ALVEOLAR BONE DENSITY

Pelton W. Wheeler, D.D.S.

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INTRODUCTION

The term density, when referring to bone, has been imbued with a variety of meanings. To some, density is the quality of radiopaqueness of roentgenograms. To others, it is the mass-volume ratio of the organ bone. Still, to others, it is the mass-volume ratio of the tissue bone, or of the inorganic fraction. Density in the strict sense is the ratio of the mass of a substance per unit of volume. Precise use of the term is a prerequisite for accurate scientific communication.

Interest in bone tissue has increased tremendously in recent years, yet relatively little work has been done in the area of physical properties of dental bone. This interest has been stimulated by greater activity and research in skeletal tissue biology, metabolic bone disease and increased demands in the field of geriatrics.

Dentistry has for some time made untested assumptions about alveolar bone. It has been implied that the bone tissue of the retromolar area is more dense than the bone of the alveolar areas and, thus, more resistant to tooth movement.(1) Reitan described from histologic sections three types of bone of varying density.(2) The density variance was based on the relative number of marrow spaces present. He then correlated these three grades of density to rate of tooth movement. In these examples, density has been used to indicate x-ray mass or a histologic evaluation of volume rather than absolute bone density.

It is the purpose of this paper to investigate the absolute density of mandibular alveolar bone of the human, whale and miniature swine. The calcified matrix of bone tissue may encompass various spaces: osteocyte lacunae, canaliculae, haversian, Volkmann, primary longitudinal canals and

marrow cavities. To determine the absolute density of bone, these spaces cannot be included in the measured volume. This study will use the water displacement method and the air pycnometer to measure the absolute volume of bone to calculate its absolute density. The results will then be related to previously mentioned assumptions concerning bone density and compared with the cell count per unit area of alveolar bone in these mammals.(3)

REVIEW OF LITERATURE

Dental interest in alveolar bone density began as early as 1891 when Guilford made gross observations of the human alveolus.(4) He observed that the alveolus had a spongy inner structure contained within a cortical layer. This cortex was more dense and harder on the outer surface than on the inner layer.

In 1924, MacMillan x-rayed serial cross-sections of the mandible and maxilla of man for a study of internal structure of alveolar bone.(5) He showed variations within each arch and between arches. The areas that were more resistant to the rays were labeled as the more dense bone. The spongy internal structure was least resistant. As the "closeness of the spacing" increased in the cortex, the bone became more resistant to x-rays.

Strang used histologic investigations of bone to relate density to the magnitude and the direction of occlusal masticatory force.(6) His histologic interpretation indicated that the bone against which the force was exerted was the most dense but least in bulk. The bone on the side from which the force was applied was least dense and greater in bulk.

Salzmann, using histologic and roentgenographic data, stated that the density, hardness and texture of osseous structure of the mandible varies.(7) Variations in the trabecula between cortical plates are produced by the normal functional requirements of the jaw. The lability of bone tissue allows variation of density to be produced under the influence of systemic control mechanisms, such as nutrition and endocrine function. The manner in which bone quality is affected by these mechanisms is dependent in which direction equilibrium is disturbed, resulting in apposition or resorption of bone.

Provenza illustrated with roentgenograms and parasagittal whole bone section of maxilla quantitative differences in trabeculation and variations in caliber of the alveolar bone within the arches and between the mandible and maxilla.(8)

It is evident in the above reports that observed density variations are the basis for the statements. They also illustrate the varied meanings of the term density to the authors. The majority of the authors do not define the term density with consistency or precision.

Some early work on a method of precisely measuring density was done by Todd.(9) This was a radiographic comparison method, utilizing a density gauge to compare with the degree of radiopaqueness of the film. The step wedge was an aluminum bar of calibrated varying densities which was placed on the film holder and exposed at the same time skeletal tissue was x-rayed. The degree of radiopaqueness was then reported as density, dependent upon its correlation with an area of the gauge.

Other investigators have used a gravimetric method of water displacement to determine bone volume in a density calculation.(10, 11) The specific gravity of the bone sample is calculated in metric units according to formulae from its weight in air and loss of weight in water. The specific gravity is the ratio of the density of the body to that of water at four degrees centigrade. The density and specific gravity are equivalent in the metric system at four degrees centigrade since the density of water is greatest at that temperature. And, therefore, the mass of the sample divided by its volume is the relationship used.

Trotter et al considered density to be the weight per unit volume and the volume of the organ bone was determined by the amount of millet seed displaced from a graduated cylinder, or calibrated container of sufficient size to accommodate the bone.(12) The weight in grams was then divided by the measured volume in cubic centimeters to give the density of the sample.

