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On Mineral and Water Content in Bovine Bone

Siddiq Mohiuddin

Dept. of Applied Medical Sciences
Riyadh Community College, King Saud University
Riyadh, Saudi Arabia

Abstract: ***Water plays an important role in influencing the physical properties of biological tissues. On the basis of water content, biological tissues are differentiated as soft and hard. Water is comparatively high in scapula and low in femur when compared to the rib. The paper presents a comparative account on density of wet; oven dried and decalcified bovine bones. The elements present, in traces, in bovine scapula, rib and femur bone also presented. The study reveals that the magnesium plays a major role in the deposition of apatite in the matrix of proteins present in the hard tissues. Among the bones studied, the calcium deposition is comparatively high in femur than rib and scapula, while the magnesium content is low. It can be concluded that the magnesium regulates the deposition of calcium phosphate in the tissue. The calcium deposition decreases as the concentration of magnesium increases in the tissue.***

Keywords: density, bovine bone, mineral, water content

1. Introduction

The composition and structure of mineral component of bone aroused the interest of physical scientists more than century. Bone tissue is in a stage of continuous remodeling. While new bone is being deposited by cells called osteoblasts. The anabolic and catabolic processes account for the growth of the skeleton, for maintenance of its architecture and for the participation of bone mineral in the regulation of mineral metabolism in the body. The fibrous organic material in the bone is collagen, which belongs to the group of fibrous proteins containing while connective tissue, tendons, cartilage, etc.

The fibers of collagen are visible in the optical microscope with diameters ranging from 20 to 200µ. These fibers can be seen to be divided as primitive fibers, which have diameters in the range 2 to 10µ. Primitive fibers have thinner components, fibrils, which may be too thin (a few hundred Angstroms) to be observed under the optical microscope, and hence it is necessary to use an electron microscope, Fibrils, however , are composed of still thin 'filaments', and it is the arrangement of the molecular polypeptide chains in these filaments which is revealed by X-ray diffraction studies on collagen. The X-ray diffraction pattern of crystal is unique, and it is only necessary to measure the times of the pattern on an X-ray power diagram and compares the results with these from a sample of the pure substance. The difficulty with bone arises from the fact that the lines are so diffuse and that there are very few. If one bone is subjected to any treatment such as heating in order to improve the diffraction diagram, than it is not possible to be absolutely certain that no change has taken place.

The chemical and physiochemical analysis of the mineral part of bone have not succeeded in identifying with any certainty the exact substance or substances present. That the main constituent as an apatite-like material is easily established the difficulty arises because there are several closely related substances, such as carbonate apatite, α-tricalcium phosphate, and hydroxyl apatite, which would have the necessary properties for the bulk of the inorganic bone substance.

Lee and Young [1] determined the composition of hard tissue proteins extracted from bovine horn, water buffalo horn rhinoceros horn to elucidate and compare the composition of the various keratin derivatives. Analysis for amino acids, monosaccharide, hexamine, uronic acids and sialic acids were found in 3 keratin derivatives and there was no difference in their composition. Marshall and Gillespie [2] examined the keratin proteins of wool, horn, and hoof from sheep. The constituents of low and high sulphur proteins, isolated from 3 hard keratins of wool. Horn and hoof of sheep were compared. Horn and hoof were more similar in nature to each other than they were to wool.

The complex role of various components of bone mineralization is sufficiently exposed by various workers. A role of magnesium in the ordinary events accompanying bone mineralization ahs been suggested. In the adult rat, intake for 48 days of abnormal amounts of magnesium, either too much or too little, arrested the growth of the mineralization system of tibia [3].

Smith et. al. [4] used the computerized tomography and photon absorption has also used to determine bone cross-section or bone mass. However, these techniques also have limited sensitivity and they have not been used in monitoring fracture healing. This suggests that there is a definite need for additional means of detecting the in vivo condition of bone. Hidea Yano et. al. [5] developed hydroxyl apatite ceramics as the artificial bone a alternative material and studied their characteristics.

The density of the bone is related to mineral content, water and also the organic composition. It is obvious from the study that the deposition of calcium phosphate in the matrix of collagen is more or less homogeneous for femur, whereas in the case of scapula and rib, the deposition is inhomogeneous. However, the variation in density is less in the case of decalcified and oven dried bones [6].

2. Materials and Methods

Fresh scapula, rib and femur bones of bovine were collected from the slaughter house for the present investigations. Fleshly material present on the bone is removed. Specimens were cut from the mid region of the bone. Some bones specimens were decalcified by keeping them in 9% nitric acid for 24 hours, and then washed in running water for 24 hours. Fresh specimens were dried by keeping them in oven at 100°c for 24 hours.

