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Comparative thermogravimetric, x-ray diffraction and electron microscope investigations of burnt bones from recent, ancient and prehistoric age

Istochimica e mineralogia. — *Comparative thermogravimetric, x-ray diffraction and electron microscope investigations of burnt bones from recent, ancient and prehistoric age* [*]. Nota di Ermanno Bonucci e Giorgio Graziani, presentata [**] dai Corrisp. A. Ascenzi e C. Lauro.

Riassunto. — Sono state studiate comparativamente, in campioni di osso fossile musteriano, di osso del vi–v secolo A.C., e di osso recente, le modificazioni strutturali causate nel tessuto osseo dall'esposizione al fuoco.

A tal fine i provini di osso sono stati analizzati mediante analisi termiche, diffrattometriche ai raggi *x* ed elettroniche, ed al microscopio elettronico.

I risultati hanno dimostrato che nel tessuto osseo, esposto in aria a temperatura elevata, la componente inorganica subisce profonde modificazioni strutturali, già evidenti alla temperatura di circa 300 °C. Queste modificazioni, del tutto tipiche, permettono di dedurre la temperatura alla quale un frammento d'osso è stato esposto.

Introduction

Thermogravimetry or thermogravimetric analysis is a techinque used for registration of weight changes produced in a body by volatilization of chemical compounds as consequence of heating (Duval, 1963). Useful information can be obtained with this technique about the structure and composition of the heated body.

Thermogravimetry has been used to study compact bone by Civjan *et al.* (1971), and enamel and dentine by Holager (1970). In all of these tissues three distinct weight losses have been recorded during heating, the first presumably corresponding with loss of absorbed and bound water (about 500 °C), the second with volatilization of organic substance (about 600 °C) and the third with disappearance of carbonates (600–800 °C). Between 600 and 800 °C the single hydroxyapatite crystallites undergo deep changes and their dimensions drastically increase (Andreatta and Fonni, 1952; Carlstrom and Engfeldt, 1954; Dallemagne and Richelle, 1973), probably because hydroxyapatite is transformed to tricalcium phosphate (Civjan *et al.*, 1971). Very probably, the loss of weight occurs together with ultrastructural changes, but to our knowledge no electron microscope investigations have been carried out on heated and calcinated bone, dentine and enamel.

Recently, we have had the opportunity of studying fossil bone (musterian period) and ancient bone (vi–v century B.C.), all of which appeared to have been exposed to fire. Because the analysis of the structural and conformational changes produced in calcified tissues by heating can be of obvious

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anthropological, archeological, biological and paleontological interest, we have studied fossil and ancient bone with thermal methods of analysis, electron microscopy, electron and x-ray diffraction and have applied the same methods to recent compact bone, with the main aim of:

a) Establishing by means of thermogravimetry (TG) and differential thermal analysis (DTA) the thermal behaviour of normal bone and by means of electron microscopy and x-ray and electron diffraction the ultrastructural changes eventually produced by heating;

b) Investigating the thermal (TG and DTA) behaviour of burnt fossil and ancient bones and their ultrastructural changes;

c) Comparing the results obtained in recent bones with those concerning fossil and ancient bones;

d) Examining the possibility of deducing the temperature at which fossil and ancient bones had been heated from their ultrastructural conformation.

MATERIAL AND METHODS

Three types of bones have been studied: *a*) fossil bone; *b*) ancient bone; *c*) recent bone.

a) Specimens of *fossil bone* have been obtained from a long, not better identifiable bone found by A.C. Blanc (1938, 1939) together with remnants of Rinoceros, Bos, Cervus and Equus in the musterian layer which formed the ground of the north-west wall of the Fossellone Cave (Latium, Italy). One end of this bone showed a relatively preserved epiphysis, the other end was fractured. The zone near the eiphysis was black probably as a consequence of carbonization, while the zone near the fractured end was whitish, as if it had not been exposed to fire. An intermediate zone was ochraceous, as if exposed to a moderate degree of heating. Specimens have been taken from each of the three zones and marked " black ", " white " and " ochraceous ", respectively.

b) Specimens of *ancient bone*, datable to the 6 th-5 th century B.C., have been obtained from skeletal remnants (ribs, vertebrae, skulls) of cremated young children, whose ashes were contained in cinerary urns found in the 2nd sheet of the 3rd layer of the " tofet ", the phoenician sanctuary of Mozia (Sicily, Italy) (Ciasca, 1972-73, 1973). These osseous specimens have been chosen because there was a strong probability that, as shown by their colour and extreme brittleness, they had been exposed to very high temperatures, certainly higher than those concerning fossil bone. Among these cremated bones two types have been selected, one consisting of black-grayish, the other of white-grayish specimens. They have been marked " black-grayish " and " white-grayish ", respectively.

c) Specimens of *recent, compact bone* have been obtained from the diaphysis of ox tibiae soon after the death of the animals.

All of the bone specimens have been studied with the following methods:

1) *Thermic analyses*: these analyses have been carried out on bone powder (granule size 53-37 μ) obtained by grinding the very superficial bone, i.e., bone which had been exposed directly to fire, from areas of different colour. The bone powder of the single specimens has been comparatively examined by TG and DTA using a Cahn electrobalance model RG (sensitivity 0.1 μg) and a BDL micro-thermodifferential equipment with a Platinel II probe. The registration of ponderal and differential signals has been obtained by a properly modified multichannel system " Laben Correlatron 4096 ". After amplification, the signals have

been digitalized according to pre-established constants and the numerical values, memorized for adequate time intervals, have been recorded and, if necessary, selected on printing paper. This made it possible to easily operate on experimental data, eventually by calculating derivations and integrals, correcting the instrumental background, and visually comparing the thermograms.

The thermal analysis has been carried out in standard conditions by operating in a constant flux of dried air (3 l/min) and with linear temperature increments of 5 °C/min. The weight of the material examined in each experiment was of about 25 mg.

2) *Diffractometry*: the bone powder of all of the specimens has been examined by x-ray diffraction according to the technical modalities reported by Carlstrom (1958). Moreover, specimens from recent ox bones have been examined by x-ray diffraction after heating at different temperatures. Temperature intervals have been chosen on the basis of the slopes of both TG and DTA diagrams. On this basis, specimens of ox bone heated at 105°, 200°, 300°, 350°, 450°, 550°, 650°, 750° and 900 °C have been chosen.

The crystallites of tricalcium phosphate having hexagonal symmetry, it is possible to calculate their dimensions from the reflections $(00l)$ and $(hk0)$. Since the x-ray diffractograms have been carried out using not oriented samples of bone, and since the reflections were rather weak with unsharp definition in respect to the irregularities of the background, calculations have been performed by considering only $(00l)$ reflections and particularly (002) reflection which is the best defined. The relation between the dimension of this reflection and the length of the crystallites has been calculated according to Scherrer's formula (Klug *et al.*, 1969); the values of the equation factors have been drawn from Carlstrom's paper (1958).

Selected area electron diffraction has been carried out using a field-limiting diaphragm of 50 μ at 60 KV and 10 mA.

