Bone Diagenesis and Taphonomic History of the Paso Otero 1 Bone Bed, Pampas of Argentina

María A. Gutierrez

INCUAPA, Departamento de Arqueología, Facultad de Ciencias Sociales (UNCPBA), Avda. del Valle 5737, B7400JWI Olavarría, Argentina

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Samples of guanaco bone from an archaeological site in the Pampas of Argentina have been analysed to understand the diagenetic profile of the bone assemblages that characterized the taphonomic history of the site. Two archaeological occupations of Paso Otero 1 were investigated, encompassing similar landscape settings, climates, and depositional environments. The time span is a *c*. 2000 year period from *c*. 4800 to 2800 years BP. A total of 30 bone samples taken from both occupations were used to provide a preliminary characterization of the diagenetic pathways at the site. The parameters investigated provide a comprehensive account of how both mineral (hydroxyapatite) and bone protein (collagen) have been altered. In order to compare the two bone assemblages in terms of their diagenetic parameters, multivariate analyses were conducted. Results indicate two different diagenetic analyses indicate that protein is less preserved in the bone assemblage from the middle stable landscape. Alternative interpretations of the diagenetic profiles are discussed in light of the taphonomic history of the site, and palaeoenvironmental information of the region. One hypothesis stresses the importance of the role of climate in defining the different diagenetic pathways, and the other the continued action of the combined diagenetic factors along time as the main explanation for the variability in the state of preservation of the bones in Paso Otero 1.

Keywords: GUANACO BONE BED, BONE DIAGENESIS, DIAGENETIC PARAMETERS, TAPHONOMIC HISTORY, STATE OF PRESERVATION.

Introduction

ertebrate taphonomy is the study of the processes occurring to bones from the time of the death of the organism, through burial, and up until the bones are recovered and studied. The preburial processes are widely studied and understood (Behrensmeyer & Hill, 1980; Bonnischsen & Sorg, 1989; Cadée, 1991; Lyman, 1994). However, the area of taphonomic research concerning the post-burial processes, known as diagenesis, is not yet completely understood and more studies are required (Pike, 1993; Nielsen-Marsh, 1997). Combined with the information on macroscopic bone modification, the diagenetic measurements provide an additional contribution to understanding the structure and preservation of the archaeological record.

The diagenetic analysis of bone material recovered from the archaeological site Paso Otero 1, Argentina (Figure 1), is undertaken to investigate the broad variety of post-burial processes responsible for the state of preservation of the bone deposit. The aims of this study are to understand how post-burial processes affect the integrity of the archaeological record, and to estimate the role played by these processes for the given structure of the site, both diachronically and synchronically. This paper further attempts to identify which factors were crucial in determining the diagenetic pathways followed by the Paso Otero 1 bone collection.

State of preservation is the result of past events. In some cases, the biological information contained in the living organisms is either obscured or destroyed as a result of the complex physical and chemical changes that usually take place in bones after burial. If differential preservation is not understood, it can lead to misinterpretation in faunal analysis, dietary reconstruction, radiocarbon dating, and bone pathology. The study of bone alteration at the microscopic level provides additional evidence to macroscopic effects (e.g., root etching, weathering, geological abrasion, etc.) for identifying probable agents and processes responsible for bone modifications. Chemical and structural alteration and/or loss of both the organic and inorganic components provides a potential source of information on taphonomic history of bone assemblages (Bell, 1990; Garland, 1987a, 1987b, 1989; Hedges, Millard & Pike, 1995).



Figure 1. Map of the Pampean Region of Argentina showing the Interserrana Area and Paso Otero Locality.

Study Area and Site Information

Paso Otero 1 (Politis, Gutierrez & Martínez, 1991; Gutierrez et al., 1999; Martínez, 1999; Johnson et al., 1997) is located in the Intersarrana Bonaerense area (Necochea District, Buenos Aires Province) within the humid Pampa sub-region of the Pampean Region (Figure 1). Twelve buried archaeological sites, known as the Paso Otero locality, have been recorded in the middle basin of the Río Quequén Grande and represent terminal Pleistocene to middle Holocene times (Martínez, 1999). Paso Otero 1 is located on an ancient floodplain of the left bank of the river. The stratigraphic sequence at the site, from bottom to top, consists of aeolian sediments of the Pampiano Formation (Fidalgo & Tonni, 1978, 1981; Fidalgo, De Francesco & Colado, 1973). Overlying this Formation are the fluvial sediments of the Luján Formation with two members, the late Pleistocene Guerrero Member and the early to middle Holocene Río Salado Member. The Río Salado Member at Paso Otero 1 is a stratified fluvial deposit in which three major periods of stable landscape environments in the river valley have been recorded (Gutierrez et al., 1999; Johnson et al., 1997). Overlying the Luján Formation are the late Holocene aeolian sediments of La Postrera Formation (Fidalgo & Tonni, 1978, 1981; Fidalgo, De Francesco & Colado, 1973).

The stable landscapes are represented by buried A horizons of soils that developed in the sediments (Johnson *et al.*, 1997, 1998) and represent very local-

ized environmental conditions. These A horizons are noted as upper, middle, and lower stable landscapes. They developed within a very moist setting that was producing a large amount of organic matter (1.0-1.5%)under poorly-drained conditions. At Paso Otero 1, the Río Salado Member records an alternating pattern of alluviation-stability-alluviation. This setting is interpreted as a wet meadow and its typical vegetation would have been grasses and reeds. The bone remains in Paso Otero 1 have been recovered from the middle and upper stable landscapes of the Río Salado Member (Johnson *et al.*, 1997).

Field seasons between 1989 and 1991 yielded about 3500 bone elements from a 22 m^2 area (Politis, Gutierrez & Martínez, 1991). Except for a few small rodent bones, the bone remains come from guanaco (Lama guanicoe). These remains come from at least four piles, one pile from the upper A horizon and at least three piles in the middle A horizon (Gutierrez et al., 1999; Johnson et al., 1997; Gutierrez, 1998). The stratigraphic position of the findings indicates two separate events, supported by differential bone preservation, colour, weathering patterns, and radiocarbon dating (Gutierrez et al., 1999; Johnson et al., 1997, 1998; Gutierrez, 1998; Martínez, 1999). Six small lithic flakes, a bipolar-reduced pebble, and a bezoar (stomach stone) with three polished faces have been recovered in association with the bone piles.

