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CURRENT PROBLEMS IN RESEARCH

The Ultrastructure and Function of Bone

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Within recent years there has been an increasing understanding of the nonmechanical functions of bone, and this field is now under intensive cultivation, from many points of view. The present article deals with current trends in research, with special reference to the relation of the fine structure of bone to its function.

The particular function of bone with which this paper is concerned is the role of the skeleton in the homeostatic regulation of the ionic composition of the circulating fluids of the body. It is now clear that the bones serve as a reservoir for the all-important calcium ion, and to some extent for other ions as well. For control of the concentrations of these ions in the blood, which is essential to the maintenance of a steady state in the internal environment, a combination of ion transfer and exchange, in both directions between blood and bone, is in constant operation. Such transfers and exchange are partly purely physicochemical, but they are aided and regulated by biologic processes, including hormonal control and cellular activity.

Current understanding of these processes is dependent upon a variety of experimental approaches to the subject. This paper, while emphasizing the relation of structure and more particularly of fine structure, to function, will draw upon other methods of observation and of experiment, in order, in so far as possible, to present a unified account of the current state of research on the interchanges between blood and bone. Individual statements are not documented, but a short list of references of general interest is appended.

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Structure of Bone

The most striking characteristic of bone is that it is hard; this hardness results from the deposition, within a soft, organic matrix, of a complex mineral substance, composed chiefly of calcium, phosphate, and carbonate. While the structure of bone, as observed by the light microscope, has been well described for many years, the introduction of new biophysical techniques, such as x-ray diffraction and electron microscopy and the use of radioactive isotopes, has provided much new information concerning the fine structure of both the organic matrix and the mineral of bone and has added to our knowledge of the interrelationships between these components. These newer methods, as well as refinements of those longer in use, continue to give insight into the relation of ultrastructure to function of bone, and they are being actively pursued.

The unit of structure of bone, at the microscopic level, is the haversian system, or osteon. This is, when fully formed, an irregularly cylindrical and branching structure, with thick walls and a narrow lumen, the haversian canal. The canal carries one or more capillaries and venules. The cylindrical osteons are usually oriented in the long axes of the bones, and their basic structure consists of concentric layers, or lamellae, the fibrils of each lamella running spirally to the axis of the canal. The osteon, in addition to its canal and its fibrillar structure, includes large numbers of lacunae, housing the cells of bone (osteocytes) and interconnected with one another and with the lumen by means of branched canalicules. This circulatory system, poor as it may appear, is the only means for transfer of fluids and dissolved substances between the hard tissue of bone and the fluids of the body. In cross section the osteons average approximately 150 microns in external diameter, while the canal may not measure more than 20 microns in diameter. The length of the osteons, however, commonly extends up to several millimeters.

An osteon is formed by deposition of layers, or lamellae, of fibrillar bone matrix on the walls of a cylindrical cavity or tunnel, and subsequent mineralization of these lamellae. After an osteon has been fully mineralized it loses some of its ability to react with the fluids of the body, mainly because its constituents are less accessible to the circulating fluids. Throughout the lifetime of the individual, however, compact bone undergoes a process known as haversian remodeling. This insures the availability of a constant supply of reactive bone, which accounts for less than one percent of the total mass of bone, and it is this reactive bone that gives the skeleton its function as a tissue.

Techniques for Study of Ultrastructure

At the electron-microscope level much information has been obtained concerning the fibrillar structure of bone and the characteristics of collagen fibers, as well as about the form of the crystals of the bone mineral and their relation-

