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The Application of Differential Thermal Analysis and Thermogravimetric Analysis to Dating Bone Remains

Dating material is a difficult problem in legal medicine, no matter what substance is being tested. Concerning bone remains, the classic works of Orfila and Lazaretti [*1*] showed the difficulty of establishing the time since death. Since then, little research has been done in this field; mistakes can be made in dating bone material.

The problem has been approached from three points of view: morphologic state of bone remains [*2,3*]; histological studies of haversian canals [*4,5*], the morphological elements of cells, and putrefaction of medulla [*6–8*]; and alteration of dental pulp [*9*]. In addition, other authors have considered the problem from a chemical point of view: mineralization state according to solubility in hydrochloric acid [*4,5*]; persistency of lipids shown by staining with saturated copper acetate [*2,* p. 437] or osmic acid solutions [*7.8*]; antigen-antibody reactions [*10*]; organic-mineral matter relationship [*11*]; and mineral salts [*12*] or lead or zinc [*13*] enrichment.

Knight [*14–16*] approached the problem with more amplitude, studying total and amine nitrogen as well as fluorescence and benzidine reactions. Bass [*17*] employed a complete test involving morphological, histological, and physicochemical aspects. Physical methods such as strontium-90 isotopic studies [*18*], scanning electron microscopy [*19*], and X-ray procedures [*20*] have also been used.

In the last few years we have considered the problem [*21*] by making a systematic study of the most relevant organic and inorganic compounds. When putrefaction starts, there is a loss of organic material and a subsequent relative enrichment in mineral substances which is completed after a long time. We believe that differential thermal analysis (DTA) and thermogravimetric analysis (TGA) can be useful to show the mineralization process in postmortem bones, not only statically but also dynamically by recording how the loss of weight evolves (TGA), how long it takes (derivation of TGA), and the physicochemical process involved (DTA curve).

Research in forensic sciences has not used DTA very much, with the exception of DeHaan's works [*22*] concerning explosives and the criminalistics research of Rynearson and DeHaan [*23*]. In this report we show our results with this new technique, but we do not try to draw definitive conclusions at this time.

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Material

We analyzed 34 bone samples of known date, most of them less than 30 years since death because these are the only ones of forensic interest in Spain. Bones with a short time since death came from autopsy or from amputated legs; therefore, some of them were pathologic.

Sometimes the bones were left to be aged in the laboratory; others were buried for different times. Samples with a long time since death belonged to skeletons inhumed in the same cemetery, but in places with different humidity and ground composition. As a mean, in this cemetery bones take 4 to 5 years to skeletonize.

Table 1 shows a summary of characteristics of the studied bones, including two problem bones. Sample HR-25, an arm in an advanced state of putrefaction, was found in a package inside a plastic bag; it was estimated that amputation had occurred 15 to 20 months before. Sample H-4 was part of a skeleton found in a cave 60 m deep and of high humidity; the elapsed time since death was estimated to be between 20 and 40 years. Both samples showed a large amount of unidentified coleoptera in the medulla.

TABLE 1—*Characteristics of the bones.*

Sample	Type	Source	Time Since Death	Environment
H-1	fragment	adult	millennial	buried
H-2	long bone	adult	millennial	buried
H-4	long bone	adult	20–40 years	cave
H-5	long bone	adult	centenary	buried
H-40	long bone	adult	40 years	buried
H-40-b	long bone	adult	40 years	buried
H-40-c	long bone	adult	40 years	buried
H-20	long bone	adult	20 years	buried
H-19	long bone	adult	20 years	buried
H-19-b	short bone	young	20 years	buried
H-19-c	short bone-plane bone	young	20 years	buried
H-18	short bone-plane bone	young	18 years	buried
H-15-3	long bone	child	15 years	buried
H-15-4	long bone	child	15 years	buried
H-15-5	long bone	child	15 years	buried
H-10-1	long bone	adult	10 years	buried
H-10-2	long bone	adult	10 years	buried
H-10-3	long bone	adult	10 years	buried
H-10-4	long bone	adult	10 years	buried
H-10-5	long bone	adult	10 years	buried
F-15-E	fragment	adult	2 years	buried
HR-16	fragment	adult	16 months	laboratory
HR-15	fragment	adult	1 month	laboratory
HR-25	fragment	adult	15–20 months	plastic bag
F-26-E	fragment	adult	6 months	buried
HR-17	fragment	adult	6 months	laboratory
HR-18	fragment	adult	6 months	laboratory
HR-19	fragment	adult	6 months	laboratory
HR-20	fragment	adult	6 months	laboratory
HR-26	fragment	adult	immediately	...
HR-27	fragment	adult	immediately	...

