BONE DISEASES

in Medical Practice

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Preface

In recent years extensive investigation in the field of skeletal diseases has clearly shown that bone, like other tissues, enters into the vital metabolic processes of the body. The skeleton no longer can be considered to act solely as a static, supporting framework of the body. The latter function, of course, is of paramount importance for health and well-being, but we must not overlook that bone lives, is constantly changing, and markedly influences many of the vital functions of the organism.

The viable portion of bone is made up of a characteristic cellular population living in an intricate and specific protein-carbohydrate environment referred to as the bone matrix. In this matrix the inorganic bone salts are deposited. The metabolic importance of the large amounts of calcium and phosphate that are liberated during pathologic resorption of bone has been known for several decades. We now recognize that in both the healthy and the diseased skeleton the protein bone matrix, too, is the site of many metabolic processes that are just as important as the well-known changes of calcium and phosphorus metabolism. In this way the metabolic role of the skeleton may be considered to be as significant as its supportive function.

Recently, the availability of radioisotopes has afforded rich opportunities to explore the normal and pathologic physiology of bone and bone marrow, previously inaccessible to investigation. Moreover, improved laboratory techniques have markedly refined the clinical differential diagnosis of bone diseases and new therapeutic measures have been introduced. This may, at least partly, be responsible for the recent wave of enthusiasm of the general medical profession for this chapter of medicine. It was only a few years ago that the following sardonic observation seemed justified:

> When budding physicians are first introduced to osteology, the skeleton seems to be only a collection of oddly shaped tubes and plates consisting of calcium carbonate and calcium phosphate. Most members of the medical profession cherish this first impression for the rest of their lives.

Fortunately this attitude has radically changed and the general physician is no longer of the opinion that the knowledge of skeletal diseases represents an esoteric chapter of medicine, falling within the province of the orthopedist, the radiologist, or the "medical bone specialist."

The author has become vividly aware of the mounting interest shared by all members of the medical profession for this phase of medicine. He has had numerous opportunities to share this interest with varied audiences, and it is because of such general enthusiasm for this subject that the present book was written. In its actual format this volume has one major objective: to present the subject in such a way that it can be used in the daily practice of medicine, emphasizing that in many skeletal diseases a clear-cut diagnosis can be made which will usually point the way to rational and often remarkably rewarding therapy.

As is always the case in the preparation of a book on bone diseases, the writing of the manuscript is difficult, the reproduction of the representative roentgenograms a heartbreaking task. The x-rays illustrating the text have been culled from collections of many different hospitals located in three different continents. I am deeply obliged to many of my colleagues who have helped me find the roentgenologic material that would satisfy the technical requirements necessary to obtain good, sometimes even excellent, reproductions. The technical part of the new photo-electronic methods employed for the improvement and the reproduction of roentgenograms was in the able hands of Mr.

Robert Carlin. The publisher has gone to great lengths to find an adequate method, in this case the gravure process, for the reproduction of the roentgen photos in book form. Only the reader can decide whether such additional effort has been rewarded.

This book, therefore, is the end result of the efforts and the interest of many medical and non-medical colleagues and friends. The author can only assure all of them that he will never forget their valuable help and their continuous encouragement.

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Chapter 1

Physiology of Bones

FOR MANY YEARS IT WAS GENERALLY believed that the skeleton, in the depths of which living bone marrow is hidden, served solely as a support for surrounding organs. Today, however, it is clearly recognized that only 70 per cent of the bone substance consists of inorganic salts. The other 30 per cent—some authors label it 50 per cent—is made up of living tissue that has a complicated metabolism. This is the so-called bone matrix, consisting of an amorphous ground substance and highly differentiated fibers. In the bone matrix both specific proteins and carbohydrates are present. Bone is formed by deposition of bone salts in this matrix.

Apart from bone matrix and bone salts, the bone substance contains living cells—osteoblasts, osteocytes and osteoclasts.[9] The osteoblasts are found on the surface of the bone trabecules; the osteocytes are osteoblasts surrounded by calcified bone matrix; and the osteoclasts are multinuclear giant cells which nowadays are considered to be formed by the fusion of osteoblasts. Osteoblasts form bone matrix, osteocytes serve to maintain the bone and osteoclasts participate in the dissolution or resorption of bone. Osteoclasts live only a short time, probably 36 hours; the life span of osteoblasts is much longer. One osteoclast destroys in 36 hours the amount of bone which ten osteoblasts can produce in 10 days. It follows that if, in histologic bone sections, one osteoclast is present for every few osteoblasts, excessive bone destruction must be going on. Osteoblasts form an alkaline phosphatase, while osteoclasts allegedly produce an acid phosphatase.

