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Amino acid racemization in bone and the boiling of the German Emperor Lothar I

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Abstract—We have used the extent of aspartic acid racemization in bone samples from three 12th century German burials to determine that the corpse of one individual, Emperor Lothar I, was boiled in water for about 6 h before burial. Boiling was evidently used to deflesh Lothar's corpse to prevent postmortem decay during transit from the place he died, which was 500 km from his castle where he was buried.

INTRODUCTION

AMINO acid racemization involves the conversion of chiral amino acids into a racemic mixture in which the ratio of D- and L-enantiomers is unity. This process was first studied in aqueous solutions over a century ago (see BADA, 1985a and references therein). During the last two decades it has been demonstrated that this reaction has important geochemical and biological implications. Because racemization is a chemical process the extent of racemization in a system is dependent not only on the time which has elapsed since the chiral amino acids were synthesized, but also on their exposure temperature. Other parameters, such as pH, humidity and position in a peptide linkage, are also important. Geochemical uses of amino acid racemization include the dating of fossils, or in the case of specimens of known age, the determination of their temperature history. Fossil types ranging from bones, teeth, and shells have been extensively studied. For recent reviews see BADA (1985b, 1987), MASTERS (1986a), WEHMILLER (1982, 1984) and MILLER and MANGERUD (1985). *In vivo* aspartic racemization has also been detected in metabolically inert tissues such as molar crown dentine and the proteins in the nucleus of eye lens (BADA, 1984; MASTERS, 1986b). The extent of racemization in dentine can be used to determine the age of living mammals and to ascertain the age of an individual at death, provided there has been no extensive postmortem racemization (MASTERS, 1986c).

As an example of the application of the racemization reaction to anthropological bone material, we present here the results of the analyses of the German Emperor Lothar I, who died on 5 December 1137 in Upper Bavaria and was buried 500 km north of the place of his death at his castle in Königslutter (20 km west of Braunschweig). Using 20–30 km/d as the estimated transportation speed for his corpse, we calculate that an interval of several weeks is likely to have occurred between the time of his death in Bavaria and his subsequent burial at the castle. This long period would have obviously presented problems of postmortem decay and putrefaction of his remains. Medieval historians believe that in situations such as this, the corpse was first defleshed before transport, probably by boiling in water. In order to test this hypothesis, we have carried out aspartic acid racemization analyses of Lothar, and two of his relatives, his wife Richenza and brother-in-law Duke Heinrich der Stolze, both of whom died at Königslutter within a few years of Lothar's death and were buried in the castle along side him (see Fig. 1).

EXPERIMENTAL METHODOLOGY

Small bone fragments (~1–2 g) were collected when the sarcophagus of each individual was opened in 1978. These bone samples were prepared and processed for racemization analyses as described elsewhere (BADA, 1985b). Two different hydrolysis procedures were used. One set of samples were hydrolyzed 4 h in constant-boiling 6N HCl under reflux (110°C), while another set was hydrolyzed in 6N HCl for 6 h at 100°C in sealed glass tubes. After hydrolysis, the samples were evaporated to dryness, redissolved in double distilled water, and desalted by cation-exchange chromatography. Aspartic acid was next isolated from the amino acid extracts by anion-exchange chromatography. The aspartic acid was then derivatized to form the *N*-trifluoroacetyl-diisopropyl esters and the aspartic acid enantiomers were separated using a Perkin–Elmer gas chromatograph with a chiral capillary column as described by PAYAN *et al.* (1985). Each

