RELATIVE DATING
OF
MOUNDVILLE BURIALS

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Contents

Introduction

Relative dating of archaeological remains is well known as a method for ordering a group of samples chronologically. It cannot provide exact ages, as can absolute dating, but it can provide a relationship between samples, as long as they have been preserved under the same conditions. Both dating methods are usually based on how the content of some element or compound changes over time. The difference lies in the fact that the substances important for relative dating methods are greatly effected by environmental conditions, while those important for absolute dating methods are assumed to have a constant rate of change everywhere. Thus a control for environmental conditions is necessary when doing relative dating. The most obvious control is that samples to be compared come from the same archaeological site.

In the summer of 1970, I undertook a nitrogen analysis of bone samples from fifteen gravelots at the Moundville site in Alabama, on the suggestion of Vincas P. Steponaitis. He has made a seriation of gravelots at Moundville based on the pottery found in association with the burials (1). He wished to have a radiocarbon analysis done on the fifteen skeletons in order to place them in time. Unfortunately, all of the bones, which were unearthed by C. B. Moore before 1941, are coated with the preservative Alvar. This preservative, a carbon based polymer, would throw off greatly any values obtained by radiocarbon analysis. For this reason, he decided that a relative dating technique that does not involve any of the elements contained in Alvar would be more suitable for his purposes. Although it would not give absolute dates, it would provide a chronological check on the pottery seriation. And so, in August of 1970, I went to the Smithsonian Institute in Washington, D. C. to get the bones from Mr. Steponaitis, and a procedure for nitrogen analysis from Dr. David von Endt.

During the course of my analysis, I had the excellent

fortune of recieving an offer to have a fluorine analysis done on the bones by Dr. Albert L. Hanson at Brookhaven National Laboratory in Upton, New York. He was aware of my project, and of the utility fluorine dating would have as a cross check for nitrogen dating. His technique is a highly sophisticated analysis utilizing proton inelastic scattering. In March of 1980, I went to Brookhaven and spent a week preparing samples and helping to set up the analysis procedure. So with the generous aid of Dr. Hanson, I was able to make my project complete.

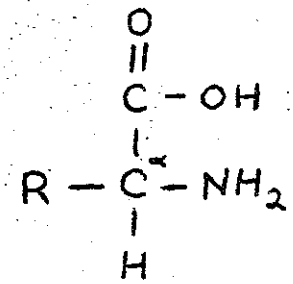
In the following study, I will present a complete account of my work with the relative dating of the fifteen Moundville skeletons. In addition, I will give a brief review of the theory and history of nitrogen and fluorine dating.

Nitrogen Dating

Nitrogen dating is based on the fact that buried bones and teeth lose nitrogen over time. Thus the older the bone, the less nitrogen per total weight the bone will contain.

Fresh human bone contains about five percent nitrogen by weight (2). Almost all of this is contained in the fibrous protein collagen. Like all proteins, collagen is made up of α-amino acids linked by peptide bonds. All α-amino acids have the basic structure shown in Figure 1 below.

Figure 1

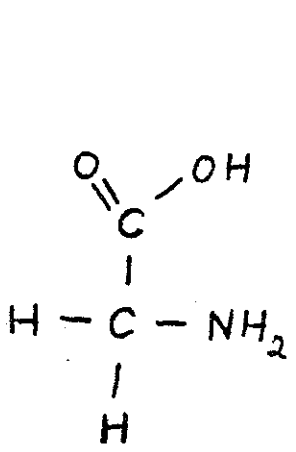


α-amino acid

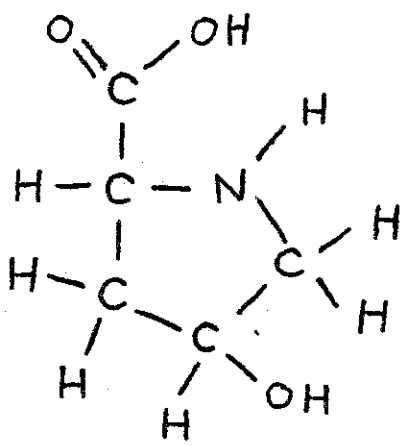
Each consists of a carboxyl group and an amino group on the same carbon atom, designated the α-carbon atom. The side chain, different for each of the twenty-odd biological amino acids, is represented by the R. Most of the protein nitrogen is contributed by the amino groups. A much smaller amount may be contributed by side chain nitrogen atoms found in some of the more basic amino acids such as lysine and arginine.

The collagen molecule is made up of three peptide chains which are particularly high in the amino acids glycine and hydroxyproline, shown in Figure 2. The peptide chains are held together by hydrogen bonds. Bundles of

Figure 2



glycine



4-hydroxy proline

these molecules are arranged in fibrils which have a characteristic 640 Å banding, created by the overlapping molecules.

Collagen is especially long lasting. In its unaltered state, it is resistant to most proteolytic enzymes. Collagenase, the collagen degrading enzyme, is one of the few that effect it. However, when collagen is exposed to hot water, the hydrogen bonds between peptide chains break and the chains separate, producing a gelatin that is easily broken down into its constituent amino acids by proteolytic enzymes.

The major event in the loss of nitrogen from buried bone is protein hydrolysis in which peptide bonds are broken by the insertion of a water molecule, freeing the amino acids to leach out of the bone. During the first years after burial, this is most likely to depend on the presence of collagenase producing micro-organisms, mainly of the species Clostridium, and on the environmental suitability for them (3). Over long periods of time decomposition

takes place in other ways. One of the most influential factors is temperature. Ortner et al. have demonstrated experimentally that the higher the mean annual temperature, the greater the rate of nitrogen decay in bone (2). Another important factor is soil pH; the lower the pH, or the more acidic the soil, the faster hydrolysis takes place. Of course, hydrolysis cannot take place without water, and the more water present (up to saturation), the more accelerated the decay rate.

All chemical reactions can be described by mathematical equations expressing the relationship between the reaction rate and concentration of one or more of the reactants. For example, reactions with rates that depend only on one of the reactants are known as first order reactions. Unfortunately, the hydrolysis of proteins and the disappearance of the constituent amino acids from buried bone is a process so complex and dependent on so many factors (only a few of which are mentioned above) that it cannot be expressed accurately in such a simple manner. But for practical purposes, nitrogen loss from bone appears to be a first order reaction dependent on the concentration of nitrogen present in the bone (2, 4), and is therefore called a "pseudo-first order" reaction. As such, this process can be expressed by the first order rate equation

Equation 1 $$dx = k(a - x)dt$$

where dx is the change in the reactant nitrogen, dt is the change in time, a is the starting concentration of nitrogen, x is the amount lost at time t, and k is the rate constant, particular to each reaction. Experimental data is easier to work with using the integrated form of the above equation:

Equation 2 $$\ln(a/a - x) = kt$$

Thus when ln(a/a - x) is plotted against time, a straight line with slope k results (in reciprocal time units). This means that the loss of nitrogen from bone is a logarithmic function of time.

Analytical Methods

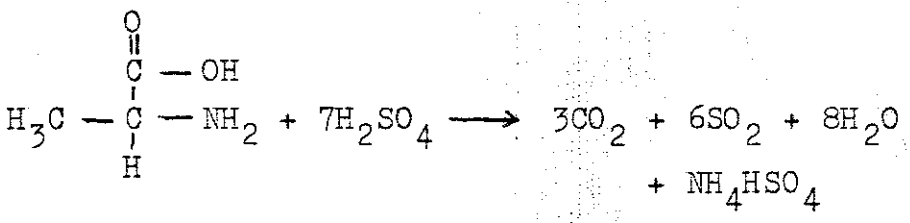
Perhaps the earliest method for organic nitrogen determination was first published by Dumas in 1831 (5). In this procedure, the sample is burnt in a carbon dioxide atmosphere, oxidizing all nitrogen present. Everything is then reduced, converting the nitrogen into its gas, the form in which it is measured directly (3). Although this method must be done carefully to obtain reliable results, when done properly it is an excellent way to determine total nitrogen content, since it converts essentially all nitrogenous compounds. It is still considered to be an indispensable technique.

The most commonly used method for organic nitrogen was first developed by Johann Kjeldahl and published in 1883 (6). While working in the Carlsberg Laboratory, he wished to trace protein changes in grain during fermentation (7). Unlike the Dumas method, the Kjeldahl method was an immediate success since it is easier to use and more reliable. It is, however, limited to a much lower range of compounds; it cannot convert oxidized nitrogen well, such as nitro compounds. But for many organic materials such as proteins, it is very well suited and has been a standard procedure for nitrogen determination in the biological sciences for decades. Over the years, the technique has been improved and modified so much that there are now countless variations on the theme.

In general, the Kjeldahl method involves acid digestion of the sample and then measurement of nitrogen. Digestion usually takes place in boiling sulfuric acid, often with the addition of an oxidizing agent or catalyst and a salt for elevation of boiling temperature. This

converts the nitrogen into ammonium ion. Although the exact mechanism of this conversion in protein is unknown, it certainly involves hydrolysis of peptide bonds and complete protonation of the nitrogen atoms. A typical reaction that might take place is shown below for the conversion of the amino acid alanine in sulfuric.

Reaction 1



The boiling time required for complete digestion varies widely from one substance to another, lasting up to twelve hours or more for stubborn materials. Characteristically, the digestion solution clears, at which time almost all of the nitrogen has been converted. Many experimenters prefer to continue boiling past this point to ensure conversion of any intermediate compounds that may have formed.

Digestion of the sample is followed by analysis. In some methods, the ammonia is first recovered by addition of excess caustic and distillation or aeration. The nitrogen is then assayed by titration, colorimetrically, or by some other means. Some methods do not include the ammonia recovery step, but involve measurement on the neutralized digest. An interesting method for assaying ammonia uses a gas chromatographer (8). An excess of hydroxide is added to the ammonium sulfate produced during digestion, releasing gaseous ammonia into the chromatographer.

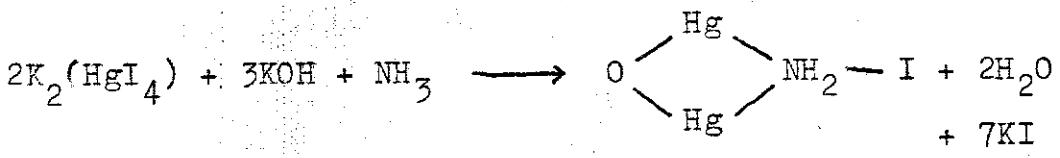
One of the more common methods for determining microgram quantities of material uses direct colorimetric determination with a Nessler's reagent (2, 6). This is the sort of Kjeldahl method used in this study. The Nessler's reagent is an alkaline potassium mercuric iodide solution which

produces a yellow color when combined with small amounts of ammonia. The color complex is produced by the following reactions (7):

Reaction 2 $HgCl_2 + 2KI \longrightarrow 2KCl + HgI_2$

Reaction 3 $HgI_2 + 2KI \longrightarrow K_2(HgI_4)$

Reaction 4



$$2K_2(HgI_4) + 3KOH + NH_3 \longrightarrow O(Hg)(Hg)NH_2 - I + 2H_2O + 7KI$$

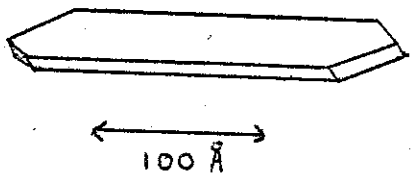
There are some problems to be wary of when using the Kjeldahl method. During digestion, it is important that the temperature is high enough to effect complete digestion, but too high a temperature can result in loss of nitrogen. Loss may also occur if the digestion period is too long. This is due to the fact that if there is much acid lost by boiling, there may not be enough hydrogen ions to keep the ammonia in its liquid soluble ammonium form (7). There can also be problems with the Nessler's reagent. Many experimenters have reported turbidity or cloudiness during color development, caused by improper pH or multivalent cations. It has also been found by Moore (10) that turbidity may be caused by various organic solvents such as chloroform, ethanol, acetone, and many other alcohols and ketones.

Fluorine Dating

In 1844, J. Middleton (11) pointed out that the mineral portion of bone, hydroxyapatite takes up fluorine, and suggested that the amount might indicate geological age. By 1803, Carnot (12) showed conclusively that bone exposed to fluoride ion for long periods of time in fact accumulates it irreversibly. These men laid the foundation for what is today known as fluorine dating.

The structural basis of bone is made up of crystals of hydroxyapatite (Figure 3). These crystals grow along the collagen fibrils. They are extremely small; individual crystals are too small to be seen by a light microscope. They are rarely larger than 200 angstroms long and 70 angstroms wide (13).

Figure 3 (14)



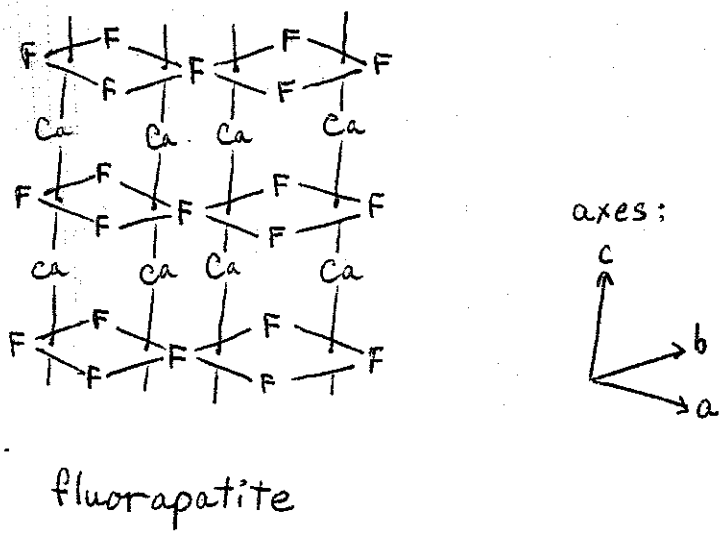
hydroxyapatite crystal of bone

The chemical formula for a unit cell of hydroxyapatite is $Ca_{10}(PO_4)_6(OH)_2$. However, in bone this mineral is very impure. Perfect apatite has a Ca/P ratio of 1.67, but this ratio in bone mineral is 1.5 (15). The composition of mineral in bone is actually a varying mixture of calcium phosphates, including other ions, such as magnesium and carbonate.

Most apatites are too small or poorly developed to examine structurally on an atomic level. Only fluorapatite occurs naturally in crystals suitable for accurate x-ray diffraction analysis. Thus the basic structure for

all apatites has been deduced from studies of this mineral. It is a rather complex structure. As shown in Figure 4, the atoms seem to be arranged in diamond shaped planes with fluorine atoms at the corners. Five calcium and five phosphorus atoms lie in one of these planes. Other calcium atoms seem to link the planes together.

Figure 4 (16)

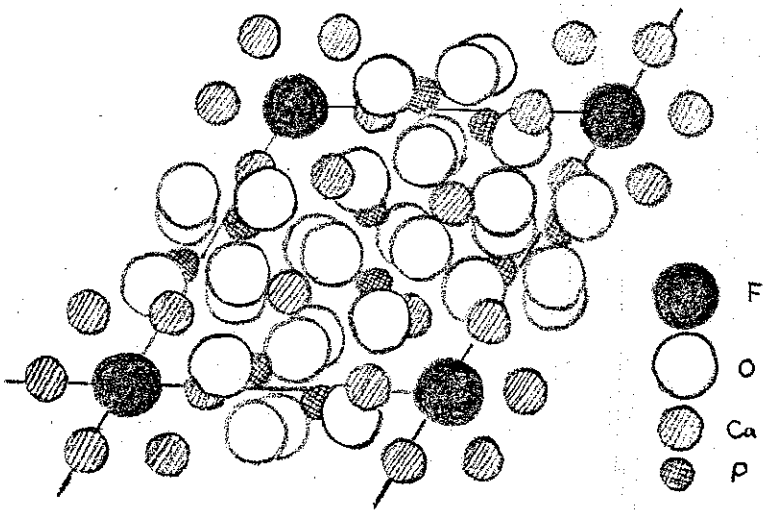


fluorapatite

In Figure 5, a view of one unit cell is shown, looking along the c-axis. This includes two of the planes shown in Figure 4. Each fluorine atom has a triangle of calcium atoms around it; these triangles "spiral" about the c-axis from one plane to the next.

Hydroxyapatite is assumed to have the same structure as fluorapatite, with hydroxide ions replacing fluoride. The hydroxide ions are all oriented in the same direction along the c-axis (17). In fresh human hydroxyapatite, there are trace impurities of fluoride ions, taking position as in pure fluorapatite. In adult farm animals, and probably in most human populations, the concentration of fluorine is 300-600 ppm in bone, 100-270 ppm in enamel and 240-625 in dentine (18). These concentrations are

Figure 5 (19)



much higher than anywhere else in the body.

Since Middleton it has been known that fluorine accumulates in bone to strengthen it. This fact has been taken advantage of where teeth are concerned by the fluoridation of water. An increased amount of fluoride in the teeth helps to prevent tooth decay. The recent addition of fluoride to drinking water in the United States has certainly raised the trace amount of the element in modern bones well above the level mentioned for farm animals, perhaps into the range of 1000 ppm. When fluorine amounts reach abnormally high levels, a condition known as fluorosis arises. This is first recognized by a visible mottling of bones and teeth at about 2500-3000 ppm. At about 5000-6000 ppm, the condition becomes critical (18).