BONE MATRIX

Part of the collagen—the specific protein moiety of the bone matrix—is present in amorphous form. The rest of the collagen is arranged in fibers which, when studied with the electron microscope, prove to consist of bundles of fibrils. These collagen fibrils, with a diameter varying between 200 and 2000 Å (angstrom units*), have a characteristic band structure. The distance between the principal bands is always the same—640 Å.

Collagen contains considerable amounts of glycine, glutamic acid, proline, hydroxyproline and small quantities of other amino acids. Hydroxyproline and hydroxylysine are the characteristic components of collagen, because these two amino acids are found only in collagen and not in blood serum. No cystine, cysteine or tryptophane are present in collagen. Collagenases, enzymes which can break down unaltered collagen, are rarely encountered in nature and have not been demonstrated in the skeleton. The only known sources of collagenases are B. subtilis and two different strains of clostridium. Fresh unaltered collagen is resistant to proteolytic enzymes, but the latter can digest collagen which has first been denatured by acid, alkali or heat.

The carbohydrates of the bone matrix belong to the group of the mucopolysaccharides. The prevailing ones are chondroitin, chondroitin sulfate and hyaluronic acid, but the presence of other mucopolysaccharides has been proven. In the ground substance of the bone matrix, compounds of collagen and mucopolysaccharides are present which are

*One angstrom unit is one ten millionth of a millimeter.

able to initiate calcification, both in bone matrix and in cartilage.[13,21]

Whereas the primitive collagen fibril itself does not contain any mucopolysaccharides, the latter substances are found in the collagen fibers, which consist of hundreds of fibrils.[17] These fibrils are kept together by a cement substance, and the mucopolysaccharides are probably present in the latter material.

In histologic preparations, the bone matrix presents in the form of osteoid. Due to the presence of mucopolysaccharides, osteoid in fixed preparations stains metachromatic; with toluidine blue, the osteoid appears reddish, whereas the rest of the tissue stains blue. The metachromasia disappears under the influence of hyaluronidase.

BONE SALTS

The main part of the bone salts consists of calcium and phosphate, in a relation of 1:2. About 99 per cent of the total body stores of calcium, 90 per cent of the citric acid, 30 to 40 per cent of the sodium, 25 per cent of the body water, and considerable amounts of magnesium, fluoride and other essential electrolytes are found within the skeleton and the teeth.

The precise chemical constitution of the calcium phosphate compound of bone is still debatable. Formerly, it was surmised that bones consists of tertiary calcium phosphate to which magnesium phosphate, calcium carbonate and calcium fluoride had been adsorbed. Later, it was believed that the bone salts closely resemble the mineral apatite, a hexagonal calcium phosphate. Recent evidence indicates that the molecules of the bone salts contain a considerable number of hydroxyl groups. For the time being it is accepted that the bone salts consist of a hydroxyapatite, that is, a hexagonal tricalcium phosphate with hydroxyl groups fixed on the corners of the molecule. In addition, fluoride, phosphate, carbonate, bicarbonate, citrate, sodium, magnesium and potassium are adsorbed to the hydroxyapatite molecule. Other mineralogists, however, have vigorously defended other structural formulas for the bone salts—hydrated tricalcium phosphate, for example.

In bone, the hexagonal hydroxyapatite microcrystals, measuring 200-300 by 20-50 Å, are arranged in long columns within the ground substance and form rings around the collagen fibers.[19] Ultimately, a lattice structure results in which one gram of bone salt is spread over a tremendous surface, which is said to vary between 106 to 200 square meters. Inasmuch as the calcium content of the skeleton of the adult varies around 1500 grams, the whole surface area of an adult male skeleton must amount to more than 100 acres. This vast surface is bathed by a few liters of body fluids. It was generally believed that the entire outer surface of the crystals—about 14 per cent of the total crystalline mass—could partake in surface chemical exchanges. Thus, the opportunity for a rapid transport of different ions from the extracellular fluid to the surface layers of the tremendously expanded bone substance seemed practically unlimited. Recent measurements, however, indicate that in a normal adult man the total amount of skeletal calcium available for exchange does not exceed five to six grams. Since this quantity amounts to less than one half of one per cent of the total calcium of the skeleton, it is apparent that only a fraction of the theoretical crystal surface can participate in the rapid exchange of calcium from the extracellular compartment to the surface of the skeleton.[2]