Methods

Instruments

We used a METTLER-2 thermoanalyzer with DTA-TGA and derivative-TGA equipment. It had a five-channel recorder with simultaneous plotting of temperature, DTA,

TGA, and a record of the first derivative of TGA (dG/dt). The sensitivity was 200 μV (full scale) for DTA, 10 μV for temperature, 100 and 10 mg for the two plots of TGA ($\times 1$ and $\times 10$ sensitivities), and 5 mg/min for derivative-TGA. The heating rate was 10°C/min, and the recorder speed was 12 in./h (0.3 m/h).

Samples and inert material (calcined alumina) were placed on platinum crucibles, supported by capsules of the same metal, in close contact with the platinum thermocouples (90% platinum-10% rhodium).

Preparation of Samples

The bone surface was washed with mechanical friction and rinsed with distilled water until free of foreign material. The bones were transversely sectioned with an electric saw and crushed in a mortar until uniformly granulated. Approximately 10 mg of the powder was placed in a dessicator until testing.

Results and Discussion

Qualitative analysis showed a general outstanding exothermic effect, and only bones with short time since death exhibited an endothermic effect (marked with parentheses in Table 2). Table 2 summarizes the more relevant effects. Multiple phenomena appear at 50 μV sensitivity, but because it is difficult to make a quantitative estimation at that level they have not been considered.

The areas of main effects were obtained by weighing the initial sample and then measuring the corresponding weight loss, which was determined by measuring in the TGA curves the weight loss that takes place while the effect is occurring (Table 3). The ratio of the area of DTA peak S to loss of weight has been calculated. This relation, even though established for all the effects in which the quantitation of S was possible, was only applied to the more outstanding effects.

Table 4 is a summary of the ratio of S to weight loss for effects at 350 and 450°C. The ratio between these two values is also shown. The three curves obtained are shown in Fig. 1. The ratio of the effects in the 450 to 470°C region to the effects in the 340 to 350°C region increases as time since death decreases, being 18.27 for those bones of shortest time since death (Table 4). This effect does not occur for the oldest bones.

Even though the number of samples studied is not sufficient, we can reach some conclusions. This method is suitable for a dynamic study concerning bone degradation processes. The results can be reproduced if the bones being compared have remained buried in the same place for a similar period of time. As Table 2 shows, the highest effect for the DTA/TGA ratio within each group presents little dispersion and permits analysis of the anomalous degradation that takes place in buried bone fragments because of mechanical action or in unburied ones showing an abnormal putrefaction by the influence of weather or insects. This is demonstrated in Samples HR-25 and H-4, which were preserved with optimal humidity and confinement conditions.

The TGA values are highly significant for the extreme series (oldest and most recent), with a weight loss of 55% (standard error of the mean $S_m \pm 3.74$) for recent bones and 25.09% ($S_m \pm 5.15$) for the oldest ones, but they are not significant in the intermediate series.

From a qualitative point of view DTA patterns can be divided into three different groups. Old bones (Fig. 2) show an exothermic effect in the 340°C region, with the peaks showing a good configuration because of the homogeneously prepared samples. Recent bones (Fig. 3) show a complex curve, being the only ones with endothermic effects in the 380°C region; the exothermic effects appear along the whole pattern, with low tem-