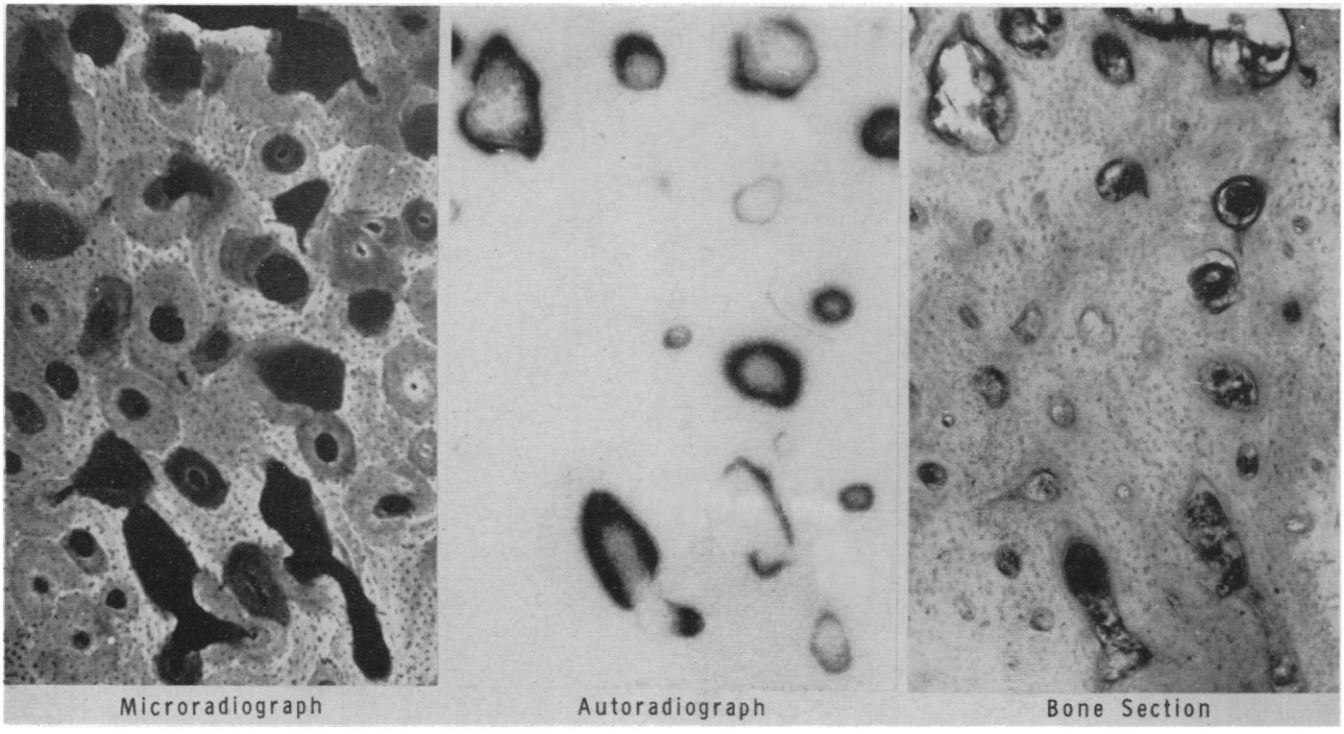

Fig. 1. Microradiograph, autoradiograph, and photomicrograph of the same section, from the shaft of the tibia of a young adult dog, sacrificed two weeks after intravenous administration of 250 microcuries of strontium-90 per kilogram. Undecalcified, unstained, ground transverse section (×62). [Courtesy of Jenifer Jowsey].

ship to the collagen fibers and to the ground substance. A beginning has been made on the ultrastructure of ground substance, until recently described as amorphous. The potentialities of the electron microscope for elucidating the fine structure of bone, and thus permitting further insight into its functions, have by no means been exhausted.

Electron microscopy of bone has been aided by x-ray diffraction, both high-angle and low-angle. High-angle diffraction, particularly with microdiffraction techniques, has been of much assistance in studying the crystallographic properties of bone tissue. Low-angle diffraction has given information about the dimensions of the particles and their orientation.

Microradiography requires the passing of x-rays through thin sections and the recording of differences in absorption of the radiation on photographic film, thus permitting enlargement of the image. Application of this technique to bone has given results of great interest. Whereas ordinary histologic sections of bone do not reveal differences in the density of individual osteons, such differences are clearly demonstrated in microradiograms, the younger osteons being less mineralized, and consequently less dense than the older ones. This has focused attention on the mineralization of osteons, and it is readily demonstrable that the new and less dense osteons account for most of the uptake of radioactive calcium, strontium, and phosphorus.

