H1 The Temporal Degradation of Bone Collagen: A Histochemical Approach to Postmortem Interval Estimation

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After attending this presentation, attendees will gain knowledge regarding the development and application of a simple histological method that selectively stains collagenous and non-collagenous proteins to determine whether a predictive rate of collagen loss occurs over a scale of months and years.

This presentation will impact the forensic science community by providing a potentially useful technique for estimating the Postmortem Interval (PMI) of skeletonized remains over a range of months to years. As collagen degrades over time, the pattern of degradation may be used to predict the PMI of skeletonized remains in forensic cases. The relative simplicity of this procedure and its easily obtainable equipment (already available in many forensic laboratories) potentially allow for the future widespread use of this method in forensic anthropology.

Forensic anthropologists are currently unable to reliably estimate the PMI of skeletonized remains.[1,2] This pilot study was conducted to determine if bone collagen degrades at a predictable rate over time and if so, to determine if this pattern could contribute to the creation of a quantitative method for estimating the PMI. The sample used in this study consisted of one fresh pig long bone and an additional ten pig long bones from subjects with known PMIs ranging from two to twelve months. The method involved the use of a histochemical stain which, when applied to embedded sections of bone, selectively stained collagenous proteins pink and non-collagenous proteins green. Excess stain was rinsed from the section, and the remaining stain was eluted and analyzed using a spectrophotometer at optical density frequencies of 540°nm and 605°nm to yield an Optical Density (OD) value of each dye. A standard curve was created such that a given OD value at a specific frequency would correspond to a known concentration of collagenous or non-collagenous proteins in the unknown section. The ratios of these concentrations were then calculated and statistical analyses were conducted to determine if a significant change or degradation in the ratio of bone protein concentrations occurred over time. Correlation and regression analyses also were performed to determine if the bone protein ratios were significantly correlated with time and, if so, how much of the correlation (or variance in the sample) could be attributed to this correlation.

A statistically significant change took place, with the largest change occurring in the first two months. The Pearson's correlation analyses revealed a significant negative correlation between the ratios of protein concentrations and time; however, the results of the regression analyses yielded only a moderate R^2 value, indicating that while time has some predictive value in determining the age of a bone, additional factors also appear to have a significant effect in predicting the age of an unknown bone.

Via visual assessments of pre-eluted sections, it was evident that the first regions of a bone section to lose collagenous proteins and stain green were the endosteal and/or periosteal regions. The exception to this pattern was found in those bones whose structural integrity had failed and the cortical bone had cracked. In these cases, green staining appeared along the margins of the site of structural failure. Among the modern samples, there was a clear change in the ratio of the pink and green dyes ranging from almost entirely pink in the fresh sections to predominantly green in most of the 10- and 12-month sections.

In summation, while it is apparent that collagenous proteins are significantly correlated with time, other factors also clearly play a role in the rate and process of bone degradation, or diagenesis. As a result, PMI estimations cannot currently be made solely upon the ratio of remaining collagenous and non-collagenous proteins in bone. Rather, further research which monitors the rate and pattern of diagenesis with respect to time and extrinsic factors, such as soil pH values and microbial activity, should be conducted.

References:

1. Goff M. Early post-mortem changes and stages of decomposition in exposed cadavers. Exp Appl Acarol 2009;49:21-36.
2. Schwarz H, Agur K, Jantz L. A new method for determination of postmortem interval: citrate content of bone. J Forensic Sci 2010;55:1516-22

Bone Histology, Collagen, Postmortem Interval

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