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Osteoporosis in longstanding acromegaly: characteristic changes of vertebral trabecular architecture and bone matrix composition

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Summary. Although it is now 60 years after Erdheim's (1931) detailed description of vertebral alterations in severe acromegaly, it is still unclear whether osteoporosis is a consistent feature of acromegalic bone disease or not. We studied the vertebral trabecular bone of a 44-year-old woman who had suffered active acromegaly for more than 20 years, and compared it with 17 normal as well as 2 osteoporotic controls. Histomorphometry revealed a very low trabecular bone volume and thus documented the presence of osteoporosis. The mean trabecular plate thickness was strikingly increased in acromegaly (possibly caused in part by a low-dose fluoride treatment), whereas it was normal or reduced in the osteoporotic controls. The meticulous analysis showed islands of cartilaginous tissue in the core of the acromegalic trabeculae which were not present in any other sample. In these areas collagen II was detected by immunohistochemistry. Biochemical analysis revealed that collagen II accounted for 7% of the total collagenous matrix. The degree of hydroxylation of lysyl residues of collagen I was close to the average value of all control samples studied. Our data show that osteoporosis can occur in acromegaly and that it is characterized by unusual architectural and compositional features. These findings challenge the prevailing view that the matrix of osteoporotic bone always shows a normal composition.

Key words: Acromegaly – Osteoporosis – Collagen type II – Lysyl hydroxylation

Introduction

Sixty years ago, in *Virchows Archiv*, Erdheim (1931) gave the classical and detailed description of the changes in vertebral bone which occur in longstanding acromegaly. Since that time a number of investigators have studied the bone of acromegalic patients, mainly by analysing biopsies from the iliac crest (Delling and Schulz 1977; Halse et al. 1981; Riggs et al. 1972).

However, some issues are still controversial. Does the excess of growth hormone lead to osteoporosis, and if so, does the osteoporosis in acromegaly differ from other forms of osteoporosis with respect to architectural or biochemical features?

The difficulty in answering these questions may arise from the possibility that bone changes could vary both in early and late phases of the disease as well as in different regions of the skeleton. Histomorphometrical data on acromegalic vertebral trabecular bone are lacking in the literature. However, this bone represents the localization where probably the most severe changes occur. Therefore, we analysed the spine of a case of longstanding acromegaly and compared it with spines from patients without bone disease and with osteoporosis. In addition to a detailed morphometrical analysis of the trabecular structure we studied the composition of the collagenous bone matrix. To our knowledge a combined histomorphometrical, immunohistochemical and biochemical investigation of acromegalic bone tissue has not been previously performed.

Materials and methods

Spines were obtained from a total of 20 subjects at autopsy. Of these, 17 patients, 22–91 years of age, had no evidence of bone disease. Two patients suffered from osteoporosis according to the definition of Nordin (1987), in 1 (22-year-old male) presumably due to long-term treatment with corticosteroids for Crohn's disease, in the other (67-year-old female) probably of post-menopausal type. All these patients served as controls. Their bone structure has been described in detail elsewhere (Diebold et al. 1990).

A case of a 44-year-old woman with longstanding acromegaly was analysed. The onset of the disease dated back to the age of 13, when gigantism developed and a pituitary adenoma was found. Several operations, radio-gold inlays and telecobalt irradiations followed until the age of 19 and caused amaurosis on the left side. However, the disease remained clinically active until the age of 35, when medical treatment with bromocriptine was instituted.

