Trabecular bone changes in the greater trochanter after fracture of the femoral neck

Bone samples were taken from the trabecular part of the greater trochanter in 32 patients who had had a fracture of the ipsilateral femoral neck, and from 24 patients who had coxarthrosis. 42 cadavers served as controls. The samples were sectioned, stained and examined histologically.
The coxarthrosis cases differed only slightly from normal, whereas the fracture cases had increased osteoid volume and surface. Osteoclasts were also increased in number, as were active osteoblasts.

Karl J. Obrant

University of Lund, Department of Orthopaedic Surgery at Malmö General Hospital, S-21401 Malmö, Sweden

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Herfarth (1924) and Beck (1925) found increasing numbers of osteoclasts in bone biopsies following trauma. Rieder (1936) also described an increasing fraction of unmineralized bone after fracture in man. Freudiger (1950) and Geiser & Trueta (1958) found the same in animal experiments. Landoff (1942), however, in rabbits found no increase in the amount of unmineralized bone. None of these early investigators had quantified the histological changes in bone. In addition, all the examinations were undertaken before the technique of cutting and staining undecalcified sections became available.

The histomorphometric analysis method described by Merz & Schenk (1970) has made it possible to quantify histological entities in an undecalcified bone section. Although this histological technique has been available for decades, most investigations on post-traumatic osteopenia have been non-invasive. In immobilized rats, Eichler (1970) found increasing osteoid volume, increasing numbers of osteoclasts and decreasing numbers of osteoblasts. On the other hand, Minaire et al. (1974) in iliac crest biopsies from immobilized patients found osteoporosis and no increasing amount of osteoid. Baud & Pouëzat (1973) described two cases of post-traumatic osteopenia in which, using the quantitative micro-radiographic technique, they found a lowered mineral content in the trabeculi. Dempster et al. (1980) have shown by the electron-probe technique that the concentration of calcium differs between parts of the trabeculi. There was a difference between not only osteoid and mineralized bone, but also within the osteoid and within the mineralized bone. In addition, the low correlation between the absolute volume of trabecular bone and ash-weight in iliac bone samples (Melsen & Mosekilde 1981) indicates variations in the degrees of mineralization of trabecular bone also in man.

The aim of the present investigation was to examine morphologic changes in the trabecular bone after fracture.

Patients and methods

Fracture cases

The 32 patients selected for the investigation had all fractured the femoral neck 8±10 months earlier. Their age at the time of the biopsy was 75 (52–93) years (Table 1) and they were all scheduled for reconstruction surgery.

Table 1. Age and sex

	Men		Women	
	N	Age	N	Age
Femoral neck fracture	10	75±12	22	75± 8
D:o controls (AVTB%)	11	75± 7	12	75±11
Coxarthrosis	9	71± 5	15	70± 7
D:o controls (AVTB%)	14	71±10	13	70±12
Controls, all	25	53±23	17	63±23

AVTB%: Absolute volume of trabecular bone.

Coxarthrosis

Twenty-four consecutive patients with primary coxarthrosis were selected. Their age at the time of the biopsy was 71 (56–79) years, and they were all to have total hip replacement procedures.

Controls

Bone samples were taken from 42 necropsy cases in the Department of Forensic Medicine, all from individuals who had suffered sudden death. In each case, samples were obtained from both greater trochanters. The age was 57 (16–95) years. Cases with known alcoholism or malignant disease were not included in either group.

Biopsy

A biopsy was taken from the trabecular bone in the greater trochanter (Figure 1). In the control cases the biopsy technique and the instrument described by Burkhardt (1966) were used, resulting in a bone cylinder 15 mm long and 5 mm in diameter. In the coxarthrosis and fracture cases the biopsy was obtained from the same site with a chisel in conjunction with a hip arthroplasty.

Histological methods

The bone samples were fixed in 10% buffered formalin for at least 24 h and then dehydrated with alcohol in increasing concentrations (40, 70, 96, 99.9%), 24 h for each concentration. Defatting was carried out in a 3:1 solution of 99.9% alcohol and chloroform for another 24 h, followed by rinsing in xylol. The bone samples were then ready for embedding in methylmethacrylate, adding benzylperoxide and plastoid N in 37° for 48 h. Four of the 32 post-fracture cases were immediately fixed in 99% ethanol instead of formalin. These were samples from patients who had been given tetracycline preoperatively for a parallel study. The samples were then sectioned in a hard sectioning Jung microtome, model K, making sure that the sections for morphometric evaluation were collected from the central part of the specimen. Sections, 5 μm thick, were prepared and stained according to Goldner's modification of Masson trichrome staining (Goldner 1938).

