Virchows Arch. A Path. Anat. and Histol. 366, 249—256 (1975)


# Structural Changes of Cortical Bone in Secondary Hyperparathyroidism

## Replacement of Lamellar Bone by Woven Bone

B. Krempien*, G. Geiger, and E. Ritz

Department of Pathology, Head: Prof. Dr. W. Doerr, and Department of Internal Medicine, Head: Prof. Dr. G. Schettler, University of Heidelberg

Received March 16, 1974

*Summary.* In femoral cortical bone of 16 uremic patients with long standing renal insufficiency an increased fraction of woven bone was found both in Haversian and in interstitial bone. Either partly resorbed Haversian systems were replaced by non lamellar woven bone or single Haversian systems showed partly well organized lamellar bone and partly disorganized non lamellar texture without signs of antecedent resorption. The replacement of lamellar bone by woven bone was measured morphometrically in undecalcified ground sections. Woven bone was defined by its lack of structural birefringence under polarized light. In advanced cases of secondary hyperparathyroidism more than 60% of cortical bone were composed of woven bone. The substitution of immature less organized woven bone for mature well organized lamellar bone has important implications for the biomechanical properties of the skeleton.

The skeleton of human adults is composed of lamellar bone. The structure of lamellar bone shows some principles that are used in technology as composite material (Knese, 1958). The combination of collagen fibers, resistant to traction, and mineral crystals, resistant to compression, allows to meet static demands with a minimum of material. The light-weight construction of the skeleton is made possible by the skeleton's ability to adapt its architecture to functional demands (Kummer, 1971). This implies continous skeletal remodelling (i.e. removal of superfluos or damaged elements with modelling of new structures required by mechanical or metabolic needs).

The biomechanical properties of the skeleton depend on the structure of bone tissue: bone geometry, number and spatial arrangement of lamellar systems, direction and quality of collagen fibers and mineral density. Secondary hyperparathyroidism induces increased bone turnover with the appearance of woven bone. Woven bone is primitive bone with an irregular haphazard arrangement of collagen fibers. Lack of directional orientation of collagen is the cause of its inferior biomechanical properties. In patients with chronic renal failure and advanced secondary hyperparathyroidism, mechanical insufficiency of the skeleton causes severe clinical problems (Uehlinger, 1955; Krempien *et al.*, 1973, 1974; Mehls *et al.*, 1973; Ritz *et al.*, 1973; Tschöpe *et al.*, 1973; Griss *et al.*, 1974). It may be partly the consequence of the appearance of woven bone. It was therefore the purpose of the present investigation of femoral cortical bone of patients with

* With support of Deutsche Forschungsgemeinschaft.