Several attempts (both standard and AMS) were made to date the bone beds, but failed due to the state

of preservation of the collagen in the bones. Organic sediment samples from each of the three buried A horizons were radiocarbon dated (Johnson *et al.*, 1998). The middle stable landscape, associated with the first occupation of the site, yielded a date of 4855 ± 105 BP (DRI-2829) and 4750 ± 60 BP (DRI-2830) and the upper stable landscape, where the second occupation of the site was registered, is dated at 2720 ± 40 BP (DRI-2837) (Johnson *et al.*, 1998).

Paso Otero 1 bone assemblages are not the result of water transport and accumulation process (see discussion in Johnson et al., 1997; Gutierrez, 1998). They represent two diachronic kill/butchering sites where guanaco was butchered and the meat from the carcasses was filleted from the bones. A minimum number of 27 guanaco were killed in the older assemblage and a minimum number of nine guanaco were killed in the younger assemblage (Gutierrez, 1998). These bones (essentially the entire carcass) were discarded at the site and only the meat itself was taken back to the campsite. The piles differ in their skeletal part composition. As part of the butchering strategy, hunter-gatherers at Paso Otero 1 accumulated bones by discarding anatomical units (forelimbs, rearlimbs) selectively and spatially in piles. Long bones from the upper portions of the limbs were discarded separately from the short bones from the lower portions of the same limbs. Cultural modifications to bone (e.g., helical fractures, cut marks) indicate that the origin of the pileconfiguration is related to human processing techniques. Therefore, even though the lithic material recovered around and within the piles is scarce, the association is clear and primary (Gutierrez, 1998).

Although the water velocity and load capacity of the river are not known, proxy data are concordant with an expected very low velocity and low energy of the river in general. Freshwater molluscs, such as *Biomphalaria peregrina* and *Littoridina parchappii* in the Río Salado Member and at Paso Otero 1 in particular (Fidalgo, 1981), and the topographic setting where the bone assemblages were deposited (ancient floodplain), suggest that the water away from the main current in the river was very slow moving.

Palaeoenvironmental reconstruction, based on biostratigraphic studies, indicated that in the eastern part of the pampean region, semiarid to arid conditions with lower mean temperatures developed during the late Pleistocene and early Holocene (c. 18,000 to 8500 years BP (Alberdi et al., 1993; Tonni, 1992). These conditions were accompanied by changes in the geographic distribution of the pampean fauna (Tonni & Fidalgo, 1978; Fidalgo & Tonni, 1981). During middle Holocene times, warmer and more humid conditions than today existed in the pampean region (Iriondo & García, 1993; Tonni, 1992). This humid and warm period persisted until c. 5000 to 3500 years BP, and consequently, prevailed when the bone assemblage from the middle stable landscape was deposited at Paso Otero 1. Cooler and arid conditions were reestablished during late Holocene times and lasted until *c*. 1000 years BP (Tonni, 1992). Hence, the bone assemblage from the upper stable landscape of Paso Otero 1 occurred under these conditions.

The palaeoclimatic model based on the pollen record of the pampean region presents an alternative interpretation for palaeoenvironmental investigations, as the material studied (pollen), has a different degree of resolution comparable to models based on mammalian fauna or wind circulation. Even though slight differences exist between these models, a main trend is recognizable and compared. The palaeoclimatic model based on pollen recognizes a subhumid-dry period prior to 10,500 years BP. A subsequent change towards environments with locally more effective moisture occurred at 10,500 years BP until 8000 years BP. This subhumid-humid to humid climate persisted until c. 5000 years BP. The late Holocene vegetation suggests subhumid-dry conditions, but not as extreme as those of the late Pleistocene (Prieto, 1996; Zárate & Blasi, 1993).

Materials and Methods

Bone diagenesis is detected analytically by chemical and microscopic analyses (Hedges et al., 1995). A clear consensus has not been reached for the particular chemical change involved in any given diagenetic process (Millard, 1993; Pike, 1993; Hedges & Millard, 1995; Hedges, Millard & Pike, 1995; Nielsen-Marsh, 1997). Consequently, choosing the most relevant and useful type of measurements for representing the diagenetic state of the bone is a matter of continuous investigation (Hedges, Millard & Pike, 1995; Nielsen-Marsh et al., 2000). Seven different types of bone chemical and structural measurements are analysed following methodological procedures established by Hedges, Millard & Pike (1995). These measurements are known as "diagenetic parameters", defined as "... a single measurable aspect of a bone sample which reflects the degree of diagenesis which the bone has recognisably undergone" (Hedges, Millard & Pike, 1995: 201). The diagenetic variables are: (1) microporosity; (2) macroporosity; (3) crystallinity (IRSF); (4) carbonate content (C/P); (5) nitrogen content (%N); (6) calcite content (%calcite); and (7) histological integrity.

The skeletal material used for the diagenetic analysis consists of 30 metapodials of guanaco, 20 coming from the middle stable landscape (older bone assemblage) and 10 from the upper stable landscape (younger bone assemblage). The sampling criteria are based on representative and preservational issues of the particular bone element. The guanaco skeletal body has four metapodials, i.e., two metacarpals and two metatarsals. They are commonly present at most pampean archaeological sites and have a thick cortical surface, appropriate for carrying out diagenetic analysis. In

addition, a single metacarpal of a modern guanaco has been processed and used as a control sample.

Diagenetic analysis encompasses a variety of techniques that require a segment of bone to be removed from the element. Initially, the specimens were cut in 3–4 cm thick transverse sections from the metapodial mid-shaft. However, archaeological bones usually are incomplete and exhibit a high degree of fragmentation. For these particular cases, decisions were made upon sample availability. The diagenetic parameters were measured from the same bone sample. Bone sections were cut using a p-shaped hand saw and sand blasted to remove the surface dirt. The diagenetic analysis is a destructive process impacting the archaeological bone and destabilizing it.

