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FINE STRUCTURE OF THE OSTEOCYTE CAPSULE AND OF THE WALL OF THE LACUNAE IN BONE

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With 12 Figures in the Text

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Summary. Bone tissue from femura of adult and old rats and from young mice, as well as from dogs, were fixed in osmium tetroxide, potassium permanganate, in an osmium tetroxide — potassium permanganate combination, and in glutaraldehyde followed by osmium tetroxyde — potassium permanganate. The results of the different fixatives were found to complement one another in such a way that existing controversies and uncertainties concerning the fine structure could be settled. This was especially true of the question whether or not the so-called capsule of the osteocyte contains collagen fibrils. Notwithstanding considerable variations in the structure of the capsule it was definitely shown that cross-banded fibrils are present in a mucopolysaccharide-containing ground substance. The material of the capsule corresponds, therefore, to the matrix of connective tissue in general, and its ground substance is, as in any connective tissue, the medium of transport between the blood and the tissue. In respect to the organic structures the "intralacunar" matrix is similar to the "interlacunar" mineralized matrix. In sections of demineralized bone, especially after osmium tetroxide fixation, the wall of the lacuna and canaliculi is marked by a dark line which is described as a special osmiophilic lamina. Since the same line, although thinner and less distinct, was found also in tissue fixed with agents other than osmic acid it was concluded that the osmiophilic lamina is a true structure which must be permeated by substances passing to and from the interlacunar matrix. The osmiophilic lamina belongs to a wider border zone which differs from the bulk of the mineralized matrix by its thinner and less tightly packed fibrils. Accordingly, the bone crystals were found to be less orderly arranged than those deeper inside the mineralized matrix. Bordering directly on the intralacunar pathway they were described as the coastal crystals and are believed to represent the labile bone minerals which are metabolically available without any change in the bone structure. The findings about the fine structure of the capsule of the osteocyte and of the wall of the lacunae were discussed in terms of the transport problems in bone. The osteocyte itself, by its fine structure and relationship to the intralacunar matrix seems to be engaged not only in the maintenance of the open pathways in bone but also in the transport mechanism itself.

One of the questions relating to the fine structure of bone which has not as yet been answered satisfactorily concerns the relationship of the osteocyte to the wall of the lacuna. Light microscopic sections of both undemineralized and demineralized bone show the osteocytes surrounded by the non-mineralized material of the capsule (Rouget-Neumann sheath, Grenzscheide, la gaine limitante), which stains with basic dyes and gives a positive P.A.S. reaction (WEIDENREICH, 1930; LORBER, 1951; LIPP, 1954). In the electron microscope, a space around the osteocytes and

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their processes in the canaliculi is regularly observed and identified with the capsule (BAUD, 1960, 1962; BAUD and WEBER-SLATKIN, 1961; WASSERMANN, 1962; DUDLEY and SPIRO, 1961; CAMERON, 1963). MJÖR (1962), however, whose material consisted only of undemineralized bone, was mainly interested in an electron dense zone separating the lacunae from the rest of the matrix. He believed his findings to be in disagreement with those of DUDLEY and SPIRO (1961), and he declared that the layer of non-fibrillar material of moderate density shown by them could not be demontsrated in his material. We will show that MJÖR's dense zone is quite different from the capsule of the osteocyte, and that the disagreement between this author and the majority of observers, including DUDLEY and SPIRO, does not exist. MJÖR's dense zone may be identical with WASSERMANN's osmiophilic lamina, or with the zone of the wall of the lacuna in which KNESE and VON HARNACK (1962) found the collagen fibrils arranged in a way different from the rest of the matrix („Eigenfaserung" of the wall of the lacuna).

Among the observers who have studied the capsule with the light or electron microscope, the opinions concerning the material which occupies the pericellular space are divided. Some found it homogeneous (CAMERON, 1963; DUDLEY and SPIRO, 1961; WEINMANN and SICHER, 1955), while others (WEIDENREICH, 1930; BAUD and MORGENTHALER, 1963), recognized fibrils within the capsule. This point was given special attention in the present study. If the capsule contains collagen fibrils in addition to the polysaccharide-protein complexes shown by the P.A.S. reaction, it would indeed correspond to a more or less fibrous connective tissue, and in particular to preosseus tissue. One might then correctly call the capsule a zone of non-mineralized matrix separating the bone cells from the mineralized matrix. From a functional point of view such non-mineralized matrix, being continuous with the connective tissue of the Haversian canals, would be the medium of transport between blood and bone, as in any connective tissue (WASSERMANN, 1962; BAUD and MORGENTHALER, 1963). It seemend, therefore, of special importance to examine the structures in the immediate vicinity of the osteocytes with the electron microscope. It was found in the course of the present study that the aspect of these structures varies somewhat with age and probably other factors not yet known. Disagreements among investigators concerning the occurence of fibrils in the osteocyte capsule can be resolved in the light of our observations.

Material and Methods

All of the bone used was chipped from the mid-shaft region of femurs. Bone from mature dogs, guinea pigs and rats was fixed for one hour in solutions of osmium tetroxide at 4° C (PALADE, 1962) and decalcified for 1—3 days at 23° C in an unbuffered, saturated solution of disodium ehtylene diamine tetracetate (EDTA) or in 0.5 M EDTA adjusted to pH 7.3 with 20 % sodium hydroxide. Bone from mice a few weeks old was fixed for two hours at 4° C in solutions of potassium permanganate (LUFT, 1956), osmium tetroxide (PALADE, 1952) and osmium tetroxide-potassium permanganate combination (TAHMISIAN, 1964) or in 2 % glutaraldehyde (GORDON, MILLER and BENSCH, 1963) followed by post-fixation in the osmium-permanganate solution. This material was not demineralized. Finally, bone from mice a few weeks old and rats a few months old was fixed in 2 % glutaraldehyde (GORDON, MILLER and BENSCH, 1963), demineralized for 1—3 days 4° C in 0.05 M EDTA with 0.13 M sucrose and 0.08 M cacodylic acid adjusted to pH 7.3 with 20 % sodium hydroxide, and post fixed in osmium-permanganate. All of the bone was embedded in Epon and sectioned with a Porter-Blum microtome using a diamond knife. Most of the sections were stained with lead citrate

(REYNOLDS, 1963), a few were stained with uranyl acetate (WATSON, 1958). The electron micrographs were made in an RCA EMD-3F microscope.

Observations

The osteocyte. In studies of the fine structure of bone, the osteocyte in its relation to the wall of the lacuna is of predominant interest. If the cell were allowed to shrink during fixation, dehydration and embedding, no reliable statements could be made in this respect. Especially important is the preservation of the plasma membrane of the osteocyte, in order to distinguish correctly between cellular and

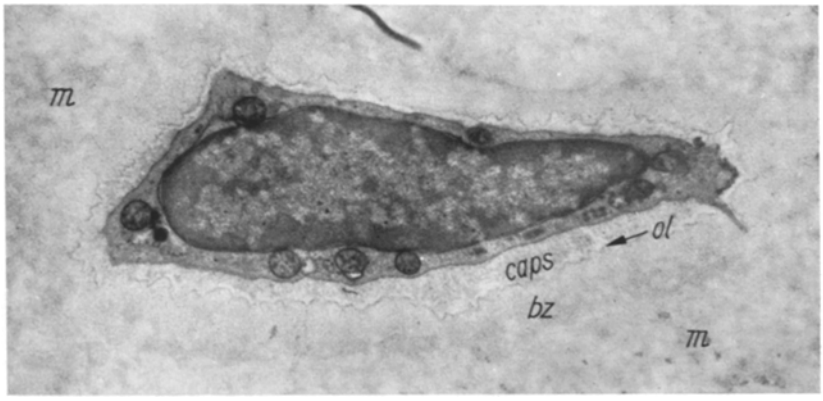

Fig. 1. Electron micrograph of a relatively old osteocyte from a mature rat. Specimen was fixed in glutaraldehyde, demineralized with EDTA, and post-fixed in osmium permanganate. The section was stained with lead citrate. Cell membranes are well preserved. The less dense intralacunar matrix (*caps*) is separated from the interlacunar matrix (*m*) by a thin osmiophilic layer (*ol*). A broader perilacunar zone of matrix (*bz*) surrounding the osmiophilic layer can be differenciated from the bulk of the matrix (*m*). Magnification × 10,500

extracellular material. These requisites are not always met in specimes fixed in osmium tetroxide, potassium permanganate, or glutaraldehyde. In addition, preparing unltrathin sections, particularly of undemineralized bone, was a difficult problem.