3) *Electron microscopy*: all of the specimens, including those from ox bones heated at different temperatures, have been studied with electron microscopy. To this end, small blocks (3 × 2 × 1 mm) have been taken from the bone specimens in such a way that their external surface is easily recognizable. After acetone dehydration, these blocks have been embedded in Araldite and their external surface has been suitably oriented so that it is possible to cut sections only from the most superficial layers. Ultrathin sections (500-800 Å thick) have been examined without staining, so that only the inorganic substance is visible because of its intrinsic electron density.

Results

1) *Thermal analysis* (TG, DTA).

The results are summarized and graphically reported in figs. 1, 2, 3, 4, 5 and 6. Each figure refers to a single bone type and shows the thermogravimetric diagram (TG), the corresponding electron derivative (DTG) and the differential thermal diagram (DTA).

The comparative analysis of the thermogravimetric diagrams shows that, although of rather similar shape, the curves reveal obvious differences due to different weight loss. If the total weight loss at 900 °C is considered, it is of about 38.6 % in recent bone and considerably decreases in the " white ", " ochraceous " and " black " parts of the fossil bone, where it reaches a value of 30.6, 18.8 and 18.3 %, respectively. The total weight loss further decreases in the " black-grayish " part (11.8 %) and in the " white-grayish " portion (2.4 %) of ancient bone.
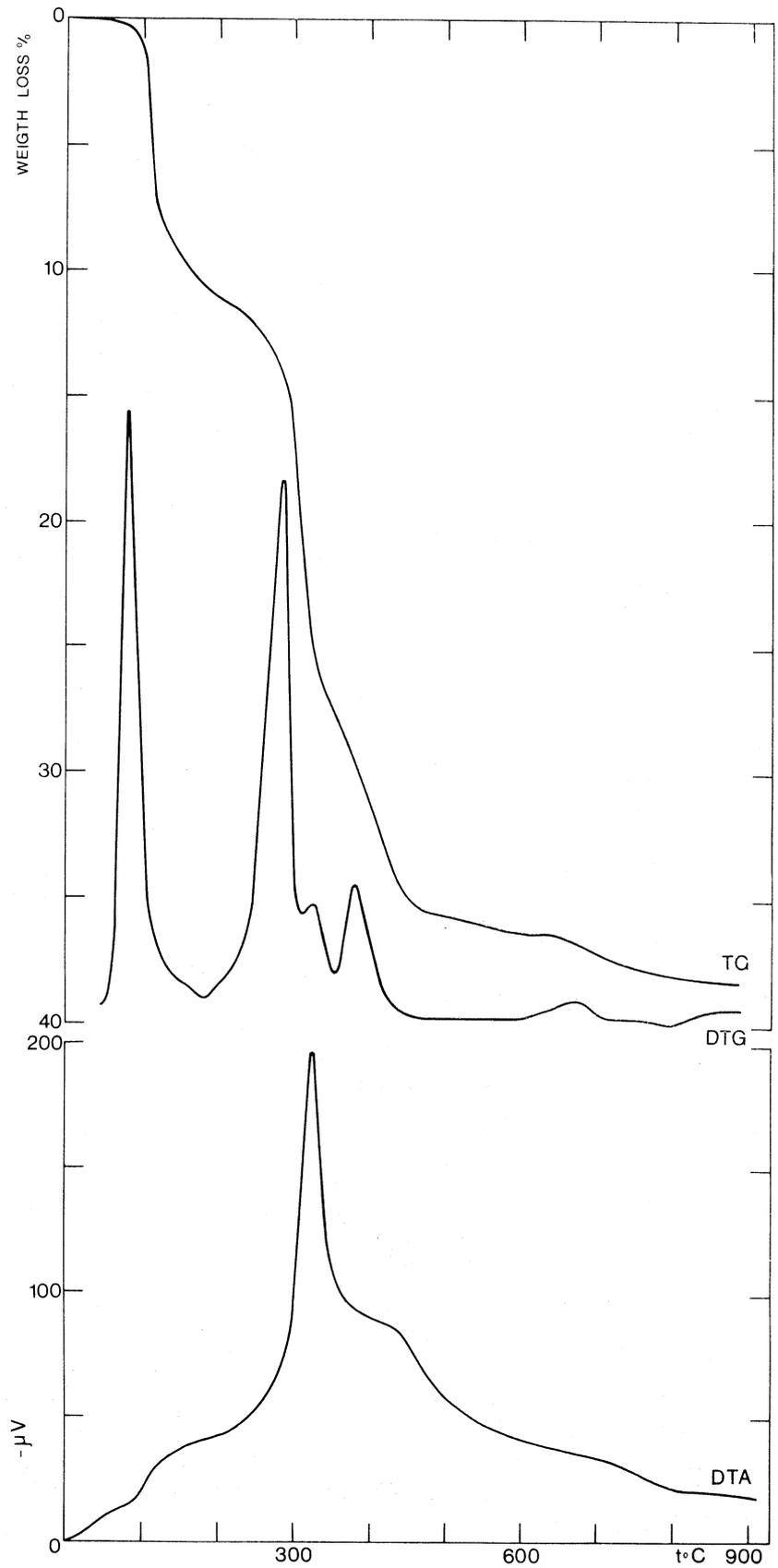
Fig. 1. – Recent bone. Thermal analysis.
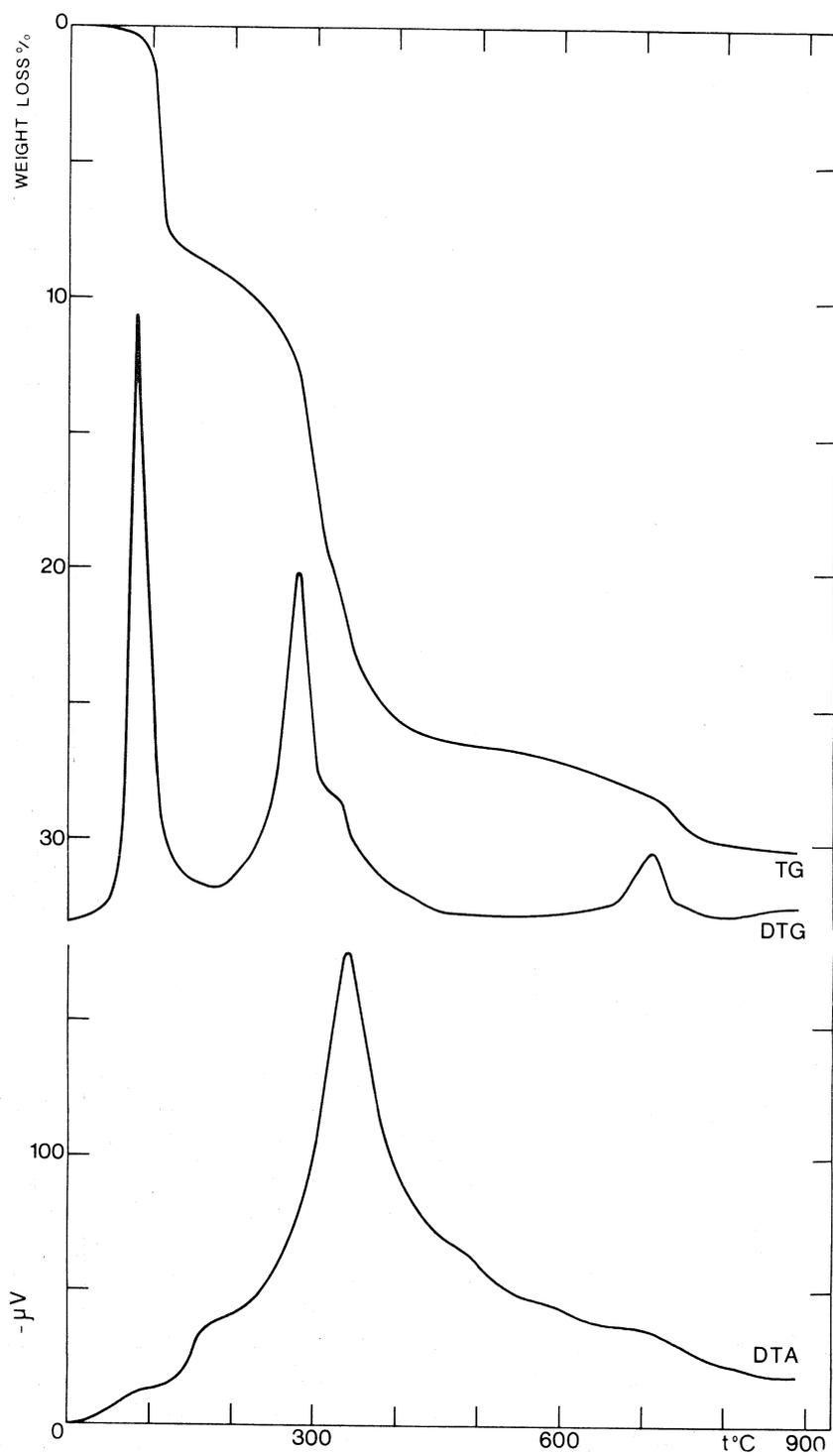
Fig. 2. – Fossil bone: " white " portion. Thermal analysis.
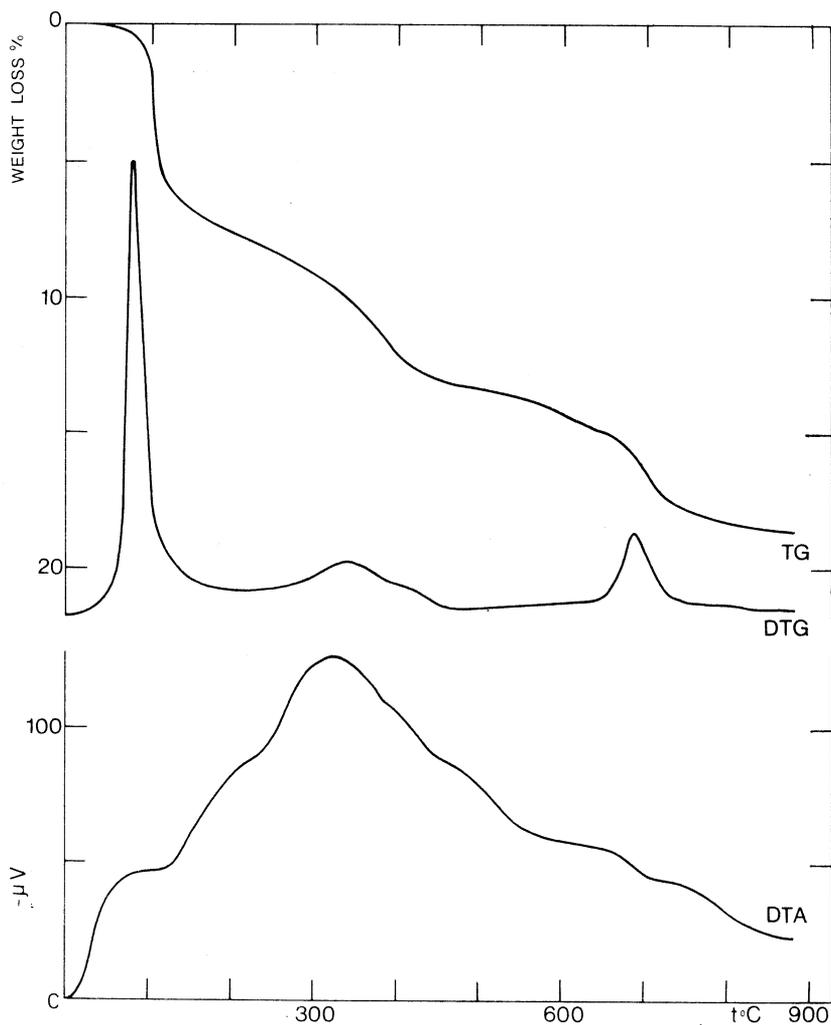
Fig. 3. – Fossil bone: " ochraceous " portion.   Thermal analysis.

