Virchows Arch. A Path. Anat. and Histol. 375, 169–183 (1977)

Virchows Archiv A

Trabecular Bone Morphometry in Beagles with Chronic Renal Failure

Robert W. Norrdin, Philippe Bordier, and Charles W. Miller

André Lichwitz Research Unit (I.N.S.E.R.M.), Hôpital Lariboisière, 75010 Paris, France and the Department of Pathology and Collaborative Radiological Health Laboratory, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colorado 80523, USA

Summary. A morphometric study was performed on undecalcified sections of trabecular bone from the ribs of adult dogs that were controls ($n=8$), or had various degrees of renal failure as a result of perinatal irradiation ($n=16$). In a group identified as markedly uremic there was an increase in the proportion of surface with osteoid seams ($P<0.01$), osteoblasts ($P<0.01$) and osteoclastic resorption ($P<0.01$) as well as the % osteoid volume ($P<0.01$). Aside from a decrease in the % trabecular bone which was of unclear significance, the results agree well with those of similar studies in man. Within this group, 6 of the samples had a histologic pattern that was principally that of hyperparathyroidism and in the remaining 2 there was evidence of a mineralization defect and osteomalacic changes. These 2 dogs had the poorest renal function and one that had been biopsied 5 months earlier, when its renal function was less impaired, had had a hyperparathyroid pattern.

Key words: Dogs – Kidney failure, chronic – Hyperparathyroidism – Osteomalacia.

Introduction

The bone lesions that accompany chronic renal failure involve one or more of a variety of changes that include osteitis fibrosa, osteomalacia, osteosclerosis and osteopenia (Ellis and Peart, 1973; Duursma et al., 1974; Bordier et al., 1975). This variety apparently reflects disturbances in several metabolic systems. Of particular importance is the retention of phosphate associated with declining renal function (Kleeman et al., 1970) which is thought to depress serum ionized calcium and lead to secondary hyperparathyroidism (Slatopolsky et al., 1971). Also involved is interference with the conversion of 25 hydroxy-vitamin D3 to

For offprints contact: Dr. R.W. Norrdin, Department of Pathology, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colorado 80523, USA

1,25 dihydroxy D3, its biologically active metabolite, by the diseased kidney (Stanbury et al., 1973). This would seam to be related to disturbances in the absorption of calcium and its metabolism in bone leading directly or indirectly to a failure of mineralization and the development of osteomalacia. In addition, metabolic acidosis (Barzel, 1969) and toxemia (Gitelman, 1970) are other possible factors to be considered in the progression of renal disease. All these variables make any group of affected individuals a very heterogenous one in which to study the pathogenesis of the bone lesions. Yet, such understanding is essential to the control and treatment of the disease.

In an experimental study of the effects of irradiation on beagles it was found that many of the dogs (mostly males) that had been exposed to sublethal doses around the time of birth developed renal failure and often died with bone lesions (Phemister et al., 1973). The survivors of these groups, coming to scheduled sacrifice at 2 and 4 years of age with varying degrees of renal failure, provided a spectrum of disease in which natural progression of untreated renal osteodystrophy could be studied in a well-controlled experimental environment. The renal disease, a slowly progressive glomerulosclerosis associated with radiation damage to the developing renal cortex (Phemister et al., 1973), simulated the naturally occurring disease more closely than experimental renal failure produced by nephrectomy and infarction in which there can be variable regeneration of tissue. A tetracycline-based study of haversian bone was carried out and revealed that the uremic dogs had a greater amount of remodeling activity in which, there was an increased rate of bone formation (Villafane et al., 1977). This was attributed to the secondary hyperparathyroidism in these dogs (Ojerio et al., 1975). The findings contrasted however, with similar studies on haversian bone in man, in which decreased formation rates were found (Sarnsethsiri et al., 1969; Hitt et al., 1970) suggesting differences in the stage or nature of the bone disease. The present study was then performed on trabecular bone from 4 year old animals to compare the morphometric findings with those in man and attempt to relate them to loss of renal function.

Materials and Methods

The study was performed on beagles that were controls ($n=8$) or had various degrees of renal failure as a result of irradiation with 270–435 R ^{60}Co whole body gamma irradiation at 2 days of age or in utero at 55 days post coitus (Phemister et al., 1973). Most of the dogs was sacrificed at 4 years of age. The irradiated dogs were classified as non-uremic (blood urea nitrogen, BUN, less than 30 mg/100/ml, $n=4$), mildly uremic (BUN 30–75 mg/100/ml, $n=6$), or markedly uremic (BUN greater than 75 mg/100/ml). The latter group includes a 6 year old dog that died of renal failure and two 4.5 year old dogs that had a rib biopsy and were sacrificed 5 and 6 months later when they were thought to be in a terminal uremic state. There was thus a total of 8 observations from 6 dogs in this group. The dogs received no treatment aside from subcutaneous fluids administered to the 2 dogs in a terminal state while arrangements were being made for necropsy.

For some of the dogs, data was available from the most recent of bimonthly, non-invasive renal function tests. In these tests Na sulfanilate clearance was used as a measure of glomerular filtration in the method described by Carlson and Kaneko (1971). This substance, which has a clearance ratio with inulin of 0.93 in the dog, was injected intravenously and the half time for its disappearance from plasma, T1/2 sulfanilate, was determined from blood samples drawn 30, 60, and 90 min later.

At necropsy, the parathyroid glands from one side were dissected out for weighing. Their weights were expressed in terms of femur length to correct for differences in body size, dehydration, etc. The middle of the right 9th rib was collected for evaluation (the 11th rib had been taken in the 2 dogs biopsied). The ribs were generally fixed in phosphate buffered 10% formalin, however 70% alcohol was used in 4 dogs. They were embedded undecalcified in methylmethacrylate, and cut at 4–5 microns thickness on a Jung model K microtome. The sections were stained with toluidine blue at pH 2.8 and evaluated by routine point-counting, line intercept techniques using 25× and 40× objectives with Zeiss Integrating eye pieces I and II. For each rib, 6–8 sections were evaluated and measurements were only made on trabecular bone. The cortical-endosteal surface was not included because its remodeling activity may be different (Frost, 1969). The trabeculae were considered to end at a line through their cortical attachment that conformed to the general outline of the marrow cavity in the cross section. The "% osteoid surface" was the proportion of total surface covered by osteoid (Figs. 1–3). The "% osteoblast surface" was that proportion of total surface covered by plump osteoblasts (Fig. 2). These were cells with enlarged nuclei and cytoplasm in which a golgi could usually be seen and thus thought to be engaged in active matrix synthesis. The "% osteoclastic resorption surface" was that portion of the total surface with a Howship's lacuna that contained an osteoclast (Figs. 4–9). The number of osteoclasts encountered was also recorded and expressed as "no. of osteoclasts/mm^2" of bone tissue. The "% trabecular bone" was the proportion of bone in the marrow cavity tissue and the "% osteoid volume" was the fraction of that bone occupied by unmineralized matrix. These techniques and criteria were based on those of other studies in the literature (Schenk et al., 1969; Merz and Schenk, 1970; Bordier and Tun Chot, 1972). The "osteoid seam thickness index" was derived by dividing the "% osteoid volume" by the "% osteoid surface" and multiplying by 100. This index has been shown to be directly proportional to actual measurements of seam width (Meunier and Edouard, 1977).

