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A test of the citrate method of PMI estimation from skeletal remains☆

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ABSTRACT

Citrate content in bone has been shown to be associated with the postmortem interval (PMI), with citrate decreasing after death as a function of time. Here we test this method using porcine ribs for the period of 1–165 days after death, and also assess citrate content and variation from samples placed into two different postmortem environments (terrestrial and aquatic). Higher citrate variation, lower citrate recovery, and a weaker association with time were found in this study as compared to others. Citrate content, however, was found to decrease with increasing PMI, and the method was found to be easy and inexpensive to apply. No significant differences were found in citrate loss between terrestrial and aquatic environments. Although more research is needed, citrate content appears to be a promising new approach in estimating PMI from skeletal remains.

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1. Introduction

Accurate methods of postmortem interval (PMI) estimation continue to elude researchers and practitioners, particularly for cases where the PMI is greater than several days. Early postmortem changes such as *algor mortis*,*livor mortis* and *rigor mortis* can be useful in estimating PMI with considerable accuracy within the first hour and days after death. With increasing PMI, however, there is an associated decrease in the accuracy of PMI estimation. This is largely due to the numerous intrinsic and extrinsic factors that can affect the rate of postmortem changes. Decomposition (or the decay of remains through the combined processes of autolysis and putrefaction) results in considerable and easily observed changes in the soft tissue such as discoloration, bloating, skin slippage, and tissue reduction. Historically these changes have been assessed rather qualitatively, with the states of decomposition being very broadly categorized (*e.g.*, [1]) providing only vague indicators of the likely time of death. Recent studies have improved on these approaches by developing methods that involve applying more objective measures such as scoring systems and calculations [2,3]. Moreover, other studies have approached variation quantitatively, showing, for example, that temperature accounts for around 80% of the variation seen in decomposition rate, due primarily to its relationship with the activity of both microbes and insects [3,4].

The estimation of PMI from skeletal remains (*i.e.*, once the soft tissues have decomposed) is even more problematic because even less is known about the PMI-related changes that bone experiences, particularly those that affect bone in the timeframe of medicolegal significance. Changes to the bone's physical appearance with time as a result of weathering were among the first to be associated with PMI (*e.g.*, [5]). The time ranges for observed features, however, are so broad as to be of little predictive value for PMI estimation. Several analytical methods have been examined for estimating PMI from skeletal remains including physiochemical and serological changes as well as changes in radioisotope concentrations [6,7]. Microflora from the surrounding environment cause perceptible changes in the quality of osteons preservation as observed histologically [8,9], but such changes are not strongly associated with time. Nitrogen and amino acid content in bone have also been shown to decrease with time [10], but are most useful in assessing whether remains are likely recent enough to be of medicolegal significance. Bone's reaction to luminol (which reacts with blood remnants) as well as its fluorescence when exposed to certain wavelengths of light (likely a function of collagen content) are also known to diminish with time, but also can only be used to make broad claims of modernity or antiquity [6,11–13]. Radiodecay testing of carbon-14 is highly accurate for assessing the antiquity of skeletal remains, but is not very useful for more recent materials [14] and is also rather

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expensive. Radiocarbon bomb peak testing can accurately place the time of death prior to or after above-ground thermonuclear testing [15,16], but is not as accurate for more recent timeframes. Methods of PMI estimation from skeletal tissue therefore remain under-developed.

One recent study [17] found a strong correlation with PMI and bone citrate content, with citrate decreasing linearly as a function of time. Citrate is a well-known but poorly understood citric acid derivative involved in bone biochemistry and mechanics, with about 90% of the body's citrate being found in bone at a level of about 1–2 wt.% [18–23]. *In vivo* citrate content apparently does not vary significantly by the individual's sex or age [24], and citrate decrease is claimed to be largely independent of decomposition environment. In Schwarcz et al.'s [17] study, very consistent initial citrate content for bones was found across numerous sample groups (1.958 ± 0.061. wt.%), with a very strong association with time reported (r^2 = 0.919). This would seem to be a highly reliable and accurate method for estimating PMI from skeletal remains, and several equations were offered for estimating PMI based on a bone's citrate content. Additional conclusions included that detectable citrate loss began after about four weeks postmortem, the method could be useful up to a PMI of about 100 years, citrate loss was diminished below 0 °C, and it was suggested that moisture/humidity may play an important role in the decrease of citrate.

Subsequent studies, however, have failed to replicate Schwarcz et al.'s [17] results both in terms of the level and consistency of initial citrate content, as well as the strength of the correlation between citrate content and PMI [25–28]. In Zimmer et al.'s [25] study which attempted to replicate Schwarcz et al.'s [17] method using infant pigs, high variation in initial citrate was found, and there was no association noted between citrate level and PMI. Since citrate may be related to mineral content in bone, however, the use of infant specimens in that study may have been a factor. A study by Dunphy [26] aimed in part at developing a reliable method for citrate measurement, used two different methods and found much lower extraction and recovery rates of citrate than Schwarcz et al. [17] (1.26–1.69 wt.%). Dunphy [26] also noted high standard deviations and variances suggesting that techniques for reliably quantifying citrate need further development. Similarly, Pysh [29] detected citrate levels much lower and more variable than Schwarcz et al. [17] (1.12 ± 0.63 wt.%).

