Pre-Symposium Workshop
Bone Histology, Morphology and Pathology

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POSSIBILITIES AND LIMITS OF UNDECALCIFIED BONE HISTOLOGY

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The introduction of polymers as embedding media for histolocigal specimens has opened the way for processing of calcified tissues and presumably bone. Consecutively, microtomes and special microtome knives have been developed and many procedures for differential staining of cells and various matrix components have been adapted to the requirements of the new embedding media.
This technology, however, still has its limitations. Microtomes can only cut without fragmentation through cancellous bone, or cortical bone in the longitudinal direction. Polymerization temperatures often inactivate enzymes and restrict the application of enzyme histochemistry. Finally, specimens containing implants made of metals or ceramics can not be sectioned. These drawbacks have reactivated the interest in sawing and abrasive technologies. Besides grinding and polishing, ultramilling was introduced in order to produce faultless surfaces. Improvements in surface staining provide satisfactory and even extended information in regard of the components of the calcified matrices, and most implant materials can be preserved in situ. There is also a wider choice of plastics, since their mechanical properties become less critical. In view of histomorphometric evaluations, ground and milled sections are no longer subject to any deformation, and ultramilling allows the production of step-sections at known intervals, a prerequisite for the application of improved stereologic estimations.
The gaining importance of enzyme histochemistry, immunohistochemistry and in situ hybridization asks for a combination of such techniques with cryomethods. In this respect, the cryo-miller may become an alternative to cryo-microtomes if sections of heavily mineralized lamellar bone are required.

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HISTOMORPHOMETRY OF BONE REMODELLING

G. Boivin

Histomorphometry consists of counting or measuring the tissue components (cells, extracellular constituents, or both). Bone tissue from adults is continuously remodelled throughout life, and this remodelling corresponds to a precise cascade of coupled events (activation, resorption, formation). A local system of specialized bone cells (basic multicellular unit = BMU) is responsible for the remodelling process which culminates in a basic structural unit (BSU = intermediary level of bone organization), i.e. an osteon in compact bone or a cancellous bone packet. Histomorphometry is particularly suited to evaluate tissue and cell changes at this level of intermediary organization, to better estimate the diagnosis of bone diseases and the effects on bone of new drugs and/or therapeutic approaches.
Histomorphometry of undecalcified bone samples not only permits the measurement of static parameters but also of dynamic parameters with the use of tetracycline double labelling. Transiliac biopsies taken at the standard site, are fixed in alcohol then embedded in methylmethacrylate. Histological observations are done either on stained (solochrome cyanin R, Goldner's trichrome, toluidine blue) or on unstained sections. Histomorphometric parameters are measured by a manual method (integrating eyepieces) as well as with either semiautomatic or automatic image analyzers.
The main changes in bone remodelling may be evaluated using : a) structural static parameters measured in compact bone (cortical width, cortical porosity), b) structural static parameters measured in cancellous bone (bone volume, mineralized volume, wall width), c) remodelling static parameters measured in cancellous bone (eroded perimeter, osteoid volume, perimeter, and width), d) cellular parameters measured in cancellous bone (number of osteoclasts, osteoblast perimeter), e) dynamic parameters measured in cancellous bone (mineral apposition rate, single and double labelled perimeters, bone formation rate, adjusted apposition rate, mineralization lag time, formation period).

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QUANTITATIVE ANALYSIS OF TRABECULAR BONE STRUCTURE

M. Vogel*, M. Hahn**, G. Delling**

Trabecular bone structure depends on the 3- dimensional configuration of trabeculae. Therefore, it is quite questionable wether a quantitative analysis can be done in 2-dimensional histological sections. For this reason we developed a new preparation technique which allows combined 2- and 3-dimensional inspection of the bone specimens. After removal of bone marrow by rinsing with water and hydrogen-peroxide the specimens are dehydrated, immersed in a special plastic embedding medium and when polymerisation is completed they will be ground on a grinding machine down to a thickness of 1 mm. The polished surface of the specimens can be stained with all conventional stains. The result is that trabeculae can be analysed at the stained surface like normal histological sections. Furthermore, in the depth of the preparations the 3-dimensional configuration and connectivity of the trabeculae can be studied. If we do not remove bone marrow it is possible to analyse the vascular vessels in relation to the trabecular network.

Age dependent bone loss may be the result of a decrease in trabecular thickness or the complete loss of individual trabeculae. We can demonstrate that in the human spine (in contrast to the femoral neck e.g.) the main mechanism of bone loss are trabecular perforations. It is discused wether Osteoporosis Type I and II are the result of different mechanisms of trabecular bone loss due to perforations or trabecular thining. Perforations can be visualized and analized quantitatively. This leads to a progressive destruction of trabecular plates which are transformed to strut like elements. The ongoing loss of trabecular elements preferes horizontal oriented trabeculae and results in a decrease of intertrabecular connectivity which can be quantitated by a new parameter, TBPf (Trabecular Bone Pattern Factor). Microfractures and microcallus formations are mechanisms which show a close relation to the extend of intertrabecular connectivity. Their contribution to the reconstruction of trabecular bone can be very important . It seems as if there are typical locations in the trabecular network for the occurrence of microcallus formations. Furthermore, microcallus formation might be a mechanism for the generation of completely new trabeculae by its ability to build bridges of woven bone between existing trabeculae.

