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Review

Bone Water

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Summary. A short review is given of the water in bone. Various analyses of bone water content are discussed, and its possible location is considered in relation to the behaviour of water in isolated components of bone. Some of the difficulties encountered in examining such microscopic phenomena as water structure in a heterogeneous system such as bone are also discussed.

Key words: Water — Bone — Homeostasis — Structure.

Introduction

Interest has recently increased concerning the role of water in determining the structure and behaviour of biological systems. In this connection several comprehensive reviews have appeared (Kuntz and Kauzmann, 1974; Tait and Franks, 1971; Berendsen, 1968; Ling, 1965) and symposia have been held (Ann. N.Y. Acad. Sci., 1973). Most of the work described has been centred on the hydration of fibrous proteins such as collagen (Berendsen and Michgelsen, 1966), intramuscular or brain water (Cope, 1969) or cell membrane water (Ling, 1965). A few investigations into the state of water in dental tissue have been described (Carlstrom et al., 1963; Söremark et al., 1973; Little and Casciani, 1966; Dibdin, 1972) but little work has been done to determine the state of water in other calcified tissues, notably bone. The object of this paper is to look critically at the available information on bone water, to analyse the difficulties involved in studying such a system and to make tentative suggestions regarding possible roles and structures adopted by water in bone.

Water Content of Bone

In general, investigations on the water content of bone have only been carried out as part of more extensive quantitative analyses for other constituents. Such studies have been carried out on cortical bone from man, dog, monkey and ox (Gong et al., 1964; Eastoe and Eastoe, 1954; Campo and Tourtellotte, 1967; Mueller et al., 1966; Robinson and Elliot, 1957; Woodard, 1964). In addition, Blitz and Pellegrino (1969) have reported data on the comparative composition of bone from sixteen different mammals including man and mammoth. Some analyses of cartilage have also been reported (Campo and Tourtellotte, 1967).

The results of these analyses show a general similarity in water content between species when expressed as a weight/volume ratio of bone. There are however, significant variations within a single species dependent on various parameters as described below.

Variations within a Single Species

Variations between Types of Bone. Cortical bone is generally of a higher density and hence of a lower water content than trabecular bone. This may be correlated with the increased mineralization of cortical bone.

Variations within a Single Tissue Specimen. Atkinson and Weatherall (1967) have shown that the density and porosity of compact bone varies along the length and around the circumference of the femoral shaft. This almost certainly implies a variation of water content since an increase in porosity has been shown to be allied to an increase in the water-plus-lipid content of bone (Klein et al., 1968).

Variation with Age. As porosity increases with age, the amount of water associated with such porosity would

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also be expected to increase. This is not so and the decrease must therefore be due to the decrease in water content associated with the tissue itself. This is explicable in terms of increased mineralization producing larger crystals with a smaller surface-to-volume ratio and hence a proportionately smaller amount of mineral bound water.

In general, the water content of bone decreases with age as a result of increased mineralization. A quantitative study of this variation by Mueller et al. (1966) suggests that the water content of human cancellous bone decreases from birth to 30–35 years of age and thereafter remains fairly constant. Our own work on human cortical femoral bone (Timmins, unpublished results) implies a rather steadier decrease in water content from birth to death (Fig. 1). These preliminary results must, however, be viewed with caution in view of the small number of experimental data.

Variation with Sex. Most studies have included both male and female subjects but there may be marked differences between the bone of the two sexes, particularly after the age of about 50 years. Meema et al. (1965), in a study of postmenopausal osteoporosis, concluded that this was not primarily due to ageing or to calcium deficiency, but to an increased production of ovarian hormones. As mentioned earlier, a change in porosity will reflect an alteration of the water plus lipid content of bone (Klein et al., 1968).

Pathogenic Condition. Various pathogenic conditions have been shown to produce large changes in bone composition. For example, in cases of osteomalacia the mineral content of the matrix is abnormally low and the water content correspondingly high (Mueller et al., 1966).