It was our experience that adequate thin sections of osteocytes in adult bone could be obtained only when the specimes was demineralized in EDTA before embedding. Figures 1 and 2 show the results of this procedure while Figure 3 demonstrates the result of using the same routine with young bone. Young bone from the femora of weanling mice could be used in this study both as demineralized and undemineralized specimes. Potassium permanganate, glutaraldehyde and osmium-permanganate proved to be excellent fixatives, as Figures 4—6 demonstrate. Osmium tetroxide was the only fixative used which did not give the same satisfactory resluts (Fig. 10).

Figures 1, 2, 3, 5 and 6 were printed at the same magnification to facilitate comparison. Three factors contribute to the differences in osteocyte size seen in electon micrographs: 1. there are normal variations in the size of osteocytes in the same bone; 2. old osteocytes possess much less cytoplasm than young ones; 3. the cells appear larger or smaller depending on the plane of sectioning relative to their long axis.

The osteocytes showed a well developed endoplasmic reticulum and numerous mitochondria (Figs. 4—6), and the Golgi apparatus was frequently demonstrated (Fig. 6). The plasma membrane outlined the cell surface distinctly. It is of interest that the cell surface is often seen to project out in small pseudopodia-like protru-

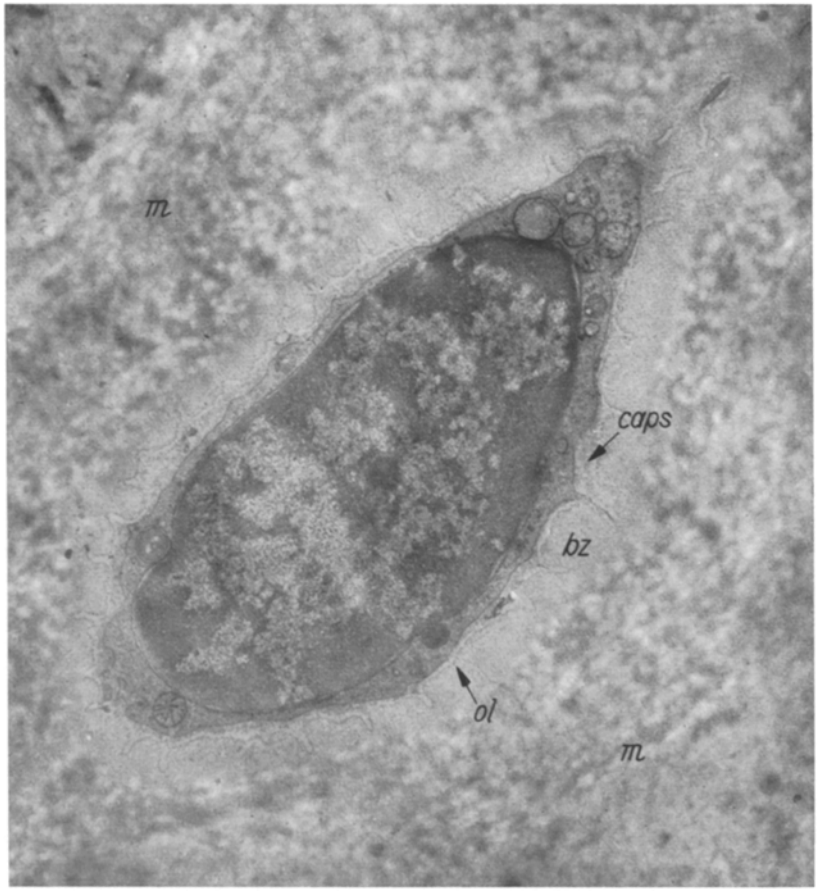

Fig. 2. Electron micrograph of a relatively old osteocyte from a mature rat. Specimen was prepared as that in figure 1. As in figure 1, the intralacunar matrix (*caps*) is about 0,7 μ thick. It is separated from the relatively homogeneous border zone of perilacunar matrix (*bz*) by the osmiophilic layer (*ol*). Collagen fibres are indicated in the interlacunar matrix (*m*). × 10,500

sions. Some cell processes were seen to arise from conical elevations of the cell surface and to enter canaliculi (Figs. 3—6). The nuclei of the osteocytes possessed distinct double membranes with occasional pores, best seen in sections after permanganate fixation. In osmium, or glutaraldehyde-fixed cells, the nucleus contained heavy chromatin aggregates (Figs. 1, 2, 3, 5, 10), while they had a more homogeneous appearance after permangante fixation (Fig. 4).

As previously described by KNESE and KNOOP (1958), osteocytes in bone from young animals had more abundant cytoplasm than osteocytes in bone from older animals (compare Figs. 1 and 2 with Figs. 3 and 4). Although the age of any particular cell was obviously not known, it is reasonable to assume that bone from younger animals contained predominantly young cells, while the bone from older animals contained predominatly old cells.

Considering the elaborate cytoplasmic structure, and the frequent signs of cell unrest, both also demonstrated by BAUD and his co-workers (1962), we believe that the osteocyte is a metabolically very active cell.

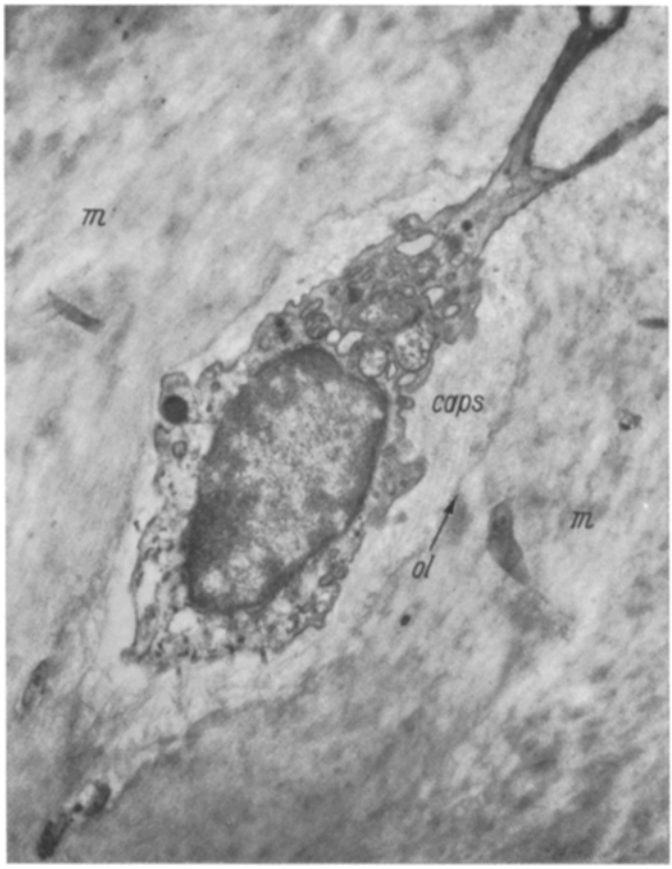

Fig. 3. Electron micrograph of a relatively young osteocyte from a young mouse. Specimen prepared as that in figure 1. The less dense intralacunar matrix (*caps*) is separated from the interlacunar matrix (*m*) by a thin layer (*ol*, see figure 10). Collagen fibrils are visible within the intralacunar matrix, but indistinct in the interlacunar matrix. Magnification × 10,500