Results

The markedly uremic dogs as a group had a mean BUN of 119.5 ± 27.7 mg/100 ml (Table 1). Creatinine was moderately elevated at 4.2 ± 2.6 mg/100 ml and the parathyroid gland weights were greater than twice that of the control group. In the mildly uremic group the BUN was the only one of these three parameters that was significantly different from controls.

The results of the morphometric analysis are given in Table 2. In the calculation of % osteoid surface, very thin seams such as that illustrated in Figure 3 were included. This parameter, as well as % osteoblast surface was significantly elevated in the mildly uremic group ($P<0.05$) although this was primarily attributable to 2 individuals. The markedly uremic group had a greater % osteoid surface ($P<0.001$), % osteoblast surface ($P<0.01$), % osteoclast resorption surface ($P<0.01$), number of osteoclasts/mm^2 ($P<0.01$), % osteoid volume ($P<0.001$) and osteoid seam thickness index ($P<0.01$). The % trabecular bone volume was lower for the group, and this was also true of the mildly uremic dogs ($P<0.01$).

Within the markedly uremic group there appeared to be a spectrum of change. In 6 of 8 samples the proportion of surface covered by osteoid varied from slightly above normal to 80%. In the ribs with the mildest lesions, the increase in osteoid surface was a principle finding (Fig. 4). A increase in % osteoblast surface was associated with an increase in osteoid volume to between 6 and 18%. This reflected a longer, uniformly widened osteoid seam that had a discrete margin at the surface of the mineralized bone (Fig. 5). The osteoid seam thickness index was 15–20. In these ribs the osteoclastic resorption surface was increased up to 6%. Osteoclasts tended to be larger with a greater number of nuclei and more

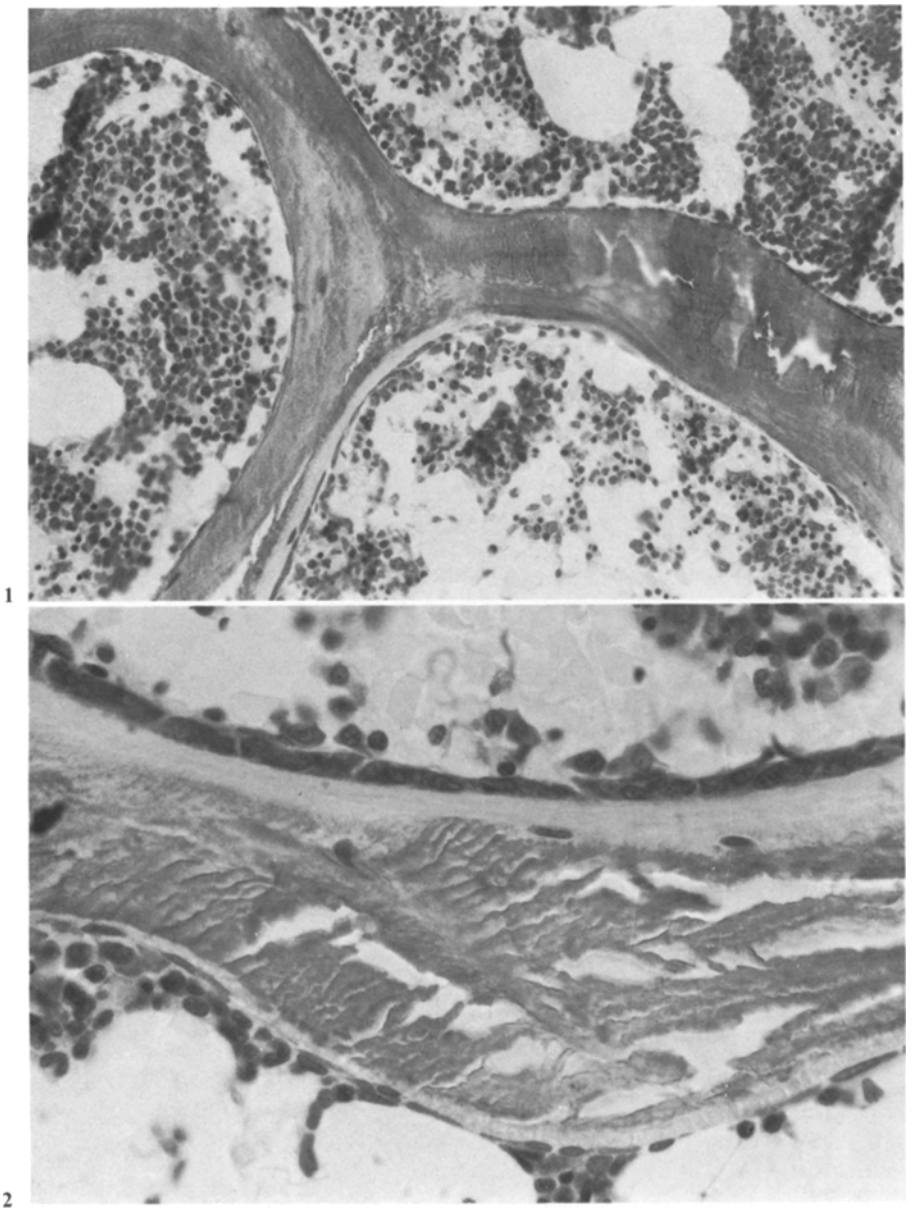


Fig. 1. Trabecular bone in control dog. Note orderly lamellar orientation of bone and normal width of osteoid seam along bottom spicule. (All photomicrographs are undecalcified bone with toluidine blue stain.)

