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## Experimental production of local osteomalacia[1]

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*Summary.* The newly deposited bone which was laid down on necrotic bone in the experimentally produced osteochondral chips in the knee joint of 16 sheep and rabbits remained unmineralized and undermineralized in which respect it resembled osteomalatic bone. Local factors which interfere with the mineralisation of a new covering bone should be considered in the pathogenesis of osteomalacia, in healing of aseptic bone necrosis and fractures, and incorporation and fate of bone transplants.

The recently developed histological staining techniques for osteoid tissue and individual bone matrix components in ordinary decalcified paraffin bone sections[2-4] allowed the author in the past 2 years to screen and evaluate the bone mineralisation process in a large number of specimens from human conditions and experimental material. Since the techniques are simple to perform and make it easy to distinguish between unmineralized osteoid tissue and calcified bone (figure 1), a number of interesting observations were made in a relatively short time. One of these - the existence of 'local osteomalacia'[5] forms the background of the experiments reported in the present communication.

In principle, it was found during our studies of human and experimental bone necrosis and bone-rebuilding processes that when the bone dies, the newly formed bone which after revascularisation of the area is deposited on the necrotic bone remains unmineralized or incompletely mineralized. The new wide seams and areas of unmineralized or hypomineralized osteoid tissue on the surface appear identical to those in human osteomalacia and metabolic bone disease. The existence of purely local factors which might be able to influence the normal mineralization process producing an osteomalacic bone seems to be a new and important element since at the present time it is commonly believed that osteomalacia is a systemic condition in which there is a general incapability to mineralize the newly formed osteoid tissue either due to the deficiency or malabsorption of the bone mineral or due to the deficiency of general humoral factors responsible for this process.

In order to confirm that lost or diminished vitality of the underlying bone is a factor responsible for 'local osteomalacia' the following experiment was set up: in 8 rabbits and 8 sheep bone necrosis was achieved under general anaesthetic by producing an osteochondral chip from the lateral femoral condyle, which was then left to float freely as a loose body in the knee joint. The bone in this fragment died between 4-12 days (as judged by the gradual degeneration and dissappearance of the bone cells from their lacunae) but at the same time revascularisation occurred, via adhesions from the synovia and joint capsule and layers of new bone were deposited on the dead trabeculi of the original bone. The animals were sacrificed at intervals between 8 h and 8 weeks after operation. The revascularized fragments were dissected out of joints, photographed and X-rayed and divided into 2 parts: one was decalcified in formic acid or EDTA and then stained by haematoxylin-eosin, toluidin blue and by the author's tetrachrome and PTAIH (phosphotungstic acid-iron haematoxylin) methods[2-4]; from the other half undecalcified sections were cut and stained by haematoxylin-eosin and von Kossa's silver method, and viewed under the fluorescent microscope for bone autofluorescence (Zeiss' fluorescent microscope, exciter filter = I, barrier filter = 53/44).

3 undecalcified sections from each group (from the specimens with most advanced bone-rebuilding process) were ground down to 80 µm thickness for the purpose of contact microradiography (Softex apparatus, 7.5 kV, 3 mA, 90-240 min, Kodak ES film V-6028).

The results have shown that, as it has been previously observed in human local osteomalacia[5] wide seams of newly deposited lamellar bone and to a certain extent woven bone which were laid down on cores of the old dead bone were unmineralized or grossly undermineralized (figure 2). This defective bone was found in all specimens 9 days after the operation and its microscopical appearance was identical to one type of the malacic bone seen in human metabolic bone disease. Because its incidence was locally limited being confined to the old dead trabeculi and because its undermineralisation was not caused by the lack of calcium and phosphorus in the circulating blood as witnessed by the normal calcification of the newly formed bone in other parts of the same specimen, this phenomenon is called local osteomalacia.

The new defective bone was represented on the surface by 7-9-µm wide seams of the unmineralized, 'normal' osteoid tissue underneath which, down to the junction with the dead bone, it had throughout the character of 'osteoid bone'[4]. This latter is a pathological undermineralized or demineralized bone tissue, neither osteoid nor bone, recently recognized as a common feature in human osteomalacia and numerous other conditions[4]. From about the 28th day after the experiment the mineralisation of this osteoid bone slowly advanced. At 8 weeks the calcification made a further progress though it was still incomplete. However, a 10-30-µm wide linear area at the site of the