When a radioisotope—for example, strontium-90—has been administered to an animal and undecalcified sections of bone have been made, it is possible to prepare photomicrographs, microradiograms, and autoradiograms, all of the same section. These may be enlarged to the same size and compared (Fig. 1). The radioisotope is not only taken up chiefly by the newer and less dense osteons; by far the greater portion of it is taken up by a thin layer of new and only slightly mineralized matrix, which may not be seen at all in microradiograms, but which may be visible in the section under the light microscope. When this layer is absent, no appreciable amount of radioisotope may be taken up by a particular osteon, even though the osteon as a whole may be incompletely mineralized, and hence of a density comparable with others which do take up the isotope. These combined techniques afford a record of the sequence of events in the growth and mineralization of bone, not seen by any one of the techniques alone.

The results obtained on normal bone tissue by microradiography have been confirmed by microinterferometry. In addition, microinterferometric measurements made on decalcified sections have shown that the content of organic material varies but little from one osteon to another, indicating that the differences seen on microradiographs depend solely upon the degree of mineralization. The polarizing microscope also gives new and valuable information about the ultrastructural organization of calcified tissues.

Organic Matrix of Bone

Collagen. Collagen is a fibrous protein, common to all forms of connective tissue. Most of the protein present in bone is collagen, which is responsible for about 95 percent of the dry fat-free organic content. It occurs in fibers about 800 angstroms wide, of indeterminate length, characterized by dense cross-banding at intervals averaging about 640 angstroms, and is generally oriented in the long axes of the bones. It is in an organic crystalline form, capable of refracting x-rays. In addition to a wide-angle pattern, it gives a low-angle pattern with a fundamental repeating unit of about 660 angstroms; this is in good agreement with the cross-banding observed with the electron microscope.

Collagen from certain sources, such as rats' tail tendon, can be dissolved and reconstituted by appropriate treatment. When reconstituted it may assume one

of a number of forms, according to the method of treatment, including the native form as seen in connective tissue. From electron microscopic observations it is believed that bone collagen is secreted by osteoblasts—the bone-making cells—in soluble form as molecular units, with the capability of self-aggregation.

Ground substance. The remainder of the organic matter of bone, slightly less than 5 percent, is termed the "ground substance"; it fills the spaces between the collagen fibers and the crystals of bone mineral. In connective tissue in general, ground substance has commonly been described as amorphous, but recent electron microscopic observations indicate that it has an organization and ultrastructure of its own. It varies in consistency from that of the interstitial fluid to that of the basement membrane. It is coextensive with both; they represent its fluid and condensed portions, respectively. The interconnection of the ground substance with the tissue fluid, by virtue of which the two exist as a continuum, permits exchange of ions and other substances with the blood. Chemically the ground substance consists of protein and carbohydrates, some of which exist as polymers of glucuronic acid and hexosamines, in both sulfated and unsulfated forms.

Mineral of Bone

Ultrastructure. There have been differences, not wholly resolved, in the reports of the form of the crystals of bone mineral. All observers are agreed that the crystals are extremely minute, and estimates of the dimensions vary only within comparatively narrow limits. The crystals have been described as hexagonal tabular forms, of variable size, but in general only a few hundred angstroms in length and breadth, and with a thickness of only a few unit cells, averaging a total of perhaps 20 to 50 angstroms. Low-angle x-ray diffraction results have been interpreted to indicate that the crystals are rods or hexagonal prisms, about 200 angstroms long and 75 angstroms in diameter. That the crystals are rod- or needle-shaped, with a length of 200 to 700 angstroms and a diameter of 30 to 50 angstroms has been the finding most commonly reported, from electron microscopy, in recent publications. All of these findings are in agreement about the tremendous surfaces of the crystals in relation to their mass and about the importance of surface phenomena in determining the composition and reactivity of the mineral phase of bone.

A subject of much current interest is the interrelationship between the collagen fibers, with their cross-banding, and the mineral of bone. It has been suggested, but not proved, that the collagen fibers, or some part of them, are responsible for seeding and thereby initiating the deposition of the crystals of hydroxyapatite, perhaps by the phenomenon known as epitaxy. A variant of this is the proposal that the mechanism of nucleation involves a specific stereochemical configuration resulting from a particular state of aggregation of collagen macromolecules. In any event it is clear that the long axes of the crystals are commonly oriented in the long axes of the collagen fibers, that they are arranged for the most part around the fibers or fibrils, forming a sort of a sheath, and that they have a special relationship to the cross-banding.