PHYSICAL CHARACTERISTICS OF BONE

The collagen fibers of the bone matrix are organized in the form of regular lamellae, which fit snugly between the columns of calcium salts. This arrangement is similar to the structure of reinforced concrete, plywood and laminated plastics. The remarkable re-

sistance of bone to both compression and tension is due mainly to this spatial relationship between the bone matrix and the plates of hydroxyapatite. Not only is bone three times stronger than wood, but the tensile strength of bone is nearly as great as that of cast iron. In addition, lumbar vertebrae can withstand a pressure of 600-900 lbs. per square inch. This rare combination of marked resistance against both tensile and compression forces is hardly ever found in building materials.[5]

In this connection, the remarkable mechanical characteristics of the intervertebral disks may be mentioned. Increasing loads up to 100 kilograms cause only negligible changes in the configuration of the lumbar disks.[11] Under such a load, the compression of the disk does not exceed 1.4 millimeter, the expansion, 0.75 millimeter. There is a fundamental difference, however, between the results of a continuous compression and a sudden trauma of short duration to the vertebra. After a sudden blow, an intervertebral disk starts to oscillate. These remarkable oscillations occur even if a sudden short blow is directed against a disk which previously had been placed under a static load of 130 kilograms. In this way, the intervertebral disks represent a shock-absorbing apparatus that protects the structure of the vertebral column. In the higher age groups the ratio between the amounts of collagen and polysaccharides present in the disks changes. This leads to decreased water content, which impairs the shock-absorbing capacity of the intervertebral disks.[12] Thus, in older individuals the vertebral column becomes more and more vulnerable to trauma.

USE OF RADIOISOTOPES FOR THE INVESTIGATION OF BONE PHYSIOLOGY

The introduction of isotopes into experimental medicine has provided extremely valuable tools for the investigation of the physiology of the skeleton. Both radioactive calcium (Ca^{45}) and radioactive phosphorus (P^{32}) have introduced new approaches to the investigation of the deposition and absorption of the inorganic bone salts. Investigation of other "bone-seeking" isotopes has led to the conclusion that both Sr^{85} and Ba^{140} can be used for the same purpose. Since chondroitin sulfuric acid contains sulfur, radioactive sulfur (S^{35}) has been employed to obtain a better understanding of some of the problems connected with the mucopolysaccharide component of bone matrix. Radioactive carbon (C^{14}) has been used in animal experiments to analyze the collagen part of the bone matrix.

The first experiments with oral administration of radioactive calcium to rats showed that within 45 hours 50 per cent of the total skeletal calcium was exchanged for calcium of the body fluids.[14] These exchanges of calcium were much less marked in rats 15 to 20 weeks of age than in rats 6 to 8 weeks old. The phosphate and the sodium of the bone also are subject to comparable rapid changes. Carlsson, Bauer and associates found that the greater part of the tracer substances which can be discovered within the skeleton after injection are only adsorbed on the surface of the bone crystals.[3] At best, the tracer substances are dissolved in the watery phase, which is always present within the crystals. These exchange reactions are reversible; thus, a large part of the rapidly absorbed tracer substances leaves the skeleton nearly as quickly as it had been adsorbed. This rapid exchange of tracer substances between bone and surrounding tissue fluid must be distinguished from true crystallization of the injected Ca^{45} in the form of hydroxyapatite. The latter process—the participation of injected radiocalcium in the formation of bone salt—has been designated "accretion."

In the same way, the rapid liberation of the loosely adsorbed injected mineral must be distinguished from actual bone resorption or dissolution of bone salt. It was found that in the tibia of young rats, 2.0 milligrams of calcium per hour were adsorbed in exchangeable form. In contrast to this large quantity, the actual accretion of calcium—that is, the

deposition of calcium in nonexchangeable form—amounted to only 0.17 milligram per hour. The net increase of the total calcium content of the tibia was 0.04 milligram per hour. It follows that in the tibia of the young rat, when 0.17 milligram of calcium per hour was deposited, 0.13 milligram of calcium was resorbed.