Kanz et al. [27] also attempted to replicate the Schwarcz et al. [17] study using specimens from different environments as well as sampling different bones. Kanz et al. [27] noted different citrate levels in different bones from the same individual (temporal and femur) in certain environmental contexts. Less citrate was found in flat bones, and in general they found it was not possible to obtain reliable citrate concentration measurements from temporal bones. They suggested that initial citrate in bone may related to the number of osteons and that it may therefore be preferable to use elements with more cortical bone, and further recommend using bones with a thick cortical layer. Even using the femora, however, Kanz et al. [27] report an overall low accuracy of the Schwarcz et al. [17] equation, which consistently underestimated PMI. Kanz et al. [27] did agree with Schwarcz et al. [17] on the apparent need for moisture for the decrease in citrate to occur. Kanz et al. [27] also noted that the technique requires affordable lab equipment and should be fast, easy and inexpensive for appropriately trained personnel.

Brown et al. [28] also attempted to validate and optimize the Schwarcz et al. [17] method, finding results similar to Kanz et al. [27]. Citrate recovery rates were found to have good precision and low error using two methods, but similarly to Dunphy's [26] results, Brown et al. [28] found significantly lower initial citrate in their samples (1.21 ± 0.03 and 1.19 ± 0.04 wt.%). Moreover, they report that the correlation between citrate and PMI in their sample was much weaker than that reported by Schwarcz et al. [17]. Brown et al. [28] suggest that citrate content may serve as a valid method to sort medicolegally significant remains from ancient remains.

Overall, results of previous studies seem to suggest citrate content in bone can be measured with reasonable accuracy, and that the citrate content decreases with time. However, this relationship does not seem to be as strong or as independent of the postmortem environment as initially proposed. While possibly still a promising approach to PMI estimation using skeletal remains, much more research is needed to clarify the best method of assessing citrate content in skeletal samples, the relationship of citrate content with time, and the effect of environmental conditions.

This study aims to test the reliability and validity of the method of PMI estimation using bone citrate content. Specifically we attempt to replicate results using the Schwarcz et al. [17] method in terms of both citrate recovery and correlation with PMI. Due to the apparent importance of water, we also assess the effect of terrestrial *versus* aquatic environments on citrate measurements and decrease over time. We also assess the ease with which the citrate method of PMI estimation can be applied.

2. Materials and methods

Ribs from a single domestic white pig (*Sus scrofa*) were purchased from a local butcher. The time of death of the pig was approximately 24 h prior to purchase. The ribs were defleshed using hemostats and cut into 1.5–2 cm sections, resulting in a total of 35 specimens (Fig. 1). Five control samples were immediately placed into a freezer within a freezer bag.

The remaining 30 specimens were divided into two environmental sample groups: "terrestrial" and "aquatic." The artificial "environments" consisted of plastic containers, one of which was empty except for the ribs, and one of which contained three liters of distilled water. Fifteen rib specimens were placed into each container in a 3 × 5 arrangement, the lids were affixed, and the containers were maintained indoors at room temperature (∼70 °F). At days 55, 110 and 165, five specimens were removed from each of the environments and placed into a freezer within labeled freezer bags. All samples remained frozen until the time of citrate extraction, which was 116 days after the last specimens were removed from the environment containers.

Citrate extraction and measurement largely followed the procedure outlined by Schwarcz et al. [17]. All specimens were removed from the freezer and allowed to reach room temperature. The specimens were defatted by soaking in a solution of

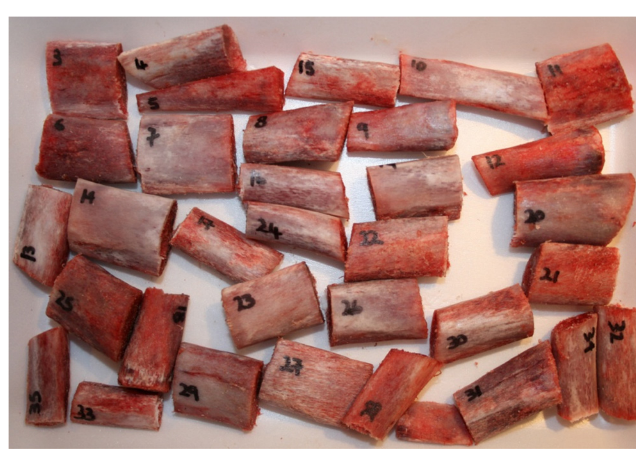

Fig. 1. Defleshed, sectioned rib portions.