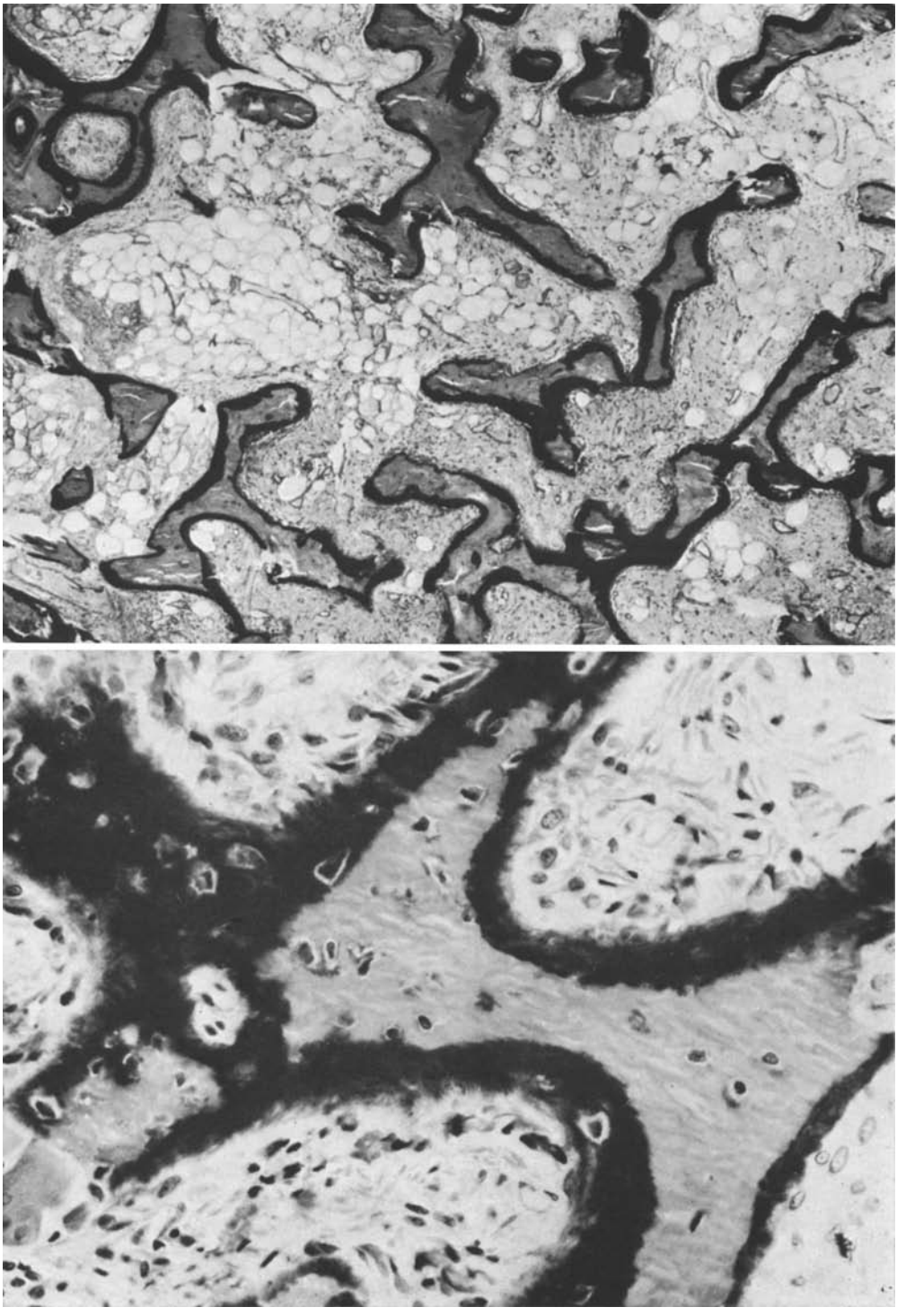


Fig. 1a and b

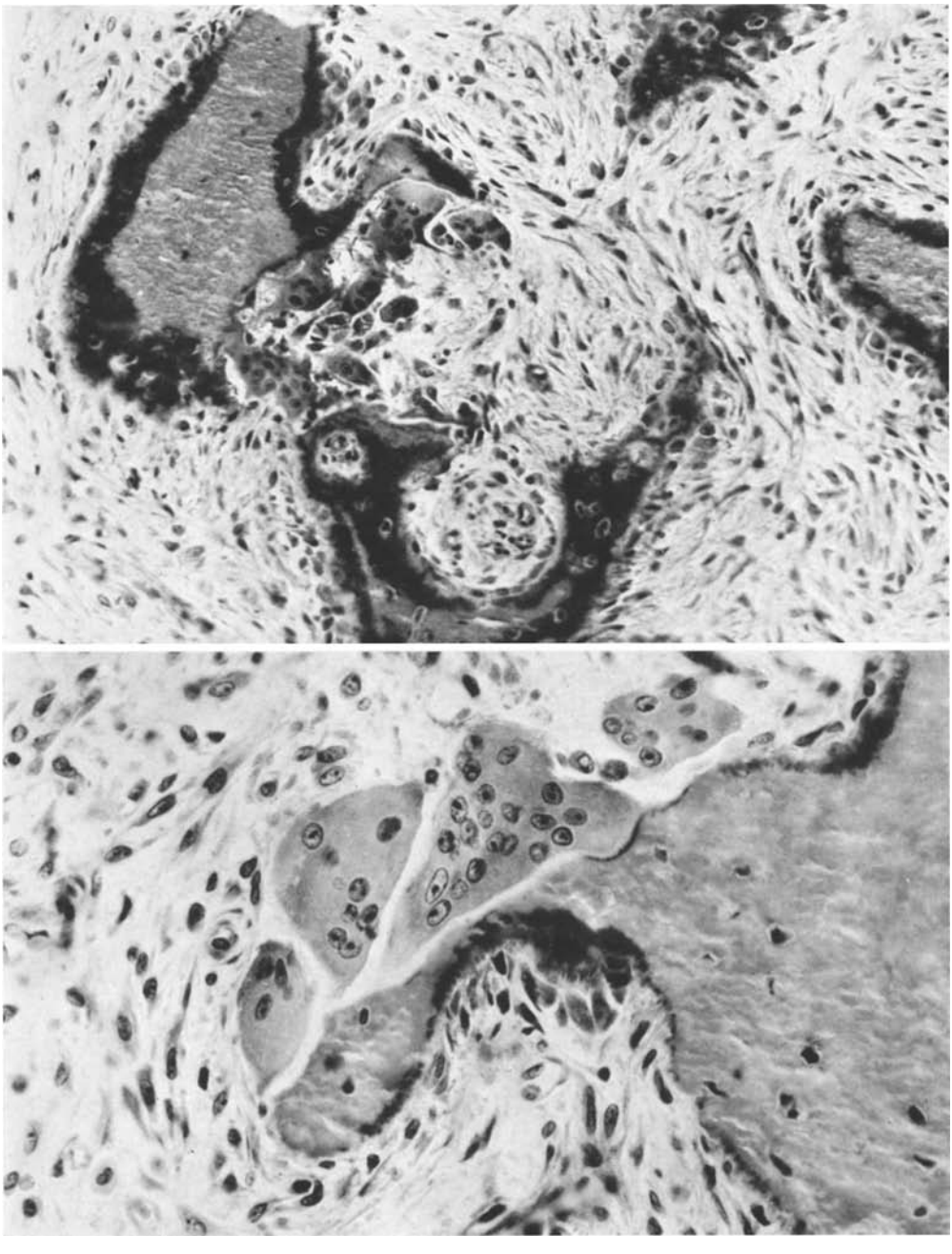
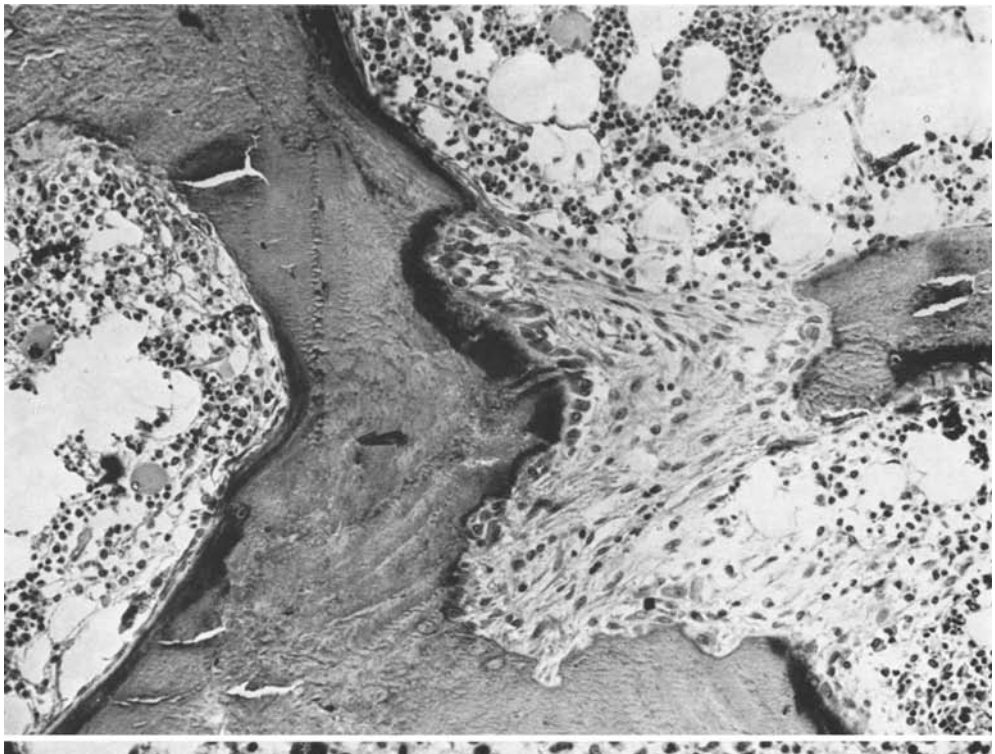


Fig. 1a—d. Iliac crest spongiosa. 35 y. old woman. Chronic renal insufficiency without hemodialysis. a Severe renal osteopathy with marked marrow fibrosis, coarse trabeculae and enlarged osteoid seams. Enl. 1:32. b Atypical woven bone and osteoid with large osteocytes and high osteocytic density. Enl. 1:250. c Marrow fibrosis with dense cellularity and metablastic bone formation Enl. 1:150. d Large multinucleated osteoclasts and immature "fibroblastic" osteoblasts. Enl. 1:180. Microphotographs. Methylmetacrylate. Masson-Goldner stain