Fig 2. Trabecular bone with osteoid lined surfaces in a control dog. Osteoblasts on upper surface represent the plump, active-appearing cells that were included in the counts

Table 1. Blood urea nitrogen, creatinine, and parathyroid gland weights in uremic and nonuremic beagles

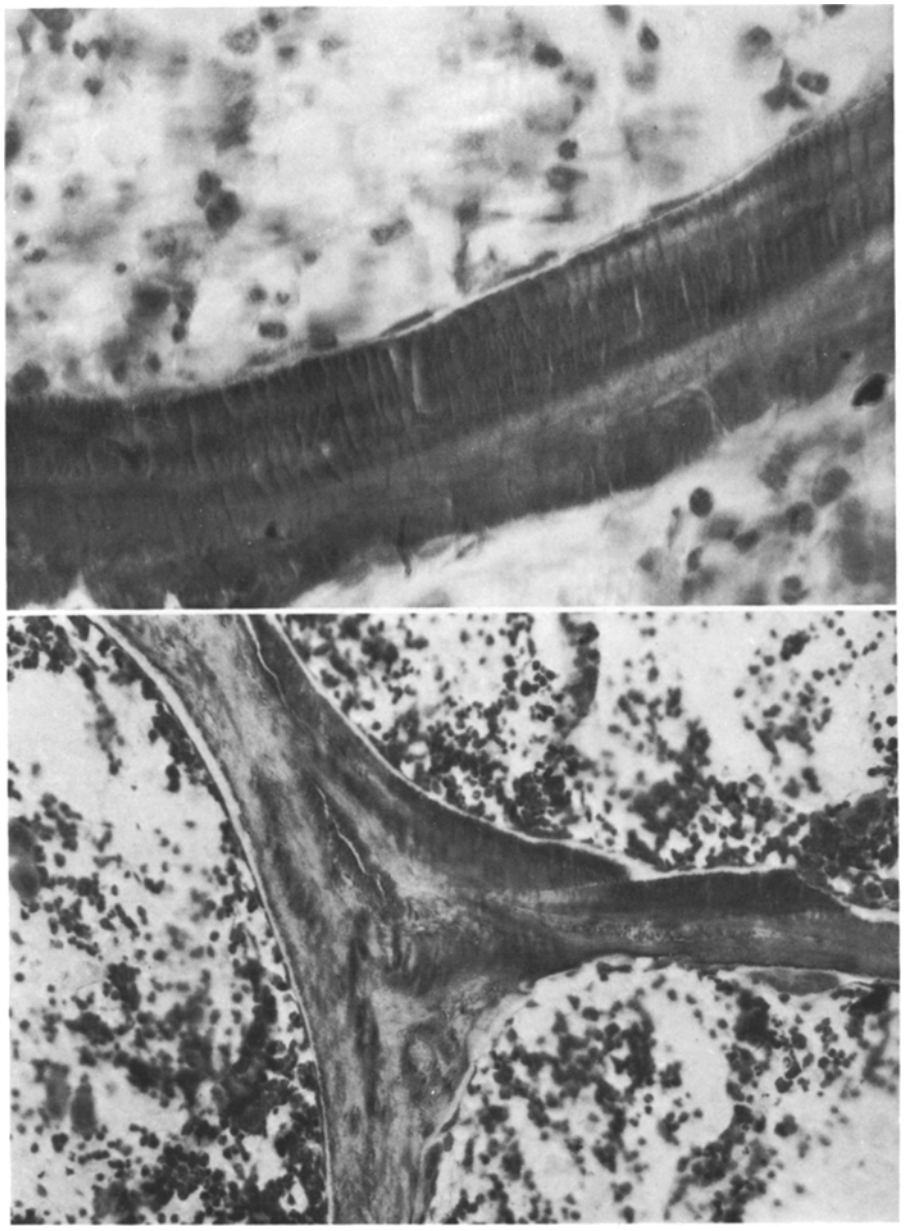
	Controls	Perinatally irradiated		
		Nonuremic	Mildly uremic	Markedly uremic
	($n=8$)	($n=4$)	($n=6$)	($n=8$)
Blood urea nitrogen (mg/100 ml)	16.6[a] ±5.5	15.5 ±2.4	34.2** ±2.2	119.5*** ±27.7
Creatinine (mg/100 ml)	1.0 ±0.2	1.0 ±0.2	1.3 ±0.2	4.2** ±2.6
Parathyroid gland weight (mg/cm femur length)	1.56 ±0.22	1.25 ±0.67	1.81 ±0.71	3.62[b] ±1.15

** $P<0.01$;
*** $P<0.001$
[a] Mean ± SD
[b] Sample size for parathyroid gland weight is six since data were not available in two dogs undergoing biopsy only

Table 2. Results of trabecular bone morphometry in uremic and nonuremic beagles

	Controls	Perinatally irradiated		
		Nonuremic	Mildly uremic	Markedly uremic
	($n=8$)	($n=4$)	($n=6$)	($n=8$)
Osteoid surface %	26.4 ±12.2	34.5 ±9.6	44.1* ±13.0	73.3*** ±16.9
Osteoblast surface %[a]	3.7 ±2.3	6.4 ±6.3	8.9* ±5.7	16.0** ±12.2
Osteoclast resorption surface %	1.1 ±0.2	1.4 ±0.7	1.5 ±0.8	4.6** ±3.2
Osteoclasts (No/mm^2)[a]	0.87 ±0.45	1.10 ±0.70	0.88 ±0.16	4.36** ±2.82
Trabecular bone volume %	19.9 ±2.7	19.0 ±5.6	15.2** ±2.4	15.2** ±2.9
Osteoid volume %	2.3 ±1.4	2.9 ±1.3	3.9 ±1.5	18.9*** ±10.5
Osteoid seam thickness index	8.3 ±2.2	8.4 ±1.6	9.2 ±2.9	23.9** ±11.0

* $P<0.05$; ** $P<0.01$; *** $P<0.001$
[a] For these two parameters poorly-fixed (70% alcohol) tissue from four dogs was excluded. Thus there is one less dog in the markedly uremic and nonuremic groups and two less in the mildly uremic group