A method of mathematically computing the volume of the holes in a serial section of a bone sample was used by Johnson.(13) The area of all spaces was computed and this figure was multiplied by their respective depths. This computed volume of the spaces was subtracted from the total external volume of the slides to give the absolute bone volume which was then used to compute the density.

Felts and Spurrell utilized a photodensiometer scanning of standardized radiographs of whale humeri to express structural density - bone per unit volume - of whale serial sections.(14) The density determined by this method was compared with the absolute density, actual weight per volume of the serial sections.

It is evident in the majority of the reports dealing with alveolar bone and supporting structure that a varied meaning of the term density exists between authors. These authors do not define the term density with consistency or precision.

METHODS AND MATERIALS

A. Sample Description.

The human samples were obtained from the mandibles of ten adult cadavera in the University of Oregon Dental School anatomy laboratory. A sample was taken from each of the mandibles in the right and left alveolar and retromolar areas. These samples were then prepared by removing the periosteum and cortical bone prior to any determinations. The alveolar samples included alveolar bone proper and supporting alveolar bone from the cuspid area. The retromolar samples included supporting alveolus from the third molar areas. Each test on human material in this study was performed on ten samples.

The whale samples were obtained from six alveolar areas of adult sperm whale mandibles obtained from a commercial whaling station. The samples were prepared in the same manner as the human material. Each test on whale material was performed on two samples from each of the six areas.

The swine samples were obtained from the alveolar and retromolar areas of the mandibles of three miniature swine used in the animal experiment section of the University of Oregon Dental School. These samples were prepared in the same manner as the human samples. Each test in this study was performed on two samples from each of these areas.

B. Definitions.

1. Absolute bone density - The ratio of the mass of bone to its absolute volume.

2. Absolute bone volume - The external volume of a bone sample minus the volume of its contained spaces.
3. Alveolar bone - The portion of bone in the maxilla or mandible which contains the teeth.
4. Alveolar bone proper - Cribriform plate of the alveolus.
5. Alveolar trabeculation - Spongy diploe of the alveolus.
6. Alveolar cortex - External cortical plate.
7. Inorganic fraction - The calcified portion of the bone that remains after all lipid and protein material has been removed.
8. Organic fraction - The elements enclosed in lipid free bone that are not calcified.
9. Organ bone - A structural unit of the skeleton.
10. Tissue bone - The organic matrix with the cells of bone and cementing substance.

C. Sample Handling.

Lipid material was extracted from the samples in a Soxhlet extractor (Figure 1) using petroleum ether. Each extraction consisted of 150 cycles followed by a distilled water rinse of 150 cycles.(15)

Protein material was extracted in the Soxhlet apparatus with 80% aqueous solution of ethylene diamine. The extraction sequence consisted of two 150 cycle extractions separated by one and followed by two distilled water rinses of 150 cycles each after the method of Gong.(10)

Dehydration of the samples was carried out in a 100°C. oven until the samples reached a constant weight after two weighings agreeing within limits of the mass weighing error.

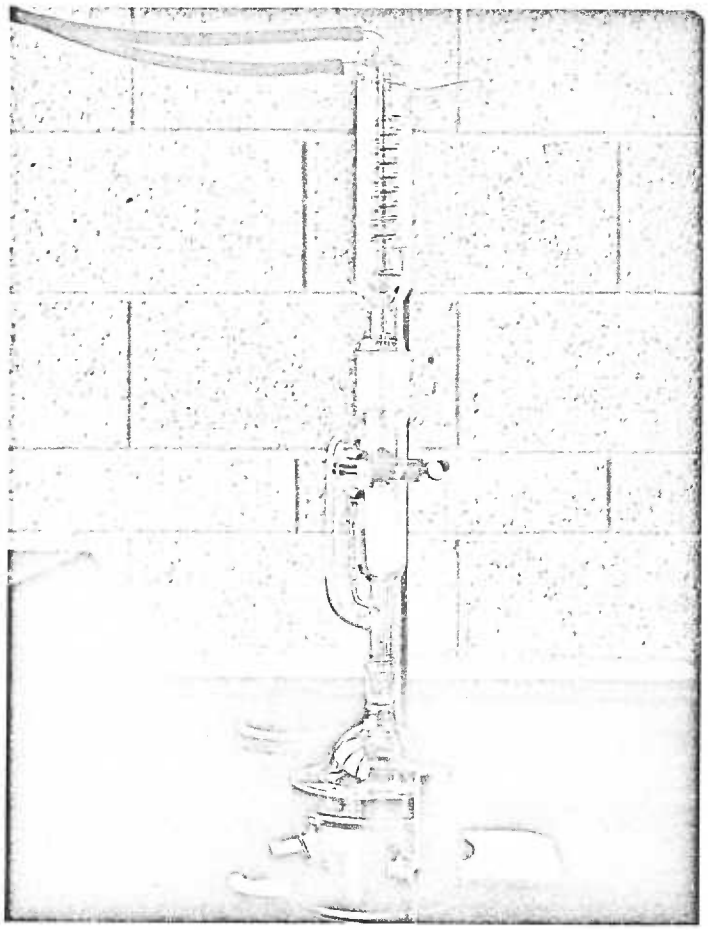

Figure 1. Soxhlet extractor.

