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EVIDENCE FOR THE EXISTENCE OF TWO PHYSICAL STATES OF BONE MINERAL

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INTRODUCTION

Our concepts of the chemical and physical nature of the bone mineral have undergone drastic changes in the last 25 years.

Originally the bone mineral was thought to be tricalcium phosphate. Then appreciation of the presence of differing ratios of calcium to phosphorus, and the presence of adventitious ions such as carbonate, citrate, sodium, and chloride, made this concept appear inadequate. After much work and much disagreement, a new concept of the bone mineral has evolved.

The new concept is that the mineral is present as hydroxyapatitie crystals. The crystals have attached to them hydration shells of water, and by various means noted elsewhere, the amounts of calcium and phosphorus, and the variable amounts of other ions, are accounted for. The route by which hydroxyapatites are formed in the bone mineral are at present unknown and the subject of considerable investigation.

In this paper I will present evidence which suggests strongly that in addition to the hydroxyapatite present in bone, for which compelling evidence exists, there is another physical state of mineral salts present. The structure of these salts and their chemical composition are unknown.

OBSERVATIONS

Several publications of this laboratory deal with the presence of a quasipermeable state of the bone surrounding living osteocytes. This quasipermeable state has been termed "halo volume." Another peculiarity of halo volume has been noted in versene decalcified, haematoxylin stained sections.

The oustanding technical peculiarity of the quasipermeability of the bone in halo volume is its disappearance in vivo after death of the osteocyte, and its rapid disappearance in vitro after drying and other manipulations that are common in histological work.

The peculiar sensitivity of the halo volume permeability was not recognized at the same time as the halo volume feature itself. The variability in demonstration of halo volume resulting from this ignorance necessitated delay of publication for a full 5 years.

DISCUSSION

The reproducibility with which halo volume may be demonstrated in perfectly fresh bone, and ablated by such a simple thing as drying sections for a few minutes in air, indicates that something about the physical state of the mineral in the halo volume moiety of the skeleton is different from the mineral in the rest of the skeleton.

Then non-halo volume skeletal moiety does not exhibit the permeability or loss of permeability characterizing halo volume.

If halo volume mineral were all hydorxyapatite in vivo, the peculiar behavior of halo volume would not be expected and could not be explained. If however there were present a mineral state — in adidtion to hydroxyapatite — which spontaneously converted to hydroxyapatite upon death, drying and other manipulations known to ablate the halo volume permeability, the observed phenomena would be accounted for.

Hydrated calcium phosphate immediately comes to mind since it is known to follow this observed behavior in vitro.

This line of speculation may be solidified somewhat by some additional observations. Publications in this laboratory have already noted the normal existence of halo volume in perfectly fresh human bone, and the fact that halo volumes in vitro enlarge in acid pH and shrink in alkaline pH. As Nordin has postulated, the pH of the living, normal, human osteocyte's lacunar fluid may well be about 6.8, rather than the 7.4 characterizing the rest of the body fluids. The high degree of diffusion impedence existing between the average osteocyte and its average source of blood supply supports this contention because it explains how such a variation would be possible.

Is the non-hydroxyapatite mineral fraction in the halo volume really a calcium phosphate? It will take considerable additional work to answer this question.

SUMMARY

The existence of halo volume and its peculiar behavior in vitro and in vivo make it necessary to infer a difference in halo volume mineral composition as compared to non-halo volume mineral.

KNOWN QUALITATIVE VARIABLES IN HUMAN BONE

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Not long ago it was possible to state that bone was either normal or abnormal on the basis of measurement of the ratio of its weight dried at constant temperature, (on the order of 110 C) to the weight of ash remaining after burning off the organic part of the bone sample (at temperatures on the order of 800 C). Qualitative abnormalities of the bone were considered present if there were variations in the weight/ash ratio beyond certain empirically determined limits.

Recent work has brought to light a group of abnormalities in quality of bone which escaped detection by the above and other methods. In this paper some of these qualitative abnormalities are presented, in addition to the originally known abnormalities. The purpose of the presentation is to acquaint investigators of qualitative features of bone with these variables. While it is unfortunate that the additional variables will complicate future qualitative measurement of bone, the additional knowledge gained thereby may be expected to shed more light on skeletal physiology in the future.

QUALITATIVE VARIABLES

Some of the variables which are presented may be readily quantitated, others not, at least at present.

1) *Variation in mineral density.* A given unit volume of bone matrix may contain very little mineral, as in osteoid seams, or a great deal of mineral, as in micropetrosis, these being extremes. A given moiety of bone undergoes a definite and orderly progression of mineral accretion during its biological lifetime. A number of diseases may affect this orderly progression, leading to qualitative abnormalities.

