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S.F. Cook and R.F. Heizer *

Introduction

In a recent paper (Cook, 1951) the fossilization of bone was considered from the standpoint of the behavior of the principal components: calcium, phosphate, and carbonate. These substances may increase or decrease with the duration of fossilization and in detail are subject to wide fluctuation, depending on the chemical nature of the soil matrix in which the bones are imbedded. Leaching, accumulation, and ion exchange may raise or lower the level of any of the inorganic constituents in such a way as to make the analytical values in a single specimen of little use in estimating the age of the bone. The organic matter, on the other hand, appears to undergo a consistent secular reduction which is much less dependent upon the chemical and physical environment. The water content likewise decreases quite uniformly with age.

The present paper reports the results of recent investigations of the organic components and water in fossil bone. Application of the results to dating is not attempted here in detail but will be dealt with in a separate paper.

Fresh compact bone of man or of other large mammals consists of approximately 22.5 per cent ignitable organic matter and 11 per cent water. The organic matter is made up of the bone proteins (primarily ossein), a little fat, and perhaps traces of carbohydrate and there has as yet been scant investigation of its occurrence in fossil bone.[1] Something is known about the fat content, and this will be discussed forthwith. The protein of normal, or fresh bone has been considered by various authors, but its chemistry still needs to be clarified. It has been possible in this investigation to analyze only elementary organic nitrogen in fossil bone; its presence creates the presumption that protein is also present but by itself it tells us little if anything concerning the nature of the nitrogenous compounds.

Fat

Several years ago Gangl (1936) suggested that a slow reduction with age occurs in the fat content of fossil bone. This he ascribed to a gradual nonenzymatic oxidation, in the presence of atmospheric oxygen, of the fatty acids. In 1947 the present authors reported a series of preliminary analyses of California bones in which the fat content appeared to be of negligible value. Recently another series has been subjected to a more thorough examination.

We first tested six samples of freshly ground, clean bone from the solid shaft of a beef femure. The samples were extracted with acetone at a temperature of 70°C for forty-eight hours followed by a six-hour extraction with a mixture of ethyl ether and alcohol. The mean weight loss, which is equivalent to total lipid, was 3.8 per cent. The corresponding value found by Gangl[2] was 5.5 per cent. He does not explain, however, what part of the bone he used or whether he was careful to remove all traces of marrow fat before the analysis. If allowance is made for minor differences in technique, his results and ours are reasonably similar.

Gangl analyzed three fossil human bones, their ages estimated as 2,500, 2,500, and 3,700 years, respectively. He does not state from what part of the skeleton they came or how they were prepared. His corresponding values for the petroleum ether extract were 0.01, 0.01 and 0.02 per cent by weight.

For our fossil bone analyses we selected twelve samples, each from the shaft of a femure. The samples came from a wide range of archaeological sites, one sample from each site. Each bone was thoroughly cleaned, ground, and analyzed by the standard micromethod of Bloor (1928). The results are expressed as the per cent by weight of equivalents of tripalmitin, substantially the same mode of expression as that used by Gangl. Our results are as follows. [3]

Site	Burial no.	Tripalmitin equivalents as per cent of bone weight
CCO-141	12-5428A	0.0032
CCo-138	12-6104	0.0131
Sac-6	12-7135	0.0070
Mesa Verde	. . .	0.0033
Tul-18	. . .	0.0045
Marin Co. (Mrn-232b, 242, 266)	12-6483	0.0063
Sac-104	12-7434	0.0064
Fre-48	12-6073	0.0084
LAn-1	12-8206	0.0095
Sac-107	12-5598A	0.0130
SJo-68	. . .	0.0125
SJo-142	12-5675	0.0150

Our petroleum ether extracts contained almost exactly the same amount of lipid as those reported by Gangl; hence we can confirm his findings. However, the quantities involved are so extremely small that our published figures for total organic matter are not appreciably affected.

In the accompanying tabulation the bones used in the analyses have been arranged in approximate order of age. Culturally, the first four sites may be reckoned as relatively recent, the next three as intermediate, and the last five as early. Thus, the Mesa Verde bones are 1125 ± 50 years old according to the dendrochronological date of the ruin, and site SJo-68 has a radiocarbon (Carbon 14) age of 4052 ± 160 years. The fat equivalents of the individual samples show no tendency whatever to decrease with the age of the specimen. Indeed, if there is any actual trend at all, it is in the direction of an increase. Hence the fact content of fossil bone may be immediately discarded as a practicable criterion of age.

Exactly what happens in the bone during interment is not wholly clear. Gangl assumes, probably correctly, that the fat of the fresh bone undergoes direct oxidation and he implies that this process is relatively slow. If it were very slow, then we should find the quantity of fat gradually decreasing over a period of centuries or perhaps millenia. Our data, however, contradict this assumption. The most recent and best preserved bones, some of which cannot be more than a few hundred years old, contain even less extractable material (fat) than those which are certainly several thousand years old. We are therefore forced to assume that the aerobic, nonenzymatic oxidation phase is completed within a relatively short period, leaving faint traces of some breakdown product that is extraordinarily stable. Of this product's nature we have at present no knowledge whatever.

An alternative hypothesis, which may be merely suggested, is that the material extracted by petroleum ether from fossil bone is not derived from the original bone fat at all. Gangl describes the extract from fresh beef bone as "salbig" (salvelike) in contrast with the extract from fossil bone, which he calls "kristallinisch." This agrees with our own observations. Moreover, we noted that the dried extract had a peculiar musty or moldy odor, not at all characteristic of a fat. It is entirely possible that the extracted material represents an accumulation of some organic compound derived from the soil or the mound matrix. Over hundreds of years small traces of this organic compound may, under correct conditions, penetrate the bone from outside. If this theory is valid, then the substance, though having some theoretical chemical interest, loses most of its significance for the determination of age through the analysis of the fossilization process.

Organic Carbon and Nitrogen

To measure the total quantity of organic matter in fossil bone we have used direct analyses for elementary carbon and nitrogen.

Nitrogen was determined as total nitrogen by the standard micro-Kjeldahl procedure. Each sample was finely ground and digested in hot sulphuric acid and selenium oxychloride. The ammonia was then distilled off from concentrated NaOH, collected in boric acid, and titrated against standard HCl. The results were expressed as per cent nitrogen per unit weight of bone. The values thus obtained for total nitrogen may be taken as equivalent to organic nitrogen since the quantity of ammonium salts or nitrates in the bone is exceedingly small.

