ANALYSIS OF THE FLUORIDE CONTENT OF HUMAN REMAINS FROM THE GRAY SITE SASKATCHEWAN

by

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ABSTRACT

Fluoride analysis of bone from archaeological sites is one of the oldest proposed dating methods. However, techniques for determining fluoride content were not sufficiently refined until the mid-twentieth century to be of use for this purpose. Since the development of adequate micro-chemical techniques, fluoride analysis has been used with varying degrees of success. One of the main criticisms of fluoride dating has been the intricacy and expense as opposed to production of information. The work presented here describes a simple, inexpensive technique for fluoride determination and applies it to an archaeological problem which maximises the information gained.

The micro-chemical techniques utilize ion-specific electrode and photospectrometer equipment. The subject is the Gray Site, an Archaic Oxbow cemetery in southwestern Saskatchewan. The techniques are used to seriate temporally 101 individuals from the cemetery. This seriation is coupled with radiocarbon dates to give estimates of the beginning, peak and termination of the use of the cemetery.

Results of this analysis include not only the seriation. The analysis provides a guide for choosing future radiocarbon samples, demonstrates the absence of any hiatuses in the use of the site and shows that morphological variation in the skeletal material is not sensitive to time. Further the temporal framework allows many other aspects of the Oxboe culture to be examined.

In conclusion the work here achieves its goal. That is a simple, inexpensive technique for fluoride analysis is provided. The technique is applied to a problem which makes the greatest use of the derived information.

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CHAPTER 1:

INTRODUCTION

The analysis presented here is designed to demonstrate a technique for analysing fluoride content in human remains for purposes of archaeological dating. The technique used here is relatively simple in comparison to those more commonly used and the necessary instruments are readily available. A review of the literature during an earlier study (Callaghan, 1976a; 1976b) indicated that fluoride analysis, and chemical analysis for dating in general had been largely ignored by North American archaeologists.

The disuse of these dating methods in North America may be attributed to such highly critical reviews as that of McConnell (1962). The failure of fluoride analysis to produce valuable information in McConnell's (1962) review stems not so much from a general fault of the concept of chemical dating, but from the type of problem to which this type of analysis had been applied. The examples cited by McConnell (1962) are all "problematic" to begin with. The potential for fluoride analysis in conjunction with C^{14} dates to sequence individuals from ossuary sites seems to have been overlooked until recently. I felt that if the value of this type of analysis could be demonstrated at a low cost this tool, useful to both the archaeologist and physical anthropologist, would be taken advantage of more often.

The ion specific electrode techniques used here are all reported in the literature of analytical chemistry (Bagg, 1976; Frant and Ross, 1968; Mesmer, 1968; Singer and Armstrong, 1968). With the exception of Singer and Armstrong (1968), none of the reports are concerned with calculating fluoride contents in bone and no report is concerned with

archaeological materials. Two key problems in technique arose because reports were not concerned with the specific problem presented here. Methods used to put bone fluoride into solution and to overcome effects of contaminants picked up during interment had to be developed through experimentation. It also had to be established what constituted a contaminant when using this particular analytical technique. Fortunately, the required information was, for the most part, in the literature. Experimentation then was largely a matter of incorporating a number of modifications into the basic fluoride determination technique used by Singer and Armstrong (1968). The result of those modifications presented here is specifically designed for analysing bone recoveries from archaeological sites.

The Gray Site is an ossuary site belonging to the Archaic Oxbow Complex of the northern Plains in southwestern Saskatchewan (Figure 1). The site was considered as a subject because of its large sample of human remains, the number of differing lines of research being conducted which could benefit from a sequencing of the skeletal remains, and its accessibility. The prospect of comparing a time sequence with morphological data was particularly intriguing.

In order to fully appreciate the value of this analysis a brief discussion of the Oxbow Complex and the Gray Site is included. The type site for the complex is the Oxbow Dam Site (Nero and McCorquodale, 1958) in southeastern Saskatchewan. The complex is defined largely by the presence of the eared Oxbow projectile point and while Gibson (1980) calls this point type "distinctive" there is some disagreement. Shutler (1980) regards Dyck's (1977) illustration of the Oxbow projectile point type as a non-diagnostic style of point.

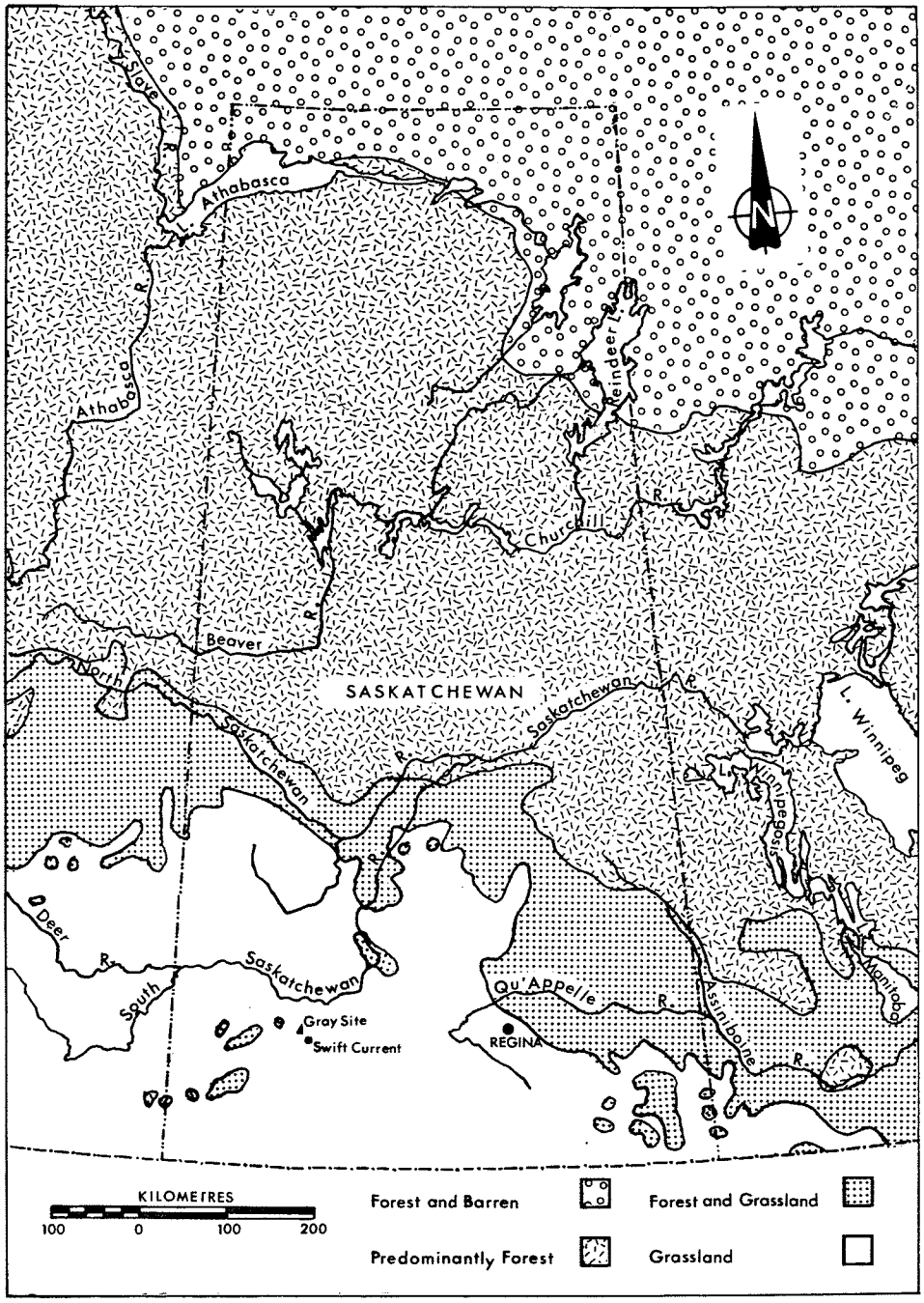

FIGURE 1 : SITE LOCATION AND VEGETATION ZONES

The Oxbow Complex is found spread across the northern Plains. Some "Oxbow" projectile points have been found in the boreal forest and some, which Millar (1978) considers acceptable, from North Carolina. Much of the distribution is based on the Oxbow projectile point type alone and given Shutler's (1980) argument should be considered tentative. The temporal range is from 3150 B.C. to 965 B.C. and Millar states that, "(t)here are no evident geotemporal clues that would give any hint of movement or expansion ..." (1978). Buchner (1980) would disagree with this view, seeing "... a grassland orientation for earliest Oxbow with a trend towards increasing utilization of the forests to the north over time." This view is based on securely dated Canadian sites from the grasslands, aspen parkland and boreal forest.

The bison, hunted primarily by means of jumps or traps, was the major food source of the Oxbow peoples with remains of elk, wolf, coyote, dog, fox, rabbit, marten, goose, frog and clams also found in habitation sites (Buchner, 1979; Dyck, 1977; Haug, 1976; Wettlaufer and Mayer-Oakes, 1960; Nero and McCorquodale, 1958). Some evidence for seasonal fishing also exists (Millar, 1978). The seasonal round of the Oxbow peoples ranged from hunting bison on the grasslands during summer to hunting bison on the forest margins of the aspen parkland in the winter. Buchner (1980) hypothesises that shifts in climate from the Atlantic Episode to the Sub-Boreal Climate prevented bison from gaining the shelter of the forest fringe during "anomalous winters". This may also have prevented Oxbow peoples from returning to the grasslands in order to obtain bison. This may have led to an increasing adaptation to forest conditions making it unnecessary to return to the grasslands during the summer season.

The Gray Site is the only excavated cemetery site belonging to the Oxbow Complex (Millar, 1978). The general environmental setting (Figure 1) of the Gray Site is as follows.