2) *Variation in water content.* A given unit volume of bone matrix may contain only so much water. When the matrix is first formed and is completely unmineralized the water content is maximal. When maximum mineralization has been achieved the water content is minimal. In other words, water and mineral vary inversely, the amount of and volume of matrix remaining constant as far as is known at present. The significance of this shift is that water content may be used to study mineral content of bone. Water content is more easily studied than is mineral content.

3) *Average biological age.* In any given skeleton there is an average age of the myriad of bone moieties which comprise the whole. Recent measurements indicate that in ribs and clavicles this age is about 15 years at age 30, and 8 years at age 60 in man. The significance of the average skeletal age is that the average diffusion impedence in the mineralized matrix depends on the average mineralization of the bone. Increasing mineralization occurs with increasing age of any given bone moiety, and increasing mineralization increases diffusion impedence between blood and bone. The average age of the skeleton is dependent on the rate of remodelling.

4) *Average diffusion impedence.* As noted above, this is directly related to the age of a moiety of bone, older bone being more highly mineralized and more

impermeable to ions and molecules of all sizes. Diffusion impedence and degrees of mineralization are in turn affected by other variables, among them being osteocyte death in vivo, feathering, micropetrosis. Exchange between bone and blood of a number of bone seeking radioisotopes would be governed in large part by the average diffusion impedence of the skeleton.

5) *Feathering.* A newly recognized bone affection characterized morphologically by an abnormally low *degree* of mineralization of the affected matrix, and dynamically by markedly retarded *rate* of mineralization of the affected matrix. Feathering is not usually related to any known osteomalacic process. Presumably feathered bone is unusually permeable in vivo. On occasion over 75% of the skeleton is composed of feathered bone.

6) *Micropetrosis.* Another newly recognized bone affection, this time characterized morphologically by death of the osteocytes in the affected bone and occlusion of the canaliculae by dense, impermeable deposits of mineral. This disease is found with increasing incidence in older patients and in the presence of vascular insufficiency. The affected bone is highly mineralized and in vitro exhibits high diffusion impedence.

7) *Osteocyte Death in Vivo.* With advancing age an increasing proportion of one's store of osteocytes die off, leaving behind them empty lacunae. There are sound reasons for anticipating that bone in the region of such empty lacunae exchanges to a lesser degree with the blood than bone which contains live osteocytes. In addition the diffusion impedence in bone, most of whose osteocytes are dead, may be expected to be high because one of the dynamic factors normally opposing a high diffusion impedence is the system of concentration gradients produced by living osteocytes.

8) *Osteoblastic Activity.* It has recently become possible to measure human osteoblastic activity by two methods, both possessing considerable precision when compared to the methods previously available. It is now possible, in man and with certain limitations, to measure the amount of new bone formed per unit volume of skeleton per unit time. This is a true measurement of rate.

9) *Osteoclastic Activity.* It has recently become possible to measure osteoclastic activity in man also, again with useful precision. The method, and the results thereof, are not published but will be the topic of the next large group of publications from this laboratory. When a measurement of both osteoblastic activity and osteoclastic activity has been made, the results may be expressed both as the remodelling rate and in terms of skeletal balance.

10) *Mineral Composition and State.* Study of this aspect of bone is withering but is due for a renaissance. The amount and composition of the bone mineral are affected, among others, by the factor of osteocyte metabolism. There is reason to believe there are variations in mineral composition of the bone in certain diseases, among them being severe and prolonged cardiac failure. There is sufficient evidence to conclude that there is a different physical state of bone mineral present in vivo in addition to the widely known and studied hydroxyapatite. The high degree of instability of this new state, and the fact that it readily converts to hydroxyapatite after death, have prevented its wide recognition in the past.

11) *Osteoid Seams per Cubic Millimeter·* Recently it has been possible to make a true measurement of osteoid seams per unit volume of bone. This came about through the combination of new methods and new physiological understanding of bone. The result is that much finer degrees of qualitative disturbance in osteoid seams are detectable and measureable than previously. We may anticipate that osteomalacia will be found to be the extreme of a continuous spectrum of qualitative disturbances extending from normal bone to Milkman's skeleton.

DISCUSSION

The informed reader has already noted that in the case of many of the preceding factors a variation has not been associated with a known physiological or histological disturbance of bone.

He is correct.

He is correct because these things are too new to have been correlated by previous workers with disease. The presence of the variables has been noted by the writer in one or more pathological states of the skeleton in the course of 7 years' work with fresh, undecalcified bone sections and two years' work with tetracycline labelled human material. The factors are real, but as yet we do not quite know what to do with all of them!

In the future, in stating the quality of osteoporotic bone, or in stating the quality of bone in a testing machine, it will be necessary to evaluate some or all of the variables listed. Other variables not included would require consideration for special purposes.