In this way it could be ascertained that ingested calcium and phosphate do participate in the growth of the crystal lattice of the bone minerals, and that continuous resorption and crystallization of part of the bone salts take place. Experiments with growing animals have led to the conclusion that after 50 days, 29 per cent of the bone salt of the femoral and tibial epiphyses were renewed. In the diaphyses this value amounted to only 7 per cent. Measurements of accretion and resorption rates of Ca^{45} thus enable us to make a study of metabolic processes that would not be possible by the use of measurements of net increase of calcium content alone.

The weak beta-radiation and long half-life (about 150 days) of the commonly available calcium isotope Ca^{45} makes it impractical to use this isotope in humans. Not only is it hard to measure the weak radiation following low dosage, but, in addition, Ca^{45} may be dangerous because of possible long-term irradiation damage following high dosage. In the near future, another calcium isotope (Ca^{47}) may become available, and, since this isotope has a six-day half-life and is a pure gamma-emitter, it may permit isotope studies of skeletal calcium metabolism in humans on a routine basis.

P^{32} has been used for studies of bone salt metabolism in normal and rachitic children. For such a purpose, the accretion rate in the upper part of the tibia of children who had received P^{32} was determined from bone biopsies of this region. It was found that the accretion rate of phosphorus of the bone sample decreased with age. Moreover, in vitamin D–deficient children, the accretion rate was lower than in normal children of the same age. Following the administration of vitamin D to the rachitic children, the accretion rate rose to about normal values.

Considerable caution must be exercised before the results of experiments on rats can be used to explain physiologic and pathophysiologic observations in humans. The structure of the bones of adult rats is different from the skeleton of other animals, because in the epiphyses of the adult rat—and even the old rat—cartilage always persists. Thus, the turnover of the minerals in the epiphyseal part of the bones of the adult rat is more intense than that of other animals. This applies also to man, in whom the epiphyses no longer contain cartilage. The experiments of the Swedish scientists show that the accretion of bone salt in the proximal part of the tibia of normal infants is five times slower than it is in the epiphyses of the tibia of young rats.

Both the accretion rate of calcium and the deposition of exchangeable bone salt are higher in young individuals than in old persons. The same differences are encountered in a fractured bone, as compared to an intact control bone. It seems possible that some of the exchangeable calcium, located on "crystal surfaces," becomes unavailable for exchange later in crystal life.

By the use of modern techniques, roentgenograms can be made of the microscopic structure of bone disks that have been carefully ground to a thickness of 50 micra. Such microroentgenograms permit precise study of differences in the calcium content of the osteons.* This method reveals that about 10 per cent of the osteons of adult dogs are incompletely calcified. These presumably are the young osteons. When the animal has previously been injected with Ca^{45}, the radioactivity of the osteons of the bone disks can

*An osteon or a haversian system consists of a narrow haversian canal containing blood vessels, surrounded by a thick wall of concentric bone lamellae.

be determined with the help of radioautographic images. Combined studies of microroentgenograms and radioautographs of the same areas demonstrate that radioactivity appears only in the radioautographic images of the young, partly calcified osteons, but not in the completely ossified older osteons of compact bone.[4,15] These incompletely calcified osteons represent the metabolic part of the skeleton where calcium and phosphorus derived from the intercellular fluids are deposited. The other 90 per cent of the osteons are completely calcified and actually serve as the solid mainstay of the body. This part of the skeleton influences calcium and phosphorus metabolism only when abnormal local erosion of bone occurs.

Ingestion of radioactive phosphorus (and of radiocalcium in selected cases) reveals that in the skeleton of a normal adult man the accretion-resorption rate of the calcium averages about 0.5 gram per day. If the skeleton contains about 1500 grams of calcium and accretion-resorption took place at a uniform rate in all parts of the skeleton, it would take about 3000 days to replace all skeletal tissue. Most of this accretion-resorption process of calcium must occur in the young and active osteons of the adult skeleton. Since these osteons comprise about 10 per cent of the total amount of osteons of adult bone, the calcium of the active osteons may be renewed in about 300 days. These active osteons are especially concentrated in the metaphyses,* which is the reason the metabolic turnover is considerably faster in the epiphyses than in the shafts of the long bones, even after closure of the epiphyseal line. It is therefore probable that in adult humans, rebuilding of the entire diaphysis of the femur as a result of the normal accretion-resorption processes must take 25 years or more. In contrast, more than 50 per cent of the sodium stored in the skeleton is renewed within a period of 10 days.

