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THE USE OF Sr/Ca RATIOS IN THE DETERMINATION OF TROPHIC LEVELS OF CONTEMPORARY AND RECENT HUMAN AND FAUNAL POPULATIONS

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Intro to Research

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Abstract.--Previous studies have shown that Sr/Ca ratios were good indicators of trophic levels of human and faunal populations in a given geographical region. These studies have operated under the assumption that removal of debris and post-depositional matter from the bone by using a wash protocol will bring the Sr/Ca ratios to their original state. this study concludes that that is an erroneous assumption. Wildebeest samples collected at death and ten years later were analyzed for Sr/Ca ratios and the results were negative for the return of the tenth-year sample to the ratios of the first-year sample after washing. Andrew Sillen's recent Sr/Ca study is followed in this present study and his conclusions and assumptions are discussed.

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Introduction

"Bone," Pate and Brown (1985) have said, "has two major functions in living organisms. It provides structural support and acts as a mineral reservoir which assists in the maintenance of proper electrolyte concentrations in extracellular fluids." This last function makes bone of great interest in biochemical and anthropological study.

Fresh bone, by dry weight, is about 30% organic and 70% mineral material, with 90% of the organic made up of collagen (Price et al. 1985; Hare 1980). In fossilized bone the mineral portion has become hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$] crystals. These crystals contain information about the background of the animal and the diagenetic changes that have taken place (Pate & Brown 1985; Price et al. 1985; Parker & Toots 1980).

Different elements are incorporated into or leached out of the formed crystal at different rates, and some elements present at death are thought to be incorporated into the crystal as it is formed without much change in concentration (Price et al. 1985; Parker & Toots 1980). It is these last elements that are of interest to scientists who are trying to study the trophic levels of previously living animals. It would be well to enumerate which elements belong in each of the above categories.

After an animal has died and the existing bone minerals have crystallized, most authorities agree that sodium (Na) and calcium (Ca) tend to be leached out of the bone, aluminum (Al), Manganese (Mn), potassium (K), and iron (Fe) tend to enrich the bone and strontium (Sr) and zinc (Zn) tend to be relatively stable with time (Lambert et al. 1984, 1985; Parker & Toots 1980; Pate & Brown 1985).

The concentrations of the above elements in crystallizing bone tissue are affected by individual variations present at the time of death, by environmental changes such as a change in the soil water solution, pH, or temperature, and by diagenetic changes such as "dissolution, precipitation, mineral replacement, and recrystallization" (Pate & Brown 1985; Price et al. 1985).

Most attention has focused on the element strontium because it seems to remain relatively unaffected by the above changes. Zinc also remains stable, but strontium is focused on for yet another reason, which will be discussed below.

Strontium can replace calcium in most tissues, but it is discriminated against by the body. Any strontium not passed through the body collects in the skeletal system because of the easy ion-exchange between

strontium and calcium. This collection of bodily Sr in the skeletal system causes meat to have little or no Sr (Schoeninger 1979). Plants, which can tell no difference between Sr and Ca, readily incorporate environmental Sr into their systems and thus have the highest Sr content of foods (Sillen 1981).

The above pattern has been used to discriminate between carnivores, herbivores, and omnivores on the basis of Sr/Ca ratios. Theoretically, herbivores, which eat only plants, would have the highest Sr/Ca ratios. Carnivores, which eat only low strontium meat, would have the lowest Sr/Ca ratios, and omnivores, which eat both plants and meat, would have ratios intermediate between the other two, depending on the balance of meats and plants in their diets (Price et al. 1985; Schoeninger 1979; Pate & Brown 1985). This theory is based on the assumption that the Sr ratios in post-depositional bone remain relatively stable and are approximately the same in all the bones of one body, as well as being incorporated similarly in all individuals. The results would only be valid for animals in the same geographical region and thus no overall standard could be set up to determine trophic levels. For good results, the crystals must also be formed in quite non-acidic soils (pH above 6.5) since acidity promotes the incorporation of diagenetic Sr into the fossil (Lambert et al. 1985).