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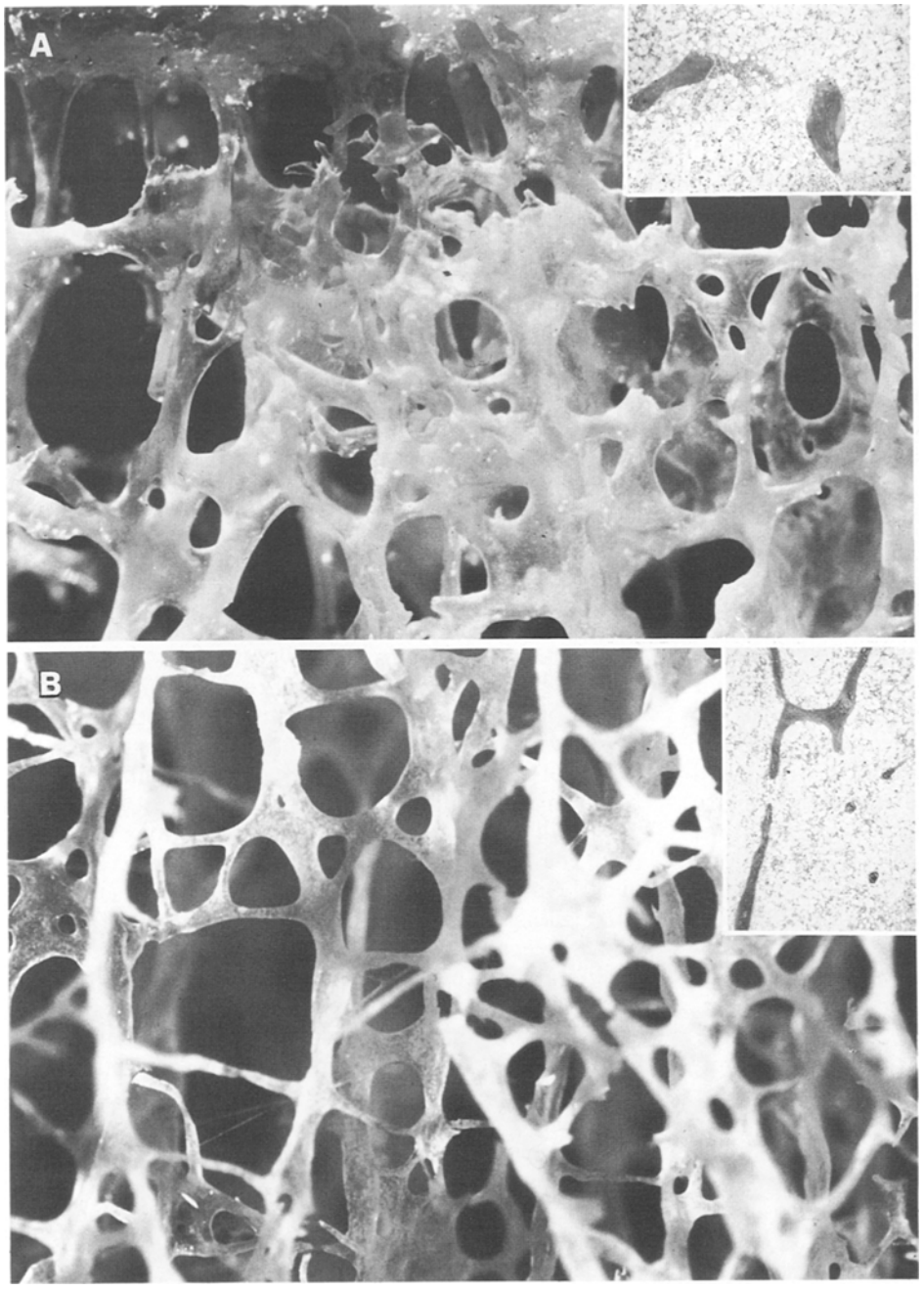

Fig. 1. Comparison of vertebral trabecular architecture in acromegaly (**A**) and in a osteoporotic control (**B**). Macroscopic appearance and representative microscopic fields (*insets*), on which morphometry was performed. Masson-Goldner, ×12

At that time the basal level of growth hormone was 101 ng/ml and showed a paradoxical increase during an oral glucose tolerance test. (An insulin-like growth factor 1 assay was not available a decade ago.) The patient was euthyroid (T4 61 ng/ml, T3 0.8 ng/ml, TSH 1.0 μU/ml) and had normal levels of cortisol both at base line (111 ng/ml) and after adrenocorticotropic hormone (214 ng/ml). The prolactin level was in the normal range as well (85 mIU/ml). Basal levels of luteinizing hormone (1.8 mIU/ml) and follicle-stimulating hormone (1.0 mIU/ml) were normal, but responded insufficiently to luteinizing hormone releasing hormone (2.1 and 1.1 mIU/ml, respectively) and thus revealed a latent hypogonadism. Bromocriptine treatment was started with a maximal dose of 50 mg/day and the growth hormone level fell to 7.9 ng/ml. It remained low under continuous medication until death. Diabetes mellitus was never present. During a respiratory infection weakness and lethargy developed, which were interpreted as clinical signs of adrenal insufficiency. Subsequently cortisol was substituted regularly with hydrocortisone (20/10/5 mg/day). A control at the age of 37 revealed a growth hormone level of 14 ng/ml. The prolactin level was again in the normal range.