The thermogravimetric diagrams show a first weight loss at 105 °C, followed by a greater loss between 300 and 500 °C. The slope corresponding with this last weight loss is rather deep in recent bones and in the " white " part of fossil bone, and becomes flat going from the " ochraceous " to the " black " part of fossil bone, and from these fossil specimens to the " black-grayish " and " white-grayish " part of ancient bones. Moreover, the termogravimetric diagrams of recent bones and of the " white " portion of fossil bone show a slope at about 400 °C which is not present in the thermogravimetric diagrams of the other bone specimens. On the contrary, all of the diagrams show a weight loss between 650° and 850 °C.

Similar qualitative results are shown by the derivatives electronically and simultaneously drawn from the thermogravimetric analysis. A first peak is evident in all of the specimens at 105 °C. A second and a third peak

appears at about 300º and 400 ºC; these, however, decrease and change their shape in the " white ", " ochraceous " and " black " part of the fossil bone, and completely disappear in ancient specimens. A fourth peak at about 700 ºC is present in all of the specimens, but its height progressively decreases going from the " white ", " ochraceous " and " black " samples of fossil bone to the " black-grayish " and " white-grayish " specimens of ancient bone.

Similarly, the DTA diagrams show three interpenetrated peaks which occur at about 105º, 350º and 450 ºC and another peak at about 700 ºC. The peak at 350 ºC is very evident in recent bone. Its height progressively decreases and its width progressively increases in the " white ", " ochraceous " and " black " parts of the fossil bone, and it completely disappears in the " white–grayish " part of the ancient bone.
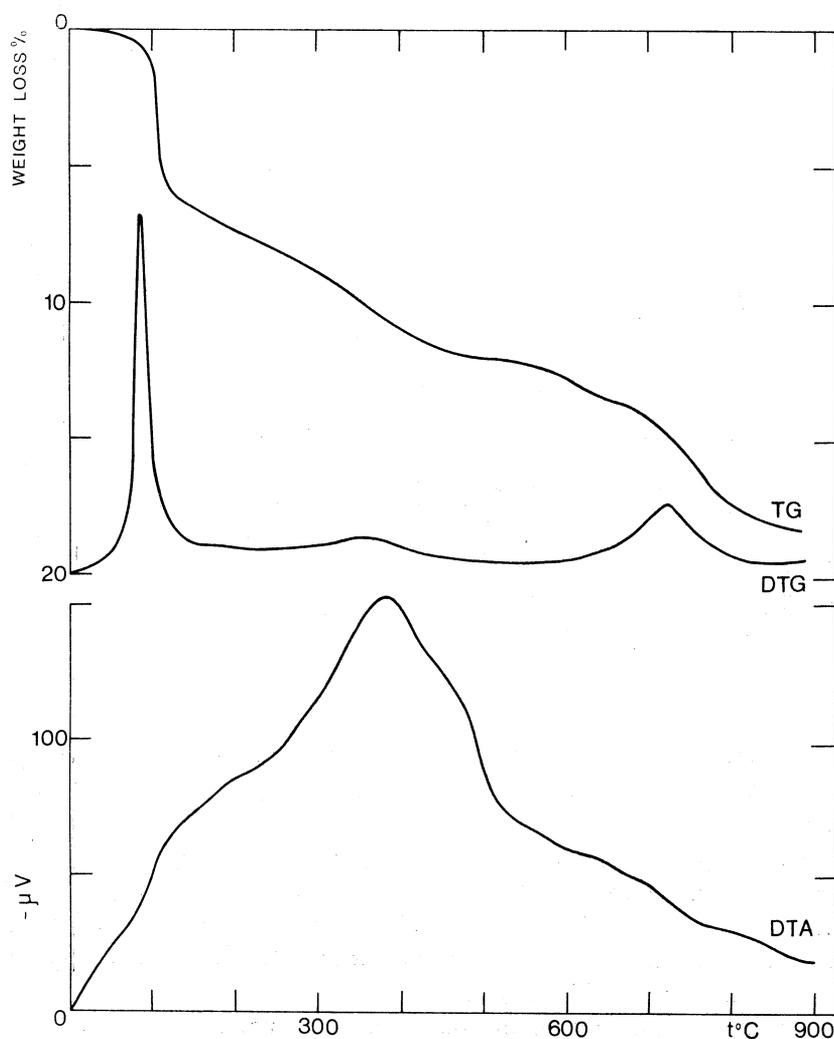
Fig. 4. – Fossil bone: " black " portion. Thermal analysis.
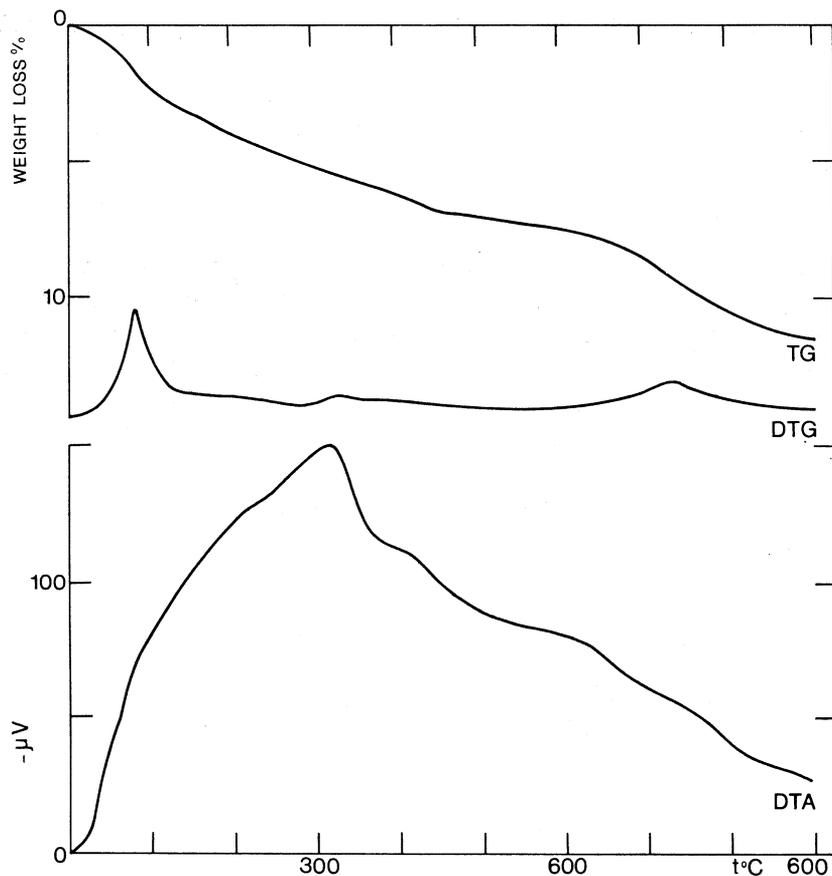
Fig. 5. – Ancient bone: " black-grayish " portion. Thermal analysis.

Interestingly, the powder of recent bone changes its colour during heating. It is initially white and than changes to yellowish between 200 and 250 °C, becomes brownish at 250–300 °C, black at 300–350 °C and grayish at 550–600 °C, and finally acquires a white colour at 650 °C which does not further change for higher temperatures.

2) *x-ray diffraction.*

A comparison of the diffractograms concerning recent bone shows that the temperature increment induces a progressively better definition of the reflections, chiefly of the triplet (231–321), (140–410), (402) placed between the reflections (123–231) and (004). The definition of this triplet increases with increase in temperature and it is better defined in the " black " than in the " ochraceous " and " white " portions of fossil bone and reaches the best definition in ancient bone, where it is better defined in the " white-grayish " than in the " black-grayish " portions.

Fig. 7 shows the portion of the diffractograms, between 2 Θ values of $30°50'$ and $34°50'$, in which the most intense reflections are found. It is
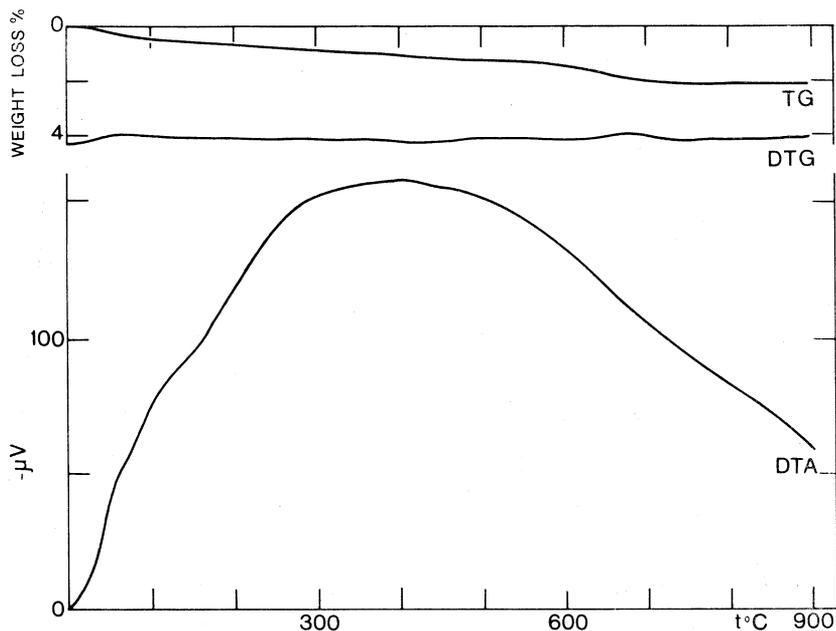
Fig. 6. – Ancient bone: " white-grayish " portion. Thermal analysis.

evident that the definition of the reflections increases proportionally to the temperature. This figure refers to recent ox bone; however, the same phenomenon is observed in fossil and ancient bone, as shown in fig. 8.