3

4

Fig. 3. Thin osteoid seams which were also included in counts of osteoid lined surfaces. Collagen fibers could be found in such seams with polarized light

Fig. 4. Bone from markedly uremic dog showing an increase in the osteoid covered surface that was uniform and of normal width. There are osteoclasts at the bottom and at right. This was the milder degree of change seen in this group

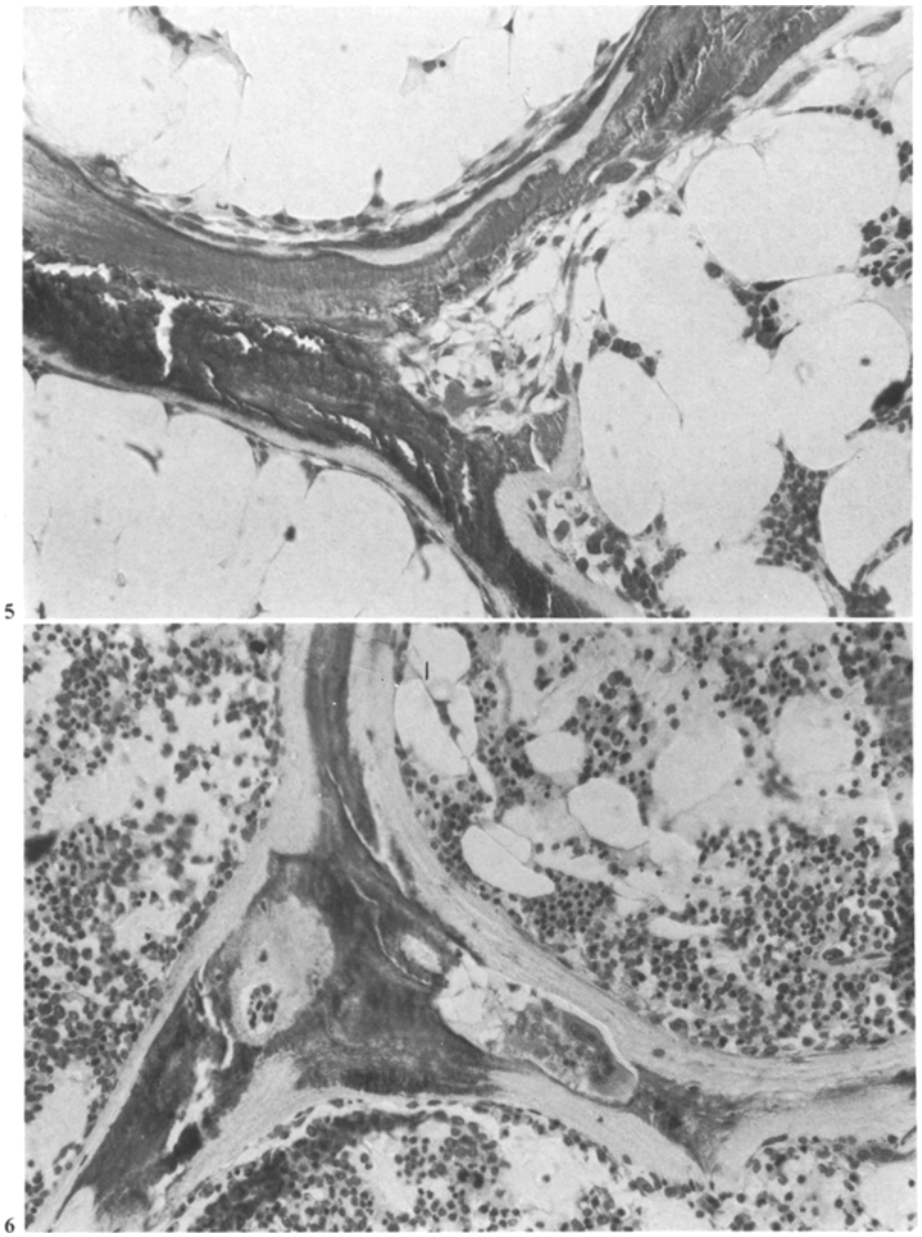


Fig. 5. Trabecular bone from dog in markedly uremic group showing an increased extent of osteoid covered surface, slightly wider seams and a greater proportion of surface with osteoblasts or osteoclastic resorption sites. Note loose fibrous connective tissue over resorption site. These changes were interpreted as increased remodeling activity of hyperparathyroidism

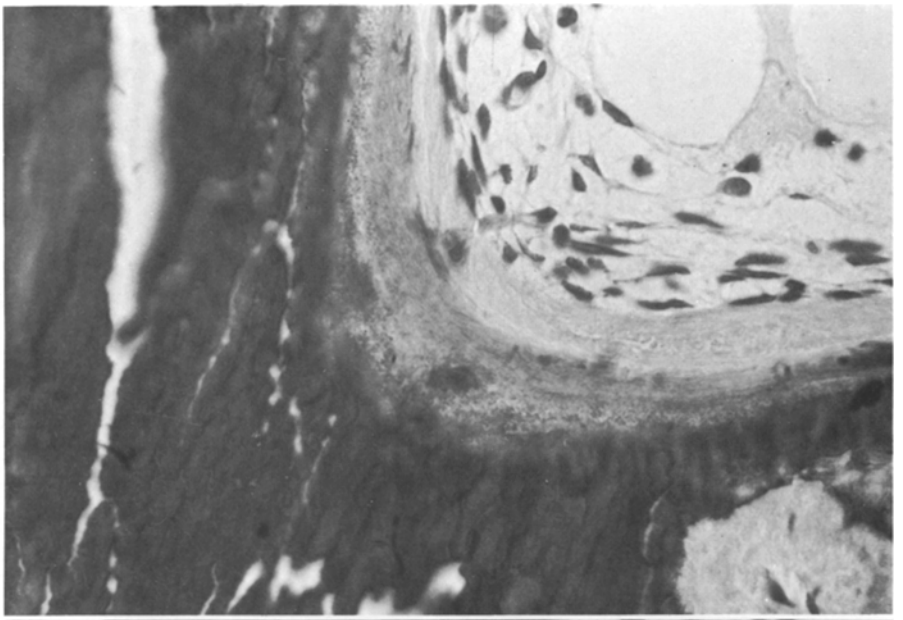
Fig. 6. One of the 2 dogs in the markedly uremic group that had the most severe bone changes. Note the irregularly widened osteoid borders and greatly increased percent osteoid volume

deeply staining cytoplasm (Figs. 5, 10). The number of osteoclasts/mm^2 more or less paralleled the % osteoclastic resorption surface and most of those counted were in resorption sites on the bone surface. Only infrequently were osteoclasts found apart from a Howship's lacuna, i.e. not in a position of active resorption. Mild, early marrow fibroplasia was seen in some ribs with plump fibroblasts in a loose connective tissue along trabecular surfaces, but most often over sites of resorption (Fig. 5).