The Pericellular Space. In light microscopic sections of fixed and demineralized bone, one regularly finds a relatively wide space between the surface of the osteocytes and the previously mineralized matrix (Fig. 7). In or sections it measured about 2.7 μ in thickness. Thin strands of material with a low density passed through this pericellular space, extending from the surface of the cell to the dark lining of the lacunae. The dark lining stained with hematoxylin and the P.A.S. reaction was described by numeros previous investigators (WEIDENREICH, 1930; LORBER, 1951; LIPP, 1954), and has been designated the capsule of the osteocyte or the Rouget-Neumann sheath. Its thickness is about 1 μ or less. The wider, unstained space, however, is an artifact caused by shrinkage of the cell during fixation, dehydration and embedding. In sections of fresh frozen bone taken from the sternum of a guinea pig (which can be sectioned without demineralization) and observed under the phase contrast microscope in Ringer's solution within 10 minutes after removal from the anesthetized animal, a narrow pericellular zone was apparent (Fig. 8). Its thickness, about 0.7 μ, corresponded with that of the stained layer in Figure 7. It ist, therefore, clear that the entire space in the fresh-

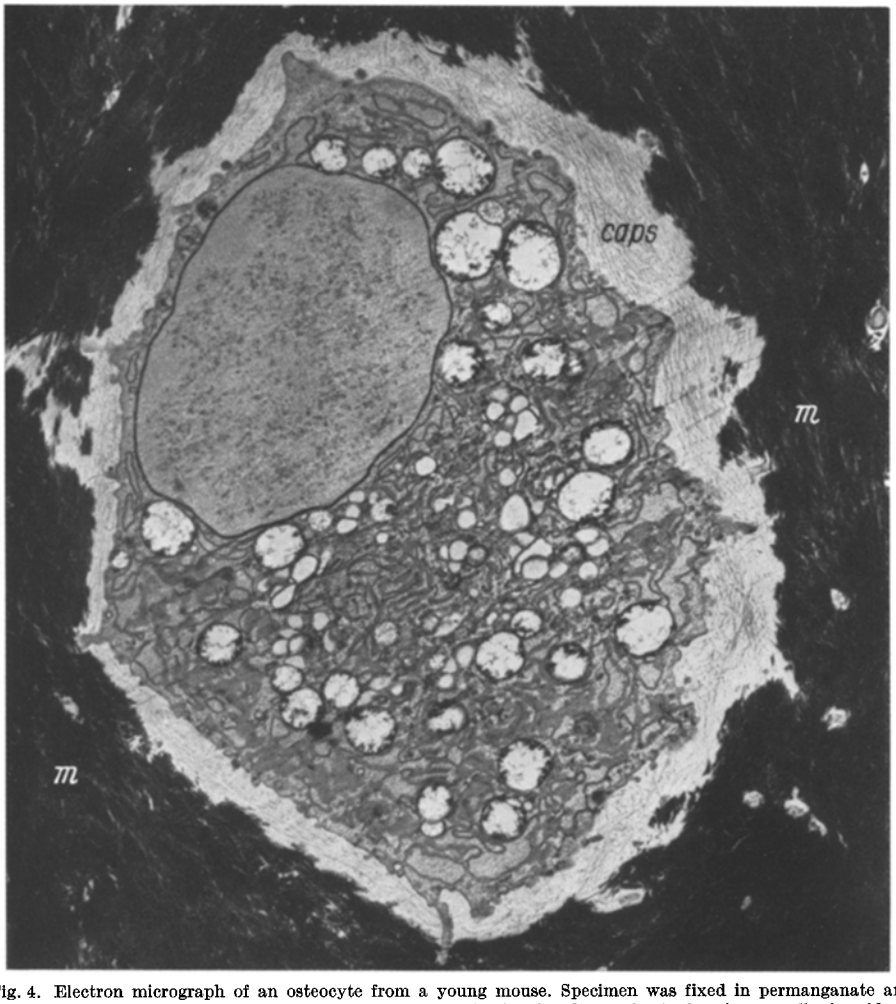

Fig. 4. Electron micrograph of an osteocyte from a young mouse. Specimen was fixed in permanganate and sectioned without demineralization, stained with lead acetate. An abundance of cytoplasmic organelles is evident. A relatively thick layer of unmineralized intralacunar matrix (*caps*) separates the cell from the mineralized matrix (*m*). The intralacunar matrix contains collagen fibrils and an amorphous interfibrillar material. Magnification × 6,600

frozen section represents the capsule. That the shrinkage space is absent is understandable, since fixation and dehydration were avoided. LIPP (1954), using similar techniques, anticipated the conclusion that the pericellular space in fresh frozen sections in equivalent to the capsule. He succeeded in staining the contents of the space with the P.A.S. reaction, and with lipid stains.

Our electron micrographs of undemineralized sections (Figs. 4—6) came very close to the frozen sections in that the pericellular space was found to be 0.1—2.0 μ thick, with a mean value for 21 lacunae of 0.7 μ. According to these measurements, the pericellular space in the electron micrographs is, as in the frozen sections, identical with the capsule. Noticeable shrinkage was evidently not caused by the techniques used for electron microscopy. As seen in Figure 9 the cell processes are

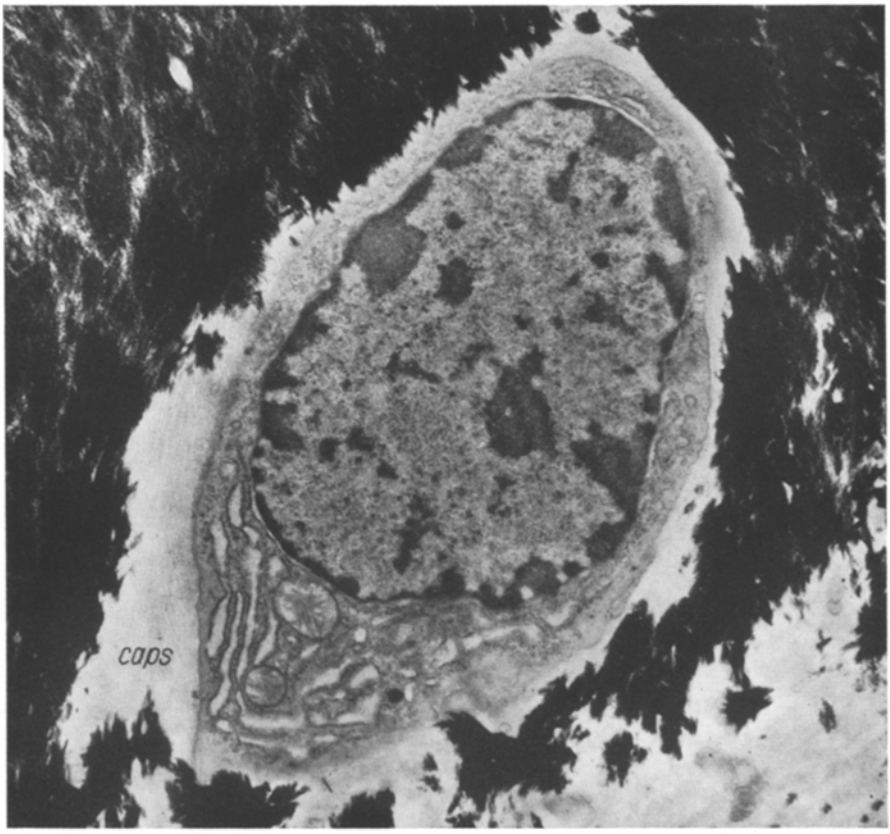

Fig. 5. Electron micrograph of an osteocyte which has been recently trapped in bone of a young mouse. Specimen was fixed in glutaraldehyde, post-fixed in osmium permanganate and subsequently treated as that in figure 4. Fibrils in the intralacunar matrix (*caps*) are again evident, some of them, at the left in lower half of picture, show cross-striation, while the material immediately at the cell surface is rather homogeneous. This indicates fibrillogenesis. Magnification × 10,500

separated from the walls of the canaliculi by a space that corresponds with the capsule of the osteocyte in the lacunae.