In fig. 9, the (002) reflection obtained from recent bone is compared with the same reflection obtained from samples of fossil and ancient bone. It is evident that also this reflection is better defined at high than at low temperature, and in ancient than in fossil bone.

The (002) reflection has been used for calculating the length of tricalcium phosphate crystallytes according to the c axis, as specified before. Although the calculation of the crystallite length is approximate to some extent owing to the experimental conditions, it has been possible to show that the length changes with the variations of the temperature and that the crystallite lengthening increases proportionally to the heating degree.

In recent ox bones, the length of the crystallites results to be of about 190 Å. This value increases to 200 Å at 200-300 °C, to 220 Å at 350 °C, to 230 Å at 500-600 °C and finally progressively reaches about 530 Å at 900 °C.

The same calculations drawn from the diffractograms of ancient and fossil bones have shown that the length of the crystallites of the " white " portion of fossil bone has about the same value, 190–200 Å, as that of crystallites of recent, unheated ox bone. On the contrary, in the " ochraceous " and " black " parts the length of the crystallites has a value of about 220 Å, and ancient specimens have given values of 230 and 430 Å for the " black-grayish " and " white-grayish " parts, respectively.
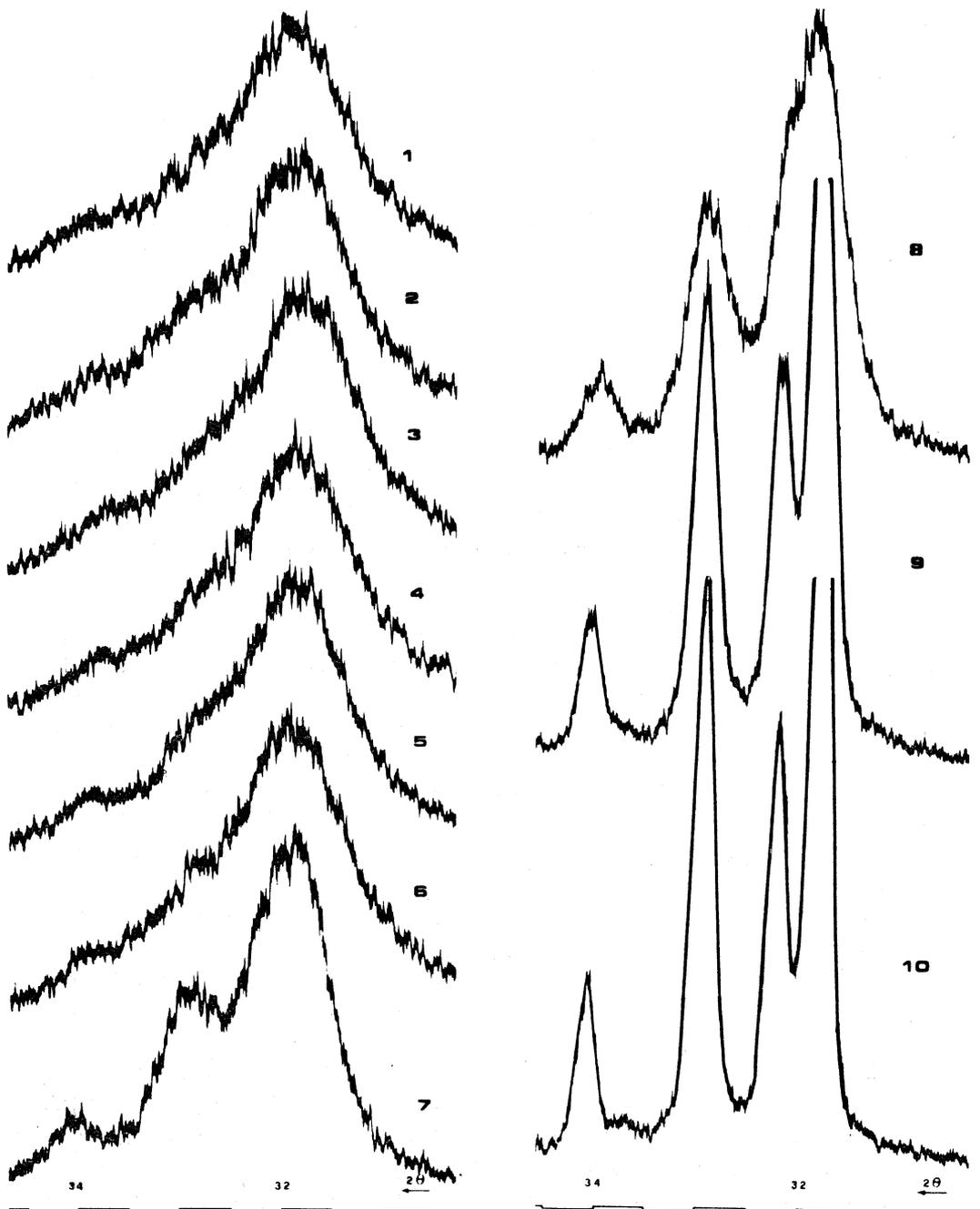
Fig. 7. – Recent bone.   X-ray diffractograms carried out using not oriented samples.
2 Θ : 30°50′–34°50′. 1) Room temperature; 2) 105 °C; 3) 200 °C; 4) 300 °C; 5) 350 °C;
          6) 450 °C; 7) 550 °C; 8) 650 °C; 9) 750 °C; 10) 900 °C.
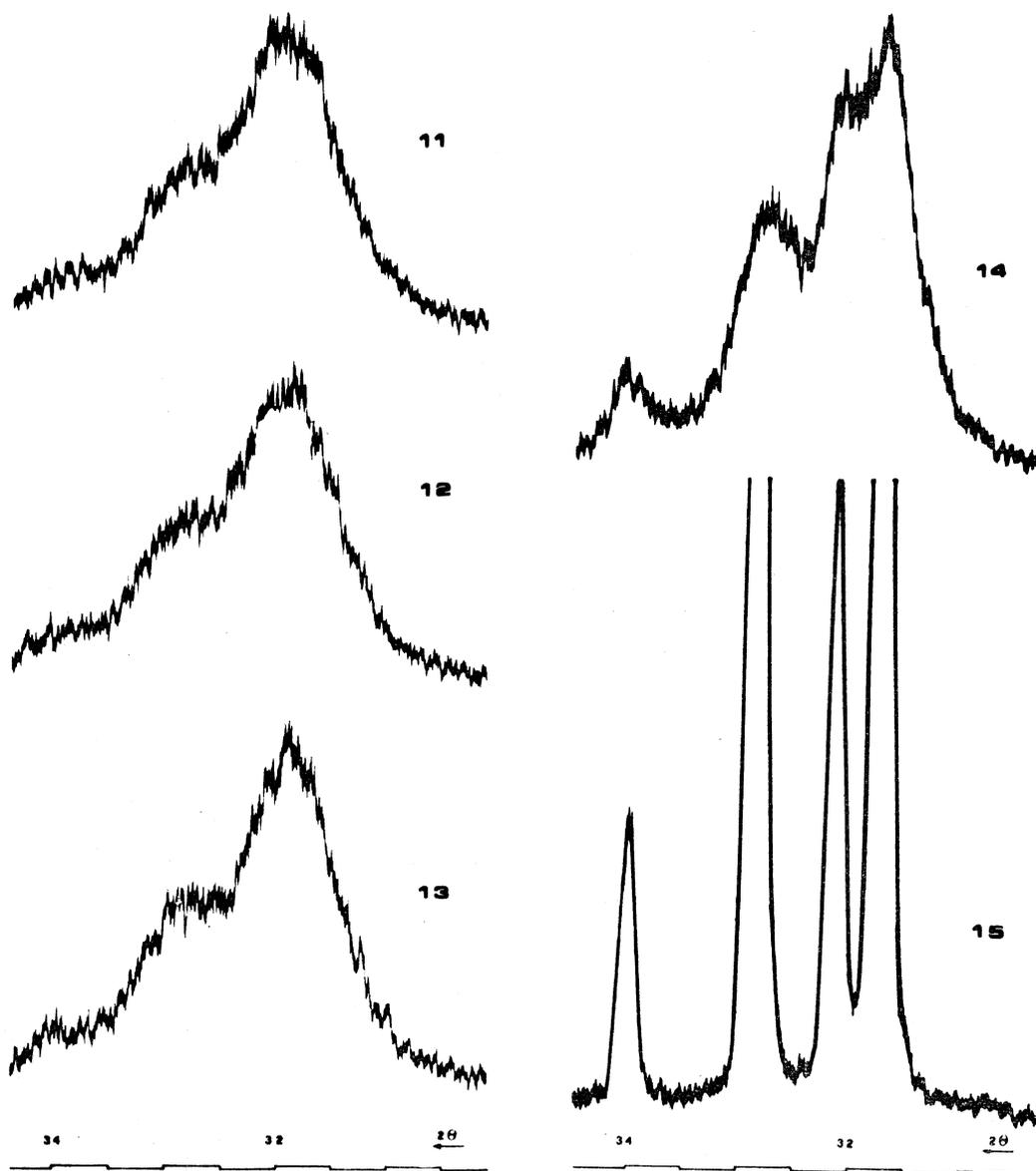
Fig. 8. – Fossil and ancient bone.   X-ray diffractograms carried out using not oriented samples.   2 Θ : 30°50'–34°50'.   Fossil bone: 11) " white " portion; 12) " ochraceous " portion;   13) " black " portion. Ancient bone: 14) " black-grayish " portion;   15 " white--grayish " portion.
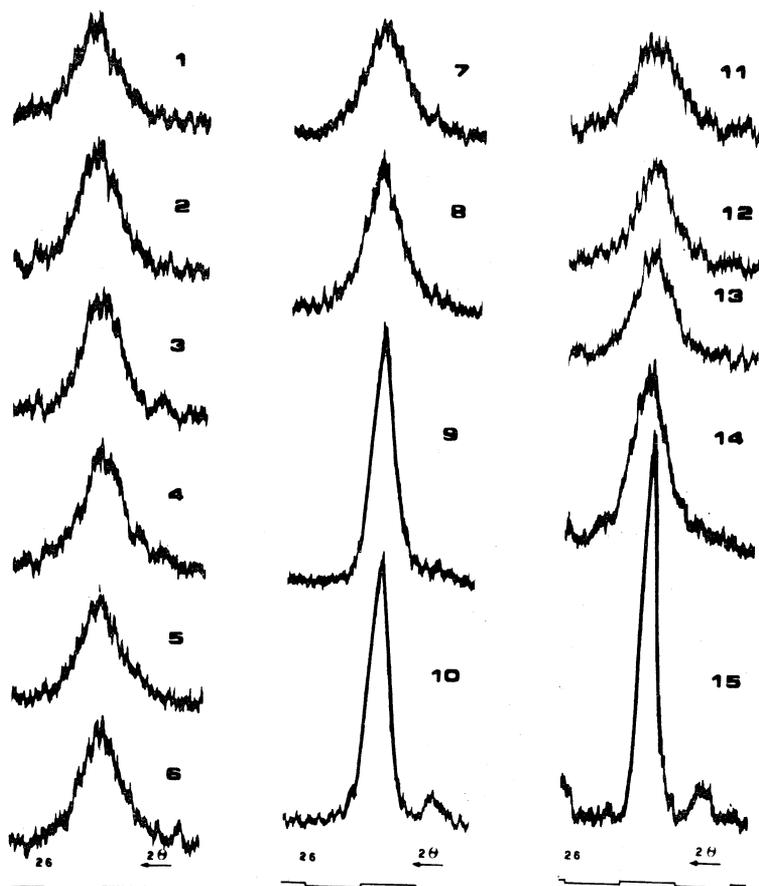
Fig. 9. – Recent, fossil and ancient bone. X-ray diffractograms carried out using not oriented samples. 2 Θ : 24°50′–26°0′. Variation of the (002) reflection with increase in temperature. Recent bone: 1) Room temperature; 2) 105 °C; 3) 200 °C; 4) 300 °C; 5) 350 °C; 6) 450 °C; 7) 550 °C; 8) 650 °C; 9) 750 °C; 10) 900 °C. Fossil bone: 11) " white " portion; 12) " ochraceous " portion; 13) " black ,, portion. Ancient bone: 14) " black-grayish " 15) " white-grayish " portion.