*The metaphysis is the part of the diaphysis adjacent to the epiphysis.

Studies of this type give some quantitative background to radiographic observations on the rate at which the skeleton loses and gains in density during development and eventual reversal of osteoporosis (p. 21). They also show why the bone-seeking fission products are more dangerous than those which are taken up by the soft tissues. Once Sr^{90} (half-life about 25 years) is incorporated in the skeleton, it stays there virtually for life.

After injection of radioactive sulfur (S^{35}) in adult dogs, only the incompletely calcified osteons become radioactive.[16,22] This indicates that not only calcium deposition but also the greater part of the new formation of mucopolysaccharides takes place in young osteons. The latter conclusion can be confirmed by direct metachromatic staining of the bone disks. Again, the incompletely calcified young osteons contain by far the largest part of the metachromatic staining substance, presumably chondroitin sulfuric acid.

Introduction of radioactive carbon in growing rats is followed by the appearance of radioactive granules below the periosteum of the diaphyses and the endosteum of the metaphyses. The radioactive granules, found after introduction of radiocarbon, do not disappear under influence of either the diastatic enzymes from saliva or hyaluronidase. This excludes the possibility that the C^{14} has been deposited either in glycogen or in mucopolysaccharides. It is therefore accepted that the C^{14} is introduced into the growing collagen fibers.

In view of the results of these experiments, it is now believed that the rapidly growing part of the bone matrix (and therefore of the bone) is spread over a funnel-shaped area. The wide part of the funnel is situated in the subperiosteal part of the diaphysis; the stem of the funnel is located in the subendosteal part of the metaphysis. This funnel-shaped area of young osteons is surrounded by old osteons in which under normal circumstances metabolic changes no longer take place.

BONE FORMATION

For many decades, bone formation was believed to depend upon the supersaturation of the calcium and phosporus content of the intercellular fluid, which would favor the precipitation of calcium phosphate. As a matter of fact, serum as such is an undersaturated solution of secondary calcium phosphate, but this undersaturation exists only in the absence of a solid phase. If serum is in contact with a solid phase, i.e., with hydroxyapatite crystals, it is supersaturated.[19a] This fact helps to explain the growth of bone.

As far as endosteal, periosteal, and membranous bone formation are concerned, the protein component of the bone matrix plays an important role in bone formation. It has been shown over and over again that when collagen preparations are brought in contact with serum, hydroxyapatite precipitates. Since the deposition of hydroxyapatite takes place exclusively in the matrix, it follows that bone formation is possible only when a sufficient amount of bone matrix is present. Thus, when no organic osseous matrix is manufactured, the daily wear and tear gradually leads to atrophy of the bone.

The bone matrix is also responsible for the physical orientation of the inorganic crystalline components of the bone. Feitelberg,[7] using x-ray diffraction, demonstrated that the longitudinal direction of the collagen fibers of a partially decalcified femur runs parallel with the crystallographic c-axis of the hydroxyapatite crystals. He then completely decalcified a femur, sealed one end with paraffin, filled the lumen of the decalcified tubular bone with calcium chloride solution and sealed the other end of the bone with paraffin. Thereafter, the whole preparation was immersed in a sodium phosphate solution for thirty days. Calcium diffused slowly into the bone matrix from within, phosphate diffused from without, and calcium phosphate precipitated in the decalcified bone. After thirty days the femur was washed in water. X-ray diffraction at that time revealed that the calcium had precipitated in the form of hydroxyapatite, just as it precipitates in living bone. In addition, the c-axis of the hydroxyapatite crystals again ran parallel to the axis of the fibers of the bone matrix. Both the form of crystallization and the physico-chemical structure of the calcium salts of the bone followed a special pattern which directly depends upon the specific qualities of the bone matrix.