Electron microscopy of a series of specimes fixed in various ways permitted us to decide the still-debated question about the structure of the capsule. The major disagreement among previous investigators concerns the material of the capsule, wheter it is "argyrophobic" as WEINMANN and SICHER maintain, a non-fibrillar material according to DUDLEY and SPIRO, or wheter it contains fibrils as WEIDENREICH and BAUD claim. We found that the nature of the capsule showed some variation, depending not so much on fixation as on factors as yet unknown. The age of the animal and of the bone may be among these factors. We found in some lacunae only amorphous material surrounding the osteocytes (Fig. 6), in some the pericellular space contained rather thick fibrils (Fig. 10), but in the majority of specimes, thin fibrils were present (Figs. 3—5). Their thickness of about 20 mμ (range, 5—50 mμ in 15 sections) and cross-banding characterized them undoubtedly as collagen fibrils. In contrast to these intralacunar fibrils, those in the previously mineralized matrix (Figs. 10, 11) averaged 55 mμ in thickness (range, 22—100 mμ in 15 sections). It is clear that in the light microscope, the very fine

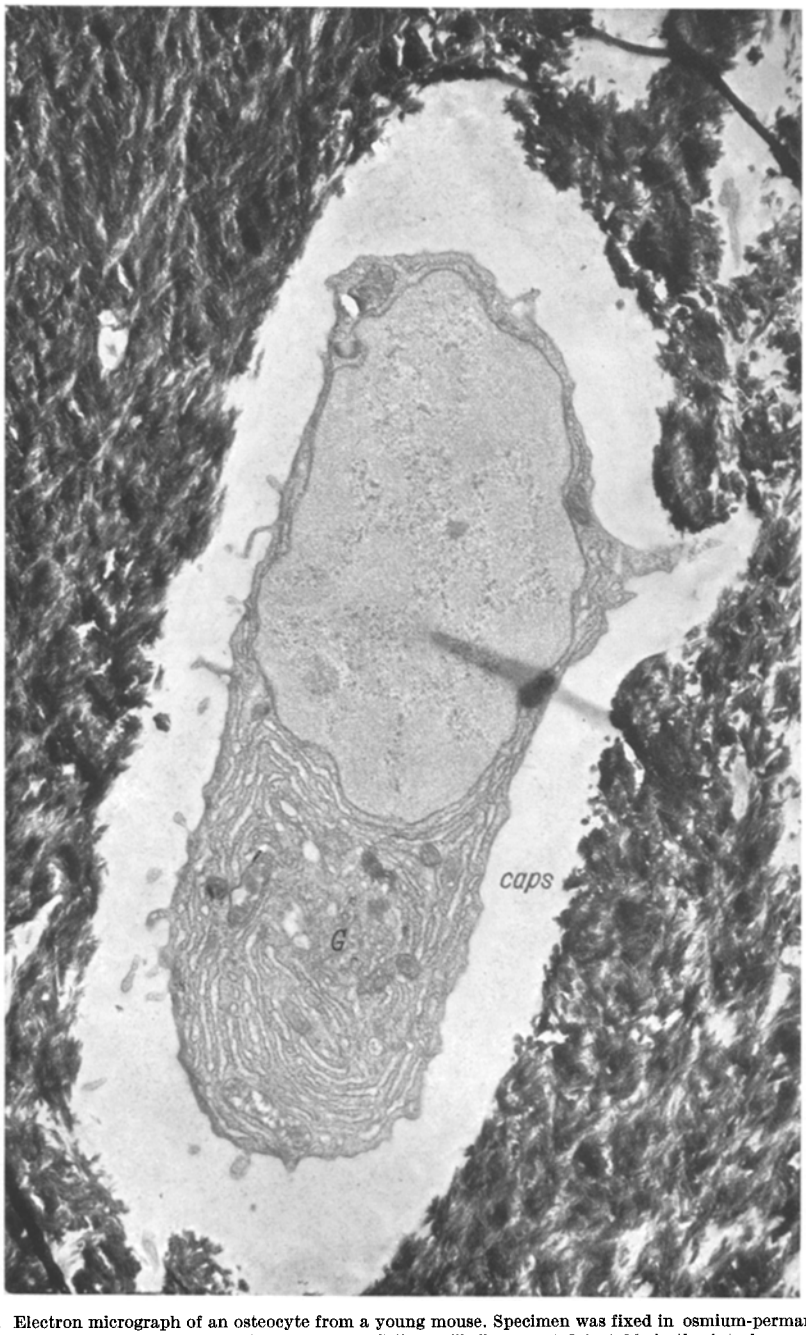

Fig. 6. Electron micrograph of an osteocyte from a young mouse. Specimen was fixed in osmium-permanganate, and subsequently treated as that in figures 4 and 5. Collagen fibrils are not detectable in the intralacunar matrix (*caps*) although the cytoplasm is better preserved than in the previous figures; at *G*, Golgi apparatus. At the right a process of the osteocyte is seen entering a canaliculus. Notice the pseudopodia-like formations at the cell surface. Magnification × 10,500

fibrils cannot be recognized, and that the coarser fibrils, although not distinguishable as such, may give the impression of a fibrous material. Between the fibrils

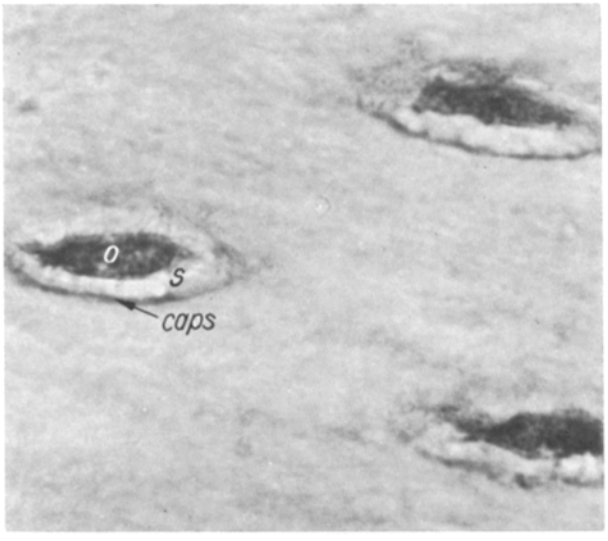

Fig. 7. Photomicrograph of osteocytes (*o*) in bone from a mature guinea pig. Specimen was fixed in formalin-talcohol, demineralized, and sectioned in paraffin. The section was stained with the periodic-acid-Schiff procedure. The spaces between the surfaces of the cells and the dark lining (pink in the section) of the lacunae are about 2—3 μ wide. The dark layer at the wall of the lacunae which consists of PAS-positive material measures about 0.7 μ in width, and corresponds therefore to the intralacunar matrix of the elctron micrographs (*caps*) while the wider space (*s*) resulted from shrinkage of the cells. Magnification × 1,200