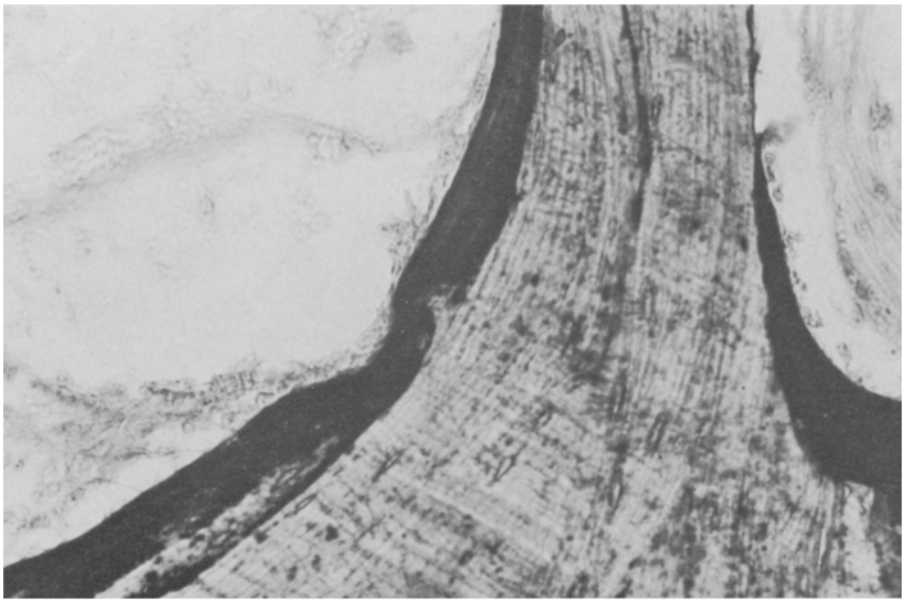

Fig. 1. Osteomalacic bone in a 53-year-old woman. The wide unmineralized and undermineralized seams of dark osteoid and osteoid bone (deep blue in the method) are clearly distinguishable from the pale (red-orange) mineralized bone (author's tetrachrome method). × 175.

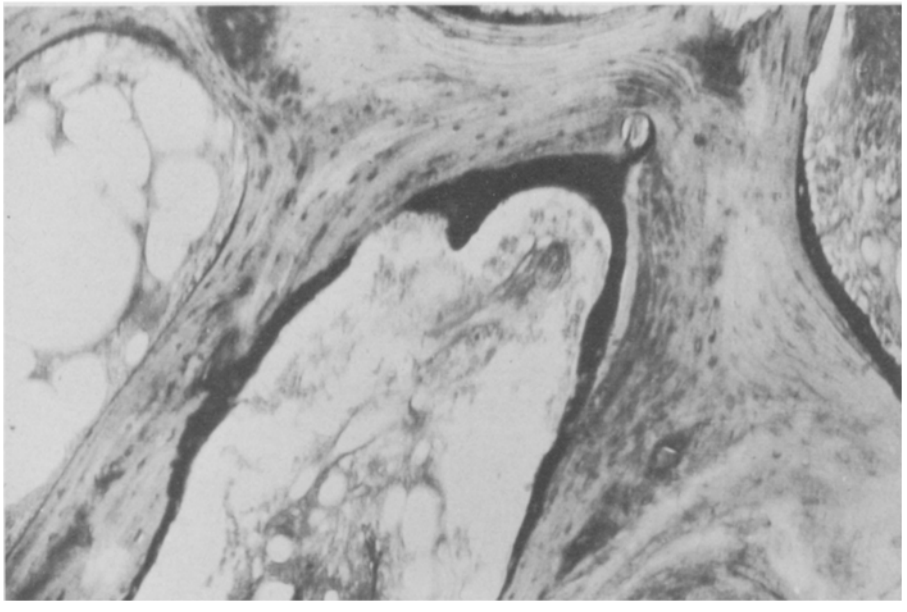

Fig. 2. Experimental 'local osteomalacia' in sheep 9 days after the bone necrosis was induced. The wide seams of unmineralized osteoid tissue (dark) covering the bone trabeculae (pale have the same appearance as in human osteomalacia (author's tetrachrome method). × 80.

original junction with the dead bone, appeared to be even at this late stage completely unmineralized.

While the delayed and slow calcification of the new post-necrotic covering bone is probably a temporary phenomenon, this unmineralized junctional area which also showed increased basophilia, irregular texture and multiple cracks and separations thus demonstrating lack of cohesion and mechanical stability between the 2 types of bone tissue, is a more permanent feature. The author had the opportunity to observe it many times since in various conditions, this stigma being still detectable a long time after the necrosis of trabecular bone and its repair by a new covering bone has occurred.

Results of these observations and experiments therefore strongly suggest that not only metabolic disorders but also local factors in the bone tissue, such as the morphological and functional state of the osteoblasts and bone cells, the status of the pre-existing bone matrix and other possible local conditions must be considered, not only in the pathogenesis of osteomalacia but also in the normal process of bone mineralisation. In addition, the partial non-mineralisation and delayed mineralisation of repairing and covering new bone and its inadequate consolidation and cracks in the interface with the pre-existing dead bone has basic implications for the problem of healing of bone infraction, aseptic bone necrosis and fractures and also for the behaviour and fate of bone auto- and homotransplants.

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