Main complications were caused by the changes of the skeletal system. Severe kyphoscoliosis of the spine and generalized osteoarthrosis, particularly severe in the knees, had developed. On conventional radiography the whole skeleton was radiolucent. Six years prior to death the right lower leg fractured and healing was prolonged. From that time calcium (250 mg/day) and sodium fluoride (25 mg/day) were given. The patient was wheelchair-bound during the last years of her life and lived in a nursing home. Clinically she died of pneumonia.

At autopsy gigantism and acromegaly, with characteristic coarsening of facial features, were seen (body weight 132 kg, body length 214 cm). Visceral enlargement was remarkable (heart 610 g, liver 3200 g, spleen 350 g, kidneys combined 570 g). Residual tumour tissue of the pituitary adenoma was seen in the basal cisternae

Table 1. Morphometrical results

	Acromegaly $n=1$	Osteoporosis $n=2$	Controls $n=17$
TBV (%)	4.7	3.1[a]/6.0[b]	9.6±2.3
Sv (mm^2/mm^3)	0.674	0.860/1.350	1.933±0.330
S/V (mm^2/mm^3)	14.34	27.74/22.50	20.75±3.73
MTPT (μm)	139.5	72.1/88.9	99.7±20.3
MTPD (l/mm)	0.337	0.430/0.675	0.966±0.165
MTPS (μm)	2795.8	2253.5/1392.6	966.4±194.8

TBV, Trabecular bone volume; Sv, bone surface density; S/V bone surface to volume ratio; MTPT, mean trabecular plate thickness; MTPD, mean trabecular plate density; MTPS, mean trabecular plate separation

[a] First value relates to the case of post-menopausal osteoporosis, [b] second value to the case of steroid osteoporosis

demonstrating regressional changes. Immunohistochemically growth hormone and prolactin were detected in tumour cells. Frontal sinuses were excessively enlarged and the brain swollen (weight 1380 g). The cerebellum protruded into the foramen magnum. The main other findings were severe pulmonary emphysema, chronic bronchitis, severe kyphosis of the thoracic spine, osteoarthrosis of the knees and hips, moderately stenotic coronary atherosclerosis, diffuse myocardial fibrosis, mild atherosclerosis of the aorta and the main arteries and urolithiasis. Gastrointestinal haemorrhage, presumably of gastric origin, and bronchopneumonia were regarded as immediate causes of death.

Samples from the central part of the second lumbar vertebral body at least 1 cm away from the intervertebral disc were used for morphometrical analysis. Undecalcified sections (4 μm) were stained according to Goldner's trichrome method and an area of 20–30 mm^2 was analysed with a Zeiss Universal microscope (Zeiss, Oberkochen, FRG) at a magnification of ×160. The microscope was connected to a camera (DXC-101P, Sony, Japan) and a digitizing tablet (Videoplan, Kontron, Eching, FRG). Trabecular bone area as a fraction of total trabecular tissue area (marrow and bone) and the perimeter of marrow-bone interface were determined in the same microscopic field. In addition, the mean osteoid seam width was measured. In accordance with normal stereological practice no attempt was made to control the plane of sectioning. The following three-dimensional indices were calculated according to Parfitt et al. (1983): trabecular bone volume (TBV), bone surface density (Sv), bone surface to volume ratio (S/V), mean trabecular plate thickness (MTPT), mean trabecular plate density (MTPD), and mean trabecular plate separation (MTPS).

Bone tissue from all parts of the second and third lumbar vertebrae was fixed in 10% formalin, decalcified in ethylenediaminetetra-acetic acid (EDTA), embedded in paraffin, cut at 5 μm, dewaxed and stained with haematoxylin and eosin, and alcian periodic acid-Schiff (PAS).