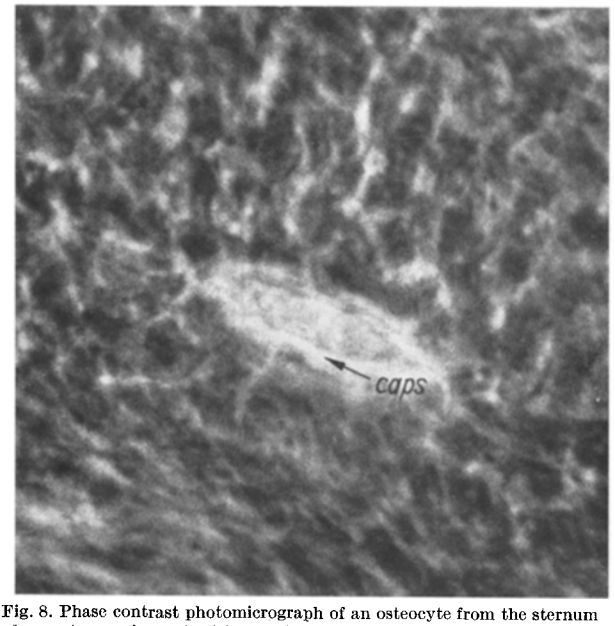

Fig. 8. Phase contrast photomicrograph of an osteocyte from the sternum of a mature guinea pig. The specimen was sectioned fresh on a freezing microtome and mounted unstained in Ringer's solution. The space between the cell surface and the wall of the lacuna (*caps*) is about 0,7 μ wide and corresponds therefore to the PAS-positive structure in figure 7, and to the intralacunar matrix in the electron micrographs. No noticable shrinking of the cell in the fresh specimen. Magnification × 1,200

one sees a finely granular material which in demineralized specimes can be compared with the corresponding interfibrillar material of the matrix proper, and appears to be less dense than the latter. Fundamentally, the material of the capsule and of the mineralized matrix are comparable since both are composed of polysaccharide-protein-containing ground substances and collagen fibrils. Stressing the absence of bone crystals from the capsule, one might quite correctly distinguish a non-mineralized or intralacunar matrix from the mineralized interlacunar matrix. This distinction will be re-examined in the discussion.

The Osmiophilic Lamina. In sections from bone fixed in osmium tetroxide and subsequently demineralized in EDTA, a dark layer was regularly found (Fig. 10) between the intralacunar and intracanalicular matrix and the interlacunar matrix. The senior author introduced this layer as an osmiophilic lamina (WASSERMANN, 1962). It was found to be up to 50 mμ thick. In undermineralized sections, the lamina cannot be distinguished, because it is masked by bone crystals. Using undecalcified material for the study of osteocytes and their cap-

sules, BAUD and his co-workers did not notice the osmiophilic layer (BAUD and MORGENTHALER, 1963). In one of the electron micrographs of DUDLEY and SPIRO (1961, their Fig. 12), it can be seen, but the authors did not refer to the structure. As long as it was demonstrated only in specimes fixed in osmium tetroxide, one could not be sure whether or not it was a structure present *in vivo*; the osmium tetroxide, diffusing out from the lacuna into the mineralized matrix, might accumulate at the border between mineralized and non-mineralized matrices, thereby producing the image of an osmiophilic structure. However, the dark line was also detectable in material fixed in glutaraldehyde and demineralized before post-fixation in osmium-permanganate (Fig. 1), although it was thinner and less conspicous tahn in the material fixed in osmium tetroxide before demineralization. This may well be due to an adverse effect of glutaraldehyde, which was manifested also in the indistinctness of the collagen fibrils in the same sections (Figs. 1—3). We believe that the osmiophilic lamina is a special area at the surface of the mineralized matrix, which is detectable when the masking crystals are removed.

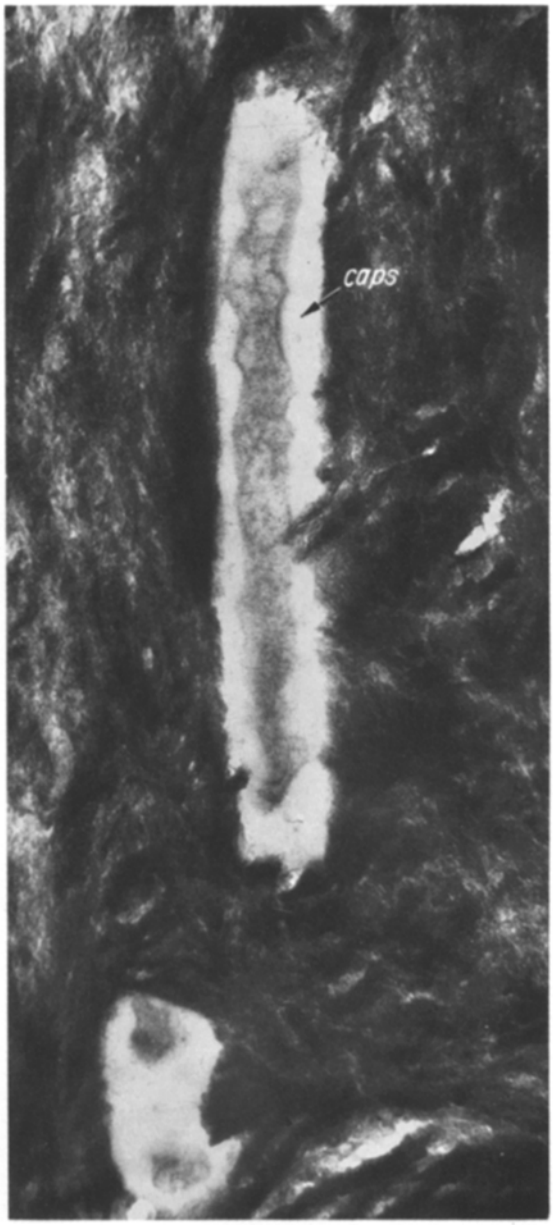

Fig. 9. Electron micrograph of section of undemineralized bone from a young mouse showing processes of osteocytes within canaliculi. The processes are surrounded by non-mineralized intralacunar matrix (*caps*). No collagen fibrils could be observed in these areas. Magnification × 37,000

The crystals in the osmiophilic lamina are of special interest because of their location at the edge of the mineralized matrix. They are in contact with the intralacunar matrix and are therefore "coastal crystals" (WASSERMANN, 1962), likely to be the most readily available part of the mineral in bone. Crystals which border