For organic carbon it was necessary first to estimate the inorganic carbon, since all fossil bones contain appreciable to large amounts of carbonates. Each bone sample was divided into two parts. One was subjected to treatment with weak acid, and the carbon dioxide was determined gravimetrically. The other part was then subjected to wet combustion in hot concentrated sulphuric acid -- bichromate mixture. The CO_2 produced was determined gravimetrically after absorption in a commercial preparation known as "Ascarite." The carbonate carbon was then subtracted from that obtained by combustion, the difference being the carbon derived from organic matter.

Four hundred and fifty-four bone samples were analyzed for both carbon and nitrogen. When the values are plotted on a single graph, a scatter diagram is obtained which apparently indicates a direct proportion between the two elements. At the same time, there is an extensive dispersion of the individual points, produced by local variability in the bones as well as by experimental error.

The simplest method for establishing the relation between the two elements is to calculate the correlation coefficient. This is found to be +0.836, a value which, for such a large sample is highly significant.

Some of the random variation may be eliminated by segregating the individual analytical values into groups or classes. The range for carbon throughout the entire series runs from approximately 1 to somewhat over 10 percent. We then subdivide this range by intervals of 0.4 percent from 1 to 6.6 percent and by larger intervals above this value. Finally we compute the average percent of nitrogen centering around the midpoint of each carbon group. Thus, as seen in table 1, where these values are aligned, the mean value for nitrogen is 0.42 percent for all samples whose percentage of carbon falls within the range of 0.8 to 1.2 percent (midpoint 1.0 percent). There are nineteen such pairs of values and their coefficient of correlation is +0.966, a value which is highly significant.

Another simple procedure is to calculate the carbon-nitrogen ratio. This is best done with the consolidated class values shown in table 1, in the third column of which the appropriate ratios are given. For the nineteen values the mean is 2.296 with a standard deviation of ±0.266, or 11.6 percent of the mean. A further test is to use the chi-square method. The hypothesis may be proposed that there is a progressive change in the carbon-nitrogen ratio as the carbon percentage in the bone increases. However if, for the nineteen points the values of the ratio are plotted against those for the carbon percentage at the midpoints of the class intervals, the value of chi-square is 1.42. This is utterly without significance and indicates that there is <u>no</u> change in the ratio.

It is thus sufficiently evident that the carbon and nitrogen disappear from the bone in a parallel fashion and that the figures for the two elements constitute a clear index to the disintegration of the organic matter as a whole. Concerning the mechanism of this process no theories can be advanced at the present time. Nevertheless it may be pointed out that the constancy of the carbon-nitrogen ratio implies that we are dealing primarily with a single type of compound, most probably protein, which disintegrates <u>in toto</u> in such a way as to release carbon and nitrogen simultaneously, instead of undergoing conversion into such a substance as carbohydrate or ammonia. If there are such reactions, then the end products must be sufficiently soluble to be removed from the bone relatively easily by leaching.

With respect to the time element there appears to be a consistent reduction with age in both carbon and nitrogen. In table 2 are shown the mean values for each element, expressed as percent by weight of the bone, the samples being listed according to archaeological sites. For each site the standard deviation and standard error are given in terms of percent of the mean for that site. The latter figures indicate that there is considerable (an unavoidable) variation between the individual bones. However a general idea may be obtained concerning the trend if we take the average of all the sites within each age group, Late, Middle, and Early horizons, and use the average standard error for each group. We may then compute the value of <u>t</u> (or critical ratio) for each pair of groups and thus derive some notion of the significance of the differences between them. These results are summarized very briefly as follows.

Mean of the mean values	Carbon	Nitrogen
Late horizon sites	5.99 ± .496	3.00 ± .256
Middle horizon sites	4.38 ± .337	1.99 ± .156
Early horizon sites	2.03 ± .181	0.73 ± .084
Value of *t* for:		
Late and Middle	2.68	3.36
Middle and Early	6.18	7.11
Late and Early	7.50	8.42

Although no great accuracy can be claimed, nevertheless the high values for *t* are strong evidence that the broad differences between the three horizons are significant. There thus appears to be a clear reduction in both carbon and nitrogen as one progresses from the later to the earlier cultural periods.[4]

Water

One of the major constituents of bone is water.[5] Hence this substance should not be neglected in a study of the chemical changes undergone by bone during fossilization. In fact we are able to show that the water content of fossil bone varies in time in a manner parallel with that of the organic material.

Since all comparative data for ancient bone must be based upon weights obtained with specimens preserved in museums and laboratories at ordinary room temperatures, it was first considered advisable to determine the constancy of water content under such conditions. A few preliminary tests quickly established the fact that the weight of a bone indeed is not constant but varies from day to day by a factor of as much as 0.2 percent. This fluctuation is due to the alternate loss and recovery of water as the relative humidity of the ambient atmosphere changes. Hence it is clear that a considerable error will be introduced if a sample of bone is merely taken off a shelf and weighed as the starting point for the estimation of water content. It must first be equilibrated with a standard and reproducible relative humidity.

For all water analyses of our fossil bones we have accordingly placed each sample in a dessicator over a saturated solution in contact with solid ammonium chloride. This procedure establishes a fixed relative humidity in the air space of 80 per cent and eliminates the small fluctuations in weight due to uncontrollable weather conditions.

The actual determination of water content may be carried out by either of two general procedures. The first is to expose the sample to drying in a vacuum at ordinary, or room temperature and the other is to heat the material to a temperature at or just below the boiling point of water. Both methods were tested and both were found to give consistent results. After exploring several variations of the general technique the following method was adopted as combining the greatest simplicity, convenience and accuracy. The sample is placed in a small, weighed glass beaker and subjected to a temperature of 90°C for twenty-four hours, at the end of which time it has come to substantial equilibrium. It is then removed from the oven, allowed to stand for twenty-five minutes and rapidly weighed. Admittedly, this method is arbitrary, but for purely comparative purposes and with a long series of samples it yields consistent, uniform and reproducible results.

Another technical problem pertains to the size of the particles into which the bone is divided, since one might anticipate that the quantity of water eliminated would depend upon the mass of material and the distance through which the vapor was forced to diffuse in order to escape. In order to investigate this point a single sample was ground into four sizes of fragments: 6-8 mm., 2-4 mm., 1-2 mm., and from 1 mm. to impalpable powder. All were exposed to a temperature of 90°C for forty-eight hours. The percentages for weight loss (i.e. water content) were respectively as follows: 7.58, 6.90, 6.78, 6.68. The small differences observed can be ascribed to errors in measurement. At any rate there is no significant tendency for water to be lost in greater quanity, within the limited time in question, from one size than from another. We therefore selected the fourth category, that is, the finest size, as the standard for comparative purposes.