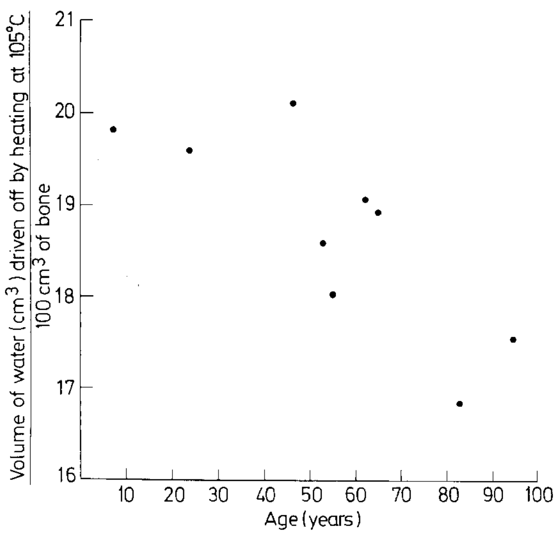

Fig. 1. Variation in water content of human cortical femoral bone with age

The Location of Water in Bone

No reports have yet identified the location of water in bone tissue although Blitz and Pellegrino (1969) have attempted to identify two components classed as "crystalline" water and "osteoid" water. Table 1 shows the nature and size of some of the pores in which one might expect to find water.

Robinson (1960) noted that water in adult compact bone of the dog could be divided into two fractions, one driven off at 50° C and the other at 100° C, corresponding to water associated with the marrow-vascular-osteoid spaces and with the calcified matrix respectively. Our own results on human cortical femoral bone suggest that the removal of water is rather more gradual than this and that the separation into two discrete fractions by the technique of thermal dehydration is rather arbitrary. Arnold and Tont (1967) have shown that a certain reproducible proportion of water from trabecular bone is removable by centrifugal fields above 1000 *g*, this water being associated with the surface. They also show, however, that water would be unlikely to be lost from canaliculi at fields below 1.5×10^6 *g*, whereas water loss from Haversian systems should be almost complete at 2.5×10^4 *g*. Studies by Neuman et al. (1953) on cortical ox bone ground to 425 μm (40 mesh) indicated that an insignificant amount of water was lost below 4.0×10^4 *g*.

One of the most thorough investigations of bound water in bone is the dielectric study of Marino et al. (1967). When dielectric constant and dielectric loss measured at three different frequencies are plotted against hydration, the points lie fairly well along two straight lines of differing slopes. These two lines intersect at hydration values of 37–49 mg H_2O/g bone. This indicates a critical hydration value (h_c) below which water is assumed to be bound and above which it is free. This is a better criterion for identifying two separate fractions than that of Robinson (1960) discussed earlier.

Marino et al. also show that their critical hydration value is consistent with a simple mixture of two BET-type absorbers, synthetic hydroxy apatite and hide col-

Table 1. Nature and dimensions of water-containing pores

Pore	Dimensions (μm)	Number/mm³
Haversian canal	20–100	
Lacunae	10 × 15 × 25	20,000
Canaliculi	0.3	10^6 (50–100/lacuna)

lagen which in the proportions found in bone (65% mineral, 35% collagen) would be expected to have $h_c = 44.8$. They therefore postulate that water absorption by bone may be considered as a summation of the individual absorptions by collagen and by hydroxyapatite. This is clearly an oversimplified picture, as other material with primary absorption sites is also present (e.g., mucopolysaccharides) but considering the range of hydrations found it probably identifies the chief absorbing species correctly.

Katz and Li (1973a,b) estimate that of the water in rat tibia, some 27% is located in the extrafibrillar spaces, whilst the other 73% is located in the intrafibrillar spaces.

Carlstrom et al. (1963) have investigated the state of water in dental enamel using the birefringence properties of the tissue and conclude that it is bound in two ways. They propose that a small amount of water is loosely bound, probably by the organic matrix, whereas the greater part is bound firmly to the mineral phase.