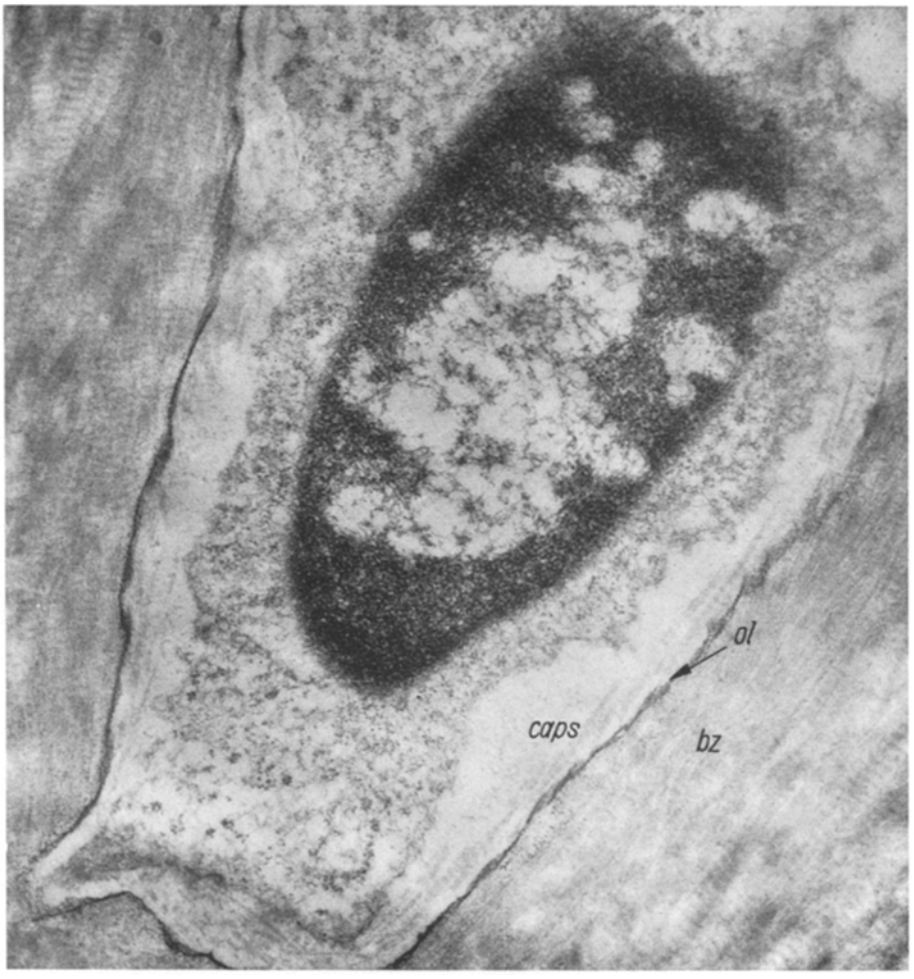

Fig. 10. Electron micrograph of an osteocyte from an old rat. Specimen was fixed with osmium tetroxide and demineralized with EDTA. The section was stained with uranyl acetate. Collagen fibrils are evident in the intralacunar matrix (*caps*) and the osmiophilic layer is very distinct after osmium tetroxide fixation. The border zone (*bz*) contains fibrils (at the right) which are much thinner than those in the matrix proper. Magnification × 21,000

directly on the intralacunar matrix are often seen to form spikes of various sizes which give the border of the mineralized matrix an irregular contour (Figs. 4—6). This indicates a less strictly ordered arrangement of the coastal crystals than is found in the interior of the matrix where the crystals are intimately attached to and aligned by the collagen fibrils (ROBINSON and WATSON, 1955). Figure 11 demonstrates this situation in a section of undecalcified bone.

The Zone of Interlacunar Matrix Adjacent to the Osmiophilic Lamina. Although the osmiophilic lamina was distinct in osmium tetroxide-fixed and demineralized material, we believe that it shares certain characteristics with a broader, adjacent border region. The structure of this thicker layer, as seen after demineralization, varied considerably (Figs. 1 and 2). Even around the circumference of one lacuna, as in Figure 10, one sees, at the right, fibrils which are much thinner than those

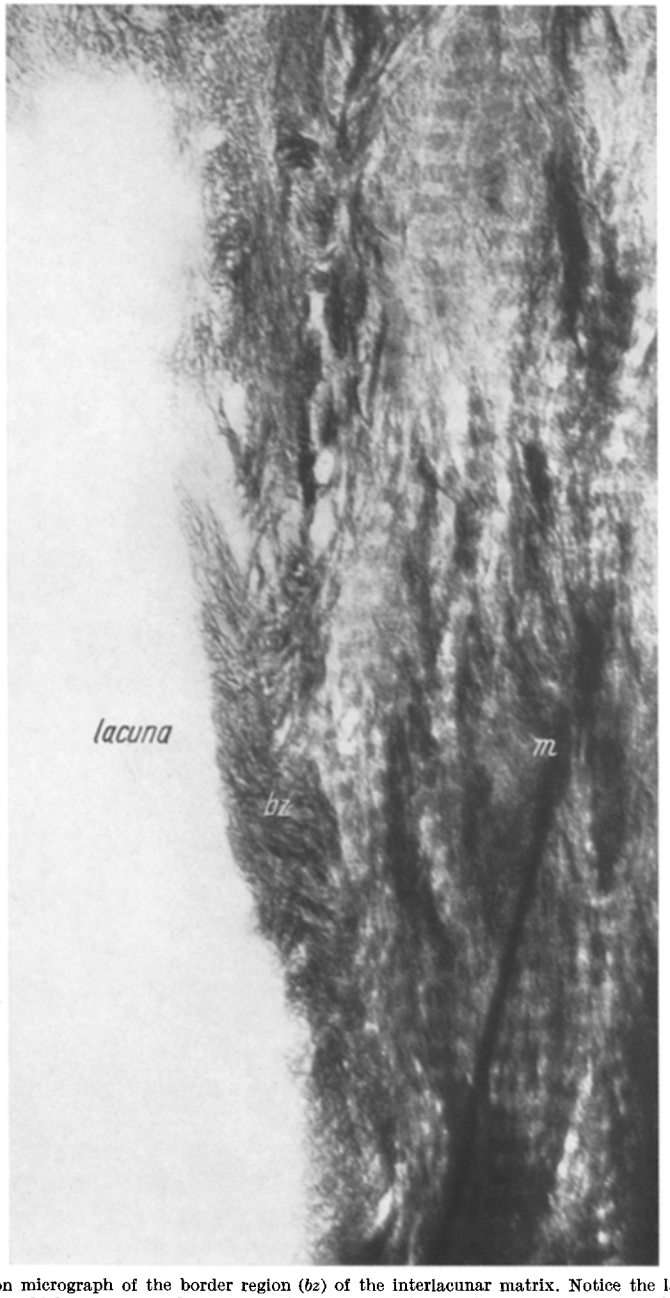

Fig. 11. Electron micrograph of the border region (*bz*) of the interlacunar matrix. Notice the layer of coastal crystals (see text) which are not as orderly arranged as those attached to the mature collagen fibrils in the matrix proper (*m*). The fine fibrils in the border zone (figure 10) cannot be seen in the undecalcified section. However, the cross-banded fibrils in the matrix proper are visible where they are not covered by the bone crystals. Magnification × 52,000

in the bulk of the matrix, and at the left, typical matrix fibers immediately on the osmiophilic lamina. At some places fibrils of the border region of the previously mineralized matrix were seen to continue into the intralacunar matrix.

In some of the sections of material fixed in glutaraldehyde, the perilacunar zone appeared to be relatively homogeneous (Figs. 1 and 2). Although this is probably the effect of the fixative, which does not preserve the fibrils well, the difference between the border region and the rest of the matrix under these conditions indicates that real differences exist between them.