Since Ca amounts are very similar in all individuals, the ratio of Sr/Ca has been used to more accurately determine trophic levels based on Sr differences (Pate & Brown 1985). Sillen and others have used this method of trophic level determination and have overall been confident with the results, although there are others who have questioned the reliability of the method (Sillen 1981; Parker & Toots 1980; Lambert

et al. 1979, 1985).

Background

Andrew Sillen's 1986 study using the Sr/Ca procedures, enumerated in his paper "Biogenic and diagenetic Sr/Ca in Plio-Pleistocene fossils of the Omo Shungura Formation" was brought to the attention of these researchers. Some of Sillen's assumptions and conclusions were questioned, so a few hypotheses were formulated and his study was repeated using a different but similar group of humans, and adding a control which he had not used. As shall be seen, the control was the key to the conclusions.

Sillen's study revolved around two groups of humans from geographically different areas in Africa. Sillen assumed that by washing powdered bone with weak acid 24 times, each time drawing off the wash liquid for analysis, that one could wash off the diagenetic elements and return to the original Sr content of the bone. This content could then be analyzed and compared to the others in the group to determine the trophic level of the animal. In his results, Sillen saw trophic differences in the group of humans, based on the Sr/Ca ratios of washes 10-24 of the widely-used solubility profile. He divided the washes into three compartments which he considered significant.

It was hypothesized that Sillen's trophic levels could have been predicted after only a few cleansing washes, and it was also hypothesized that if a control were used, one would find that washing would not extrapolate the Sr/Ca levels to their original ratios. The study reported in this paper revolves around these hypotheses.

Method and Materials

Previous methods of elemental analysis have consistently used atomic absorption (Sillen 1981; Schoeninger 1979; Lambert et al. 1979; Sillen 1986), but the easier and more accurate method of inductively coupled plasma (ICP) elemental analysis was used in this study. Several controls were also added, one being that of two wildebeests' bones collected each year for approximately ten years from and including the year of death, and the other being two modern human hip bones. Other modifications to Sillen's procedure included reducing the number of washes to 20 and combining, for analysis, washes 1 and 2, 3 and 4, 5 and 6, etc. so that each sample would have the required two ml to be run on the ICP.

The skeletal material chosen for this project is human bone of early American inhabitants obtained from the San Diego Museum of Man, San Diego, California. These were chosen for their geographical and cultural similarity. Bones from two wildebeests found in central Africa were also used as well as two modern hip bones.

In the laboratory, each specimen was milled in a Spex model 6700 freezer-mill using a metal rod in a tube with the sample, enclosed and submerged in liquid nitrogen while magnetics were used to move the steel rod, powdering the sample. Fifty mg of each powder was then weighed on a Perkin-Elmer microbalance and placed in a one ml Eppindorf micro-centrifuge tube. One ml of 100 mM acetic acid/sodium acetate buffer, adjusted to pH 4.5, was added to the powder by Eppindorf syringe, and the sample and acid were vortexed for 15 seconds, then centrifuged for 40 seconds on an IEC-M (16,000 x g) microcentrifuge. The buffer thus separated was decanted from the powder and saved for elemental analysis.

This extraction procedure was repeated 20 times on each sample and every two extractions were added together for the analysis procedure on the ICP.

The concentrations of six elements were measured simultaneously in each of the two-wash mixtures using the Jobin Yvon-70 Type II Inductively Coupled Plasma Elemental Analyzer made by ISA. The elements analyzed were strontium, calcium, magnesium, copper, phosphorus, and zinc. Reference solutions were obtained from the ICP supplier. A 5% error in all of the above named procedures was accepted.

The results of these solubility profiles were reported in ng Sr/ug Ca, figured by the following formula:

$$\left[\frac{\left(\frac{\text{ICP Sr Conc. X 1000}}{\text{MW Sr}}\right)}{\left(\frac{\text{ICP Ca Conc.}}{\text{MW Ca}}\right)}\right] = \text{Reported Ratio}$$

Discussion and Conclusions

As in Sillen's graphs, the curves in Figure 1 drop off at first, and then level out after the initial dirt and post-depositional elements have been washed off. The relative positions of the samples on the graph vary only slightly after the initial several washes. No distinct compartments are seen, just a general downward trend with a gradual leveling off.