Role of Water in Bone

The role of bone in the mammalian skeleton is twofold. It provides a rigid weight-supporting structure and also a mineral reservoir for many ions vital to the metabolism. The water in bone plays a significant part in both these functions.

Structure. It is well known that the mechanical properties of bone samples are affected by their degree of hydration. Sedlin and Hirsch (1966) found that the tensile and compressive strength characteristics, the modulus of elasticity and the hardness of bone all increase with decreasing water content. This is possibly due to a deficiency of water in the collagen fibres causing them, and hence the bone as a whole, to be stiffer and therefore stronger (Wall, 1973). However, the energy absorbed before failure is greater for wet than for dried bone (Evans and Lebow, 1951).

It has been suggested by McPherson and Juhasz (1964) that bone may be hydraulically strengthened. They suggest that if the muscles of the thigh suddenly contract, say in jumping off a wall, then blood will be forced into the femur and the marrow pressure will be increased considerably. At the same time the single muscle contraction will restrict the venous outflow. Thus one would then have a fluid-filled vessel with the fluid being unable to escape. When such a vessel is loaded in compression the fluid would transmit the load equally in all directions and thereby increase the weight-bearing potential of the vessel. Frost (1964) suggests that this internal hydraulic effect is of importance only if the loads are brief and cyclic as is the case in vivo. However Swanson and Freeman (1966) have experimentally investigated the possibility of trabecular bone being thus strengthened. The results of their in vitro study show that trabecular bone is not hydraulically strengthened and they further suggest that neither is tubular bone.

Mineral Homeostasis. In 1953, Neuman et al. considered how the surface ions of bone crystals relatively removed from the circulation can undergo exchange with ions in the circulating fluid. The location and structural nature of the bone water may be central to the final solution of this problem.

The State of Water in Isolated Components of Bone

Bone is a heterogeneous system with a number of major constituents in addition to water. These are the mineral phase, approximating in composition to hydroxyapatite, and the organic matrix consisting of collagen and mucopolysaccharide. Capillaries and surfaces are formed by the interactions of these compounds. The investigation of one component (e.g. water) within such a complex system as bone is impossible using most techniques. However, by examining the state of water in these isolated systems clues may be found as to the nature of water in the more complex system which constitutes true bone. This approach is at best only a crude approximation which ignores the role of water in the interaction of the separate components of the system.

Collagen. The nature and role of water in noncalcified collagenous tissue has been the object of much investigation in recent years (Berendsen, 1962; Chapman and McLauchlan, 1969; Dehl and Hoeve, 1969; Bell and Breuer, 1971). Berendsen (1962) has noted that there exists a repeat distance in collagen of almost exactly six times the nearest neighbour distance in bulk water. Using this information in conjunction with NMR evidence the author proposes that there exists an epitaxial relationship between water and collagen. (A similar relationship exists between water and crystalline hydroxyapatite and hence between water, hydroxyapatite and collagen.) Whether or not the postulated relationship actually exists it is clear that considerable amounts of bound and ordered water are present.

Bone Mineral. An excellent review of the chemistry of bone mineral has been published by Posner (1969) in which he concludes that bone mineral contains two phases, an amorphous calcium phosphate and a crystalline (apatite) phase. The latter is thought to be a calcium-deficient apatite approaching stoichiometric perfection with age but probably never becoming true hydroxyapatite. Studies of water-binding in synthetic hydroxyapatite have shown that as a result of the high electrostatic force around the crystal there is a shell of at least 35 mg H_2O/g hydroxyapatite. There is a progression of water binding from the crystal surface to the bulk solution (Neuman and Neuman, 1958). It has been shown that monovalent ions (K^+, Na^+, F^-) diffuse from solution into the hydration shell but are not concentrated there. More highly charged ions (Mg^{2+}, Sr^{2+}, Rb^{2+}) and highly hydrated and/or polarized cations tend to be concentrated in the hydration shell. Thus almost any ion may enter the hydration shell but only certain ones are concentrated there and hence be available for exchange. Clearly the structure of the water in this hydration shell may be of importance in this differential concentration. Glimcher (1959) has noted that there is likely to be a fairly large amount of "capillary water" associated with crystals of colloidal dimensions and this water will not behave as solvent water.