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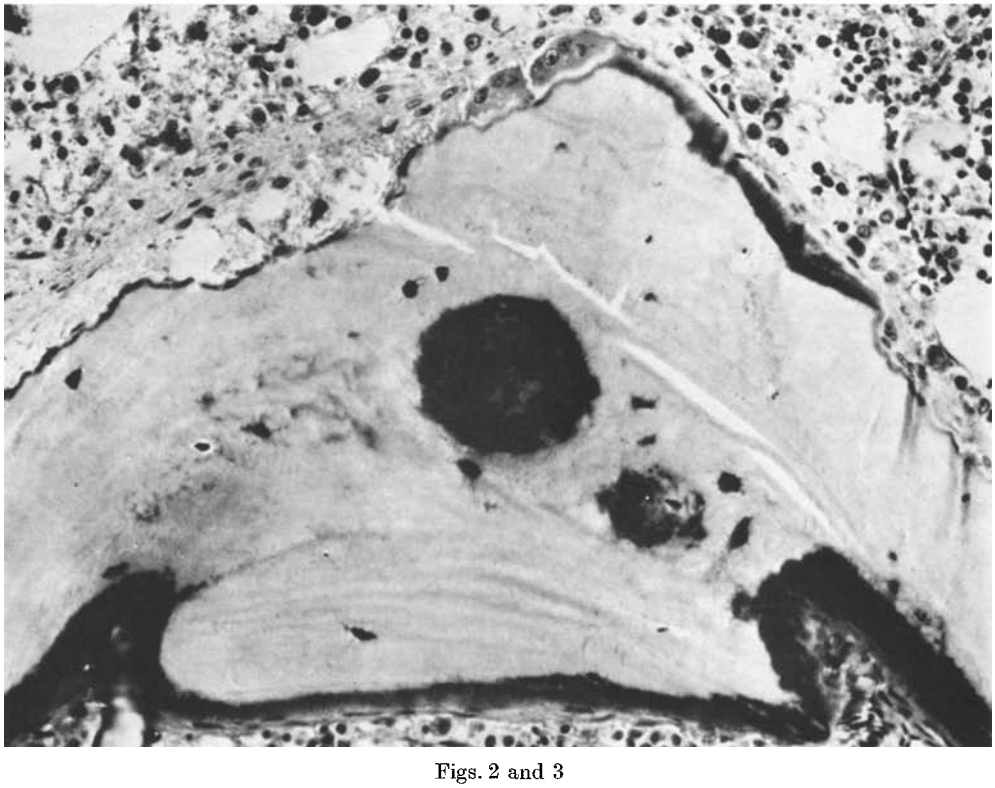

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Figs. 2 and 3

seams and the number of empty lacunae was abnormally high. Although the shape of spongiosa trabeculae may be bizarre in excessive renal osteitis fibrosa, usually trabeculae were coarse and thick (Fig. 1 and 2). In severe cases of azotemic osteopathy, both with and without hemodialysis, there were occasional foci of metablastic bone formation in the marrow spaces.

2. Micromorphometric Measurements. All micromorphometric measurements are given in Table 1 and 2. Volumetric density (Vv), i.e. the fraction of total spongiosa volume which is represented by bone matrix volume is increased in uremic

Table 1. Micromorphometric measurements

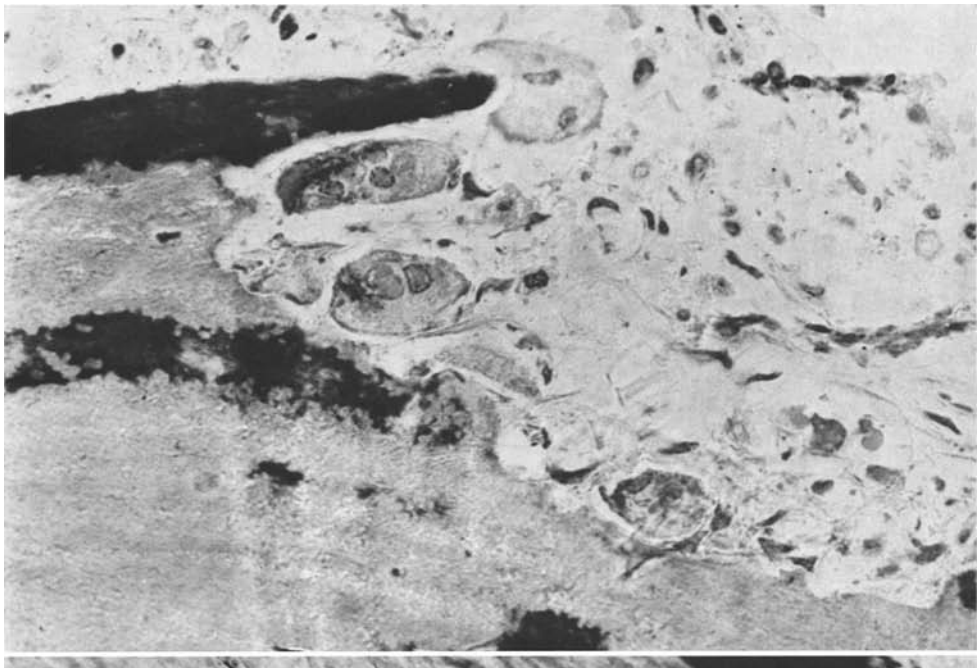
	Control $n = 20$	Uraemic (non dialysed) $n = 20$	Uraemic (dialysed) $n = 50$
Volumetric density (V_v)	26.0 ± 7.11	31.5 ± 7.3	32.0 ± 9.37
Osteoid volume (V_o)	0.841 ± 0.739	4.62 ± 0.793	5.19 ± 5.51
Osteoid volume (V_{ob})	3.09 ± 2.26	15.4 ± 12.7	15.8 ± 16.1
Osteoid surface (OS)	18.0 ± 8.8	51.4 ± 22.7	52.0 ± 24.3
Active osteoid (OS_a)	20.3 ± 20.4	28.9 ± 20.6	37.8 ± 24.5
Active Howship's lacunae (HO)	1.90 ± 1.67	8.03 ± 5.50	8.84 ± 4.76
Mean thickness of osteoid seams (μ)	9.54 ± 4.41	16.6 ± 7.2	18.7 ± 13.3
Specific surface (S/V)	17.3 ± 6.19	17.4 ± 6.54	19.0 ± 5.81