All statistical tests selected to test the hypotheses in this study were performed using a MatLab statistical software (version 4.2c.1) routine.

Porosity

The pore structure of the bone (distribution of porosity for a given pore radius) can determine how it interacts with groundwater (Pike, 1993; Hedges & Millard, 1995; Nielsen-Marsh, 1997) and, as a consequence, the extent of diagenesis. In this study porosity is measured using an adaptation of the standard soil science method described by Marshall & Holmes (1988) where the distribution of pore size is determined by measuring the mass change of the bone sample equilibrated at specific relative humidity (%RH) (controlled with dilute sulphuric acid). This method is discussed at greater length in Nielsen-Marsh (1997).

Porosity measurements were made using fragments of bone with a weight range between 200–300 mg. Three different porosity parameters were measured at varying relative humidities to estimate the total porosity, microporosity (pores<4 nm radius), and macroporosity (>4 nm radius) of the bone (Nielsen-Marsh, 1997).

Crystallinity

Crystallinity is considered an important feature of the inorganic diagenesis of bone (Sillen, 1989; Tuross, Behrensmeyer & Eanes, 1989; Weiner & Bar-Yosef, 1990). The small crystals of biologically formed bone mineral become thermodynamically unstable after death, leading to the formation of larger crystals measurable in archaeological bone (Weiner & Price, 1986).

Fourier Transform Infra-red (FTIR) spectrometry was conducted to determine the degree of alteration in bone mineral crystallinity and any alteration in the biogenic carbonate content (between 3-5%) in the bioapatite. The FTIR requires only a very small amount of bone powder (~1 mg). The presence of calcite can also be determined via this technique (Weiner & Bar-Yosef, 1990; Weiner, Goldberg & Bar-Yosef, 1993). The infra-red spectra were collected using OMNIC software. The crystallinity indices were estimated from a simple equation, using the phosphate v_4 doublet at 567 and 605 cm⁻¹ peaks (Weiner & Bar-Yosef, 1990; Hedges, Millard & Pike, 1995).

Carbonate content

The amount of carbonate (CO_3^{2-}) present in the bioapatite was measured using the infra-red spectra obtained for the crystallinity measurements. Two different measurements were taken in order to determine the presence of CO_3^{2-} in the bone sample: (1) overall CO_3^{2-} content; and (2) calcite content. Both measurements help to identify if there has been diagenetic exchange and/or loss of biogenic carbonate, and if this has been replaced by calcite from the surrounding environment. Moreover, and, to some extent.

The overall CO_3^{2-} content was estimated using the PO_4^{3-} v_3 (1035 cm⁻¹) and CO_3^{2-} v_3 (1415 cm⁻¹) peaks in the bone spectrum as described by Wright & Schwarcz (1996). Carbonate content was calculated from the ratio of the absorbencies of the CO_3^{2-} and PO_4^{3-} peaks (C/P) (Wright & Schwarcz, 1996).

The calcite content was calculated following Nielsen-Marsh (1997). The calcite content was estimated by measuring the height of the 713 cm⁻¹ peak in the bone spectrum. Spectra obtained from pellets using analytical grade CaCO₃ and modern bovine bone powder combined in different proportions (i.e., 5, 10, and 20% of CaCO₃) were used as a comparative reference with the archaeological bone spectra in order to estimated semi-quantitatively the amount of calcite present in the bones (see Nielsen-Marsh, 1997 for further discussion).

Protein content

The quantity of collagen decreases as bone degrades (Hedges & Law, 1989). In order to determine the amount of protein that survives in subfossil bone, the nitrogen content (%N) in the whole bone is estimated using a CHN analyser (Europa, ANCA, Roboprep). This technique requires a small bone sample (~10 mg). The percentage of nitrogen left in the subfossil sample is measured by direct combustion of the bone powder (Hedges, Millard & Pike, 1995; Nielsen-Marsh, 1997). In this study modern bone was found to possess a % N value of approximately 4.8%.

Histological integrity

The histological examination of archaeological bone gives important information about the state of preservation and, in particular, about microbial post-mortem destruction (Stout, 1978; Hackett, 1981; Garland, 1985, 1987*a*, 1987*b*, 1989; Bell, 1990). Thin-section optical microscopy was conducted in order to describe the qualitative features of the diagenetic changes.

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The examination involved designating a "histological diagenesis index value" that better describes the state of preservation of the microscopic features. A sampling method was developed in order to examine the whole section for signs of bone diagenesis (see Gutierrez, 1998). A histological diagenesis index scale was created on the basis of various descriptive exotic histological features (Gutierrez, 1998). They represented features that are unusual for the normal histology of unaltered bone. Similar histological alteration was documented by Marchiafava, Bonucci & Ascenzi (1974) and Hackett (1981). The causes of these changes were attributed to bone invaders such as bacteria and fungi.

The parameters used for describing the stages of the histological diagenesis scale are different from the histological index defined by Hedges, Millard & Pike (1995). Paso Otero 1 bones possess histological features not described in Hedges, Millard & Pike (1995). Therefore, a new histological diagenesis scale has been defined (Gutierrez, 1998). However, the order of the scale (i.e., stage 1 being the worst preserved and stage 5 the best preserved) is maintained for comparative purposes.

Hypotheses

Given the environmental and depositional conditions of the bone assemblages, a general hypothesis is proposed to understand the state of preservation of the Paso Otero 1 collection.

Hypothesis 1: the intensity of the microscopic taphonomic effects varies significantly between bone assemblages.

In order to compare the two bone assemblages in terms of their diagenetic parameters, multivariate analyses were conducted. Principal component analysis (PCA) and discriminant function analysis (DFA) were selected among the multivariate analyses because PCA is an exploratory way to find the major pattern of variation in the data (Manly, 1997) and DFA answers the questions of how the groups are different and which characters most contribute to this difference (Kachigan, 1991; Sokal & Rohlf, 1995; Manly, 1997).

The histological integrity of the bones formed part of the set of diagenetic parameters measured in the Paso Otero 1 collection, but the results of this analysis are discrete data (i.e., histological stages). For this reason, the histological integrity was tested separately from the rest of the diagenetic parameters (continuous data).