In Figure 2, the modern bone controls, one sees series 1 climbing in Sr/Ca while series 2 stayed stable, as had been expected. It should be noted that both bones were from hip operations on elderly, healthy persons.

In Figure 3, the wildebeest controls, the two wildebeests are compared

Solubility Profiles
San Diego Museum

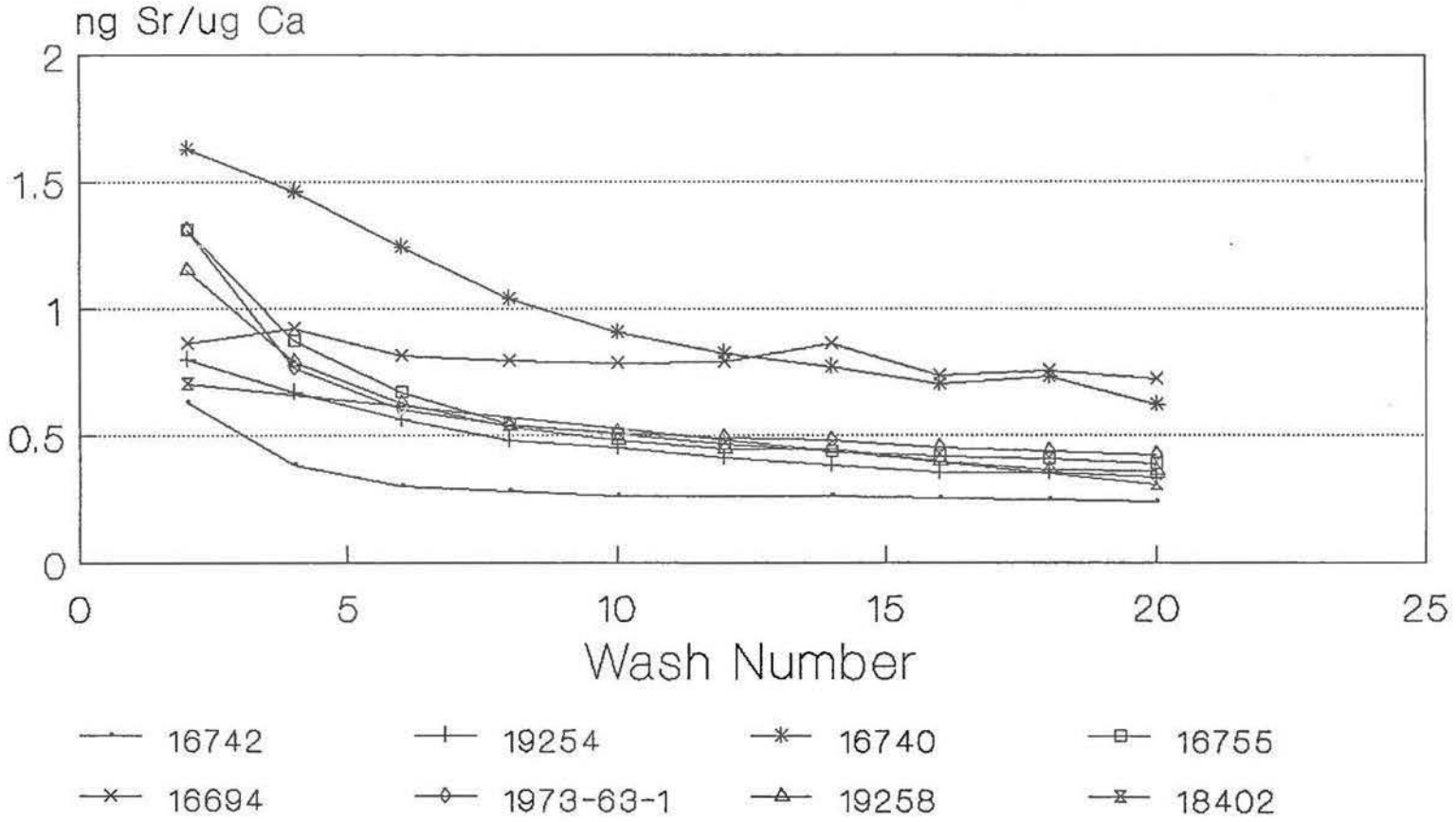

FIGURE 1

SDM 16694: 10x Mg values

Solubility Profiles
Modern

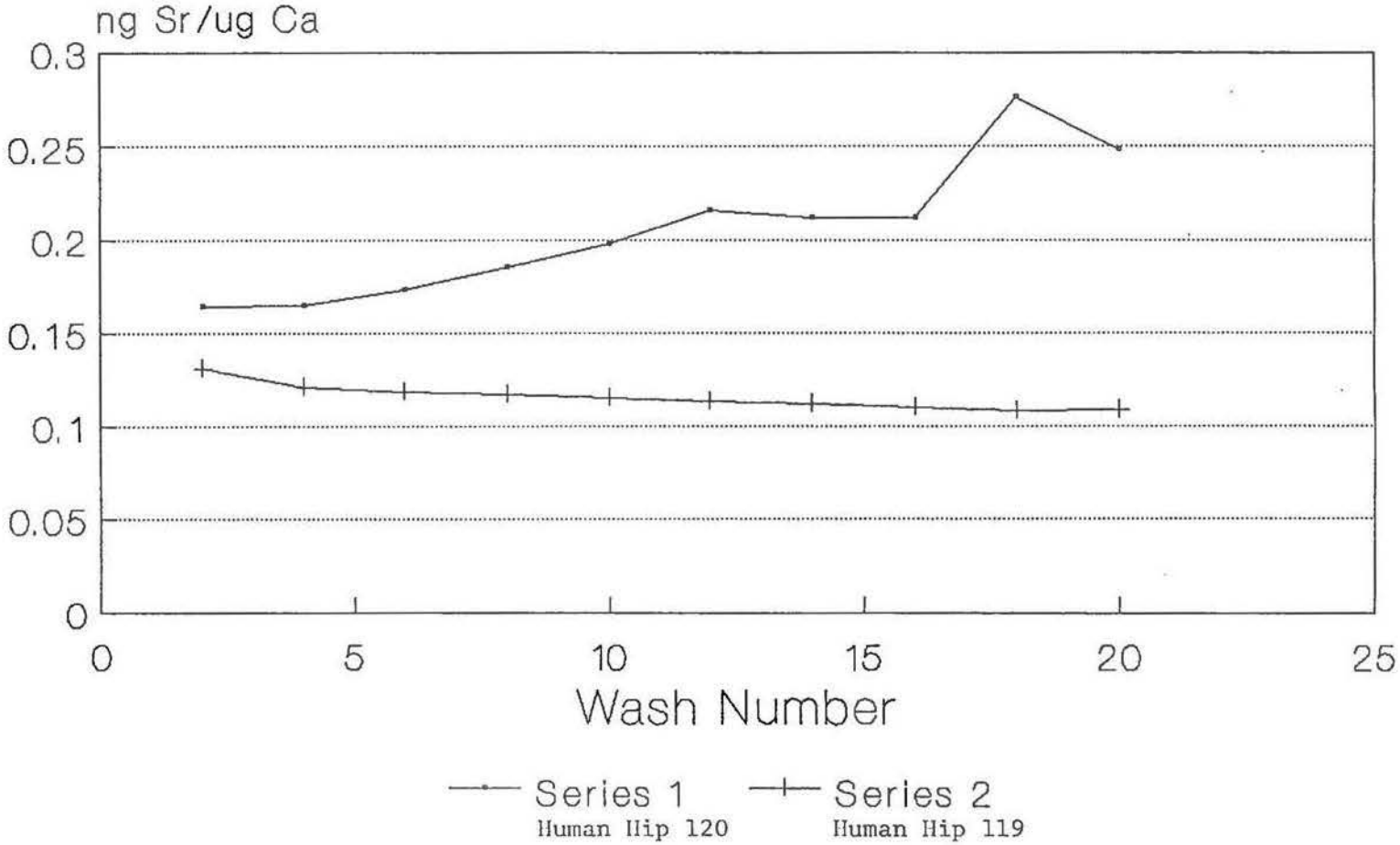

FIGURE 2

Wildebeest Strontium Levels

C75-1 and C75-9