Random samples were selected for multiple determinations to determine the accuracy of the calculated density. A dried bone sample was used for mass error determination. A hydrated bone sample was used for mass volume error determination. A standard steel ball reference unit was used for the air pycnometer volume error determination. (See Table 6.)

D. Description of Air Pycnometer Operation.

The air pycnometer used to obtain direct volume measurements of the samples was a Beckman Air Pycnometer Model 930 (Figure 2). This is a manually operated instrument that measures the volume of porous, irregularly shaped solids, rapidly and precisely. After a short period of familiarization, an operator can obtain measurements repeatable to better than $\pm$ 0.05 cubic centimeters. Calibrated steel balls supplied with the instrument are the standards on which this was attained.

The principle of operation is a direct reading gauge that is an indicator of the differential in pressures between the reference cylinder and the measuring cylinder.

There are two cylinders, reference and measuring (Figure 3). For descriptive purpose suppose there is no sample in either cylinder and the volumes are equal. If the coupling valve is closed, any change in one must be duplicated by an equal change in the other to obtain equal pressures on each side of the differential pressure indicator. If, however, a sample is introduced in the cylinder and both pistons are advanced the same amount, the pressure on each side of the indicator will not be equal. However, the pressure can be equalized by withdrawing the piston of the measuring chamber a distance equal to the volume of the sample.

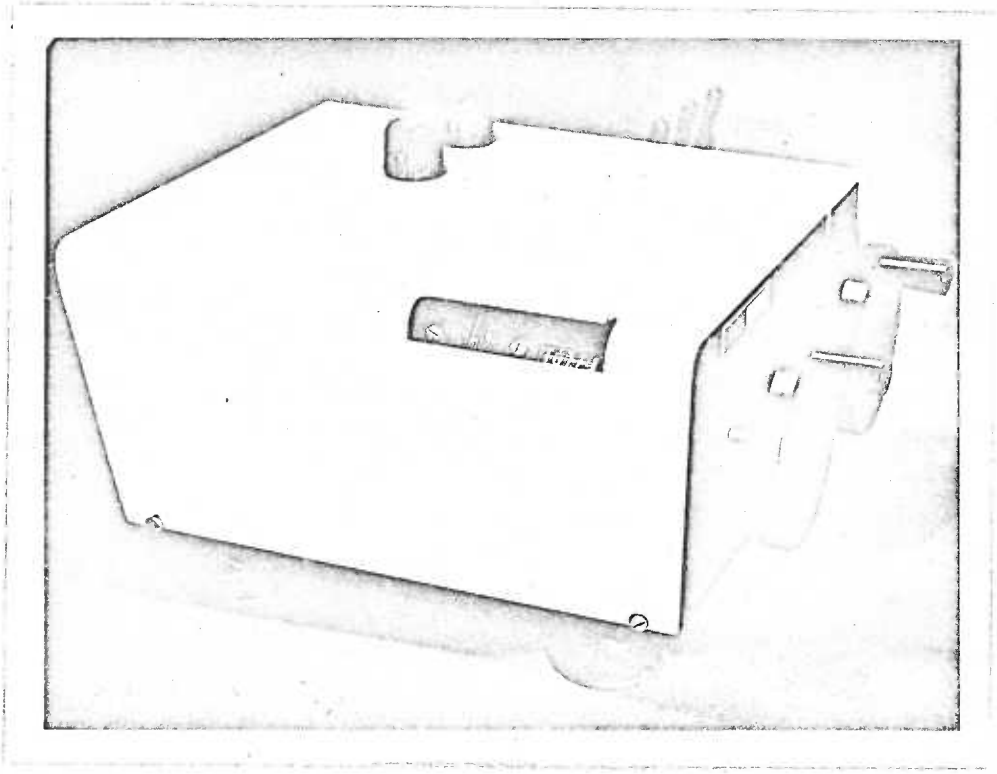

Figure 2. Beckman Air Pycnometer Model 930.