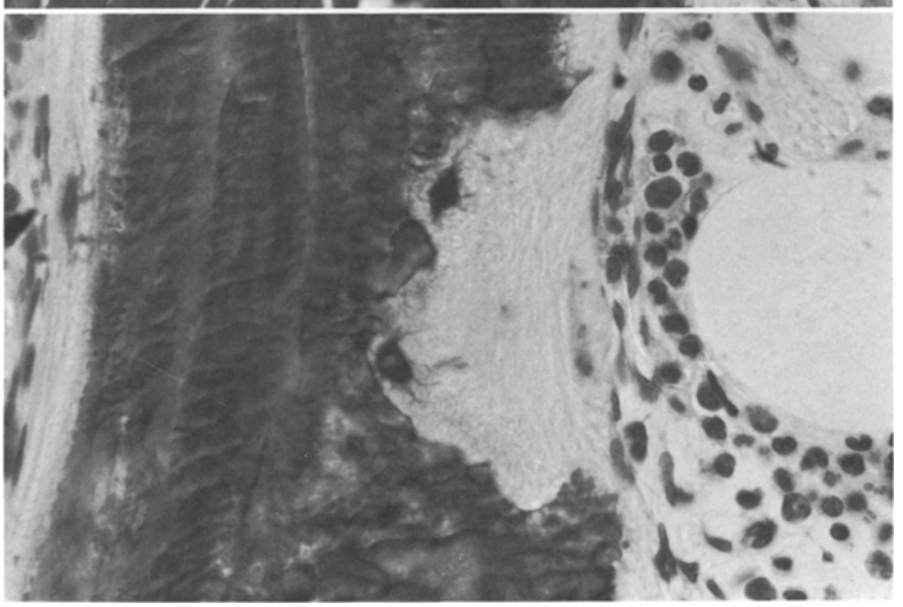
In 2 of the samples from the markedly uremic group the bone lesions were considerably more severe. In these ribs the % osteoid volume had increased to 30 and 35% and the seam thickness index up to 30 and 44. Moreover there was considerable irregularity in the width of osteoid borders (Fig. 6). The margin with the mineralized bone was not discrete and where the mineralization front could be identified with the toluidine blue stain it was wide and fuzzy (Fig. 7). Often there were irregular, finely granular deposits of mineral in the widened seams or around the osteoid osteocytes nearest the mineralized bone. Frequently there were places found where the scalloped border of the mineralized bone indicated there had been Howship's lacunae, and the osteoid filling this resorption site contained little or no mineral (Fig. 8). Another feature of the 2 samples was an almost complete absence of osteoblasts. Although 80.4 and 90.2% of the trabecular surface was covered with osteoid, the surface cells for the most part were thin, flat lining cells (Figs. 7 and 8). In some places these were spindle-shaped cells with slightly more plump, elongated nuclei. Where osteoblasts were found they were relatively small cells with a cuboidal outline. Marrow fibroplasia was still quite limited in extent but tended to be somewhat more dense than in the other dogs. In 1 of the 2 dogs there was markedly increased % osteoclast resorption surface (10.5%), while in the other it was below control levels (0.7%), while in the other it was below control levels (0.7%). In the former dog, the osteoclast resorption surface accounted for half of the non-osteoid lined surface and osteoclasts often appeared to be tunneling through the mineralized core of the trabeculae (Fig. 9). In the osteoid matrix the collagen had a coarse, more loosely-packed appearance in polarized light (Fig. 10), and the less intense staining of the bone suggest a decreased mineral content. Osteocyte lacunae were generally somewhat enlarged and often had irregular, dark staining margins.

In general, thoughout the study, patterns on the trabecular bone surface were carried over into the cortical bone. Thus, in the uremic dogs with an abundance of plump osteoblasts on trabecular bone, the same type of cells were found lining developing haversian systems and in the 2 samples with greatly widened osteoid seams and flat lining cells, the same was found on haversian bone surfaces.

When the principal findings were plotted against declining renal function as measured by the clearence of sulfanilate in those dogs for which the data was available, the patterns of change correlated fairly well (Fig. 11). The 2 bone specimens with the most severe lesions were from the 2 dogs with the most prolonged clearance time athough one was considerably worse than the other. This was the dog that had a decrease in both osteoclast resorption surface and osteoblast surface. It had been biopsied 5 months earlier when its bone morphology corresponded to that of most of the dogs in the group with moderately impaired renal function, i.e. an increase in osteoblast and osteoclast resorption surface.



7



8

Fig. 7. One of 2 dogs in markedly uremic group with greatly widened seams. Note wide band of partially mineralized osteoid, and indiscreet margin with mineralized bone. There are flat lining cells and loose fibrous tissue on the surface of the osteoid

Fig. 8. Dog rib with irregular wide seams. Here osteoid fills a resorption site and there is only faint mineralization in the deepest osteoid