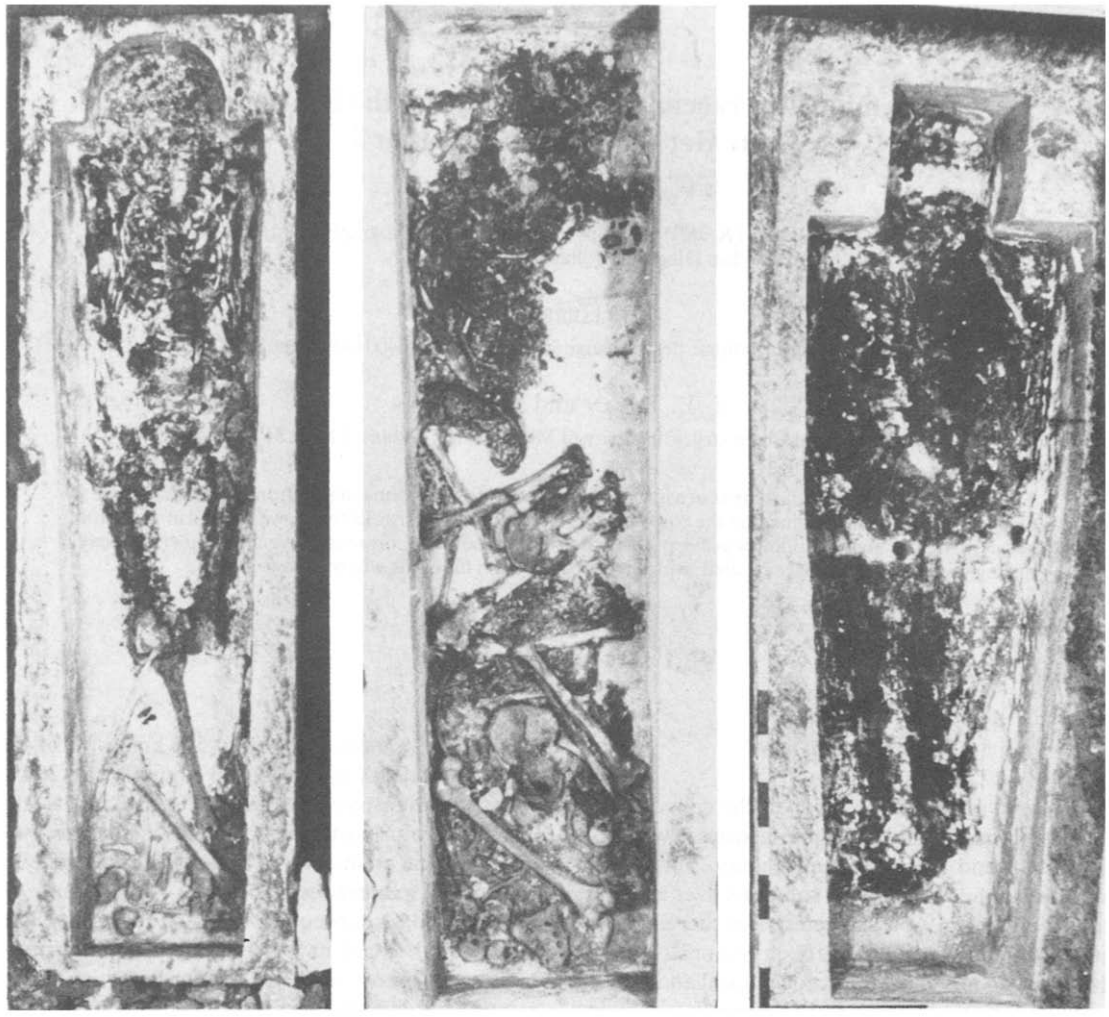

FIG. 1. Photograph of the three medieval (12th century) burials analysed in this study. The jumbled state of Lothar's skeleton in comparison to those of his two relatives is due to the opening of the sarcophagus in 1620. The year each individual died is given under their name.

sample was analyzed twice and an average *D/L* ratio determined. The amino acid composition of each sample was determined on a Beckman 119 amino acid analyzer interfaced with a DEC PDP-11 computer used for data acquisition and analysis.

RESULTS AND DISCUSSION

The bone sample of each individual had similar amino acid compositions which were identical to that of modern bone collagen. This does not rule out the possibility that Lothar was boiled because it has been found that modern bones boiled for up to 24 h retain their original amino acid composition (SKELTON, 1983). The extent of aspartic acid racemization determined in bone from the three burials are given in Table 1. The different enantiomeric ratios for the two hydrolytic procedures can be attributed to the different time and temperature conditions. The two hydrolysis series clearly show that the extent of aspartic acid racemization for the Richenza and Heinrich bone samples are the same, whereas Lothar's bones contain more extensively racemized aspartic acid.

Table 1. Aspartic acid enantiomeric ratios in bones from the three burials

	D/L aspartic acid ratio*	
Burial	A	B
Richenza	0.059 ± 0.002	0.028 ± 0.004
Lothar I	0.090 ± 0.001	0.056 ± 0.001
Heinrich	0.059 ± 0.002	0.029 ± 0.004

*A = hydrolysis 4 h, 110°C; B = hydrolysis 6 h, 100°C.

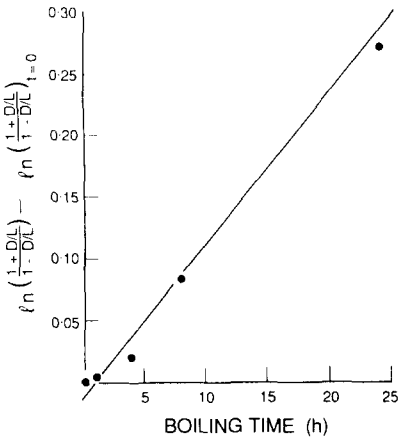

FIG. 2. Aspartic acid racemization in bone (deer) during boiling in water plotted in the form of reversible first order kinetics (see BADA, 1985a). The data were taken from SKELTON (1983). The straight line was obtained by a least squares fit of the data.

The small extent of racemization in Richenza and Heinrich are consistent with their age of only about 850 a and the cool temperatures (~10°C) of the burial locality.

Can the excess racemization in the Lothar bones be attributed to boiling, and if so, how long was his corpse boiled? In order to answer these questions, we have used the results of SKELTON (1983) who studied the kinetics of aspartic acid racemization in blacktail deer femur bones (with flesh attached) under boiling conditions. These results are shown in Fig. 2. A least squares fit of these data yields the following equation for the rate of aspartic acid racemization in bone as a function of boiling time:

$$\text{boiling time } (h) = (Q - 0.01)(0.016)^{-1}$$

where

$$Q = \ln\left\{\frac{1 + D/L}{1 - D/L}\right\} - \ln\left\{\frac{1 + D/L}{1 - D/L}\right\}_{t=0}.$$

As can be seen in Fig. 2, the linearity is excellent ($r = 0.996$) demonstrating that this equation provides a good indication of the extent of racemization which would be produced for various boiling periods.

Using the average D/L aspartic acid ratio of the Richenza and Heinrich bones for the $t = 0$ term in the above equation yields the following results for Lothar:

boiling time = 6 h 15 min for hydrolysis procedure A

boiling time = 5 h 45 min for hydrolysis procedure B.

Thus, the extent of aspartic acid racemization in the Lothar bones suggests that his corpse had been boiled for 6 h ± 30 min.

CONCLUSIONS

We have used the amino acid racemization reaction to examine whether the corpse of the German Emperor Lothar I, who died in the 12th century, was defleshed by boiling in water before transit to his burial site. The answer is yes; Lothar was apparently boiled for about 6 h. We feel these results are a nice illustration of the application of the amino acid racemization reaction to anthropological problems.

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