The primary question here of theoretical interest associated with the water found in fossil bone is whether the water is attached to or bound with the inorganic, mineral framework or with the organic matter which persists for a long time after the demise of the individual. In any event, the water must be rather loosely held, since it can be released by quite moderate heat and by simple dessication. Moreover, it can be shown with great consistency that if, after dessication, the bone is restored to an atmosphere of high humidity (e.g., 80 per cent) it reverts to its original weight, thus demonstrating that the original loss is entirely reversible.

It is not easy to ascertain by direct methods whether the water is associated with the organic or the inorganic matter, since these two fractions cannot be separated _in vitro_ unless by such drastic procedures as to alter completely the water relations. Hence we are forced to depend upon an indirect approach. The basis for such an approach is the series of changes with age in the inorganic and organic bone constituents which have been discussed previously. The organic matter, as indicated by carbon and nitrogen, tends to decrease as the bone ages. Simultaneously the inorganic part, expressed in terms of calcium, phosphorus, and carbonate, must necessarily undergo a corresponding relative increase. If the water is primarily or exclusively attached to the mineral part, then it likewise should show a relative increase with the age of the bones. Conversely, if tied to the organic fraction, it should decrease. Actually, from analyses of several hundred specimens it can be shown to undergo an unequivocal decrease. Its relationship with the other bone constituents can also be seen in the following small-scale experiment, which was, in principle, repeated several times.

Five bone samples were selected from five sites of widely varying antiquity. Each sample was ground, sieved, and analyzed for all constituents (see table 3). Then from each sample three aliquots were taken, placed in small beakers, and exposed to a temperature of 60°C for one day. For each sample the water loss of the three aliquots was averaged. A similar procedure was then followed at 90°C and 110°C and subsequently two series were tested in a dessicator *in vacuo* over P_2O_5. The results from the five types of treatment were then averaged for each sample. From simple inspection of the data it appears that the water content is more or less directly proportional to the carbon and nitrogen content of the bones. The relation to the calcium, phosphorus, and carbonate is more obscure but shows some indication of being inverse.

In order to clarify the situation there are several indirect procedures, or expedients which may be adopted. Although none of these is wholly satisfactory yet in combination they enable us to reach reasonable conclusions with respect to the water content and distribution in fossil bone.

We have a total of approximately 450 bone samples for which there exist analytical values for water, organic carbon, nitrogen, calcium, phosphorus and inorganic carbon. It is possible therefore to get direct correlations between the water and each of the other five components. The coefficients are as follows:

Water with	carbon................	+.746
	nitrogen..............	+.787
	calcium...............	-.547
	phosphorus	-.622
	carbonate.............	-.416

With 450 samples, despite considerable individual variation, these are all highly significant values for *r*. The presumption is thus strongly supported that the water content runs parallel to the organic fraction of the bone and opposite to the inorganic fraction. Moreover, if the preliminary assumption be allowed that the water is closely associated with the organic matter, then one would expect it to be an inverse function of the inorganic substance, since the latter clearly increases as the former decreases.

A somewhat different avenue of approach is by plotting the graphs of water against carbon and water against nitrogen. As we found when carbon was plotted against nitrogen, the direct plots of individual points show very marked scattering. This scattering is due to experimental error plus inherent variation between different bones and different sites. Many of these effects of minor variations, however, can be eliminated if we average the values on the *y* axis for class intervals on the *x* axis. If this operation is performed for the two graphs involving water, the resulting points, in each instance, take the form of a curve rather than a straight line. The regression is hence curvilinear and not linear. If, on the other hand, we plot the logarithms of the points mentioned above, they fall with remarkable precision in straight lines, as may be seen in figures 1 and 2. Thus the correlation of water with carbon and with nitrogen is as nearly perfect as can be required.

A simple formulation of the mathematical relationship of carbon, nitrogen, and water is now possible. Since there is a straight line function of the logarithms we may write:

$$\log C = k_1 \log W$$

$$\log N = k_2 \log W$$

where C denotes carbon, N, nitrogen, and W, water. Removing the logarithms, we get:

$$C = W^{k_1}$$

$$N = W^{k_2}$$

which is the standard exponential function. The values for k_1 and k_2 can be calculated with sufficient accuracy from the slopes of the curves and are respectively 2.27 and 2.50. If the graph of carbon with reference to nitrogen is plotted in a similar manner in the logarithmic form, a straight line is also obtained, but the slope as computed from the graph is 1.075.

In these calculations there is an error of probably less than 10 per cent, but this error does not appear great enough to invalidate the general conclusions. The slope of the carbon-nitrogen line is close enough to 1.00 to make it clear that these two components are directly proportional to each other, as would be expected if both elements are liberated simultaneously from slowly decomposing organic matter. If water is tied to the organic matter in some form, even though its precise mode of combination is as yet unknown, the exponents k_1 and k_2 should be identical. Their computed values differ by roughly 10 per cent, a relatively small divergence under conditions of experimental measurement.

Although the foregoing discussion indicates that water is primarily associated with the organic part of fossil bone, this conclusion may not be in all respects correct. A somewhat anomalous association presents itself when we consider very old bones from which the organic matter has almost entirely disappeared.

Most of our analyses -- those upon which the previous computations have been based -- have been with bones from the Late and Middle cultural horizons together with a considerable number from the Early horizon of the Central Valley. These, as a whole, still contain appreciable quantities of nitrogen and carbon. Some are almost devoid of these elements. Now if we adhere without qualification to the hypothesis that the water is tied exclusively to the organic part of the bone, then the specimens with little or no nitrogen or carbon should likewise contain little, if any, water. This does not appear to be true.

Table 4 gives the analyses of carbon, nitrogen, and water for thirty-two human bones which were selected as having the least carbon and nitrogen of all those examined. The mean for carbon was 0.682 per cent, for nitrogen 0.253 per cent. The mean value for water, however, was 4.015 per cent. Similar data for ten Late Pleistocene animal bones gave corresponding means of 0.042, 0.510, and 3.837 per cent nitrogen, carbon, and water respectively.

From the graphs shown in figures 1 and 2, as well as from the equations proposed, it is possible to calculate the expected water content, provided the relationships found to hold for moderate and high organic content hold also when the organic matter has substantially disappeared from the bone.