Hypothesis 2: the distribution of the histological stages among the two bone assemblages from Paso Otero 1 does not differ more than expected by chance alone.

Rejection of Hypothesis 2 (H2) would indicate that the distribution of the histological stages differs significantly between the bone assemblages (alternative hypothesis H_A), and would provide evidence of differential preservation of the histological features between the upper and middle stable landscapes.

The frequency distributions of the histological stages from the metapodials coming from the upper and middle stable landscapes (H2) were compared using a two-level mixed model nested ANOVA (Sokal & Rohlf, 1995). A nested ANOVA was selected because: (1) it uses discrete data; and (2) it is a two-level comparison. The two bone assemblages represented a fixed treatment effect and constitute the highest level of comparison. At the first level, the nested ANOVA tested whether or not differences exist in the mean distribution of the histological effects between the two bone assemblages. The bones uncovered at Paso Otero 1 represented a randomly chosen sample and the second hierarchical level of the nested ANOVA. At this level, the nested ANOVA tested whether or not the added variance of the histological stages is significantly different among each sample due to the fact that the observations come from different bones.

The potential variation introduced by use of different bone elements (metatarsals and metacarpals) for the diagenetic analysis was also investigated.

Hypothesis 3: the mean differences of the diagenetic parameters among metatarsals and metacarpals from Paso Otero 1 do not differ more than expected by chance alone.

Rejection of Hypothesis 3 (H3) would indicate that the distribution of the diagenetic parameters differs significantly between bone-types (alternative hypothesis H_A), and would provide evidence of the preservational differences between these two bone elements in the Paso Otero 1 bone collection.

In order to test H3, and specifically to investigate whether a combination of the diagenetic parameters varies as a function of the type of bone being analysed, multivariate analysis of variance (MANOVA) has been calculated. The strength of the analysis was tested by randomization using bootstrapping (1000 iterations). In this study, MANOVA tests whether the mean differences of the diagenetic parameters among metatarsals and metacarpals are likely to have occurred by chance. The hypothesis is tested by comparing variances. A new variable is created by MANOVA that is a linear combination of the set of original variables, combined in such a way to maximize group differences and separate the groups as much as possible (i.e., a discriminant axis; Tabachnick & Fidell, 1989). The sample size consists of 13 metatarsals and 13 metacarpals. The rest of the bones (four) that constitute the diagenetic sample were not included in this test because they could only be identified as metapodials. Diagnostic features were not present on the bone fragments to distinguish them as metacarpal or metatarsal.

Several statistical assumptions are made. The MANOVA is based on the multivariate normal distribution. This assumption indicates that the sampling distribution of the means of the diagenetic variables and all linear combinations of them are distributed

	Sample	Total porosity	Macro porosity	Micro porosity	IRSP	C/P	N	% calcite
Younger Occupation	PO.2.201	0.4221	0.3506	0.0715	3.7	0.331	0.17	2
	PO.2.166	0.3933	0.3751	0.0182	3.2	0.333	0.15	3
	PO.2.6	0.3801	0.3104	0.0698	3.4	0.3	0.17	2
	PO.2.131	0.4069	0.3382	0.0688	3.3	0.346	0.16	5
	PO.2.9	0.3369	0.2703	0.0666	3.3	0.319	0.17	2
	PO.2.85	0.3808	0.3055	0.0753	3.3	0.291	0.22	2.5
	PO.2.88	0.3602	0.2877	0.0724	3.2	0.356	0.15	2.5
	PO.2.143	0.3975	0.3265	0.0710	3.3	0.304	0.17	0
	PO.2.237	0.3303	0.2543	0.0759	3.2	0.346	0.18	0
	PO.2.135	0.4197	0.3328	0.0869	3.3	0.35	0.17	0
Older Occupation	PO.3.2.SE.16	0.4025	0.3321	0.0704	3.3	0.335	0.14	3
*	PO.3.SE.2a	0.3699	0.2958	0.0741	3.3	0.406	0.14	8
	PO.3.3.SO.0k	0.3335	0.2683	0.0653	3.3	0.377	0.15	7
	PO.1.124	0.3726	0.2952	0.0774	3.6	0.357	0.16	4
	PO.1'.75.NO	0.3149	0.2431	0.0718	3.7	0.35	0.14	10
	PO.1'.4.SE	0.3034	0.2213	0.0821	3.3	0.373	0.24	10
	PO.4.4.SE.4	0.4319	0.3543	0.0776	3.3	0.363	0.15	3.5
	PO.5.1-2.SO.12	0.3985	0.3266	0.0719	3.3	0.331	0.15	3.5
	PO.6.3.NO.11	0.3454	0.2773	0.0681	3.4	0.35	0.14	3.5
	PO.5.4.SO.1	0.3912	0.3183	0.0729	3.4	0.329	0.15	7
	PO.1.102	0.3981	0.3214	0.0767	3.4	0.371	0.14	10
	PO.1.103	0.3906	0.3192	0.0714	3.2	0.431	0.12	12
	PO.1.104	0.3700	0.2924	0.0776	3.1	0.39	0.15	2
	PO.1.129	0.3192	0.2420	0.0772	3.2	0.417	0.15	2
	PO.1.230	0.3239	0.2516	0.0723	3.4	0.404	0.1	10
	PO.1.235	0.3121	0.2349	0.0772	3.2	0.409	0.13	3.5
	PO.3.3.SO.36a	0.3905	0.3142	0.0763	3.2	0.439	0.15	5
	PO.3.2.NO.7	0.3718	0.2930	0.0788	3.3	0.392	0.14	3.5
	PO.3.4.NO.3	0.4012	0.3236	0.0776	3.4	0.395	0.14	4
	PO.3.3.SE.14	0.3363	0.2561	0.0802	3.4	0.38	0.14	6
Modern Guanaco		0.1332	0.0740	0.0592	2.6	0.369	4.72	

Table 1. Data from the diagenetic parameters

IRSF=Infra-Red Splitting Factor; C/P=Carbonate/Phosphate; % N=Percent of Nitrogen.