The density of wet, decalcified and oven dried specimens was measured by taking their masses in air (m_1) and when immersed in water (m_2). Then density (d) is calculated as

$$d = \frac{m_1}{m_1 - m_2} \text{ gm/cm}^3$$

Percent water content of bones (w) was determined by taking masses before and after oven drying and using the formula

$$w\% = \frac{m_1 - m_3}{m_1} x100$$

where m_1 = mass of the bone, m_3 = mass of oven dried bone.

Fractional change (d_f) in the density of decalcified and oven dried bones was determined by using the formula

$$f_d = \frac{d_1 - d_2}{d_1}$$

where d_1 = density of wet bone, d_2 = density of oven dried or decalcified bone.

For the estimation of trace elements by atomic emission spectroscopy, smooth powder of the bone samples was prepared by grinding. The powder was collected in polythene bags.

For the estimation of calcium in the bone samples, 1 gm. of powder sample was dissolved in conc. HNO_3 and kept for 24 hours. Then the solution was diluted and made up to the mark in 100 ml standard flask. 20 ml of the solution was pipette out and further diluted up to 200 ml. Then the solution was heated to boiling and 25 ml of 6% ammonium solution was added to precipitate ions into calcium oxalate. This is filtered by using Whatman No. 40 filter paper. The precipitate was washed with cold distilled water and transferred into a conical flask. 25 ml of diluted H_2SO_4 was added and titrated against potassium permanganate solution of 0.0098 molar. By noting the end point the amount to calcium present in the given sample was calculated. Elements present in bovine bones, in traces, were estimated by atomic emission technique. For this purpose, Jewell Ash 3.4 m. Ebert grating spectrograph was used, in which the arc current is 8 amps.

3. Results and Discussion

Table 1, presents a comparative account on density of wet, oven dried and decalcified bovine bones. Fractional change in density is also presented. It can be noticed that the density of femur is more than that of rib, while in the case of scapula; it is less whatever may be the physiological condition of the bone, whether it is wet, decalcified or oven dried. The fractional change in density is high in scapula, low in rib and in between for femur, when decalcified bones are considered. In the case oven dried bones, the fractional change in density is more or less the same but it is more than that of femur.

It is found that the water content of bovine bones is comparatively high in scapula (12%) and low in femur (10%) when compared to the rib (11%). Water plays an important role in influencing the physical properties of biological tissues. On the basis of water content, biological tissues are differentiated as soft and hard. In the present investigation, the influence of water on the elastic and electrical properties of bovine bone has been studied.

Table 2, reports the data on the elements present, in traces, in bovine scapula, rib and femur bone. It is found that phosphorous is less than 1%; Si, Fe, Cu, Al, are less than 0.1%, while Ba, Sr, Pb, Zn are very much less than the traces i.e., less than 0.001%. The element – Magnesium is present in bones studied in the range of 1 to 3%, more in scapula (3%) less in femur (1%) when compared to rib (2%).

The magnesium plays a major role in the deposition of apatite in the matrix of proteins present in the hard tissues. Among the bones studied, the calcium deposition is comparatively high in femur than rib and scapula, while the magnesium content is low. It can be concluded that the magnesium regulates the deposition of calcium phosphate in the tissue. The calcium deposition decreases as the concentration of magnesium increases in the tissue. Similar report is available in the literature, Clark and Belanger reported that in the adult rat, intake for 48 days of abnormal amounts of magnesium arrested the growth of tibia.

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Table 1: A comparison of density of wet, oven dried and decalcified bovine Scapula, rib and femur

Bone	Density (gm/cm^3)			Fractional change in density	
	Wet	decalcified	oven dried	decalcified	oven dried
Scapula	1.613 ± 0.235	1.342 ± 0.089	1.439 ± 0.073	0.168	0.108
Rib	1.623 ± 0.204	1.409 ± 0.166	1.449 ± 0.243	0.132	0.107
Femur	2.097 ± 0.058	1.784 ± 0.098	1.896 ± 0.057	0.149	0.083

Table 2: Percentage of elements analyzed in bovine bones

Bone	Mg	P	Si	Fe	Cu	Al	Ba	Sr	Pb	Zn
Scapula	3	<1.0	<0.1	<0.1	<0.1	<0.1	<0.001	<0,001	<0.001	<0.001
Rib	2	<1.0	<0.1	<0.1	<0.1	<0.1	<0.001	<0,001	<0.001	<0.001
Femur	1	<1.0	<0.1	<0.1	<0.1	<0.1	<0.001	<0,001	<0.001	<0.001