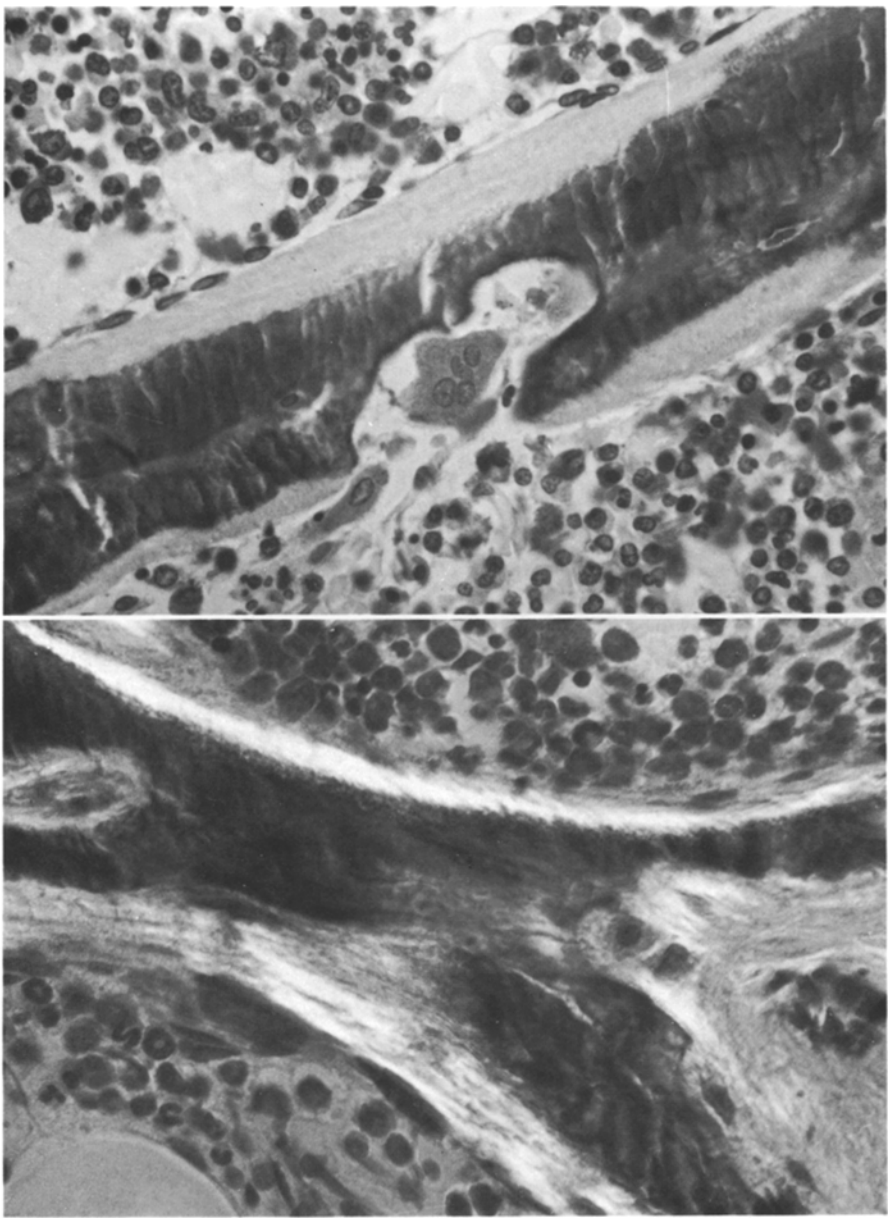


Fig. 9. Bone, markedly uremic dog. Where osteoid covered 80% of the trabecular surface, osteoclastic resorption was often found in the mineralized bone core

Fig. 10. One of 2 most severely changed ribs. Polarized light demonstrates the coarse, loosely packed collagen in osteoid

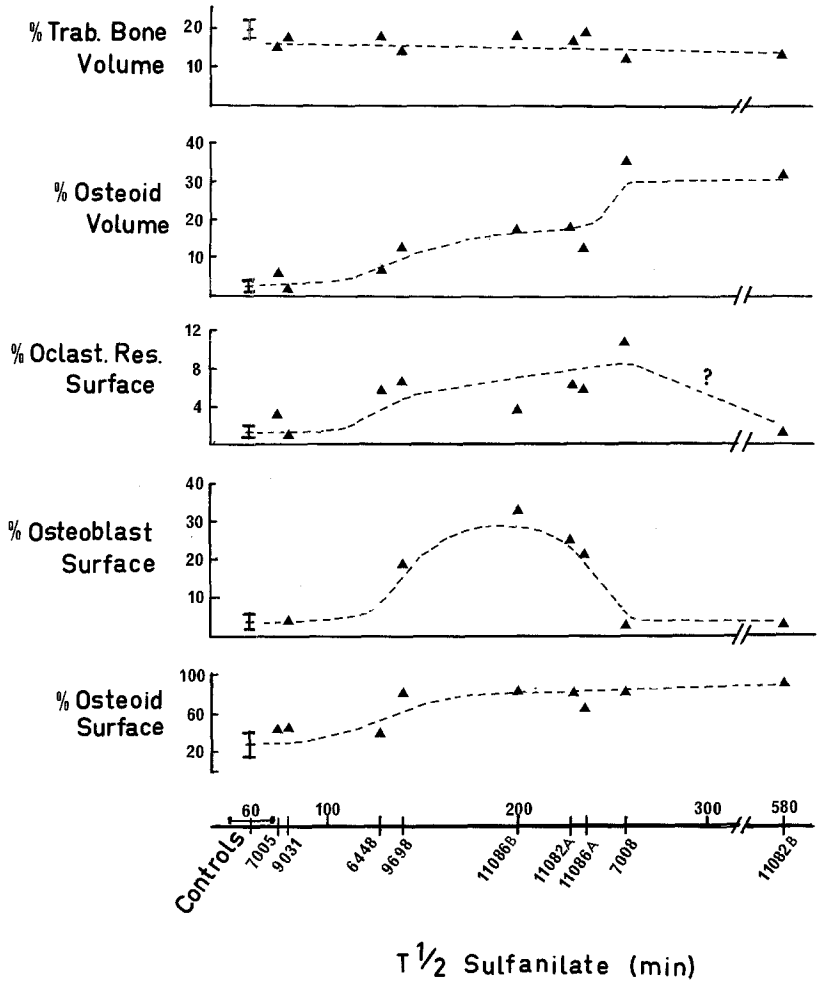


Fig. 11. Plot of morphometric parameters against renal function as measured by sulfanilate clearance, Data was available for 2 mildly uremic dogs (7005, 9031) and 5 markedly uremic dogs, two of which had biopsies (labeled A) 5–6 months earlier

In the other dog that had been biopsied 6 months earlier, renal function and bone morphometry had remained about the same. It should be noted however that this dog also was sacrificed because it had stopped eating and appeared toxic, even though it had started to respond to nursing care.