Table 2. Correlation matrix of the original diagenetic variables

	Microporosity	Macroporosity	IRSF	C/P	% N	Calcite
Microporosity	1.0000	-0.0867	-0.1414	0.3104	0.2191	- 0.0830
Macroporosity	-0.0867	1.0000	0.0912	-0.2939	-0.0574	-0.2406
IRSF	-0.1414	0.0912	1.0000	-0.3235	-0.0237	0.2105
C/P	0.3104	-0.2939	-0.3235	1.0000	-0.5140	0.4476
% N	0.2191	-0.0574	-0.0237	-0.5140	1.0000	-0.2887
Calcite	-0.0830	-0.2406	0.2105	0.4476	-0.5887	1.0000

IRSF=Inra-Red Splitting Factor; C/P=Carbonate/Phosphate; % N=Percent of Nitrogen.

normally. The second assumption indicates that more elements should occur than variables in every cell in order to avoid lowering the powder of the analysis. The third assumption is the homogeneity of variance for each of the original variables measured (Tabachnick & Fidell, 1989).

Results

Hypothesis 1

The results of the diagenetic parameters are shown in Table 1. These data are used for the multivariate

analyses. Based on these data, the correlation matrix is shown in Table 2. After the correlation matrix was calculated, a PCA was carried out (Tables 3 and 4; Figure 2). The eigenvalues (Table 4) represent the percentage variance accounted for by each principal component. The results from Table 4 and Figure 2 imply that a pattern exists within the data. The loading values (coefficients) represent the amount of variation that each diagenetic variable is contributing to the observed pattern.

Out of the six principal components, PC1 is the axis that accounts for the largest proportion of total variation among the data (33%); PC2 represents 24% of the

	PC1	PC2	PC3	PC4	PC5	PC6
Microporosity	0.1208	0.5990	0.1751	0.6773	- 0.1371	- 0.3441
Macroporosity	-0.3212	-0.2098	-0.5965	0.5473	0.4261	0.1259
IRSF	-0.1540	-0.5502	0.4813	0.4578	-0.3864	0.2883
C/P	0.6554	0.1703	-0.1491	0.1297	-0.0069	0.7088
% N	-0.4468	0.3844	0.4787	-0.0650	0.4768	0.4380
Calcite	0.4789	-0.3431	0.3611	0.1052	0.6504	0.2974

Table 3. Loadings of the variables on the principal components

IRSF=Infra-Red Splitting Factor; C/P=Carbonate/Phosphate; % N=Percent of Nitrogen.

Table 4. Percentage variance accounted for by each principal component

Principal component	Eigenvalues		
PC1	32.5787		
PC2	23.9434		
PC3	17.8348		
PC4	14.3322		
PC5	8.6422		
PC6	2.6686		

 $\mathbf{2}$ 0 1.5Group 1 1 С 0 0.50 PC2 0 С C -0.50 -1Group 2 -1.5-2-2.50 -2 -1 1 2 PC1

Figure 2. Plot of the PC1 versus PC2 for the two bone assemblages at Paso Otero 1. 1=Upper stable landscape, younger occupation; 2=Middle stable landscape, older occupation.

variation within the data; and PC3 accounts for 18% of the residual variation. Although all the PCs obtained were reported (Table 4), only those PCs that represent the majority of the variance (PC1 to PC3) are considered further. The total variation accounted for PC1 to PC3 is approximately 74%.

For PC1, microporosity, C/P, and calcite content are positive and macroporosity, IRSF, and % N are negative. The C/P, % N, and calcite content have the higher loadings values. However, % N contributes negatively to the pattern shown by the data (Table 3). The results indicate that PC1 axis accounts for the majority of the Table 5. Loadings of the variables on the discriminant factor

Variables	DF1		
Microporosity	0.2170		
Macroporosity	-0.3296		
IRSF	0.0075		
C/P	0.8556		
% N	-0.6376		
% Calcite	0.7673		

variation of the data and that the rest of the variation is within each occupation (Figure 2). As one moves along the PC1 axis, C/P and calcite content variables increase while % N and, in lesser proportion, macroporosity decrease.

For PC2, microporosity, C/P, and % N have positive loading values while macroporosity, IRSF, and calcite content are negatives. The most significant contribution to PC2 is from microporosity and IRSF. However, IRSF has a negative loading value (Table 3). As one moves along the PC2 axis, towards increasingly positive values, the pattern of variation is within instead of between assemblages (Figure 2). Microporosity and, in lesser proportion, % N increase while IRSF decreases (Figure 2).

For PC3, microporosity, IRSF, % N, and calcite are positive and macroporosity and C/P are negative. Three variables contribute most to the variation of PC3: macroporosity, IRSF, and % N. With these three diagenetic parameters, macroporosity presents a negative loading value (Table 3). This result implies that when IRSF and % N values are high, macroporosity is low.

The results for the Discriminant Factor Analysis are shown in Table 5 and plotted in Figure 3. The total variation of the pattern within the data is represented in DF1 (100%). The most significant discriminatory power of the original diagenetic variables is provided by C/P, % N, and calcite content (Table 5). As one moves along the DF1 axis, towards increasingly positive values, C/P and calcite content increase while % N and, in lesser proportion, macroporosity decrease (Figure 3). In conclusion, the representation of these results (Figure 3) shows that the two groups are almost completely distinguishable, and these groups (1 and 2



Figure 3. Plot of the DF1 versus DF2 for the two bone assemblages at Paso Otero 1. 1=Upper stable landscape, younger occupation; 2=Middle stable landscape, older occupation.

Table 6. Frequency distribution of histological stages by bone assemblage

	Upper Stable Landscape Younger Bone Assemblage		Middle Stable Landscape Older Bone Assemblage		
Stages	Obs	% Freq	Obs	% Freq	
1	0	0	2	0	
2	5	2	19	3	
3	62	21	214	36	
4	178	59	275	46	
5	55	18	90	15	

in Figure 3) represent the upper and middle stable landscapes, respectively. H1 has been accepted, the two groups do possess distinctive diagenetic profiles, which can be identified from the multivariate analysis, indicating that the intensity of the microscopic taphonomic effects varies between bone assemblages.