Thermodynamic studies have shown repeatedly that fluorapatite is a more stable compound than hydroxyapatite. Comparisons between entropies, enthalpies and heat content for the two compounds support this conclusion. For example, Hagen (20) has made a study of the exchange reaction

Reaction 5 $Ca_5(PO_4)_3OH_{solid} + F^- \rightleftharpoons Ca_5(PO_4)_3F_{solid} + OH^-$

He calculated an equilibrium constant of $K_{eq} = 10^{1.26}$, decidedly in favor of the righthand side of the equation. This implies that fluorapatite will form from hydroxyapatite, where given the opportunity and sufficient time. Furthermore, the reaction is promoted when the pH is low.

The accumulation of fluorine in bone is by no means a simple diffusion in, as is the decrease in nitrogen diffusion out. Although diffusion does play an important part in the process, it does not present an explanation. First, diffusion of fluoride and hydroxide ions does not take place in the three directions. Rather, these ions move in one direction, along the c-axis of the crystal (21). Second, there is probably no exchange of ions, but an interchange of ions with vacancies (21). Thus there is no random mixing; there is ordered movement. And finally once the fluorine is in, it is stably bound.

Although the accumulation of fluorine in bone is much more ordered than the loss of nitrogen, it is still very dependent on environmental variables. All bones start out with a tiny amount of flourine, and this amount is more age dependent than that of nitrogen in fresh bone. Fluorine content increases with age, reaching a plateau at about age 55 (18). Fluorine in fresh bone is probably evenly distributed, deposited through internal routes. But fluorine taken up by buried bone must enter through the surface. One would thus expect a diffusion gradient of fluorine on the surface of buried bone. The rate of fluorine accumulation is of course very dependent on the amount present in the soil. This factor varies widely throughout the world, from almost none to 7070 ppm, with an average for surface soils of 202 ppm (22). Other factors such as temperature, water and pH are also important. It is interesting that a lower pH accelerates fluorine uptake as well as nitrogen decay.

Analytical Methods

Chemical methods for analysing fluorine accurately have not been easy to develop. They are often tricky and tedious to work with. Fluorine is hard to recover quantitatively and other halogens, such as chlorine, can interfer. Especially where fluorine is in trace amounts do these problems become evident.

One of the first reliable wet chemical methods to become standard procedure was proposed by Willard and Winter in 1933 (23). Their method involves separation of fluorine by distillation in the form of hydrofluosilicic acid and titration with thorium nitrate. Later, Armstrong (24) simplified the method by using an aqueous sodium alizarin sulfonate solution as indicator instead of the zirconium-alizarin mixture used by Willard and Winter. Hoskins and Fryd further improved the method in order to measure the fluorine in the notorious Piltdown skull (25). They evaluated fluorine content for each sample by adding known increments of fluoride ion and titrating these progressively enhanced aliquots. Extrapolation backwards provided the fluorine contribution from the bone. This modification enabled them to measure smaller amounts and also to eliminate interference from other substances.

Another more recent gas chromatographic method which has been applied to bone is described by Groff (8). Digestion takes place in sulfuric acid, releasing fluorine as hydrofluoric acid. This is injected into the chromatographer and converted into silicon tetrafluoride as it passes over silica sand.

During the past decade, some newer methods have come into use involving nuclear reactions with fluorine. Although these techniques require highly sophisticated equipment and expertice, the advantages for trace measurements are as yet unsurpassed. Fluorine can be detected in any chemical form. Materials to be analysed require only minimal preparation, or no preparation at all. And the method

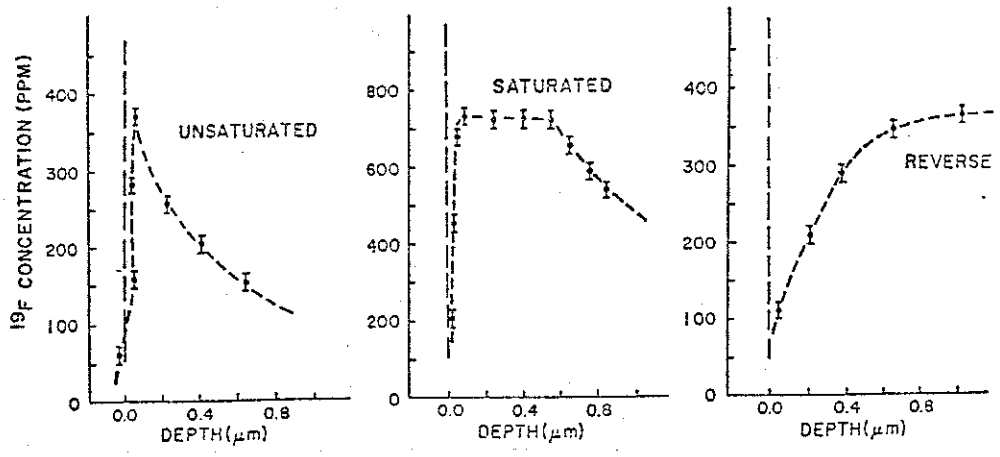
is much more precise.

Taylor has used such a method to study the diffusion of fluorine in chipped lithic material (26). The reaction used for his study is

Reaction 6 $$^{19}F(p,\alpha\gamma)^{16}O$$

in which the sample is bombarded by protons, exciting the fluorine into emitting first an alpha, and then a gamma ray at a characteristic frequency, and leaving an oxygen nucleus behind. The amount of fluorine is proportional to the rate at which gammas are produced. By varying the energy of the proton beam, Taylor was able to take measurements at various points just below the surface of the stone, obtaining a depth profile of fluorine diffusion. He found three types of diffusion shown in Figure 6, unsaturated, saturated, and reverse. This study suggested that the amount of fluorine diffusion on a lithic surface may be used to judge the passage of time since that surface was exposed.

Figure 6 (26)



Another reaction that has been employed for fluorine

studies is

Reaction 7 $^{19}F(p, p'\gamma)^{19}F$

in which the bombarding proton is deflected by the fluorine nucleus with a lower energy than it had coming in. The fluorine nucleus so excited then emits a gamma ray, and falls back to its ground energy state. There are two characteristic gamma frequencies from this reaction, one at 110 keV and the other at 197 keV, their rates of emission being directly proportional to the amount of fluorine present. This method of proton inelastic scattering has been used recently to study fluorine in vegetation samples (27) and food samples (28, 29). It has been shown by these investigators that this analysis is superior to chemical analyses in both recovery and precision.

The technique described above is exactly that used for the present study. Ground bone samples were activated using a Van de Graff accelerator at Brookhaven National Laboratory. Dr. Albert Hanson, who carried out the work, has been kind enough to write the following primer to aid in a better understanding of this method.

A Primer on the Analysis of Fluorine by Inelastic Scattering of Energetic Protons

by Albert Hanson

The purpose of this primer is to provide a basis for the understanding by non nuclear scientists of the relatively new technique of proton inelastic scattering for the analysis of fluorine in materials. This technique has been developed and applied at Brookhaven National Lab mostly to the analysis of fluorine in food samples (1). Recently this technique has been used to analyze fluorine in Indian bone samples from the Moundville, Alabama archaeological site. The amount of fluorine in the bone samples should provide a relative dating of the bones since fluorine in the soil will diffuse into the sample, replacing the hydroxide ion in the calcium phosphate hydroxyapatite.

When a beam of energetic particles interacts with a solid, several different interactions can take place. These different interactions compete with each other; the probability for each interaction being dependent on the type of particles involved (in this case ions) and the energy. At the energies of interest the types of interactions between ions and solids can usually be divided into two major groups; the first being between the ions and the electrons of the target atoms and the second between the projectiles and the nuclei of the target atoms. The interactions between the ions and the electrons of the target atoms usually provide the largest contribution to the slowing of the beam within the target and can result in the production of characteristic x-rays. There are three basic interactions between the ions and the nuclei of the atoms: 1) elastic scattering, 2) inelastic scattering, and 3) nuclear reactions. Elastic scattering obeys the classical laws of energy, momentum and mass conservation. Inelastic scattering is similar to the elastic scattering in that there is no change in the number of nucleons of

neither the ion nor the target, however the target nucleus is left in an excited state. This excited nucleus will decay emitting a characteristic gamma ray. Nuclear reactions result in changing the number of nucleons of the particles involved.

The interaction used in this analytical technique is the inelastic scattering of protons off fluorine, which is written:

$$^{19}F(p, p')^{19}F^* \qquad (1)$$

With the 3.4 MeV protons used in the analysis the first two states of the fluorine are excited. These states decay resulting in the emission of the 110 and 197 keV characteristic gamma rays (2). The number of gamma rays produced in a target which is thick enough to stop the beam is given by the expression:

$$\gamma_T = \int_0^{E_o} N_B \, N_F(E) \, \sigma(E) dE \qquad (2)$$

where:

E_o = incident beam energy

N_B = the number of protons that struck the target

$N_F(E) = N_F(x)/(dE/dx)$, and $N_F(x)$ is the number of fluorine atoms in the sample as a function of depth. Therefore if the sample is homogeneous, $N_F(E) = N_F/(dE/dx)$. The term dE/dx is the stopping power in the target.

$\sigma(E)$ = the cross section for the production of the gamma rays.

For a homogeneous sample equation (2) becomes:

$$\gamma_T = N_B \, N_F \int_0^{E_o} \sigma(E)/(dE/dx) \, dE \qquad (3)$$

The number of gamma rays produced as a function of incident beam energy has been calculated and is shown in Figure 1 (3). The line marked 6.13 MeV is for the production of the 6.13 MeV gamma rays from the competing reaction:

$$^{19}F(p,\alpha)^{16}O^{*} \qquad (4)$$

The residual $^{16}O^{*}$ nucleus decays to the ground state by emitting 6.1 (along with the smaller intensity 6.0 and 7.1) MeV gamma rays.

Equation (3) describes the production of the total number of gamma rays. The total number counted by a detector will then be:

$$\gamma = \gamma_T \, k_g \, k_e \qquad (5)$$

The term k_g is the geometrical calibration constant which includes the solid angle subtended by the detector. This means that if all gamma rays are emitted isotropically (that is equal in all directions) only a fraction will be emitted in the direction of the detector. Once the gamma ray reaches the detector it has a certain probability of being counted which is k_e.

The gamma rays were counted with a teflon free detector placed at 90° roughly 2 cm from the target (Figure 2). The special teflon free detector is to reduce contributions from inelastic scattering of neutrons off the fluorine which results in the same 110 and 197 keV gamma rays. A mylar lining was placed in the glass "Tee", which held the targets, since mylar is low in fluorine content. The signals from the preamp were shaped and amplified with an Ortec 472 spectroscopy amplifier and analyzed with a Nuclear Data 6660 analyzer and minicomputer (Figure 3).

A typical gamma ray spectra is shown in Figure 4, the 110 and 197 keV gamma ray lines identified. The height of the peaks, minus the background radiation is proportional to the number of fluorine atoms in the samples, as described by equation 3.

References

1. R. E. Shroy, H. W. Kraner, K. W. Jones, J. S. Jacobson, and L. I. Heller, Nuclear Instruments and Methods 149 (1978) 313-316
2. F. Ajzenberg-Selove, Nuclear Physics A190 (1972) 1-196
3. R. E. Shroy, H. W. Kraner, K. W. Jones, J. S. Jacobson, and L. I. Heller, (1979), BNL-27153

Other Readings:

1. K. W. Jones, Ph. Gorodetzky, and J. S. Jacobson, Intern. J. Environ. Anal. Chem. 4 (1976) 225-231

Figure Captions

Figure 1. Calculated production of 110 and 197 keV gamma rays from the $^{19}F(p, p')^{19}F^*$ reaction and, for comparison, of the 6.13 MeV gamma rays from the $^{19}F(p,\alpha)^{16}O^*$ reaction are given as a function of proton energy. The following parameters were assumed: a thick organic target containing 1 ppm ^{19}F, and integrated beam current of 1 μC, and a solid angle of 4π sr. (Reproduced vertatum from ref. 3 without permission of the authors.)

Figure 2. Experimental setup

Figure 3. System electronics

Figure 4. Typical gamma ray spectrum

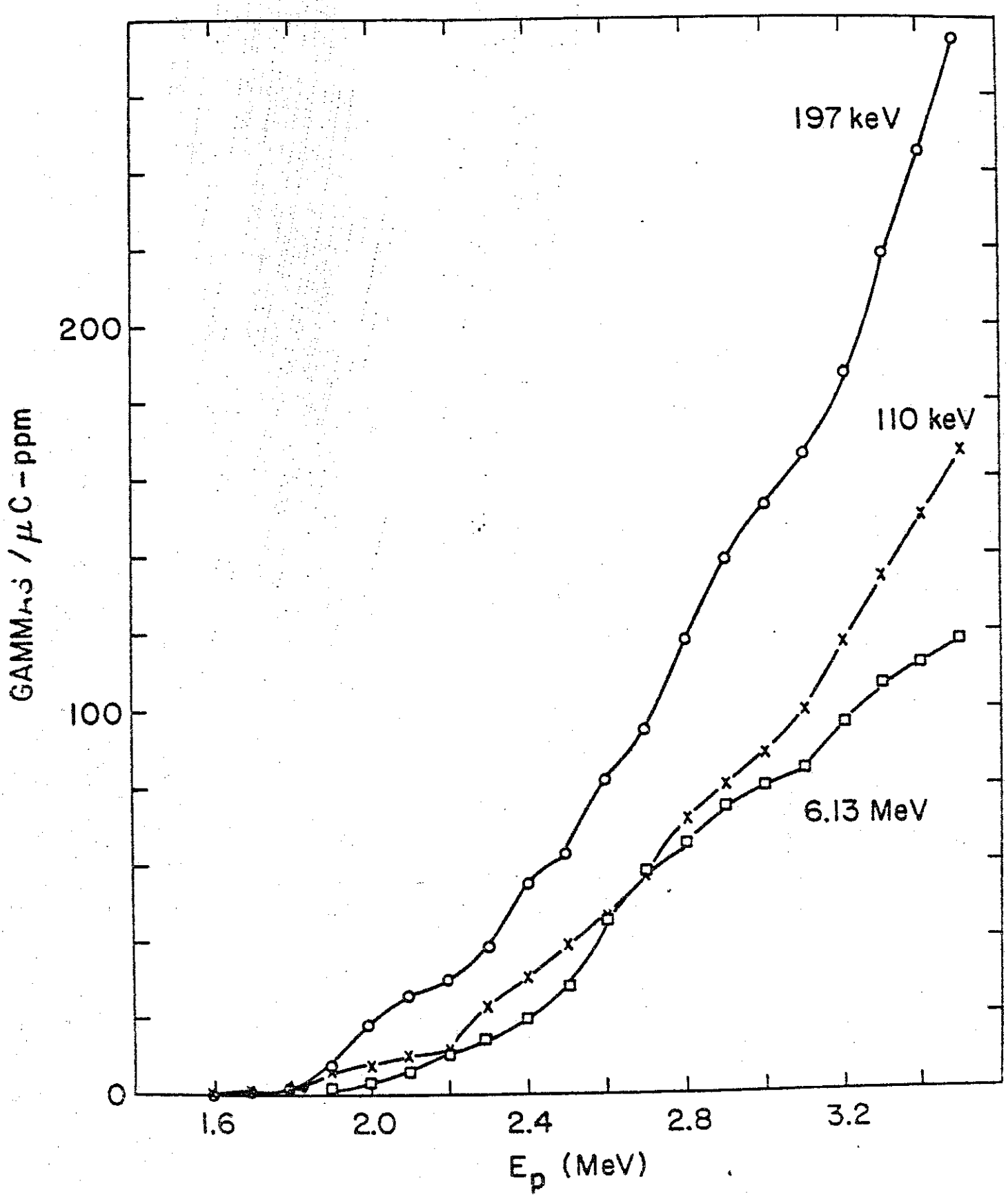

197 keV
110 keV
6.13 MeV
200
100
0
GAMMAS / μC - ppm
1.6
2.0
2.4
2.8
3.2
E_p (MeV)

Figure 1

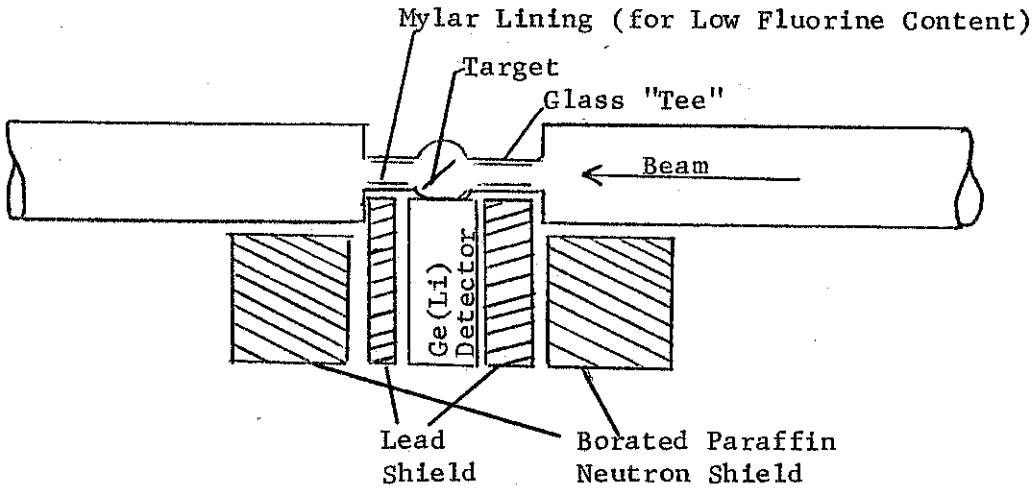

Mylar Lining (for Low Fluorine Content)
Target
Glass "Tee"
Beam
Ge(Li) Detector
Lead Shield
Borated Paraffin Neutron Shield

Figure 2.