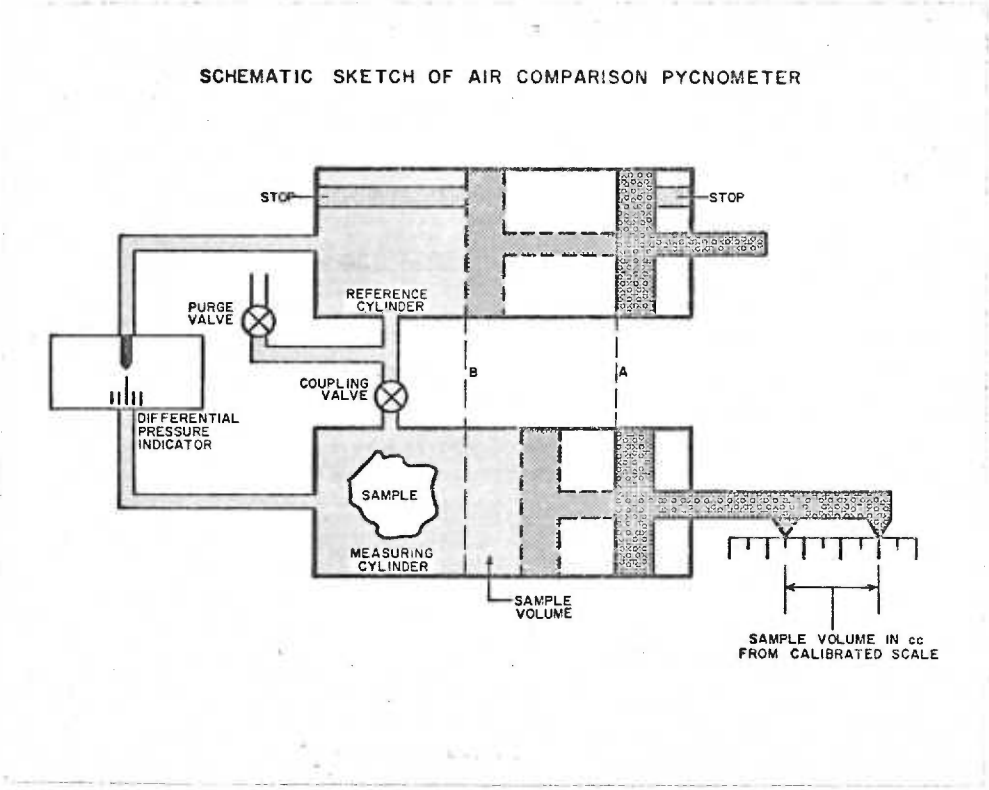

Figure 3. Schematic diagram of the Beckman Air Pycnometer Model 930.

If the piston of the reference chamber is always advanced to the same position, then the distance the measuring piston differs from it when the pressures in both chambers are equal will be proportional to the volume of the sample.

This distance between pistons is calibrated and made to read directly in cubic centimeters on a digital counter.

E. Description of Runs.

The samples were subjected to the following procedures:

1. Samples were selected and prepared by removing periosteum and cortical bone.
2. Lipid material was extracted with petroleum ether in the Soxhlet apparatus.
3. Samples were weighed in and out of water of known temperature suspended on fine wire.
4. The density of the hydrated sample was computed with the following formulae.

   Mass of hydrated bone = weight of blotted sample on Mettler Balance Type H6T

   Volume of hydrated bone = weight in air minus weight in H20

   Mass divided by volume times spec. gravity temp. factor = density
5. Samples dried to a constant weight in a 100°C. oven.
6. Weights of dehydrated samples determined.
7. Density of dehydrated sample computed with the formula:

   Volume of Hydrated Bone - Mass of H20 loss = Vol. dehyd. Bone
   (Mass of Hydrated bone - Mass of dehyd. bone = Mass of H20 loss)

   Mass of dehyd. bone divided by volume of dehyd. bone = Density of dehyd. bone

8. Volume of dehydrated bone measured with the air pycnometer.

9. Protein material extracted in the Soxhlet apparatus with ethylene diamine. Figures 4 through 8.

10. Samples weighed in and out of water and density computed as in Step 4.

11. Samples dried to a constant weight in 100°C. oven.

12. Dehydrated samples weighed and density computed as in Step 7.

13. Volume of dehydrated samples measured with air pycnometer.

14. Density of organic fraction computed with the formula.

Lipid extracted dehydrated mass minus dehydrated weight of protein extraction divided by volume of lipid extraction minus volume of protein extraction.

