



Luigi Capasso, Gabriella Di Tota

## ATOMIC ABSORPTION STUDIES ON ANCIENT HUMAN DENTAL CALCULI FROM AN IRON AGE POPULATION IN CENTRAL ITALY (SULMONA, ABRUZZO, ca IIIrd CENTURY B.C.)

**ABSTRACT:** The authors analyse the chemical composition of tartar detached from the teeth of 11 subjects from the pre-Roman Iron Age necropolis in Sulmona (Abruzzo, dated to ca the 3rd century B.C.). The analysis has been carried out spectrometrically in order to standardize a possible analytic method for this organic archaeological material. The results obtained, although preliminary, offer the possibility of using data regarding the chemical composition of dental calculi even for paleoalimentary reconstructions.

**KEY WORDS:** Human dental calculi — Atomic absorption studies — Iron Age population — Sulmona, Abruzzo (central Italy).

### INTRODUCTION

Tartar calculi are the result of precipitation of calcium phosphate ( $\text{Ca}_3(\text{PO}_4)_2$ ) and magnesium phosphate ( $\text{Mg}_3(\text{PO}_4)_2$ ) salts within the bacterial plate. This plate is a mucoproteic gel of salivary origin with the contribution of food particles and substances originating from the activity of bacterial colonies.

Tartar calculi, as a whole, contain a proteic fraction (derived from salivary glycoprotein, from oral bacterial activity, and directly from food) and a mineral fraction that comes from the deposition of ions (mainly Ca) on carboxylic groups of glycoproteins. The mineralization activity on glycoproteins of the plaque depends, qualitatively, on salivary pH also (Ravisanda 1984).

The bioarchaeological interest of tartar calculi depends on their long preservation after death (the result of being saturated with mineral salts), and they can easily be individualized in human graves from archaeological excavations.

The chemical composition of tartar depends on the diet in a direct way (as the chemical composition of food influences the chemical habitat of the mouth where the deposition of tartar occurs), as well as in an indirect way (as, certainly, the chemical composition of saliva is influenced by the chemical composition of plasma, which also depends on the diet). The chemical study of tartar from archaeological excavations could therefore open a new field of application, especially when correlated with the composition of diet in ancient populations.

Although it has been clearly established that trace elements in bone have a concentration strictly related to the composition of the diet (Bisel 1980), this has not so far been considered in tartar. Theoretically, the chemical composition of tartar (and, consequently, the absolute and relative quantity of trace elements present in it) should be influenced by diet in the same way as the chemical composition of bones. This is principally due to the fact that not only substances present in saliva (and, therefore, in plasma) but also some residues derived from food introduced into the oral cavity accumulate in tartar.

In order to verify this connection between the composition of diet and that of ancient tartar, we have started a research protocol in our laboratories of paleopathology on these points:

A) to determine the chemical composition of tartar found in archaeological excavations in relation to the chemical composition of tartar taken from living beings;

B) to investigate the possible qualitative correlations between the concentrations of trace elements in tartar with respect to bone;

C) to codify the possible use of tartar analysis as a basis for palaeoalimentary reconstruction.

In this preliminary paper we explain the results of our first investigation, carried out to ascertain the relationship between the chemical composition of ancient tartar and that of today.

### MATERIALS AND METHODS

The material utilized for this preliminary study consists of 11 samples of tartar taken from the tooth surfaces of 11 subjects from the necropolis of Sulmona (Aquila, Central Italy), and dating back to the Iron Age (about 3rd century B.C.).

There were 5 females and 6 males, all adults between 30 and 40 years of age.

Eleven samples of tartar taken during ultrasonic ablation in the Clinica Odontoiatrica of Chieti University, from 5 females and 6 males between 30 and 50 years of age, have also been examined. The same processes of preparation and analysis have been utilized for both archaeological and modern samples. Tartar calculi have been taken through ultrasounds, then carefully washed in distilled water for two days, and dehydrated in a drying oven at 50 °C for 48 hours, after which the tartar has been reduced to ash at a temperature of 600–700 °C (calcination).

The ash has then been treated with nitric acid and the solutions totally evaporated.