> The Gray Site is located approximately eight kilometers northwest of the city of Swift Current in the southwestern corner of the province of Saskatchewan, Canada. It lies within the grasslands zone of the northwestern plains, well south of the ecotone with the parkland zone which forms the northern limit of the grasslands from southwestern Manitoba, across Saskatchewan and through central Alberta. (Millar, 1978)

The soils of the area belong to the Brown Soil Zone, typifying semi-arid temperate grasslands. The site is in a band of sandy soils that extends in a southeasterly direction. The sand deposits are the product of aeolian reworking of outwash sediments. The flora of the area is grassland between true prairie and short-grass prairie. There is considerable local variation in ground cover.

Topographically, the Gray Site is located on a very gently sloping hillside, with a southern exposure, on the west side of an extinct glacial meltwater channel. The sandy soils of the site have undergone deflation at an exaggerated rate over the last fifty years. In some places arcuate sand dunes are formed along fence lines.

The estimated number of burials recovered is 308. Millar's (1978) estimate of the cemetery area is 134 square meters. The density of burials within this area ranged from one individual in two or three square meters to three or four individuals per square meter. Disturbance on the site is due to four factors: intrusions of later burials into earlier ones, rodent burrowing, cultivation, and "potting". The last two factors, being very recent in terms of the overall time scale, are unlikely to have affected F^-/P_2O_5 ratios to any detectable degree.

Intrusive burial-pit excavation should not have an effect either, as any exposed burials seem to have been immediately covered. Neither does it seem possible for rodent activity to have affected F^-/P_2O_5 ratios. The only way any of these factors could significantly affect this analysis is through mixing of remains. Having a great deal of confidence in the osteologists who separated individuals and by including only the most complete individuals in my sample, I am sure that post-interment disturbance is not a problem.

Several factors have been postulated by Millar (1978) as reasons for the site location. The general region was favourable to Oxbow peoples as evidenced by nearby Oxbow habitation sites. The southern exposure, sandy soil, and gentle slope are conducive to early spring thawing. Soils allow easy excavation. The hillside is above ponding water. It is visible from much of the surrounding area but not from known habitation sites of the period. It is the closest area of sandy soil located down-wind from the habitation sites.

Some preliminary consideration of conditions at the Gray Site had to be made before full scale analysis began. It had to be established that the soil matrix was fairly consistent and that the burials were not widely separated. Fluoride activity can vary greatly with different soil matrices. This is largely due to the mobility of fluoride in solution. For instance, though buried at the same time, bone interred in clay will have less fluoride than bone in sand simply because fluorides in solution cannot move through the non-porous clay as easily as through sand. Therefore, it is crucial that all materials considered for this type of analysis come from soil matrices with similar porosity. In the same manner interments at a distance from each other may be in soils

with greatly differing drainage patterns. This again would result in different fluoride environments making comparisons of age impossible. Since both conditions of homogeneous soil matrix and close proximity are met at the Gray Site, analysis proceeded. At this time other forms of chemical analysis, nitrogen in particular, were investigated should fluoride have proven inapplicable. Fluoride analysis was found to be the better test as pre-burial treatment of exposure on scaffolds and recent exposure by wind deflation are not significant factors when using a ratio of fluoride to phosphate. Nitrogen content, on the other hand, could be greatly influenced by exposure.

Some idea of the actual range of fluoride levels in the Gray Site materials had to be obtained. This range had to be wide enough to show differences within the limits of sensitivity of the instruments used. Nine individuals were chosen for a preliminary calculation of fluoride levels. The results ranged from .021% F^- to .265% F^-. This range was considered very satisfactory for a full scale analysis.

The sample was chosen to coincide as closely as possible to samples used in morphological analysis (Vyvyan, 1977). A sample size of 100 was decided upon; large enough to demonstrate trends without being too large to handle while some experimentation was still in progress. One individual, #84, was added to the sample because a C^{14} date became available during the later part of the study.

CHAPTER 2:

A GENERAL DISCUSSION OF FLUORIDE ANALYSIS

I. The Fossilization Process

Upon interment, bone matter is subject to changes in total quantity of its constituents. The changes may involve either an increase or decrease of constituent substances, or the introduction of substances generally foreign to living bone. The factors producing changes are the chemical and physical environments within which the bone is interred and the concentrations of the reactants themselves. Fossilization is the blanket term for these processes of change. It has been shown (Cook and Heizer, 1953; Overweel, 1965) that fossilization processes are a logarithmic function of time, but that quantitative generalization is not possible. The three main environmental factors responsible for the rate of fossilization are concentration of solutes, available water and the temperature. There exist numerous types of chemical analysis available to the archaeologist attempting to determine the antiquity of bone. All of these are of use only for relative dating due to the complexity and localization of fossilizing processes; that is, an absolute temporal location cannot be assigned to a particular specimen with any degree of certainty. Given very similar environmental conditions and the same original constituents at the time of interment, bone material can be assigned a sequential position in relation to other specimens. In listing the main elements used in dating, one must draw particular attention to nitrogen, uranium, and fluorine, as the most widely relied upon by archaeologists. Besides these three substances, fats, citric acid, organic carbon, amino acids, and water content have been used with somewhat less success (Cook, 1960). No examination of

one constituent of bone is definitive in itself. This type of analysis is based on a statistical concept and only trends can be demonstrated. It is however, a useful first approximation of age. For an excellent review of chemical analysis of prehistoric bone, S.F. Cook (1960) should be consulted. Though two decades have passed since its presentation, the report is still one of the best general discussions of the subject in print. It is largely from that source, that the above information has been summarized.

II. Historical Sketch of Fluoride Analysis

The chemical analysis of fluoride content in prehistoric bone has a very long history in relation to most archaeological dating techniques. Probably the only technique older is stratigraphic observation. Oakley (1953) attributes the discovery that fluoride is deposited in buried bone material (ivory) to two French chemists (Foueroy and Vauquelin) in 1806. Cook (1960), credits the first work on bone fluorine to Morichine in 1802. Regardless of the exact date, fluoride analysis of bone is nearly two centuries old. While scientists in other countries, notably France and the United States, worked on the problem, the greatest nineteenth century contribution was that of the English chemist, Middleton, who in 1844, published "On Fluorine in Bones: Its Sources and Its Application to the Determination of the Geologic Age of Fossil Bones", in the Proceedings of the Geological Society of London 4: 431-433 (cited in Cook, 1960, and Christie, 1973). At this time Middleton assumed a linear relationship between fluoride content and geologic age. It was later found that the relationship was not linear and the archaeological use of fluoride analysis fell into disuse.

In 1948, Oakley revived the technique. He had at his disposal

analytical techniques capable of reliably determining very small concentrations of fluoride in bone (Willard and Winter, 1933). The original technique of Willard and Winter was improved upon by Armstrong in 1936. It was principally Oakley and Hoskins' 1950 publication, "New Evidence on the Antiquity of Piltdown", that stimulated further modifications of the basic technique (Hoskins and Fryd, 1955) and the use of radiographic techniques (Niggli, E., C.J. Overweel and I.M. Van der Vlerk, 1953).

Since Oakley revived fluoride analysis of bone, it has been widely applied, though to a much greater degree by European and British archaeologists than by their American colleagues. A few of the best known cases follow.

Piltdown Man:

> The disclosure of the Piltdown fraud is undoubtedly the most celebrated use of fluoride analysis in archaeology. The fraud involved the discovery of a human cranium with a simian-like mandible in association with extinct elephant, hippopotamus, horse, and beaver bones. In 1913, knowledge of fossil hominid variation was limited. As more and more early Pleistocene hominid finds were documented, Piltdown Man became increasingly anomalous. By 1953, it was, as put by McCown and Kennedy, "... with considerable relief to the scientific community that the discovery of its fraudulency forced its removal from the human family tree. Its loss has grieved no one .... (1972)". Fluoride analysis showed the questionable materials to have a much lower fluorine content than the Middle Pleistocene mammal bones. The actual

figures were 0.1-0.4% and 1.9-3.1%, respectively (Oakley and Hoskins, 1953). Further tests concluded that the skull was human, dating from the Neolithic or Bronze Age, the mandible was that of a modern orangutang, while the elephant and hippopotamus remains were from Tunisia and Malta (Oakley, 1955). Finally both calvaria and mandibles were submitted for radiocarbon dating showing both to be of Holocene origin. The dates are 620 ± 100 BP (GrN-2203) for the calvaria and 500 ± 100 BP (GrN-2204) for the mandibles (Oakley, Campbell and Molleson, 1971).

Fontechevade:

These discoveries were made in France in 1946. The skull fragments were recovered in association with Acheulean tools from a stratified deposit dating to the third interglacial. Above the Acheulean strata was a Mousterian assemblage with an Upper Paleolithic assemblage above that. A comparison of fluoride contents of the skull fragments and remains of animal species from the third interstadial demonstrated contemporaneity. Bones from the upper strata had a lower fluorine content thus ruling out the possibility of the skull fragments being intrusive. (Howell, 1957)

Trinil:

Here the dispute has taken two directions. The first question is whether a Homo erectus skull cap and several femora from the Trinil beds of Java are contemporary. The second question is whether the femora actually belong to Homo erectus. Studies by Day and Molleson (1973) have shown the femora closer to

Homo sapiens than to undisputed Homo erectus finds at Olduvai (Hominid 28) and Homo erectus pekinensis. Fluoride analysis, however, seemed to indicate contemporaneity of the skull cap and femora with the Middle Pliestocene fauna at Trinil. Further investigation indicates that in the very high fluoride environment of the volcanic soils high levels of fluoride are reached quickly making it impossible to confirm or dispute contemporaneity. These consistantly high fluoride levels do add some strength to the common provenience of specimins, another disputed point since some of the femora were assigned to the Trinil beds some 30 years after excavation (Day and Molleson, 1973).

Galley Hill:

This find is from Swanscombe gravels near the Thames River in Britain. These gravels also contain Acheulean hand-axes. This led to the conclusion that "modern" Homo sapiens existed in the Middle Pleistocene. Fluoride analysis by Oakley and Montague in 1948 showed that the human remains had a much lower fluoride level than other fossil mammalia from the same gravels. The conclusion that the human remains were intrusive and probably of Post-Pleistocene age was borne out by subsequent radiocarbon dating.