The computation is performed by graphic extrapolation in the figures and by substitution of the appropriate constants and observed values in the formulae. Thus we get the following calculated quantities of water, in per cent by weight from the corresponding experimental or assumed values of carbon and nitrogen.

According to graphic extrapolation 0.253 per cent nitrogen, 0.042 per cent nitrogen, 0.682 per cent carbon, and 0.510 per cent carbon should be associated respectively with 2.77, 1.34, 2.68, and 2.36 per cent of water. By substitution in the formulae 0.100 per cent carbon and 0.100 per cent nitrogen require respectively 2.50 and 2.75 per cent water. Conversely, if we assume the presence of 1.00 per cent water (log water equals 0.000), then the values of nitrogen and carbon should be zero. Clearly neither the graphs nor the equations give a true picture of the relationships of these components when the level of organic matter is very low for, if they were completely accurate, we should find 2.00 per cent or less water. Actually, as table 4 shows, the bones contain on the average approximately 4.00 per cent water.

It thus appears evident that, when the nitrogen and carbon -- hence the organic part as a whole -- has disappeared from the bone, a considerable amount of water remains. Since there is no appreciable organic material with which it could be combined, it is unquestionably associated with the inorganic part. It can be assumed to be present as loosely bound water of crystallization or as water of hydration attached by weak molecular forces to the mineral framework.

The magnitude of the residual water can be guessed at from the rather rough calculations given above. Analytical values of 0.1 per cent or less of nitrogen and carbon lie within the limits of experimental error and hence may be regarded as approaching zero. The water content theoretically should be 1.00 per cent. For a water content of 2.00 per cent the corresponding calculated values of nitrogen and carbon are respectively 0.556 and 0.483 per cent or, say, for convenience, 0.50 per cent. Hence in the lowest practicable range of analysis (i.e., from 0.1 to 0.5 per cent nitrogen and carbon) the water bound to organic matter will range from 1.00 to 2.00 per cent or, as a working approximation, 1.50 per cent. Now the figures in table 4, which in general lie within the indicated range, show a mean water content of nearly 4.00 per cent. The difference, therefore, (2.50 per cent) must be considered as the water bound to inorganic matter.

In the whole series of fossil bones the total quantity of inorganic matter, irrespective of the individual components, varies relatively little, say from 75 to 100 per cent by weight of the bone. Hence the quantity of inorganically bound water, which is always a fixed proportion of the total inorganic material, will vary in a parallel fashion, or roughly from 2.00 to 2.50 per cent of the entire bone weight. The difference indicated is of the same magnitude as that expected from experimental error and inherent random variation. Consequently, with a large range of samples we may neglect it and for purposes of numerical analysis treat the inorganically bound water as having a constant value; i.e., 2.50 per cent of the bone weight. The corollary follows naturally: the organic water is given by the total removable water, minus 2.50 per cent.

These considerations not only help to clarify the puzzling relationship experimentally found to exist between the water content and the organic matter itself but also will introduce the necessary correction if the water content is to be used as a criterion of the relative age of the bone. It is clear that only the water bound to the organic matter decreases with age and hence is of interest to the problem of dating.

Density

Heretofore in this report all constituents of fossil bone have been evaluated in relative terms, usually as per cent by weight of the bone. It is possible, however, to determine their absolute values and thus learn more about the process of fossilization. This may be done by considering the apparent density of the bone itself.

Fresh bone is compact and superficially homogeneous. Actually, however, it consists of a mineral framework, the interstices of which are filled with organic matter and water. In addition there are trabeculae and canals -- some microscopic, some of visible dimensions -- which in life are filled with fluid, blood vessels, and cellular elements. After burial most of these elements disappear, and when the bone is dry, the spaces formerly occupied by them are filled with air. The overall density of the bone thus tends to decrease.

If we determine for a particular piece of fossil bone the per cent of a certain constituent -- let us call it X -- and do not take into account the change in density, we may get a somewhat distorted picture of what has happened. To illustrate, we will assume a hypothetical fresh bone, having a volume of 1 cu. cm. and weighing 2 gm. The density, referred to water, is thus 2.0. Our constituent X is present to the extent of 10 per cent. There are therefore 200 mgm. of X in the bone. A fossil bone is now examined and found to contain 10 per cent of X. We would immediately say that, on a purely relative basis, there had been no change in the quantity of X during fossilization. But the density of the fossil bone is discovered to be 1.5, that is 1 cu. cm. of the bone weighs only 1.5 gm. Constituent X consequently is present only to the extent of 150 mgm. and actually 50 mgm. have been lost during the period of burial.

The actual estimation of density is relatively simple. A fragment of the solid bone (the size as large as possible but varying according to the material available) is weighed to the nearest milligram. It is then evaluated under water in order to expel the air, for the appearance and release of bubbles during subsequent measurement of volume conduces to error. The bone is then wiped carefully to remove all free liquid adhering to the surface and its volume is measured under water in a graduated cylinder. Several determinations may be made and the average taken. The error in volume is much less than 1 per cent. The density is then reckoned as the ratio of the weight in grams to the volume in cubic centimeters.

The density was determined for most of the bones for which data have previously been given. The principal omission is the series of bones from the Southwestern sites. All the material from this source had been used up before it was decided to measure density and no more was readily available. From the densities it was then possible, as described above, to compute the absolute values for various bone constituents. These included organic carbon, nitrogen, organically bound water, inorganically bound water (taken as 2.5 per cent of the bone weight), calcium, phosphorus, and acid-extractable carbon dioxide. The results, averaged by sites to conform to the data given in table 2, are given in table 5. The values given for each site are the averages for all samples analyzed from that site. The densities are expressed as the specific gravity of whole bone when water is taken as 1.0. All other values are in terms of milligrams per cubic centimeter of bone. To obtain a basis for comparison with the fossil bone an entire set of analyses was performed with a series of six samples of fresh beef femora, sections of the solid shafts being used. Before analysis each sample was thoroughly cleaned and freed of periosteum and all other nonosseous tissue. The results of the analyses of these beef bones are also included in table 5.

The data in table 5 support the conclusions derived from examination of the relative quantities of bone constituents. Some added information may, however, be obtained. With a single exception the bones from all burial sites show a reduction in density. Moreover, there appears to be no significant difference between the material from recent (Late) and relatively old (Early) sites. This fact implies that the most rapid change takes place during the first period of interment, perhaps during the first few decades after burial. On the other hand, there is great variability among sites of each cultural age group, indicating that local conditions exert influence irrespective of time.