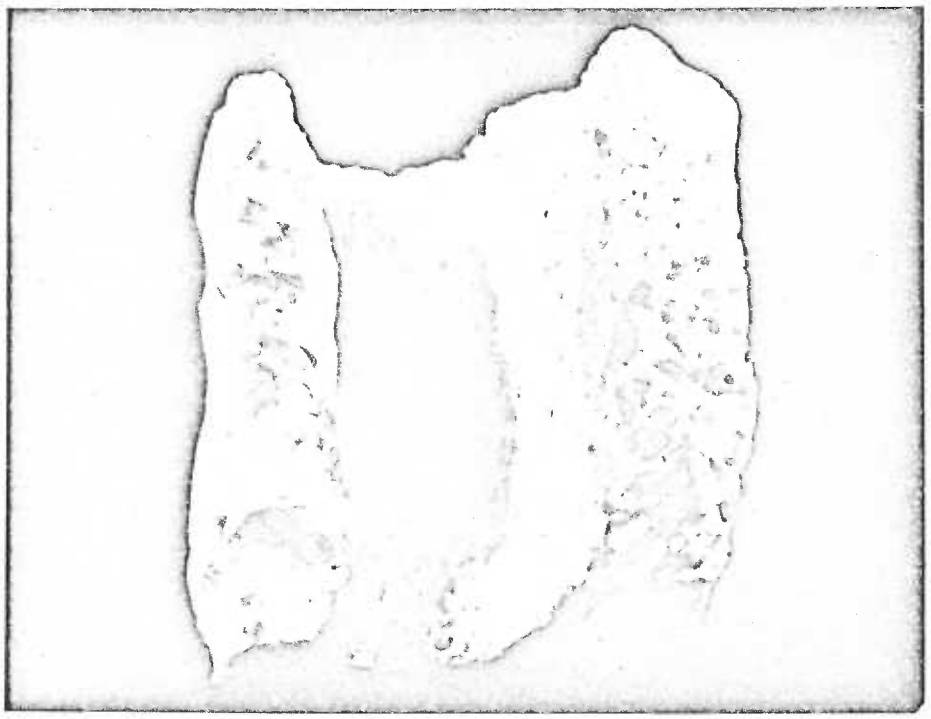

Figure 4. Human inorganic aleolar bone. Section of right mandibular cuspid viewed from the mesial.

Figure 5. Human inorganic alveolar bone. Section of right retromolar area viewed from mesial.

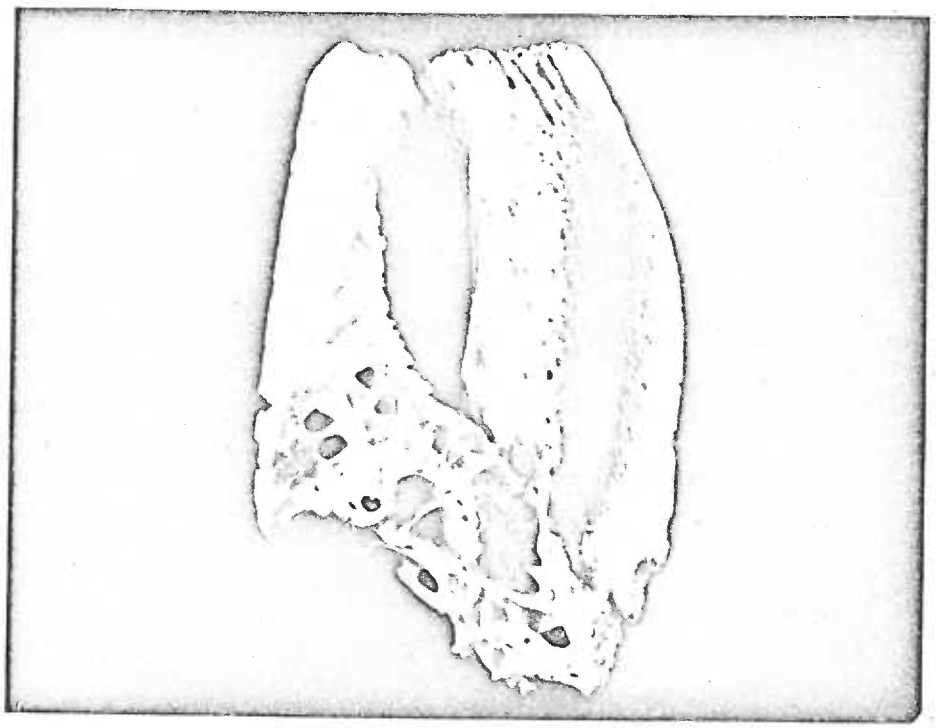

Figure 6. Swine inorganic alveolar bone. Section of molar viewed from mesial.