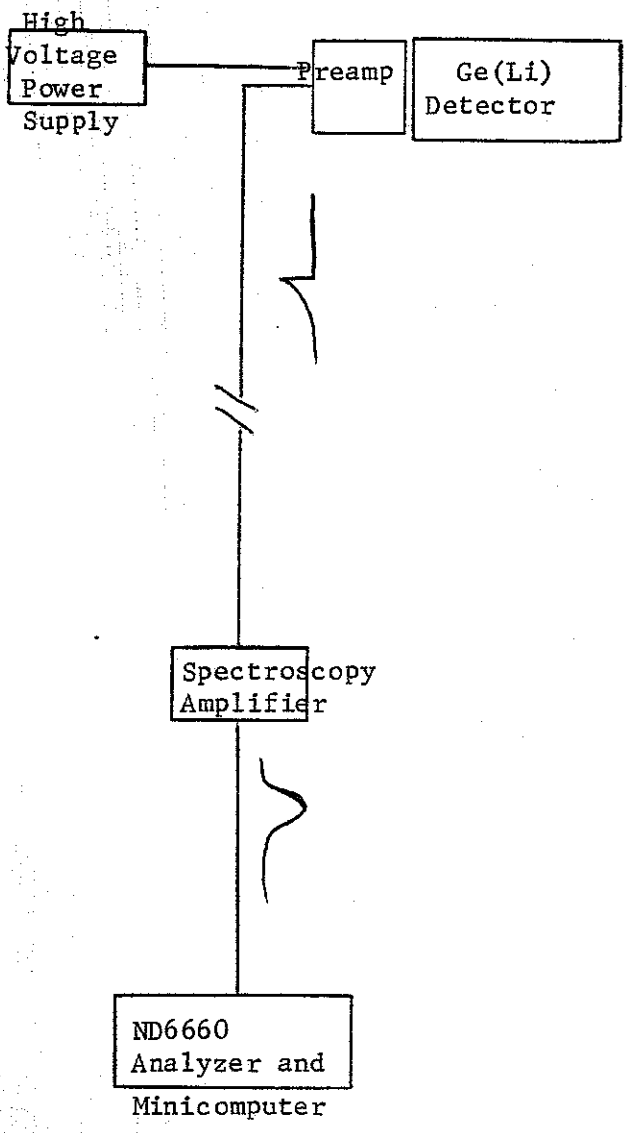

High
Voltage
Power
Supply
Preamp
Ge(Li)
Detector
Spectroscopy
Amplifier
ND6660
Analyzer and
Minicomputer

Figure 3.

ID NO. START TIME + DATE STOP TIME + DATE DESCRIPTION
20 10:19 APR 01,'80 10:19 APR 01,'80 BONE SAMPLES FOR FLUORINE. #1788

SYSID (AT TIME OF PLOT) = 14
EVENT LINE NO. 1

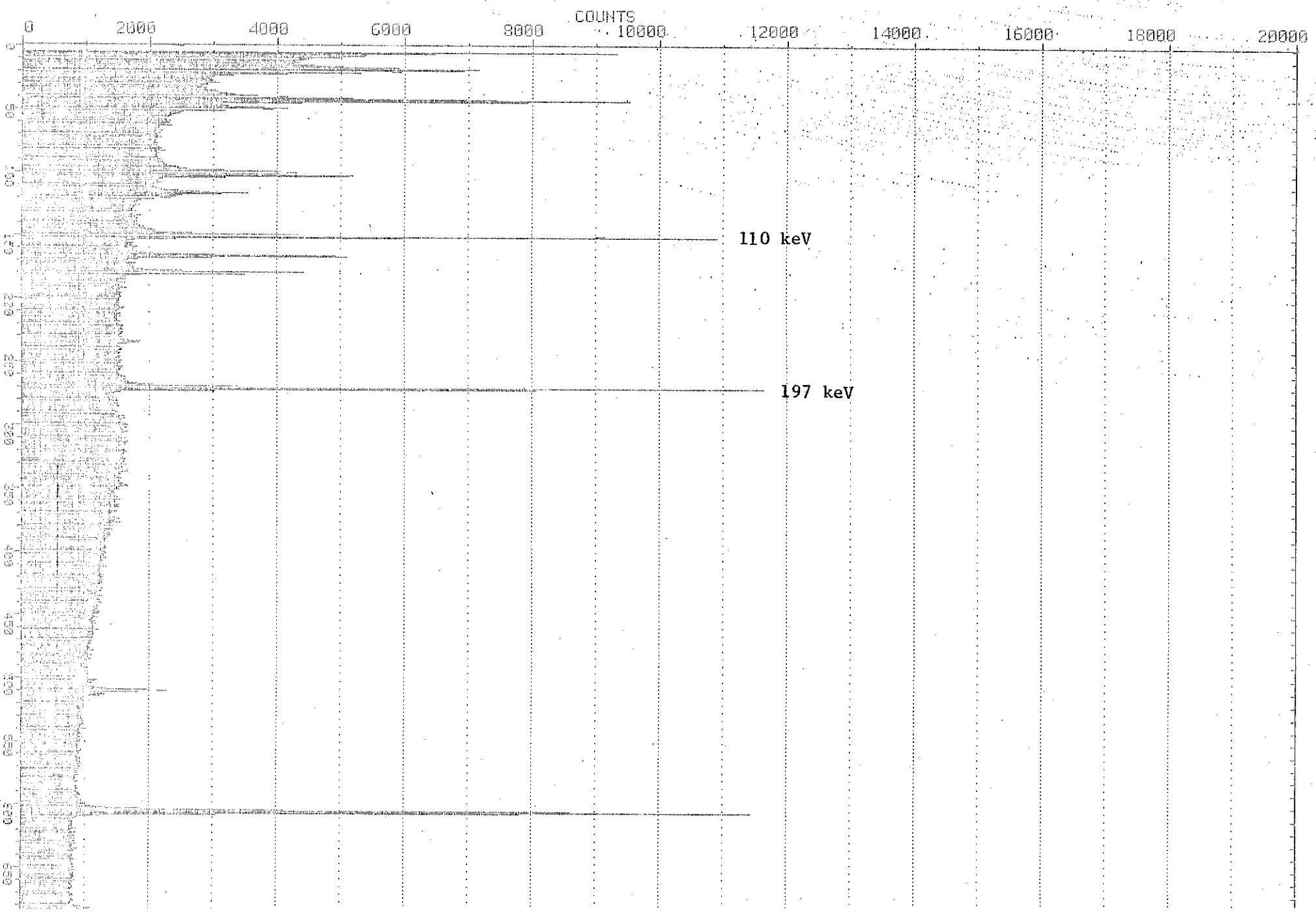


Figure 4.

Some Past Studies

By the late 1940's, there had accumulated many fossils that presented problems in finding the correct time periods to place them in. Stratigraphically, such specimens would claim a place in one era, but skeletal features would indicate another. Around 1947, W. F. Libby discovered the chemical changes in organic matter that form the basis for radiocarbon dating. But at that time, this potentially powerful technique was not well developed and even today is limited to a rather recent time period. Still, the need for some Explanations had reached a critical level and so investigators began to employ relative dating methods more seriously.

One of the foremost proponents of relative dating at this time was Kenneth P. Oakley. And one of the biggest puzzles was Piltdown Man. By this time, of course, the mandible and cranium pair were held with suspicion since such a combination of anthropoid and hominid characteristics was otherwise unheard of. One of the more popular explanations was that it was simply a chance association between two creatures of very different geological age. But the "skull" had been shelved by many anthropologists until someone could find the right answer. In 1950, Oakley and Hoskins opened the first door to the truth when they published their fluorine results for "Eoanthropus" and the associated fauna (30). Their study showed that both the mandible and cranium were contemporaneous, and belonged to a much more recent period than the associated Villefranchian fauna. Then what was a recent ape jaw doing in Sussex? In the next few years the evidence came pouring in (31, 32): the nitrogen content, the filing of the teeth, the staining. The answer was now horrifyingly obvious. Someone had intensionally prepared and placed the bones in the gravel at Piltdown, creating the greatest hoax the scientific community has ever seen. The result of the episode is that Piltdown Man became infamous, while fluorine dating

became famous.

Another problem solved by Oakley, this time in collaboration with M. F. Ashley Montagu, was that of the Galley Hill Skeleton. This specimen was found eight feet below the soil in Swanscombe, Kent, and for this reason was placed by some in the Paleolithic period. But the morphology of the skeleton was quite recent, with no substantial differences between it and modern day skeletons. The fluorine analysis published by Oakley and Montagu in 1940 (33), showed that the bones were in fact fairly recent, confirming that it was actually an intrusive burial. Later, Oakley did a comparative study of Galley Hill and the famous Swanscombe remains. Chemical analyses showed 0.5% fluorine and 1.6% nitrogen for Galley Hill, and 1.7% fluorine and only traces of nitrogen for Swanscombe (34). This study proved the reliability of the nitrogen-fluorine cross check for remains from the same geographical region.

Other more recent studies have been done using relative dating, although carbon-14 is usually the preferred method. Doberenz did a study of nitrogen content on a large variety of faunal bones ranging from early Pleistocene to modern times (35). Among this selection were several Pleistocene Rancho La Brea samples that contained almost as much nitrogen as modern bones. This study demonstrated the great influence environment has on the state of preservation of bones. Relative dating also confirmed the antiquity of Arlington Springs Man from California (36). Carbonaceous material found near the bones of this man had been dated as approximately 10,000 years old by radiocarbon analysis. Relative dating by nitrogen and fluorine analysis showed that the bones were also of great antiquity, and it could be concluded that they were of the same age. Also, nitrogen analysis can be used to assess the organic carbon content of materials as a preliminary to radiocarbon analysis (37). Thus, although carbon-14 analysis is by far the more powerful technique, relative dating is still a useful method alone or in conjunction with absolute dating.

The Moundville Bones

The bones used for this study are from the Moundville site in Alabama on the Black Warrior River. They are all human bones from the mounds themselves, unearthed by C. B. Moore over a twelve year span covering the 1930's. In 1941, all of the bones were dipped in Alvar to keep them preserved (38).

I obtained the bones in August of 1970 from Vincas P. Steponaitis at the Smithsonian Institute in Washington, D. C., who in turn had gotten them earlier that year from Kenneth R. Turner at the University of Alabama. Mr. Steponaitis had originally intended on using the samples for radiocarbon analysis, but the Alvar prevented the possibility of obtaining reliable results by this method. They therefore came into my possession for relative dating analysis.

Mr. Steponaitis' intention in having the bones dated was to confirm his own seriation of the Moundville gravelots based on pottery types. A reproduction of this seriation is presented in Figure 7 (1). The figure shows the pottery traits each gravelot exhibits as coded in Table I (1), and its relative position in time based on these traits (best fit position). The letters following the gravelot numbers describe the mound each sample was found in. For example, 2417-WP means that gravelot 2417 was found west of Mound P.

Twelve of the fifteen samples used for this study were included in this seriation and are marked on Figure 7 with a dot. The other three are unseriated. Only one sample, 830, has been assigned to the Moundville I period based on pottery traits. Moundville I is believed to have flourished between the years 1100 AD to 1250 AD (30, 40). Four of the samples, 1587, 1406, 1530 and 1788 have been assigned to the early Moundville III period by this seriation and the other seven, 1181, 1800, 1563, 1065, 1515,

Figure 7 (1)

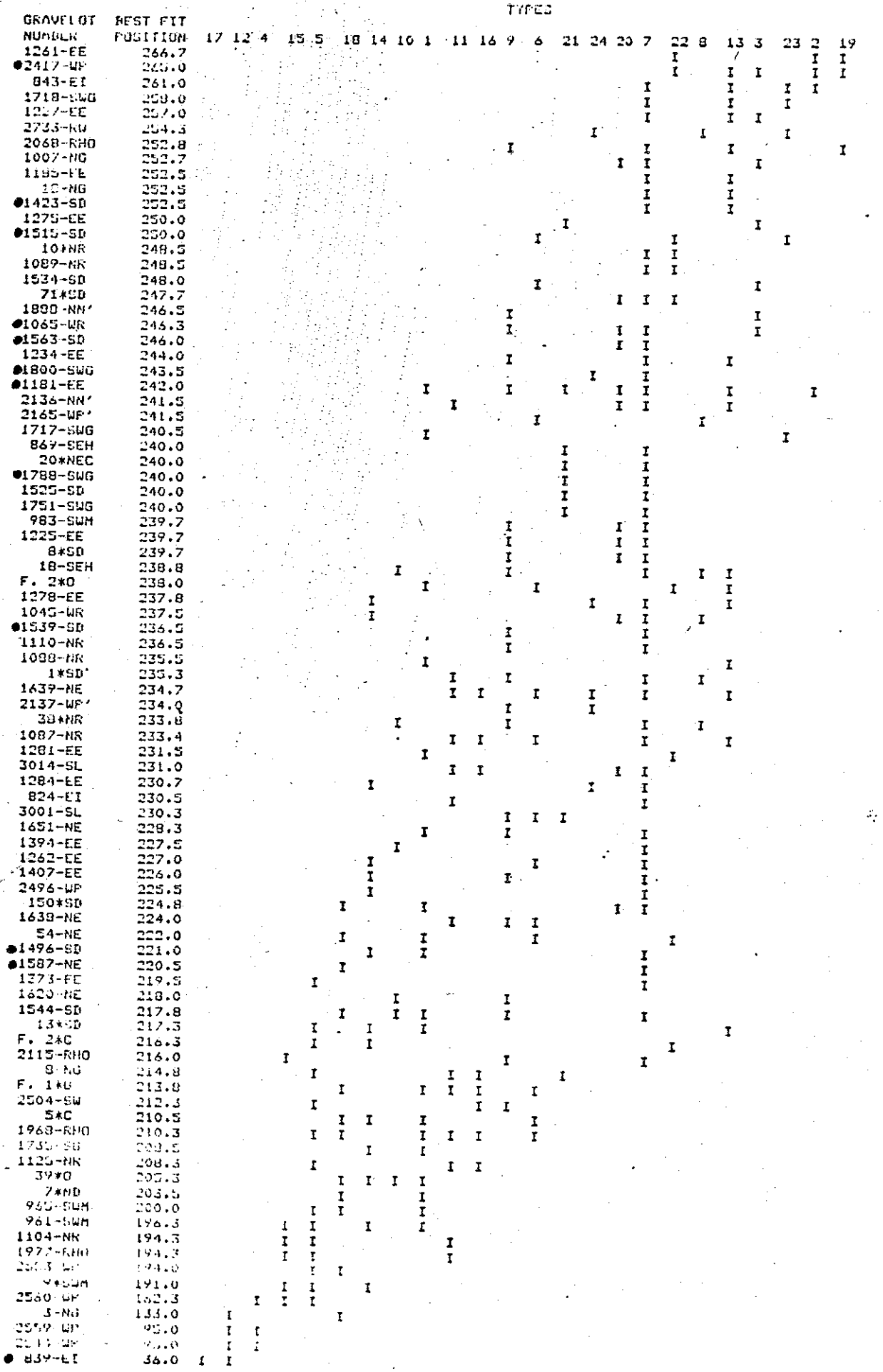

TYPES
GRAVELOT NUMBER
BEST FIT POSITION
17 12 4 15 5 18 14 10 1 11 16 9 6 21 24 20 7 22 8 13 3 23 2 19
1261-EE 266.7
●2417-WP 265.0
843-EI 261.0
1718-SWG 259.0
1227-EE 257.0
2733-NW 254.5
2068-RHO 252.8
1007-NG 252.7
1185-EE 252.5
10-NG 252.5
●1423-SD 252.5
1275-EE 250.0
●1515-SD 250.0
10*NR 248.5
1089-NR 248.5
1534-SD 248.0
71*SD 247.7
1800-NN' 246.5
●1065-WR 246.3
●1563-SD 246.0
1234-EE 244.0
●1800-SWG 243.5
●1181-EE 242.0
2136-NN' 241.5
2165-WP' 241.5
1717-SWG 240.5
869-SEH 240.0
20*NEC 240.0
●1788-SWG 240.0
1525-SD 240.0
1751-SWG 240.0
983-SWM 239.7
1225-EE 239.7
8*SD 239.7
18-SEH 238.8
F. 2*O 238.0
1278-EE 237.8
1045-WR 237.5
●1539-SD 236.5
1110-NR 236.5
1088-NR 235.5
1*SD' 235.3
1639-NE 234.7
2137-WP' 234.0
38*NR 233.8
1087-NR 233.4
1281-EE 231.5
3014-SL 231.0
1284-EE 230.7
824-EI 230.5
3001-SL 230.3
1651-NE 228.3
1394-EE 227.5
1262-EE 227.0
1407-EE 226.0
2496-WP 225.5
150*SD 224.8
1638-NE 224.0
54-NE 222.0
●1496-SD 221.0
●1587-NE 220.5
1373-EE 219.5
1620-NE 218.0
1544-SD 217.8
13*SD 217.3
F. 2*C 216.3
2115-RHO 216.0
9-NG 214.8
F. 1*O 213.8
2504-SW 212.3
5*C 210.5
1968-RHO 210.3
1750-SD 208.5
1125-NR 208.3
39*O 205.3
7*ND 203.5
960-SWM 200.0
961-SWM 196.3
1104-NR 194.3
1977-RHO 194.3
2563-WP 194.0
4*SWM 191.0
2560-WP 182.3
3-NG 133.0
2599-WP 95.0
2613-WP 92.0
● 839-EI 36.0

Table I: Key to Attributes of Shape and Design Used in Seriation (1)

1. cylindrical/semicylindrical bowl
2. short neck bowl
3. flaring rim bowl (deep)
4. slender ovoid bottle
5. subglobular bottle, pedestal base
6. subglobular bottle, slab base
7. subglobular bottle, simple base
8. burnished jar with 2 handles
9. beaded rim
10. widely spaced nodes (on bowl)
11. indentations
12. lug and inward facing effigy (on bowl)
13. 2-5 line scroll (engraved or incised)
14. 2-5 line scroll, crosshatched background (engraved)
15. 4-10 line vertical scroll (engraved)
16. 15-40 line scroll (engraved)
17. arch (incised)
18. multilinear band at rim (engraved)
19. red on white (painted)
20. winged serpent (engraved)
21. paired tails (engraved)
22. hand and eye (engraved)
23. jar with 8 or more handles
24. fish effigy (on bowl)

1423 and 2417, have been assigned to the late Moundville III period. Moundville III is believed to have covered the years 1400 AD to 1550 AD (39, 40). The three unseriated samples, 1647, 1648, and 1840, are also believed to belong to the Moundville III period. It should be kept in mind that these assignments are not restrictive, rather they represent the best placement within a range.