Table 2. Micromorphometric measurements: duration of dialysis

	<1 year $n = 20$	1–3 years $n = 23$	>3 years $n = 7$
Volumetric density (V_o)	30.0 ± 10.7	34.9 ± 9.29	27.1 ± 2.64
Osteoid volume (V_o)	4.82 ± 4.74	6.22 ± 6.40	2.41 ± 2.1
Osteoid volume (V_{ob})	15.3 ± 14.2	18.1 ± 18.3	8.79 ± 7.51
Osteoid surface (OS)	55.0 ± 25.9	54.3 ± 24.0	38.4 ± 20.5
Active osteoid (OS_a)	35.2 ± 34.4	30.9 ± 15.9	49.5 ± 26.5
Active Howship's lacunae (HO)	8.95 ± 5.78	7.97 ± 4.16	10.9 ± 4.97
Mean thickness of osteoid seams (μ)	14.3 ± 9.0	20.5 ± 13.5	13.1 ± 7.68
Specific surface (S/V)	20.0 ± 6.68	18.3 ± 5.82	18.7 ± 3.02

Fig. 2. Iliac crest biopsy, 35 y. old man, maintenance hemodialysis since 2 years. Focal marrow fibrosis overlying resorption cavities and active osteoid seams. Methylmetacrylate. Masson-Goldner stain. Microphotograph. Enl. 1:70

Fig. 3. Iliac crest biopsy, 25 y. old woman, maintenance hemodialysis since 8 months. Slight endosteal fibrosis and "activated" osteocytes surrounded by bone matrix stained like osteoid. Methylmetacrylate. Masson-Goldner stain. Microphotograph. Enl. 1:250



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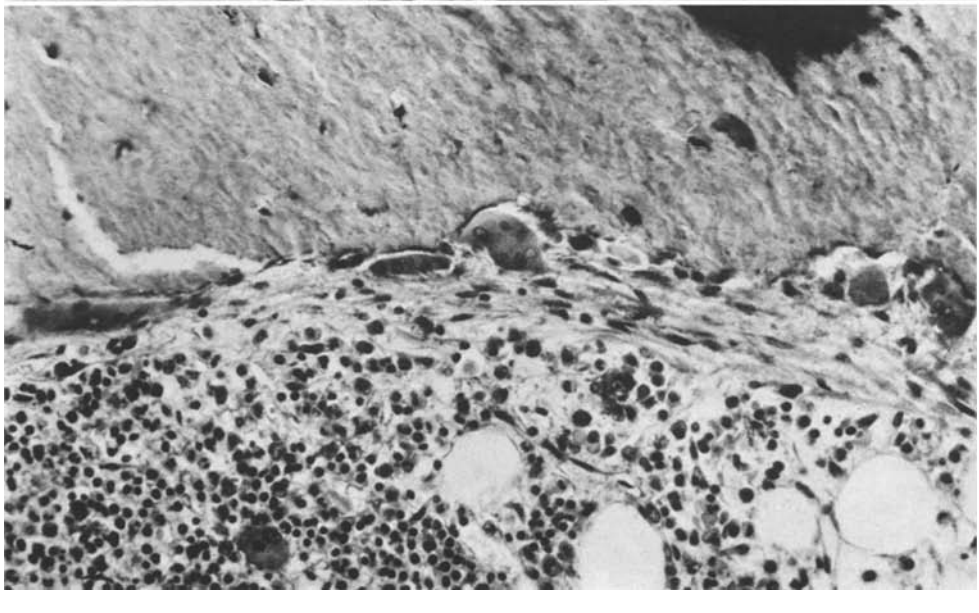

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Fig. 4. Iliac crest biopsy, 55 y. old man. Chronic renal insufficiency without dialysis. Burried osteoid and osteoid spur overlying dissecting bone destruction by multinucleated osteoclasts. Methylmetacrylate. Masson-Goldner stain. Microphotograph. Enl. 1:200

Fig. 5. Iliac crest spongiosa. 41 y. old woman, maintenance hemodialysis since 2 years. Trabecular surface covered by numerous active Howship's lacunae with small osteoclasts and endosteal fibrosis. Methylmetacrylate. Masson-Goldner stain. Microphotograph. Enl. 1:160