Small particles, approximately 100 angstroms in diameter, with no preferred orientation relative to the fibril axis, have been described between the bands in the earliest stage of calcification. In fully calcified bone the crystals, oriented in the fibril axis, tend to form bands around the collagen fibrils, leaving gaps, as observed with the electron microscope, between the bands (Fig. 2); these gaps correspond to the spacing of the collagen fibrils between the periodic cross bands. In some instances the crystals are long enough to span two or more major periods, obscuring the fiber period. The question of the possibility of crystalline or amorphous mineral substance, situated *within* the collagen fibers, remains undecided. The close physical association between the fibers and the crystals of bone mineral lends weight to the belief that there is a chemical interaction between the two phases; it has been proposed that the close association of the organic matrix with the crystals serves to preserve an instability in crystal structure, responsible for the reactivity of the crystals.

Chemistry. Despite intensive efforts over the past century, and despite the great advances made in recent years, the chemistry, dynamics, and ultrastructure of bone mineral remains a fertile field for inquiry.

It is now firmly established, by x-ray diffraction and by other methods, that the bone salt belongs to the apatite series of minerals, of which the prototype is fluorapatite:

$$3Ca_3(PO_4)_2 \cdot CaF_2,$$

which is abundant in nature. The weight of evidence is to the effect that the crystalline material found in bone corresponds most closely to hydroxyapatite:

$$3Ca_3(PO_4)_2 \cdot Ca(OH)_2$$

although there are differences in interpretation of the evidence now available. While the bone mineral contains considerable amounts of carbonate and citrate, current opinion is to the effect that these substances are related to the

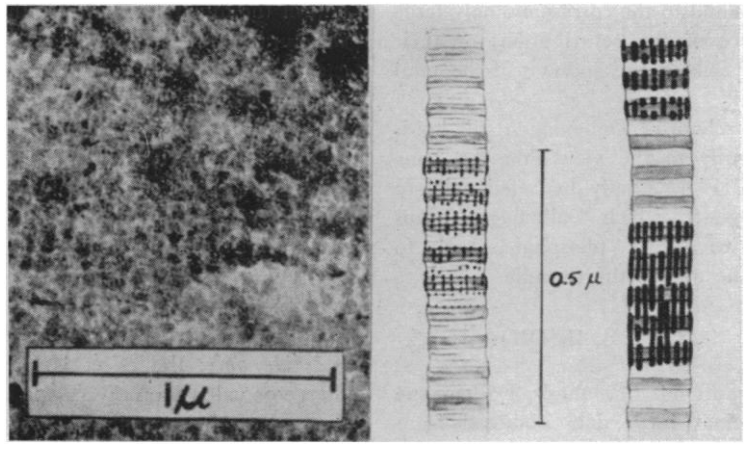

Fig. 2. (Left) Electron micrograph of a section of an undecalcified human rib, illustrating the pattern of arrangement of crystals of bone mineral in relation to collagen fibers. The underlying collagen is barely visible, but the crystals are easily seen in the regularly arranged bands corresponding to the main collagen striation. The longitudinal direction of the fibers is horizontal. [From Fig. 1, W. F. Neuman and M. W. Neuman, *Chem. Revs.* **53**, 4 (1953) (courtesy of R. A. Robinson and M. L. Watson). Reproduced by courtesy of Williams and Wilkins Co.] (Right) Course of calcification in bone matrix, showing the relation of inorganic crystals to cross banding of collagen fibers. [From Fig. 21, R. A. Robinson and M. L. Watson, *Ann. N.Y. Acad. Sci.* **60**, 620 (1955). Reproduced by courtesy of the publishers.]