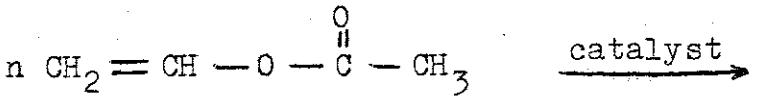
The approximate age of these bones makes them excellent candidates for nitrogen and fluorine analysis. They are recent, and would be expected to have fairly high and easily measurable amounts of nitrogen. Yet they are old enough to have accumulated fluorine beyond the trace levels found at death. Unfortunately, the period they cover is short, only a few hundred years. Thus, the question is whether or not the environmental conditions are sufficiently extreme to have created detectable differences during the time lapses between burials.

Alvar

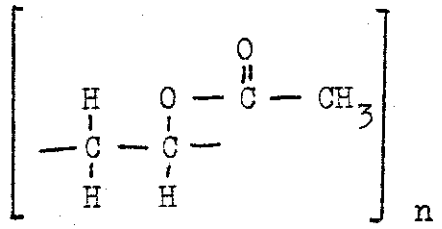
Alvar is the commercial name for a polymer which has been commonly used for preserving bones. It is usually sold as flakes or chunks which can be dissolved in acetone. It is then spread on the bone or the bone is dipped in it and allowed to dry.

It is made from vinyl acetate by catalysis:

Reaction 8



vinyl acetate



polyvinyl acetate (Alvar)

Being a compound with a carbon base, Alvar introduces too much carbon into a bone sample to allow carbon-14 analysis to be useful for dating. I was lucky enough to obtain a sample of Alvar from Dr. C. Loring Brace at the University of Michigan. Analysis showed no detectable nitrogen by the micro-Kjeldahl method used for this study. Fluorine analysis by Dr. Hanson showed nothing above the normal background presence of fluorine. Therefore, the Alvar in the bone samples could not interfer in these relative dating methods beyond throwing off the weight percentages by a small amount.

Materials and Methods

Sample Preparation

All bone samples used for this study appeared to have been cleaned before they were treated with the preservative Alvar. But there were spots, especially on the insides of long bones, where grains of dirt were glued on with Alvar. Such visible dirt was removed using a thin steel spatula. Any chemical treatment of these bones to remove the Alvar was concidered strictly out of the question for two major reasons. First, there was the risk of removing fluorine and especially nitrogen during such a treatment. Second, almost any organic solvent suitable for dissolving Alvar would interfer in the Nesslerization step of the nitrogen analysis by causing turbidity. (An unfortunate and unexpected confirmation of this fear was discovered during the nitrogen analysis of one sample, ulna 1183. It had apparently been chemically cleansed prior to my obtaining the sample. The solvent used destroyed any spectrophotometric readings.)

All bones were ground to a fine powder using a porcelain mortar and pestal. A few samples, on which the Alvar was especially thick, were difficult to grind to a consistent powder; some of the preservative remained in flakes. Such samples were not "filtered" to remove the flakes, since fluorine near bone surfaces could be lost in this way. Error is higher for these samples.

For fluorine analysis, the samples were further prepared by pressing approximately 2 gm of each bone into an aluminum planchet at 1500 lb/in^2 pressure. This formed a disk 2.6 cm in diameter and about 2 mm thick.

Nitrogen Analysis

The procedure for micro-Kjeldahl analysis of bone was borrowed from Dr. David von Endt at the Smithsonian Institute's Department of Anthropology.

Reagents: The digestion mixture was prepared by dissolving 15.0 gm of anhydrous sodium sulfate (Na_2SO_4) in 100 ml of double distilled water. 70 ml of concentrated sulfuric acid (H_2SO_4) was added very slowly to this salt solution while swirling. 2.5 ml of 20% perchloric acid ($HClO_4$) was added as an oxidizing agent. Finally 1.0 gm of cupric sulfate ($CuSO_4 \cdot 5H_2O$) was added as a catalyst.

The Nessler's reagent was prepared by dissolving 45.5 gm of powdered mercuric iodide (HgI_2) and 34.0 gm of crystal potassium iodide (KI) in a small amount of double distilled water in a one liter volumetric flask. In a separate vessel, 112 gm of potassium hydroxide (KOH) was dissolved in about 140 ml of double distilled water. This was added to the mercuric potassium iodide solution and brought to one liter. After preparation, it was not used for several days in order to allow the characteristic sediment to settle to the bottom of the storage bottle. This reagent was filtered before use. It was stored in an amber glass bottle in the dark. This reagent remains stable for a year or more (7).

Standard Curve: A standard curve was prepared for the Nessler's reagent as follows. The stock solution for standardization was prepared by dissolving 0.7643 gm of granular ammonium chloride (NH_4Cl) in 100 ml of double distilled water. A second dilution was made by bringing 4.0 ml of the first solution up to 100 ml in a volumetric flask. Aliquots of this second ammonium chloride solution were placed in 50 ml volumetric flasks. Three separate flasks were prepared for each aliquot size. These were filled about halfway with double distilled water, along with an extra flask to be used as a blank. 2.5 ml of filtered Nessler's reagent was added to each flask, starting the yellow colored reaction. The flasks were then brought to volume and inverted to mix. After 30 minutes, readings were taken against the blank at wavelength 410 nm on a McPherson double beam spectrophotometer, model EU-700.

The data obtained from this standardization is tabulated in Table II. The amount of nitrogen in each aliquot was calculated from the molecular weight of ammonium chloride (53.5 gm/mole), the atomic weight of nitrogen (14.0 gm/mole) and the dilution volumes as follows:

Equation 3

$$(7.643 \text{ mg/ml } NH_4Cl)\frac{(14.0 \text{ gm/mole})(4 \text{ ml})}{(53.5 \text{ gm/mole})(100 \text{ ml})}(1000 \ \mu g/mg)$$

$$= 80.0 \ \mu g/ml \text{ nitrogen for the second standard solution}$$

From the nitrogen amounts and corresponding absorbance readings, a linear regression analysis was done giving a y-intercept of -7×10^{-4} and a slope of 4.22×10^{-3} for the best fit line. Pearson's correlation coefficient, r, was calculated to be 0.9994 (see Figure 8)

Sample Digestion: Approximately 10 mg of ground bone was placed in a 10 ml round bottom flask with 1.0 ml of digestion mixture. This was boiled on an electric heating mantle while the sample turned from dark brown, to amber, to yellow, to green and finally to a clear blue green. After some initial experimenting, digestion time for a sample was worked down to 30 minutes or less. After the sample cooled, it was neutralized with a few drops of double distilled water. It was then transferred to a 10 ml volumetric flask and brought to volume.

Nitrogen Determination: Three 1.0 ml aliquots of the diluted sample were placed into 50 ml volumetric flasks. The flasks were half filled with double distilled water along with an extra flask as a blank and 2.5 ml Nessler's reagent was added to each. The flasks were then brought to volume. After 30 minutes, absorbance readings at 410 nm were taken on a McPherson double beam spectrophotometer, and compared with the standard curve

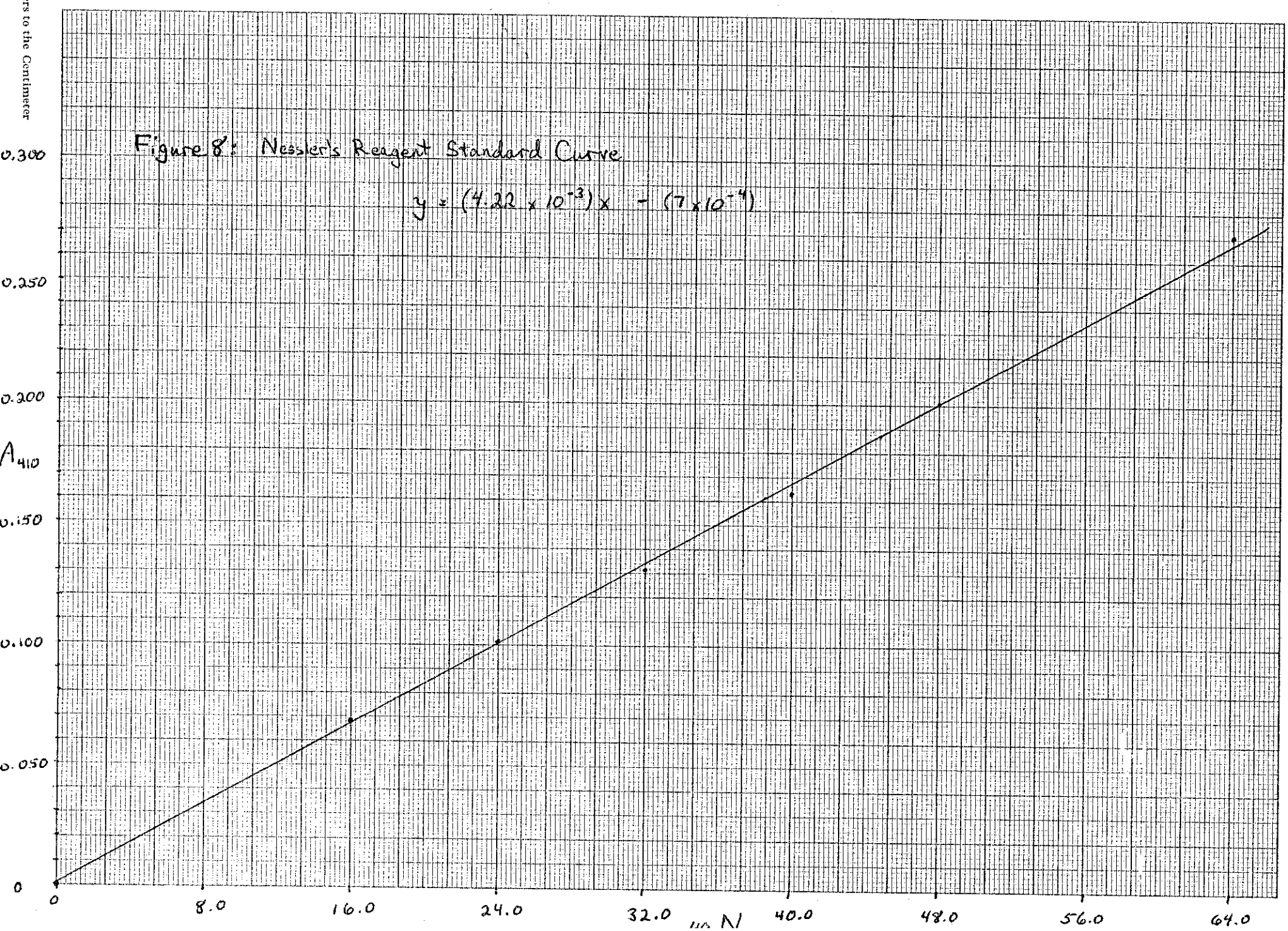
Equation 4 $$y = (4.22 \times 10^{-3})x - (7 \times 10^{-4})$$

Table II: Standard Curve for Nessler's Reagent

aliquot size	nitrogen	A_{410}	average A_{410}
0.20 ml	16.0 µg	0.066	0.0683
		0.071	
		0.068	
0.30	24.0	0.103	0.1017
		0.100	
		0.102	
0.40	32.0	0.135	0.1317
		0.131	
		0.129	
0.50	40.0	0.160	0.1630
		0.164	
		0.165	
0.60	48.0	0.205	0.2060
		0.208	
		0.205	
0.80	64.0	0.271	0.2707
		0.270	
		0.271	

10 Millimeters to the Centimeter

NATIONAL
12-188



to obtain the amount of nitrogen. Nitrogen by weight was then divided by the total sample weight to obtain the percentage of nitrogen.

Fluorine Analysis

Bone samples were analysed using a Van de Graff accelerator at Brookhaven National Laboratory by Dr. Albert L. Hanson. Pressed planchets of bone were irradiated with a proton beam of energy 3.4 MeV. The glass "Tee" sample holder was lined with mylar for protection from fluorine contamination. Gamma rays were counted at a deflection angle of 90° using a teflon free Ge(Li) detector. An Ortec 472 spectroscopy amplifier was used to shape and amplify signals from the preamp. Data was analysed using a Nuclear Data 6660 analyser and microcomputer.

When a sample is bombarded by a proton beam such as that used for this study, excitation will take place over a diameter of a few millimeters and to a depth on the order of micrometers, depending on the stopping power of the material. In order to determine whether the pressed samples were thick enough, several of the samples were analysed after a second layer of ground bone was pressed on top of the first. The same results were obtained. This confirmed that the thickness of our samples was sufficient.

Analyses were also done on an empty aluminum planchet and on a planchet filled with dissolved and resolidified Alvar, using the solvent acetone. Both gave fluorine counts on the level of normal background radiation, indicating that effects from these sources are negligible.

The absolute concentration of fluorine in each sample was calculated by comparison with a standard, National Bureau of Standards certified phosphate rock. This comparison is done using the equation

Equation 5

$$\frac{N_{\gamma_1}}{N_{\gamma_2}} = \frac{N_{F_1}}{N_{F_2}} \frac{(dE/d\rho x)_2}{(dE/d\rho x)_1}$$

where N_{γ_i} is the number of gamma rays emitted from sample i, N_{F_i} is the concentration of fluorine in sample i, and $(dE/d\rho x)_i$ is the stopping power for protons of sample i in keV/(atoms/cm^2). Stopping powers for protons are known for all elements. But for compounds, stopping powers must be approximated using the Bragg rule, shown here for the compound A_nB_m.

Equation 6 $$(dE/d\rho x)_{A_nB_m} = n(dE/d\rho x)_A + m(dE/d\rho x)_B$$

Thus the total stopping power of a material must be calculated from its component elements. For the standard phosphate rock, percentages of carbon, oxygen, fluorine, aluminum, silicon, phosphorus, calcium and iron are very accurately known. However, bone is a different story. The composition of bone has been approximated by Engstrom and Finean (41) as shown in Table III.

Table III: Bone Composition (41)

	weight percent
organic material	30.0
Ca	27.0
P	12.0
CO_2	4.0
Mg	0.5
	73.5%

For calculation purposes, the "organic material" in the table has been assumed to be carbon and the remaining 26.5% has been assumed to be oxygen. This is obviously a very inaccurate characterization for bone since it completely disregards hydrogen and nitrogen. Furthermore, it is not known how well these figures for fresh bone compare with bone that has been buried for centuries. However, it will suffice for our present purposes.

Dr. Hanson calculated the stopping powers of phosphate rock and bone to be 3.2 and 2.6 keV/(10^{18} atoms/cm^2) respectively for the proton energy of 3.4 MeV. It can be noted here that although stopping power decreases with increasing proton energy, the ratio of stopping powers for rock to bone remains fairly constant, around 1.2.