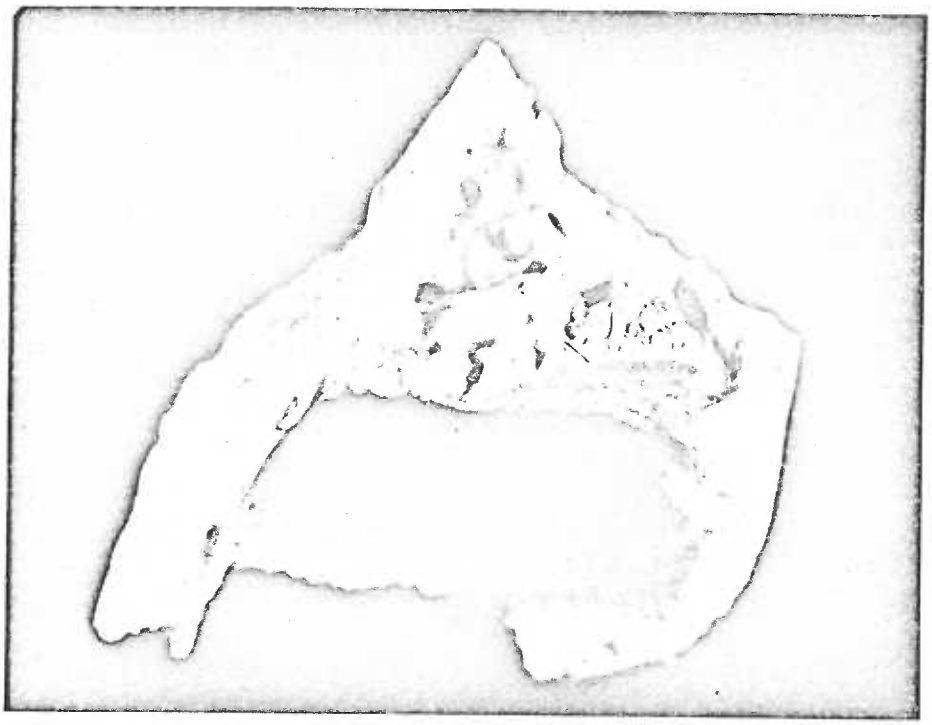

Figure 7. Swine inorganic alveolar bone. Section of retromolar area viewed from occluso-mesial.

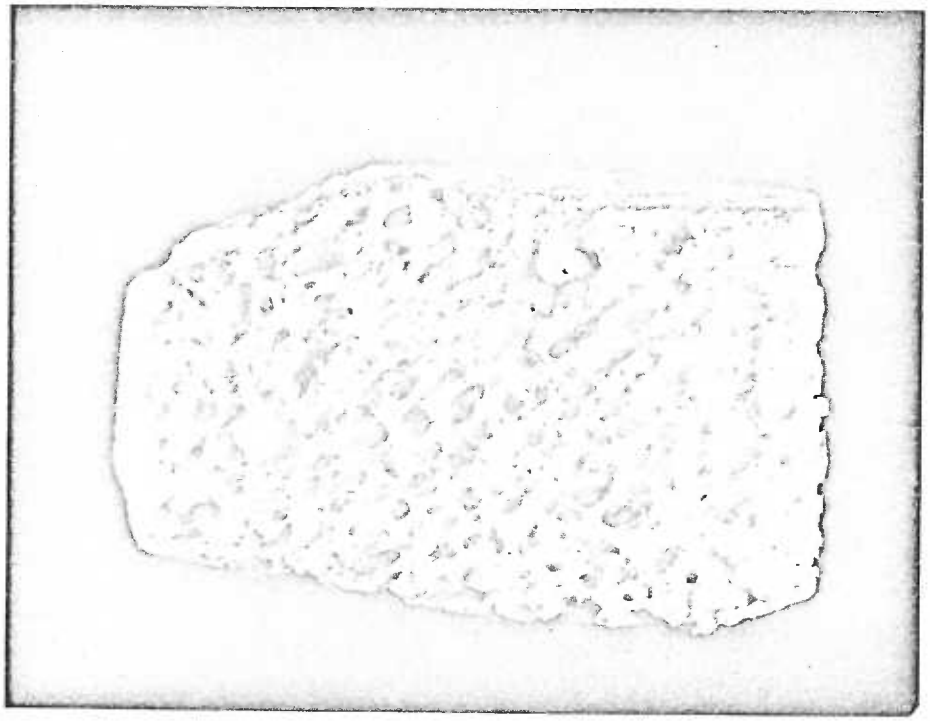

Figure 8. Whale inorganic alveolar bone. Section not oriented.