More recently the use of fluoride analysis in problematical cases has become common in the Netherlands. A current report by D.P. Boisscha Erdbrink, C. Meiklejohn and J. Tacoma (1979) shows probable contemporaneity between human and mammoth remains dredged from the Loo-Waard flatlands of the Rhine. Both fluoride and nitrogen analysis produced

remarkably close estimates. One caution in this particular instance is that the tests were conducted on bone and enamel; two materials which may not have the same rate of fluorine uptake.

In the United States, success with fluoride dating has been less impressive. This is probably more due to use in inappropriate cases than such factors as lack of time depth, as has been suggested by some authors (Christie, 1973). Very high fluoride contents can be arrived at in relatively short periods of time (Fleming, 1976). Two of the more classic New World cases are presented here.

Tranquility:

This find consists of five human burials in association with horse, camel and bison remains. Fluoride analysis conducted by Heizer and Cook (1952) demonstrated an average of 0.15% fluorine for the non-human bone and an average of 0.20% for the human bone. This, along with several other chemical tests, led to the conclusion that the Tranquility human remains date from the Late Pleistocene.

Calaveras Quarry:

Again reported by Heizer and Cook (1952), this find consisted of human bone in association with extinct horse. Fluoride analysis on a human ulna showed concentrations 26 to 30 times less than in the associated horse remains. The conclusion being, that the human bone became associated with the horse much later than the extinction of horse in North America.

While fluoride analysis seems to have been abandoned until recently in North America, it is widely used in the Old World. This may be due

to a preference for the development of generally applicable absolute dating techniques and a failure to make full use of relative techniques. Meiklejohn (1973), using examples from his work with the Paleolithic/ Neolithic transition in Europe, has urged a greater use of such chemical dating techniques as fluoride in North America. A discussion of controversial fossil hominids that have had their position clarified by fluoride and other forms of chemical analysis is contained in Oakley (1964).

III. Techniques Used in Archaeology for the Determination of Fluoride Content

The technique used in Oakley's Piltdown work was developed by Willard and Winter (1933). This technique separates the fluoride from the rest of the bone matrix by distillation with perchloric acid. By the addition of a silicate, hydrofluorosilicic acid is formed. A precipitate of thorium fluoride is then produced when the released fluoride ion reacts with thorium nitrate in an aqueous solution. Using sodium alizarin sulfonate as the titration indicator, Armstrong (1933) measured amount of displaced nitrated by the intensity of the resulting pink color. Armstrong (1936) further demonstrated the use of this technique for microchemical analysis with almost no change in procedure. The error in this technique does not exceed 0.2% in a 5 mg sample. A final modification was made by Hoskins and Fryd (1955), allowing the detection of 0.0005 mg of fluoride. Thus the accuracy depends on the size of the sample used. Cook (1960) describes this technique as "... intricate, tedious and difficult .." as well as "... extraordinarily sensitive to the effects of acids, salts, and of course, extraneous fluoride ..."

Taking a totally different approach to the measurement of fluoride in bone, some researchers have turned to X-ray diffraction (Baud, 1960;

Niggli, Overweel and Van der Vlerk, 1953). The technique is based on differences in the cell size of the crystal lattice; i.e., fluorapatite and hydroxyapatite cells are of differing sizes. While the technique has seen much use, it has been criticized strongly by McConnell (1962) (erroneously cited as McDonnell in Fleming, 1976). Several other chemical changes may give the same results as the substitution of F^- for OH^-. Hancox (1972) states that, besides these other changes, poor crystalline structure may also be affected by alkaline solutions. Hancox also points out the difficulty in distinguishing between X-ray diffraction patterns of octa-calcium phosphate, tetra-calcium phosphate and calcium hydroxy-apatite, all of which may occur in fresh bone.

Gas chromatography has been used by Groff with great success and described by him as follows:

> To the organic chemist, the gas chromatograph has been a chemist's dream - a continuous magical tube into which a complex group of chemicals is placed and from which a completed analysis may emerge (1971).

Despite the above description and the rapidity of the actual analysis, Groff goes on to point out its drawbacks. The machinery must be kept running throughout the analysis. The separation column must be purged with the carrier gas, helium, for a minimum of thirty minutes after each sample is run. The drying columns must also be serviced or replaced frequently. Still, gas chromatography, as Groff states, is faster than the technique used by Willard and Winter (1933). Other disadvantages are the expense of the machine itself and the skill necessary to use it (Bodin and Cheinisse, 1970).

A simpler, less expensive, technique requiring less skilled personnel is desirable for archaeologists to take greater advantage of

fluoride analysis. The use of ion-selective electrodes seems to be the answer. These are now available for the detection of a wide range of elements. Superficially they resemble standard electrodes for the measurement of pH. They are a tube filled with a specific ion solution sealed by a membrane, permeable only to that specific ion. The electrical contact in the sample solution is completed by a standard reference electrode. The fluoride ion-specific electrode was announced in 1966 by Frant and Ross. The operating principle is an ionic semi-conductor crystal of lanthanum fluoride, producing a potential in the millivolt range, proportional to fluoride ion activity in the sample solution. The electrode system is coupled with a pH meter having an expanded millivolt scale. The actual operation of the instrument is the same as in regular pH testing systems. (Anonymous, n.d.)

The necessary pH meters and electrodes are now common in most chemical laboratories. The cost of such equipment is small in comparison to other analytical instruments. The preparation of bone sample is also quite simple. The greatest problem involved is the extreme care necessary in the measurement of samples and reagents. This is due not to any complexity in procedure, but to the small amounts of fluoride being dealt with.

IV. Environmental and Chemical Factors Influencing Formation and Measurement of Fluorapatite

The basis of fluoride analysis in archaeology is the replacement of the hydroxyl ion (OH^-) in hydroxyapatite by the fluoride ion (F^-) from the environment. The resultant fluorapatite is harder to break down without the actual destruction of the apatite crystals. The theoretical formulae for hydroxyapatite and fluorapatite are

$Ca_{10}(PO_4)_6(OH)_2$ and $Ca_{10}(PO_4)_6(F)_2$. Fleming (1976) has mistakenly given these formulae as $Ca_5(PO_4)_3OH$ and $Ca_5(PO_4)_3F$ (Hancox, 1972). The proper expressions are the former as these are formulae for the "unit cell" or "... smallest arrangement of the crystal that can be found repeated in the same arrangement and ratio throughout the whole crystal" (Hancox, 1972). Environmental factors strongly influence fluorine uptake. Not all factors operate the same at all sites. These differences in effect necessitate a careful consideration of the conditions at each site individually.

The consistency of the soil matrix is the greatest factor operating on fluorine uptake. As mentioned above, the mobility of the fluoride ion in solution differs widely from one soil type to another. Soil types may change several times over a few hundred feet horizontally and changes may occur within centimeters vertically.

Interment below the watertable for a substantial period of time has been assumed prerequisite for fluoride analysis. This is obviously not the case at the Gray Site. Some means of accounting for the high levels of fluoride found in preliminary testing of Gray Site material was necessary. Consultation with Dr. W. Rannie, a hydrologist with the University of Winnipeg's Geography Department provided an answer. Capillary action would concentrate fluorides near the surface. The best conditions for this would be a hot dry climate with silt-like soils. An alkaline soil would indicate that such capillary action has been taking place.

Several chemical properties of soil may effect fluoride uptake. In basic soils chlorine as well as fluorine can replace the hydroxyl ion (Cook, 1960) reducing fluoride content. Volcanic soils may be so

rich in fluorine that bone fluorine quickly reaches very high levels, obscuring differences due to time.

The homogeneous nature of the soils (Millar, et al., 1972) at the Gray Site eliminates or reduces the effect of these factors. There are no differences in soil type either horizontally or vertically. The compact spacing and similar depths are also favourable to the analysis. The soil at the Gray Site is basic but chlorine interference would have acted as a constant over time and can be ignored. The range of fluoride contents obtained for the preliminary tests given above shows that these factors are not a concern in this particular case. Were the soils volcanic, the preliminary test would have indicated the applicability of the analysis. Sample selection and treatment can also affect results. In the measurement of fluoride content, bone density can affect results. Differences in density may be due to variations in the type of bone, the process of fossilization, and the method of ashing the samples. The choice of bone should be consistent. All samples should be either compact or cancellous bone as slight differences in fluoride content may be due to differences in bone density. In this analysis, all samples were compact bone. The author (1976a) conducted preliminary tests between cancellous and compact bone from a single individual and found no significant differences. This result however, may have been because the individual tested was a young child. Hancox (1972) states that bone composition in newly forming bone is not the same as in mature bone. This is the reason for attempting to use only adult skeletons in the analysis presented here. Fossilization alters the density of bone, primarily through replacement of organic matter and mineralization. Nothing can be done in the selection of a sample to compensate for this.

Finally, ashing of the sample causes loss of organic material, water, and carbonate. It seems reasonable that weights of bone given must be for ashed bone rather than whole bone, and that ashing be done at precisely the same temperature and for the same duration. Cook (1960) advocates the use of F^-/P_2O_5 ratio in order to compensate for differences in bone density. This ratio has also been recommended by Brothwell (pers. comm.) to provide "... an index of age relative to stratigraphic environment." The maximum theoretical value for this ratio in fresh bone is 9, but in some cases this may be exceeded (Flemming, 1976). Values greater than 9 are probably due to dilution of the phosphate concentration by the effects of CO_3 present in alkaline soils as found at the Gray Site.

Consideration must be given to the particular limits and conditions for optimum use of the equipment. The fluoride ion electrode measures fluoride concentrations in the range of $1\text{-}10^{-6}$ molar (M). It must be pointed out that the electrodes actually measure ion activity rather than concentration. Because of this a concentration curve is usually calculated from the standard solutions and from the activity coefficients provided by the manufacturer with the electrode. However, in this case fluorine contents were in the $10^{-4}\text{-}10^{-6}$ M range. At these levels the activity coefficients are 1.0. The activity curve and concentration curve coincide, eliminating the need for calculation. Electrode manuals must be consulted for this information.