The certified phosphate rock contains 3.84% or 38,400 ppm fluorine. The 110 keV line yielded 104,880 counts and the 107 keV line yeilded 116,470 counts.

Equation 5 can now be solved as follows:

Equation 7
$$N_{F(bone)} = \frac{N_{\gamma(bone)}}{N_{\gamma(rock)}} N_{F(rock)} \frac{(dE/d\rho x)_{bone}}{(dE/d\rho x)_{rock}}$$

where $N_{\gamma(rock)}$ = 104,880 counts (110 keV line)
= 116,470 counts (107 keV line)
$N_{F(rock)}$ = 38,400 ppm
$$\frac{(dE/d\rho x)_{bone}}{(dE/d\rho x)_{rock}} = \frac{1}{1.2}$$

So that

Equation 8
$$N_{F(bone)} = N_{\gamma(bone)} (0.3051 \text{ ppm/count}) \text{ for the 110 keV line}$$
$$= N_{\gamma(bone)} (0.2747 \text{ ppm/count}) \text{ for the 107 keV line}$$

The uncertainty in this calibration is extremely hard to assess as explained to me by Dr. Hanson for the following reasons:

1. Equation 5 is an approximation.
2. The Bragg rule is an approximation.
3. The stopping power has never been measured at these energies for protons incident of fluorine, magnesium, silicon, and phosphorus.
4. The composition of bone is assumed.

Results

Nitrogen

Each sample was analysed two or sometimes three times. Percentages, individual and averaged, standard deviations and uncertainties at a 95% confidence level are tabulated in Table IV. Uncertainties were calculated using the Student's t test, $t_{05\%,\nu}\, s_{\bar{x}}$, where $\nu = N - 1$ and $s_{\bar{x}} = s_x/\sqrt{N}$. Uncertainties calculated in this manner are extremely high. It must be noted that this is because most are calculated from only two repeat samples. Thus these figures are misleading and should not be taken as the actual ranges for these samples. A larger number of repeat analyses would be desirable, especially where ranges are high (for example, ulna 1647 and femur 1788). High ranges for such samples is probably most attributable to inconsistencies in the powder where the Alvar was particularly stubborn.

It was not possible for me to find the same bone to use for each sample. It would of course be preferable to analyse all tibias or all ulnas, since differences in bone dimensions would be expected to result in differing nitrogen loss rates. But since this was not possible, I analysed mostly those bones which were in the most abundance, namely tibias and ulnas. Both a tibia and an ulna were analysed for sample 1647. As can be seen from the table, nitrogen amounts for these samples are fairly comparable. In fact, the slightly higher amount contained in the ulna is probably due to error, since being a thinner bone, the ulna would actually be expected to lose nitrogen faster. (See Appendix for bone dimensions and other notes.) For sample 1788, there was only a femur for my use. The femur would be expected to have a slightly higher nitrogen content than its tibia or ulna, being a thicker bone. For 1406, there were only foot bones for my use. I analysed a metatarsul, an extremely thin bone. Its nitrogen content

Table IV: Nitrogen percentages by weight

sample	individual %N	average %N	standard deviation	$t_{.05,\nu} s_{\bar{x}}$
1648 tibia	1.08	1.07	0.07	0.17
	1.00			
	1.14			
1647 tibia	1.87	1.87	0.01	0.01
	1.87			
	1.88			
1840 tibia	1.52	1.52	0.01	0.02
	1.51			
	1.53			
1515 tibia	1.42	1.39	0.05	0.44
	1.35			
1065 tibia	1.04	1.03	0.01	0.13
	1.02			
2417 ulna	1.64	1.59	0.08	0.70
	1.53			
1539 ulna	2.87	2.86	0.01	0.13
	2.85			
1563 ulna	2.03	2.03	0	0
	2.03			
1423 ulna	2.44	2.47	0.04	0.32
	2.49			
1800 ulna	2.08	2.10	0.03	0.25
	2.12			
1647 ulna	2.10	1.94	0.23	2.10
	1.77			
1788 femur	1.85	1.78	0.11	0.95
	1.70			
1496 metatarsul	0.76	0.83	0.09	0.86
	0.89			
839 clavicle	2.86	2.85	0.01	0.13
	2.84			

is very low, too low to be compared to tibias and ulnas. It will therefore not be taken into account in the data analysis. For sample 830, a clavicle was analysed. Its dimensions are approximately that of an ulna, and will therefore be used for comparisons. An ulna was analysed for sample 1183, but this bone was contaminated, probably with an organic solvent used to remove the Alvar, and it caused turbidity in the Nesslerization step of the analysis. It is therefore not shown in the table. One more sample, 1587, was not analysed for nitrogen. It consisted largely of extremely thin ribs and dirty vertibrae, unsuitable for analysis, and an ilium, which I felt to be too uncomparable to the other bones to be used.

Fluorine

Raw data and the data in ppm from the fluorine analyses is tabulated in Table V for the 110 keV line and in Table VI for the 107 deV line. Analyses were repeated for some of the samples on the same pressed planchet. In some cases, extra ground bone was repressed into the planchet before repeating the analysis; these are marked with an (rp) after the amount.

In Table VII, fluorine values, averaged from both 110 and 107 keV lines, are tabulated with standard deviations and errors calculated at a 95% confidence level. Errors are based on the Student's t test, $t_{95\%,\nu}\, s_{\bar{x}}$, where $\nu = N - 1$ and $s_{\bar{x}} = s_x/\sqrt{N}$. As for the nitrogen data, these errors are very high where only two measurements have been compared. Again these are misleading and should not be taken as actual ranges. It should also be noted that these statistical analysis does not reflect the error in the calibration factor used for conversion to ppm. (See section on Materials and Methods.)

Fourteen of the fifteen samples were analysed. Tibias analysed for nitrogen were analysed for fluorine, excluding 1840. All ulnas analysed for nitrogen were analysed for

Table V: Fluorine Data, Raw and in ppm for 110 keV Line

sample	fluorine counts	fluorine ppm
1648 tibia	2610	706
	2600	706
	2462 (rp)	751
1647 tibia	507	155
	548 (rp)	167
	546 (rp)	167
1515 tibia	1670	512
	1463	446
	1700 (rp)	521
	1565 (rp)	477
1065 tibia	1408	430
2417 ulna	1616	493
	1644	502
1530 ulna	1030	317
1563 ulna	616	188
1423 ulna	1641	501
	1587 (rp)	484
1800 ulna	716	218
	707	216
	704	215
1183 ulna	765	233
	851 (rp)	260
1788 femur	1050	320
	1070	326
1788 femur (outer part)	2224	670
	2247	686
1406 metatarsul	735	224
1515 metatarsul	1848	564
830 clavicle	866	264
	880	268
1183 clavicle	891	272
	880	268
1587 ilium	2224	670
2417 rib	2020	616

Table VI: Fluorine Data, Raw and in ppm for 107 keV line

sample	fluorine counts	fluorine ppm
1648 tibia	2874	780
	2863	786
	2668 (rp)	734
1647 tibia	600	167
	617 (rp)	160
	620 (rp)	173
1515 tibia	1822	501
	1846	507
	1806 (rp)	496
	1800 (rp)	494
1065 tibia	1578	433
2417 ulna	1803	495
	1707	494
1530 ulna	1112	305
1563 ulna	666	183
1423 ulna	1760	486
	1827 (rp)	502
1800 ulna	780	214
	815	224
	781	215
1183 ulna	803	221
	900 (rp)	247
1788 femur	1171	322
	1103	328
1788 femur (outer part)	2418	664
	2463	677
1406 metatarsul	827	227
1515 metatarsul	1038	532
830 clavicle	948	260
	934	257
1183 clavicle	990	272
	996	274
1587 ilium	2300	650
2417 rib	2180	500

Table VII: Fluorine in ppm

sample	N	$\bar{x}$	s_x	$t_{0.05,\nu}\, s_{\bar{x}}$
1648 tibia	6	775 ppm	26	27
1647 tibia	6	166	6	6
1515 tibia	8	404	24	20
1065 tibia	2	432	2	18
2417 ulna	4	406	4	0
1530 ulna	2	311	8	72
1563 ulna	2	186	4	25
1423 ulna	4	403	10	22
1800 ulna	6	217	4	4
1183 ulna	4	240	17	37
1788 femur	4	324	4	0
1788 femur (outer part)	4	677	0	10
1406 metatarsul	2	226	2	18
1515 metatarsul	2	548	23	206
830 clavicle	4	262	5	11
1183 clavicle	4	272	3	6
1587 ilium	2	660	14	126
2417 rib	2	608	12	108

fluorine, plus 1183, the one which was contaminated for Nesslerization. The femur from 1788 was analysed for fluorine as for nitrogen. In addition, a thin layer from the surface of this bone was removed and analysed separately. As can be seen from the table, the fluorine content of this outer part is much higher than throughout the entire bone, as would be expected given that fluorine must diffuse in from the surface. Two metatarsuls, 1407 and 1515, were analysed. The fluorine content of metatarsul 1515 is higher than in the ulna, which is to be expected given the higher surface area to volume ratio of the metatarsul. Two clavicles were analysed, 830 and 1183. The fluorine content of the clavicle can be compared to that of the ulna for 1183. I would have expected a closer concentration between the two; it is possible that there was some fluorine removed from the ulna when it was cleansed of Alvar. The rib from 2417 was analysed and can be compared to the ulna from this sample. Its fluorine content is much higher than in the ulna, probably due again to the thinness of the bone in comparison. The ilium from 1587 was analysed. This bone is very high in fluorine despite the great amount of Alvar included in the sample. (See Appendix for bone dimensions and other notes.)

Comparison of Fluorine and Nitrogen Data

A compilation of data from both sets of analyses, nitrogen and fluorine, is presented in Table VIII. There are a total of eleven bones which were analysed for both elements, including four tibias, five ulnas, a femur and a clavicle. (The metatarsul from sample 1406 as not included in the analysis, since it is concidered uncomparable to the other bones where nitrogen is concerned.)

As discussed earlier in this paper, the oldest bones are expected to have the lowest nitrogen and the highest fluorine amounts, while newer bones will have high nitrogen and low fluorine amounts. To demonstrate whether or not the analyses cross check in this way, a rank correlation was performed based on the method of Olds (42). This consists of

Table VIII: Compilation of Nitrogen and Fluorine Data

sample	nitrogen (%)	fluorine (ppm)
1648 tibia	1.07	775
1647 tibia	1.87	166
1840 tibia	1.52	——
1515 tibia	1.30	404
1065 tibia	1.03	432
2417 ulna	1.50	406
1530 ulna	2.86	311
1563 ulna	2.03	186
1423 ulna	2.47	403
1800 ulna	2.10	217
1183 ulna	——	240
1647 ulna	1.04	——
1788 femur	1.78	324
1406 metatarsul	(0.83)	226
1515 metatarsul	——	548
830 clavicle	2.85	262
1183 clavicle	——	272
1587 ilium	——	660
2417 rib	——	608

assigning a number of rank, one through eleven in this case, to both sets of data, and squaring the difference, d, between ranks for each member. Squares are then summed and compared with a significance table as presented by Olds. Table IX shows the rank correlation for nitrogen vs. fluorine. A rank of one has been assigned to older specimens, as indicated by either low nitrogen or high fluorine amounts.

Table IX: Rank Correlation between Nitrogen and Fluorine

sample	nitrogen rank	fluorine rank	d	d^2
1648 tibia	1	1	0	0
1647 tibia	5	11	6	36
1515 tibia	2	3	1	1
1065 tibia	6	5	1	1
2417 ulna	3	2	1	1
1530 ulna	11	7	4	16
1563 ulna	7	10	3	9
1423 ulna	9	4	5	25
1800 ulna	8	9	1	1
1788 femur	4	6	2	4
830 clavicle	10	8	2	4
				$\Sigma d^2 = 98$

From Olds' Table V ('42), for N = 11 there is a probability, P, of 0.90 of Σd^2 having a value between 105.6 and 334.4 (or for a single tail distribution, a P = 0.95 of Σd^2 having a value greater than 105.6). There is a probability of 0.96 of Σd^2 having a value between 77.1 and 362.9 (or for a single tail distribution, a P = 0.98 of Σd^2 having a value greater than 77.1).

Statistical significance here is defined as a probability, P, of 0.05 or less for a single tail distribu-

tion. In Table IX, $d^2 = 08$, which means that there is a probability between 0.05 and 0.08 of this ranking occuring randomly; it is therefore statistically significant.

The data from the two analyses for the alaven samples is plotted in Figure 9. Linear regression analysis gives a y-intercept of 2.66 and a slope of -1.75×10^{-3} for nitrogen vs. fluorine. The Pearson's correlation coefficient, r, for this plot is -0.5642. A perfect negative correlation would have a value of r = -1.

If loss of nitrogen from bone is a "pseudo-first order" reaction, as discussed in the section on nitrogen dating, it would follow first order reaction kinetics as expressed by the integrated rate law

Equation 9 $$\ln(a/a - x) = kt$$

where a is the original amount of reactant present, x is the amount lost at time t, and k is the first order rate constant for the reaction.

In the case of this nitrogen loss "reaction"

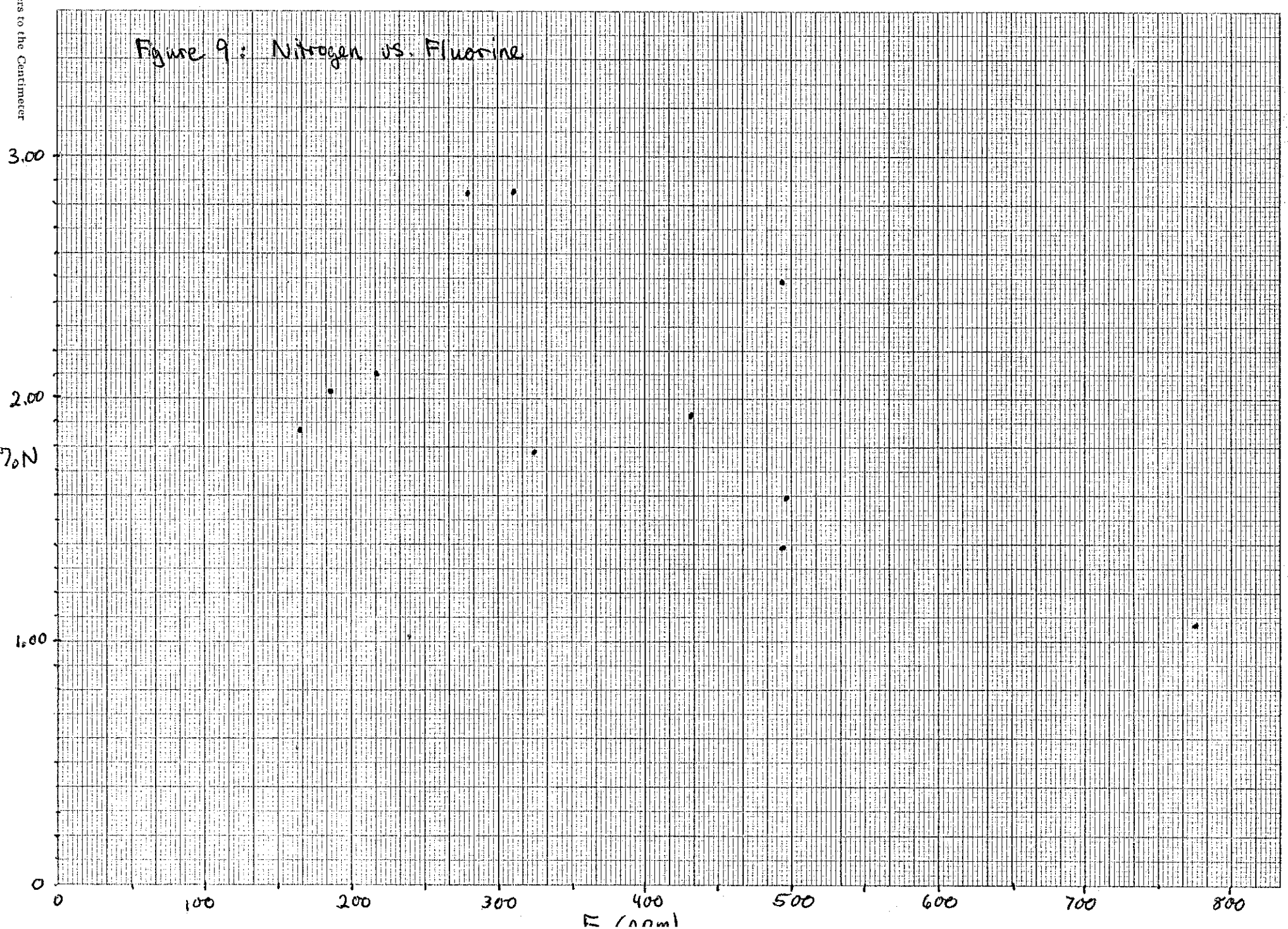
Equation 10 $$\ln(a/a - x) = \ln\left[(\%N)_{t=0}/(\%N)_t\right]$$
$$= \ln\left[(\%N)_{t=0}\right] - \ln\left[(\%N)_t\right]$$

In order to simplify the demonstration of whether or not such kinetics exist, without attempting to calculate k, the term $\ln\left[(\%N)_{t=0}\right]$ can be disregarded. It is a constant and would have the effect of merely shifting the plot along the $\ln(\%N)$ axis.