RESULTS

The data in Table 1 gives the mean densities of the inorganic bone from the whale, human, and miniature swine. As these samples were treated with ethylene diamine and all protein removed, the remainder represents the inorganic fraction and not bone in the sense of bone tissue.

Table 2 contains the mean densities of the human alveolar and retromolar inorganic bone. These samples were likewise treated with ethylene diamine.

Table 3 shows the mean densities of lipid extracted human and whale alveolar bone. This sample represents complete bone tissue less the fat content.

Table 4 represents the mean density of human and whale inorganic bone. This sample is the same as that in Table 3 and this step was necessary to calculate the density of the organic fraction as shown in Table 5.

Tables 6 through 10 represent error determinations and the statistical survey used in this report.

DISCUSSION

The term density in the dental literature is used in many different ways with different meanings. One must carefully check each author's definition of the term, or if not defined, what is implied before drawing conclusions. To complicate matters, the determination of absolute skeletal density is made more difficult by the extremely small size of the canaliculi spaces in bone which average about 0.5 micron in diameter. Hence, it seemed desirable to search for a method other than water displacement for a volume determination. The air pycnometer offers a rapid means of volume determination with little difficulty of sample preparation.

The greatest source of error in the study was the inherent error of the air pycnometer. This was too large to give accurate measurements of small samples, so the samples were pooled for measuring volumes with this instrument.

Failure to maintain a constant water level, thus a constant weight of the wire carrier, in the water displacement weighings can produce large errors. This was controlled in this study by using a pipette to regulate the water level to give a constant carrier weight.

Loss of part of the samples due to their extreme fragility could be a source of error. It is very important to use the utmost care in handling the samples to alleviate this loss.

Dehydrated samples were kept in a desiccator jar at all times they were not in the oven or on the balance. Neglect of this precaution will produce erroneous mass determinations due to absorbed water.

All procedures were performed whenever possible under controlled or identical conditions to minimize error. If the conditions were altered on subsequent procedures the appropriate correction factors were applied. For example, all density values determined by the water displacement method were adjusted for water temperature according to published standards.(17)

The absolute densities of alveolar bone of three mammals, human, swine and whale were determined by two methods, water displacement and the air pycnometer. The densities of the inorganic bone of the three mammals in the hydrated and dehydrated state were reported. The density of the tissue bone and the organic fraction of the human and whale were determined. The values of all these mean densities are reported in Tables 1 through 5.

The values of the densities determined in this study are in general agreement with the values reported by other authors.(10, 11, 13, 14) The values of the densities computed using volumes measured with the air pycnometer are consistently higher than those determined by the water displacement method. This could be expected considering the measuring principle of the instrument.

The air pycnometer has a greater potential than water of penetrating the small holes in bone. This factor would decrease the value of the absolute bone volume. The bone mass is equal in both methods. Using the density formula, density equals mass divided by volume, the lower volume values would give a higher absolute density value.

A statistical analysis using the t-test at the 1% level was performed on the mean densities of the inorganic bone determined by the water displacement and air pycnometer (Table 7). The hypothesis that these means were equal

was tested. Six of the eleven means fell within the critical region, rejecting the hypothesis. The remaining five means were not significant and the hypothesis was accepted.

The inconstancy of the findings of the statistical analysis and the inherent error of the model 930 air pycnometer reduce the value of this instrument in determining volumes in this type investigation. The advantages of the air pycnometer, direct and rapid measurement of volume, are not sufficient to make it the method of choice over the water displacement method. Investigations of the density of human alveolar bone require the use of small samples and samples of this size unless pooled fall within the range of inherent error of the air pycnometer.

A scatter diagram was prepared to show the relationship of the mean density values of the fat free and inorganic samples. It showed no consistant relationship existed and for this reason is not included in the data. This would indicate that the mass to volume ratio of the organic fraction was not constant.

It has been reported that there is a 3.8 to 8 to 12 cell count per unit area ratio for whale, human and miniature swine alveolar bone.(3) If we relate this histologic data to the mean density values found in this study, we find a relationship exists for the swine and human. However the data of this study does not show this relationship for the human and whale alveolar bone. This is also reflected in a comparison of the mean density of the human and whale organic fractions.

A t-test was used to test the hypothesis that the mean density of human alveolar and retromolar bone were equal. Tables 8, 9. At the 1% level of significance none fell within the critical region, the area of rejection, so we can accept the hypothesis. Thus, in terms of the "null hypothesis", these sample differences could be selected by chance. A difference in bone densities cannot be demonstrated. Also, this could allow us to erroneously reject a true hypothesis by chance only one time in one hundred. This allows rejection of an observed or assumed clinical density difference. Neither our empirical x-ray observations nor clinical tooth rate movement observations could be influenced by mean density differences this small.

