The Lability of bone*

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For a long time it has been fashionable to pay lip service to the idea that bone is a plastic tissue and yet it appears that the total implications of such a view have not been really explored. You had probably anticipated from my title that I would review the question of mineral exchanges in bone, since the emphasis for many years has been placed on these components. I do not propose to follow that well worn path. It is much more profitable and exciting to consider the reactions of bone in terms of its organic or protein components and to draw parallels between the behavior of this matrix or ground substance and the ground substance of less specialized connective tissue.

Our understanding of the chemical and physical structure of connective tissue is currently undergoing an extensive revision. The influence of connective tissue plasticity on the most vital functions of the organism are only now being recognized. One of the most important contributions to this knowledge was made in 1949 by Gersh and Catchpole.[3]

Connective tissue contains cellular and extracellular components. The extracellular portion is composed of fibers, extracellular fluid and an optically homogeneous ground substance. The ground substance is formed largely of a protein-carbohydrate complex. The nature of the linkage between these substances is not understood nor is there much information about the protein. The pioneering effort of Karl Meyer and his colleagues[5] of Columbia University has yielded some information about the sugars involved. Two of the most important of these are chondroitin sulfate and hyaluronic acid. Because of the acidic character of these carbohydrate substances the proteins to which they are linked are negatively charged in the environment of the body. This may be an important factor in explaining many of the physiologic activities of the ground substance, particularly its reactions with positively charged ions.

These carbohydrate-protein molecules are believed to be aggregated together to form larger molecules, macromolecules. The degree of aggregation, often referred to as the degree of polymerization, varies with, and may even determine, the physical state of the tissue. Thus loose connective tissue as in umbilical cord jelly or young dental pulp is thought to display a low degree of polymerization with a more random arrangement of molecular units. On the other hand, hard substances as cartilage, dentin and bone are thought to display a more definite submicroscopic orientation and to be highly polymerized.

While the highly polymerized substances are only slightly soluble in water and relatively inert in the presence of

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many chemical reagents, they are, nevertheless, highly reactive in a different sense. In many physiologic and pathologic states they become altered. For example, the giant molecules may be fragmented or depolymerized. Hard or dense connective tissue structures can be softened or, on the other hand, loose structures can become dense or hardened — even calcified and highly polymerized. These changes of tissue state are in part regulated by some endocrine secretions. The potent effects of such hormones as ACTH and cortisone are in part due to their effects on the connective tissue ground substance. While the specific nature of these effects are still unknown they may occur because the hormones (1) influence the secretion of ground substance or its precursors by the connective tissue cells and (2) regulate the activity of enzymes having to do with the polymerization and depolymerization of the carbohydrate-protein molecules.

With this introduction to the general nature and properties of the ground substance we can begin to understand bone in a different manner. I intend to proceed, not hesitating to continue to introduce occasional speculations, as this is a license one should be permitted among friends.

In areas of bone formation one can observe, with careful microscopy, exceedingly small intracellular granules of glycoprotein (carbohydrate-protein) in the connective tissue cells (osteoblasts).[4] These may be the precursors of the bone matrix. The glycoprotein macromolecules are conceived to be more densely aggregated in bone than in most other connective tissues, that is, the submicroscopic structure is characterized by a high degree of polymerization.[1] The manner in which this is induced is not understood. Perhaps the way in which this particular matrix protein combines with calcium may be the key clue. The following tentative explanation is offered: the negative charge of the macromolecules has been mentioned; as a consequence of this charge positively charged elements, as the ions of calcium, (each carrying a charge of 2+) are attracted to their structure. The negatively charged macromolecules are thus discharged, and the repulsion between like charged macromolecules is reduced, permitting a greater degree of aggregation. This results in the formation of a highly polymerized, hard mineralized structure, bone.

The carbohydrate-protein complex can be demonstrated in preparations for the microscope. To do so accurately requires special fixation by freezing and then drying from the frozen state — (in a manner similar to that used for preparation of dry plasma in blood banks), use of undecalcified tissue for sections, and treatment with chemical reagents which produce insoluble colored products which are specific for carbohydrate containing substances. With these methods young bone stains intensely red indicating the presence of readily available reacting chemical groups. As bone ages the intensity of the stain diminishes indicating the presence of fewer available reacting groups. It is assumed that older bone is more highly polymerized than young bone, that is, the groups which were seen to react with the staining reagent in young bone are involved in cross linking, in developing a more highly aggregated structure as the bone ages. They cannot, then, react, with the microchemical reagent.

The use of special methods which lie within the domain of both histology and chemistry (histo-chemistry) give additional information about some of the cellular processes accompanying ossification. During active bone forma-

tion the connective tissue cells, osteblasts and osteocytes, are packed with large aggregates of glycogen. Lesser quantities are seen in relatively quiescent areas. What role does the glycogen play? In the presence of phosphate, and catalyzed by the enzymes phosphorylase and phosphatase, glycogen could assist in making phosphate available and in supplying energy for the synthesis of bone matrix. It is also possible that a part of the carbohydrate involved in the formation of the protein-carbohydrate complex is derived ultimately from these intracellular stores of glycogen.

Bone resorption can be thought of as a process involving the concurrent solution of the bone protein and mineral. There may be a reversal of the events described in synthesis. It is convenient to suggest that the carbohydrate-protein ground substance is being depolymerized and that its ability to bind calcium and other minerals is remarkably reduced.

What evidence is there to support such an hypothesis? For over twenty-five years it has been maintained that the parathyroid hormone regulates calcium and phosphorous metabolism. Large doses of the hormone cause the rarefaction of the bones and it was presumed that this was due to calcium and phosphorous withdrawal from bone. Recently completed experiments in this laboratory showed that the blood levels of carbohydrate-containing protein (glycoprotein) were elevated in rats injected with parathyroid extract.[2] Histologic, histochemical and chemical studies of the bones show bone resorption accompanied by the release of soluble carbohydrate-protein complex from the bone matirx. Presumably this is material which then appears in the blood causing elevated glycoprotein values. This blood change under the influence of parathyroid extract is consistent. We have thus been led to postulate that parathyroid extract mobilizes the carbohydrate-protein complex of the bone and of epiphyseal cartilage.

The precise manner in which the bone is dissolved is obscure. It is probable that cells are involved in some way. These may be the multinucleated osteoblasts of the classical morphology or other differentiated connective tissue cells. I am suggesting that these connective tissue cells liberate enzymes which break down or depolymerize the macromolecules of the bone ground substance thus reducing its ability to bind materials. In physiological states, a balance or equilibrium is maintained between the activities of synthesis and resorption of bone.

How could the cells be stimulated to produce greater amounts of bone depolymerizing enzymes? In some instances this may stem from local factors, as for example pressure in orthodontic tooth movement. Under other circumstances the cells may be stimulated through hormones, as with parathyroid hormone. Thus far, such enzyme activity has not actually been proven but I consider it an attractive possibility.

By these considerations of the changes in bone matrix one may begin to offer simple explanations which are useful for understanding bone physiology and pathology.

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