

bodies nonburied (found outdoors) and from not well defined burial conditions (church burials and cemetery exhumations).

Exchanges of elements, anyway, between bone and the surrounding soil after burial have been reported (3–5) but never examined systematically. To the best of our knowledge the most recent paper on this topic has been published in 1998 by Shinomiya et al. (6). In 1980 the inorganic substance content of skeletal remains was used by Foldes et al. (7) as a means of determining the duration of burial in the ground. In this latter paper the authors demonstrated that trabecular bone is highly susceptible to post-mortem absorption of inorganic contaminant substances than compact bone. They also observed that the metals content in bones gradually increases with age measuring higher concentrations in archaeological skeletal remains than in recent bone samples; differences in metal content were observed between bones and soil samples collected from the surroundings of the burial site. Variation of trace metals in ancient and contemporary bones were also discussed by several other authors in relation to the mineralization of bones and the surrounding environment (8–10).

Actually, we are going deeper in our research analyzing soil samples from the cemetery where the buried bones come from, and even if the study is still going on, we can anticipate that no clear false positives seem to have occurred to the femura of the “modern” group. This is supported from the mineral content of bone and soil samples measured by atomic-absorption-spectrophotometry. Between the “modern” bone samples and several contemporary controls no significant differences in the Fe, Zn, Pb, Cu, Cb, Mg and Mn content have been observed—unlike the high concentration of metals measured from cemetery soil samples according to the results illustrated by Foldes et al. (1980).

However, regarding the postmortem absorption of inorganic substances such as salts of heavy metals by the skeleton, we think that much more has to be investigated. Since several substances can cause variation in the fluorescent intensity of luminol, we agree with you, of course, that controls, such as soil samples collected from the area surrounding the skeletal remains, clothing associated with them and bone cleaning materials should be tested when available in conjunction with the luminol test. This procedure of testing could exclude occasional false positives or negatives and validate the results obtained from the analysis. Since our JFS paper is a preliminary attempt to classify the correlation between the intensity and distribution of chemiluminescence from bone powder (compact tissue) and postmortem interval (PMI), our results provide only a glimpse of the potential of a luminol test as a chemical and physical method for dating human skeletal remains. We hope in the future to share our experience with other investigators and that our efforts continue to stimulate research and open discussions in this field. Further comments or suggestions are welcome and they are helpful to us.

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Commentary on Hochmeister MN, Budowle B, Sparkes R, Rudin O, Gehrig C, Thali M, Schmidt L, Cordier A. Validation studies of an immunochromatographic 1-step test for the forensic identification of human blood. *J Forensic Sci* 1999;44:597–602.

Sir:

In their validation of a device for identification of human hemoglobin in bloodstains, Hochmeister et al. (1) tested bloodstains from a variety of animal species. The domestic ferret (*Mustela putorius fero*) was not among the animals tested. Examination of a database of amino acid sequences of proteins (2) reveals that the hemoglobins from humans, several primates, and ferrets share a common amino acid sequence from residues 67 to 73 of the alpha chain, namely TNAVAHV.¹ This sequence differs from that of the corresponding segment of hemoglobin from mouse (ASAAGHL) and rabbit and goat (both TKAVGHL) and is therefore potentially immunogenic for the production of monoclonal and polyclonal antibodies. Such antibodies are the critical components of immunochromatographic devices. Among the amino acid differences between mouse and human hemoglobin, the TNAVAHV sequence shows maximal discrimination between human and other commonly encountered animal hemoglobins and is the likely candidate for contribution to the epitope recognized by a monoclonal antibody selected for this purpose.

Ferrets are occasionally encountered as companion animals in the United States and are potential sources of bloodstains. Because of this hemoglobin sequence homology, it is necessary to indicate the reactivity toward ferret blood in validation studies of immunoassays for identification of human hemoglobin in bloodstains.

References

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2. PIR-International Protein Sequence Database. National Biomedical Research Foundation, Georgetown University Medical Center (DC), 1999. The “PROWL” search program (<http://prowl1.rockefeller.edu>) was used.

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¹ Single-letter abbreviations for the amino acid residues are: A, Alanine; G, Glycine; H, Histidine; K, Lysine; L, Leucine; N, Asparagine; S, Serine; T, Threonine; and V, Valine.