Whole Bone Samples

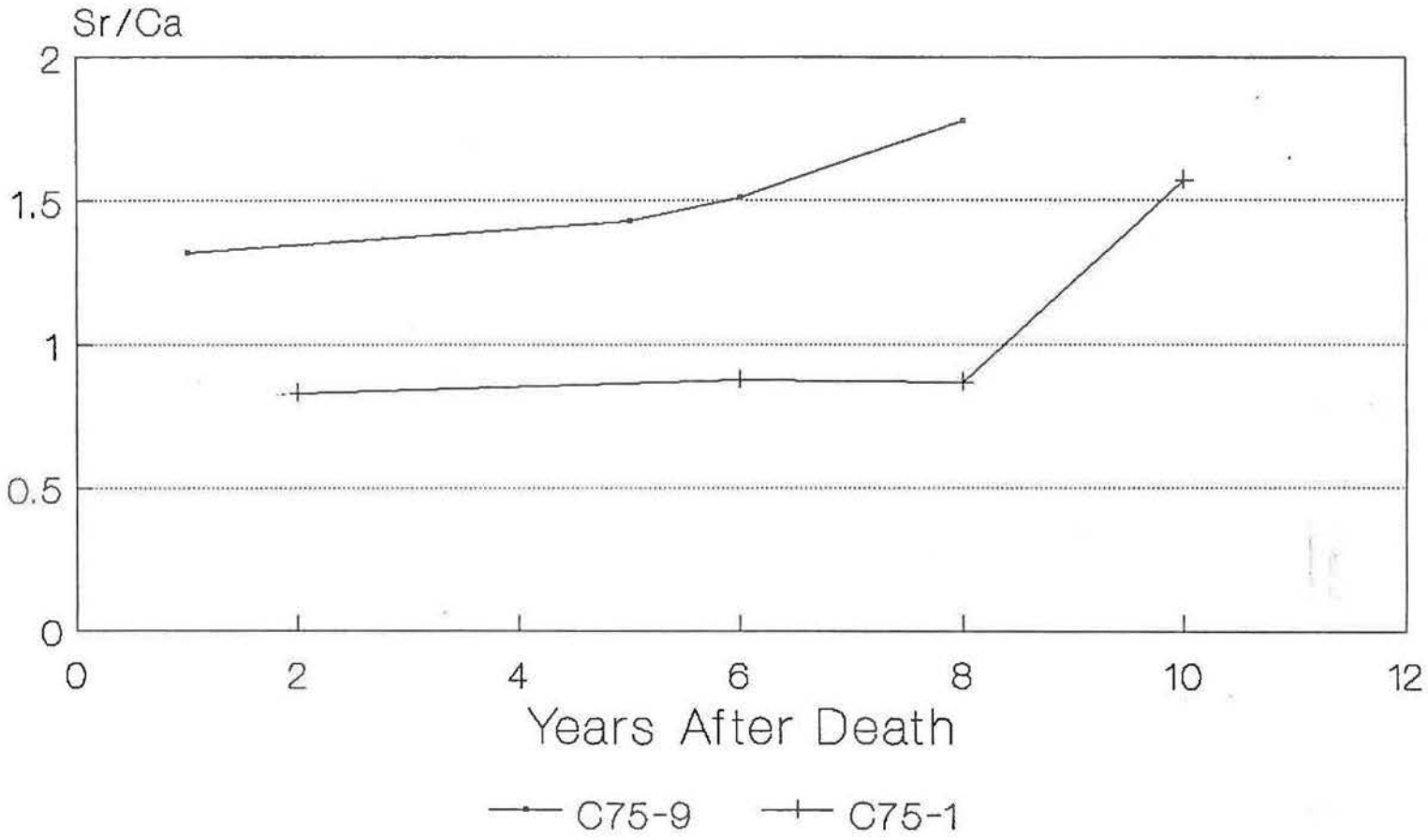

FIGURE 3

Wildebeest Profiles
C75-1

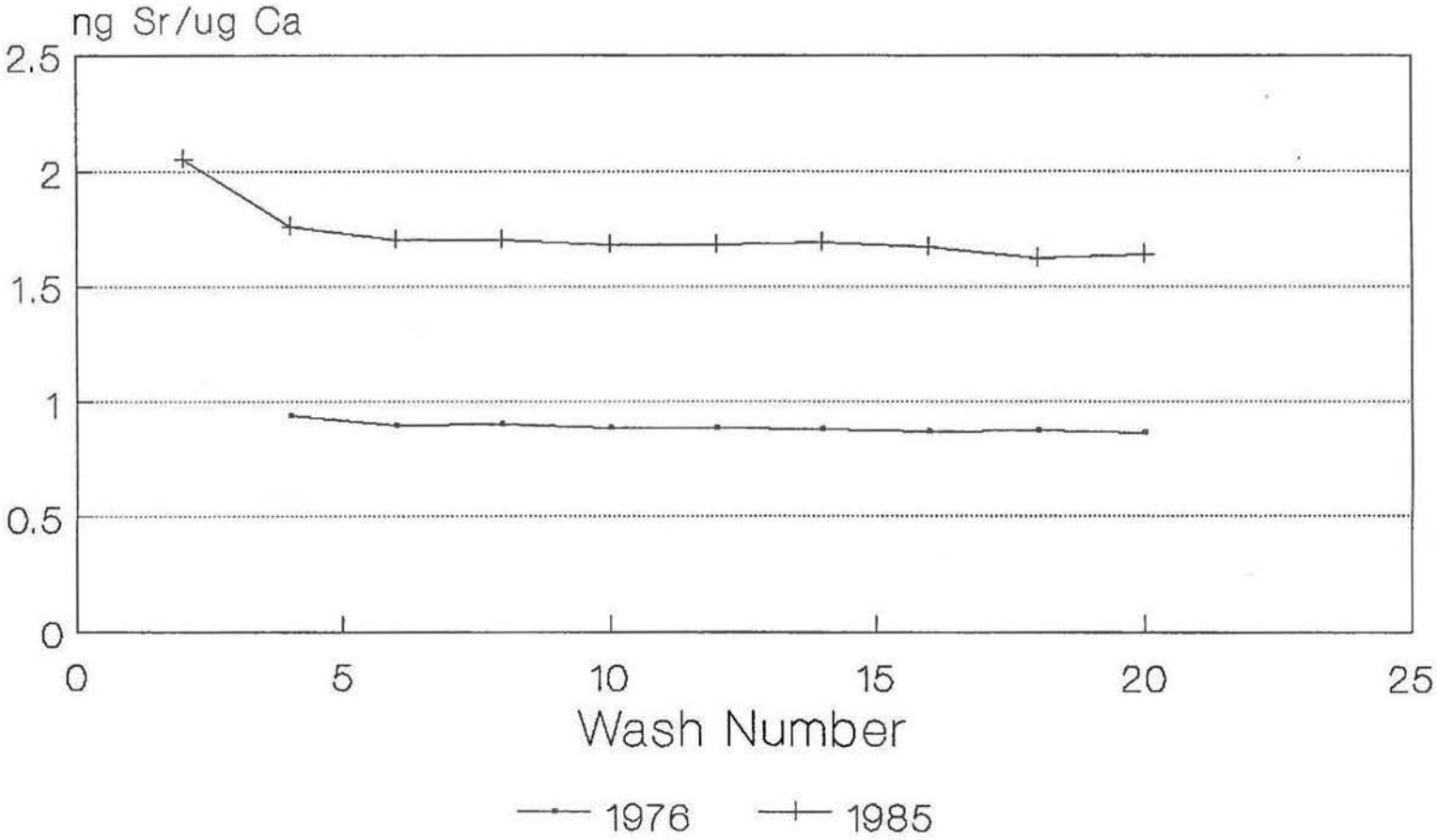

FIGURE 4

Wildebeest Profiles
C75-9

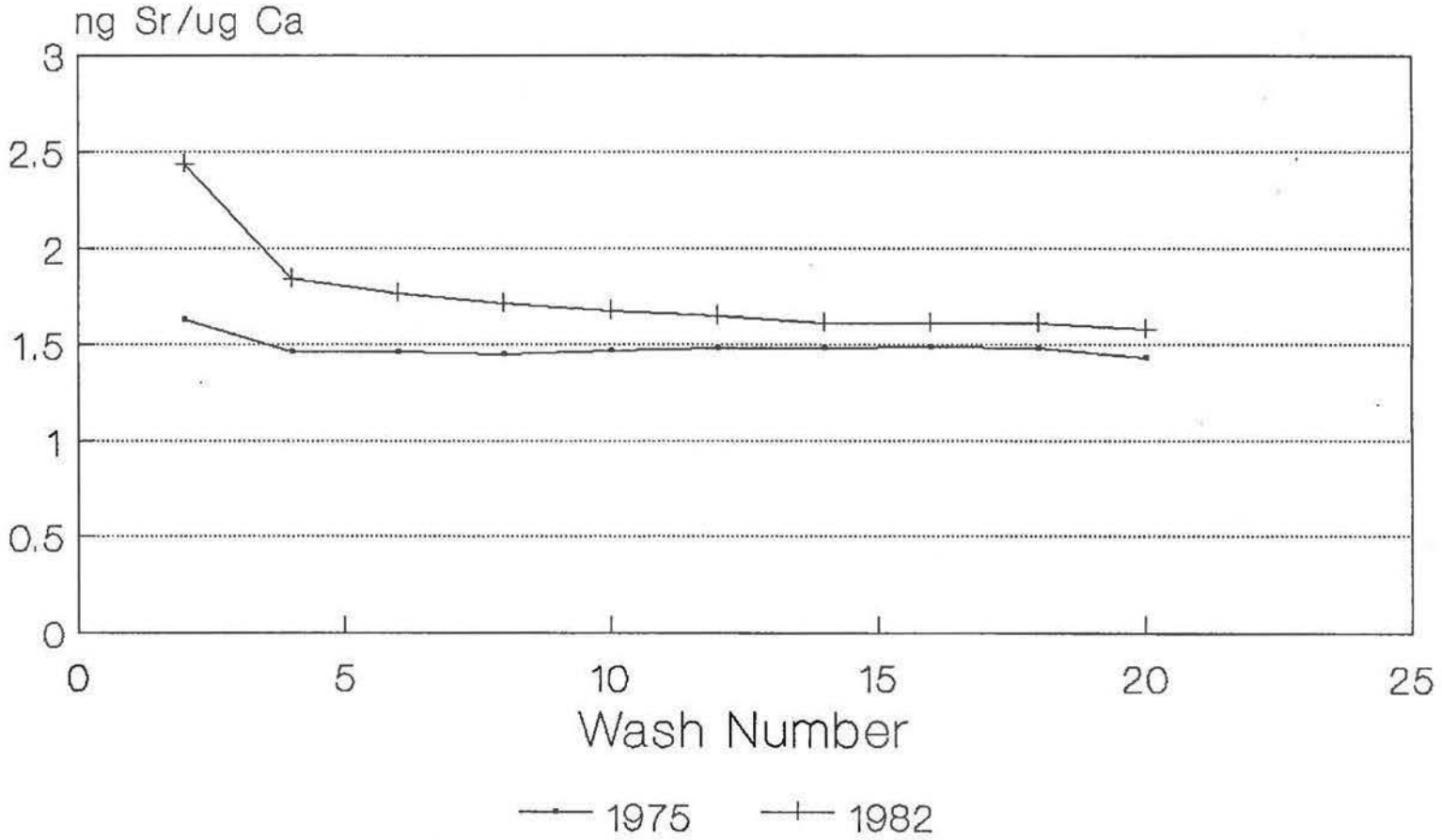

FIGURE 5

ng Sr/ug Ca FOR SAMPLES IN FIGURE I

SAMPLE	Wash # 1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19-20
16742	.632	.386	.303	.284	.265	----	.261	.254	.250	.240
19254	.799	.671	.565	.483	.451	.413	.384	.354	.353	.333
16740	1.63	1.46	1.24	1.04	.906	.826	.771	.703	.731	.620
16755	1.31	.873	.672	.544	.506	.464	.439	.420	.408	.391
16694	.863	.922	.814	.798	.784	.790	.864	.737	.755	.724
1973-63-1	1.31	.769	.603	.540	.511	.490	.479	.454	.436	.425
19258	1.15	.791	.624	.536	.483	.447	.446	.396	.362	.358
18402	.703	----	----	----	----	----	----	----	----	.305