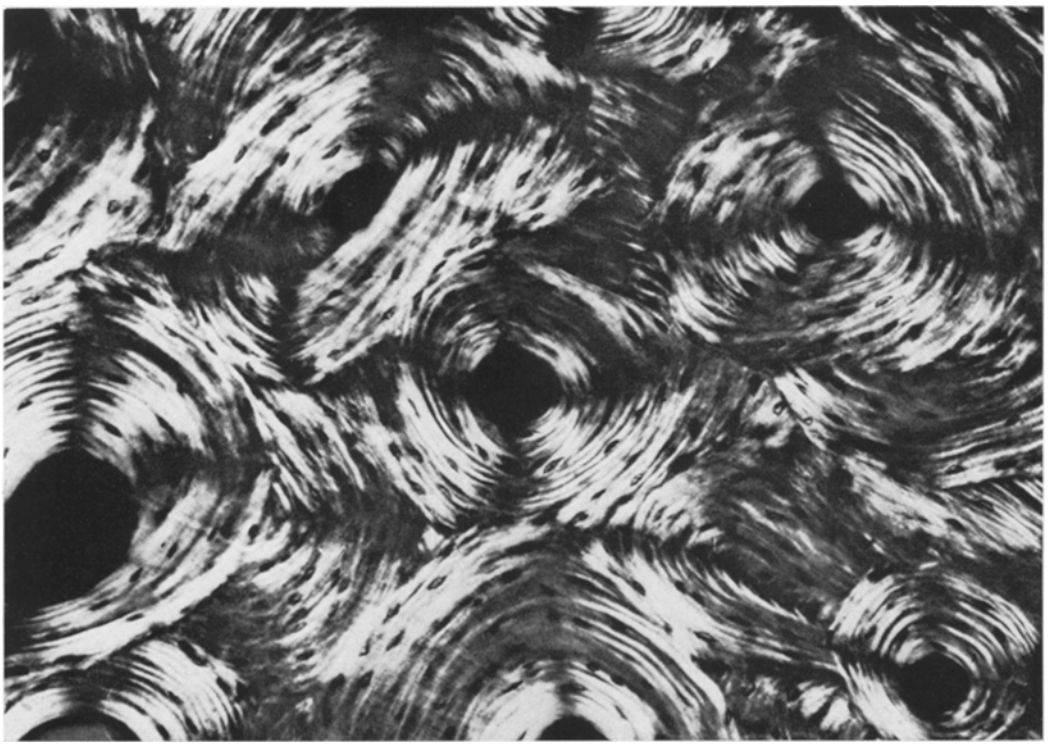

Fig. 1a

Fig. 1. (a) Femoral diaphysis. Lamellar bone with regular birefringence of Haversian systems and of interstitial lamellae under polarized light. 40 year old man, sudden death, no skeletal disease. (b) Femoral diaphysis. Severe secondary hyperparathyroidism. Structural birefringence is considerably lost due to subtotal replacement of lamellar bone by sharply demarcated woven bone in osteones (middle) and in interstitial bone. 43 year old woman, chronic uremia. (c) Femoral diaphysis. Several Haversian systems with gradual transition from lamellar texture of collagen to non-lamellar woven texture within a single osteon. 49 year old woman, chronic renal failure. Undecalcified ground sections. Microphotograph. Enl. 1:125

secondary hyperparathyreoidism, to quantitate the fraction of woven bone, to study the involvement of Haversian and non Haversian bone and to investigate whether differences of collagen texture (lamellar, woven) are only between or also within single Haversian systems.

## Material and Methods

16 patients with chronic renal insufficiency and a serum creatinine above 5 mg-% (mean age: 38.4 ± 16.6 y.) and 8 control patients without skeletal disease were studied. Undecalcified bone samples taken from the anterior part of the right femoral diaphysis were fixed in ethanol (70%). Without prior embedding slices were taken with a saw and ground sections with a final thickness of 70 μ were prepared by grinding with carborundum paper. All specimens were polished, freed from abrasions particles in an ultrasonic bath and studied under polarized light to distinguish lamellar and woven bone. Woven bone was identified by its lack of structural birefringence due to the irregular spatial arrangement of the collagen fibers (Fig. 1a–c). Point