The reduction in density appears to be associated with a loss of organic rather than inorganic material. It will be noted that the carbon, nitrogen, and organically bound water, in almost every instance, show a strong reduction in the Late cultural group and that this reduction is intensified as one passes to the Middle and Early groups. The inorganic material, on the other hand, is only slightly reduced in the Late and Middle groups and is materially increased in the Early group. These facts corroborate the hypothesis already advanced, that is, that there is a quite consistent loss of organic matter with age whereas the behavior of the inorganic matter depends largely, if not exclusively, on the local soil conditions.

It will be observed that the sites within each cultural group vary quite widely in the quantities of single components. Part of this variation, may be ascribed to sampling error and inherent differences in skeletons and bones. Another part may be due to the fact that the cultural grouping possibly does not reflect with absolute accuracy the true age grouping. A third part must be attributed to anomalies in the chemical influences to which the various sites have been subject.

For instance, let us consider sites CCo-141 and Fre-48. The bones from the former site (supposedly of the Middle culture period) show quite high nitrogen and organically bound water and excessively high carbon. Indeed there is more of this last component present in the fossil bone than there is in fresh bone. On the whole, the inorganic matter has decreased. Apparently these bones underwent a primary loss of material in the usual manner but were secondarily subjected to an infiltration of dissolved organic substances of a nonnitrogenous nature, which accumulated until the total carbon content exceeded that originally characteristic of the bone. It would therefore be reasonable to disregard the carbon and assess the age of the bone upon the basis of nitrogen and water alone.

The bones from Fre-48 are very old, if we are to judge by the extremely low content in organic matter and water. On the other hand, the calcium, phosphorus, and carbonate are far in excess of that contained in fresh bone. Here, again, we must be dealing with the phenomenon of accumulation, a process which extraordinarily high bone density shows has been carried almost to the point of petrifaction. The Early Horizon sites in the Central Valley (SJo-68, SJo-142, Sac-107, and SJo-56) all indicate that the same process was in operation in them, although to a lesser extent. We may surmise that among the causes were the climate of the region and the topography of the sites. They are all, with the possible exception of Sac-107, situated in the level valley and hence subject to semiannual rise and fall of ground water. Lime salts, particularly carbonate, could thus readily be deposited in the bones. Quite different is site LAn-1: here the bones contain a good deal of calcium and phosphorus but very little carbonate. This site lies at the crest of a ridge or knoll where the drainage is continually downward. There might therefore have been continuous leaching rather than deposition with attendant loss of carbonate.

It is unnecessary, and indeed it would be tedious, to embark upon a detailed discussion of the minor variations in bone components from one site to another. It may, however, be again emphasized that the primary value of expressing the quantity of bone constituents in absolute rather than relative terms is that a much clearer view of the actual changes undergone by the bones may be obtained, although either method is theoretically valid. Furthermore, the comparison of bones for the purpose of estimating the probable age is more satisfactory if the absolute, not the relative, values for the components are used. This theme will be developed in a subsequent paper.

Summary

The analyses and experiments reported in this paper reveal the following facts concerning the chemistry of fossil bone.

1. The existence of very small traces of fat, or at least of some lipoid material in fossil bone, is confirmed, but there is no association between the quantity of this material and the probable age of the bone.

2. The presence of organic carbon and nitrogen can be demonstrated in all fossil bones analyzed, thus indicating the persistence of organic matter long after the burial of the bone. Since these elements occur consistently in a 1:1 ratio, we may conclude that we are dealing with the remnants of the original bone protein. A clear reduction in amount of both elements parallels the cultural age of the bones, which indicates a gradual disintegration of the organic matter over many centuries, relatively independent of external factors.

3. Fossil bone contains varying quantities of loosely bound water which can be removed by heat or dessication and can also be replaced. There is an apparently consistent relation between this water and the carbon and nitrogen, from which it may be argued that at least part of the water is bound to the organic compounds. At the same time a constant quantity of water (2.5 per

cent by weight) seems to be attached to the inorganic framework. The part organically bound varies inversely with the presumed age of the bone and can therefore be employed with carbon and nitrogen in the measurement of antiquity.

4. Determination of the density of whole bone (weight of dry bone divided by volume) makes it possible to compute the absolute, as well as the relative, amounts of all bone constituents in fossil bone and to compare these with analogous values for fresh bone. This in turn furnishes an improved set of data for use in the investigation of both relative and absolute age.