As immunohistochemistry of bone matrix collagens is not possible on undecalcified tissue (Gay et al. 1976) it was also performed on the paraffin-embedded material. Antisera against collagen types I, II, III and IV were generated in rabbits. After digestion with hyaluronidase (5 ng/ml; Boehringer, Mannheim, FRG) and protease K (2 mg/ml; Merck, Darmstadt, FRG) the deparaffinized tissue sections were incubated for 2 h with the primary antisera (dilution 1:200–1:500). Incubations with biotinylated polyvalent antibody and with a preformed avidin-biotinylated horseradish peroxidase complex (ABC Vectastain, Burlingame, Calif., USA) followed. As colour substrate aminoethylcarbazole was added. Negative controls were performed by omitting the specific antiserum and using normal rabbit serum, instead.

Slabs of vertebral bone (2 cm thick) including both thoracic and lumbar vertebrae were taken for biochemical analysis. Cortical bone, intervertebral discs and adherent connective tissue were carefully removed. The remaining trabecular bone was cut into small cubes, which were washed extensively in distilled water and 96% ethanol to remove blood and residual fat. Using a steel homogenizer and a conventional mortar bone samples were powdered under liquid nitrogen.

Details of the further processing of the bone powder (2–10 g) have been published elsewhere (Bätge et al. 1990a). Briefly, the material was demineralized with EDTA in the presence of phenylmethansulphonylfluoride as a protease inhibitor. Collagens were extracted by repeated, but limited pepsin digestion. In order to enrich type III and to isolate type I collagen sequential neutral salt precipitations were performed. Electrophoretic separation of the collagens was carried out on sodium dodecyl sulphate-polyacrylamide gels, which were stained with Coomassie blue. The relative amounts of the different compounds were measured by densitometry and identified by immuno-blotting using antisera against collagen types I, II, III and V. The α_1 chain of collagen II was identified by cleavage with cyanogen bromide in 70% formic acid (30° C, 4 h). The α_1 and α_2 chains of collagen I were isolated on a reverse phase HPLC column. Subsequently, an amino acid analysis of the separated alpha chains was performed. The extent of hydroxylation of lysine residues was expressed as the ratio of hydroxylysine/(hydroxylysine + lysine)×1000. Data are expressed as means ± standard deviation.

Results

On macroscopical examination the vertebral trabecular bone of the acromegalic woman differed in several ways from the control samples. The number of trabeculae seemed to be reduced as in the osteoporotic cases. However, the bone structure was coarsened due to trabecular thickening (Fig. 1). Compression fractures were found in thoracic vertebrae 9, 10 and 11.

The changes were quantified by microscopical morphometry performed on central parts of the vertebrae (insets in Fig. 1, Table 1). The reduction of TBV, Sv and MTPD was similar in the acromegalic and in the osteo-