Hypothesis 2

The results of the two-level mixed model nested ANOVA show that the distribution of the histological stages differs significantly between the two bone assemblages (P < 0.001). The null hypothesis is rejected at 0.05 level of significance. The frequency distributions of the histological stages from the metapodials are shown in Table 6 and Figure 4. The younger bone assemblage shows higher relative frequencies in stages 4 and 5 (better preserved) than the older assemblage (Figure 4).

Hypothesis 3

The result of the MANOVA for testing whether or not a combination of the diagenetic parameters varies as a function of the type of bone being analysed was not



Figure 4. Relative frequency of the histological stages by bone assemblage. \Box , Younger bone assemblage; \blacksquare , Older bone assemblage.

significant (lambda=0.7604; F=0.9979; P=0.4400; df=24 and 25). The null hypothesis has failed to be rejected at 0.05 level of significance indicating that the differences in preservation do not depend on the type of bone (metacarpals and metatarsals) selected for the analysis.

Discussion

Depositional environment

In order to interpret the diagenetic pathways followed by the bones in Paso Otero 1, the conditions of the microenvironmental deposition need to be reconstructed. The floodplains of the middle basin of the Río Quequén Grande may have attracted animals over a long period of time, as water was a natural resource associated with the river. Although floodplain soil formation is a very localized process and soils are weakly developed (Holliday, 1992), the buried A horizons at Paso Otero 1 indicate reducing environments, relatively slow depositions, and surface stability during the occupations of the site.

After deposition, the natural postdepositional processes started to act on the bones. Culturally-induced modifications such as dismemberment, defleshing, fracturing, distribution, and transportation were among the first processes that the guanaco bones recorded in their early taphonomic history. The combination of intrinsic factors such as the initial condition of the bone (e.g., size, shape, and porosity of the skeletal tissue, age of the individual at death, rate of decay of soft tissues), plus extrinsic factors such as the depositional microenvironment (e.g., vegetation, microbial action, sediment pH, hydrology, and temperature) may all determine the pathway followed by the natural processes that constituted the rest of the taphonomic history of the assemblages at Paso Otero 1. The duration of exposure, and consequently, the intensity of the pre-burial processes, was another factor determining the diagenetic pathways after the bones were buried. The spatial distribution of the bone in piles created a differential immediate microenvironment

Table 7. Percentage of taphonomic effects in younger and older bone assemblages

Variable	Upper Stable Landscape Younger bone assemblage	Middle Stable Landscape Older bone assemblage
Manganese staining	40%	37%
Weathering	29%	35%
Root etching	67%	46%
Geological abrasion	24%	26%

with unique characteristics for each pile, and which may help to explain the variability in the frequencies of the taphonomic effects found among piles. Human processing decisions and techniques, therefore, were responsible for the immediate depositional microenvironment of the bones by selectively discarding the skeletal parts in piles.

Manganese staining recorded on the bone cortical surface from both stable landscapes (40% in the upper stable landscape versus 37% in the older one) (Table 7) suggests that the depositional microenvironments of the piles, at least during an important part of the deposition, was sufficiently rich in water (Rapp & Hill, 1998). Whether this water availability was a consequence of seasonal fluctuations or minor local fluctuations associated with the middle basin of the Río Quequén Grande is still a matter of investigation. The buried A horizons constituting the stable landscapes are defined as wet meadows and reducing soils (Johnson *et al.*, 1997, 1998). Reducing soils, due to the lack of oxygen, decreases the activity of aerobic microorganisms.

Pre-burial processes

The differential diagenetic pathways depend upon the conditions of the surrounding environment at burial (Child, 1995; Hedges, Millard & Pike, 1995; Nielsen-Marsh, 1997; Nielsen-Marsh et al., 2000). The analysis of the cortical surface features on the bones indicates that a variety of diagenetic events occurred affecting the bones, the bone piles, and the two occupations. Soft tissue and bone deterioration started immediately after the death of the guanaco. After the carcasses were abandoned, these tissues degrade initially biochemically by autolysis (Garland & Janaway, 1991; Janssen, 1984; Walker et al., 1988) followed by microbiological activity. This soft tissue degradation by autolytic mechanisms facilitates bone alteration (Child, 1995). In Paso Otero 1, burial did not occur immediately after guanaco death. This situation means that microorganism population growth was favoured by higher temperatures generated by the autolysis process than it otherwise would have been if the carcasses were rapidly buried and cooled off.

Weathering features occur more frequently on bones from the middle stable landscape (35%) than on the upper one (29%) (Table 7). The degree of weathering at which bones enter the burial context may affect the quality and intensity of diagenetic processes. Bones that had already experienced organic and inorganic degradation before burial would be weakened and, consequently, more susceptible to diagenetic processes and less resistant to compression forces (e.g., sediment pressure and soil mechanics).

The cortical surface alteration is not severe in either of the stable landscapes, indicating either a protective microenvironment, or a relatively rapid burial of the bone assemblage, or both. The intensity of the preburial taphonomic processes would have differed between bones that still had some soft tissues attached to them and those lacking soft tissue. The presence of remnant flesh would have protected the bones from exposure to weathering agents. In addition, the spatial arrangement of the bones would have favoured the preservation of the cortical surface against superficial weathering agents. Bones located for example, at the bottom, or within the interior of the piles, were probably more protected than bones placed at the top of the pile. However, all the bones would have been exposed to post-burial weathering.

Geological abrasion occurs at approximately the same frequency (24% versus 26%) in both bone assemblages (Table 7). Water did not transport the bones (Gutierrez *et al.*, 1999; Gutierrez, 1998). However, the temporary water that covered the floodplains probably abraded the bones *in situ*. The evidence of abrasion indicates that water was a common extrinsic factor present in both assemblages.

Post-burial processes

The percentage of root etching (used as an indicator of root presence at the site) is relatively high in both assemblages, suggesting that roots have played an important role in the taphonomic history of the site. Between bone assemblages, root etching occurs more frequently on bone from the upper stable landscape than bone from the middle landscape (67% versus 46%) (Table 7). Accordingly, the upper stable landscape registers the lowest percentage of weathering (29%).