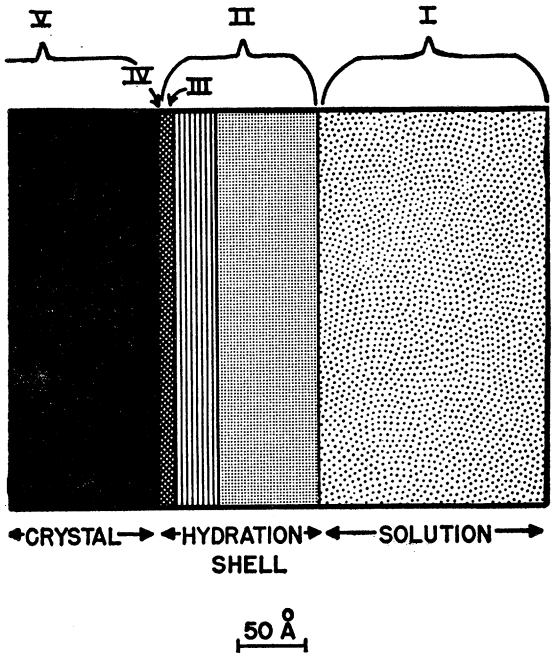

Fig. 3. Diagrammatic representation of the crystal-solution interface. I, the bulk solution (intercellular fluid): II, the hydration shell, comprised of three layers — loosely held water, tightly bound water, and the hydrated ion layer (III); IV, the lattice surface; and V, the crystal interior. [From Fig. 1, W. F. Neuman and M. W. Neuman, *Am. J. Med.* **22**, 124 (1957). Reproduced by courtesy of the publishers.]

surfaces of the crystals of hydroxyapatite and that they are not part of the lattice structure. The small amounts of such ions as Na^+, K^+, Mg^{++}, Cl^-, and F^- found in the mineral are regarded as impurities, resulting from the formation of the mineral from the body fluids, from which these ions are deposited during the process of crystallization. More difficult to account for is the fact that the molar ratio Ca/P, theoretically 1.66, varies from 1.3 to 2.0 in a series of solid calcium phosphates which give the characteristic apatite lattice pattern on x-ray diffraction. This variation is now attributed to the extremely small size of the crystals and to the correspondingly large influence of the contributions of their surface ions to the analyses of the total mineral.

The school of Dallemagne still holds tenaciously to the view that the bone mineral is essentially hydrated calcium triphosphate, which Dallemagne terms alpha tricalcium phosphate, and to which he assigns the formula

$$3Ca_3(PO_4)_2 \cdot H_2(OH)_2$$

In support of this thesis Dallemagne now brings forth data obtained from observation of ion-exchange reactions, which he interprets to indicate (i) that the mineral of living bone is in an unstable form; (ii) that it is kept in this unstable form by its close association with the organic matrix; (iii) that the characteristics responsible for its instability are also responsible for its reactivity in the living organism; and (iv) that on removal from the bone, except under very special precautions, it is transformed into the stable hydroxyapatite, which then is identical with preparations of this mineral prepared synthetically.

Crystal complex. An understanding of the phenomena of ion transfer or ion exchange between the crystals of bone mineral and the circulating fluids of the body requires as accurate information as possible concerning the ultrastructure of the reactive crystals and of their surroundings. The descriptions here used are derived from those of Neuman and Neuman. It is convenient to regard the unit of the mineral of bone, at the ultrastructural level, as a crystal complex, each such complex being based upon a microcrystal of colloidal dimensions, only a few hundred angstroms in length and with a thickness of only 20 to 50 angstroms. Owing to their minute size, these crystals have an enormous surface per unit of mass—of the order of 200 to 300 square meters per gram. Each ion held on the surface of the crystal is surrounded by oppositely charged ions *on one side only*; the effect is a residual charge or valency on the crystal surface. Because of their small size, these crystals can attain greater stability almost exclusively by chemisorption; this leads to a highly variable surface composition. It results also in increased reactivity of the crystal complex. Around the crystal and partly bound to its surface there are also a layer of hydrated ions and a shell of water which may exceed the dimensions of the crystal itself. Synthetic apatite crystals, for example, may bind 0.8 gram of water per gram of crystals; because of the difference in density, this represents a volume of water greater than that of the crystal.

Each crystal complex, therefore, where adequate hydration is possible, may be viewed as a series of layers, as illustrated in Fig. 3. Any ion from the surrounding fluids can diffuse into the outer layers of the hydration shell. At the crystal surface there is a bound layer of hydrated calcium, phosphate, and hydroxyl ions, constantly interchanging with similar ions in the surface layers of the lattice. Owing to vacant lattice positions there is also a constant though much slower ion interchange within the crystal itself. Of the ions in the fluids of the body, some can penetrate the hydration shell only (K^+ and Cl^-), some can penetrate the surface-bound ion layer of the lattice surface by displacing calcium, phosphate, or hydroxyl ions (Na^+, UO_2^{++}, CO_3^{--} and citrate^{-3}) and some can pentrate still further—that is, into the crystal interior (Ca^{++}, Sr^{++}, Ra^{++}, PO_4^{-3}, F^-). All of the possible ion transfers and movements may be assumed to be of constant occurrence wherever the fluids of the body are in close contact with the crystal complex of reactive mineral.