Morphometric evaluation

For the morphometric evaluation, a template consisting of a point-wave pattern in the eye-piece of the microscope (Merz & Schenk 1970) was used. For the calculation of osteoid parameters, an objective magnification of 13× and an eye-piece magnification of 10× were used. With this magnification, 30 fields including only trabecular bone could be measured in most sections (Johnell et al. 1979). For the purpose of counting the osteoclasts, the margins of the sections were taped in order to obtain a straight-line rectangular area of trabecular bone. All osteoclasts in the area were counted per mm^2 with 40× objective and 10× eye-piece magnification. The osteoclasts were identified by the light pink, often vacuolar cytoplasm and the distinct nucleoli. Only osteoclasts with two or more nuclei and in contact with the bone surface were counted (Johnell et al. 1977). Active osteoblasts were defined as cuboidal cells in contact with osteoid, often with their nuclei in the part of the cytoplasm facing away from the trabeculum. For the counting of active osteoblasts an objective magnification of 40× and an eye-piece magnification of 12.5× were used, again with a point-wave template. Counting sites were selected at random. Intersections lined with active osteoblasts and intersections with other lining cells were counted.

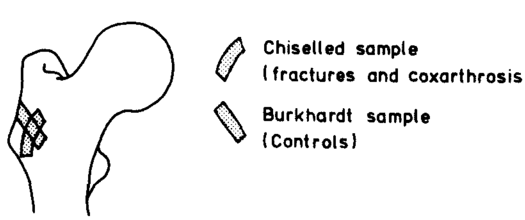

Figure 1. Sample sites.

The following variables were calculated:

1. Absolute volume of trabecular bone (AVTB%): the percentage of bone trabeculae, including mineralized as well as osteoid tissue, of the total bone volume, including medullary and vascular space.
2. Relative osteoid surface (OS%): the bone surface covered by osteoid expressed as a percentage of the total trabecular bone surface.
3. Relative osteoid volume (OV%): the percentage of AVTB% occupied by osteoid.
4. Osteoclast count: the number of osteoclasts/mm^2 section surface covered by trabecular bone.
5. Osteoblast ratio: the ratio of osteoid surface covered by active osteoblasts/osteoid lined by other cells.

For comparison of AVTB% only, age- and sex-matched controls were used. Since, for other variables no age or sex differences were suspected or could be demonstrated, all available controls were used.

Table 2. Absolute volume of trabecular bone, per cent

	Men	Women
Femoral neck fracture	13.8±5.9 n.s.	17.6±10.8 n.s.
D:o controls	17.8±4.8	12.6± 4.7
Coxarthrosis	11.1±4.4 $p<0.01$	14.6± 8.7 n.s.
D:o controls	17.4±4.3	13.0± 4.9

The number of osteoclasts was significantly higher ($p < 0.01$) in the post-fracture cases compared with the control cases (Table 3).

Finally, active osteoblasts also occurred more often in fracture patients than in controls ($p < 0.01$).

A frequent finding was that osteoid and bone (n.b. layers more and less mineralized) could be observed; this was altogether different from the appearance in, for instance, osteomalacia (Figure 2).

Results

Since in the control cases there was no left-right difference in any of the variables, the data from both sides were pooled.

The absolute volume of trabecular bone was significantly lower in men with coxarthrosis than in controls; otherwise, there were no differences between the groups. No correlations with age were found. If all controls were included and compared between men and women, the AVTB% was significantly less in women ($p < 0.01$) (Table 2).

The osteoid surface in the fracture cases was increased by a factor of 14, but also in the coxarthrosis patients there was a significant increase ($p < 0.05$). Similarly, the osteoid volume was increased in the fracture cases ($p < 0.001$) (Table 3).