Of course, $\ln\left[(\%N)_t\right]$ cannot be plotted against time in this case since such data does not exist. A possible (although poor) substitute would be to plot it against the fluorine data. This has been done and is shown in Figure 10. Linear regression analysis gives a y-intercept of 1.054 and a slope of -1.06×10^{-3} for $\ln(\%N)$ vs. fluorine, with a correlation coefficient of -0.6516.

10 Millimeters to the Centimeter

NATIONAL 12-188



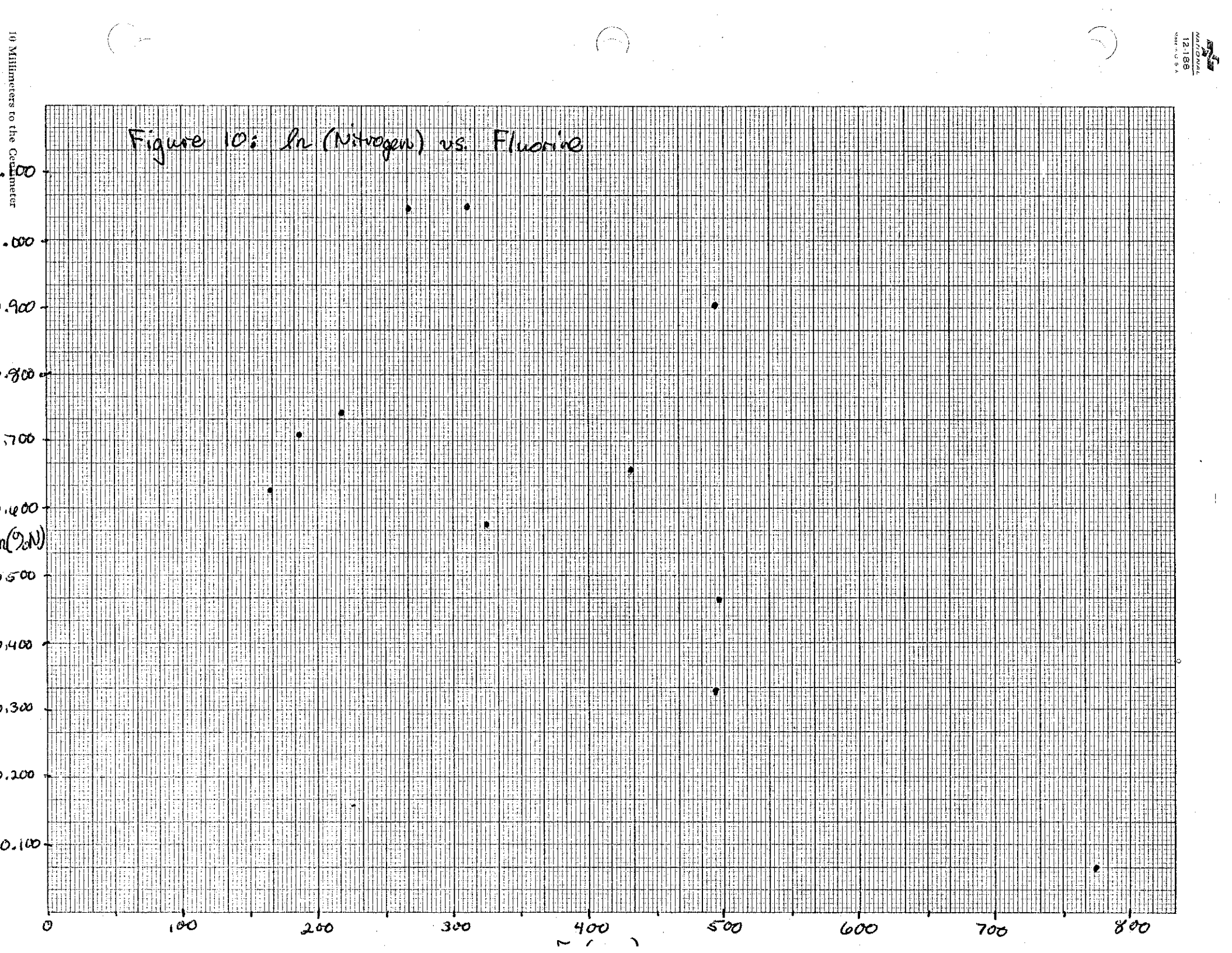


Figure 10: ln (Nitrogen) vs. Fluoride
.000
.900
.800
.700
.600
ln(%N)
.500
.400
.300
.200
0.100
0
100
200
300
400
500
600
700
800

Although this sort of data treatment cannot be taken as conclusive evidence, there is a substantial increase in the absolute value of the correlation coefficient, r, when ln(%N) rather then %N is plotted against fluorine data. This seems to indicate that nitrogen decay may indeed be a "pseudo-first order" reaction. Other similar manipulation of the data did not have the same effect of improving the correlation coefficient. In fact, all other such treatments attempted actually decreased the absolute value of r.

Discussion

As shown in the previous section on results, nitrogen data and fluorine data show similar chronological trends for these bone samples. In fact, the cross check between the two analyses came out remarkably well concidering the high number of uncontrollable variables a study such as this involves.

Although the burials are believed to have taken place over a period of only a few hundred years, there is great variation in the amounts of nitrogen and fluorine present. Nitrogen percentages show a change of almost threefold, from 1.07% for tibia 1648 to 2.86% for ulna 153°. Even more notable is the range of fluorine content which shows a change of almost fivefold from 166 ppm for tibia 1647 to 775 ppm for tibia 1648. Thus it appears that environmental conditions for the Moundville region are extreme to the point where chemical changes occur rapidly in buried bone over only a few years. (Or else the time span these burials cover is actually much longer than we have believed.) It is these large chemical differences that have made this study possible. If such a large range were not present, I would not know whether I was seeing true differences.

Despite the indications of success of the dating methods in this study, there are still short comings. Although nitrogen and fluorine trends support one another, the agreement is not as complete as I might prefer. For example, tibia 1065 has moderate amounts of both fluorine and nitrogen, while tibia 1647 has approximately the same amount of nitrogen but only one third the amount of fluorine. Thus, by one method they are quite contemporary and by the other, not at all. This kind of skewed data is almost certainly due to the confusion wrought by our old enemy Alvar. Given two bones with the same amounts of fluorine and nitrogen, if one has its total weight increased by an Alvar bath, it will appear older by nitrogen

analysis and younger by fluorine analysis. Other possible factors which might skew the data in this way include a miriad of environmental variables, all of which I have assumed constant from mound to mound, but which may not actually be so (for example, local ground water). Another possibility is variations in chemical composition upon death, which I have again assumed constant. In the case of nitrogen, this is a valid assumption, since all individuals were adults except 83°, which has been classified as sub-adult. However, this is not so valid for fluorine, since older individuals may have accumulated more fluorine during their lifetimes. There is also the problem with finding comparable bone types for analysis. And a last possible source of skewed data would be if some bodies were kept above ground for extended periods before burial. Such treatment would not effect nitrogen loss greatly, but fluorine accumulation would be delayed until a source of the ion were available. Fortunately, records indicate that all bones used for this study were articulated upon unearthing, indicating that they were buried shortly after death (38).

The ultimate goal in dating these samples was to provide confirmation of a gravelot seriation based on pottery styles by Vincas P. Steponaitis (1). In Table X, samples and their values for nitrogen and fluorine have been arranged by best fit position based on pottery traits, from the single Moundville I sample through the Moundville III samples. Unseriated samples, all classified as late Moundville II or Moundville III, are at the end of the table. An examination of the table reveals that the chemical analyses do not support this ordering of gravelots based on pottery styles. The chemical values, when ordered this way seem to have a random distribution. And if there is any order present at all, it is in the backwards direction. However, the best fit position is not meant to be an exact placing, but rather an intermediate of a range. The range assignments in the table have been given in terms of a six phase sequence: Moundville I (denoted by I), early Moundville II (eII),

Table X: Comparison of Ceramic and Chemical Data

sample	best fit position	range	%N	fluorine
830 clavicle	36.0	I	2.85	262
1587 ilium	220.5	lII,eIII	——	660
1406 metatarsul	221.0	lII,eIII	(0.83)	226
1530 ulna	236.5	eIII	2.86	311
1788 femur	240.0	eIII	1.78	324
1183 ulna	242.0	lIII	——	240
1183 clavicle	242.0	lIII	——	272
1800 ulna	243.5	eIII,lIII	2.10	217
1563 ulna	246.0	eIII,lIII	2.03	186
1065 tibia	246.3	eIII,lIII	1.93	432
1515 tibia	250.0	eIII,lIII	1.30	404
1515 metatarsul	250.0	eIII,lIII	——	548
1423 ulna	252.5	lII,eIII, lIII	2.47	403
2417 ulna	265.0	lIII	1.50	406
2417 rib	265.0	lIII	——	608
1647 tibia	——	lII,eIII	1.87	166
1647 ulna	——	lII,eIII	1.94	——
1648 tibia	——	lII,eIII	1.07	775
1840 tibia	——	eIII,lIII	1.52	——

late Moundville II (lII), early Moundville III (eIII), late Moundville III (lIII), and Alabama River. When the picture is viewed in this way, the chemical data is not at all surprizing, and may actually aid in narrowing down the ranges.

However, there is one particularly outstanding sore thumb revealed in the table. This is with sample 83°. There is no doubt that this gravelot is the oldest one ceramically. However, chemical data implies that it is one of the youngest of the bone samples. I can offer no explanation as to why this is so, except to point out the possibility of mislabeling of either pottery or skeletal material.

There is also an item of particular interest concerning samples 1647 and 1648. These two skeletons were found side by side in Mound E (38). The two burials have been assumed to contemporary, and have been classified together as late Mounville II or early Moundville III. But from the chemical data, the two are obviously far from contemporary. The one, 1648, is certainly the oldest of any of the samples, while the other, 1647, is fairly new (the newest by fluorine analysis, and intermediate by nitrogen analysis). Assuming that these samples have not been mislabeled, the evidence indicates that 1648 was a very early burial, and that 1647 was placed beside it at a much later date. The pottery associated with the two was most likely introduced during the second burial.

The above discovery leads one to wonder about the contemporaneity and relative ages of other gravelots. Table XI shows the samples grouped by mound. The letters with the gravelot numbers represent the direction in relation to the mound in which each gravelot was found. The table includes the amounts of nitrogen and fluorine, and a relative classification for each sample (early, moderately early, moderate, moderately late, and late) based on these amounts. All of the five samples from Mound D are from a moderate to late time period, except for 1515, which is earlier. There is

Table XI: Comparison of Gravelots Arranged by Mound

sample	%N	fluorine	comment
Mound D			
1406S metatarsul	(0.83)	226	late
1530S ulna	2.86	311	mod. late to late
1563S ulna	2.03	186	moderate to late
1515S tibia	1.30	404	early to moderate
metatarsul	——	548	
1423S ulna	2.47	403	moderate to mod. late
Mound E			
1648N tibia	1.07	775	early
1647N tibia	1.87	166	moderate to late
ulna	1.04	——	
1587N ilium	——	660	early
1183E ulna	——	240	late
clavicle	——	272	
Mound G			
1788SW femur	1.78	324	mod. early to mod. late
1800SW ulna	2.10	217	moderate to late
Mound I			
830E clavicle	2.85	262	late
Mound P			
2417W ulna	1.50	406	mod. early to moderate
rib	——	608	
Mound R			
1065W tibia	1.03	432	moderate
Mound W			
1840N tibia	1.52	——	mod. early

not as much variation there as there is in Mound E, that of 1647 and 1648. Mound D has another pair of samples which vary in age. These are 1587, which is early, and 1183, which is much later. However, both were analysed only for fluorine. Furthermore, there was only an ilium analysed for 1587, a bone not known to be comparable to the others. Two other rather early burials are from Mound P, a mound where many Moundville I and II burials have been found, and Mound W. Mound W, though, is particularly close to the Black Warrior River, which may have accelerated nitrogen loss.

There is no way of knowing how much nitrogen and fluorine would be in a Moundville I sample. The single sample that was classified as Moundville I is certainly not that old. It could be, however, that others are, in particular 1648. The chemical data for this sample is so extreme that an age dating back to Moundville I is easily credible. An obvious solution to this question would be to analyse more samples that have an early pottery classification, or to analyse a set of faunal bones from the well stratified living areas of Moundville.

Conclusion

This study has shown that nitrogen and fluorine dating will give similar chronological trends in a group of bones recovered from the same archaeological site. This fact has long been known from previous work, but I am not aware of any such study which includes a set of samples covering so short a time period as this one. From the earliest stages of this study, I had many doubts that the time lapses between burials at Moundville were long enough to show up in the analysis. I can now conclude that they were indeed long enough, and that despite several complications, nitrogen and fluorine dating provide a reliable cross check for one another. This is the major success of this study.

Unfortunately, the comparison of chemical ages and ceramic ages has not proven to be such a success. Some of the bones seem to fall within the assigned ranges, while others do not. Results point out many areas for further work, and are hence far from conclusive. Thus once again a new analysis has created more questions than it has answered.

Appendix: Notes on the Bones

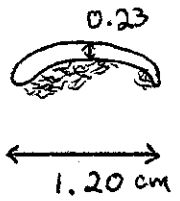
The following notes are observations on the particular bones used for analysis in this study. They include precise measurements made on the parts of the bones used, a factor important for determining the comparability of the bones. (All dimensions are in centimeters.) There are also observations on the condition of the bones, including degree of post-mortem erosion and estimation of Alvar saturation. Comments on pathological conditions have been taken from an inventory on the bones intended for radiocarbon analysis prepared by Kenneth R. Turner from the Laboratory of Human Osteology, University of Alabama (43).

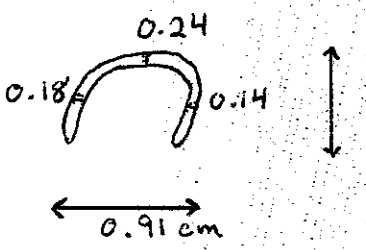
All bones were originally labelled with numbers beginning with SK (for skeleton). At some point, usually after the bones were coated in Alvar, these numbers or sometimes just the SK were scratched off the bones and replaced with numbers beginning with MD (for Moundville).

All bones were sampled where indicated at the cross sections shown for each bone. No trabecular bone was sampled.

<u>83º clavicle</u>: left, sub-adult

cross sections of the two ends





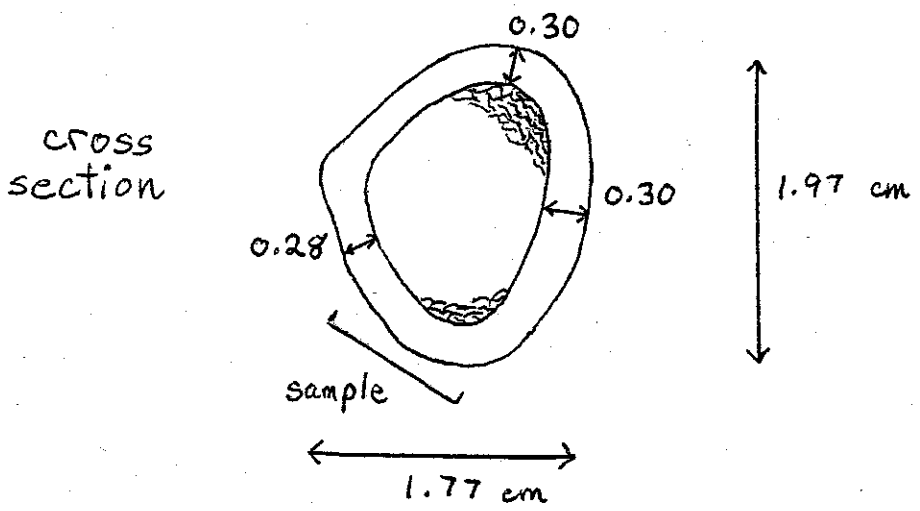
This fragment consists of a small and very incomplete portion of the bone. There is a small amount of trabecular bone. There is slight post-mortem erosion.

The sample analysed was taken from most of the fragment.

No known pathologies.

Length: 5.5 cm (approx.)
Alvar: heavy

1065 tibia: left, adult



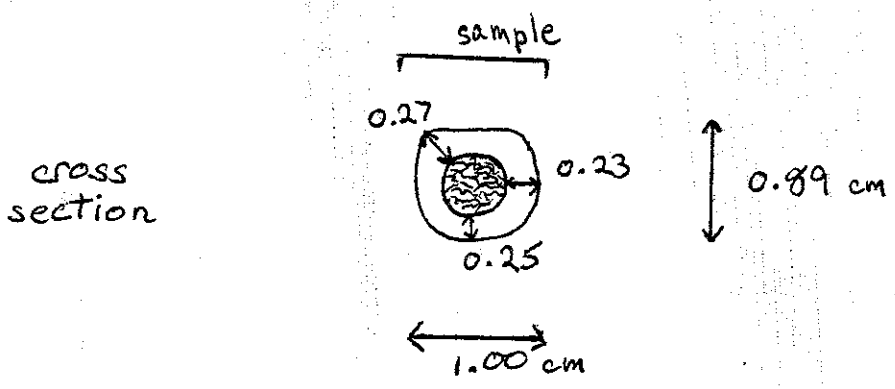
This fragment consists of most of the shaft of the bone. This bone is smooth with no erosion.