Several sources of interference are possible with the fluoride electrode. The only direct ionic interference with the determination of fluoride is from the hydroxyl ion (OH^-). It is however only in solutions with a pH above 8 that this interference becomes a problem.

Further, even given a pH of 8, no interference results in solutions with greater than 10^{-6} M fluoride concentrations. (Anonymous, n.d.)

Because the electrode measures only the activity of free fluoride ions, agents which bond with fluoride must be considered. In acid solutions H^+, Al^{3+} and Fe^{3+} reduce fluoride ion activity. Hydrogen ions do not bond fluoride ions when the solution pH is greater than 4. (Anonymous, n.d.)

In order to overcome these problems a buffer (Total Ionic Strength Adjustment Buffer, "TISAB") was used (Frant and Ross, 1968). The buffer prevents aluminum and iron interference and balances the pH of solutions within bounds where OH^- and H^+ ions are not a problem. Aluminum could be introduced into the bone from the soil, particularly when interred in clay. Iron may also be introduced from the soil, but the ritual use of red ochre (ferric oxide) in many burials is likely to be the larger contributor. The use of the buffer necessitated several changes in procedure. The standards were prepared with distilled water rather than a solution of sodium chloride, sodium acetate, and acetic acid. The samples were dissolved and partially neutralized as described by Singer and Armstrong (1968), but the pH was not adjusted further. The 1:1 mixture of both standards and samples with the buffer standardizes the pH as well as eliminating interference from iron or aluminum contaminants.

Measurements of the association between Na^+ and F^- suggest the formation of significant quantities of NaF^o in "TISAB" (Robinson, Duer, and Bates, 1971; Butler and Huston, 1978). The result is again a lowering of fluoride ion activity. Bagg (1976) suggests that 15-20% of fluoride in "TISAB" solutions prepared from sodium salts is present

in the form NaF^{o}. Potassium salts greatly reduce this problem. Further, Mesmer (1968) found less fluoride contamination in potassium salts than in sodum salts. These experiments prompted the replacement of all sodium compounds with potassium compounds in the analysis.

CHAPTER 3:

TECHNIQUES AND PROCEDURE

I. Combustion of Bone Samples

Previous work by the author (Callaghan, 1976a) utilized the technique described by Singer and Armstrong (1968) with only slight modifications. Sample weights and reagent volumes were all increased by a factor of ten in order to simplify handling. It was found that burning the bone samples at 550° C overnight was inadequate. A temperature of 800° C for 8 hours was found to be sufficient. The higher temperature is still below the range where changes in relevant chemical composition would take place (Overweel, 1965).

II. Determination of Phosphate Content

A colorimetric technique for the determination of P_2O_5 was decided upon after consultation with Dr. Racs from the University of Manitoba's Soils Sciences Department. The technique is described by Cornwall (1958) and makes use of the molydenum blue reaction. While the technique is intended for soils analysis, Dr. Racs affirmed its suitability for analysis of bone ash, the only caution being that the relatively high phosphate content, 42% in fresh bone (Fleming, 1976), calls for considerable dilution. A Bausch and Lomb Spectronic colorimeter was used for the analysis of phosphate.

(a) Preparation of Lorche's developer

Ammonium Molybdate	12.0000 g
Sodium Sulphite	10.0000 g
Hydroquinone	0.5000 g
Sulphuric acid (conc.)	45 ml

The reagent grade, Ammonium molybdate, sodium sulphite, and hydroquinone were dissolved in approximately 500 ml of distilled water. Added to this was the reagent grade sulphuric acid. The solution was brought up to 1 liter with distilled water in a volumetric flask.

(b) Preparation of phosphate standards

Standards were prepared from Fisher 1000 ppm phosphate stock solution diluted with Lorche's developer in the following manner:

2.5 ml P stock dilute to 250 ml = 10 ppm

80 ml 10 ppm dilute to 100 ml = 8 ppm

60 ml 10 ppm dilute to 100 ml = 6 ppm

40 ml 10 ppm dilute to 100 ml = 4 ppm

20 ml 10 ppm dilute to 100 ml = 2 ppm

10 ml 10 ppm dilute to 100 ml = 1 ppm

(c) Test for linear relationship of standards

The wavelength selector on the Spectronic 20 was set at 621, this setting being optimal for phosphate determination as given by Cornwall (1958). The amplifier control was set at 0% with the cuvette receptacle closed. The light control was set at 100% with the receptacle holding a cuvette of Lorch's developer. Each standard solution was then tested in the colorimeter using the same cuvette for each solution in order to avoid variation in cuvette structure. The results read from the absorption scale were as follows:

1 ppm	=	.092 absorption
2 ppm	=	.232
4 ppm	=	.459
6 ppm	=	.71
8 ppm	=	.97
10 ppm	=	1.28

When graphed, an acceptable degree of linearity was demonstrated.

(d) Sample preparation

Because of the large sample size, it was not possible to prepare or test all the samples at one time. Also when large numbers of samples are tested the standards must be rerun periodically. Rerunning the standards checks for internal drift in the instruments themselves. This is true for both phosphate and fluoride determinations. For those reasons the sample was broken down into sets. Set size only reflects the maximum number of individual samples that could be prepared or tested per day. Rather than 1 g of sample bone ash as indicated by Cornwall (1958), 0.5 g was used and all solutions likewise halved to avoid excessive use of materials. By halving both sample size and liquid measures the same sample/solution ratio is maintained. A solution of 3N H_2SO_4 was made by adding 300 ml reagent grade 10 N H_2SO_4 to approximately 500 ml distilled H_2O and brought up to 1 liter with distilled H_2O in a volumetric flask. Each bone sample was crushed in a mortar and 0.5000 g weighed into a test tube. To each sample 10 ml 3N H_2SO_4 was added, followed

by immersion in a boiling waterbath with occasional shaking for 15 minutes. The sample was then filtered and 2.5 ml of the filtrate mixed with 10 ml Lorche's developer. The samples were returned to the waterbath for 15 minutes. A suitable dilution factor of 1/200 was determined by trial and error. Using a pipette 0.1 ml of each sample was diluted to 20 ml with Lorche's developer.

(e) Testing of samples

After preparation of each set of samples, the colorimeter was adjusted and the standards tested as in section (c). Again the same cuvette was used for each set to avoid optical variation. Tables 1-4 contain the absorption figures for each standards test and the corresponding set of samples. While in all sets the higher standards fell outside the upper limits of the Spectronic 20's scale, this was of no consequence as none of the samples were within this range.

(f) Calculation of phosphate content

A graph (figure 2) was drawn from the standards and the phosphate content for the corresponding set of samples read from this. Two of the samples fell below the lower limit of the standards but were so close that they were retained. These two samples were 76II and 36II. The calculation of P_2O_5 in 1 g bone ash is as follows:

(1) Cx = solution concentration read from graph

(2) Cx X 200 = solution concentration multiplied by dilution factor

(3) Cx X 200 X (12.5/2.5) = ppm in 2.5 ml filtrate

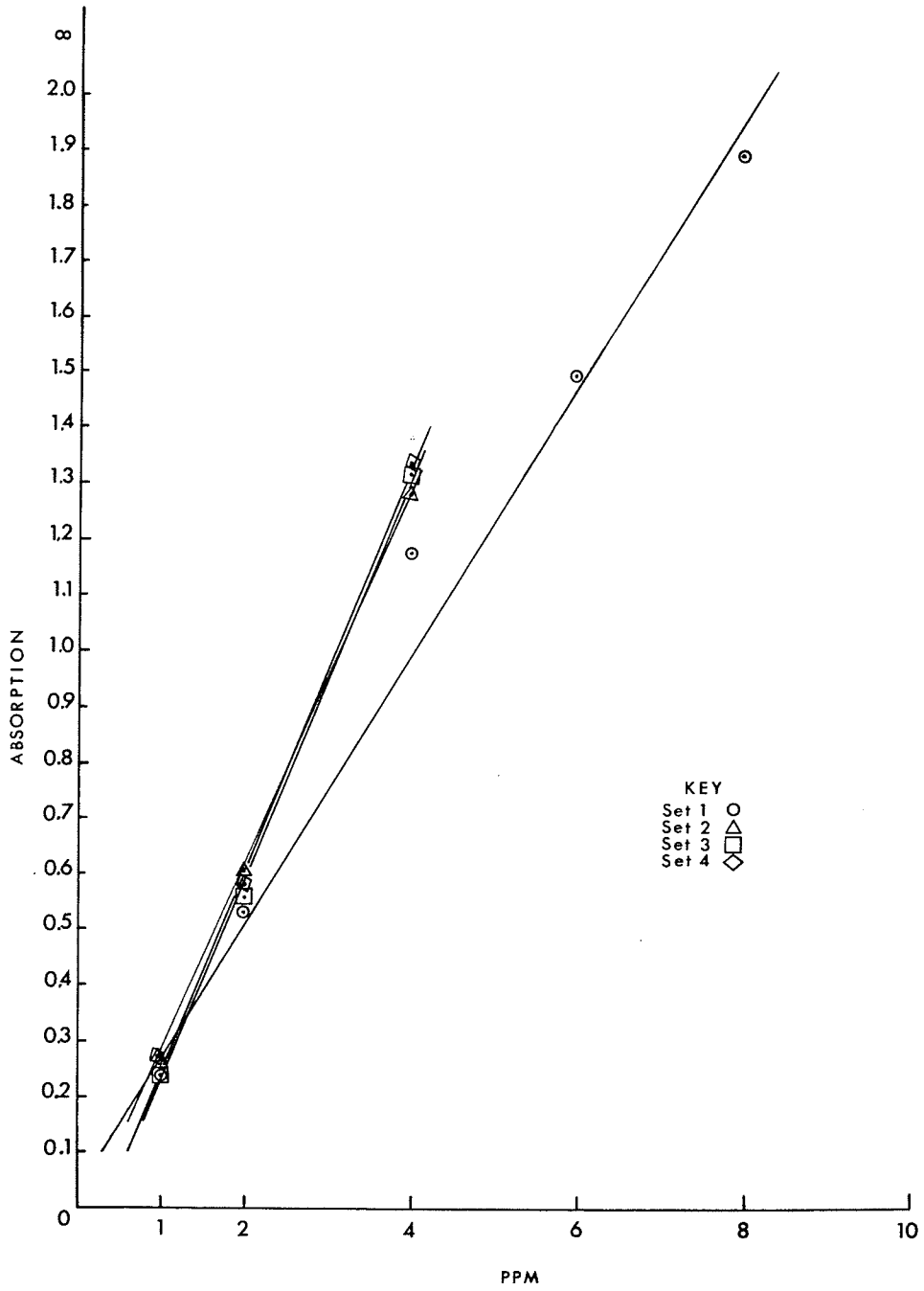

FIGURE 2 : PHOSPHATE STANDARDS

(4) Cx X 200 X (12.5/2.5) X 10 = wt. P_2O_5 in mg. in 10 ml H_2SO_4

(5) Cx X 200 X (12.5/2.5) X 10 X 2 = wt. P_2O_5 in 1 g bone ash

This reduces to Cx X 20,000.