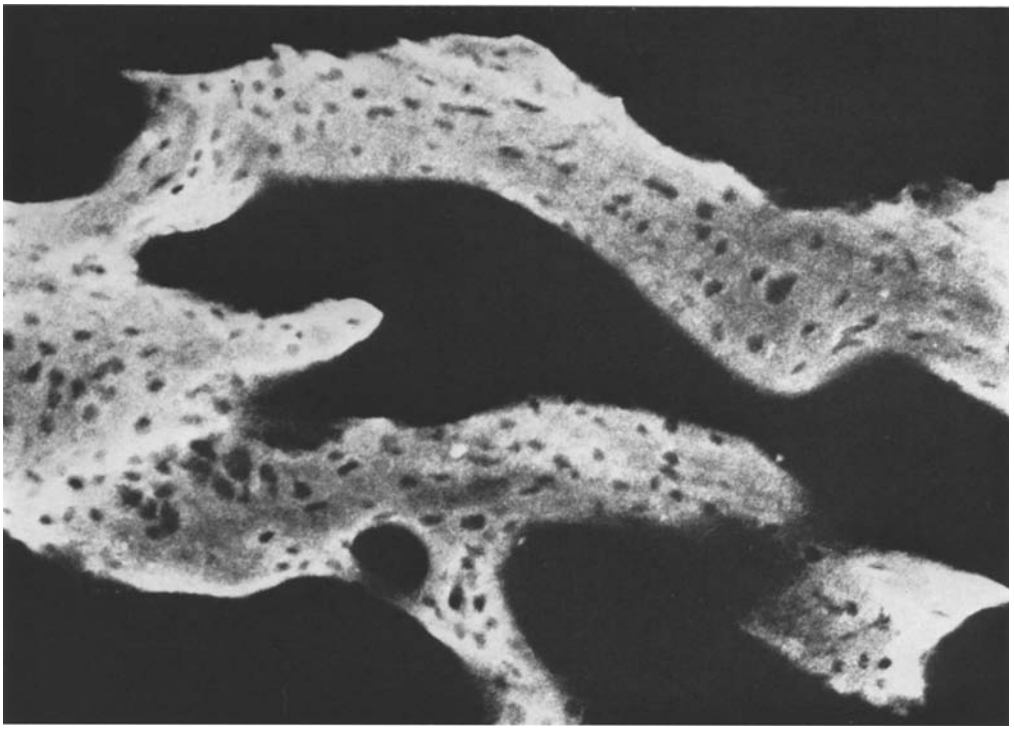

Fig. 6. Microradiography of iliac crest spongiosa. 30 y. old man with maintenance hemodialysis since 2 years. Marked inhomogeneity of bone with periosteocytic osteolysis and low and high density areas of surrounding bone matrix. Blurred outline and scalloping of trabeculae. Undecalcified ground section, 50 μ. Microphotograph. Enl. 1:160

patients both with and without hemodialysis. There is an increase both of the fraction of total spongiosa volume (VO) and of the fraction of bone matrix volume (VOb) represented by unmineralized osteoid. In addition the fraction of trabecular surface covered by osteoid seams (OS) was markedly increased. The mean thickness of osteoid seams was clearly elevated in uremic patients both with and without hemodialysis. The percentage of osteoid seams covered by osteoblasts (active osteoid, OSa) was slightly but not significantly increased both in uremia and under maintenance hemodialysis when compared with normal controls. However, since the absolute amount of osteoid was higher in uremic patients, the absolute amount of active osteoid was increased likewise. An elevation of the number of osteoclastic resorption centers was evident from the finding of an increase of the fraction of trabecular surface that was covered by osteoclasts (active Howship's lacunae, HO). The specific surface (S/V), that reflects the complexity of the structure of bone trabeculae, was unchanged in uremic patients both with and without hemodialysis when compared with non uremic control subjects.

3. Microradiographic Findings. In contrast to the well defined outline of trabeculae in normal bone the outline of uremic bone was usually indistinct and blurred (Fig. 6). The fraction of the trabecular surface covered by Howship's

Table 3. Relation of serum PTH levels and micro-morphometric bone parameters in dialysed patients

	Serum PTH(pgEq bovine PTH/ml)		
	400–800 $n=12$	800–2000 $n=14$	>2000 $n=7$
Volumetric density (V_v)	35.2 ± 14.4	33.9 ± 6.4	33.1 ± 6.8
Specific surface (S/V)	17.7 ± 5.97	18.1 ± 4.78	19.6 ± 9.49
Osteoid volume (V_o)	2.44 ± 2.91	7.58 ± 7.8	8.09 ± 6.23
Osteoid surface (OS)	37.1 ± 24.4	55.9 ± 24.1	52.9 ± 27.3
Active Howship's lacunae (HO)	4.75 ± 3.64	9.79 ± 4.65	6.46 ± 2.44
Osteocytic area (μ^2)	—	385 ± 57.1	588 ± 115

lacunae was markedly increased. The periosteocytic lacunae were enlarged (Fig. 6). The trabeculae showed marked microheterogeneity by skeletal remodelling. In cases with severe osteopathy a bizarre deformation of trabecular structure was encountered. The same changes that had been observed in patients in terminal renal failure, were also seen in patients on maintenance hemodialysis. In contrast to non dialysed patients, there seemed to be an increase of low mineral density zones, which were localized predominantly in areas close to enlarged osteocytic lacunae (Fig. 6). These findings were not quantitated however.

4. Correlation between Micromorphometric Data and Serum PTH, Serum CT, Efficiency of Dialysis and Duration of Dialysis. Serum PTH levels were markedly elevated in our uremic dialysed patients (1268±736 pgEq bovine PTH/ml; normal 400 pgEq/ml, Table 3).

There was a correlation between serum PTH levels (x=pgEq bovine PTH/ml) and

1. osteoid volume Vo (y=% bone volume)

$$y=15.9+0.00158\ (x-1377);\ p<0.05.$$

2. osteocytic area ($y=\mu^2$)

$$y=413+0.102\ (x-1411);\ p<0.05.$$

3. active Howship's lacunae HO (y=% surface)

$$y=7.8+0.00656\ (x-1295);\ p<0.1.$$

Serum CT levels of dialysed patients were in the normal range (285±11.3 pg HCT/ml; normal 395 pg HCT/ml). Micromorphometric parameters were not correlated to serum CT levels and to efficiency of dialysis (predialytic creatinine levels). We found no correlation between micromorphometric data and duration of dialysis (Table 2).