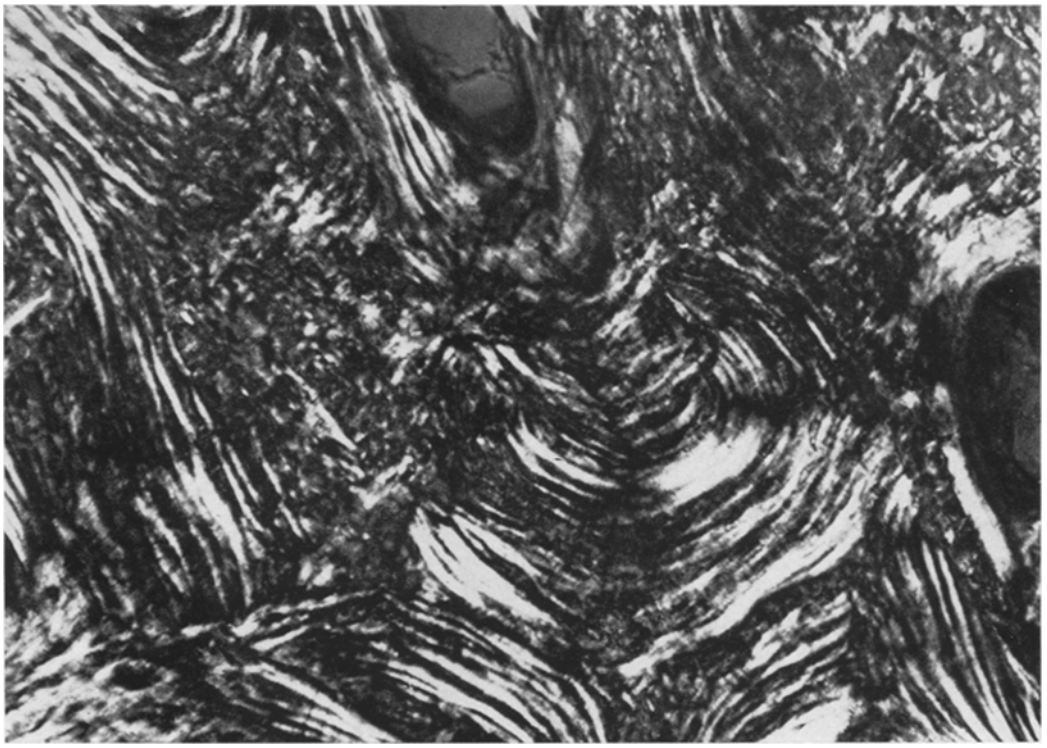

Fig. 1b

Fig. 1c

counting procedures could not be applied since under polarized light the grid could not be identified microscopically in unlit parts. Therefore, in each specimen, 8 areas ($200 \times 200$ µ) of the outer, middle and inner cortex were photographed at a magnification of 1:125. The photographs were enlarged (1:10). The outlines of the areas with lamellar and non lamellar bone in interstitial bone as well as in Haversian systems were marked with a pencil. Non osseous areas (Haversian channels, porosity) were subtracted from the total area. The areas with lamellar and non lamellar bone were cut out and weighed. Non lamellar bone was calculated as fraction of total Haversian or total interstitial area.

## Results

As shown in Fig. 2a, in all uremic patients the fraction of Haversian bone represented by woven bone exceeded the 95% confidence limits of the control patients without skeletal disease. The difference between the two groups was highly significant ($p<0.001$) with the non parametric Wilcoxon-test for random samples. Whether in normals the small fraction of bone that did not show up as lamellar bone under polarized light, represents true woven bone or whether its non-lamellar appearance must be explained by the limited accuracy of the method cannot be decided. Similar in almost all uremic patients the fraction of interstitial bone represented by true woven bone exceeded the 95% confidence limits of normals (Fig. 2b). Again the difference between the two groups was significant ($p<0.01$). Substitution of woven bone for lamellar bone in Haversian systems occurred in two different ways. Either partly resorbed Haversian systems were replaced by non lamellar woven bone sharply demarcated from lamellar bone (Fig. 1b); or single Haversian systems showed partly well ordered lamellar and partly disorganized non-lamellar texture without signs of antecedent resorption of preexisting lamellar bone (Fig. 1c).

## Discussion

In human bone two types of osseous tissue can be found: lamellar bone and woven bone. They can be differentiated histologically by their behaviour under polarized light (v. Ebner, 1874). In adults, lamellar bone forms almost the entire skeleton. Lamellar bone replaces the fibrous bone of the fetal skeleton during the first three years of postnatal life (Aeby, 1877; Kölliker, 1886; Steendijk, 1971). Beyond that age woven bone is found in growth, repair and reaction. In adult bone, major amounts of woven bone appear in rapid bone formation (fracture healing, Paget's disease, hyperparathyroidism; v. Recklinghausen, 1891; Ascenzi and Marinozzi, 1961). Woven bone is also a characteristic feature of renal bone disease (Garner and Ball, 1966; Binswanger *et al.*, 1971). Presumably as a consequence of the replacement of lamellar bone by woven bone the resistance of the skeleton to mechanical deformation is reduced in uremic patients. Inferior strength of bone tissue together with cortical thinning and increased cortical porosity leads to bone fractures (Uehlinger, 1955; Tschöpe *et al.*, 1973; Griss *et al.*, 1974) in the adult skeleton and to epiphyseolysis in the growing skeleton (Krempien *et al.*, 1974).

There is a clear correlation between the structure of bone and its biomechanical properties: as in steel reinforced cement, the interaction of mineralized ground substance and spiralling collagen fibers confers upon bone a remarkable resistance to axial compression, axial tension, bending and twisting (Knese, 1958, 1960). On