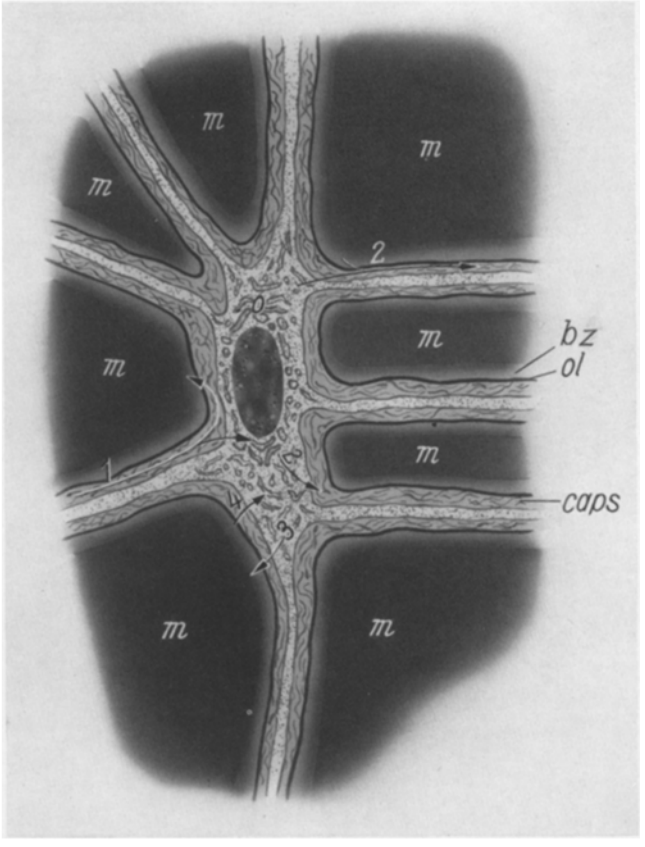

Fig. 12. Diagramatic drawing showing an osteocyte (*o*) in a lacuna with processes in canaliculi, the capsule (*caps*) with fibrils, the osmiophilic lamina (*ol*), the border zone (*bz*) and the bulk of the mineralized matrix (*m*). The arrows indicate the possible intra- and extracellular pathways of substances in transport from the blood to the matrix and vice versa, and from the cells to the matrix and vice versa. Arrow 1, pathway via capsule to the cells and the matrix; arrow 2, from the matrix and the cells to the capsule; arrow 3, from the cells to the matrix; arrow 4, from the matrix to the cells. See discussion

Discussion

Bone has often been described as a mineralized connective tissue. This is correct insofar as the intercellular organic compartment of bone consists of the same two components as all connective tissues, viz., ground substance and collagen fibers. The electron micrsoscope findings reported in this paper add a new factor to this description by the demonstration of fibrils in the capsule of the osteocytes. We recognize, therefore, the fundamental similarity between the organic components of the capsule and those of the mineralized matrix. This similarity is emphasized here by the use of the terms intralacunar und interlacunar matrix-the former

designating the non-mineralized capsule of the osteocyte, and the latter the mineralized intercellular bone matrix in the traditional sense.

Although it seems justified on the one hand to stress the unity of the intercellular compartment with regard to ground substance and fibrils, it is equally important to understand how much more complex a system bone is than the other connenctive tissues. The intercellular compartment of soft connective tissue is continuous, that of bone is discontinuous, divided into several compartments: the intralacunar matrix, the osmiophilic lamina, the adjacent broader perilacunar area and the well-organized interlacunar matrix. Figure 12 illustrates these distinctions in diagramatic form. The layers surrounding the intralacunar matrix-are, of course, mineralized. The border layer, but not the osmiophilic lamina, has been recognized by KNESE and VON HARNACK as a layer of thin fibrils („Lage dünner Fibrillen, Eigenfaserung" of the wall of the lacuna). The result of an examination by SCHMIDT (1959) of the „Grenzscheide" with polarized light apparently pertains to the border layer described here and not to the intralacunar matrix, since not only fibers but mineral crystals were demonstrated. Whether or not the osmiophilic lamina is correctly considered as a separate layer is questionable. This was taken into account in our description where it was dealt with as part of the border area of perilacunar matrix. In any case, the osmiophilic lamina may be of functional importance if it indicates the position of a barrier to be passed by substances on their way from the intralacunar to the interlacunar matrix, and vice versa. A similar osmiophilic lamina has been described in dentin matrix by ARWILL (1960).

The consideration of a barrier as a part of the intercellular system of bone emphasizes the problem of exchange of substances between blood on the one side and the cells and matrix on the other. The metabolic activity of the cells of "resting" bone no doubt plays an important role in calcium and phosphorus homeostasis (JOWSEY, RIGGS and KELLEY, 1964). The concept of "labile calcium" according to MCLEAN and Urist (1961), is simply that "a portion of the calcium, variously designated as labile, exchangeable, metabolic or reactive, is capable of moving in or out of bone without any necessary disturbance of the organic matrix with which it may be associated." The uptake of ions in preformed bone has been demonstrated also when Ca 45 or Ra 226 are administered, producing a relatively uniform low level of darkening of autoradiographs of bone (ROWLAND, MARSHALL, and Jowsey, 1959). A similar exchange has been demonstrated in dentin (WASSERMANN *et al.*, 1941), where remodeling does not occur.

It is clear that the continual exchange of substances between blood and bone involves, besides inorganic ions, all of the materials the bone cells and matrix need for their maintenance. In Figure 12, the pathways of this multiple exchange are indicated by arrows. The main route of the metabolic traffic is the intralacunar matrix, which, via the orifices of the canaliculi, is continuous with the ground substance in the Haversian canals. Because of its polysaccharide-protein complexes and collagen fibrils, the intralacunar matrix is equivalent to any other connective tissue. We can therefore say that the medium of transport between the Haversian blood vessels and the cells, as well as the matrix, is like that of any other organ, i.e., the connenctive tissue ground substance. As is any connective tissue, the permeability of the ground substance at a given time and locality will depend on many factors, among them enzymatic and hormonal actions. In this respect, the

transport problem in bone is similar to that in connective tissues in general. But in addition, the movement of materials in bone implies special problems which are as yet unsolved. One legitimate consideration concerns the state of the main pathway, the intralacunar matrix, which may differ from the interlacunar matrix by a lesser degree of polymerization of its ground substance and a higher degree of hydration (GERSH and CATCHPOLE, 1960). The lack of understanding which at present obscures the transportation problem in bone is sometimes disregarded by such over-simplifications as to declare that a tissue fluid "circulates" via lacunae and canaliculi. We believe that the electron microscopic investigation of bone structure, especially combined with autoradiographic methods. will bring these problems to the foreground.

The lack of regular orientation of the fibrils in the broader perilacunar zone as compared with that in the bulk of the matrix, the smaller diameter of the fibrils, and the relatively greater amount of ground substance, will necessarily be reflected in the orientation of the mineral crystals. They cannot be as tightly packed as they are between the more regularly arranged fibrils in the bulk of the matrix. The rough surface of the wall of the lacuna indicates a rather random arrangement of crystals, supporting this opinion. We called them coastal crystals and suggested that they may represent mineral which is more labile than that in the interior of the matrix.

Our findings concerning the fine structure of the osteocytes are in agreement with those of BAUD and co-workers. The inclusion of the osteogenic cells into the lacunae does not seem to cause significant alterations in structure. The well-developed endoplasmic reticulum, the numeros mitochondria and the Golgi apparatus are all about the same as in osteoblasts, according to the descriptions of KNESE and KNOOP (1958). This is certainly true for the osteocytes we have seen in young bone. Our experience with regard to variations in the size and structure of osteocytes is limited, but it seems evident from our sections of older bone that with age, the cytoplasmic volume decreases, and the number of cytoplasmic organelles is reduced.