Discussion

The skeletal changes in uremia have been thoroughly described by Follis and Jackson (1943), Ammann (1961) and Garner and Ball (1966), whereas the influence of hemodialysis on the natural course of uremic osteopathy has not been

clearly established so far. In order to clarify the behaviour of uremic osteopathy under maintenance hemodialysis and to examine the dependance of skeletal changes on possible pathogenetic factors (duration of dialysis, serum PTH-levels, serum CT-levels, efficiency of dialysis) we studied iliac crest biopsy specimens from dialysed and non dialysed uremic patients. Apart from patients on anticoagulants and those, who had previously undergone subtotal parathyroidectomy, all patients of 3 German dialysis centers were examined thus excluding a serious sampling error. Micromorphometric data on histopathological changes can only be reasonably interpreted when control values are at hand. Since control values obtained from literature are not strictly comparable due to methodological and interindividual differences between different examiners, we studied in contrast to Kuhlencordt *et al.* (1971) and Duursma *et al.* (1972) an own closely age-and-sex-matched control group.

Our micromorphometric measurements were done after Merz (1967) and Schenk (1967) and Schenk *et al.* (1970). This technique, using undecalcified microtome sections is in our opinion superior to quantitative microradiography, because it allows to measure unmineralized bone mass and cellular parameters at the trabecular surface. Since the extent of histological changes in the skeleton varies considerably between different uremic patients, it is essential to study a large number of patients. Some of the apparent differences in literature may well be explained by sampling errors in groups of insufficient size.

In confirmation of earlier reports mentioned above (Follis and Jackson, 1943; Ammann, 1961; Garner and Ball, 1966) our histological studies showed both osteomalacia and osteitis fibrosa in the iliac crest spongiosa of uremic patients (Krempien *et al.*, 1971). Osteomalacia as the morphological equivalent of a mineralization block, points to the resistance of the uremic skeleton to physiological doses of vitamin D (vitamin D-resistance; Chu and Liu, 1943). Defective bone mineralization in uremia is not the consequence of disturbed intestinal calcium absorption (Ritz *et al.*, 1971). The inefficiency of physiological doses of vitamin D may well be due to the inability of the uremic patient to metabolize 25-hydroxy-cholecalciferol (Stanbury, 1972). Hydroxylation of 25-hydroxycholecalciferol to 1,25-hydroxy-cholecalciferol has been shown to occur exclusicely in renal parenchyma (Fraser and Kodicek, 1970). This metabolite seems to be the active metabolite of vitamin D in the intestine. Osteitis fibrosa is caused by increased serum parathyroid hormone levels (Bodansky *et al.*, 1930; Rutishauser, 1932). The stimulus for parathyroid gland overactivity and hyperplasia seems to be the tendency of azotemic patients to retain phosphorus (Slatopolski *et al.*, 1966, 1968; Bricker *et al.*, 1969) and/or the tendency towards hypocalcemia caused by a general depression of calcium transport processes. Defective calcium transport has been shown in the intestinal tract, in the sarcoplasmatic reticulum of muscles (Spannagel *et al.*, 1971; Ritz *et al.*, 1972) and is possibly present in the cellular layer that governs the calcium exchange between the mineral phase of bone and the extracellular fluid (Neuman and Ramp, 1972). In contrast to what is seen in primary hyperparathyroidism marrow fibrosis is only infrequently encountered in secondary hyperparathyroidism We saw it only in two cases with excessive (so called tertiary) hyperparathyroidism (Fig. 1). As a rule we found a moderate fibrosis of the endosteal layer which is somewhat more pronounced underneath resorptive and appositional zones.

Enhanced cellularity with an increase of active osteoid seams and of active Howship's lacunae pointed to increased parathyroid activity. Usually only shallow resorption cavities are encountered pointing to a possible functional inefficiency of osteoclasts, that has been suggested by Villanueva *et al.* (1970) and Jaworski *et al.* (1970) for uremic patients and has been confirmed by us in animal experiments (Krempien *et al.*, 1972). An additional feature, that deserves special emphasis, is the large number of "activated osteocytes" (Vittali, 1968), which in our opinion is an early morphological sign of parathyroid gland overactivity. Marked periosteocytic osteolysis can be seen well in microradiography.

It may be mentioned that the phenomenon of periosteocytic osteolysis has been already described by v. Recklinghausen (1910) under the term of "tryptische Onkose". We were impressed by the frequency with which periosteocytic areae showed the staining characteristics of osteoid. While it cannot be entirely ruled out, that mineralized bone matrix acquires staining characteristics of osteoid by mineral loss as a consequence of periosteocytic osteolysis (Belanger *et al.*, 1963), the peculiar staining properties might also be compatible with the possibility of "periosteocytic osteomalacia", i.e. formation of non mineralizing matrix by osteocytes under the conditions of chronic renal failure. Bone matrix synthesis by osteocytes has been clearly demonstrated (Baylink *et al.*, 1972).