Even if there can be no doubt that for the formation of bone the collagen of the bone matrix is of paramount importance, it must be recognized that chondroitin sulfuric acid of the bone matrix also plays an important role. All tissues—not only cartilage and bone matrix, but also arteriosclerotic plaques, renal stones and calcified scar tissue—in which calcium is deposited contain this mucopolysaccharide.[16] It has been recently suggested that chondroitin sulfuric acid may well act as a cation exchange resin, capable of concentrating calcium and other cations. The incredibly large surface area of the skeleton certainly facilitates such cation exchange.

Even if our actual knowledge of endosteal, periosteal, and membranous bone formation is only superficial, our lack of understanding of the mechanism of enchondral ossification is still more discouraging.

Enchondral bone is formed in the metaphyseal part of the epiphyseal disks, where no matrix is present and where the pre-existing hyaline cartilage contains hardly any glycogen, phosphatase or enzymes that are able to split long carbohydrate chains—all substances which are generally regarded as indispensable for enchondral bone formation.

The appearance of glycogen in cartilage in the course of the initial stages of ossification may well be of importance, since the presence of glycogen and phosphate permits the formation of organic phosphates.[8] These data would seem to lift a tiny corner of the veil that hides the secrets of enchondral ossification, were it not that glycogen and enzymes appear only *after* ossification has actually started.

It is quite possible that Sobel's complex of collagen and chondroitin sulfate[21] may be a major factor in the dynamics of enchondral ossification. Different complicated histologic processes ultimately lead to the formation of columns of osteoid, manufactured by the osteoblasts. In these columns of osteoid hydroxyapatite crystals are deposited, bringing about the formation of parallel, vertical rows of primary bone trabecules. As soon as the infant starts to move around, these primary bone trabecules are reabsorbed and replaced by the secondary spongiosa. The new bone trabecules are arranged in the form of girders of a vault, which serve to transfer to the shaft the strains and stresses to which the skeleton is continuously subjected. Modifications of the arrangement of the pattern of the bone trabecules continue during life. Since such changes necessitate destruction and reconstruction of a certain number of trabecules, moderate quantities of bone substance must be released every day by osteoclastic resorption and replaced by osteoblastic action. This is the so-called daily wear and tear of the skeleton. This incessant resorption and deposition of bone which, at first sight, might appear aimless and even redundant, is nevertheless of the utmost importance. It safeguards the resilience of the skeleton by modifying the spatial orientation of the trabecules until the highest degree of effective solidity is obtained.

Osteoblastic activity starts in utero, but osteoclastic resorption does not occur before the infant is eight months old. At this time, the attempts to walk require the resorption of the primary bone trabecules by osteoclasts to be replaced by the mechanically better adapted secondary trabecules. Thus, a certain degree of osteopetrosis exists temporarily in young infants, because the osteoblastic bone deposition is not held in check by osteoclastic activity.

A connection between alkaline phosphatase and bone formation must exist, because phosphatases are always present when normal or pathologic bone formation takes place. However, the concept that inorganic phosphate, formed from organic phosphoric esters by the action of phosphatase, plays an important role in the ossification of cartilage, is all but proven. As a matter of fact, cartilage contains so little hexose phosphate that after hydrolysis by phosphatase, only traces of inorganic phosphate would become available.

A new approach to the problem has been developed since Engfeldt and Zetterström[6] in 1954 described a congenital skeletal disease in which retarded growth clinically resembled severe rickets. Renal damage was also present, together with hypercalcemia and a significant decrease of the alkaline phosphatase of the serum to 20-40 per cent of the normal values. The mineralization of the newly formed bone matrix was greatly defective. It is obvious that there must be a connection between the hypophosphatasemia and the defective calcification of matrix.

For years, the discussion had centered upon the question whether the action of alkaline phosphatase upon hexose phosphate esters could liberate the phosphate, this action being necessary for the initiation of the ossification of the matrix. McCance, Morrison and Dent[18] now mention the possibility that alkaline phosphatase might liberate phosphate by the hydrolysis of phospho-ethanolamine and not of hexose phosphate esters. In patients with hypophosphatasemia, the urine contained considerable amounts of phosphoethanolamine, whereas this substance was not excreted by normal persons. One could thus speculate that under normal circumstances the alkaline phosphatase hydrolyzes phosphoethanolamine and that the liberated inorganic phosphate is used for calcification of cartilage. In hypophosphatasemia the alkaline phosphatase of the body is markedly decreased, and no phosphate is split off from phospoethanolamine. In the absence of phosphate, the calcification of the matrix would be impaired, and the unchanged phospho-ethanolamine would appear in the urine.