The inorganic residue, which has finally turned white, has been treated with hydrochloric acid and nitric acid for solubility and the destruction of any organic residue. Brought to volume it underwent the necessary dilutions for the different concentrations of inorganic ions.

Readings have been taken from the atomic absorption spectrophotometer for the various elements: for potassium 565 nm, for strontium 460.7 nm, for sodium 589.6 nm, for calcium 422.7 nm; phosphorus has been determined colorimetrically with the molybdenum-stannous chloride method.

The organic substances have been determined by weight difference estimation before and after calcination. A spectrophotometric investigation has been carried out separately to determine the absorption during the interval of 260–280 nm, in correspondence with which we have obtained the absorption band of the nucleic acids and of aromatic aminoacids.

## RESULTS AND DISCUSSION

The data of the chemical investigation of the inorganic part are reported in *Tables 1 and 2*, in which the various ion percentages in ppm are evident for both the ancient dental calculi (*Table 2*) and the modern ones (*Table 1*).

It is difficult to propose any interpretation of these data because no comparison can be done yet, so we can only outline some general considerations. From the absorption curve of the organic part of the calculi, both ancient and modern, according to Piattelli et al. (1984), an absorption around 260 nm results: the absorption band of nucleic acids and phenylalanine. The absence of tryptophane and tyrosine, indicated by the lack of absorption around 280 nm, leads us to deduce the presence of collagen or a fraction of it in the proteic part. Quantitatively, however, it is necessary to emphasize that, according to what Piattelli et al. (1984) have already established, the peak at 260 nm, is clearly and constantly higher in samples of modern tartar; in those of ancient tartar the same peak, even if present, is hardly mentioned and in some of them is nearly invisible. It is difficult to guess the origin of this organic fraction in ancient tartar, but it is possible that it represents an altered residue of proteins coming directly from food and, above all, from the bacteria that formed the original plaque. The difference in content of organic substance in ancient tartar with respect to the modern one is considerable as one may imagine (*Table 1*); this means that the organic fraction present in the tartar is formed by substances highly stable in time, as happens for bone.

As far as the inorganic fraction is concerned, we have expressed the concentration of each ion both in absolute value (ppm) and in relation to the amount of calcium present in each sample. In this way we have obtained an index to which many authors who studied the trace elements have given a comparative value (Lambert et al. 1972, 1982, 1983). This index is very reliable, as the probable concentration of each single trace element in tartar and in bone is proportional to the levels of mineralization of the organic matrix and, finally, to the absolute quantity of calcium.

As far as the sodium and potassium ions are concerned, in modern calculi the major concentration of potassium with regard to sodium (*Figure 1*) is due to the fact that the potassium con-

centration in the saliva is greater than that of sodium (contrary to plasma); in fact, the salivary glands tend to concentrate potassium, calcium and phosphate, and the magnesium concentration remains unvaried. This situation is inverted in the archaeological remains of dental calculi: in this case the sodium concentration is greater than that of potassium (*Table 2*). The concentration of such ions descends to very low values, which is understandable if we keep in mind the great solubility of sodium salt and potassium.

As far as the calcium concentration is concerned, this ion is in a stoichiometric relationship with the phosphorus (e.g. in calcium phosphate called hydroxyapatite). It is also important to notice that there are no significant differences in calcium ion concentration in the sample of ancient tartar when compared with samples of modern tartar. This could be due to a relative insolubility that characterizes the calcified proteic chemical complexes (*Figure 1*).

Phosphorus, however, especially in modern samples, is in a slightly superior concentration to the stoichiometric relationship. This could derive from the fact that phosphorus could be found also as magnesium phosphate and bicalcium phosphate, besides phosphorus deriving from organic compounds like nucleic acids and phosphatides.