These calculations are given in table 9.

III. Determination of Fluoride Content

The instruments used were a Fisher Accumet model 520 pH/ion meter coupled with a Coleman fluoride ion-selective electrode and a Radiometer model 29b pH meter with a combined glass-calomel electrode, type GK 2311 C.

(a) Preparation of total ionic strength adjustment buffer ("TISAB")

This solution was prepared according to instructions given by Frant and Ross (1968), substituting potassium compounds for those containing sodium as suggested by J. Bagg (1976).

Glacial acetic acid	57 ml
Potassium chloride	74.56 (g)
Potassium citrate	0.324 (g)
5 M potassium hydroxide	140.275 (g) diluted to 500 ml H_2O

Reagent grade glacial acetic acid, potassium chloride, and potassium citrate were combined in approximately 500 ml distilled H_2O and the pH adjusted to 5.1 by the addition of 5 M KOH using the Radiometer pH meter. The solution was then topped up to 1.1 with distilled H_2O in a 1000 ml volumetric flask and allowed to cool before use.

Table 1. SET I Phosphate Absorption

Standards:	Absorption
1 ppm	.24
2 ppm	.53
4 ppm	1.18
6 ppm	1.5
8 ppm	1.9
10 ppm	(reading off scale)
Burial No.	
36 V	.461
35 I	1.05
G8B3	.67
64 I	1.16
73 II	.97
66 II	.678
G3B1 II	.505
37 VII	.58
57 I	.661
72 II	1.05
57 III	.565
G3B6 V	.46
G3B1 I	.32
G13B6 II	.5
G3B2 III	.632
40 III	.661
82 III	.658
79 I	.8
G13B5	.91
58 IV	.607
59 IX	.521
35 V	.548
G3B6 IV	1.0
70 I	1.2
G3B3 II	1.4
G13B1	.542
80 III	1.5
62 I	.306
G3B6 III	.52
67 I	.915
36 I	.72

Table 2. SET II Phosphate Absorption

Standards:	Absorption
1 ppm	.272
2 ppm	.608
4 ppm	1.29
6 ppm	(2. + off scale)
8 ppm	(reading off scale)
10 ppm	(reading off scale)
Burial No.	
73 I	.78
37 III	.70
59 V	.618
61 I	.565
61 II	.559
G13B6 III	.75
68 I	.80
37 VI	.521
41 I	.58
83 II	.681
35 IV	.50
62 II	.384
76 II	.228
35 III	.54
59 X	.49
42 II	.275
47 V	.28
88 I	.32
35 II	.63
59 IV	.66
58 II	.52
71 III	.521
76 I	.585
59 III	.535
69 I	.528
G8B1	.341
G3B4 III	.36
91 II	.64
71 I	.46
58 III	.552

Table 3. SET III Phosphate Absorption

Standards:	Absorption
1 ppm	.24
2 ppm	.56
4 ppm	1.35
6 ppm	1.75
Burial No.	
50 I	.42
34 II	.315
47 IV	.40
39	.31
51 I	.428
42 I	.238
44 IV	.27
65 II	.46
33 V	.448
87 I	.525
32 II	.202
34 III	.433
23 XII	.642
48 I	.47
55 I	.461
EcN 1a 39	.41
G13B6 I	.495
27 I	.54
33 IV	.561
81 VI	.39
53 I	.408
63 I	.419
54 IV	.67
56 V	.50
80 I	.46
36 II	.465
45 III	.25
G3B31	.381
78 I	.47
58 V	.281

Table 4. SET IV Phosphate Absorption

Standards:	Absorption
1 ppm	.272
2 ppm	.58
4 ppm	1.39
6 ppm	1.75
Burial No.	
43 I	.32
29 II	.498
65 I	.278
46	.549
52 II	.45
49 I	.58
72 I	.3
33 VII	.442
23 XIV	.4
84	.8

(b) Preparation of standards

A 1000 ppm fluoride stock solution was made from potassium fluoride. In 500 ml distilled H_2O, 4.951 g KF were dissolved and then brought up to 1000 ml with distilled H_2O in a 1000 ml volumetric flask. Dilution of the F^- stock solution to the appropriate standard concentrations with distilled water is shown below.

10 ml stock diluted to 100 ml	- 100.0 ppm F^-
1 ml stock diluted to 100 ml	- 10.0 ppm F^-
1 ml 100 ppm F^- diluted to 100 ml	- 1.0 ppm F^-
1 ml 10 ppm F^- diluted to 100 ml	- 0.1 ppm F^-

Each standard was mixed 1:1 with the "TISAB" and placed in Nalgene storage bottles.

(c) Preparation of samples

Between 40 and 60 mg of each sample was weighed into a Nalgene beaker. To each sample 10 ml 0.25 M hydrochloric acid was added. The solutions were then agitated until each sample was fully dissolved (30 - 60 min.). The samples were partially neutralized by the addition of 11 ml 0.125 M potassium hydroxide. The resultant solutions were brought up to 50 ml and diluted 1:1 with "TISAB".

(d) Calculation of fluoride content

Standards were tested with the Fisher Accumet 520 before and after each set of samples. Both samples and standards were stirred with a magnetic stirrer for 10 minutes before the electrode potential was determined. Using the average Mv reading of the two standards tests a calibration graph was

constructed on semi-log paper (figure 3 and tables 5-8). The multivolt readings were plotted along the arithmetic scale while the molarity of the standards was plotted along the logarithmic scale. The molarity of each standard is as follows:

$$100 \text{ ppm } F^- = 5.26 \times 10^{-3}$$

$$10 \text{ ppm } F^- = 5.26 \times 10^{-4}$$

$$1 \text{ ppm } F^- = 5.26 \times 10^{-5}$$

$$0.1 \text{ ppm } F^- = 5.26 \times 10^{-6}$$

Since both standards and samples were diluted 1:1 with "TISAB" compensation is not necessary. At lower levels of fluoride content some compensation may be required (Frant and Ross, 1968). The sample Mv readings were then plotted and the molarity determined. From this the actual fluoride content in 1 g of bone ash was calculated.

Sample calculation:

$$\text{Molarity} \times 19 \times \frac{50}{1000} = \text{wt. } F^-(g) \text{ per sample}$$

$$\frac{\text{wt } F^-/\text{sample}}{.0500 \text{ gm (sample wt.)}} \times 10_m^6 = \text{ppm } F^-/\text{sample}$$

These calculations are shown in table 9.

(e) Calculation of F^-/P_2O_5 ratio

The calculation of F^-/P_2O_5 is self evident from table 9. The multiplication of the fluoride content by 100 is merely to eliminate fractional values.