Capillaries. There is little convincing evidence that water structure is changed by being constrained in very small capillaries but some claims have been made. Shereshefsky and Carter (1950) found that the lowering of vapour pressure of 0.025–0.80 molal KCl solutions in cone-shaped capillaries of radius 3–10 μm was 7–80 times greater than that calculated from the Kelvin equation. Such a change would be an indication of a change in water structure as it would imply the existence of a *stable* form of water below the normal equilibrium freezing point. Whether the existence of a modified form of water at low temperature implies the existence of such a form at high temperature is unclear. In our own work (Finney et al., unpublished) no change in the melting point of ice in capillaries of diameter down to 2 μm has been detected. (Change in *freezing point* is not a good criterion for change in water structure as freezing is a nonequilibrium effect.) There may, however be some effect below this diameter as there might also be for aqueous solutions of salts rather than pure water. Evidence in favour of a structural change in water is the supercooling of pure water to $-100°$ C when held between quartz plates (Hori, 1956), whereas it is not possible to supercool bulk water below $-40°$ C.

Water has a temperature of maximum density and minimum volume (4° C) which might be expected to be affected by changes in structure. Barnes et al. (unpublished results) found no detectable change in the temperature of maximum density of water when measured in glass capillaries down to 2 μm diameter. Roberts and Northey (1972), using signal line broadening from NMR studies of water in slices of sintered glass with pore size ~15 μm, postulated long range modification of the water structure. Belfort (1974), however, from the evidence of pulsed NMR experiments, has disputed the validity of the conclusions drawn from the line broadening experiments.

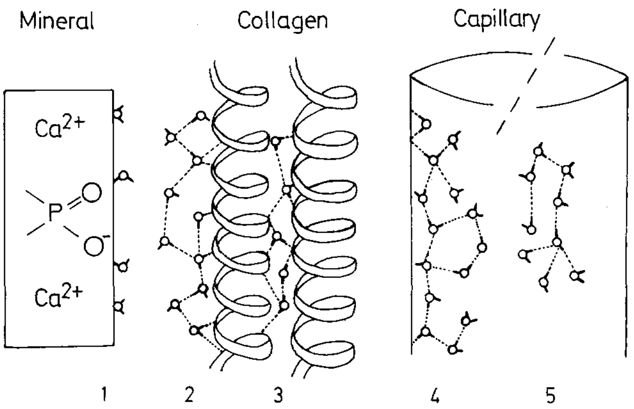

Fig. 2. Schematic diagram of possible locations for bone water: (*1*) Water associated with mineral phase; (*2*) Water on collagen fibril surface — *inter*-fibrillar water; (*3*) *Intra*-fibrillar water; (*4*) Water on surface of capillaries; (*5*) Water at some distance from capillary surface — i.e. essentially bulk water; Water molecules. Intracellular and intra-membranous water also present are not illustrated

Discussion

From published data it must be concluded that very little is understood concerning the role and structure of the water in bone, although variations in water content between species and with age and pathological conditions are fairly well documented.

Consideration of the water in isolated components of bone leads us to suggest that several types of water may be present, ranging from bulk water in the Haversian canals, to tightly bound water in the organic matrix. Figure 2 illustrates schematically some of these species. The very profusion of species with different structures and binding energies makes the identification of individual types very difficult.

Clearly the fraction of water which is most easily removed is associated with the large capillary systems, and will have the structure and properties of bulk water. A substantial proportion of the water, however, exists in much smaller volumes associated with the mineral phase, the organic matrix or capillaries of such small diameter as to radically affect the structure and properties of the water which they contain. It is this water, about which we know so little that is of most importance as a structural element of bone.

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