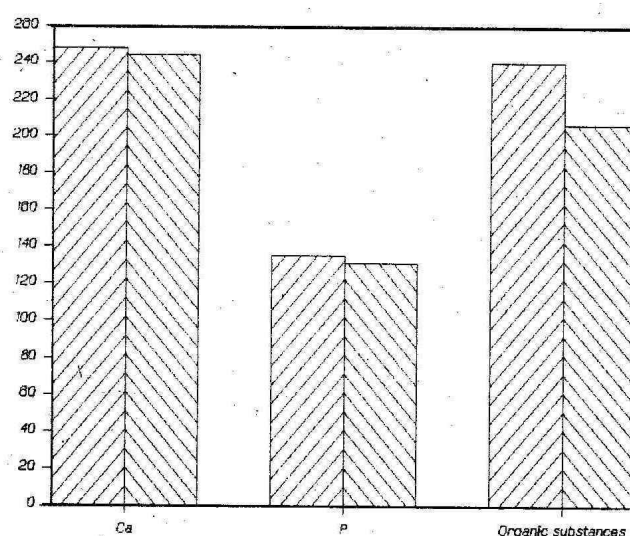


FIGURE 1.  $\diagup$  MODERN SAMPLES  $\times$  ANCIENT SAMPLES

TABLE 1. Minerals content of modern tartar samples.

Sample No.	note	Ca	K		Na		Mg		Sr		P		organic substance ppm
		ppm	ppm (K/Ca)×100		ppm (Na/Ca)×100		ppm (Mg/Ca)×100		ppm (Sr/Ca)×100		ppm (P/Ca)×100		
1	male	306.600	2210	7.208	4590	14.971	1830	5.969	50	0.163	148,400	109	261.500
2	male	200.500	3760	18.753	1950	9.726	570	2.842	80	0.399	96,100	479	195.100
3	male	215.100	4730	21.990	2730	12.692	1640	7.624	10	0.025	132,700	617	183.300
4	male	240.700	6610	27.462	3100	12.879	1180	4.902	20	0.083	180,200	749	277.700
5	male	260.600	2700	10.361	2730	10.476	930	3.569	20	0.077	110,200	423	242.100
6	male	310.400	4360	14.046	4130	13.305	2450	7.893	90	0.290	207,200	667	329.600
7	female	207.700	4980	23.977	790	3.804	1040	5.007	60	0.289	84,300	405	189.300
8	female	251.100	4920	19.594	3320	13.222	1040	4.540	10	0.040	106,000	422	204.100
9	female	187.200	1990	10.630	3140	16.774	770	4.113	10	0.053	120,500	643	171.900
10	female	298.100	4300	14.425	4760	15.968	1430	4.797	180	0.604	195,400	655	360.400
11	female	248.100	5370	21.645	3400	13.704	90	0.363	110	0.443	108,200	436	227.200
mean	—	247.827	4175	17.281	3149	12.502	1179	4.693	58	0.224	135,382	510	240.200
S.D.	—	43,264	1411	6.399	1144	3.558	643	2.107	54	0.195	41,899	180	61.890

TABLE 2. Minerals content of modern tartar sample.

Sample No.	note	Ca	K		Na		Mg		Sr		P		organic substance ppm
		ppm	ppm	(K/Ca)×100	ppm	(Na/Ca)×100	ppm	(Mg/Ca)×100	ppm	(Sr/Ca)×100	ppm	(P/Ca)×100	
1	male	226.100	590	2.609	2080	9.199	1320	5.838	100	0.442	120.100	531	183.100
2	male	248.400	210	0.845	300	1.208	1590	6.401	100	0.403	107.400	432	191.300
3	male	115.800	180	1.554	900	7.772	470	4.059	20	0.173	40.100	346	68.100
4	male	256.000	320	1.254	760	2.969	160	0.626	260	1.016	149.000	582	262.700
5	male	263.700	500	1.896	1870	7.091	2850	10.808	160	0.607	190.300	722	288.100
6	male	290.100	270	0.931	1690	5.826	3030	10.445	130	0.448	24.000	83	233.500
7	female	271.000	150	0.554	390	1.439	2020	7.454	180	0.664	158.500	585	291.000
8	female	244.500	290	1.186	1710	6.994	1300	5.317	90	0.368	96.600	395	216.400
9	female	260.000	70	0.269	1350	5.192	1970	7.577	110	0.423	138.100	531	255.800
10	female	302.300	280	0.926	1980	6.550	2080	6.881	270	0.893	103.300	342	267.600
11	female	210.700	297	1.410	1110	5.268	3350	15.899	10	0.047	315.700	1498	273.200
mean	—	244.418	287	1.221	1285	5.410	1831	7.391	130	0.499	131.191	550	206.264
S.D.	—	49.923	149	0.648	635	2.573	1007	3.978	84	0.285	78.166	357	85.241