The presence of woven bone and osteoid in the uremic skeleton is striking. In the adult skeleton immature woven osteoid is only seen when bone turnover is pathologically enhanced. The appearance of woven osteoid in secondary hyperparathyroidism demonstrates an accelerated rate of bone apposition and resorption on the tissue level (high turnover bone), although matrix synthesis and bone resorption rates are decreased on the cellular level (Villanueva *et al.*, 1969; Krempien *et al.*, 1972). Increased amounts of woven osteoid were usually seen in patients with high serum PTH levels. Woven osteoid is a primitive form of osteoid. Even when lamellar osteoid does not calcify, irregular mineralization can be seen in woven osteoid as shown by Garner and Ball (1966). In secondary hyperparathyroidism the shape of osteoblasts often resembles that of fibroblasts (Vittali, 1970). This was also evident in 5/6-nephrectomized rats (Krempien and Ritz, 1971) and in intact normal animals and bone cell cultures after administration of PTH (Gaillard, 1965; Owen, 1970). According to Vittali (1970) osteoblasts do not attain full maturity in the presence of extremely rapid bone formation. Possibly even preosteoblasts deposit immature bone matrix in this situation. Similarly rapid disappearance of woven osteoid could be demonstrated after parathyroidectomy because of severe secondary hyperparathyroidism (Ritz *et al.*, 1972; Binswanger *et al.*, 1971). It may be speculated, that immaturity of osteoblastic cells in uremia acounts also for the frequent finding of endosteal fibrosis overlying foci of osteoid synthesis. Conceivably the polarization of these cells, so that matrix deposition occurs only in the direction of the trabecular surface, may be lost in these immature cells and collagen fibers may be extruded both towards the trabecular surface and towards the marrow space. As an alternative possibility it should be mentioned that growth pressure of the proliferating cellular endosteal layer might cause stretching which according to Pauwels (1960) induces collagen fiber formation.

The presence of osteomalacia in the uremic skeleton has been firmly established by the studies of Follis and Jackson (1943), Ammann (1961) and of Garner and Ball

(1966). According to Frost (1963) osteomalacia is seen in the skeleton when the rate of organic bone-matrix formation exceeds the rate of bone mineralization leading to an increase of unmineralized osteoid. The presence of an increased amount of osteoid per se does not prove the existence of a mineralization block, since one expects to find increased amount of osteoid in the skeleton whenever the number of appositional fronts is elevated due to a high bone turnover rate. According to Vitalli (1970) a mineralization block is only firmly established, when the ratio of active to inactive ostoid is decreased.

An increase of osteoid has been definetively shown in our uremic cases with and without hemodialysis. Both the fraction of spongiosa volume and the fraction of trabecular surface represented by osteoid were markedly increased in non dialysed as well as in dialysed patients. In addition, the thickness of osteoid seams was considerably augmented in both uremic groups. In this context it should be mentioned that we interpreted unmineralized bone matrix as an osteoid seam only if it comprised more than one lamella or consisted of nonlamellar woven osteoid. Although the number of active osteoid seams covered by osteoblasts was elevated in absolute terms, the ratio between active and inactive seams in the skeleton of both uremic groups was not different from that in controls. We don't believe, however, that increased bone turnover and increased cellularity of bone alone explain these findings. There is reasonable evidence, that defective mineralization is involved. Villanueva *et al.* (1969), Sarnesethiri *et al.* (1970), Jaworski *et al.* (1970) could demonstrate, that the rate of matrix synthesis of the individual osteoblast is diminished in uremia. Our own experiments with 5/6-nephrectomized rats gave evidence for a decrease of the linear rate of bone apposition and of collagen synthesis in renal insufficiency (Krempien *et al.*, 1972a, b).

Since turnover rates are changed on the cellular level, the ratio between active and inactive osteoid is no longer a criterium for the presence or absence of a defective mineralization. The finding of increased osteoid seam thickness and the frequent finding of buried osteoid are good evidence for a mineralization block. In addition, the appearance of inactive resting osteoid seams after parathyroidectomy points to the presence of defective mineralization (Ritz *et al.*, 1972). Our morphometric data document the presence of osteomalacia in the skeleton of uremic patients both with and without hemodialysis. Whether the underlying cause is uremic vitamin D resistance (Chu and Liu, 1943) alone or to a certain extent also inhibition of mineralization by toxic metabolites [analogous to the circulating inhibitor of chondral calcification demonstrated by Yendt *et al.* (1961)] remains to be seen.

In contrast to Jowsey *et al.* (1969) and Kuhlencordt *et al.* (1971) who studied ground sections with quantitative microradiography our morphometric analysis in undecalcified bone sections gave no evidence of osteopenia in dialysed and non dialysed uremic patients. Volumetric density, a measure of the fraction of total spongiosa volume represented by calcified and non calcified bone matrix volume, was rather significantly elevated. Although we cannot discount sampling error through local inhomogeneity of iliac crest structure as a possible source of error, one would expect that this cancels out in large groups of patients. On the other hand, our data are in good agreement with the findings of de Veber *et al.* (1970) and of Delling (1972) in uremic patients with and without hemodialysis and with

the findings of Garner and Ball (1966) and of Binswanger *et al.* (1971) in non dialysed uremic patients. Duursma *et al.* (1972) found normal bone volume in dialysed and non dialysed uremic patients.

An increase of bone matrix mass is hardly compatible with claims that metabolic acidosis plays a major role in the pathogenesis of uremic osteopathy (Leman, 1969; Lennon, 1969). Skeletal changes similar to uremic osteopathy have never been reproduced by metabolic acidosis in animal experiments (Barzel, 1969; Barzel and Jowsey, 1969; Delling and Donath, 1971). In contrast to what is seen in uremia, chronic metabolic acidosis consistently produced osteopenia in experimental animals. Our own animal experiments show, that the changes of intermediary bone metabolism, of collagen and acid mucopolysaccharide synthesis, of bone formation and mineralisation rates produced by experimental renal insufficiency are strikingly different from those seen after ammonium chloride acidosis (Ritz *et al.*, 1971; Krempien *et al.*, 1972).