The sample analysed was taken from the area indicated by "sample" on the cross section above.

No known pathologies.

Length: 21 cm (approx.)
Alvar: light to moderate

1183 ulna: left, adult



This fragment consists of the shaft of the bone, in two parts of approximately 5 cm and 10 cm. There is slight post-mortem erosion. There is much trabecular bone throughout the interior. The bone is very clean; Alvar was apparently removed using an organic solvent which interfers with nitrogen analysis.

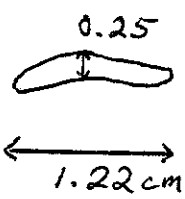
The sample analysed was taken from the area indicated by "sample" in the cross section above.

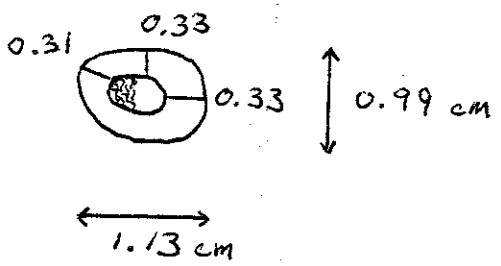
No known pathologies.

Length: 15 cm total (approx.)
Alvar: almost absent (removed)

1183 clavicle: left, adult

cross section of the two ends





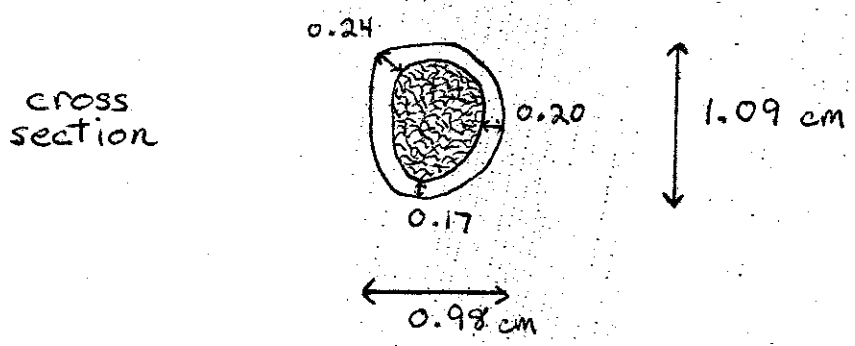
This fragment consists of a small and very incomplete part of the bone. There is some trabecular bone. The bone is clean (possibly cleansed with a solvent?).

The sample analysed was taken from the entire fragment.

No known pathologies.

Length: 7.5 cm (approx.)
Alvar: light

1423 ulna: right, adult



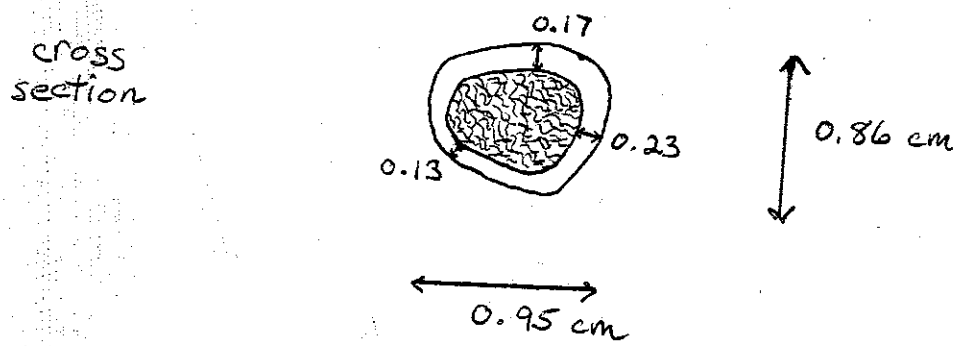
This fragment consists of most of the bone including the proximal end. There is much trabecular bone throughout the interior. There is much post-mortem erosion.

The sample analysed was taken from the entire cross section as shown above.

No known pathologies.

Length: 20 cm (approx.)
Alvar: heavy

1406 metatarsul: V left, adult



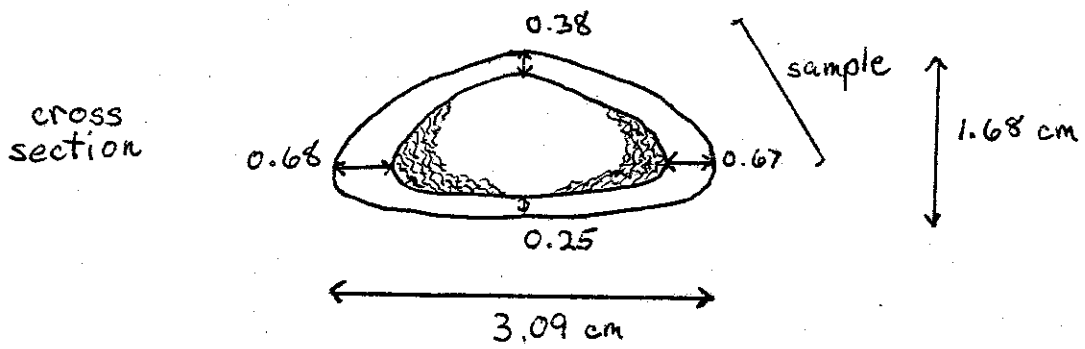
This fragment consists of the entire bone. There is trabecular bone throughout the interior. The bone is smooth.

The sample analysed was taken from the entire cross section as shown above from the middle of the shaft.

No known pathologies.

Length: 7.25 cm
Alvar: light to moderate

1515 tibia: right, adult



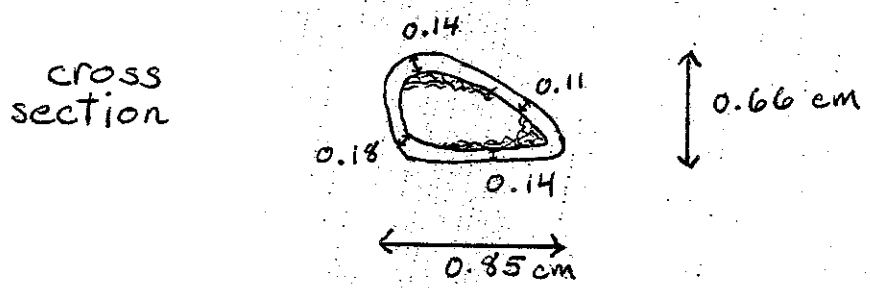
This fragment consists of approximately half of the proximal end of the bone. There is some post-mortem erosion, and a trace of rodent gnawing on the tibial crest. There is much trabecular bone.

The sample analysed was taken from the area indicated by "sample" on the cross section above.

There is the possible pathology noted by Turner as follows: "cortical resorption spaces characteristic of old age are visible macroscopically and there is a small amount of cancellous bone lining the medullary cavity at mid-shaft. The trabeculae at this site are of unremarkable dimensions. Other bones from this burial show no traces of osteoarthritis."

Length: 25.0 cm
Alvar: moderate

1515 metatarsul: III right, adult



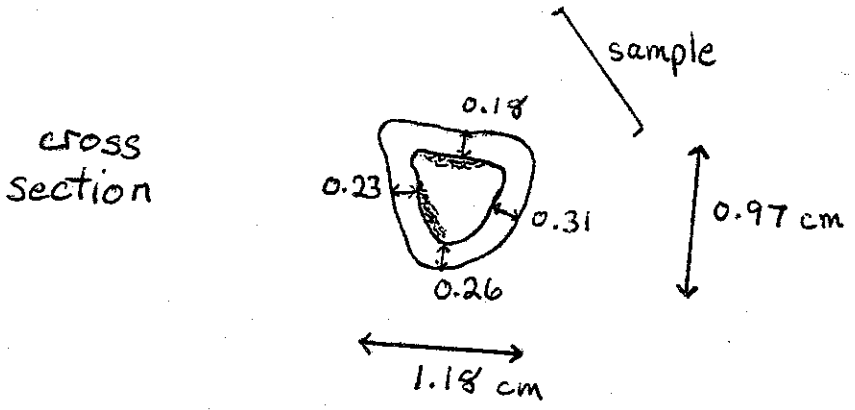
This fragment consists of all of the bone, excluding a small portion at the proximal end. The bone is smooth and well preserved. There is some trabecular bone.

The sample analysed was taken from the entire cross section shown above at the middle of the shaft.

No known pathologies.

Length: 6.8 cm
Alvar: moderate

153º ulna: left, adult



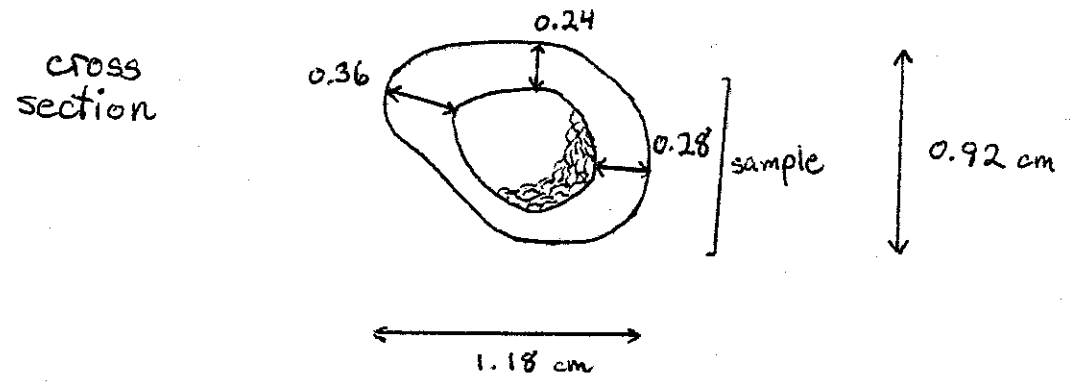
This fragment consists of approximately one third of the distal end of the bone. There is a small amount of trabecular bone, and much dirt plastered to the interior. There is slight post-mortem erosion.

The sample analysed was taken from the area indicated by "sample" on the cross section above.

No known pathologies.

Length: 10 cm (approx.)
Alvar: heavy

1563 ulna: right, adult



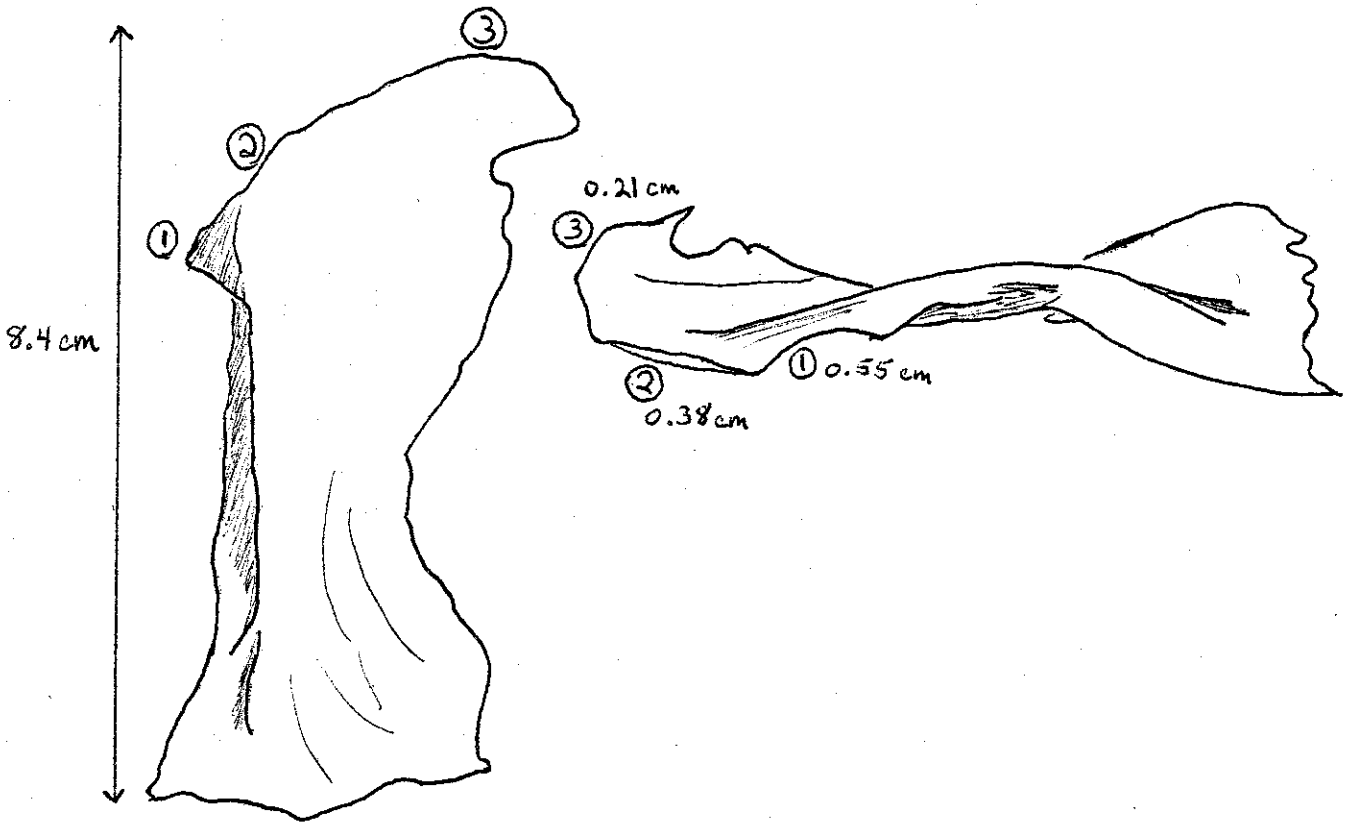
This fragment consists of most of the shaft of the bone. The bone exhibits severe post-mortem erosion. There is some trabecular bone. Alvar is heavy, but it seems to have been scraped off before the MD number was painted on.

The sample analysed was taken from the area indicated as "sample" on the cross section above.

No known pathologies.

Length: 20 cm (approx.)
Alvar: heavy

1587 ilium: left, adult



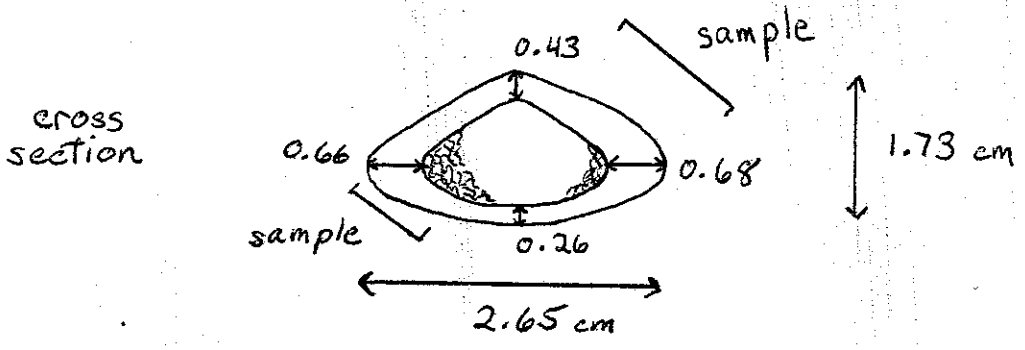
This fragment consists of part of the blade of the bone. The bone is very dirty with a layer of dark material approximately 0.08 cm thick on the exterior.

The sample analysed was taken from three relatively clean areas indicated by "①, ②, ③" on the figures above.

No known pathologies.

Alvar: heavy

1647 tibia: right, adult



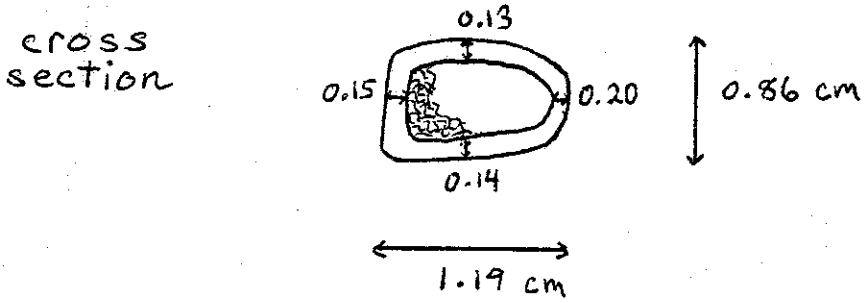
This fragment consists of approximately half of the distal end of the bone. There is trabecular bone and some dirt plastered to the interior. The bone is smooth.

The sample analysed was taken from the areas indicated by "sample" on the above cross section.

No known pathologies.

