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Short Communication

The effects of acid and alkaline solutions on cut marks and on the structure of bone: An experimental study on porcine ribs

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ABSTRACT

Among taphonomical modifications during decomposition processes, little is known about the action of high or low pH to human tissues and bones. Moreover, acid or basic solutions are seldom used to ease decomposition and wrecking of the body. In this study a total of 60 samples of porcine bones on which two cut marks were produced before the beginning of the experiment, were put in six different solutions with different pH (1, 3, 5, 9, 12, 14) and analyzed every five days over a period of 70 days. Surveys were carried out macroscopically, with stereomicroscopy and with light microscopy on thin sections. Only the specimens exposed to extremely acid (<1) or basic (>12) pH showed evident modifications of the bone's structure, as witnessed by the analyses with stereomicroscopy as well. Many samples showed a detachment of the periosteum; cut marks became soon unrecognizable with pH 14 but still detectable in all the other samples. The information gained from the present study can be of great help in detecting the exposure of human tissues to high or low environmental pH and in understanding the effects that these solutions can exert on human bones.

1. Introduction

Among the different methods that are seldom used with the aim of destroying a corpse and thus preventing discovery or at least identification, the use of highly acidic or basic solutions is something forensic pathologists and anthropologists have sometimes to deal with, especially in some criminal scenarios where corrosive substances are actually thought of as a means of hindering personal identification, completely destroying human remains and removing proof of murder. However, there is a question that has never found an answer, and that is what the real effect of these solutions can be and thus their whole potential. Moreover, determining whether a bone (or even a single fragment) was in contact with an acidic/basic solution could be a crucial question posed to the anthropologist. The main issue is: are these solutions able to make a cadaver completely "disappear"? and when human tissues come in contact with these substances, what kind of changes do they undergo? How are they recognizable? Few studies have focused on this issue and refer only to macroscopic surveys. Most of the previous studies have been addressed on teeth that were destroyed after immersion in hydrochloric acid, phosphoric acid and sodium hydroxide at concentration reasonably found in household cleaning products [1–3]. In a further study performed on teeth, [4] the corrosive capabilities of 37% hydrochloric acid, 65% nitric acid and 96% sulfuric acid was tested in a 8-h time interval. The only study available on this kind of issue on teeth and other tissues (bone, hair, nails and soft tissue) was performed by Hartnett et al. [5] with hydrochloric acid, sulfuric acid, lye, bleach, organic septic cleaner and Coca-Cola® soda at various concentration and for different immersion times. Further studies have been performed on the effects that different pHs may exert on human bone in freshwater environments [6] and in different types of soil [7].

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To our best knowledge, the few previous studies focused mainly on the capability of these substances to destroy human tissues and the time needed to make such effects develop. Furthermore, from the analysis of the times of consumption of a small sample, like a tooth, what might be indeed the time for larger samples or even for a whole body is difficult to determine. Moreover, what has never yet been investigated are the microscopic alterations of tissues such as the human bones (and signs of injuries as well) if posed in prolonged contact with acid and basic agents. The present study aimed at focusing on the macro and microscopic effects of acids and bases on bone and trauma, in cases of accidental or voluntary exposure of body parts to these agents.

2. Materials and methods

The experiment was performed on porcine material, which has already been used in many studies as an animal model to best imitate human bone, and whose structure and behavior can be considered comparable to human bone [8–11]. A total of 63 samples of porcine bone (ribs of *Sus* scrofa) 4 cm in length, were completely skeletonized and cut manually with the use of a mechanical saw without any chemical treatment, in order to best preserve the outer and inner structure of the samples. Furthermore, on each sample two parallel cut marks (approximately 1 mm wide and 1 mm deep, along the entire length of the fragment) were produced with a linear-edged knife (named L1) and with a scalpel (named L2) in order to evaluate the modifications of signs of injury in case of stressful environmental conditions. Specimens were then divided in six groups of ten each, named from S1 to S60 and each piece, of the approximate weight of 2 g, was put in 80–100 ml of six different liquid solutions with different pH (1, 3, 5, 9, 12, 14) prepared by adding sulfuric acid, acetic acid or sodium hydroxide to distilled water (at concentrations indicated in Table 1), and a pH 9 buffer solution (composed of 0.05 M boric acid, 0.05 M potassium chloride brought at pH = 9 with sodium hydroxide [12]). Reagents used were obtained from Sigma–Aldrich and purest grades available were used (usually ACS grade reagents). pH (3–12) was checked using a combined glass electrode (Orion) and an Orion potentiostat.