Discussion

The control values for trabecular bone in the dogs rib were similar to those of the same type of study on iliac trabecular bone in man (Merz and Schenk, 1970; Krempien et al., 1972; Olah, 1973; Ellis and Peart, 1973; Duursma et al., 1974;

Sherrard et al., 1974; Meunier et al., 1975; Bordier et al., 1975). The osteoid surface was greater because thin seams were included in our count whereas others, generally working at lower magnification, usually included only seams of 1 lamella or greater in thickness. Because of this difference, our values for osteoid seam thickness index are considerably lower than those of Meunier et al. (1975). The osteoclastic resorption surface reported here and by others (Krempien 1972; Duursma et al., 1974; Bordier et al., 1975) is much less than the total scalloped surface, with or without osteoclasts, that is also used (Sherrard et al., 1974; Meunier et al., 1975). The mean value for % osteoblast surface, 3.7 ± 2.3 %, is slightly lower than others whose figures are generally between that and the 6.4% mean in our irradiated non-uremic dogs. Considerable variation was found in this parameter, even though the criteria used were quite conservative in evaluating the various forms of osteoblasts (Woods, 1976).

The findings in the markedly uremic dogs as a group also agree with those of similar studies of chronic renal failure in man (Krempien et al., 1972; Olah, 1973; Ellis and Peart, 1973; Duursma et al., 1974; Meunier et al., 1975; Bordier et al., 1975). One place of disagreement appears to be in the % trabecular bone which has been reported to be increased, decreased, or unchanged in the above studies. Ellis and Peart (1973) found a decrease in 5 % of their cases and an increase in 30 %; they associated the increase with woven bone formation and wide osteoid seams. Meunier et al. (1975) found an increase in 11 % of their cases and a moderate decrease in 21 %. In the dogs a decrease in % trabecular bone in the markedly uremic group ($P < 0.01$) was associated with often striking bone lesions, but the % bone was also decreased in the mildly uremic group ($P < 0.01$) where it was not. This suggests that the osteopenia seen here may not be related to the uremic bone disease or may represent an inter-action with other factors, perhaps an effect of irradiation on the amount of trabecular bone present prior to the development of bone lesions. Additional animals will be needed to clarify this point. There is other data from this laboratory (Rutherford and Bordier, unpublished) of a study in dogs uremic from nephrectomy and infarction, in which the % trabecular bone remained unchanged or decreased moderately.

The increase in % osteoid surface was a principle finding in all the above cited studies and appears to be an early change judging from its presence in our mild group ($P < 0.05$) and patients with renal disease of short duration (Sherrard et al., 1974). These morphometric studies have also shown an increase in osteoblast-covered surface (Krempien et al., 1972; Olah, 1973; Duursma et al., 1974; Bordier et al., 1975). However, the correlation between osteoblast surface and osteoid volume that is present in normal individuals (Merz and Schenk, 1970) is lost in chronic renal failure (Olah, 1973; Bordier et al., 1975) indicating that formation is not responsible for all the osteoid present. Moreover, tetracycline-based dynamic studies on harversian bone found that formation of mineralized bone was decreased (Sarnsethsiri et al., 1969; Hitt et al., 1970). Sherrard et al. (1974) separated patients into fibrotic and osteomalacic groups histologically and found, using tetracycline labeling, that trabecular bone formation was increased in the fibrotic group and decreased in the osteomalacic group. Meunier et al. (1975) pointed out the importance of osteoid seam thickness index and tetracycline-based calcification rate in distinguishing those patients with a mineralization defect.

Using the thickness index they found only 19% of their cases had seam widths comparable to true osteomalacia.

Within the 8 observations in our markedly uremic group, 6 fit the pattern described as hyperparathyroidism: an increase in % osteoblast and % osteoclast resorption surface associated with a moderate increase in % osteoid volume. The latter was attributable mostly to an increase in the % osteoid surface with a mild but uniform widening of seams. This would suggest an increase in the amount of remodeling activity in which a sequence of resorption is followed by formation (Frost, 1969). In other studies, the % osteoblast and % osteoclast surfaces have been correlated with each other (Duursma et al., 1974; Bordier et al., 1975) and with serum parathormone (PTH) levels (Bordier et al., 1975). The extent of the osteoid surface has also been correlated with PTH levels (Bordier et al., 1975). Of these 6 dogs, 4 had been included in a tetracycline-based study of haversian bone remodeling and had increased bone formation associated with an increase in bone remodeling sites (Villafane et al., 1977). Although the response in trabecular bone may be quite different from that of haversian bone (Frost, 1969), the histology in the 2 envelopes was qualitatively very similar in these dogs. The other 2 specimens had morphologic changes more like osteomalacia with a marked increase in % osteoid volume and osteoid seam thickness index. A mineralization defect was suggested by the lack of a calcification front and the partial or patchy mineralization in the irregularly widened seams. No tetracycline data was available on these 2 dogs. The low % osteoblast surface contrasted sharply with the others in the group and suggested that there was little apposition of matrix. This might be explained as the result of a terminal toxemia in these 2 dogs, one of which died and the other sacrificed in a moribund state. The presence of an increased % osteoclastic resorption surface in 1 of the dogs might then represent the uncoupling of formation from resorption (Rasmussen and Bordier, 1974) as another effect of toxemia. It must be remembered however, that one of the other dogs was also sacrificed in a toxic state and still had marked increases in % osteoblast and % osteoclast resorption surface, and only moderately impaired renal function. This would indicate that other factors may be involved in the death of these untreated dogs, that don't necessarily affect bone morphology. Although data was missing for 1 dog in this group, renal function was most impaired in the 2 dogs in which the morphometry indicated a mineralization defect was also involved. Moreover, 1 of these dogs had had a hyperparathyroid pattern when biopsied 5 months earlier. This would support the concept that hyperparathyroidism appears first and osteomalacia is superimposed at a later stage (Ellis and Peart, 1973; Bordier et al., 1975). Evidence along this line is presented by Malluche et al. (1975) who have reported wider osteoid seams and a decrease in the number of sites taking a tetracycline label in those of a series of patients that had the lowest glomerular filtration rates. The failure of conversion of 25 hydroxy-D3 to 1,25 dihydroxy-D3 has also been seen mostly in patients with advanced renal disease (Stanbury et al., 1973). It is also possible that the defect in mineralization and lack of normal osteoblasts in the two dogs with the most severe lesions represents a later developing fall in 25 hydroxy-D3 levels, if this is the metabolite of key importance as proposed by Bordier et al. (1975). The histology in these 2 cases suggests there are qualitative differences in the osteoid being formed

that also could affect its ability to be mineralized. In any case the observations in these experimental dogs indicate that the bone lesions can present a variety of histological forms, as they do in man, and our previous finding of an increased remodeling activity in haversian bone (Villafane et al., 1977) probably corresponds to an early hyperparathyroid stage in the pathogenesis of the disease.