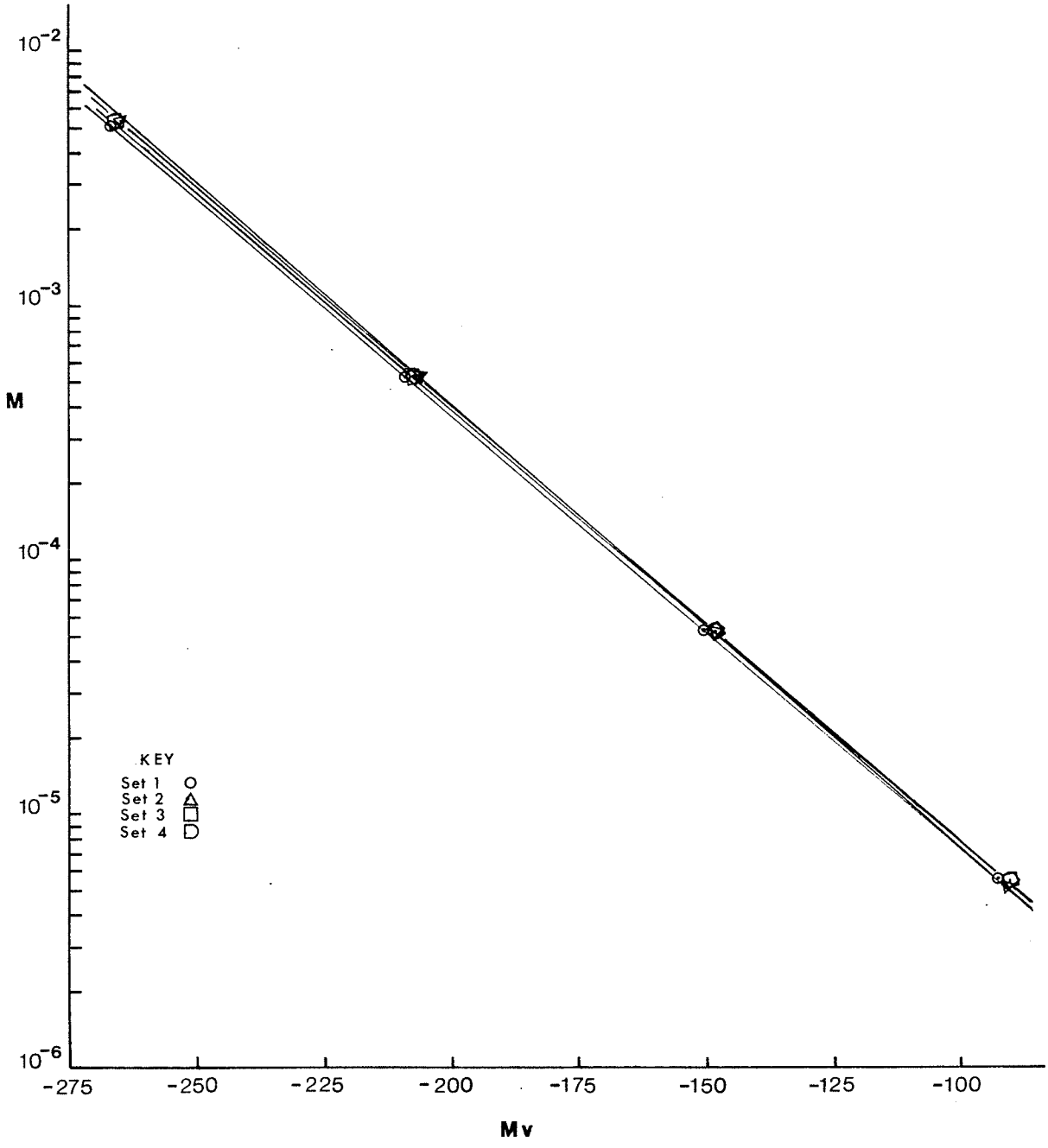

FIGURE 3 : FLUORIDE STANDARDS

Table 5. SET I Fluoride Concentration and Sample Weights

F^- Standards	-Mv	Molarity	Sample wt. (g)
100 ppm	265.9		
10 ppm	208.8		
1 ppm	150.6		
C.1 ppm	95.0		
Burial No.			
G8B3	195.5	3.1×10^{-4}	0.0442
57 I	176.4	1.35×10^{-4}	0.0440
G13B1	164.5	9.2×10^{-5}	0.0462
G3B1 II	156.8	6.6×10^{-5}	0.0400
57 III	171.5	1.2×10^{-4}	0.0412
70	160.5	7.6×10^{-5}	0.0472
G13B5	165.4	9.4×10^{-5}	0.0480
G3B1 I	169.9	1.1×10^{-4}	0.0479
80 III	185.5	2.1×10^{-4}	0.0446
G3B2 III	180.2	1.7×10^{-4}	0.0562
62 I	150.6	5.25×10^{-5}	0.0528
73 II	183.1	1.85×10^{-4}	0.0441
59 IX	164.8	9.2×10^{-5}	0.0572
66 II	156.1	6.4×10^{-5}	0.0441
79 I	157.7	6.8×10^{-5}	0.0452
G3B6 IV	162.7	8.2×10^{-5}	0.0485
G3B6 V	183.7	1.9×10^{-4}	0.0465
82 III	189.8	2.5×10^{-4}	0.0400
72 II	174.0	1.3×10^{-4}	0.0598
61 II	157.7	6.8×10^{-5}	0.0485
64 I	160.8	7.6×10^{-5}	0.0446
G3B3 II	156.9	6.6×10^{-5}	0.0444
67 I	150.9	5.28×10^{-5}	0.0411
35 V	153.5	5.8×10^{-5}	0.0438
G13B6 II	170.3	1.1×10^{-4}	0.0511
35 I	156.1	6.5×10^{-5}	0.0417
58 IV	198.2	3.4×10^{-4}	0.0543
40 III	201.7	3.8×10^{-4}	0.0430
F^- Standards			
100 ppm	265.7		
10 ppm	208.4		
1 ppm	150.8		
0.1 ppm	96.1		

Table 5 continued.

Average of F^- Standards

100 ppm	265.8
10 ppm	208.6
1 ppm	150.7
0.1 ppm	95.55

Table 6. SET II Fluoride Concentration and Sample Weights

F^- Standards	-Mv	Molarity	Sample wt. (g)
100 ppm	264.7		
10 ppm	207.1		
1 ppm	149.0		
0.1 ppm	91.1		
Burial No.			
83 II	181.5	1.9×10^{-4}	0.0570
58 III	188.5	2.5×10^{-4}	0.0492
59 III	189.4	2.6×10^{-4}	0.0438
37 VI	191.1	2.8×10^{-4}	0.0575
71 I	177.1	1.6×10^{-4}	0.0590
91 II	184.3	2.1×10^{-5}	0.0420
35 III	159.1	8.8×10^{-5}	0.0430
71 III	156.4	7.1×10^{-5}	0.0479
35 IV	180.3	1.8×10^{-4}	0.0411
73 I	165.5	1.0×10^{-4}	0.0495
76 II	172.9	1.35×10^{-4}	0.0471
33 VII	167.1	1.05×10^{-4}	0.0469
29 II	170.6	1.25×10^{-4}	0.0407
59 IV	173.6	1.45×10^{-4}	0.0518
41 II	156.9	7.1×10^{-5}	0.0483
23 XIV	155.2	6.65×10^{-5}	0.0432
72 I	179.7	1.8×10^{-4}	0.0432
37 III	190.1	2.7×10^{-4}	0.0582
46	180.1	1.8×10^{-4}	0.0454
69 I	191.2	2.8×10^{-4}	0.0423
43 I	169.1	1.2×10^{-4}	0.0400
59 V	157.0	7.2×10^{-5}	0.0498
58 II	184.0	2.1×10^{-4}	0.0532
52 II	161.9	8.8×10^{-5}	0.0425
G13B6 III	160.4	8.2×10^{-5}	0.0415
49 I	174.3	1.4×10^{-4}	0.0400
35 II	153.5	6.2×10^{-5}	0.0456
36 V	166.2	1.05×10^{-4}	0.0414
59 X	161.1	8.2×10^{-5}	0.0436
65 I	172.8	1.35×10^{-4}	0.0526

Table 6 continued.

F^- Standards	-Mv
100 ppm	265.6
10 ppm	207.8
1 ppm	149.5
0.1 ppm	91.9

Average of F^- Standards	
100 ppm	265.15
10 ppm	207.45
1 ppm	149.25
0.1 ppm	91.50

Table 7. SET III Fluoride Concentration and Sample Weights

F^- Standards	-Mv	Molarity	Sample wt. (g)
100 ppm	265.5		
10 ppm	207.4		
1 ppm	150.6		
0.1 ppm	92.2		
Burial No.			
34 III	169.9	1.15×10^{-4}	0.0466
34 II	167.1	1.0×10^{-4}	0.0516
23 XII	162.6	8.6×10^{-5}	0.0412
58 V	148.4	4.9×10^{-5}	0.0421
63 I	166.5	1.0×10^{-4}	0.0554
G13B6 I	138.6	3.3×10^{-5}	0.0417
44 IV	141.4	3.7×10^{-5}	0.0500
47 IV	169.4	1.1×10^{-4}	0.0411
32 II	155.1	6.4×10^{-5}	0.0562
51 I	136.9	3.1×10^{-5}	0.0409
33 IV	143.8	4.2×10^{-5}	0.0574
36 II	135.2	2.9×10^{-5}	0.0467
EcN 1a 39	142.9	3.9×10^{-5}	0.0529
27 I	153.4	6.0×10^{-5}	0.0462
81 VI	129.8	2.4×10^{-5}	0.0433
80 I	139.9	3.5×10^{-5}	0.0476
53 I	161.6	8.1×10^{-5}	0.0500
48 I	154.9	6.4×10^{-5}	0.0468
54 IV	143.1	4.0×10^{-5}	0.0541
65 II	147.6	4.8×10^{-5}	0.0400
33 V	147.6	4.8×10^{-5}	0.0560
87 I	138.5	3.3×10^{-5}	0.0516
39	165.5	9.6×10^{-5}	0.0507
45 III	135.2	2.9×10^{-5}	0.0432
55 I	138.1	3.3×10^{-5}	0.0421
42 I	124.9	1.95×10^{-5}	0.0535
G3B3 I	143.1	4.0×10^{-5}	0.0487
78 I	137.0	3.1×10^{-5}	0.0552
56 V	132.7	2.6×10^{-5}	0.0464
50 I	151.2	5.3×10^{-5}	0.0425

Table 7 continued.

F^- Standards	-Mv
100 ppm	265.1
10 ppm	206.6
1 ppm	151.2
0.1 ppm	90.2

Average of F^- Standards	
100 ppm	265.3
10 ppm	207.0
1 ppm	150.9
0.1 ppm	91.0

Table 8. SET IV Fluoride Concentration and Sample Weights

F^- Standards	-Mv	Molarity	Sample wt. (g)
100 ppm	264.9		
10 ppm	206.5		
1 ppm	148.4		
0.1 ppm	90.5		
Burial No.			
76 I	194.6	3.3×10^{-4}	0.0584
47 V	188.7	2.6×10^{-4}	0.0400
68 I	176.3	1.6×10^{-4}	0.0532
G3B4 III	179.6	1.8×10^{-4}	0.0470
88 I	174.4	1.5×10^{-4}	0.0479
G3B6 III	166.0	1.1×10^{-4}	0.0453
36 I	184.7	2.2×10^{-4}	0.0472
37 VII	166.8	1.1×10^{-4}	0.0495
G8B1	168.7	1.2×10^{-4}	0.0483
62 II	137.9	3.4×10^{-5}	0.0447
61 I	156.1	7.0×10^{-5}	0.0400
41 I	151.7	6.0×10^{-5}	0.0565
84	176.9	1.62×10^{-4}	0.0408
F^- Standards			
100 ppm	265.3		
10 ppm	206.1		
1 ppm	148.4		
0.1 ppm	90.3		
Average of Standards			
100 ppm	265.1		
10 ppm	206.3		
1 ppm	148.4		
0.1 ppm	90.4		