In contrast, as the crystals age, and as they increase in numbers, they gradually displace the water essential to their reactivity, until eventually there remains a solid, inert, almost unhydrated mass of crystals and fibers, incapable of more than a very slight degree of ion exchange or transfer with the fluids of the body. Such stable bone tissue makes up more than 99 percent of the mass of compact bone, and it can be made available to the organism as a whole only by cellular action, resulting in resorption. This conversion of reactive to nonreactive bone, which is a necessary concomitant of mineralization of the organic matrix, would eventually lead to a skeleton composed entirely of stable mineral—a condition incompatible with the life of the organism—were it not for the constant haversian remodeling of compact bone that continues throughout the life of the individual. In this respect the state of the mineral of bone differs sharply from that of most mineral deposits in nature; a condition of instability is a *sine qua non* of life; stability of the bone mineral, as well as of other components of the body, is a state that can be attained only after the death of the organism.

Dynamic aspects. A kinetic approach

to the interchange of ions, more especially of calcium and phosphate, between blood and bone, has been made possible in recent years by the introduction of radioisotopes. Not until the first papers appeared from Hevesy and his group in 1935, describing the behavior of radioactive phosphorus, phosphorus-32, administered to animals as inorganic phosphate, was it possible to appreciate the magnitude of the rate of turnover of skeletal phosphorus. Owing to the more limited availability of calcium-45, demonstration of its uptake by the skeleton was delayed until 1940, and in fact it was not until after 1948 that calcium-45 could be used in biological work in quantities adequate for the elucidation of the many problems relating to calcium metabolism. Still more recently a beginning has been made in Sweden with calcium-47, which is as yet not in general use; because of its short half-life and high-energy gamma radiation, it can be traced by external counting methods, and used safely in man. Strontium-85, with much the same properties, is available and has been used more extensively, but since the body discriminates between calcium and strontium the latter is not an entirely reliable indicator of the behavior of calcium.

The use of radioisotopes has not yet progressed to the level of ultrastructure. Calcium-45 and phosphorus-32 have been used for evaluation of over-all accretion, resorption, and exchange reactions in the skeleton and they have made possible quantitative estimates of the relative magnitudes of these processes, thereby throwing light on their physiologic significance. With the use of strontium-85, the application of external counting methods, and the employment of an electronic analog computer, the principles of the studies made in animals have been extended to man. Exponential analysis of the data obtained has made it possible to study rates of movement of the isotope between four compartments (serum, extracellular fluid, intracellular fluid, and bone). Similar studies, with improved equipment and using calcium-47, are now under way and are opening a wide field for further investigation.

Homeostatic Control of Calcium Ion Concentration in the Blood

The maintenance of an approximately constant concentration of the physiologically active form of calcium, the calcium ion, in the circulating fluids of the body is essential to life. Among other physiologic activities, the calcium ion, in an appropriate concentration, is essential to the heart beat, to the coagulation of blood, and to the proper functioning of nerve tissue.

It has been known for some years that the parathyroid glands, four tiny bodies embedded in the posterior surface of the thyroid gland, play a decisive role in the regulation of the calcium ion concentration in the blood plasma. Only within recent years, however, have the details of this mechanism begun to be fully understood, and certain aspects of it are still under intensive study. These glands and their functions may be regarded as analogous to the thermostatic control of the temperature of a house. Thus the parathyroid glands are sensitive to the concentration of calcium ions in the blood; a deficiency in calcium ions leads to increased activity of the glands, while an excess leads to decreased activity (Fig. 4). In recent years such self-regulating processes have acquired the name "feedback." The condition that is being regulated is itself the stimulus activating the regulatory mechanism; information about the output is fed back to an earlier stage of the process so as to influence its action and thereby control the output. Feedback is being increasingly made use of in industrial processes; the living organism is dependent upon a multitude of such built-in feedback mechanisms.