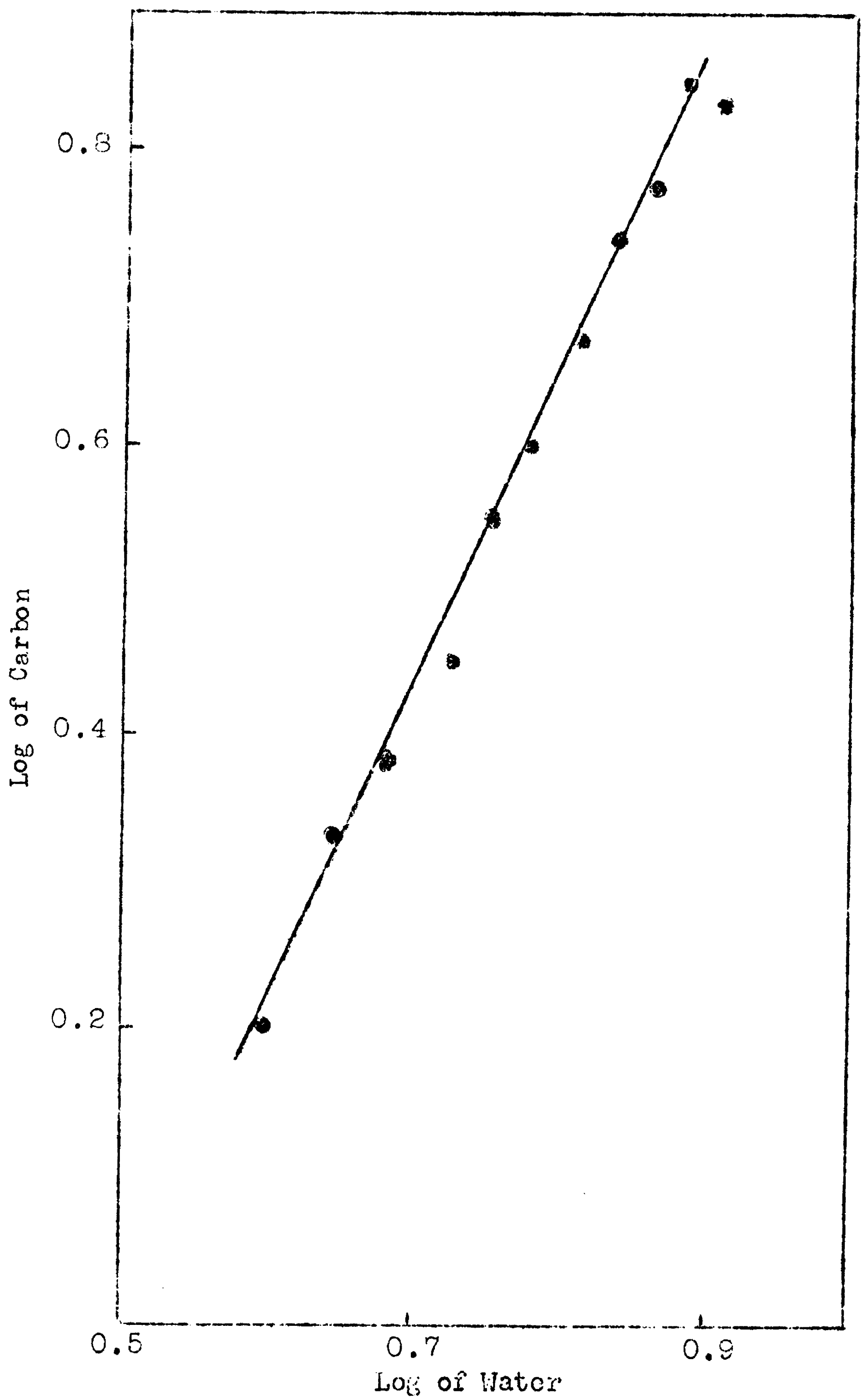

Fig. 1. Relationship of C and H_2O in fossil human bone.
For explanation see p. 7.

NOTES

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[1] The single modern investigation of carbohydrates and their derivatives in fossil bone was reported by Thunberg (1947). He found very small amounts (0.01 to 1.0 per cent) of citric acid to be present in mediaeval and prehistoric bones. There seemed to be a rough correspondence between the quantity of acid and the age of the bone. A more exhaustive examination of the carbohydrates would undoubtedly prove of value.

[2] 1936, table, p. 81.

[3] Samples with numbers prefixed by 12- are in the Museum of Anthropology, University of California.

[4] Although the primary aim of this paper is not cultural interpretation, the implications of chronology in archaeological deposits containing cultural materials make it important that the cultural data be cited at least. The following published reports (see Bibliography) contain information on the archaeology of most of the sites discussed here. Unpublished information on other sites mentioned (Sac-43, Sac-104, Sac-151, Tul-18) is on file in the UCAS records. CCo-138, Lillard, Heizer, and Fenenga, 1939, pp. 70-72; Sac-21, _ibid._, pp. 57-59; CCo-141, _ibid._, pp. 54-56; Sac-107, _ibid._, pp. 23-31, Heizer, 1949; SJo-68, Lillard, Heizer, and Fenenga, 1939, pp. 31-34, Heizer, 1949; SJo-142, Lillard, Heizer, and Fenenga, 1939, pp. 33-38, Heizer, 1949; SJo-56, Lillard, Heizer, and Fenenga, 1939, pp. 38-43, Heizer, 1949; CCo-137, Heizer, 1950; Hum-118, Mills, 1950; Sac-6, Schenck and Dawson, 1929, _passim_; LAn-1, Heizer and Lemert, 1947, Treganza and Malamud, 1950; Marin County sites, Beardsley, 1948, unpublished notes in UCAS files; Hawver Cave (Eld-16), Stock, 1918; Tranquillity site (Fre-48), G. Hewes, 1941, 1943, 1946; Santa Barbara sites, Orr, 1951, Olson, 1930, Rogers, 1929; Mesa Verde (Soda Canyon and White Mrd.), Gladwin, 1945; Elden Pueblo, Fewkes, 1927, Hough, 1932.

[5] The exact amount of water contained in fresh osseous or calcareous tissue seems to be subject to controversy. Best and Taylor (_Physiological Basis of Medical Practice_, 4th ed., 1945, p. 712) states, "Water constitutes about 25 per cent of the bone weight," but they do not specify what type of bone was analyzed or how it was prepared. We have examined, by means of the methods employed with fossil bone, six samples of fresh beef femur. Only the solid central shaft was used and before analysis the specimens were carefully freed of marrow and periosteum. The average water content was 11.1 per cent, a value which coincides reasonably well with results obtained by other investigators for dentine, enamel, and whole teeth. It is probable that dense osseous tissue contains much less water than cancellous bone or the skeleton as a whole. Since we have used dense bone exclusively, the water content of the normal tissue may be taken as from 10 to 12 per cent by weight.

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ABBREVIATIONS

A ant	American Antiquity
UC	University of California Publications
-AR	Anthropological Records
-PAAE	American Archaeology and Ethnology
UCAS	University of California Archaeological Survey
-R	Reports