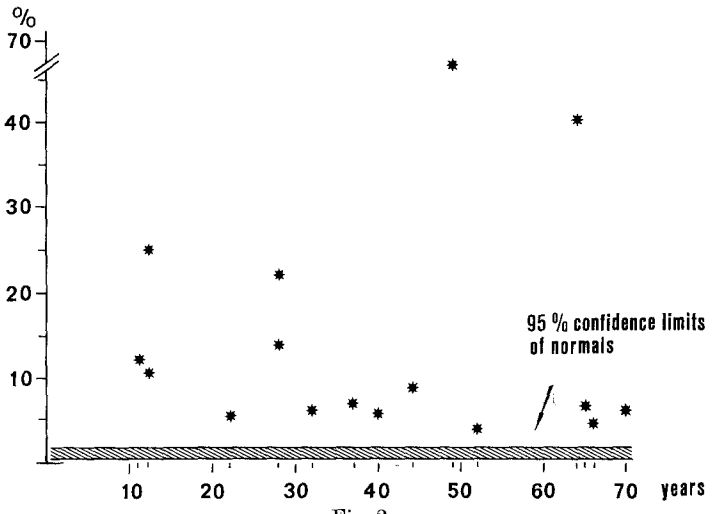


Fig. 2a

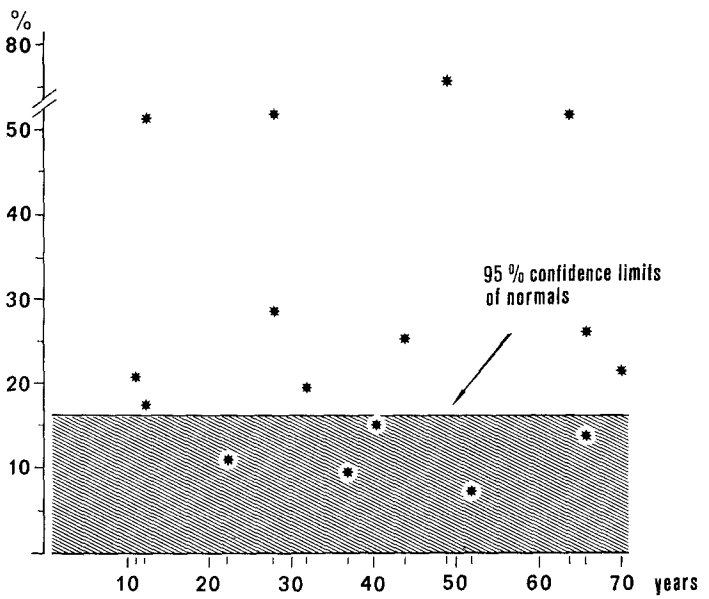


Fig. 2b

Fig. 2. (a) Quantitative measurements of the fraction of woven bone in Haversian systems of 16 uremic patients. (b) Quantitative measurements of the fraction of woven bone in interstitial lamellae of 16 uremic patients

various levels of organization (polypeptide chain, filament, fiber, lamella) collagen is arranged in spirals. On the osteone level, collagen fibers in different lamellae run in alternate directions and thus contribute to the superior biomechanical quality of lamellar bone (Ascenzi *et al.*, 1966). The angle between collagen fibers and the longitudinal axis of the osteone is different in different lamellae and parti-

cular in different osteones (Knese, 1958). According to Johnson (1963) there is suggestive evidence for a diurnal variation of osteoblast activity in lamellar bone, osteoblasts spinning collagen fibers in opposite directions on successive days. In scanning electron microscopical studies Boyd (1974) found a close association of cell and collagen orientation in bone formation. He therefore concluded that collagen orientation is controlled by cell orientation. Mechanical properties of osteones, for instance Vickers's bone hardness, clearly depend on the spatial orientation of collagen fibers (Amprino, 1958; Ascenzi *et al.*, 1966, 1973). Bone hardness is reduced in osteones of uremic patients when compared with those of normals without skeletal disease (Krempien *et al.*, 1973).

Woven bone is laid down by the simultaneous continuous and unpolarized action of cells surrounding themselves on all sides with bone matrix. Therefore collagen fibers in the woven bone lack a regular spatial pattern. This lack of spatial orientation gives rise to its lack of structural birefringency and to its inferior strength. It is probable that lamellar bone is constructed on sounder biomechanical principles than woven bone giving more strength for a given volume of material (Pritchard, 1956). Mechanical properties of bone have also been shown to depend on the degree of mineralization (Carlström, 1954; Ascenzi and Marinozzi, 1961). Since bone in chronic uremia tends to be less mineralized, its inferior strength may partially be explained by its diminished mineral content.

In rachitic animals collagen has been shown to be less extensively cross-linked (Mechanic *et al.*, 1972). Similarly in rats with experimental uremia, increased amounts of immature soluble collagen have been found by Hahn and Avioli (1970). It remains to be seen, whether one can extrapolate from this finding (obtained in an animal model of bone without Haversian osteones) to what happens in the human skeleton or whether this finding is related to the appearance of woven bone in chronic renal failure of humans.