The remaining three samples were kept as controls and immersed in distilled water (C1, 2 g was put in 100 ml water), left in ambient air (C2, 20 °C) and kept in a freezer (−20 °C) as a representative sample of the starting time 0 (C3). Starting from 5 days of immersion, up to a maximum of 70 days (end of the study) a sample from each solution was taken and subjected to the following analyses.

The study was performed with macroscopic and microscopic surveys (Table 2). Firstly, the features of periosteum, cortical bone and cut marks were investigated macroscopically for the general appearance, presence of erosion, crackings (“cracks” of cortical bone with exposure of the underlying cancellous bone) or flakings (detachment of surface portions of the cortical bone’s surface in the form of “flakes” or “grains”). Secondly, every sample underwent analysis with a stereomicroscope Wild Heerbrugg M650 (magnification 16×) aiming at testing the degree of erosion of the cortical bone and the appearance and detectability of the sharp force lesions. Finally, with the aim of further evaluating the inner structure of the bone, thin undecalcified sections were prepared and analyzed with transmitted light microscope (Leica DM 4000 B, magnification 40×) to evaluate the changes of the osteonic structures and the development of microcrackings.

Table 1
Substances used for the study.

pH	Substance used	Composition used for the study	Samples
1	Sulfuric acid	1 N	S1–S10
3	Sulfuric acid	0.01 N	S11–S20
5	Acetic acid	0.01 N	S21–S30
9	Buffer solution	0.05 M boric acid, 0.05 M potassium chloride brought at pH = 9 with sodium hydroxide [12]	S31–S40
12	Sodium hydroxide	0.01 N	S41–S50
14	Sodium hydroxide	1 N	S51–S60

Table 2
Analyses performed in the study.

Type of survey	Periosteum	Cortical bone	Cut marks
Macroscopic observation	Appearance (parcellization – detachment)	Presence/absence of erosion (loss of substance) Presence/absence of cracking Presence/absence of flaking	Degree of detectability of kerf walls and floor
Stereomicroscopy (magnification 6×, 10×, 16×)	Appearance (parcellization – detachment)	Presence/absence of erosion (loss of substance)/porous aspect – smooth surface – erosion “bubble-like” – complete erosion	Degree of detectability of kerf walls and floor
Optical microscopy (magnification 10×, 20×, 40×)	Osteons Degree of detectability of the osteons Presence/absence of radial cracking Presence/absence of circumferential cracking Presence/absence of multidirectional cracking Presence/absence of perpendicular cracking		

3. Results

3.1. pH 1

Macroscopic survey: The presence of the periosteum was macroscopically detected in all the specimens, in 60% of cases the periosteum was present over the entire surface of the sample, while in 40% it appeared fragmented, regardless of the time of immersion of the sample in the solution. From 72 h of immersion, the periosteum turned to a brown discoloration (clearly evident around the tenth day of the study, then increasingly darker) and to a grainy aspect of the surface that was kept until the end of the study. The cortical bone was kept almost intact until the end of the study, with a color that turned to light brown (10 days), then to a dark yellowish discoloration (70 days), without cracking or flaking (Fig. 1). Both lesions remained visible for the whole duration of the experiment. The only exception was the sample taken after 60 days in which some loss of substance was present to the lower third, with loss of the surrounding periosteum. *Stereomicroscopy:* A significant erosion of the cortical bone was not detected until the 25th day of the study, when a “bubble-like” erosion (Fig. 2) arose over the entire surface of the sample, each bubble being in the range 0.2–2 mm radius; from the 50th day the cortical bone was completely covered with parallel striations. *Light microscopy:* The inner microscopic structure of the osteons was clearly detectable until the 20th day of immersion, when the distinction between different osteons became more blurred and radial crackings appeared (Fig. 3). Such radial crackings were detected in all the samples between 20 and 70 days.

3.2. pH 3

Macroscopic survey: The presence of the periosteum was detected up to 50 days of immersion, while from the 50th to the