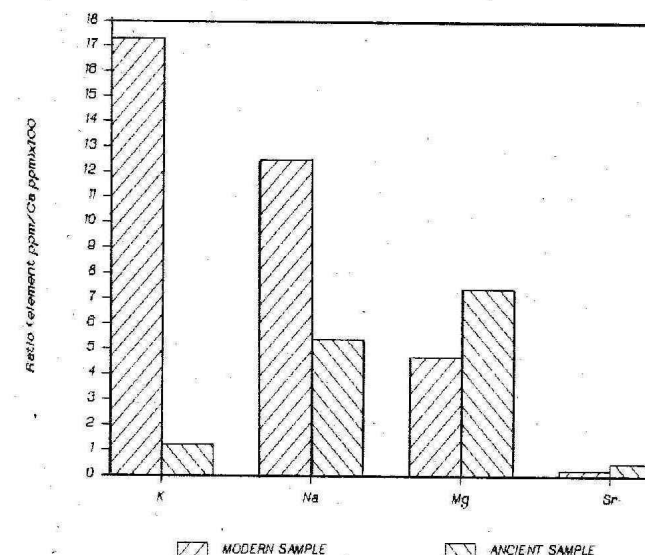


FIGURE 2.

In ancient tartar the ratio (P/Ca) × 1000 is about 550 (Standard Deviation = 357). This value is included among those typical of human bone; for example, Bisel (1980) examined a vast series of Eastern Mediterranean skeletal remains from different periods (between Neolithic and Middle Ages), and found a ratio (P/Ca) × 1000 oscillating between 596 and 424. This similarity could be related to the fact that in tartar, as in bone, most calcium and phosphorus is stoichiometrically bound, and this link is stable in time, surviving even after millennia of inhumation.

As far as research on trace elements is concerned (*Figure 2*), we have analyzed the concentration of strontium, and found that this element is always more abundant in ancient tartar than in modern, with an average ratio of about 3:1. We do not know whether this has any relationship to the composition of the diet, or to a greater percentage of vegetal food in the diet of the ancient population in comparison to the modern. In theory, there is no reason not to believe that the concentration of strontium in tartar (of salivary origin, and therefore present with a concentration proportional to that of plasma, but also of bacterial origin and of direct alimentary origin) was related to the quantity of vegetal food in the diet (Piattelli et al. 1984), just as other researches have shown in bone (Bisel 1980). However, even considering that, the information needed to establish the forms of calibration of stron-

tium dosages with respect to the site are completely lacking. It will therefore be necessary to calibrate the concentrations of trace elements present in dental calculi with those present in human bone and in herbivorous animals' bone from the same site.

A great deal of this study is still in progress in our laboratories in collaboration with the ANTARES (Australian National Tandem Accelerator for Applied Research) of the ANSTO (Sydney, Australia).

The considerations given for the strontium ion are valid for the magnesium ion as well. In modern tartar we have found that the concentration of Mg varies between 90 and 2450 ppm (average 1179; S.D. = 643); in ancient tartar, on the contrary, concentrations are much more elevated, varying between 160 and 3030 ppm (average: 1831; S.D. = 1007).

In human bone  $Mg^{++}$  ions can substitute for  $Ca^{++}$  ions in the hydroxyapatite crystalline structure, but in tartar the situation is certainly different. In fact,  $Mg^{++}$  ions present in the mineralized fraction of tartar come from saliva (where their presence is proportional to the quantity of  $Mg^{++}$  present in plasma), and directly from food, especially vegetables, legumes, seafood, nuts and cereals. However, we mention that Bisel (1980) in examining a vast sample of ancient human bones, has found a ratio (Mg/Ca) × 1000 oscillating between 1.6 and 12.2. In our sample of ancient tartar it has been found that this ratio has an average of about 7.4 (S.D. = 3.978). This would confirm that magnesium is present in dental calculi bound stoichiometrically to hydroxyapatite.

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Luigi Capasso  
 Gabriella Di Tota  
 Laboratories of Anthropology  
 National Archaeological Museum  
 Via Villa Comunale, 3  
 661 00 – Chieti (Italy)