3) *Electron microscopy.*

Specimens of ox bones heated at 105°, 200° and 300 °C do not show significative differences in respect to the fine structure of normal ox bone. The inorganic crystallites appear as thin, about 35 Å thick, needle- and filament-like structures of variable length showing a preferential orientation similar to that of the collagen fibrils (Plate I a).

Specimens heated at 350°, 450° and 550 °C show similar ultrastructural changes. The arrangement of the inorganic fraction is still the same as that of normal bone; however, the crystallites are thicker than in normal bone, about 65 versus 35 Å, and their compactness suggests that the bone matrix has been collapsed to some extent (Plate I b).

The difference in respect to normal bone is still more manifest in specimens heated at 650 °C. Grossly, the bone structure is still recognizable, but the crystallites are thicker (about 80 Å) than in normal bone and their arrangement is very irregular (Plate I c).

Specimens heated at higher temperatures, up to 900 °C, show a similar appearance. The normal bone structure is completely lost in them and their inorganic fraction consists of irregularly polygonal crystallites, whose sides measure from about 0.2 to about 0.3 μ (Plate I d).

As regards the fine structure of fossil bone, the specimen marked " white " does not show ultrastructural changes in respect to recent normal bone. It consists of thin (about 35 Å thick) crystallites of needle- and filament-like shape, which show a preferential orientation probably corresponding with that of the collagen fibrils (Plate II a). The only recognizable difference is represented by the occasional collapsed aspect of the bone matrix, probably due to fossilization.