TABLE 2—*Results of differential thermal and thermogravimetric analyses.*

Sample	DTA, °C	Weight Loss, mg	Ratio DTA/TGA	TGA, % Weight Loss
		Bones 10 Years Since Death		
H-10-1	342	4.44	2.61	35.95
H-10-2	340	4.33	2.47	...
H-10-2	373	...	...	38.63
H-10-2	440	1.58	2.11	...
H-10-3	340	4.37	2.50	...
H-10-3	370	0.60	0.71	44.83
H-10-4	340	5.26	2.34	...
H-10-4	370	3.64	2.60	40
H-10-5	340	5.38	2.83	39.32
H-10-5	375	2.44	2.87	...
Mean	340.4 ± 0.4	...	2.55 ± 0.08	39.74 ± 1.44
		Bones 15 Years Since Death		
H-15-3	350	7.86	3.74	...
H-15-3	400	...	...	49.86
H-15-3	470	7	5.38	...
H-15-4	350	7.49	3.48	...
H-15-4	400	...	...	46.92
H-15-4	480	6.84	5.06	...
H-15-5	350	7.46	3.11	48.33
H-15-5	475	7.08	4.57	...
Mean	350	...	3.44 ± 0.182	48.37 ± 0.84
		Bones 20 Years Since Death		
H-18	355	6.23	2.49	...
H-18	420	5.84	4.17	39.36
H-19	353	4.65	2.91	...
H-19	400	3.71	3.71	42.45
H-19-b	350	5.91	3.38	32.77
H-19-b	400	2.26	3.01	...
H-19-c	350	6.15	3.73	38.67
H-19-c	400	2.16	3.93	...
H-20	350	6.16	2.68	...
H-20	415	4.85	2.05	46.32
Mean	351.6 ± 1.02	...	3.03 ± 0.227	39.91 ± 2.24
		Bones 40 Years Since Death		
H-40	350	5.71	3.36	...
H-40	400	...	...	36.93
H-40	420	2.64	3.30	...
H-40-b	350	7.3	3.84	...
H-40-b	400	...	...	40.82
H-40-b	435	5.07	5.32	...
H-40-c	355	5.68	2.84	...
H-40-c	400	...	...	40.38
H-40-c	435	4.19	3.35	...
Mean	351.6 ± 1.66	...	3.34 ± 0.288	39.37 ± 1.22
		Bones > 100 Years Since Death		
H-1	340	5.26	10.52	19.69
H-2	330	2.68	2.06	20.18
H-5	350	7.94	6.62	35.4
Mean	340 ± 5.77	...	6.4 ± 2.44	25.09 ± 5.15

TABLE 2—*Continued.*

Sample	DTA, °C	Weight Loss, mg	Ratio DTA/TGA	TGA, % Weight Loss
		Recent Bones		
HR-15	355	1.35	1.13	61.8
HR-15	370			
HR-15	495	17.83	13.72	...
HR-16	360			
HR-16	(365)	1.40	0.82	51.43
HR-16	380			
HR-16	465	17.67	10.39	...
HR-17	360	0.9	0.75	...
HR-17	(370)	...	...	...
HR-17	380	0.29	0.97	51.42
HR-17	(400)	...	...	...
HR-17	480	22.66	12.59	...
HR-18	276	1.48	1.35	...
HR-18	355	1.22	0.72	...
HR-18	(365)	...	...	45.78
HR-18	380	0.28	1.12	...
HR-18	(390)	...	...	...
HR-18	480	19.85	8.82	...
HR-20	225	1.08	1.8	...
HR-20	365	22.67	6.13	46.35
HR-20	452			
HR-26	270	1.17	1.06	...
HR-26	390	2.32	0.47	73.93
HR-26	400			
HR-26	415	0.62	1.55	...
HR-26	490	19.26	6.42	...
HR-27	240	2.10	4.2	...
HR-27	360			
HR-27	(370)	1.31	0.97	54.31
HR-27	380			
HR-27	470	18.07	12.46	...
Mean	476 ± 5.63		10.07 ± 1.15	55 ± 3.74

TABLE 3—*Ratio of the area of DTA peak* S *to weight loss.*

Sample	DTA, °C	Weight Loss, mg	Ratio of *S* to Weight Loss	TGA, % of Weight Loss
F-26-E	65	0.88	1.34	51.28
F-26-E	340	8.93	3.88	51.28
F-26-E	405	2.56	3.66	51.28
F-15-E	345	7.12	7.49	...
HR-19	242	3.9	8.66	...
HR-19	370	11.73	6.90	51.52
HP-25	345	7.95	6.36	38.83
HP-25	410	1.90	6.33	38.83
H-4	355	7.85	19.63	...
H-4	390	...	...	30.65