Once it was established, only a generation or so ago, that the parathyroid glands regulate the release of calcium from the bones into the blood, it was commonly believed that this relatively slow-acting process was adequate to maintain a physiologic level of calcium ions in the blood plasma. This belief was strengthened by the fact that removal of the parathyroid glands leads to a lower concentration of calcium ions in the plasma, while hyperparathyroidism, or

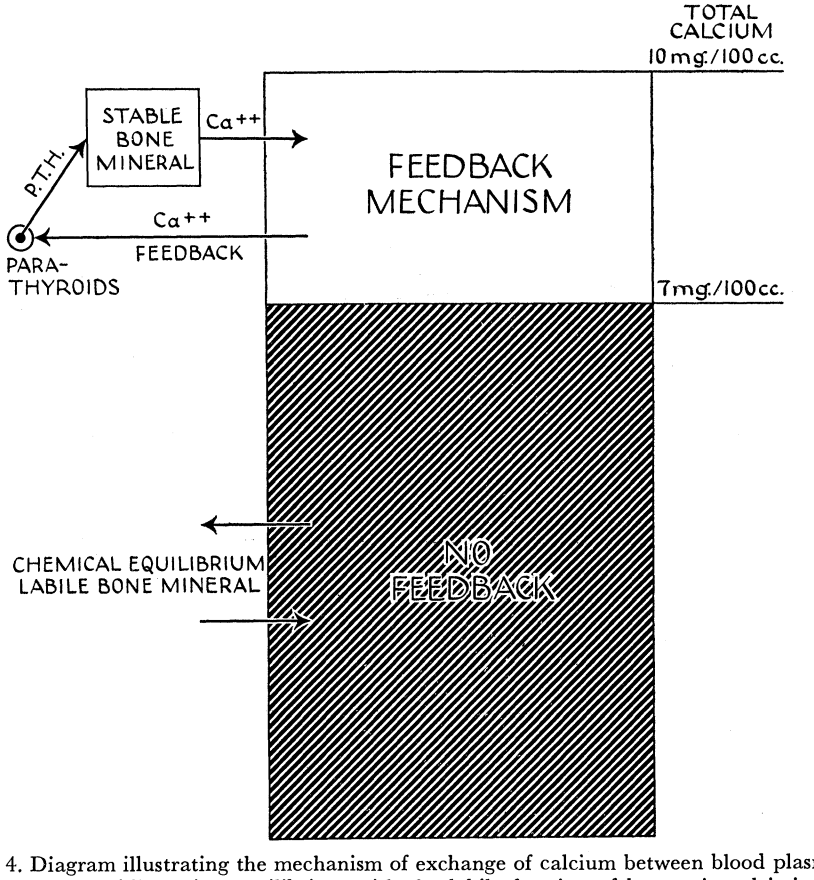

Fig. 4. Diagram illustrating the mechanism of exchange of calcium between blood plasma and bones. Rapidly acting equilibrium with the labile fraction of bone mineral is independent of the parathyroid glands and is adequate to maintain the plasma calcium level at approximately 7 milligrams per 100 cubic centimeters. Parathyroid activity is under control of feedback from Ca^{++} concentration in the plasma and regulates the slower release of calcium from stable hydroxyapatite crystals of bone mineral. The parathyroid glands monitor the calcium concentration of the plasma and maintain it at approximately 10 milligrams per 100 cubic centimeters. [From Fig. 14, F. C. McLean and M. R. Urist, *Bone* (Univ. of Chicago Press, Chicago, Ill., 1955), p. 77. Reproduced by courtesy of the publishers.]

administration of the parathyroid hormone, leads to a higher concentration of calcium ions. Introduction of tracer methods, however, and increasing understanding of the internal structure of bone have made it clear that the parathyroid mechanism, while responsible for hour-to-hour or day-to-day adjustments, is not alone adequate for the minute-to-minute interplay between blood and bone. Especially revealing in this respect is the demonstration that, in young animals, the turnover of the blood calcium may amount to as much as 100 percent per minute, which is to say that the equivalent of the total amount of calcium in the blood may be replaced every minute.