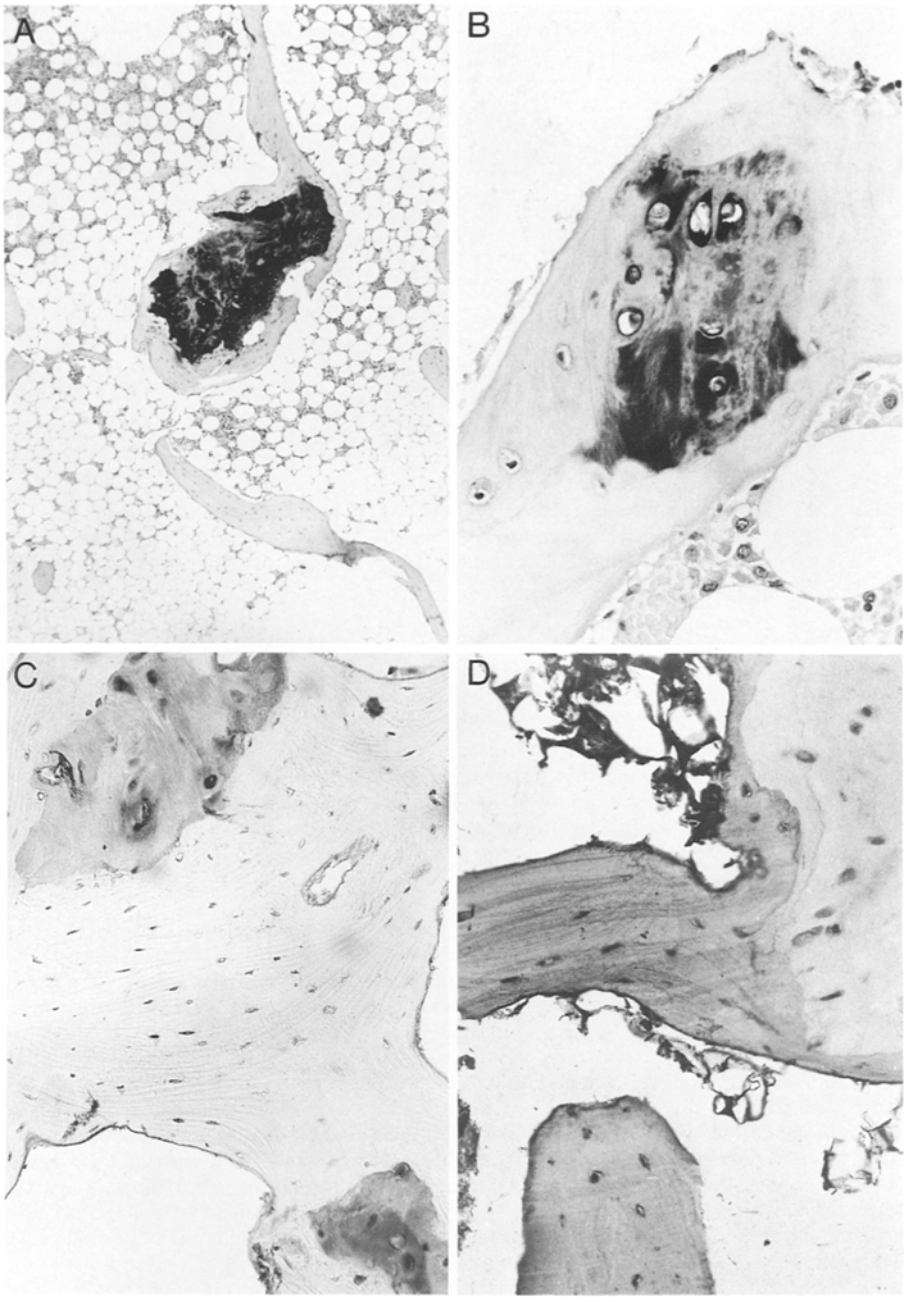

Fig. 2. Vertebral trabecular bone in acromegaly: Alcian/periodic acid-Schiff staining (**A** ×29; **B** ×290) reveals cartilaginous islands in the core of the trabeculae. By immunohistochemistry collagen type II is detected in these areas (**C** ×145), whereas collagen type I is localized in the remaining parts of the trabeculae only (**D** ×180)

porotic cases. TBV was around 2 SD below normal in acromegaly. Accordingly, MTPS was greater than normal in these cases. However, differences between acromegaly and osteoporotic controls were found with respect to MTPT and S/V. MTPT was normal or reduced in osteoporosis, whereas in acromegaly it was 2 SD higher than normal. In agreement with this, the value for S/V was low in acromegaly.

In all samples the mean width of osteoid seams did not exceed 15 μm. A specific stain for osteoclasts (Evans et al. 1979) was tested, but proved to be not feasible for post-mortem tissue. Semi-quantitatively there was no increase of osteoclasts or of resorbing surfaces in any of the cases.

A detailed microscopical examination of the acromegalic cancellous bone in decalcified specimens revealed islands of cartilage tissue in the core of trabeculae, which were found even as far as 2 cm away from the intervertebral disc. None of the control samples contained similar fields. The extracellular matrix of these areas was well stained with alcian-PAS. The extent of these regions varied from around 200 μm to 700 μm (Fig. 2a, b).

By immunohistochemistry type II collagen was exclusively detected within these areas, whereas type I collagen was present in the remaining parts of the trabeculae only (Fig. 2c, d). Small amounts of type V collagen were seen pericellularly around osteocytes, in blood vessel walls and in the endostium covering the trabeculae. All other extracellular matrix areas were devoid of type V collagen.