Acknowledgements. The authors are grateful for financial support for this research by a foreign research fellowship from the Institut National de la Santé et de la Recherche Médicale (I.N.S.E.R.M.) and U.S.P.H.S. Contract FDA 72-302 from the Bureau of Radiological Health, Food and Drug Administration, D.H.E.W., Washington D.C.

References

Barzel, U.S.: The effect of excessive acid feeding on bone. Calcif. Tiss Res. **4**, 94–100 (1969)

Bordier, P.J., Tun Chot, S.: Quantitative histology of metabolic bone disease. J. clin. Endocr. Metab. **1**, 197–215 (1972)

Bordier, P.J., Marie, P., Arnaud, C.D.: Evolution of renal osteodystrophy: Correlation of bone histomorphometry and serum mineral and immuno-reactive parathyroid hormone values before and after treatment with calcium carbonate or 25 hydroxycholecalciferol. Kidney Int. **7**, (suppl. 2), 102–112 (1975)

Carlson, G.P., Kaneko, J.J.: Simultaneous estimation of renal function in dogs using sodium sulfanilate and sodium iodohippurate – ^{131}I. J. Am. vet. med. Assoc. **158**, 1229–1234 (1971)

Duursma, S.A., Visser, W.J., Dorhout Mees, E.J., Njio, L.: Serum calcium, phosphate and alkaline phosphatase and morphometric bone examinations in 30 patients with renal insufficiency. Calcif. Tiss. Res. **16**, 129–138 (1974)

Ellis, H.A. Peart, K.M.: Azotaemic renal osteodystrophy: a quantitative study on iliac bone. J. Clin. Path. **26**, 83–101 (1971)

Frost, H.M.: Tetracycline-based histologic analysis of bone remodeling. Calcif. Tiss. Res. **3**, 211–237 (1969)

Gitelman, H.J.: Uremia toxins and mineral metabolism. Arch. intern. Med. **126**, 793–800 (1970)

Hitt, O., Jaworski, Z.F., Shimitzu, A.G., Frost, H.M.: Tissue level bone formation rates in chronic renal failure measured by means of tetracycline bone labelling. Can. J. Physiol. Pharmacol. **48**, 824–828 (1970)

Kleeman, C.R., Massry, S.G., Coburn, J.W., Popovtzer, M.M.: Calcium and phosphorus metabolism and bone disease in uremia. Clin. Orthop. **68**, 210–237 (1970)

Krempien, B., Ritz, E., Beck, U., Keilbach, H.: Osteopathy on maintenance hemodialysis. Virchows Arch. Abt. A Path. Anat. **357**, 257–274 (1972)

Malluche, H.H., Ritz. E. Kutschera, J., Krause, G., Werner, E., Gati, A., Sieffert, U., Lange, H.P.: Calcium metabolism and impaired mineralization in various stages of renal insufficiency. In: Vitamin D and problems related to uremic bone disease. (Norman A.W., Schaefer K., Grigoleit H.G., von Herrath D., Ritz, E., eds.) pp. 513–516. Berlin: Walter de Gruyter 1975

Merz, W.A., Schenk, R.K.: A quantitative histological study on bone formation in human calcellous bone. Acta. anat. **76**, 1–15 (1970)

Meunier, P., Edouard, C., Bressot, C., Valat, J.N., Courpron, P.. Zech, P.: Histomorphometrie osseuse dans l'insuffisance rénale aigue et chronique. J. Urolog. Nephrol. **12**, 931–940 (1975)

Meunier, P.J., Edouard, C.: Histomorphometry of the hyperosteoidosis states. In: Proceedings of the second international workshop on bone histomorphometry. (Meunier, P.J., ed.) In press, 1977

Ojerio, A.D., Norrdin, R.W., Jaenke, R.S.: Morphometric and ultrastructural evaluation of hyperparathyroidism in dogs with experimentally induced chronic renal failure (Abstract) Lab. Invest. **32**, 453 (1975)

Olah, A.J.: Quantitative relations between osteoblasts and osteoid in primary hyperparathyroidism, intestinal malabsorption and renal osteodystrophy. Virchows Arch. Abt. A Path. Anat. **358**, 301–308 (1973)

Phemister, R.D., Thomassen, R.W., Norrdin, R.W., Jaenke, R.S.: Renal failure in perinatally irradiated beagles. Radiat. Res. **55**, 399–410 (1973)

Rasmussen, H., Bordier, P.: The physiologogical and cellular basis of metabolic bone disease. p. 6. Baltimore: Williams and Wilkins Co. 1974

Sarnsethsiri, P., Zaworski, Z.F., Shimizu, A.G., Frost, H.M.: New bone formation, osteoid seam thickness and appositional rate in patients undergoing renal dialysis. Arch. Path. **88**, 49–53 (1969)

Schenk, R.K., Merz, W.A., Muller, J.: A quantitative histological study on bone resorption in human cancellous bone. Acta. anat. **74**, 44–53 (1969)

Sherrard, D.J., Baylink, D.J., Wergedal, J.E., Maloney, N.A.: Quantitative studies on the pathogenesis of uremic disease. J. Clin. Endocr. Metab. **39**, 119–135 (1974)

Slatopolsky, E., Caglar, S., Pennel, J.P., Taggart, D.D., Canterbury, J.M., Reiss, E., Bricker, N.S.: On the pathogenesis of hyperparathyroidism in experimental renal insufficiennecy in the dog. J. clin. Invest. **50**, 492–499 (1971)

Stanbury, S.W., Maurer, E.B., Lumb, G.A., Hill, L.E., Holman, C.A., Taylor, C.M. Torkington, P.: Vitamin D metabolism an renal bone disease. In: Clinical Aspects of Metabolic Bone Disease. (Frame, B., Parfitt, A.M., Duncan, H., eds.) p. 562–573. Amsterdam: Excerpta Medica 1973

Villafane, F., Norrdin, R.W., LoPresti, C.A., Kimmel, D.: Bone remodeling in chronic renal failure of perinatally irradiated beagles. Accepted for publication, Calcif. Tiss. Res. (1977)

Woods, C.: Why count osteoblasts? In: Proceedings of the second international workshop on bone histomorphometry (Meunier, P.J. ed.) In press, 1977

Received April 18, 1977