It is remarkable that both lamellar and woven bone may be found within one single osteon without signs of antecedent resorption of preexisting lamellar bone (Fig. 1 c). This implies that both lamellar and fibrous osteoblasts may be derived from the same clone of precursor cells. These intra-Haversian changes of collagen texture are even better visualised by scanning electron microscopy (Krempien *et al.*, 1974). A similar alteration of osteones has been observed in human fluorotic bone (Singh *et al.*, 1962; Aggarwal, 1973). It may seem surprising that the proportion of woven bone in interstitial bone exceeds that in osteones, although the latter have been formed later and presumably in more advanced stages of uremia. The data on interstitial bone are to some extent subject to artifacts, however, since the smaller dimensions in the interstitial areas may cause spuriously low structural birefringence.

The appearance of woven bone might lead to profound disturbances of bone physiology. If elastic deformation of bone plays a role in initiating bone remodelling (Pauwels, 1965), perception of elastic deformation must be decisively altered in osteocytes embedded in non-polarized matrix. This may explain why fibrous osteoblasts appear to be largely independent of the space polarizing factors that govern remodelling in accordance with the physical load of the skeleton. As a consequence spongiosal trabeculae for instance are no longer arranged in tra-

jectorially but in an irregular haphazard fashion, as is easily recognized in metaphyseal bone of uremic children (Krempien *et al.*, 1974). Also for a given static function mechanically inferior woven bone must accumulate in increased quantities giving rise to osteosclerosis. After Baylink *et al.* (1973) local provision of calcium by periosteocytic osteolysis triggers remodelling processes. One may speculate that the vastly augmented turnover of woven bone (Rasmussen and Bordier, 1974) is the consequence of activity of osteocytes in woven bone, the appearance of which is characteristically that of "activated osteocytes".

## References


Aeby, Ch.: Ueber Knochenwachsthum. Tagebl. 49. Vers. Dtsch. Naturforsch. Ärzte, Hamburg, 126 (1876)

Aggarwal, N.D.: Structure of human fluorotic bone. J. Bone Jt Surg. **55** A, 331–334 (1973)

Amprino, R.: Investigations on some physical properties of bone tissue. Acta anat. (Basel) **34**, 161–186 (1958)

Ascenzi, A., Bonucci, E., Checcuci, A.: The tensile properties of single osteones studied using a microwave extensimeter. In: Studies on the anatomy and function of bone and joints (F. G. Evans, ed.), p. 121–141. Berlin-Heidelberg-New York: Springer 1966

Ascenzi, A., Bonucci, E., Simkin, A.: An approach to the mechanical properties of single osteonic lamellae. J. Biomech. **6**, 227–235 (1973)

Ascenzi, A., Marinozzi, V.: Biophysical study of von Recklinghausen's disease of bone: study of a case. Arch. Path. **72**, 297–309 (1961)

Baylink, D., Sipe, J., Wergedal, J., Whittemore, O.J.: Vitamin D-enhanced osteocytic and osteoclastic bone resorption. Amer. J. Physiol. **224**, 1345–1357 (1973)

Binswanger, U., Fischer, J., Schenk, R., Merz, W.: Osteopathie bei chronischer Niereninsuffizienz. Dtsch. med. Wschr. **96**, 1914–1919 (1971)

Ball, J., Garner, A.: Mineralisation of woven bone in osteomalacia. J. Path. Bact. **91**, 563–657 (1966)

Carlström, D.: Microhardness measurements on single Haversian systems in bone. Experientia (Basel) **10**, 171–172 (1954)

Ebner, V. v.: Über den feineren Bau der Knochensubstanz. S.-B. ksl.Akad. Wiss., math.-nat. Kl. 1874, Abt. III, 49–138

Garner, A., Ball, J.: Quantitative observations on mineralized and unmineralized bone in chronic renal azotemia and intestinal malabsorption syndrome. J. Path. Bact. **91**, 545–561 (1966)

Griss, P., Krempien, B., Andrian-Werburg, H. v.: Urämische Osteopathie als Ursache einer zweizeitigen Hüftendoprothesenlockerung. Z. Orthop. **112**, 1157–1161 (1974)