In order to account for this rapid turnover, it is necessary to postulate a "dual mechanism" for homeostatic control. One part, which acts rapidly, requires ion transfer or ion exchange between blood and bone and is independent of the function of the parathyroid glands, being able in the absence of these glands to maintain the plasma level of calcium at approximately 7 milligrams per 100 cubic centimeters. At present there are two views about how this operates. One view is that the bone acts as an ion-exchange column, taking up calcium ions from the blood and returning others, by passive physicochemical mechanisms. By this view the labile or reactive bone mineral, or that in the newly formed and partially mineralized osteons, is in equilibrium with the ions in the fluids in contact with them. If the equilibrium is disturbed, by removal of calcium ions from the blood, additional calcium ions are transferred from the labile stores until the physiologic level has again been reached; if calcium is added to the blood, the excess is promptly transferred to the labile stores.

Another view is that this transfer mechanism may itself involve intervention by cell activity, at least in the direction of bone to blood. It has been demonstrated that both the parathyroid hormone and vitamin D, which appear to have a synergistic action in this respect, control the concentration of citric acid in the bones, presumably by influencing its formation by the cells of bone. It is then contended that the citric acid acts to carry calcium into the blood stream, by forming soluble calcium-citrate complexes, and that following this the citrate ion is metabolized in soft tissues, thus leaving the calcium in the blood. It is possible that both passive ion transfer and a mechanism depending upon citric acid formation, under the control of the parathyroid hormone and of vitamin D, operate to influence the rapid exchange of calcium between blood and bone. In any case, the transfer of ions that takes place over periods of seconds or minutes now seems to be definitely localized in the accessible, labile, and reactive bone in the new and incompletely mineralized osteons.

The possible role of the parathyroid glands in mediating this rapid transfer of mineral from bone to blood, by control of the production of citrate, cannot now be regarded as fully established. The function of the parathyroid glands with respect to the dual mechanism is, however, better understood, at least in its outward manifestations. Normally functioning parathyroid glands are essential to the maintenance of the plasma calcium at the physiologic level of approximately 10 milligrams per 100 cubic centimeters. When the parathyroid glands are removed, the plasma calcium falls to approximately 7 milligrams per 100 cubic centimeters, where it is stabilized by ion transfer; when there is hyperfunction of the parathyroid glands, the level may rise to 15 milligrams per 100 cubic centimeters, or even higher, and be maintained at abnormally high concentrations by the hyperactivity of the parathyroids.

There is abundant evidence that these glands exert their control over the level of calcium in the blood by influencing the solution of bone by osteoclastic resorption, and that this process has access to the stable, fully mineralized bone, or to the bulk of the bone not accessible to the rapidly acting transfer mechanism. Osteoclasts are giant cells, with many nuclei, often as many as 15 or 20. They are located mainly on the inner surfaces of bone—that is, on the surfaces in contact with the bone marrow. While it has not been proved that these cells erode bone, their constant occurrence in areas where resorption is taking place, together with the associated histologic picture, "suggest that their presence is more than incidental." Administration of parathyroid extract to animals leads to greatly accelerated resorption of bone, usually associated with large numbers of osteoclasts; parathyroid control of resorption is well established, even though osteoclastic participation is still conjectural.

To fit these pieces together, we may visualize the two parts of the dual mechanism, as illustrated in Fig. 4, as follows. (i) The parathyroid glands are responsible for monitoring the calcium ion concentration in the blood plasma. They respond, rather slowly, to changes in the calcium ion concentration in the internal environment, and by regulating the dissolution of stable bone mineral they serve to maintain a relatively constant level of calcium ions in the blood. (ii) What we may call the fine adjustment of the calcium ion concentration in the blood—that is, the minute-to-minute control—is effected by rapid transfers, in both directions, between the blood and the labile fraction of the bone mineral. Whether this is a purely passive chemical affair, by diffusion equilibrium, or whether it also is mediated by the parathyroid hormone (as well as by vitamin D) remains uncertain. Whatever may be the intimate details of this part of the mechanism, and however it may be related to the ultrastructure of bone, it is essential to the continued life and health of the organism, which without it would be subject to violent fluctuations in the levels of calcium ion concentration in the internal environment.

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