Table 2. Biochemical characterization of bone matrix collagens

	Acromegaly $n=1$	Controls $n=17$
Collagen types	I/V/II	I/V
Lysyl hydroxylation of α1 (I)	93	99.6±12.8
Lysyl hydroxylation of α2 (I)	178	171.8±18.8

In order to obtain some information about the origin of the cartilaginous islands, the junction between vertebral body and intervertebral disc was carefully studied. Occasionally remnants of columnar cartilage were seen adjacent to the vertebral body (not shown). In addition the bordering bone layer appeared irregular and incomplete and showed uncovered areas, where the disc was in direct contact with the bone marrow.

The results of the biochemical studies of the collagenous bone matrix are summarized in Table 2. The analysis of vertebral trabecular bone gave evidence of the predominance of type I collagen in the acromegalic woman, as well as in the osteoporotic and normal controls. Collagen type V accounted for some 8% of the extractable collagen. With regard to the relative amounts of collagens I and V, no differences were found. Collagen type II was only found in the case of acromegaly. Comigrating with the α_1 chain of collagen I, its presence was confirmed by cleavage with cyanogen bromide (Fig. 3). Collagen II accounted for 7% of the total collagen in this case.

Further analysis of type I collagen demonstrated the expected quantitative ratio of 2:1 of the two chains α_1 and α_2 in all samples studied. In the acromegalic patient the hydroxylysine/(hydroxylysine + lysine) ratio of both alpha chains was close to the average value of the normal controls. The lysyl hydroxylation data of the osteoporotic cases have been published in detail elsewhere (Bätge et al. 1990b).

Discussion

It is still unclear whether excess of growth hormone leads to osteoporosis or not. Erdheim (1931) described a "sclerosing osteoporosis" of vertebral trabecular bone, findings which were corroborated by Remagen (1965). In their textbook of bone diseases Albright and Reifenstein (1948) listed acromegaly as one of the possible causes of secondary osteoporosis. This view was challenged by some investigators who performed histomorphometry on iliac crest biopsies of patients with active acromegaly and found an increase of TBV and MTPT (Delling and Schulz 1977; Halse et al. 1981; Roelfsema et al. 1970).

Regarding these discrepancies it has to be considered that biopsies of the iliac crest showing only a very small bone area might not always reflect the situation completely one would find in the vertebral bodies. However, histomorphometric data on vertebral bone in acromegaly have not yet been published. We therefore studied the vertebral bone of a 44-year-old woman with longstanding acromegaly. Nowadays, such severe cases have fortunately become rare due to therapeutic progress in the past decades.

Our finding of a very low TBV clearly documents the presence of severe spinal osteoporosis in this acromegalic woman. The magnitude of bone loss is in agreement with the criteria for osteoporosis suggested by Nordin (1987). A loss of spinal bone mass in acromegaly has previously been described by other groups, who measured lumbar vertebral bone densities non-invasively (Diamond et al. 1989; Schulz et al. 1989).

The main structural difference between acromegalic osteoporosis and other forms of osteoporosis relates to the MTPT, which reveals changes in opposing directions. In acromegaly the trabeculae were thicker than normal, resulting in low values for the S/V ratio, as has been described by others (Delling and Schulz 1977; Roelfsema et al. 1970). In our case, sodium fluoride, which was given in lower doses than have been reported from clinical trials (Mamelle et al. 1988; Riggs et al. 1990), might have intensified the process of trabecular