Hahn, Th. J., Avioli, L.V.: Effects of chronic uremia on collagen metabolism in skin and bone. Arch. intern. Med. **126**, 882 (1970)

Johnson, L.C.: Morphologic analysis: the kinetics of disease and general biology of bone. In: Bone biodynamics. Intern. Symp., ed. H. M. Frost, p. 543–654. Boston, Mass.: Little Brown Comp. (1964)

Knese, K.H.: Knochenstruktur als Verbundbau. In: Zwangl. Abh. norm. path Anat., ed. W. Bargmann, W. Doerr, 4. Stuttgart: G. Thieme 1958

Knese, K.H.: Mechanik und Festigkeit des Knochengewebes. In: Handbuch der medizinischen Radiologie, ed. L. Diethelm, Bd. IV/1, S. 417–534. Berlin-Heidelberg-New York: Springer 1970

Kölliker, A.: Der feinere Bau der Knochengewebe. Z. Zool. **44**, 644–680 (1886)

Krempien, B., Geiger, G., Ritz, E.: Hardness of bone in various ages and diseases. Calc. Tiss. Proc., 9th Europ. Symp. Calc. Tiss., p. 95–99. Wien: Facta Publication 1973

Krempien, B.: Stoffwechsel und Struktur des Knochengewebes bei chronischer Niereninsuffizienz. Verh. dtsch. Ges. Path. **58**, 156–175 (1974)

Krempien, B., Geiger, G., Ritz, E.: Alteration of bone tissue structure in secondary hyperparathyroidism. A scanning electron microscopical study. Proc. 2nd Workshop on Vitamin D, Wiesbaden 1974

Krempien, B., Mehls, O., Ritz, E.: Morphological studies on pathogenesis of epiphyseal slipping in uremic children. Virchows Arch. Abt. A **362**, 129–143 (1974)
Kummer, B.: Trajectorial structures in the supporting apparatus; structural remodelling due to functional adaptation. 1st Colloquium of Biology and Building (1972)
Mechanic, G.L., Toverud, S.U., Ramp, W.K.: Qualitative changes of bone collagen cross links and precursors in vitamin D deficiency. Biochem. biophys. Res. Commun. **47**, 760–765 (1972)
Mehls, O., Ritz, E., Krempien, B., Willich, E., Schärer, K.: Roentgenological signs in the skeleton of uremic children. An analysis of the anatomical principles underlying the roentgenological changes. Pediat. Radiol. **1**, 183–190 (1973)
Pauwels, F.: Gesammelte Abhandlungen zur funktionellen Anatomie des Bewegungsapparates. Berlin-Heidelberg-New York: Springer 1965
Pritchard, J.J.: General anatomy and histology of bone. In: The biochemistry and physiology of bone, ed. G.H. Bourne, p. 1–26. New York: Academic Press Inc. 1956
Rasmussen, H., Bordier, P.: The physiological and cellular basis of metabolic bone disease. Baltimore: Williams and Wilkins 1974
Recklinghausen, F.v.: Die fibröse oder deformierende Ostitis, die Osteomalacie und die osteoplastische Carcinose in ihren gegenseitigen Beziehungen. Separatdruck aus der R. Virchow zum 13. 10. 1891 gewidmeten Festschrift der Assistenten
Ritz, E., Kuhn, H.M., Krempien, B., Beduhn, D.: Röntgenologische Zeichen des gestörten Calciumstoffwechsels bei Dialysepatienten. I. Häufigkeit röntgenologischer Skelettveränderungen. Fortschr. Röntgenstr. **119**, 52–63 (1973)
Singh, A., Dass, R., Harem, S.S., Jolly, S.S.: Skeletal changes in endemic fluorosis. J. Bone Jt Surg. **44** B, 806–816 (1962)
Steendijk, R.: Metabolic bone disease in children. Clin. Orthop. rel. Res. **77**, 247–275 (1971)
Tschöpe, W., Ritz, E., Bommer, J., Krempien, B., Andrassy, K., Mehls, O.: Wirbelkörperkollaps bei Dialyseosteopathie. Dtsch. med. Wschr. **98**, 1471–1474 (1973)
Uehlinger, E.: A-Avitaminase und renale Osteomalacie. Schweiz. med. Wschr. **85**, 521–527 (1955)

Priv. Doz. Dr. B. Krempien
Pathologisches Institut der Universität
D-6900 Heidelberg
Im Neuenheimer Feld 220/221
Federal Republic of Germany