METABOLISM OF CALCIUM AND PHOSPHORUS

A normal interplay exists between intestinal absorption, calcium excretion, resorption of calcium from and deposition of calcium in the skeleton. If the demands of the skeleton for deposition of calcium in new bone matrix are diminished, an increased amount of calcium may be excreted. Increased excretion of calcium must come either from the stores of calcium of the skeleton or from accelerated intestinal absorption of calcium. Thus, a direct interrelationship exists between the needs of the skeleton and the calcium balance. Calcium is normally excreted both by the gastrointestinal tract and by the kidneys. In normal persons, about 30 per cent of the calcium excreted is in the urine; 70 per cent is in the stool. Calcium is also lost from the maternal organism during the formation of the fetal skeleton and lactation. The normal value of calcium in serum varies between 9 and 11 mg. per 100 cc., the inorganic phosphorus of the serum between 3 and 4 mg. per 100 cc., and the alkaline phosphatase between 3 and 4.5 Bodansky units or 8 and 12 King-Armstrong units per 100 cc. In the absence of avitaminosis D the "threshold" for urinary excretion of calcium is about 7 to 8 mg. per 100 cc. of serum. In other words, when the serum calcium decreases below 7 mg. per cent, no calcium is excreted in the urine. Under these circumstances, all calcium is eliminated in the stool.

As a general rule, it can be stated that signs of tetany develop when the serum calcium drops below 6 mg. per cent. At the same time—at least, if the tetany is due to damage of the parathyroids—the serum phosphorus goes up to at least 6 or 7 mg. per cent. It may be added that hypocalcemia is much more important than the increase of inorganic phosphorus for the development of tetany. This follows from observations in cases of excessive losses of vitamin D and calcium in the stools, such as occur in fatty diarrhea. Here, also, signs of tetany appear when the serum calcium diminishes to less than 6 mg. per cent, although in this condition the organic phosphorus often decreases to levels below 3 mg. per cent.

The calcium of the blood is present only in the plasma; none is found in the erythrocytes. About half of the serum calcium is conjugated with protein, particularly with albumin, while the remaining serum calcium is ultrafiltrable, almost completely ionized in the form of calcium bicarbonate and calcium biphosphate. A negligible part of the ultrafiltrable serum calcium is not ionized, but is present as a complex citrate compound. Only the ionized calcium of the serum is physiologically active.

Until recently it was generally believed that the entire inorganic phosphorus content of the blood was ultrafiltrable and ionized. New experiments indicate, however, that this may hold true for only 75 to 80 per cent of the serum phosphorus. In addition, it is generally believed that when the serum calcium exceeds 15 mg. per cent, a complex colloidal calcium phosphate compound is formed which does not pass the glomerular membrane.

The relationship between the calcium ions, the carbon dioxide, the phosphates and the acidity of the blood serum has been expressed in the formula

$$\frac{Ca \times HCO_3 \times HPO_4}{pH} = K \text{ (constant)}$$

This so-called Howland-Kramer formula indicates that if the carbon dioxide and the pH of the serum remain constant, the product of the calcium and the phosphate content of the serum must always vary between 30 and 40.[1] But there are many conditions in which this formula is not valid. In avitaminosis D, for example, both calcium and phosphorus of the serum are usually low. When a patient with hyperparathyroidism develops uremia, the characteristic hypophosphatemia disappears and hyperphosphatemia results from the renal retention of phosphate. However, under these circumstances the serum calcium

does not always decrease and, in certain cases of uremia due to hyperparathyroidism, hypercalcemia persists unabated. Hypercalcemia is also frequently encountered in generalized malignancies of the skeleton and in rapidly spreading multiple myeloma. In these patients the phosphorus of the serum is either normal or moderately increased, but certainly not depressed by the hypercalcemia. It is true that the combination of decreased serum calcium and high serum phosphorus occurs frequently in chronic uremia; however, this combination of hypocalcemia and hyperphosphatemia depends upon other mechanisms, but not on the constancy of the product of serum calcium and serum phosphorus (p. 89). The formula also does not explain the characteristic biochemical changes found in hyperparathyroidism. The decrease of the serum phosphorus in this disease is due to the excessive phosphorus excretion by the kidney, the hypercalcemia to increased osteoclastic activity—both caused by increased production of parathyroid hormone.