Vegetation roots, mainly grass-roots (Johnson *et al.*, 1997) played an important role in the state of preservation of the bones as they may have contributed, by applying mechanical force, to fragmentation and destruction of the trabecular bone as they penetrated the interior via existing cortical desiccation cracks. In addition, vegetation may also have affected preservation by creating a protective microenvironment for the bone against weathering, yet provided a suitable environment for fungi and bacteria to live.

The diagenetic analysis indicate that the nitrogen content is one of the variables that accounts for the majority of variation in Paso Otero 1 (Tables 3 and 5; Figure 4). This variable measures the amount of protein left in the bones, which could be controlled by the

action of microorganisms. The presence of microorganisms in the bones of Paso Otero 1 is apparent through solution pitting (56% in the upper stable landscape and 48% in the middle one) and the alteration of the histological structure of the bones (Table 6; Figure 4). Although the processes involved are not yet completely understood, it is suggested that the solution pitting observed at the site could be related to the action of acids present in the depositional microenvironment (Johnson et al., 1997), either in the soil or excreted by fungi. In addition, microbial and fungal action associated with the fine rootlet hairs may be responsible for the chemical dissolution on the cortical surface of the bone (Gutierrez, 1998). Bone tunnelling and redeposition of bioapatite are common histological features present at Paso Otero 1 and recognized as the results of microorganism-induced diagenetic changes that altered the histological integrity of the bone.

The diagenetic analyses indicate that protein loss is greater in the bone assemblage from the middle stable landscape. This alteration is accompanied by an increase in total porosity and, as a consequence, bones from this assemblage were more susceptible to diagenetic alteration (Table 3; Figure 2). One possible interpretation of the diagenetic data is that bones from the middle stable landscape were buried in an environment of low water levels and aerobic conditions that would have facilitated protein degradation via microbial attack. But this explanation appears unlikely based on the palaeoclimatic model that indicates warmer and more humid conditions, therefore, more available water and a higher water table for the middle Holocene. Therefore, this palaeoclimatic model fails to explain the lower levels of protein from the middle stable landscape.

A second possible explanation for the diagenetic results is that they are a consequence of the combined effects of water, temperature, microorganisms, and time. The presence of water was a common variable in both occupations. Water would have been present at burial and would have affected both assemblages, with fluctuations associated with either seasonal or minor local changes. The palaeoclimatic model for the region suggests that the climate would have been warmer during the older occupation (c. 4800 years BP). Although annual mean temperatures are not known, the proxy data indicate that the temperature changes were sufficient to produce animal and plant species migration, drifting, and replacement (Tonni, 1992; Prieto, 1996; Iriondo & García, 1993). Therefore, differences in temperature could have played an important role in determining different diagenetic pathways between both assemblages by varying the rate of most of the chemical reactions, especially chemically induced collagen hydrolysis (Nielsen-Marsh et al., 2000). Climatic differences may also have affected the range of microbial species present (Von Endt & Ortner, 1984).

The activity of microorganisms at Paso Otero 1 was significant after burial. Water and temperature may have determined the range of microorganisms, mainly fungi and bacteria, that attacked the bone protein. The water from the environment surrounding the buried bones provided mineral ions that may have substituted into the bone mineral, altering crystallinity and possibly the protein-mineral relationship. Fluctuations in groundwater levels would also have contributed to protein hydrolysis. In addition, periodic floodings associated with river fluctuations supplied water to the burial site and created temporary reducing, anaerobic, and poorly-drained conditions. Anaerobic and aerobic decomposition may have been alternating depending upon the fluctuation of the water level and the rate of oxygen diffusion to the system. Once oxygen was depleted, either by putrefaction, or as part of waterlogged soil conditions, anaerobic microorganisms may have played a central role in protein decay. In order to degrade the collagen, microbiological activity at Paso Otero 1 must either have had the capacity to demineralize the bone or have grown in an environment where demineralization occurred (Child, 1995).

As a result of microbiological activity, exotic features can be seen in the histological structure of the bone, these have been recognized as tunnels. These features could be produced by dissolution of the inorganic phase (mineral part) of the bone by metabolic organic acids excreted by the microorganisms (Von Endt & Ortner, 1984). In an aerobic environment, the rate of decomposition of the bone protein is faster than in anaerobic conditions as the former environment can support a larger population of microorganisms and the decay processes are more rapid during oxidation. However, a rapid growth of aerobic microorganisms may favour anaerobic conditions when the rate of oxygen consumption surpasses the rate of oxygen diffusion (Child, 1995). Although the range of microorganisms that an anaerobic environment can support is smaller, decomposition of the bone protein still continues. Even if the microbiological activity stopped or slowed down, bone degradation may have continued by chemical hydrolysis (Nielsen-Marsh et al., 2000).

All these environmental factors are affected by time. The amount of collagen in buried bone decreases exponentially with time (Von Endt, 1979) and if the environmental conditions remain stable and constant, the rate of chemically controlled collagen degradation should be constant (Ortner *et al.*, 1972; Von Endt, 1979; Nielsen-Marsh *et al.*, 2000). It seems unlikely that both assemblages at Paso Otero 1 represent an enclosed environmental system with no changes over time.

The implications and expectations for considering either temperature or time as being the variable of major importance in the degradation of buried bones in Paso Otero 1 are the following:

(1) If temperature is considered as the most important factor in the deterioration of buried bone in Paso Otero 1, the results of the diagenetic analysis of bones recovered in the middle basin of the Río Quequén Grande would have to reflect the general climatic model inferred for the Pampa region. This model has semiarid to arid conditions with lower mean temperatures during the late Pleistocene and early Holocene (c. 18,000 to 8500 years BP) (Prado *et al.*, 1987; Tonni, 1992; Alberdi *et al.*, 1993). Warmer and more humid conditions than today existed during the middle Holocene (Iriondo & García, 1993; Tonni, 1992), which persisted until 3500 years BP. Cooler and more arid conditions were re-established during the late Holocene times and lasted until 1000 years BP (Tonni, 1992).