There are remarkable discrepancies between different authors concerning histological findings in uremic osteopathy under maintenance hemodialysis. Whereas Jowsey *et al.* (1969) found decreased bone mass, absence of bone formation zones and excessive increase of resorption zones, de Veber *et al.* (1970) found increase of osteoid and striking osteomalacia in 80% of dialysed patients. In addition bone mass was augmented in 55% of dialysed patients. Whereas Jowsey *et al.* (1969) described an increase of bone resorption rate with increasing duration of dialysis, de Veber *et al.* (1970) observed a reduction of osteitis fibrosa with increasing length of dialysis. In contrast to a group of Australian dialysed patients with predominant fibroosteoclastic bone resorption and osteopenia (Ireland *et al.*, 1970), Canadian patients (de Veber *et al.*, 1970) and our own patients showed striking evidence of both osteomalacia and osteitis fibrosa and no osteopenia. Kuhlencordt *et al.* (1971), Vitalli (1970) and Ball (1971) claimed to have found no osteomalacia in dialysed patients. Osteoid seams were small in measurements of Kuhlencordt and coworkers (1971) and the fraction of bone mass represented by osteoid was negligible. Our own results are in good agreement with the findings of de Veber *et al.* (1970). They were meanwhile also confirmed by Duursma *et al.* (1972) and Delling (1972). In nearly all cases of chronic renal insufficiency with and without hemodialysis we found evidence for an osteopathy characterized both by fibroosteoclasia and osteomalacia. Various morphometric parameters (Table 1 and 2) differed significantly from those of age-and-sex-matched controls. Apart from the prevalence of low density areas in the skeleton of dialysed patients, no significant differences between dialysed and non dialysed uremic patients were detectable. Osteomalacia did apparently not progress with increasing duration of dialysis. Our data show, that under the conditions of dialysis employed in the present series, osteitis fibrosa does not progress in the iliac crest spongiosa of the majority of patients. Since we did not perform sequential biopsies in the same patients and since the average duration of hemodialysis was relatively short, this statement is a guarded one.

Some of the discrepancies between different reports in literature may be due to differences of morphometrical methods used, to differences of the conditions of dialysis [particular dialysate calcium concentration (Goldsmith *et al.*, 1971) and serum phosphate control by oral aluminum hydroxide (Parfitt *et al.*, 1971)] or due

to differences of the vitamin D status (Lumb *et al.*, 1971) of the population studied. In addition, differences in the nutritional status and physical activity of dialysed patients, especially protein malnutrition, fluoride intoxication (Parson, 1971) use of demineralizers as opposed to deionizers, or toxic factors in tap water (possibly cadmium as suggested by Kerr (1971) for Newcastle bone disease) may explain some of the differences observed.

All quantitative measurements are disquietingly subject to the possibility of the sampling error. Although we do not have data on the variation of micromorphometric measurements in the same individual at various points of the iliac crest, we tried to eliminate sampling errors as far as possible by studying sections taken from different depths of the bone cylinder which were taken at a precisely defined point on the iliac crest. In the absence of more systematic data of the entire skeleton we do not know whether iliac crest spongiosa is representative for the whole skeleton.

The decisive influence of dialysate calcium (Goldsmith *et al.*, (1971) and magnesium (Plettka *et al.*, 1971) concentrations on the course of uremic osteopathy in dialysed patients has not been fully recognized until recently. Dialysis against inadequately low calcium concentrations leads both to activation of parathyroid gland activity and to progessive skeletal demineralization by imposing a constantly negative calcium balance. Whereas parathyroid stimulation was probably prevented in the majority of patients studied (using an average calcium concentration of 2.9 mvl/l), progressive skeletal demineralization in this patient group could be shown both by roentgenological and roentgenodensitometric methods (Ritz *et al.*, 1971). As mentioned above skeletal demineralization is probably responsible for our microradiographic finding of low density areas. Evidently, intermittent positivation of calcium balance by high dialysate calcium concentrations is necessary to overcome the negative calcium balance of the dialysed patients due to intestinal malabsorption of calcium and to fecal calcium losses (Verberckmoes *et al.*, 1971).

In support that in the PTH-radioimmunoassay not only biologically active aminoterminal material but also biologically inactive fragments of PTH are measured, there was a correlation between serum PTH levels on one hand and the surface fraction covered by active Howship's lacunae as well as the osteocytic areae measured in microradiographic specimens on the other hand. The correlation of serum PTH levels to osteoclasts was less well pronounced than that of osteocytic areae, possibly because even without PTH and vitamin D changes intermittent calcium loads during dialysis might stimulate osteoclasts proliferation by increasing cytosol calcium concentrations (Bordier, 1972).

However, there was no correlation between serum CT levels and micromorphometric parameters. Thus calcitonin evidently is not a maior factor in the pathogenesis of uremic osteopathy. In addition, the data of Delano *et al.* (1971) show that calcitonin is entirely ineffective in the treatment of uremic bone disease. This agrees with the findings, that calcitonin while possibly reacting to short term changes of serum calcium seems not to be involved in the long-term regulation of serum calcium levels in humans. There was no correlation between efficiency of dialysis and severity of bone disease. Our data show, that in the relatively short period of dialysis up to 3 years that we could study, there was no systemic trend towards improvement or worsening of micromorphometric findings of osteopathy.

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