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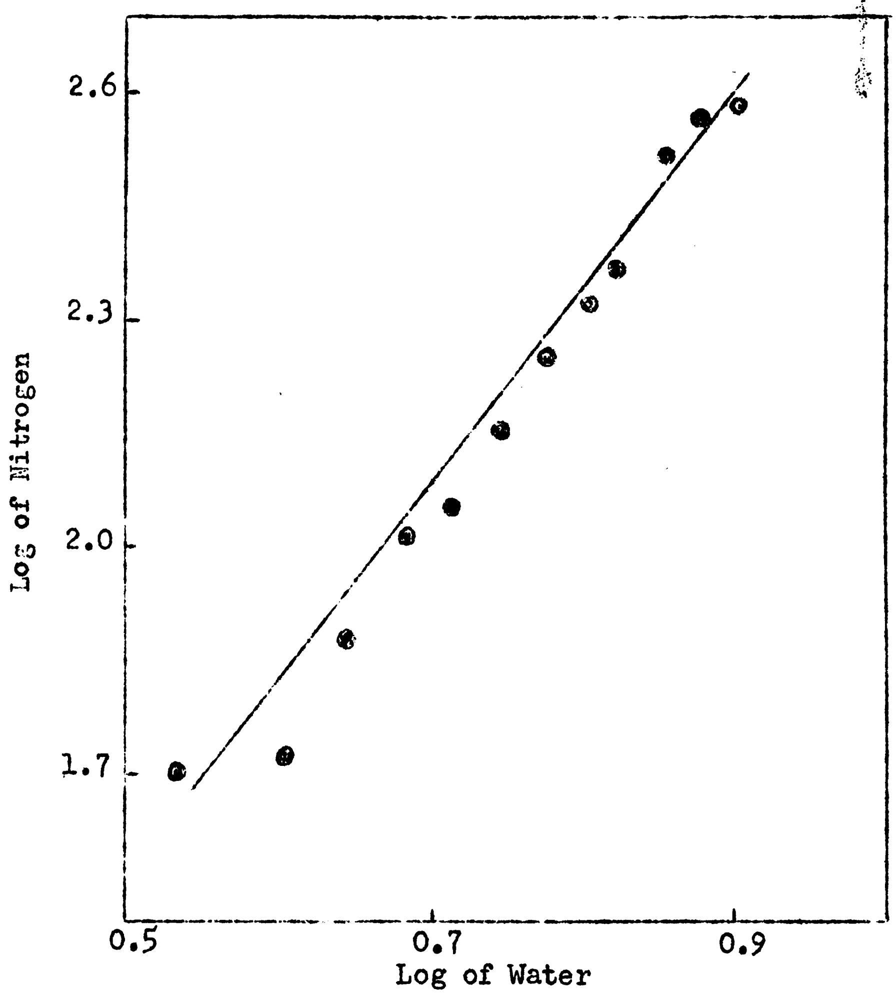

Fig. 2. Relationship of N and H_2O in fossil human bone. For explanation see p. 7.

TABLE 1

Carbon and Nitrogen in Fossil Bone

(454 samples)

	Average values		Ratio
	Carbon	Nitrogen	C/N
	1.0	0.42	2.38
	1.4	0.56	2.50
	1.8	0.70	2.57
	2.2	0.87	2.53
	2.6	1.10	2.36
	3.0	1.48	2.03
	3.4	1.55	2.19
	3.8	1.73	2.20
	4.2	1.86	2.26
	4.6	1.86	2.47
	5.0	2.19	2.28
	5.4	3.23	1.67
	5.8	3.02	1.92
	6.2	2.82	2.20
	6.6	3.23	2.04
	7.8	2.82	2.77
	8.4	3.72	2.26
	9.6	3.81	2.57
	10.4	4.27	2.43
Mean...	4.91	2.172	2.296

TABLE 2

Organic Carbon, Nitrogen, and Water in Fossil Bone from Twenty-eight Archaeological Sites

Sites	Carbon			Nitrogen			Water		
	Mean	S.D.[a]	S.E.[a]	Mean	S.D.	S.E.	Mean	S.D.	S.E.
Late horizon group									
Central California........									
CCo-138 (20)[b]...........	5.82	33.7	7.5	2.35	46.4	10.4	7.03	12.2	2.7
Sac-6 (24)..............	3.76	42.8	8.8	1.47	58.7	12.0	5.49	30.2	6.2
Marin County sites...... (Mrn-232b, -242, -266, -271, -275) (13)......	4.68	17.9	5.2	1.32	23.2	6.4	5.58	12.8	3.5
Santa Barbara area[c]									
Shalwaj (9).............	4.11	50.4	17.8	2.51	57.0	20.2	6.32	18.8	6.2
Tecolote (6)............	3.18	50.6	20.6	2.10	67.1	27.3	6.45	21.5	6.8
Las Llagas (13)..........	3.63	26.4	7.3	2.41	31.3	8.7	6.56	17.3	4.8
Santa Rosa Island (14)..	5.87	14.0	3.7	3.88	4.4	1.2	7.58	5.1	1.4
San Miguel Island (7)...	5.57	29.9	11.3	3.56	34.8	11.3	7.09	13.1	4.9
Santa Cruz Island (6)...	6.30	7.1	2.9	3.82	6.8	2.8	7.88	3.1	1.2
Southwestern United States									
Soda Canyon (10)........	10.31	7.7	2.4	4.35	3.5	1.1	7.55	2.7	0.9
White Mound (16)........	5.43	12.8	3.5	3.95	14.0	3.6	7.31	4.2	1.1
Elden Pueblo (21).......	8.56	...	...	2.99	39.8	9.1	6.91	...	...
Hawikuh (37)............	10.76	...	...	4.33	7.1	1.2	7.65	...	...

[a]Plus and minus signs are omitted from the figures for standard deviation and standard error.

[b]Figures in parentheses indicate the number of samples analyzed.

[c]The first three sites are described in Rogers, 1929; the unspecified Island sites are sites in which Orr has recently excavated; for Santa Rosa Is. sites, see Orr, 1951.

TABLE 2 (Continued)

Sites	Carbon			Nitrogen			Water		
	Mean	S.D.	S.E.	Mean	S.D.	S.E.	Mean	S.D.	S.E.
Middle horizon group									
Sac-21 (26)...............	5.61	20.3	4.0	2.37	21.8	4.3	6.20	11.9	2.0
CCo-141 (18)..............	7.86	21.7	5.3	3.66	13.9	3.3	7.23	8.7	2.1
Sac-43 (30)...............	4.05	30.1	5.5	2.04	32.8	6.0	5.96	12.1	2.2
Sac-151 (33)..............	2.73	41.0	7.2	1.15	57.0	9.9	4.34	18.1	3.2
Sac-104 (9)...............	1.52	36.8	12.2	0.64	40.2	13.4	5.51	18.0	6.4
CCo-137 (8)...............	2.64	27.6	10.0	1.70	32.1	11.3	5.74	8.9	3.3
Tul-18 (10)...............	6.33	33.6	10.6	3.33	15.2	4.8	7.95	5.9	1.9
Marin County sites (Mrn-232b, -242, -266)(10)....	4.35	20.0	6.7	1.04	28.9	9.6	5.41	14.7	4.9
Early horizon group									
SJo-68 (30)...............	1.95	53.8	10.0	1.32	55.0	10.2	5.84	23.0	4.3
SJo-142 (31)..............	1.09	75.1	13.8	0.54	53.3	9.6	4.30	14.9	2.7
Sac-107 (32)..............	1.59	35.6	6.4	0.44	67.9	12.0	4.28	15.1	2.7
SJo-56 (33)...............	4.09	25.9	4.5	1.62	42.0	7.3	5.24	17.3	3.0
Fre-48 (5)................	1.55	30.4	13.5	0.08	31.3	13.7	2.26	15.8	7.1
LAn-1 (22)................	1.32	36.3	8.1	0.42	69.5	14.8	4.30	21.2	4.6
SBa-7 (10)................	2.64	21.5	6.2	0.70	46.0	13.3	5.83	12.2	3.5
Special series: Sac-6, single skeleton									
Long bones (12)...........	...	...	...	1.47	56.8	17.4	...	...	...
Short bones (18)..........	...	...	...	2.11	46.2	11.2	...	...	...

