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PHYSICAL CHARACTERISTICS OF BONE: SHRINKAGE AND HYDRATION

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INTRODUCTION

KNOWLEDGE OF the shrinkage of bone is of interest for two reasons, one mundane and the other potentially interesting.

Accurate measurements made on dried, resin-mounted sections of undecalcified human bone might need correction for shrinkage to normalize the measurements to the fresh, hydrated state. The measurements would need to be quite accurate to justify the trouble involved in the normalization.

Physically bone is a structural material of considerable importance to all of us which however is little understood as far as its function as a structural material is concerned. Among other features which characterize bone, it is markedly inhomogeneous in its structure at all levels from macroscopic to molecular. There are known associations in physical characteristics between the obvious physical form and the histological composition on the one hand and directions of maximum strength and weakness on the other. These and other inhomogeneities are held together by adhesive mechanisms which are simply not understood.

Study of the behavior of bone's physical dimensions under varying conditions might yield some information about the nature of the adhesive that holds normal bone together. This is a preliminary report of some pilot studies done with the above thoughts in mind.

MATERIALS AND METHODS

Fresh, hydrated, undecalcified, longitudinal sections of bone 100-200 microns thick were made from varied human ribs, clavicles, femurs, and tibias. Right triangles were cut from these sections such that each side of the 90° angle was parallel to one of the major axes (longitudinal or transverse) of the bone. In this manner measurements of the sides could be made accurately and, more important, reproducibly at varying stages in the process of a series of manipulations could be obtained.

Measurements were made with a measuring microscope. The precision of the measuring procedure, as judged by the reproducibility, is slightly better than .001. The major source of error is the problem of finding exactly the same measuring point on the sections on repeated measurements.

Sections were placed in the test solutions and allowed to stabilize for 4 days before being measured. They were measured in the solutions through which they had just been passed.

Results

In summary the following points were observed:

1) On drying, sections from various sources shrank longitudinally from 0.1% to 1.6 per cent, sections from tibias and older patients shrinking the least. The mean of 10 sections was 0.5 per cent.

2) After similar treatment but measured transverse to the long axis shrinkage was from 1.8 to 4.5 per cent. The mean of 10 sections was 2.2 per cent.

3) Fresh sections placed into strong sodium or potassium chloride solutions did not exhibit significant dimensional change in the longitudinal direction. About a third of them exhibited an average of 0.2 per cent expansion, and two thirds an average 0.2 per cent shrinkage in the transverse direction.

This response to electrolyte solutions was largely ablated by prior treatment with formalin, absolute ethanol, or absolute dioxane.

4) Fresh sections placed into absolute ethanol, dioxane, or glycerine shrank as much as or, in some instances, about half as much as, sections of the same bone shrank after drying in air at 22 C.

5) No significant shrinkage was produced by pH as alkaline as ten, by sucrose, by glucose, or by urea solutions as long as the four day stabilization period was adhered to.

6) Curiously, sections transferred from solutions of electrolyte, sucrose, glucose, or glycerine to distilled water expanded temporarily and the expansion of the longitudinal axis was about 0.8 that of the transverse. In four days dimensions were normal again.

7) It has been repeatedly observed in this laboratory that alcoholated sections are extremely brittle compared to the same section thoroughly hydrated. Some of the design of our section-making procedure was dictated by this phenomenon.

8) The shrinkage produced by the dehydrating solutions is nearly always reversible simply by returning the section to idstilled water. Occasionally such sections will not return to their original dimensions.

The shrinkage produced by the electrolytes is also reversible by returning the sections to distilled water.

9) On repeated cycling of a section from water to dehydrating agent it was noted that the amount of shrinkage gradually diminished with each cycle. On testing sections prepared from bones that had been preserved in bulk in 40 per cent ethanol for a number of months it was noted that the amount of shrinkage found was always less than would have been expected.

Discussion

A. In this study no distinction was made between the radial and the tangential transverse axes. A check of this matter has revealed that this distinction must be made in the future.

B. It may be inferred that water is bound to parts of bone at the molecular level in such a manner that, first, it may be readily removed by immersion in dehydrating fluids, and, second, in such a manner and to such a degree that it normally slightly distends the bone elastically. In other words water "blows up" bone slightly as air blows up a balloon.

This hydration effect appears to be reversible.

C. It may be inferred that highly ionized electrolyte solutions exhibit binding of the electrolyte in some manner to the bone at the molecular level. The electrolyte appears to be able to displace part of the water which produces the "ballon effect" referred to above.

D. Nonelectrolytes cause far less dimensional change in fresh bone than do electrolytes or dehydrating agents. This fact adds weight to the comments in (B) and (C).

E. A change in entropy must occur with the effects noted in (B) and (C). The nature of this change and its quantity may be studied by extending and refining the studies outlined. The absolute energy change is probably close to that required to produce the volume change noted in the "balloon effect" and is related to the modulus of elasticity summed up over the (X), (Y), (Z) axes of polar coordinate space and to the 3 major structural orientations of cortical bone.

F. There is little doubt that the water "compartment" described is a hitherto unrecognized one.

G. The gradual decrease in the dehydration shrinkage, and in the dimensional sensitivity to electrolyte solutions with manipulation or with time, indicates that some unstable state within the fresh bone gradually converts to a stable state. This has a familiar ring. The writer notes the following:

The features of halo volume compelled us to consider the existence of some unstable state of bone mineral in its fresh, but not in fixed, state.

A gradual decrease in the density gradients between the bulk of a bone and its most poorly mineralized parts has been reported to occur in vitro.

There is general recognition of the fact that bone carbonate plays some essential role in bone which is not understood and which is not accounted for by the present knowledge of apatite structure or by surface and hydration-shell effects. In other words there is something in bone whose function is unknown. (Heavens!)

There is general recognition of the fact that some change occurs in bone mineral during preparation for electron microscopy and x-ray diffraction.

H. Study of shrinkage of fresh bone should prove interesting and be productive.