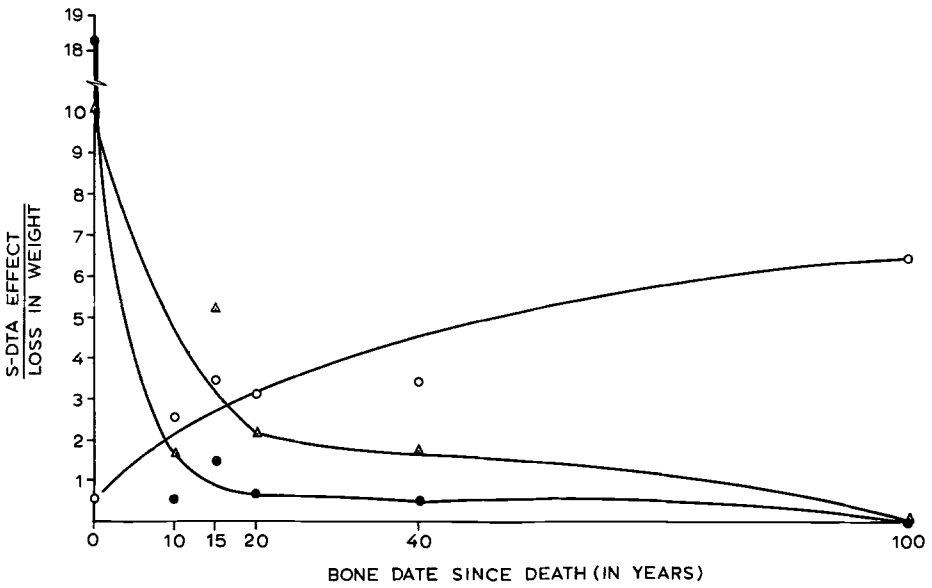

FIG. 1—*Ratio of the area of DTA peak to weight loss for the effects at 450°C* (△) *and 350°C* (○) *in bone remains of different time since death. The ratios between the 450°C and 350°C values* (●) *are also plotted.*

perature effects in the 270°C region and the highest one in the 470°C region. Bones with middle age since death present an intermediate curve (Fig. 4). As with the old bones, they have the highest effect close to the 350°C region and, like recent ones, they show a high effect near the 450°C region. They do not show low temperature and endothermic effects.

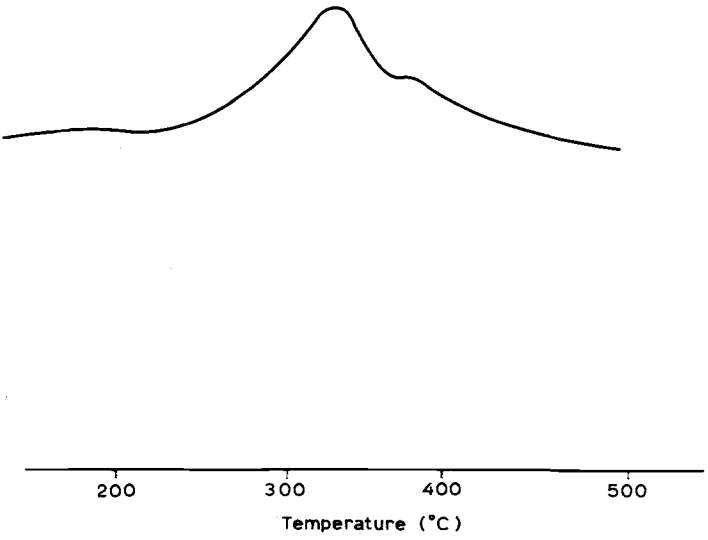

FIG. 2—*DTA pattern of old bones* (*Sample H-1*).

TABLE 4—*DTA-TGA analysis of abnormal bones.*

Sample	Mean Ratio of *S* to Weight Loss at 350°C	Mean Ratio of *S* to Weight Loss at 450°C	Ratio of 450°C to 350°C Values
Recent	0.55	10.05	18.27
10 years old	2.55	1.65	0.64
15 years old	3.44	5.18	1.5
20 years old	3.03	2.14	0.7
40 years old	3.34	1.74	0.51
> 100 years old	6.4	0	0