We know of several functions which the osteocyte performs, or may perform. Like the fibrocyte, it is the source of polysaccharide-protein complexes, which are known to have a relatively rapid turnover (SCHILLER *et al.*, 1956). Osteocytes have already been shown to contain P.A.S.-positive granules (HELLER-STEINBERG, 1951; LIPP, 1954). The presence of fibrils in the intralacunar matrix speaks strongly for the ability of osteocytes to synthesize collagen. It is further possible that the osteocyte is involved in the transport of materials in bone. Certain substances may pass through the osteocyte before they are delivered to the matrix (see, Fig. 12). Osteocytes may also serve to aid the movement of materials through the intralacunar matrix. That the cells perhaps expand and contract, at least at localized regions of the cell surface, is indicated by their frequently irregular surfaces. Such movement may mechanically support the transport of materials through the rigid tubular system of lacunae and canaliculi. The presence of the same structural components in the non-mineralized intralacunar matrix and the mineralized interlacunar matrix suggests that the osteocytes are inhibiting mineralization in their immediate enviroment. This interpretation is substantiated by the occurrence of hypermineralized lacunae after osteocyte death (ROWLAND, MARSHALL and JOWSEY, 1959; JOWSEY, 1960; SISSONS, 1962).

Finally, most important are the observations in normal, pathological, and experimentally altered bone which suggest a participation of the osteocytes in both the mobilization and deposition of mineral. JOWSEY, RIGGS and KELLY (1964) summarized their own and other investigators' findings which indicated that the osteocytes may function within the mature bone tissue as small foci of metabolic activity independent of the more commonly recognized processes of formation and resorption that take place at the surface of bones. These authors support the view, expressed in this paper, that the osteocytes are capable of metabolically regulating the nature of their enviroment.

In summary, we feel that it is now possible to be rather specific in describing the ways in which osteocytes maintain the "vitality" of bone. Their principal activity is in maintaining the patency of non-mineralized diffusion pathways throughout the intercellular matrix, and regulating the transfer of materials from blood to bone and from bone to blood by directly influencing the state of these pathways. Changing demands on the pathways are reflected in the various aspects displayed by osteocytes.

References

ARWILL, T.: Studies on the ultrastructure of dental tissues. I. Some microstructural details in the dentine. Acta. morph. neerl. scand. **3**, 147—156 (1960).

BAUD, C. A.: Observations au microscope électronique sur les canalicules du tissu osseux compact. Bull. Micr. appl. **10**, 45—48 (1960).

— Morphologie et structure inframicroscopique des ostéocytes. Acta. anat. (Basel) **51**, 209—225 (1962).

—, et P. W. Morgenthaler: Structure submicroscopique du rebord lacuno-canaliculaire osseux. Morph. Jb. **104**, 476—486 (1963).

—, et S. WEBER-SLATKIN: Aspects microscopiques et submicroscopiques des ostéoplastes du tissu osseux compact. Bull. Mikr. appl. **11**, 73—76 (1961).

CAMERON, D. A.: The fine structure of bone and calcified cartilage. Clin Orthop. **26**, 199—228 (1963).

DUDLEY, H. R., and D. SPIRO: The fine structure of bone cells. J. biophys. biochem. Cytol. **11**, 627—649 (1961).

GERSH, I., and H. R. CATCHPOLE: The nature of ground substance of connective tissue. Perspect. Biol. Med. **3**, 282—319 (1960).

GORDON, G. B., L.R. MILLER, and K. G. BENSCH: Fixation of tissue culture cells for ultrastructural cytochemistry. Exp. Cell Res. **31**, 440—443 (1963).

HELLER-STEINBERG, M: Ground substance, bone salts, and cellular activity in bone formation and destruction. Amer. J. Anat. **89**, 347—380 (1951).

JOWSEY, J.: Age changes in human bone. Clin. Orthop. **17**, 210—218 (1960).

— B. L. RIGGS, and P. J. KELLY: Mineral metabolism in osteocytes. Proc. Mayo Clin. **39**, 480—489 (1964).

KNESE, K. H., and M. v. HARNACK: Über die Faserstruktur des Knochengewebes. Z. Zellforsch. **57**, 520—558 (1962).

—, and A. M. KNOOP: Elektronenoptische Untersuchungen über die periostale Osteogenese. Z. Zellforsch. **48**, 455—578 (1958).

LIPP, W.: Neuuntersuchungen des Knochengewebes. Acta anat. (Basel) **20**, 162—200 (1954).

LORBER, M.: A study of the histochemical reactions of the dental cementum and alveolar bone. Anat. Rec. **111**, 129—144 (1951).

LUFT, J. H.: Permanganate, a new fixative for electron microscopy. J. biophys. biochem. Cytol. 2, 799—801 (1956).

MCLEAN, F. C., and M. R. URIST: Bone: An Introduction to the Physiology of Skeletal Tissue, 2nd edit. Chigaco: The Chicago University Press 1961.

MJÖR, I. A.: The bone matrix adjacent to lacunae and canaliluci. Anat. Rec. **144**, 327—339 (1962).

PALADE, G. E.: A study of fixation for electron microscopy. J. exp. Med. **93**, 284—298 (1952).
PRITCHARD, J. J.: General anatomy and histology of bone. In: The Biochemistry and Physiology of Bone, ed. by G. H. BOURNE. p. 1—25. New York: Academic Press Inc. 1956.
REYNOLDS, E.S.: The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. J. Cell Biol. **17**, 208—212 (1963).
ROBINSON, R.A., and M.L. WATSON: Crystal-collagen relationships in bone as observed in the electron microscope. Ann. N. Y. Acad. Sci. **60**, 596—628 (1955).
ROWLAND, R. E., J. H. MARSHALL, and J. JOWSEY: Radium human bone: The microradiographic appearance. Radiat. Res. **10**, 323—334 (1959).
SCHILLER, S., B. MARTIN, M. B. MATTHEWS, J. A. CIFONELLI, and A. DORFMAN: The metabolism of mucopolysaccharides in animals. III. Further studies on skin utilizing C^{14}-glucose, C^{14}-acetate, and S^{35}-sodium sulfate. J. biol. Chem. **218**, 139—145 (1956).
SCHMIDT, W. J.: Grenzscheiden der Lakunen und Kittlinien des Knochengewebes. Z. Zellforsch. **50**, 275—296 (1959).
SISSONS, N. A.: Age changes in the structure and mineralization of bone tissue in man. In: Radioisotopes and Bone, ed. by F. C. MCLEAN, P. LACROIX and A. M. BUDY, p. 359—443. Philadelphia: F. A. Davis Co. 1962.
TAHMISIAN, T. N.: Use of the freezing point method to adjust the tonicity of fixing solutions. J. Ultrastruct. Res. **10**, 182—188 (1964).
TAKUMA, S.: Electron microscopy of the structure around the dentinal tubule. J. dent. **39**, 973—981 (1960).
WASSERMANN, F.: Electron microscopic examination of the wall of the lacunae and canaliculi in bone. Argonne National Laboratory, Biological and Medical Research Division, Semiannual Report, July through December, 1961 (ANL-6535), p. 129—138. Argonne: National Laboratory 1962.
—, J. R. BLAYNAY, J. GROETZINGER, and F. J. DEWITT: Studies of the different pathways of exchange of minerals in teeth wirh the aid of radioactive phosphorus. J. dent. Res. **20**, 389—398 (1941).
WATSON, M. L.: Staining of tissue sections for electron microscopy with heavy metals. J. biophys. biochem. Cytol. **4**, 475—478 (1958).
WEIDENREICH, F.: Das Knochengewebe. In: Handbuch der Mikroskopischen Anatomie des Menschen, Bd. 1, herausgeg. von VON MÖLLENDORFF. Berlin: Springer 1930.
WEINMANN, J., and H. SICHER: Bone and bones. Fundamentals of bone biology, 2nd ed. St. Louis: C. V. Mosby Co. 1955.

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