TABLE 3

Comparison of Water Content and Other Constituents in Five Samples of Fossil Bone

(Per cent of total weight)

Constituents	Samples				
	12-5856 (CCo-138)	MV4 (Mesa Verde)	12-6702 (Sac-43)	107-S6 (Sac-107)	12-6075 (Fre-48)
Calcium...........	30.9	48.0	32.5	38.9	34.1
Phosphorus........	14.0	10.4	12.8	13.3	17.0
Carbonate.........	4.27	3.92	5.67	9.11	6.12
Organic carbon....	5.97	11.56	2.41	1.40	0.73
Nitrogen..........	1.42	4.11	1.41	0.39	0.053
Water [a]...........	7.72	8.85	6.20	4.73	2.17

[a] The figures for water are the per cent of water loss expressed as the per cent of bone weight lost. The data represent the mean of 5 water determinations.

TABLE 4

Percentage of Carbon, Nitrogen, and Water in Human and Animal Bone

Samples by sites[a]	Human Bone (32 samples)		
	Carbon	Nitrogen	Water
SJo-68			
N2	0.16	0.17	6.62
12-7600	0.00	0.10	6.81
12-7622	0.59	0.03	5.77
12-7646	0.09	0.60	7.13
Sac-107			
S1	0.80	0.09	3.98
S4	1.04	0.04	3.64
12-5650	0.76	0.08	3.46
12-5653C	0.86	0.22	3.44
12-5653D	0.85	0.38	2.82
SJo-142			
12-5664	0.11	0.63	5.49
12-5667	0.94	0.06	5.17
12-5668	0.45	0.48	5.18
12-5670	0.43	0.02	4.07
12-5674	0.33	0.01	2.51
12-5676	0.00	0.33	4.33
12-5798	0.00	0.67	4.17
12-5799	0.27	0.65	4.81
12-5801	0.69	0.29	3.87
12-5805	0.28	0.19	3.61
12-5815	0.59	0.57	3.85
12-5819	0.00	0.28	3.68
Sac-151			
X2	0.85	0.26	3.48
Fre-48			
12-6071	1.85	0.09	2.33
12-6072A	1.72	0.11	2.68
12-6072B	1.85	0.07	2.49
12-6073	1.62	0.06	1.81
12-6075	0.73	0.05	1.99
LAn-1			
T2	0.98	0.80	4.55
T8	0.83	0.24	4.24
T9	0.77	0.23	3.15
T11	0.95	0.11	3.52
T20	0.44	0.19	3.86
Mean	0.682	0.253	4.015
	Animal Bone (10 samples)		
Hawver cave (Eld-16) (sloth)			
11037	0.66	0.01	3.91
19888	0.19	0.05	4.72
19893	0.00	0.02	3.78
11062	0.26	0.11	5.21
19967	0.58	0.04	7.50
Calaveras cave (Cal-10) (horse)			
...	0.60	0.01	4.38
...	0.10	0.01	4.01
Fre-48 (horse or camel)			
61635	1.45	0.05	1.63
61979	0.37	0.07	1.76
64948	0.89	0.02	1.47
Mean	0.510	0.042	3.837

[a]Samples with numbers prefixed 12- are in the Museum of Anthropology; the animal bones from the Museum of Paleontology, University of California.

TABLE 5

Absolute Quantities of Certain Bone Constituents with Values Corrected for Density

(mgm. per cu. cm. of bone)

Source of sample[a]	Density[b]	Organic carbon	Nitrogen	Water: Organically bound	Water: Inorganically bound	Calcium	Phosphorus	Carbon dioxide
Fresh Bone								
Beef femur (6 samples).....	1.996	114	80	172	50	553	224	74
Fossil Bone								
Late sites:								
CCo-138	1.618	94	38	57	41	500	195	78
Sac-6..........	1.485	56	22	44	37	472	212	71
Marin Co. sites	1.291	60	17	40	32	450	163	45
Shalwaj........	1.541	63	39	59	39	504	196	64
Tecolote.......	1.644	52	35	60	41	531	207	95
Las Llagas.....	1.620	59	39	66	40	544	204	75
Santa Rosa I. .	1.306	106	70	92	45	538	213	56
San Miguel I. .	1.827	102	65	84	46	522	207	69
Santa Cruz I. .	1.936	122	74	104	48	573	221	69

Table 5 (Continued)

Source of sample[a]	Density[b]	Organic carbon	Nitrogen	Water: Organically bound	Water: Inorganically bound	Calcium	Phosphorus	Carbon dioxide
Middle sites								
Sac-21	1.510	90	36	56	38	470	182	57
CCo-141........	1.881	148	69	89	47	471	196	93
Sac-43.........	1.662	67	34	57	42	502	212	88
Sac-151........	1.458	40	17	27	36	488	193	90
Sac-104........	1.645	25	10	49	41	556	220	81
CCo-137........	1.572	41	27	51	39	495	179	120
Tul-18.........	1.658	39	52	85	39	570	169	82
Marin Co. sites	1.328	58	14	39	33	487	171	56
Early sites:								
SJo-68	1.738	34	23	58	43	572	233	107
SJo-142........	1.580	17	9	28	39	567	203	151
Sac-107........	1.551	26	7	28	39	551	217	150
SJo-56.........	1.783	73	29	49	45	571	225	100
Fre-48.........	2.400	37	2	0	54	950	332	152
LAn-1..........	1.421	19	6	26	36	599	205	36
SBa-7..........	1.431	38	10	48	36	594	178	93
Mean								
Late...........	1.640	79	44	67	41	515	202	69
Middle.........	1.589	71	32	57	39	506	190	83
Early..........	1.701	35	12	34	42	629	227	113

[a]Values for each site are averages for all samples analyzed.

[b]Expressed as specific gravity of whole bone when water is taken as 1.0.