BONE LINING CELLS AND THE BONE FLUID COMPARTMENT, AN ULTRA-STRUCTURAL STUDY

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Critical data has been developed in recent years that supports the hypothesis that ion concentrations in bone fluid and general extracellular fluid are modulated by a functional membrane that separates the fluid of bone from the general ECF. The evidence for the postulated functional membrane in bone is based on the experiments of Geisler and Neuman\(^1\), and Ramp and Neuman\(^2\), Scarpace and Neuman\(^3,4\). The prime points of the data are that potassium is concentrated in the bone fluid and that mineralization of the matrix is increased when bone cells are disrupted or poisoned inferring that the cells serve to partition these fluids, excluding excess calcium from the matrix surfaces. The actual "membrane" and mechanisms of its action are not clearly established. Talmage has suggested that the cells lining the bone surface (osteoblasts) serve this partitioning function\(^5\). The Talmage model proposes that calcium enters the bone fluid compartment by passive diffusion from the general ECF to the bone fluid compartment by passing between the lining cells. In the Talmage model, calcium eflux from the bone occurs by entering the osteocytes and osteoblasts whereupon it is actively transported through the cells and through the plasma membrane to reach the general ECF. Entry into the cell would not be difficult as a downhill gradient would exist, i.e., intracellular calcium levels are approximately 10\(^{-5}\). Extrusion from the cell would be an energy requiring activity as the concentration gradient would hinder eflux from the cell. Talmage has further postulated that PTH elevates plasma calcium levels by first increasing bone cell permeability to calcium allowing calcium to enter the cell more readily. It is then postulated that some "membrane pump" is activated that enhances the release of the cell calcium into the general ECF, but not into the bone ECF, a
concept that would require the bone lining cells to be polarized such that the net result of the "pump" would be flow of calcium from the bone into the general ECF. Neuman and co-workers agree that calcium enters the bone fluid compartment by passive diffusion but they differ with the Talmage model in the explanation for the calcium efflux from the bone fluid compartment. Their studies of aerobic glycolysis, ion fluxes, and bone membranes using an in vitro system indicate that significant changes in lactic acid secretion occur in bone cells as a consequence of PTH activity. Presumably, CT would diminish the secretion of lactic acid. They suggest that the resultant changes in acid secretion are sufficient in magnitude to change the bone fluid pH to levels that would yield enough free calcium and phosphate in the bone fluid to produce a gradient that would let calcium escape from bone by passive diffusion through the same intercellular spaces through which it entered. Both models suffer some criticism as the Neuman model does not provide a basis for the significant differences in potassium concentration and also does not account for the non-stoichiometric release of calcium and phosphate from the bone. The Talmage model seemingly requires a large expenditure of cell energy to maintain a constant direct calcium pump. We have thus addressed ourselves to performing some experiments to determine: are there cell compartments for calcium and do their locations or concentrations change in response to PTH or CT? If the bone lining cells do serve to partition the two fluid compartments, do they show morphologic changes consistent with the physiologic responses to PTH or CT? Do tracers such as horseradish peroxidase, Lanthanum, etc., confirm the proposed fluid pathways? Do in vitro culture systems show cell change consistent with changes in ion concentrations in the accessible compartments? Does phosphate distribution correlate with calcium fluxes?

Rapid changes in bone lining cell morphology to PTH and CT have been reported by us. These observations were made possible by the development of techniques for examining bone with the scanning electron microscope (SEM). With this instrument, large areas of bone cells can be viewed simultaneously so that large numbers of cells in different bone regions can be studied. This has the advantage of allowing the investigator to determine the extent of a given response without the tedious sampling required for transmission electron microscopy. With the SEM, the endosteal surface may be viewed following formalin fixation of the tissue and gentle washing away of the myeloid elements. Critical point drying and evaporative coating with gold-palladium are used following washing to preserve the cell integrity. With this technique the cells (bone lining cells) in immediate juxtaposition to the mineralized matrices may be viewed on their marrow aspect. The bone facing surface of these cells may also be viewed following micromanipulation as reported by Boyd. Figure 1 shows the cell

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