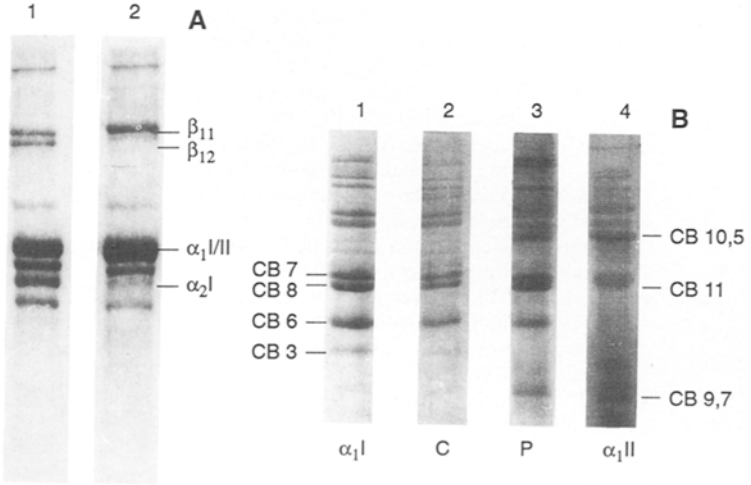

Fig. 3. **A** Pepsin solubilized and salt precipitated (0.7 M NaCl; 0.5 M acetic acid) material from vertebral trabecular bone of the acromegalic patient (*lane 2*) and of an age-matched control (*lane 1*), demonstrating a prominent α_1 band. **B** Cyanogen bromide-cleavage of isolated α_1 chains of collagen I and II (*lanes 1* and *4*) and of the α_1 chain pool of the acromegalic (*3*) as well as the control patient (*2*) reveals the presence of α_1 (II) along with small amounts of α_1 (I) in the acromegalic patient

thickening (Eriksen et al. 1985). In contrast, our osteoporotic controls exemplify that involutional osteoporosis is associated with normal or reduced MTPT (Chappard et al. 1988; Parfitt et al. 1983) and that thin trabeculae are typically seen in steroid-induced osteoporosis (Hills et al. 1989). Neither in the acromegalic patient nor in the controls was active bone remodelling noticed, indicating that all these changes had mainly occurred during a more or less distant period in the past.

The most remarkable feature of the acromegalic vertebral bone was the occurrence of cartilaginous islands in the core of trabeculae even at a great distance from the intervertebral disc. A sampling error could not account for this finding, since all spines were handled in the same way. Such lesions were found exclusively in the acromegalic spine. Collagen II was detected immunohistochemically in these regions corroborating the purely cartilaginous nature of these lesions. From our biochemical data they accounted for 7% of the total vertebral collagenous bone matrix. These lesions differed distinctly from Schmorl's nodes which can be described as herniations of disc substance into the vertebral body (Bullough and Boachie-Adjei 1988). To our knowledge such areas in vertebral cancellous bone have previously been described only by Erdheim (1931). He regarded these acromegalic changes as indicative of a "period of hasty and primitive bone formation" in the past. Formation by endochondral ossification in the intervertebral disc probably represents the most likely explanation for the origin of these cartilaginous islands, since remnants of columnar cartilage were found in the disc adjacent to the vertebral body.

The structural abnormalities observed in the vertebral trabecular bone challenge the prevailing view that osteoporotic bone tissue, which is reduced in quantity, always shows a normal composition. Further scepticism concerning this concept arises from the analysis of the lysyl hydroxylation of collagen I. From all samples of this study as a whole, it has been shown that lysyl hydroxylation was generally elevated when the TBV was low (Bätge et al. 1990b).

However, in the acromegalic patient this value was close to the average value of the normal controls. These observations suggest that the analysis of organic bone matrix might be a useful tool in distinguishing between different forms of osteoporosis. Further investigations in a larger group of patients are required to elucidate whether specific changes in the bone matrix composition or the extent of lysyl hydroxylation characterize a subset of osteoporotic disorders.

The pathogenesis of the bone loss in acromegaly has not been completely elucidated. As administration of growth hormone is known to increase rather than decrease bone mass (Rudman et al. 1990), additional factors have been implicated. Diamond et al. (1989) suggested that spinal osteoporosis in acromegaly is caused by concomitant hypogonadism. Hyperprolactinaemia (Klibanski et al. 1980) and even mild ovulatory disturbances (Prior et al. 1990) can lead to bone loss particularly in the spine, which is highly susceptible to bone resorption because of the large surface of the trabecular bone (Riggs and Melton 1986). In our case prolactin blood levels were normal on two occasions. However, prolactin as well as growth hormone were detected immunohistochemically in the tumour cells at autopsy. Thus, an at least temporary hyperprolactinaemia cannot be excluded. In addition a latent hypogonadism was noted on one occasion. Therefore, it is conceivable that apart from the growth hormone excess other factors like a mild deficiency of gonadal hormones have either acted on the bone tissue synergistically or antagonistically.

Regarding the vertebral trabecular bone as it was found at autopsy, the remarkable occurrence of cartilaginous islands and probably most of the trabecular thickening can be attributed to the growth hormone excess of over 20 years duration. A mild hypogonadism may have been an additional factor in the rarefication of the vertebral trabeculae and thus for the development of osteoporosis.

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