On the contrary, the inorganic crystallites of the portion marked " ochraceous " are thicker than those of the " white " zone of the same bone (about 55 versus 35 Å), are rather irregularly arranged and are mixed with many plate-like structures (Plate II b). This aspect is even more evident in specimens marked " black ", whose inorganic crystallites measure from about 50 to about 100 Å in thickness (mean 78 Å), are mixed with many plate-like structures and are completely disorganized (Plate II c).

Specimens of ancient bones have shown evident ultrastructural changes. The inorganic crystallites of specimens marked " black-grayish " are thicker than those of normal bone (about 80 Å thick), are irregularly oriented and arranged, and electron-transparent spaces are present between them (Plate II d). The ultrastructure of these specimens is closely comparable to that of ox bone heated at 650 °C (compare Plate II d and 1 c).

Specimens of ancient bones marked " white-grayish " contain large crystallites of rectangular or polygonal shape with a thickness of from 0.2 to 0.3 μ and a variable length, ranging from 0.2 to 1.0 μ or even more (Plate II e).

The electron diffractograms carried out on recent bones have shown that the definition of the reflections is better at high than at low temperatures. However, the reflections appear as dotted halos at 650 °C or more. Also the reflections of the " black " portion of fossil bone are better defined than those of the " white " and " ochraceous " parts. In ancient specimens, especially in that marked " white-grayish ", the reflections have a dotted appearance.

DISCUSSION

The experimental analysis carried out comparatively on recent heated bone and on fossil and ancient bones which had been exposed to different temperatures have furnished comparable results.

As far as the recent ox bone is concerned, the TG-diagrams have shown a first weight loss at about 150 °C. It is known that this phenomenon is due

to the removal of unbound water (Duval, 1963). Both x-ray diffraction and electron microscopy show that the structure of the inorganic bone fraction is not changed by this process of drying.

A second and a third slope in TG-diagrams of recent bones occur at 300–500 °C. This weight loss is considered dependent upon the elimination of bound water and pyrolysis of organic constituents (Civjan *et al.*, 1971; Holager, 1970). Interestingly, x-ray and electron diffractograms show, as previously reported by Baud *et al.* (1954), that the reflections are better defined than in normal, unheated bone, and it is possible to calculate that the length of the crystallites increases after bone exposition to these temperatures. Moreover, electron microscopy shows that the inorganic substance is more compact and more irregularly arranged, and that the crystallites are thicker, than in normal bone.

These results show that the heating of bone at 300–500 °C causes a complex of changes of the inorganic bone fraction which are sufficiently typical to be recognized. In fact, similar changes have been found in the " ochraceous " and, particularly, in the " black " part of the fossil bone, whose inorganic fraction shows x-ray diffraction and electron microscopic patterns similar to those found in recent bone heated at about 350 °C. The thermogravimetric analysis, on the other hand, shows that these portions of the fossil bone contain a reduced amount of organic components. This reduction can be a consequence of both fossilization and combustion.

The most typical and easily recognizable changes are found in specimens heated at 650 °C and at temperatures above this value, when the conversion of α to β tricalcium phosphate seems to occur. The TG-diagrams show a fourth slope for all of the samples, at about the same temperature. Electron microscopy shows that the crystallites of ox bone heated at 650 °C are thicker and more disorganized than in unheated bone. Moreover, x-ray and electron diffraction show a further better definition of the reflections and a further increase of the crystallite length. On the contrary, electron diffractogram reflections appear, at this temperature, as halos of small dots, because of the relatively small quantity of crystallites included in the field-limiting diaphragm. These results find a perfect parallelism in ancient specimens marked " black-grayish ", and the similarity is so great that it is possible to suggest that these specimens of ancient bone had been heated at a temperature of about 650 °C. Still more striking is the similarity between the ox bones heated at 650–900 °C and the ancient specimens marked " white-grayish ". Also in this case it is possible to suggest that these ancient bones had been heated at a temperature higher than 650 °C.

Both the fossil and ancient bones have shown thermogravimetrically a reduced weight loss in respect to recent bone. The reduction is already evident in the " white ", " ochraceous " and " black " portions of the fossil bone, is more manifest in the ancient bone marked " black-grayish ", and reaches the lowest values in the specimen marked " white-grayish ". It is possible to deduce from these results that the reduced weight loss during

heating is not, or at least is not completely dependent upon fossilization, but rather is proportional to the temperature to which the bone had been heated. It is evident that thermogravimetry, like x-ray and electron diffraction and electron microscopy, can furnish useful information on the temperature to which a bone fragment has been submitted.

The changes of the bone colour during heating suggest that the colour itself can be used to some extent for deducing the approximate value of the temperature. By operating in air current, the samples of recent bone become progressively ochraceous (200–250 °C), brownish (250–300 °C), black (300–350 °C), grayish (550–600 °C) and white (more than 650 °C). These colour changes are due to progressive carbonization of the organic substance (from ochraceous to black) and successive oxidation and volatilization of carbon components (from black to white). Consequently, it seems possible to roughly establish the temperature a bone has been previously submitted to on the basis of its external colour. In the present material, a good correlation has been found between the structural organization of fossil and ancient bones and that of recent bone powder having the same colouring gradation.

It is possible to conclude that, as far as the present bone specimens are concerned, the "white" part of the fossil bone had not been exposed to a temperature greater than 200 °C, while the "ochraceous" and "black" zones had been heated at about 200–250 °C and 300–350 °C, respectively. The "black–grayish" specimens of ancient bone had been heated to about 550 °C and the "white-grayish" ones to a temperature higher than 650 °C.

The experimental methods used in this investigation can be of great advantage in problems concerning archeological, anthropological and paleonthological fields. Particularly, there is a very close connection between thermal, diffractometric and electron microscopic analyses, so that electron microscopy could be used for ascertaining the temperature values to which even very small samples of bones have been previously submitted.