Length: 16.5 cm
Alvar: moderate

1647 ulna: right, adult



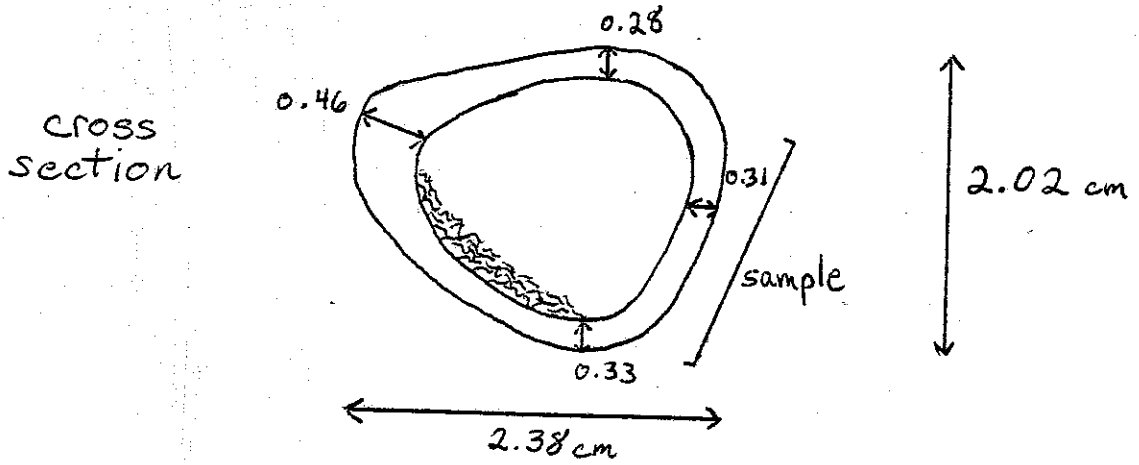
This fragment consists of a small length of the shaft near the distal end of the bone. There is some trabecular bone and slight post-mortem erosion.

The sample analysed was taken from the entire cross section as shown above.

No known pathologies.

Length: 5 cm (approx.)
Alvar: moderate

1648 tibia: right, adult



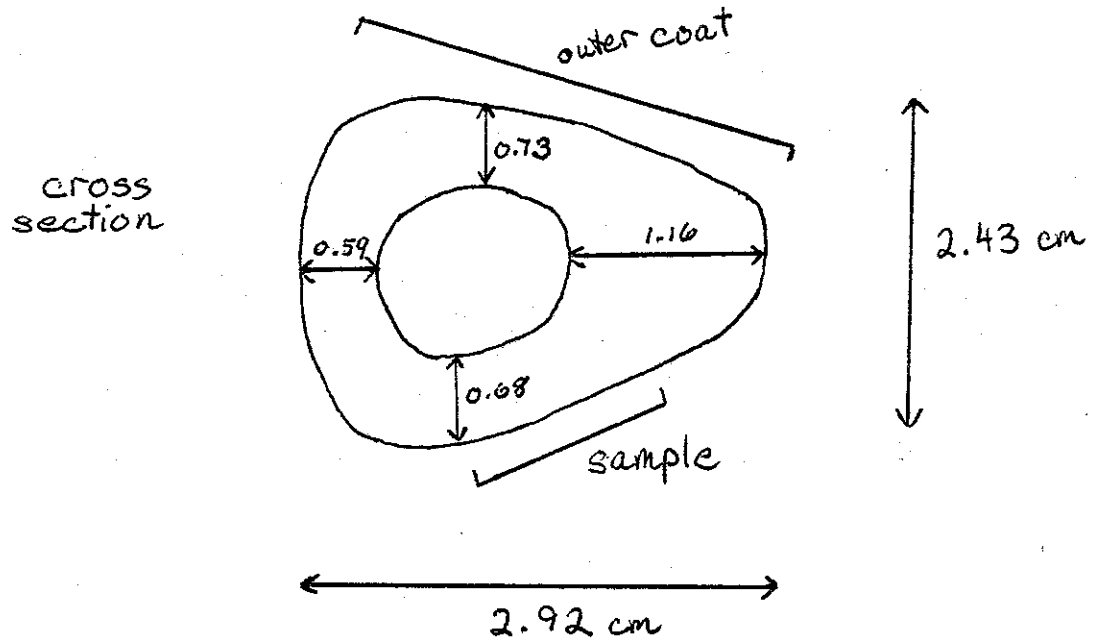
This fragment consists of most of the shaft of the bone. The fragment is mostly smooth, with only slight erosion. There is some trabecular bone.

The sample analysed was taken from the area indicated by "sample" on the cross section above.

No known pathologies.

Length: 27.5 cm
Alvar: moderate

1788 femur: left, adult



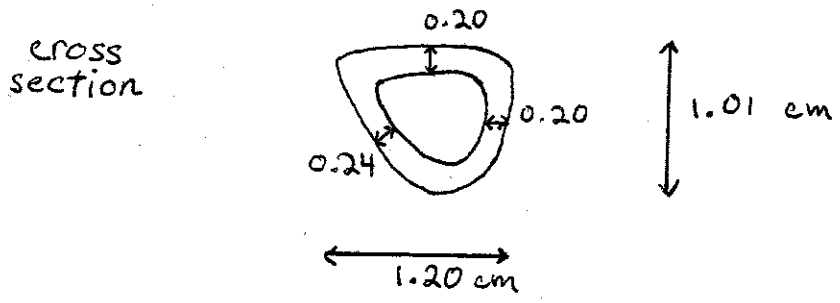
This fragment consists of approximately half of the distal end of the bone. There is widespread post-mortem erosion especially at the distal end.

The sample analysed was taken from the area indicated by "sample" on the cross section above. The outer layer analysed was approximately 0.03 cm thick, taken from the area indicated by "outer part" above. This part of the surface had no erosion.

No known pathologies.

Length: 21.7 cm
Alvar: light to moderate

1800 ulna: side uncertain, adult



This fragment consists of a small length of the shaft of the bone. There is much post-mortem erosion, and some dirt plastered inside. Alvar appears to have been scraped off in some places.

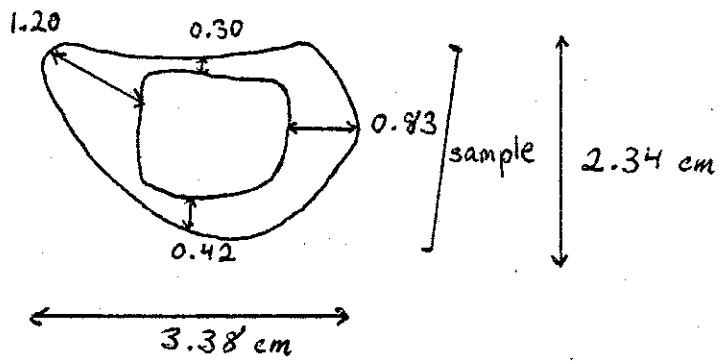
The sample analysed was taken from the entire cross section as shown above.

No known pathologies.

Length: 5 cm (approx.)
Alvar: heavy

1840 tibia: right, adult

cross section



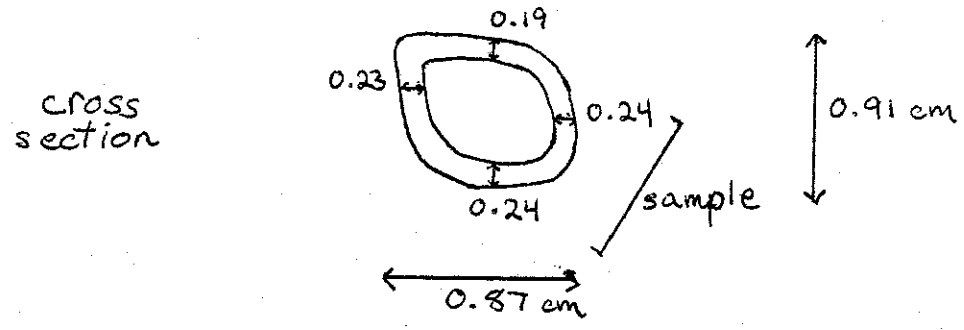
This fragment consists of a large length of the shaft of the bone. There is some post-mortem erosion.

The sample analysed was taken from the area indicated by "sample" in the cross section above.

No known pathologies.

Length: 2.38 cm
Alvar: light

2417 ulna: left, adult



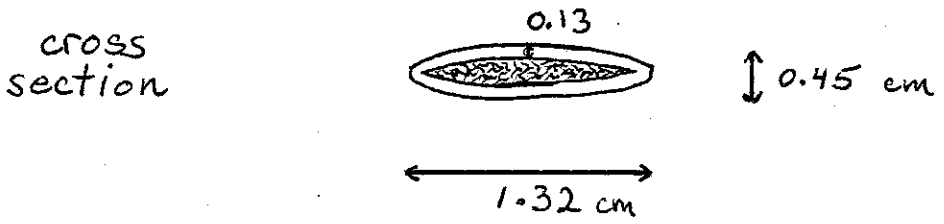
This fragment consists of only a small portion of the shaft of the bone. There is some post-mortem erosion and a small amount of dirt plastered to the interior.

The sample analysed was taken from the area indicated by "sample" on the cross section above.

No known pathologies.

Length: 5 cm (approx.)
Alvar: light to moderate

2417 rib: side uncertain, adult



This fragment consists of part of a rib shaft. The bone is smooth, although slight erosion is visible on other ribs. There is trabecular bone throughout the interior.

The sample analysed was taken from the entire cross section as shown above.

No known pathologies.

Length: 5 cm (approx.)
Alvar: moderate

Bibliography

1. Vincas P. Steponaitis "A Seriation of Moundville Gravelots" (unpublished)
2. Donald J. Ortner, David W. Von Endt, Mary S. Robinson "The Effect of Temperature on Protein Decay in Bone: Its Significance in Nitrogen Dating of Archaeological Specimens" American Antiquity, Vol. 37, No. 4, 514-20 (October, 1972)
3. J. D. Garlick "Buried Bone: The Experimental Approach in the Study of Nitrogen Content and Blood Group Activity" Chap. 43 of Science in Archaeology edited by Don Brothwell and Eric Higgs, Thames and Hudson, second edition (1969)
4. David W. Von Endt "Techniques of Amino Acid Dating" from Pre-Llano Cultures of the Americas: Paradoxes and Possibilities edited by Robert L. Humphrey and Dennis Stanford, The Anthropological Society of Washington (1979)
5. Dumas Ann. chim. phys. (Paris) (2), 47, 198 (1831) as referred to by Bradstreet (ref. 7)
6. Johann Kjeldahl Medd. Carsberg Lab. 2, 1 (1883) or Z. anal. Chem. 22, 366 (1883) as referred to by Bradstreet (ref. 7)
7. R. B. Bradstreet The Kjeldahl Method for Organic Nitrogen, Academic Press, New York and London (1965)
8. Donald W. Groff "Gas Chromatography Methods for Bone Fluorine and Nitrogen Composition" Chap. 20 of Science and Archaeology edited by Robert H. Brill, MIT Press, Cambridge, Mass. and London, Eng. (1971)
9. Calvin A. Lang "Simple Microdetermination of Kjeldahl Nitrogen in Biological Methods" Analytical Chemistry, Vol. 30, No. 10, 1692-94 (October, 1958)
10. R. F. Moore, Jr. and Dorothy L. Harris "Influence of Solvent Vapors on the Occurrence of Turbidity during Color Development with the Nessler's Reagent" Analytical Chemistry, Vol. 36, No. 1, 249-50 (January,

1964)

11. J. Middleton "On Fluorine in Bones: its Sources and its Application to the Determination of Geological Age of Fossil Bones" Proceedings, Geological Society of London, Vol. 4, 431-33 (1844) as referred to by Hoskins and Fryd (ref. 25)
12. A. Carnot "Recherches sur la composition générale et la teneur en fluor des os modernes et des os fossiles de differents âges" Annales des Mines, Memoirs, Paris, Series 9, Vol. 3, 155-95 (1893) as referred to by Hoskins and Fryd (ref. 25)
13. Kitty Little Bone Behavior, Academic Press, London-New York (1973)
14. R. A. Robinson and M. L. Watson Anat. Rec., 114, 383 (1952) as referred to by Neuman and Neuman (ref. 15)
15. William F. Neuman and Margaret W. Neuman The Chemical Dynamics of Bone Mineral, University of Chicago Press (1958)
16. P. W. Arnold Tr. Faraday Soc., 46, 1061 (1950) as referred to by Neuman and Neuman (ref. 15)
17. R. A. Young, W. van der Lugt, and J. C. Elliott "Mechanism for Fluorine Inhibition of Diffusion in Hydroxyapatite" Nature, Vol. 223, 720-30 (Aug. 16, 1969)
18. Eric J. Underwood Trace Elements in Human and Animal Nutrition, Academic Press, New York, San Francisco and London, fourth edition (1977)
19. D. Carlström Acta radiol., Suppl. 121 (1955) as referred to by Neuman and Neuman (ref. 15)
20. Arne R. Hagen "Studies on Fluorapatite: 1. Chemical Characterization and Exchange Properties" Calc. Tissue Research, Vol. 13, 259-70 (1973)
21. C. Tse, D. O. Welch and B. S. H. Royce "The Migration of F^-, OH^- and O^{2-} Ions in Apatite" Calc. Tissue Research, Vol. 13, 47-52 (1973)
22. S. F. Cook and H. C. Ezra-Cohn "An Evaluation of the Fluorine Dating Method" Southwestern Journal of Antho-

pology, Vol. 15, 276-90 (1950)

23. H. H. Willard and O. B. Winter "Volumetric Method for Determination of Fluorine" Indust. and Eng. Chem., Anal. Ed., Vol. 5, No. 1, 7-10 (January 15, 1933)
24. W. D. Armstrong "Modification of the Willard-Winter Method for Fluorine Determination" Journal of the American Chemical Society, Vol. 55, 1741-42 (April, 1933)
25. C. R. Hoskins and C. F. M. Fryd "The Determination of Fluorine in Piltdown and Related Fossils" J. appl. Chem., Vol. 5, 85-87 (February, 1955)
26. R. E. Taylor "Fluorine diffusion: a new dating method for chipped lithic materials" World Archaeology, Vol. 7, No. 2, 127-35 (1975)
27. K. W. Jones, Ph. Gorodetzky and J. S. Jacobson "Determination of Fluorine in Vegetation by Proton Activation Analysis" Intern. J. Environ. Anal. Chem., Vol. 4, 225-31 (1976)
28. R. E. Shroy, H. W. Kraner, K. W. Jones, J. S. Jacobson and L. I. Heller "Determination of Fluorine in Food Samples by the $^{19}F(p, p'\gamma)^{19}F$ Reaction" Nuclear Instruments and Methods, Vol. 149, 313-16 (1978)
29. R. E. Shroy, H. W. Kraner, K. W. Jones, J. S. Jacobson and L. I. Heller "Proton Activation Analysis for the Measurement of Fluorine in Food Samples" Analytical Chemistry (submitted manuscript) (December, 1979)
30. Kenneth P. Oakley and C. Randall Hoskins "New Evidence on the Antiquity of Piltdown Man" Nature, Vol. 165, No. 4193, 379-82 (March 11, 1950)
31. J. S. Weiner, K. P. Oakley, W. E. Le Gros Clark, F.R.S. "The Solution of the Piltdown Problem" Bulletin of the Brit. Mus. (Nat. Hist.) Geology, Vol. 2, No. 3, 139-46 (1953)
32. J. S. Weiner, W. E. Le Gros Clark, K. P. Oakley, et al. "Further Contributions to the Solution of the Piltdown Problem" Bulletin of the Brit. Mus. (Nat. Hist.) Geology, Vol. 2, No. 6, 225-87 (1955)

33. Kenneth P. Oakley and M. F. Ashley Montagu "A Re-Concideration of the Galley Hill Skeleton" Bulletin of the Brit. Mus. (Nat. Hist.) Geology, Vol. 1, No. 2, 25-48 (1940)

34. Kenneth P. Oakley "Analytical Methods of Dating Bones" Chap. 1 of Science in Archaeology edited by Don Brothwell and Eric Higgs, Thames and Hudson, second edition, (1960)

35. Alexander R. Doberenz and Philip Matter III "Nitrogen Analysis of Fossil Bones" Comp. Biochem. Physiol., Vol. 16, 253-58 (1965)

36. Kenneth P. Oakley "Relative Dating of Arlington Springs Man" Science, Vol. 141, 1172 (September 20, 1963)

37. Kenneth P. Oakley "Dating Skeletal Material" Science, Vol. 140, 488 (May 3, 1963)

38. Vincas P. Steponaitis personal communication

39. Vincas P. Steponaitis "Some Preliminary Chronological and Technological Notes on Moundville Pottery" Bulletin of the Southeastern Archaeological Conference, No. 22 (preprint) (1978?)

40. Christopher S. Peebles "Moundville: Late Prehistoric Sociopolitical Organization in the Southeastern U. S." draft for presentation at the 1979 meeting of the Amer. Ethnol. Society (December, 1978)

41. Arne Engström and J. B. Finean, Biological Ultrastructure, Academic Press, New York (1958) as referred to me by Dr. Albert Hanson

42. E. G. Olds "Distributions of Sums of Squares of Rank Differences for Small Numbers of Individuals" Annals of Mathematical Statistics, Vol. IX, 133-40 (1938)

43. Kennth R. Turner "Inventory of Human Skeletal Materials Removed for Radiocarbon Analysis in January, 1979" (unpublished)

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