There is no unanimous opinion regarding the amount of calcium which must be taken orally in order to keep a healthy individual in calcium balance. The vitamin D content of the body, derived either from food or from exposure to sunshine, is of much greater importance for the calcium balance than is the calcium content of the food. As long as the vitamin D intake is satisfactory, calcium equilibrium can be obtained even when the calcium ingested with the food is relatively small.

Whereas the National Research Council of the United States considers a daily intake of 800 to 1000 milligrams of calcium necessary for conservation of health, South African investigators are satisfied with a daily intake of 700 milligrams for men and 560 milligrams for women, French investigators, with 450 milligrams, and German nutritionists, with 400 milligrams. These differences depend mainly upon the daily calcium intake to which the peoples of the various countries are accustomed.

Since 99 per cent of the 1500 grams of calcium stored in the adult body is present in the skeleton and teeth, the growth of the skeleton must be an important factor in determining the calcium requirements of the body. At birth, the total calcium content of the body amounts to 0.8 per cent of the total body weight. This percentage rapidly increases until in the adult it has reached 1.6 per cent. Thus, the daily calcium requirement for different age groups exhibits important variations (see table 1). Children, for instance, require relatively larger quantities of calcium than adults.

TABLE 1

Age (in Years)	*Daily Calcium Requirement (in mg.)*
1	650
4	400
8	700
11	1100
14	1300
17	1000
18	700

During pregnancy, the calcium requirement is far greater than under normal conditions. The calcium intake of the pregnant woman must not only be sufficient for the normal daily wear and tear of the maternal skeleton but, in addition, it must provide the calcium necessary for building the fetal skeleton. The latter contains 23 grams of calcium at birth. Moreover, during the last trimester of pregnancy, significant amounts of calcium are deposited in the placenta. Finally, the calcium excretion in the urine of pregnant women is often remarkably high. Thus, the daily calcium requirement for pregnant women is set at 1500 milligrams, at least for the second half of the pregnancy. During lactation a considerable amount of calcium is lost in the milk (400 milligrams per liter)[20] and the daily requirement for lactating mothers is therefore increased to 2000 milligrams (p. 35).

When phosphates, fatty acids, and oxalates are present in the intestine in large amounts, absorption of calcium is inhibited because of the formation of the relatively insoluble calcium phosphates, calcium soaps and cal-

cium oxalate, respectively. The inhibition of calcium absorption by the presence of excessive amounts of phosphates in the intestine is one of the main causes for the alteration in calcium metabolism in chronic uremia (p. 89).

Even in healthy individuals, large amounts of phosphoric compounds may exert an unfavorable influence upon the absorption of calcium. During wartime in Great Britain, whole wheat bread was the only kind of bread available. The outer layers of unmilled wheat contain large amounts of phosphorus-rich phytin. The austere war diet was very low in calcium. In the intestine, the formation of large amounts of calcium phytate was sufficient to prevent satisfactory absorption of calcium from the intestine and to cause hypocalcemia. Nowadays, large amounts of phytic acid are given to patients with renal stones. The hypocalciuria ensuing from the ingestion of phytic acid decreases the tendency for renal stone formation.[10]

In most kinds of fatty diarrhea, not only fats but also fat-soluble substances, such as vitamin D, are lost in the stools. This impairs calcium absorption from the intestine (p. 40). In addition, large amounts of fatty acids, present in the intestine of patients with steatorrhea, are excreted as calcium or magnesium soaps. Due to the combined action of these two factors, fatty diarrhea of long standing usually leads to hypocalcemia and hypocalciuria.

When large amounts of oxalates are present in the food, insoluble calcium oxalate, which cannot be absorbed, is formed in the intestine. Spinach is notoriously high in oxalates but fortunately also contains considerable quantities of calcium. In certain brands of spinach excessive amounts of oxalic acid (up to 1.1 grams of oxalic acid per 100 grams of spinach) are found. When large quantities of such spinach are ingested, the intestinal absorption of calcium may well suffer.

References—Chapter 1

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