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E. Bonucci e G. Graziani – *Comparative
thermogravimetric*, ecc. – PLATE I.
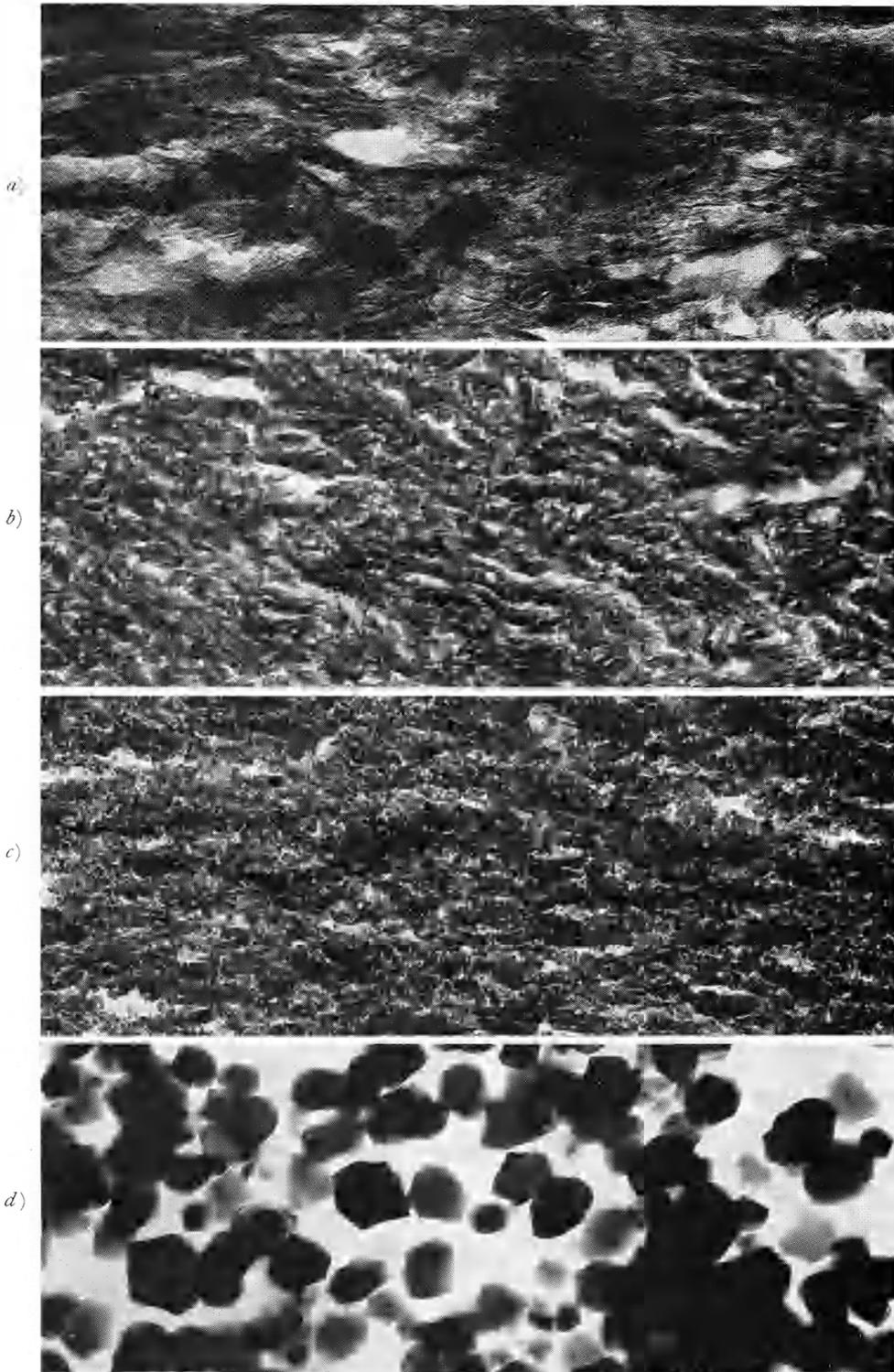
Electron microscopy, ×55,000. Recent bone: *a)* 105–300 °C; *b)* 350–550 °C; *c)* 650 °C;
d) more than 650 °C.

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E. Bonucci e G. Graziani – *Comparative
thermogravimetric*, ecc. – PLATE II.
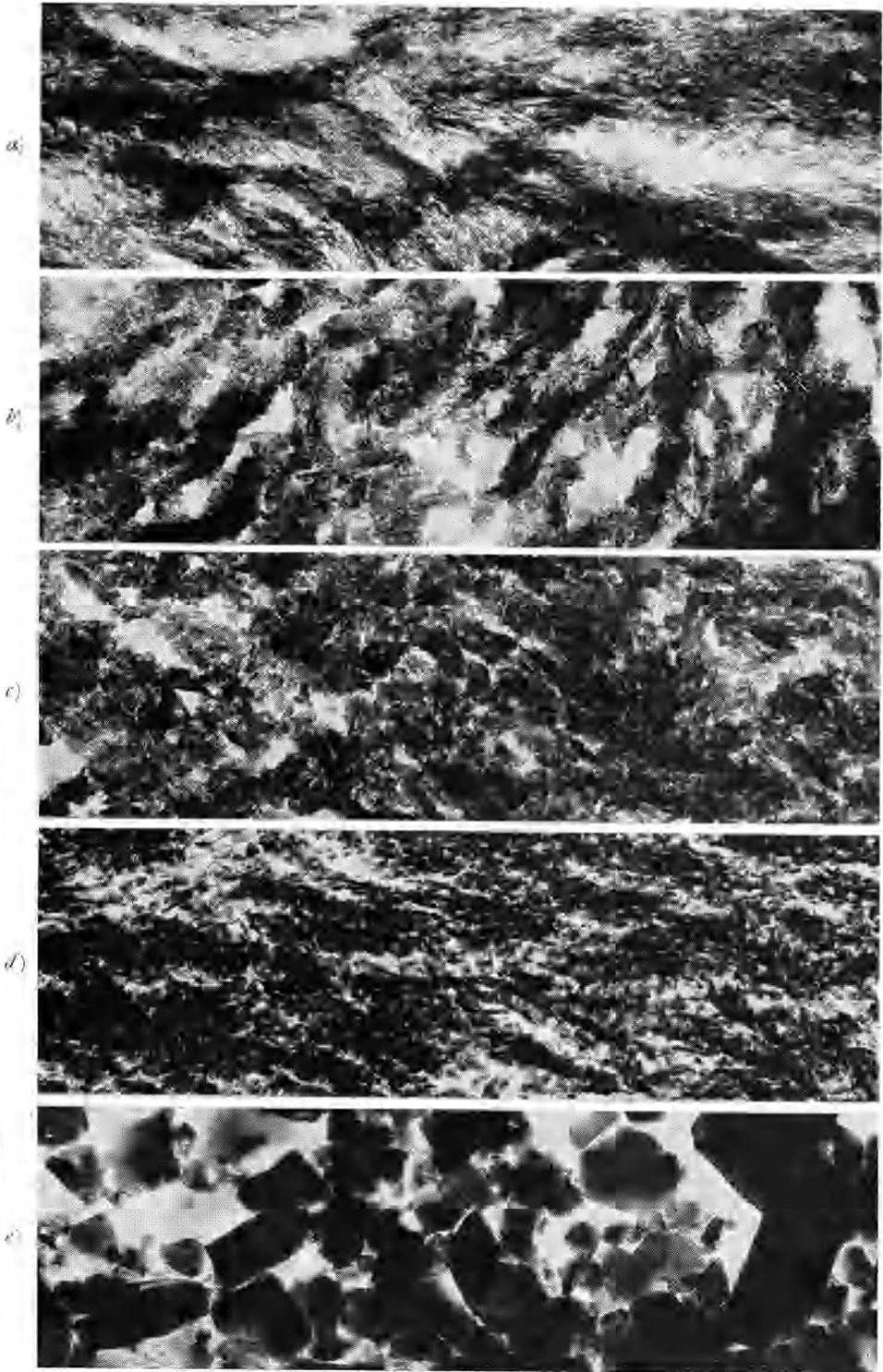
Electron microscopy, ×55,000. Fossil bone: *a*) "white" portion; *b*) "ochraceous" portion;
c) "black" portion. Ancient bone: *d*) "black-grayish" portion; *e*) "white-grayish" portion.