(2) If the deterioration of buried bone in Paso Otero 1 is considered as a result of a continued action of the different factors along time, values for bones with radiometric dates older than about 4800 BP would be plotted on the very right side of the chart for PC1 versus PC2 (Figure 2) on the diagenetic parameters. Accordingly, values for bones dated younger than 2700 BP would fit on the left side of this chart (Figure 2). The two-multivariate variables (PC1, PC2) can be interpreted as sorting the samples according to a chronological space.

In summary, one hypothesis stresses the importance of the role of climate in defining the different diagenetic pathways, and the other the continued action of the combined diagenetic factors along time as the main explanation for the variability in the state of preservation of the bones in Paso Otero 1. Two things should be kept in mind when conclusions about factors introducing variation into the diagenetic pathways at Paso Otero 1 are attempted. First, palaeoenvironmental and palaeoclimatic reconstructions that are used as a framework are general models for a larger region (pampean region). Therefore, local changes and fluctuations in the study area may be underrepresented in this general model. Second, this study only involves two distinctive points of a time continuum. A further study using samples covering a large range of time and environmental conditions would contribute to identifying the leading factors in the diagenetic process at the Paso Otero 1 site. Nevertheless, Paso Otero 1 data suggest that the continued action of the combined diagenetic factors along time played a central role in the protein degradation of the buried bones.

In the multivariate approach, the PCA results show that the data from the two assemblages can be grouped with only a few samples overlapping (Figure 2). The variables with a larger contribution to the variance of the data are C/P, % N, and calcite content. These variables also show the highest discriminatory power in the discriminant function of the DFA analysis (Figure 3). During burial, the carbonate content of the bones may have been increased by crystallization of calcite into the pore spaces and, as part of the same process, macroporosity was decreased (through blocking of pore-spaces by crystallization of the calcite). If the carbonate content is the result of the crystallization of calcite, the removal of this diagenetic carbonate should be easier than if this carbonate would have been introduced into the bioapatite lattice (structural carbonate). The failed dating attempts were due to the poor preservation of collagen. Therefore, if the original bioapatite carbonate can be recovered successfully from bones from Paso Otero 1, the C¹⁴ dating method could be applied again with improved chances of obtaining reliable dates.

The results of the distribution of the histological stages shows that the higher frequencies of better preserved histological structures are present in the younger assemblage (Table 6; Figure 4). These results are in agreement with the fact that this bone assemblage also has the higher levels of protein content. Bones from the upper stable landscape are better preserved and have higher protein values than bones from the middle stable landscape, suggesting that the continued action of the combined diagenetic processes along time played a central role in bone preservation.

An apparent correspondence does not occur between bone surface modification and microscopic bone alteration. However, the Paso Otero 1 bone collection is very fragile and susceptible to fragmentation. The cause of this current state of preservation is due to the low protein content that has left the bones more friable and also to microbial action that has altered the histological integrity of these bones.

Conclusions

The study carried out on the chemical and structural modifications occurring to the bones, as well as to the state of preservation of the bone assemblages, has provided insights into the taphonomic history of the site. In this sense, this study has helped to enhance a complete model of the taphonomic history of Paso Otero 1, both pre-burial and post-burial and has tested assumptions about the depositional environment based on macroscopic analysis of the taphonomic effects.

The taphonomic history of Paso Otero 1 is the result of the combination of human butchering techniques, climate, hydrology, microbial activity, and vegetation along time (Figure 5). Human-induced modifications such as dismemberment, defleshing, fracturing, distribution, and transportation were among the first processes that the guanaco bones recorded in their early taphonomic history. These actions determined the following taphonomic pathways of the bone assemblages. Therefore, humans played a very important role in determining the taphonomic history of the site, and consequently, the current state of preservation of the bones (Gutierrez, 1998).

Pre-burial processes were not severe in either of the stable landscapes, suggesting a relatively rapid burial



Figure 5. Model of the taphonomic history of Paso Otero 1.

for both bone assemblages. Nevertheless, the frequency values of geological abrasion and weathering supported the interpretation that bones in the upper stable landscape were buried sooner than those in the middle landscape. On the other hand, post-burial processes were intense in both assemblages. Protein content was lower in the bone assemblage from the middle stable landscape. This alteration was accompanied by an increase in total porosity. Therefore, bones from this assemblage would have been more susceptible to diagenetic change, and consequently, are less well preserved than those from the upper stable landscape, as shown by the study of histological integrity.

The results of the multivariate approach for the microscopic data suggest that the continued action of the combined diagenetic processes along time played a central role in bone preservation. Given the evidence discussed, time can be considered as the main variable that introduces variability into the diagenetic profile at Paso Otero 1. Proxy data suggest that both occupations share the same depositional environment and in order for these environments to form, certain conditions need to be met. In this sense, factors such as water availability, temperature, pH, and vegetation had to be similar during both occupations. Assuming that these factors were constant during both events, the only one that varies is time. Moreover, the current state of knowledge on palaeoenvironmental conditions of the study area does not support the hypothesis of temperature being the crucial variable that determined diagenetic pathways. Further studies on palaeotemperatures based on isotopic analysis could contribute to testing the hypothesis of which factor(s) is responsible for the diagenetic profiles in Paso Otero 1.

The state of preservation of the bones is determined by multiple factors. Distinguishing which one is the most important in determining the state of preservation is a difficult task. However, by identifying the broad spectrum of potential variables altering the chemical and physical properties of the bones in specific microenvironmental conditions, this study has contributed to a better understanding of differential preservation. This research is a pioneer study for bone diagenesis studies in Argentina. In order to determine the most crucial factor(s) in bone preservation, additional research focusing on different environments and time periods are necessary.

This study was undertaken as an exploration of the contribution of diagenetic analysis to the taphonomic history of an archaeological site. The results demonstrated that the post-burial processes were intense and greatly affected the integrity of the chemical and structural features of the bones. This approach allowed the identification of these processes and an enhanced inference of the depositional environment of the Paso Otero 1 bone assemblages. Bone diagenesis, therefore, contributed to a more complete view of the taphonomic history of Paso Otero 1.

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