Table 9. Calculation of F^-/P_2O_5 Ratio

Burial No.	Cx (solution) ppm	wt. P_2O_5 (mg)	wt. F^- (g/sample)	ppm F^-/sample	100X F^-/P_2O_5	log 100X F^-/P_2O_5
G3B3 II	5.5	110,000	6.27×10^{-5}	1410	1.3	.11
56 V	1.75	35,000	2.47×10^{-5}	530	1.5	.18
78 I	1.7	34,000	2.945×10^{-5}	530	1.6	.20
54 IV	2.2	44,000	3.8×10^{-5}	700	1.6	.20
35 I	4.45	89,000	6.175×10^{-5}	1480	1.7	.22
42 I	1.0	20,000	1.853×10^{-5}	350	1.7	.24
87 I	1.75	35,000	3.135×10^{-5}	6610	1.7	.24
36 II	1.65	33,000	2.755×10^{-5}	590	1.8	.25
64 I	4.5	90,000	7.22×10^{-5}	1620	1.8	.26
81 VI	1.45	29,000	2.28×10^{-5}	530	1.8	.26
33 IV	1.9	38,000	3.99×10^{-5}	700	1.8	.26
70 I	3.95	79,000	7.22×10^{-5}	1530	1.9	.29
G3B6 IV	3.85	77,000	7.79×10^{-5}	1610	2.1	.32
G13B6 I	1.75	35,000	3.135×10^{-5}	750	2.2	.44
80 I	1.65	33,000	3.325×10^{-5}	700	2.2	.34
67 I	2.75	55,000	5.016×10^{-5}	1220	2.2	.34
55 I	1.65	33,000	3.135×10^{-5}	750	2.3	.35
51 I	1.55	31,000	2.945×10^{-5}	720	2.3	.36
EcNx1a39	1.5	30,000	3.705×10^{-5}	700	2.3	.36
79 I	3.05	61,000	6.46×10^{-5}	1430	2.3	.36
33 V	1.6	32,000	4.56×10^{-5}	810	2.6	.41
72 II	4.05	81,000	1.235×10^{-4}	2070	2.6	.41
41 I	1.9	38,000	5.7×10^{-5}	1010	2.7	.42
G13B5	3.5	70,000	8.93×10^{-5}	1860	2.7	.42
66 II	2.55	51,000	6.08×10^{-5}	1380	2.7	.42

Table 9 continued.

Burial No.	Cx (solution) ppm	wt. P_2O_5 (mg)	wt. F^- (g/sample)	ppm F^-/sample	100X F^-/P_2O_5	log 100X F^-/P_2O_5
58 V	2.0	40,000	4.655×10^{-5}	1110	2.8	.44
62 II	1.3	26,000	3.23×10^{-5}	720	2.8	.44
G3BB I	1.4	28,000	3.8×10^{-5}	780	2.8	.44
45 III	1.05	21,000	2.75×10^{-5}	640	3.0	.48
35 II	2.05	41,000	5.89×10^{-5}	1290	3.2	.50
44 IV	1.1	22,000	3.515×10^{-5}	700	3.2	.50
35 V	1.95	39,000	5.51×10^{-5}	1260	3.2	.50
27 I	1.9	38,000	5.7×10^{-5}	1230	3.3	.51
59 V	2.0	40,000	6.84×10^{-5}	1370	3.4	.53
65 II	1.65	33,000	4.56×10^{-5}	1140	3.5	.54
61 II	1.85	37,000	6.46×10^{-5}	1330	3.6	.56
80 III	5.9	118,000	1.995×10^{-4}	4470	3.8	.58
48 I	1.7	34,000	6.08×10^{-5}	1300	3.8	.58
50 I	1.55	31,000	5.035×10^{-5}	1180	3.8	.58
73 I	2.5	50,000	9.5×10^{-5}	1920	3.8	.58
G13B6 III	2.4	48,000	7.79×10^{-5}	1880	3.9	.59
59 IX	1.9	38,000	8.74×10^{-5}	1530	4.0	.60
71 III	1.7	34,000	6.745×10^{-5}	1410	4.1	.62
G3B1 II	1.85	37,000	6.27×10^{-5}	1570	4.2	.63
61 I	1.85	37,000	6.65×10^{-5}	1660	4.5	.65
62 I	1.05	21,000	4.988×10^{-5}	940	4.5	.65
23 XII	2.15	43,000	8.17×10^{-5}	1980	4.6	.66
G13B I	2.0	40,000	8.74×10^{-5}	1890	4.7	.67
37 VII	2.15	43,000	1.045×10^{-4}	2110	4.9	.69
53 I	1.5	30,000	7.695×10^{-5}	1540	5.1	.71

Table 9 continued.

Burial No.	Cx (solution) ppm	wt. P_2O_5 (mg)	wt. F^- (g/sample)	ppm F^-/sample	100X F^-/P_2O_5	log 100X F^-/P_2O_5
23 XIV	1.14	28,000	6.318×10^{-5}	1460	5.2	.72
35 III	1.8	36,000	8.36×10^{-5}	1940	5.4	.73
59 X	1.6	32,000	7.79×10^{-5}	1790	5.6	.75
68 I	2.55	51,000	1.52×10^{-4}	2860	5.6	.75
G13B6 II	1.8	36,000	1.045×10^{-4}	2050	5.7	.76
63 I	1.5	30,000	9.5×10^{-5}	1710	5.7	.76
57 I	2.5	50,000	1.2823×10^{-4}	2910	5.8	.77
32 II	0.9	18,000	6.08×10^{-5}	1080	6.0	.78
G3B6 III	1.9	38,000	1.045×10^{-4}	2310	6.1	.79
G3B2 III	2.35	47,000	1.615×10^{-4}	2870	6.1	.79
59 IV	2.15	43,000	1.3775×10^{-4}	2660	6.2	.80
52 II	1.5	30,000	8.36×10^{-5}	1970	6.6	.82
57 III	2.1	42,000	1.14×10^{-4}	2770	6.6	.82
42 II	1.0	20,000	6.745×10^{-5}	1400	7.0	.84
33 VII	1.5	30,000	9.975×10^{-5}	2130	7.1	.85
39	1.25	25,000	9.12×10^{-5}	1800	7.2	.86
83 II	2.2	44,000	1.805×10^{-4}	3170	7.2	.86
73 II	2.75	55,000	1.7575×10^{-4}	3990	7.3	.87
36 V	1.65	33,000	9.975×10^{-5}	2410	7.3	.87
34 III	1.6	32,000	1.0925×10^{-4}	2340	7.3	.87
34 II	1.25	25,000	9.5×10^{-5}	1840	7.4	.88
84	2.5	50,000	1.539×10^{-4}	3770	7.5	.89
71 I	1.55	31,000	1.52×10^{-4}	2580	8.3	.92
47 IV	1.5	30,000	1.045×10^{-4}	2540	8.5	.93
49 I	1.9	38,000	1.33×10^{-4}	3330	8.8	.94

Table 9 continued.

Burial No.	Cx (solution) ppm	wt. P_2O_5 (mg)	wt. F^- (g/sample)	ppm F^-/sample	100X F^-/P_2O_5	log 100X F^-/P_2O_5
29 II	1.65	33,000	1.1875×10^{-4}	2920	8.8	.94
36 I	2.35	47,000	2.09×10^{-4}	4430	9.4	.97
37 III	2.25	45,000	2.565×10^{-4}	4410	9.8	.99
G8B1	1.2	24,000	1.14×10^{-4}	2360	9.8	.99
G3B1 I	1.1	22,000	1.045×10^{-4}	2180	9.9	1.00
46 I	1.8	36,000	1.71×10^{-4}	3770	10.5	1.02
58 II	1.7	34,000	1.995×10^{-4}	3750	11.1	1.04
65 I	1.1	22,000	1.2825×10^{-4}	2440	11.1	1.04
91 II	2.1	42,000	1.995×10^{-4}	4750	11.3	1.05
G3B6 V	1.65	33,000	1.805×10^{-4}	3880	11.8	1.07
43 V	1.2	24,000	1.14×10^{-4}	2850	11.9	1.08
35 IV	1.7	34,000	1.71×10^{-4}	4160	12.2	1.09
37 VI	1.75	35,000	2.66×10^{-4}	4630	13.2	1.12
58 IV	2.25	45,000	3.23×10^{-4}	5950	13.2	1.12
G8B3	2.5	50,000	2.945×10^{-4}	6660	13.3	1.12
58 III	1.8	36,000	2.375×10^{-4}	4830	13.4	1.13
88 I	1.1	22,000	1.425×10^{-4}	2970	13.5	1.13
76 I	1.9	38,000	3.135×10^{-4}	5370	14.1	1.15
82 III	2.1	42,000	2.375×10^{-4}	5940	14.1	1.15
G3B4 III	1.25	25,000	1.71×10^{-4}	3640	14.6	1.16
76 II	0.85	17,000	1.2825×10^{-4}	2720	16.0	1.20
59 III	1.75	35,000	2.47×10^{-4}	5640	16.1	1.21
40 III	2.5	50,000	3.61×10^{-4}	8400	16.8	1.23
72 I	1.5	23,000	1.71×10^{-4}	3960	17.2	1.24
69 I	1.75	35,000	2.66×10^{-4}	6290	18.0	1.25
47 V	1.0	20,000	2.47×10^{-4}	6180	30.9	1.50

CHAPTER 4:

RESULTS AND DISCUSSION

In order to interpret the results of table 9, a histogram was constructed (figure 4). Since the fossilization processes are a logarithmic function of time (Cook and Heizer, 1953; Overweel, 1965) the ratios from table 9 were plotted along a logarithmic scale while the frequencies were plotted along the arithmetic scale. As can be seen from figure 4, this results in a visually distorted graph. To eliminate distorition the ratios have been converted to their log values (table 9) allowing the histogram to be drawn on regular graph paper (figure 5).