SUMMARY

The term density has been used in a variety of ways by many authors in the dental literature. However, it appears that to date, no one has determined the absolute skeletal density of alveolar bone. This study concerned itself with density determinations within samples of human alveolus and between the human, whale, and miniature swine.

Two methods, gravimetric and an air pycnometer volume determination were used to compare the density of lipid extracted alveolar bone, the inorganic fraction, and the density of the organic substance. Consideration of errors, the ability to repeat the procedure as well as testing the mean differences was employed and reported.

On the basis of this study, it would appear that no significant density differences of clinical importance occur within the dental bone of human cadavera.

CONCLUSION

1. There is no significant difference between mandibular human retromolar and alveolar bone density that could account for previously reported clinical phenomena in the dental literature.

2. The Beckman model 930 air pycnometer will not accurately measure individual samples of alveolus but requires a pooling to allow an accurate volume measurement. If the sample is large enough, the instrument has some advantages over a water displacement method.

3. The limited sample of whale and swine appear to indicate that alveolar bone density is independent of the type and amount of spaces in the bone.

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		Water Displacement				Air Pycnometer	
		Hydrated		Dehydrated		Dehydrated	
Sample	N	Mean	S	Mean	S	Mean	S
Human Right	10	1.8922	0.120	3.2325	0.250	3.6192	0.014
Human Left	10	1.9133	0.028	3.1031	0.267	3.5555	0.182
Whale	6	1.6405	0.095	3.0891	0.117	3.3906	0.010
Swine	3	1.6426	0.081	2.5547	0.272	3.4487	0.051

Table 1. Inorganic bone mean densities of mammalian alveolar bone.

		Water Displacement				Air Pycnometer	
		Hydrated		Dehydrated		Dehydrated	
Sample	N	Mean	S	Mean	S	Mean	S
Left Alveolar	10	1.9133	0.028	3.1031	0.267	3.5555	0.182
Left Retro-molar	10	1.9179	0.064	3.1215	0.178	3.5916	0.010
Right Alveolar	10	1.8922	0.120	3.2325	0.250	3.6192	0.014
Right Retro-molar	10	1.9225	0.010	3.1828	0.258	3.3928	0.022

Table 2. Inorganic bone densities of human alveolar bone.

		Water Displacement				Air Pycnometer	
		Hydrated		Dehydrated		Dehydrated	
Sample	N	Mean	S	Mean	S	Mean	S
Human	10	1.4277	0.101	1.8140	0.154	1.8560	0.002
Whale	6	1.4292	0.113	1.9386	0.434	3.1876	0.068

Table 3. Mean densities of lipid extracted mammalian alveolar bone

		Water Displacement				Air Pycnometer	
		Hydrated		Dehydrated		Dehydrated	
Sample	N	Mean	S	Mean	S	Mean	S
Human	10	1.6824	0.057	2.5876	0.192	3.1430	0.017
Whale	6	1.6369	0.088	3.1214	0.114	3.3734	0.014

Table 4. Mean densities of human and whale inorganic alveolar bone.

		Organic Fraction	
Sample	N	Mean	S
Human	10	1.3276	0.195
Whale	6	1.2798	0.139

Table 5. Mean densites of computed organic fraction of human and whale alveolar bone.

Method	N	Mean	S
Mass error determination	10	0.1490	0.0001
Mass-volume error determination	10	0.5571	0.001
Volume error determination with air pycnometer	10	8.5745	0.030

Table 6. Error determination.

Sample	Significant at 1% level
Human Alveolar - Lipid Free	No
Whale Alveolar - Lipid Free	Yes
Human Alveolar Inorganic	Yes
Human Left Alveolar Inorganic	Yes
Human Right Alveolar Inorganic	Yes
Human Left Retromolar Inorganic	Yes
Human Right Retromolar Inorganic	No
Whale Alveolar	Yes
Whale Alveolar	No
Swine Alveolar	No
Swine Retromolar	No

Table 7. Comparison by t-test at 1% level of the mean densities of bone determined by the water displacement method and the air pycnometer.

Samples	Significant at 1% Level
Left Alveolar and Right Alveolar	No
Left Alveolar and Left Retromolar	No
Right Alveolar and Right Retromolar	No

Table 8. Comparison by t-test at 1% level of the mean densities of the hydrated human alveolar and retromolar bone.

Samples	Significant at 1% Level
Left Alveolar and Right Alveolar	No
Left Alveolar and Left Retromolar	No
Right Alveolar and Right Retromolar	No

Table 9. Comparison by t-test at 1% level of the mean densities of dehydrated human alveolar and retromolar inorganic bone.