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SCANNING MICROSCOPIES OF BONE CELLS AND BONE MATRIX

A. Boyde

Scanning microscopies have provided many opportunities to study bone matrix and mineral distribution and development, and more recently to study the bone cells themselves. Scanning electron microscopy derives its benefits from the large depth field and good resolution, the former providing the opportunity for 3-Dimensional imaging and analysis by utilising defined, contrasting projections: automated 3-D analysis has arrived. For embedded, flat, specimens quantitation of the back-scattered electron signal provides a means to study variations in mineralisation density. QBSE has substantial advantages in resolution over energy dispersive X-ray microanalysis in an SEM: Monte Carlo studies of BSE and X-ray generation volumes prove this point. To reduce the excited volume and increase the resolution it is necessary to reduce the accelerating voltage. Very high resolution is now available in very low voltage SEMs.
Confocal scanning optical microscopes have made possible the in depth study of intact bone samples both in vitro and in vivo, and in both the fluorescence and reflection modes. High scan rate CSLMs such as the latest video rate laser based instruments and the Tandem Scanning Microscopes are suitable for studying living cells. All CSLMs are making important contributions in studying immunofluorescence in prepared, fixed material. The reflection mode is important also in studying surfaces and has been important in developing new methods for the quantitation of osteoclastic bone resorption.

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IMMUNOELECTRON MICROSCOPY OF BONE FORMATION AND RESORPTION

A. Schulz, G. Jundt, B. Rau and D. Gottesbüren

Skeletal mineralization depends on the composition of bone matrix. Apart from collagen type I non-collagenous constituents play an important role not fully understood by biochemical data alone. Additional factors influencing growth and differentiation of bone cells are deposited in the matrix from internal (bone cell derived) and external (plasma transport) sources. Hence, autocrine and paracrine mechanisms seem to control the interaction of bone cells and matrix being supported by chemotactic and cell binding (cell adhesion) activities of at least some non-collagenous proteins isolated from bone.

Immunoelectron microscopy is a tool for the visualization of metabolic pathways. We applied this method to study the production, secretion, deposition and resorption of a number of non-collagenous bone proteins such as Osteocalcin (OC), Osteonectin (ON), Osteopontin (OPN), Bone Sialoprotein (BSP), Biglycan, and Decorin. Polyclonal antibodies raised in rabbits against the respective human antigens, were supplied by J.D. Termine and L.F. Fisher (NIH, Bethesda, USA).

Osteoblasts, osteocytes, osteoclasts and their interaction with osteoid and mineralized bone were studied using the immunogold method after a low temperature embedding procedure in Lowicryl (K 4 M) of human callus tissue removed during surgery for withdrawel of metallic implants.

The results show the topographic distribution of the matrix components during osteoid maturation and mineralization elucidating their possible function in initiating the mineralization process (gradual enrichment of OC, ON, OPN and BSP towards the mineralization front). Furthermore the recently discussed significance of OPN and BSP for the matrix adhesion of osteoclasts and their mononuclear precursors was examinined on a morphological basis.

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REGULATION OF OSTEOCLASTIC RESORPTION.

S. C. Miller

Bone resorption is essential for the development of bone, determination of skeletal structure and integrity and maintenance of mineral homeostasis. While many substances have been identified that will alter osteoclastic activities, the regulation of osteoclastic resorption for maintaining vital skeletal functions is rather poorly understood. At the organ level, it is apparent that there is envelope- and site-specificity to the response of osteoclasts to various challenges. Under normal circumstances the envelopes and surfaces in these envelopes most affected by increases in osteoclast activities (i.e. activation of bone remodeling and modeling in the resorption mode) are those least likely to compromise structural integrity yet simultaneously accommodate the mineral needs of the animal. Such occurs during lactation in mammals. Under 'pathological' circumstances, this does not occur. At the tissue level of skeletal organization, it is evident that osteoclast activities are regulated such that resorption occurs in domains, functioning to remove defined packets of bone. Ultrastructural studies show a network-like organization of osteoclasts within these domains, suggesting that they might act as a functional syncytium. At the cellular level, it is evident that under both physiological (i.e. egg-laying in birds) and pharmacological (i.e. administration of calcitonin) circumstances, that the activities of osteoclasts can be rapidly altered. Isolated osteoclasts tend to retain resorptive activities, perhaps indicating that in the 'nude' state, osteoclasts are active. On this basis, the hypothesis is presented that many conventional activators of resorption might act through the stimulation of osteoclast differentiation while shorter-term, physiological regulators might act by inhibiting osteoclast activities. However, these is also some evidence that novel peptides, such as PTHrP secreted during lactation, for example, might serve as regulators of bone resorption by stimulating transients changes in modeling and remodeling.

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