Discussion

The absolute volume of trabecular bone varied in an inconsistent manner. This variable is usually difficult to handle because of its large variation, but nevertheless – in the separate sample – it is a necessary correction variable. The most obvious finding in this study was the increased amount of osteoid tissue in the post-traumatic cases. In many trabeculi there was an arrangement with unmineralized bone lamellae alternating with mineralized bone lamellae. Raina (1973) described mineralized bone on the surface of osteoid in renal osteomalacia, but this phenomenon, although probably fairly common, has not otherwise been described previously. Methodological explanations may be found in the staining pro-

Table 3. Osteoid, osteoclasts and osteoblasts

	Relative osteoid surface %	Relative osteoid volume %	Osteoclast count/mm^2	Osteoblast ratio
Controls	1.2± 1.4	0.1±0.3	0.16±0.22	0.39±0.35
Coxarthrosis	2.9± 3.9	0.5±1.1	0.22±0.27	0.51±0.41
Femoral neck fracture	17.3±16.2	3.5±3.8	0.48±0.73	0.65±0.31

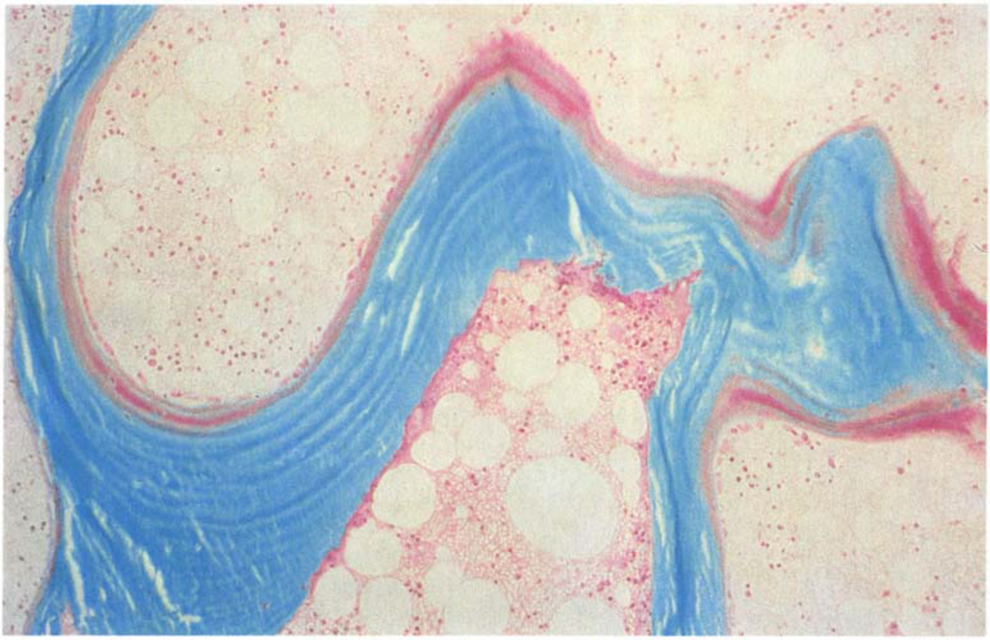

Figure 2. Goldner-stained sample from patient with femoral neck fracture (110×). Layers of osteoid (red) and mineralized bone (green). Also, the mineralized part of the trabeculum has a lamellar appearance – this type of lamellae may be seen in normal bone.

cedures, the microscopic techniques (Olah 1980) and the section thickness. However, these mineralization defects in the post-traumatic cases could not be a staining artefact as they were not seen in any of the control samples which were handled together with the fracture patient samples.

What is the significance of the increased osteoid in the fracture cases? In the past we have failed to demonstrate serious osteomalacia in hip fracture patients, at least to this extent (Hodkinsson 1971, Lips 1982). Since there are no other data on osteoid from this location, we must accept the findings at their face value.

The increase in osteoid and the unusual pattern of mineralization are probably signs of high turnover disturbance of the local bone metabolism rather than an osteomalacia lesion. Again, the presence of unmineralized bone may explain the capacity to recover lost bone mineral (Nilsson 1966, Westlin 1974, Andersson & Nilsson 1979).

The number of osteoclasts was tripled in the post-traumatic cases compared with the controls. This is most likely a sign that bone resorption is taking place, just as the increased osteoblast count indicates increased bone formation.

The coxarthrosis samples also deviated from the controls. In coxarthrosis there is an ongoing repair process in the upper end of the femur, as demonstrated by Danielsson et al. (1964) and others.

In conclusion, the data from the present study support the earlier findings of an increased metabolic rate in a fractured limb (Wendeberg 1961) in man; the cellular activities suggest that resorption and formation are both increased. In addition, there is an arrangement of more and less mineralized lamellae, which is unusual in other conditions.

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