TABLE I

ng Sr/ug Ca FOR HUMAN SAMPLES IN FIGURE II

SAMPLE	Wash # 1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19-20
Hip 120	.164	.165	.173	.185	.198	.216	.212	.212	.276	.248
Hip 119	.131	.121	.118	.117	.115	.113	.112	.110	.108	.109

TABLE II

ng Sr/ug Ca FOR WILDEBEEST SAMPLES IN FIGURES 4 AND 5

SAMPLE	Wash # 1-2	3-4	5-6	7-8	9-10	11-12	13-14	15-16	17-18	19-20
C75-1										
1976	----	.937	.893	.900	.882	.884	.878	.867	.873	.862
1985	2.05	1.76	1.70	1.70	1.68	1.68	1.69	1.67	1.62	1.64
C75-9										
1975	1.63	1.46	1.46	1.45	1.47	1.48	1.48	1.49	1.48	1.43
1982	2.44	1.84	1.76	1.71	1.67	1.65	1.61	1.61	1.61	1.58

TABLE III

as to Sr/Ca ratios in years after death. These results are from whole bone samples that had been dissolved in 12N HNO_3, nutated overnight, and then analyzed by ICP. These are straight, unwashed bone samples which show the actual Sr/Ca ratios of the bone at death and each year thereafter. In both animals there was an initial lag period after which the bone began to take on Sr and/or leach Ca.

Figures 4 and 5 are comparisons of the wash protocols of the first and last years collected on each wildebeest. According to Sillen's assumptions, the curve for the most recent year and the curve for the year of death should converge, proving that the wash protocol extrapolates the bone back to its original Sr levels. The curves do not converge on either wildebeest graph, implying that extrapolation does not take place. It is noted that the two curves formed by wildebeest C75-9 came much closer together than those of C75-1, but still did not converge.

Table I lists the actual Sr/Ca ratios for the points on the graph in Figure 1. A general decline in ratios is noted, with an initial sharper drop and then a leveling off. Tables II and III list the human hips and wildebeests, respectively. The odd gradual increase in Sr/Ca ratios of the hip 120 is noted again, and it is also noted that the wildebeests follow the same pattern as the San Diego Museum specimens.

In conclusion, it can be stated that Sr/Ca ratios cannot be accurately used to determine trophic levels using the wash profile since washing does not bring the sample back to its original Sr/Ca ratio. This conclusion is drawn from the results of the wildebeest profiles.

It can also be concluded that large numbers of washes do not enhance the accuracy of the results because the final relative positions of the samples on the graphs can be predicted from about wash 6 (after

the initial drop in ratios due to removal of debris from the bone surface).

Modern hip 119 has a relatively even curve, as was expected. Hip 120 however, is a curiosity, as was mentioned previously. This is an anomaly which might have been due to an error in the calibration of the ICP, although that is not extremely likely. It would be interesting to investigate this further, however that would be beyond the scope of this paper.

In comparing Figures 3, 4, and 5, it can be seen that wildebeest C75-9 has consistently higher Sr/Ca ratios than C75-1 in both the whole bone analysis and the wash profiles. In the profiles for the year of collection, the curves for both animals remained relatively even, while the most recents years' profiles both show a decline and then a leveling of Sr/Ca ratios. This shows that the animals are similar, although they came from different geographical regions, as is evidenced by the consistently higher Sr/Ca ratios of C75-9 over C75-1.

This study has concluded that until further concurrent methods can be used with Sr/Ca ratios, the wash protocol is useless in truly determining trophic levels of ancient populations because the original elemental level of Sr cannot be reached.

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