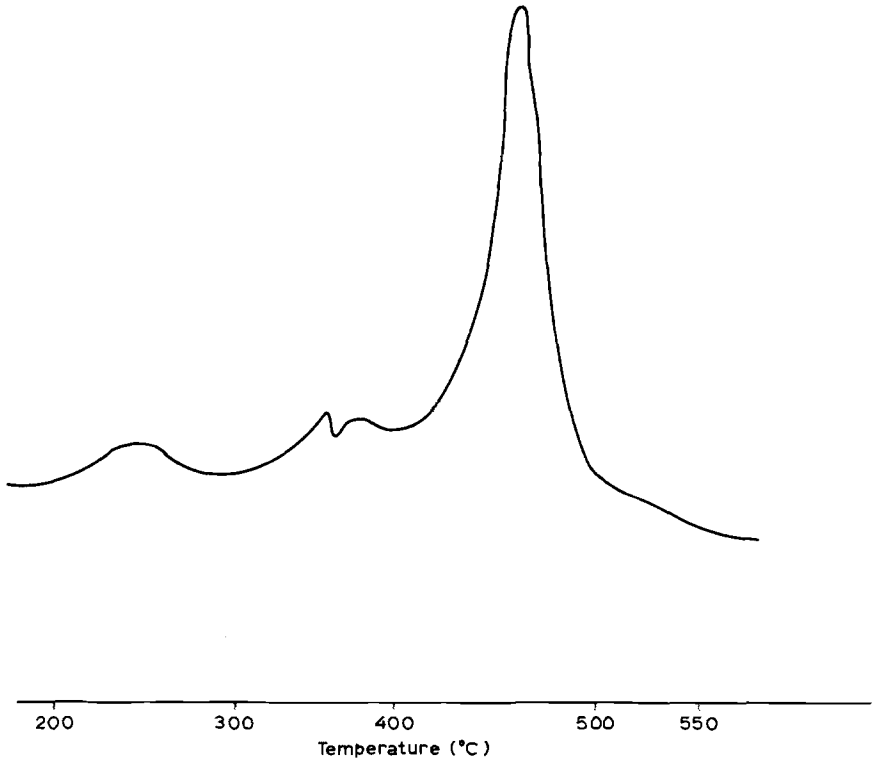

FIG. 3—*DTA pattern of recent bones (Sample HR-27).*

Simultaneous study of TGA-DTA patterns also supplies hopeful results. Acute exothermic effects produce high heat as registered by the thermocouple, indicating that the reaction takes place in situ. If this is not followed by a concomitant loss in weight, the thermal process does not take place in situ because it is not registered by the thermocouple. A correlation of both phenomena could be relevant, as Fig. 1 shows.

If we analyze the ratio between the area of the effect in the 350°C region and the weight loss while this effect goes on, we get a curve that moves away from the abscissa in proportion to increasing time since death. If we analyze the same ratio for the effect in the 450°C region, this curve reverses. This phenomenon shows great significance in the

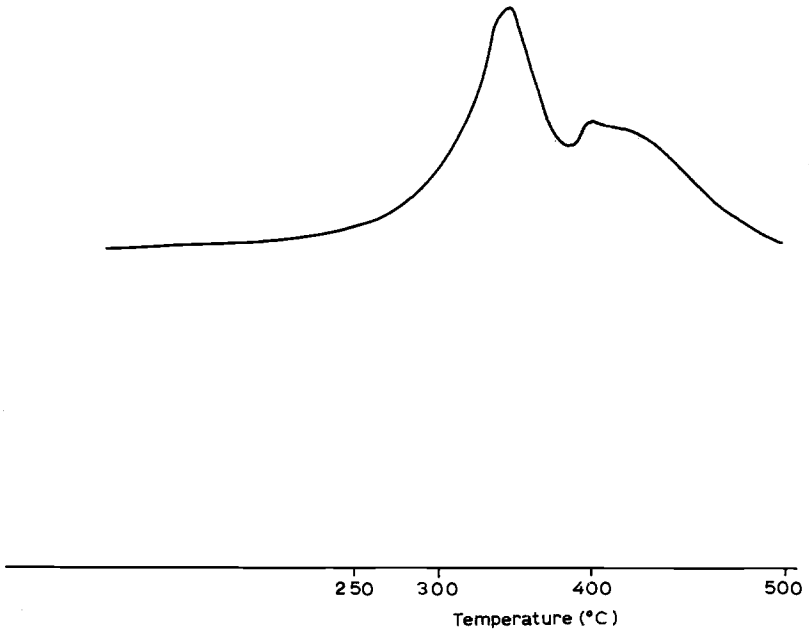

FIG. 4—*DTA pattern of bones in the middle range of time since death (Sample H-40).*

extreme series, but doubtful results with the intermediate series, as a result of the different soil composition in which each individual bone was buried.

In conclusion, at present this technique is only suitable for distinguishing between bones with very different times since death and for clarifying the aging process that takes place in postmortem bones, independent of time since death. Any factor changing the aging process, such as place of burial, age of the individual at the time of death, pathological process of bones, environment in which putrefaction takes place, or type of bone, can lead to a mistake. All information must be considered.

Correlation between the physicochemical reactions and thermal effects has not been elucidated so far. It seems that a complex phenomenon takes place, with slow overlapped degradation and oxidation process. Therefore, the bibliographic information concerning pure bone components cannot be taken into consideration. This question could probably be answered with a parallel study we are carrying out concerning bone organic and mineral compounds and X-ray spectra. We believe that the qualitative and quantitative studies of TGA and DTA curves can complete the information supplied by the other methods that are normally used, with the advantage of easy and rapid use.

Summary

Thirty-four samples of bone remains of known time since death were studied by using DTA, TGA, and derivative-TGA techniques. The DTA patterns enable us to distinguish recent from old (more than 100 years) bones. The TGA curve is also significant for an extreme series.

Both DTA and TGA curves show a correlation that allows us to obtain patterns with high significance for the extreme series. They also make evident the decomposition grade that bone organic material undergoes during the postmortem putrefactive process.

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