Figure 5 taken from Wade (1980), gives the sequential relationship of all individuals tested. By itself, however, there is no way of telling where this distribution is centred in time, nor of telling how much time is represented by the range. The ideal would be to obtain radiocarbon dates for several individuals scattered throughout the fluoride distribution. By doing the type of analysis presented here first, the archaeologist can make an optimum choice of individuals to be used in radiocarbon assay. Where a large number of individuals are involved, as at the Gray Site, it becomes very difficult to decide which specimens should be tested by radiocarbon assay to establish temporal range. In this case radiocarbon dates were obtained before fluoride analysis was initiated. Radiocarbon dates obtained before fluoride sequencing are still very useful but will not always be as productive in cross checking the sequence as they could be.

Nine radiocarbon dates were obtained for the Gray Site. Despite Millar's (1978) reliance on burial unit integrity, there exists some doubt.

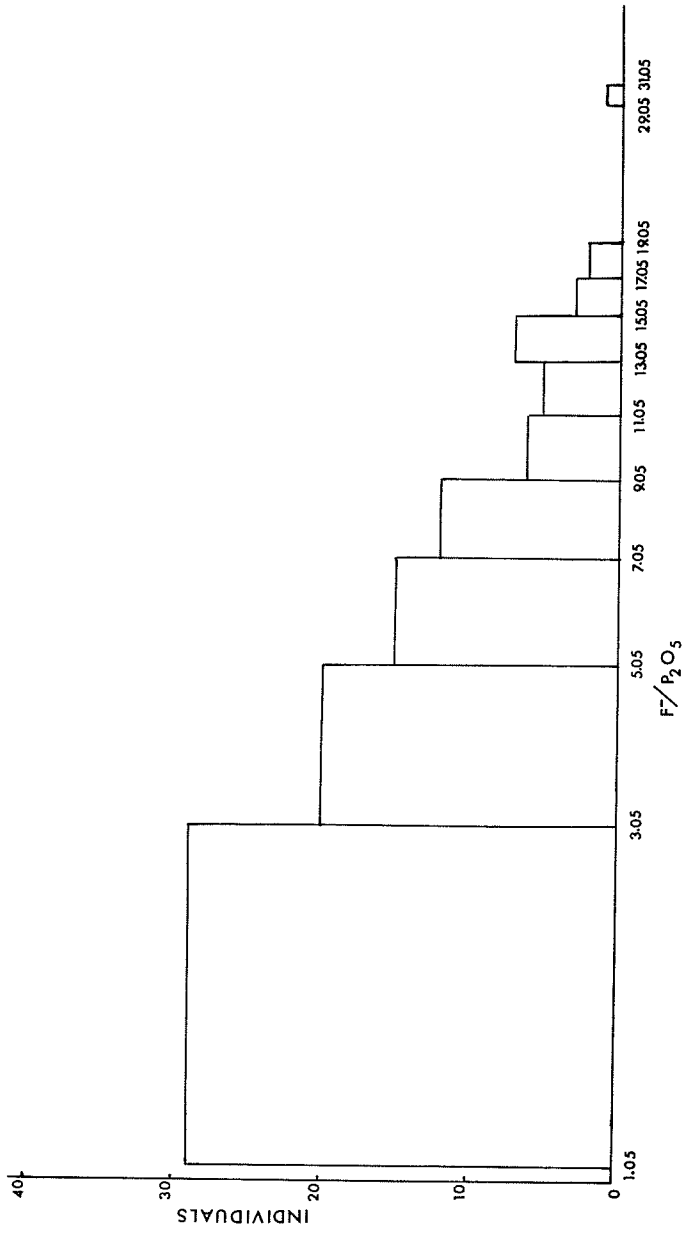

FIGURE 4 : F^-/P_2O_5 HISTOGRAM

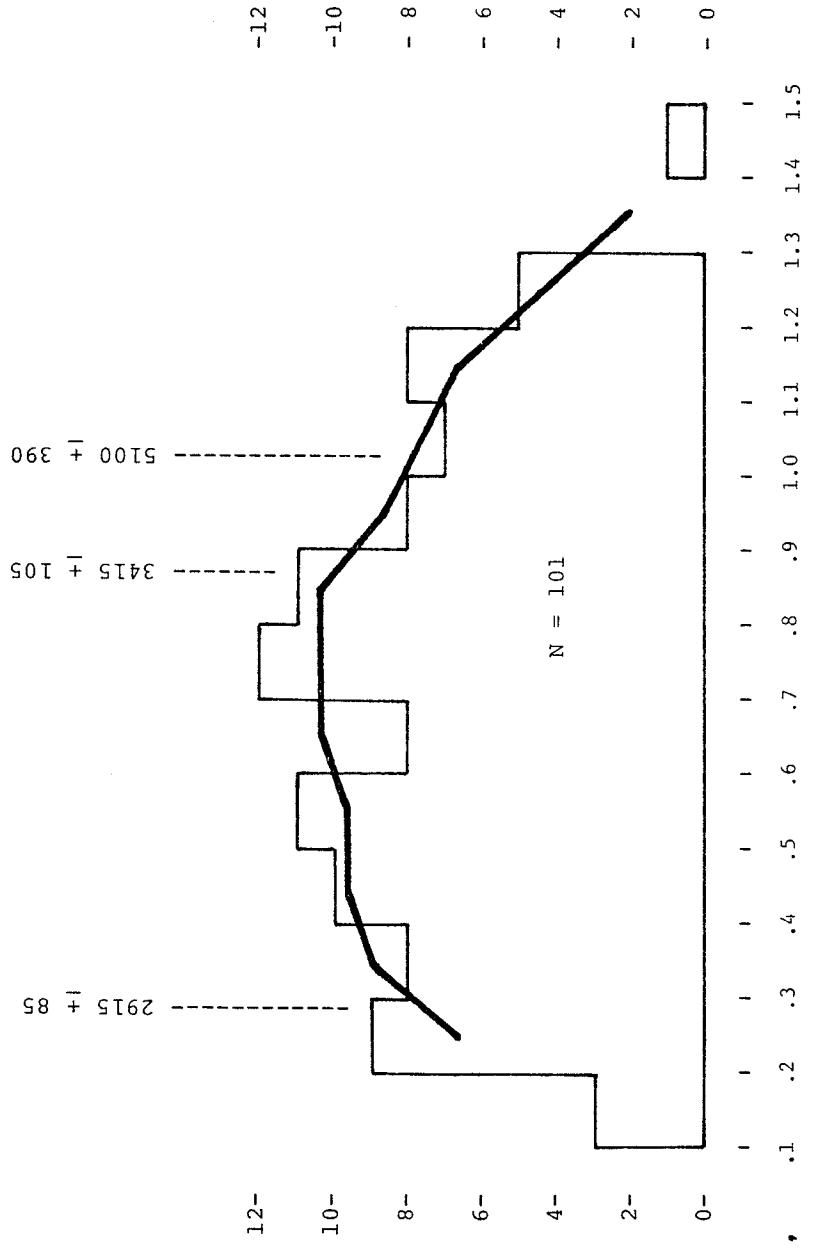

FIGURE 5: Log (x 100) Ratio F/P_2O_5 with plot of 3-interval moving average. C^{14} dates (in years B.P.) at top. (From Wade 1980)

> Except in those cases where the red ochre stain was particularly heavy or where stones were packed above the burial, the pit outlines are nowhere evidenced; even in these cases the pits are seldom distinguishable other than immediately around the burial itself. (Millar, 1978)

Because of this doubt and because, in many cases, there is no way of knowing which individual in a burial unit was used for radiocarbon assay, six of the nine C^{14} dates were unusable in this analysis. From table 9 it can be seen that individuals from a single burial unit sometimes have quite different fluoride contents. Even when burial units can be reliably defined, it is essential that C^{14} samples be from a specific individual. The three remaining C^{14} dates were retained on the basis that only a single individual was in each of these units. The three dates are: 2915 ± 85 B.P. from unit 70; 3415 ± 105 B.P. from unit 84; 5100 ± 390 B.P. from unit 46. The positions of the dates are plotted in figure 6.

W.D. Wade (1980) has shown that a correlation does exist between fluorine and radiocarbon results. This correlation is demonstrated through use of the Spearman rank correlation of radiocarbon and fluorine with multiple burial units represented by single individuals. The correlation is not strong and Wade considers the results inconclusive rather than negative.

Millar (1978) suggests four periods of use for the Gray Site based on grouping of C^{14} dates. No hiatuses are evident in the fluoride sequence. While no doubt the distribution is biased slightly by the sample of 101, testing the total site would tend to fill any gaps. At most, periods of peak usage would appear, but no real hiatus is possible. When dealing with a time span of over 2000 years and a sample in excess of 300, it seems somewhat spurious to postulate three hiatuses based on nine C^{14} dates.

Millar demonstrated that no spacial pattern is evident through time. This is based on C^{14} dates and doesn't have much weight. A spacial distribution based on the fluoride sequence does not however reveal any pattern either. No preference seems to have existed as to where on the site individuals were buried over time.

C. Pardoe (personal communication) of the University of Manitoba, Department of Anthropology has tested the fluoride sequence against cluster analysis of morphology. Two sets of morphological data were available; Pardoe's own analysis of non-metric traits and R. Vyvyan's (1977) analysis of metric traits. In neither case was there any temporal correlation to morphological grouping. There is no morphological grouping along the time line of the fluoride sequence. This lack of correlation would indicate that morphological variation is not sensitive to time.

In summary this analysis has made a number of contributions to understanding the Gray Site. A good estimate of temporal range and peak use of the site has been determined. This estimate also provides a guide for choosing future C^{14} samples that may further clarify our understanding of the site. The analysis has demonstrated that any complete hiatuses in the use of the site are extremely unlikely. That is, the site appears to have been used continuously. No pattern in spatial distribution of burials through time is evident from the analysis. Morphological variation is shown not to be sensitive to time. Also a temporal framework is provided against which many other aspects of the Gray Site can be examined, such as changes in preference for specific burial forms. Finally this analysis is a demonstration of a chemical sequencing technique that can be done quickly at a low cost per sample. The sample

chosen could not of course be the optimum one for all of the above points. However, if in the future a complete sequencing can be performed, a clearer picture will be obtained.

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