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scenarios put forth. We would certainly welcome any additional probable scenarios to explain the trauma observed.

The focus of this article was to examine the extensive injuries the individual sustained in order to ascertain the most probable manner of death. As we are aware that cause of death is a medical determination, there is no attempt in this article to ascertain cause of death. We are simply stating that the severe injuries sustained by this individual most likely seriously incapacitated him.

Finally, several of the injuries to the scapula and vertebrae have been attributed to contraction of particular muscles. However, our understanding of avulsion fractures as a result of forcible tearing or pulling suggested that these injuries could also be classified as avulsion fractures. For instance, fractures of the inferior and superior scapular angle, where there is muscle attachment, are often classified as avulsion fractures.

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Commentary on Introna F, Di Vella G, Campobasso CP. Determination of postmortem interval from old skeletal remains by image analysis of luminol test results. J Forensic Sci 1999; 44(3):535–8.

Sir:

I have a few questions for the authors followed by some comments on luminol. What was the history of the bones examined in the study? Were the bones from burials or were they from non-buried, relatively pristine bodies? Did the bones undergo any cleaning procedures prior to luminol treatment?

A forensic scientist must always be very careful when interpreting luminol results. In this study, the authors took appropriate steps to eliminate false positives that could result from plant peroxidases; however, other sources of contamination can cause false luminol positive reactions. Copper, copper salts, ferricyanide, iron ions, cobalt ions, and sodium hypochlorite (bleach) can cause luminol to fluoresce (1–3). Any of these substances could come in contact with bones, particularly bones that have been buried in mineral rich soil and bones that have been cleaned with tap water and/or bleach. I have seen luminol react with copper salts that have leached into the fabric surrounding the copper rivets of blue jeans. I have also seen luminol react with black fingerprint powder. When using the suggested method for aging bones, the scientist must be aware of other substances that can cause variation in the fluorescent intensity of luminol. Standards, such as known bone samples of varying PMI, and controls, such as a soil sample collected from the area surrounding the bone, clothing associated with the remains, and bone cleaning materials, should be used in conjunction with this type of analysis.

References

1. Albrecht HO. Über die Chemilumineszenz des Aminophthalsäurehydrazids. Z Phys Chem (Leipzig) 1928;136:321–30.
2. Grodsky M, Wright K, Kirk PL. Simplified preliminary blood testing. An improved technique and comparative study of methods. J Crim Law Criminol Police Sci 1951;42:95–104.
3. Steigmann A. Luminol light reactions. Chem Industry: 889–890 through Chem. Abstr. 1942;36:1848.

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Authors' Response

Sir:

Thank you very much for the comments regarding our article: "Determination of postmortem interval from old skeletal remains by image analysis of luminol test results." We do really appreciate them and certainly agree that forensic scientists must always be very careful when interpreting luminol results.

The goal of our study is testing a simple and easy distinction method between two broad groups of skeletal remains frequently examined during forensic investigations: "modern" (less than 50 years) and "ancient" (more than 50 years) bones. The paper is a preliminary effort to the evaluation of correlating the time since death with blood remnants in bone tissue. Luminol is very sensitive, reacting rapidly to the most minute traces of blood, but it is a presumptive test, capable of delivering both false positives and false negatives. For example it does not differentiate between human and animal blood (1).

Major sources of false positives are chemical oxidants, catalysts, and salts of heavy metals such as copper and nickel. To avoid the possible influence of the most common substances (such as iodine, rust, household bleach, formalin and plant peroxidases such as are found in horseradish, citrus fruits, bananas, watermelon and numerous vegetables), we washed in distilled water all the bone samples and heated them to 100°C for a period of 5 min prior to testing with luminol solution. This temperature does not appreciably affect the heme portion of the hemoglobin responsible for the luminescence reaction and destroys the plant peroxidases.

However, as you stated in your comments, metal surfaces such as copper, copper salts, ferricyanide, iron ions, cobalt ions and sodium hypochlorite (bleach) are particularly likely to yield false positives. To avoid the possible influence of these substances we followed procedures as reported in a previous paper on this topic (2) collecting bone powder from the inner compact tissue of the mid-shaft of each femur. Compact bone is, in fact, far less susceptible to physical and/or surface contamination than trabecular bone with its large surface area to volume ratio and multiple cavities that easily become filled with contaminating soil and clay particles. After removing the periosteal (outer) and endosteal (inner) surfaces and pulverizing the compact tissue samples into a fine bone powder using a grinder no other particular cleaning procedures were used except a second washing in distilled water.

Regarding the history of the bone samples examined, the femora belonging to the "ancient" group examined (fourth and fifth group with PMI ranging between 50 and over 80 years) were from human remains found in different ossuaries (crypts) of old Roman Catholic churches. For these latter bones the original burial conditions are still not well defined and for some skeletons completely unknown. However, based on the negative results of image analysis of luminol tests for this latter "ancient" group we can exclude manifest false positives since only one femur (PMI ranging between 50 and 60 years) revealed a very faint light-reaction (see the weaker luminance recorded from the powdered bone than the other groups). The most of femora (33 out of 60) belonging to the "modern" group (first, second and third group with PMI ranging between 1 month and 35 years) were from skeletal remains found outdoors, in open fields, during forensic investigations. The rest of femora belonging to "modern" group (27 out of 60) came from cemetery exhumations. These bodies were buried in wooden coffins embedded both beneath the soil and in cement niches for urns; actually, we do not know exactly which coffins were lined with metal (zinc) plate or which kind of clothing was associated with the remains. Consequently, it was not possible to standardize the variations caused by burial environments, since the examined material came from different sites such as

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 postmortem 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.

References

1. Grundermann KD. Chemiluminescence in organic compounds. Angew Chem Int Ed 1965;4(7):566–73.
2. Colonna M, Introna F Jr, Di Nunno C, Dattoli V. Valutazioni tanatocronologiche su resti scheletrici ed applicazione sperimentale del Luminol test. Zacchia 1987;5(4):65–80.
3. Mosenbach R. The chemical and mineral composition of inorganic substances of bones and dentin of existing and fossil vertebrates structure and size of the middle grains dependent on the geologic age. Bull Group Eur Rech Sci Stomatol Odontol 1974;17:59–85.
4. Lambert JB, Szpunar CB, Buikstra JE. Chemical analysis of excavated human bone from middle and late Woodland sites. Archaemetry 1979;21:115–29.
5. Jaworowski Z, Barbalat F, Blain C, Peyre E. Heavy metals in human and animal bones from ancient and contemporary France. Sci Total Environ 1985;43:103–26.
6. Shinomiya T, Shinomiya K, Orimoto C, Minami T, Tohno Y, Yamada M. In- and out-flows of elements in bones embedded in reference soils. Forensic Sci Int 1998;98:109–18.
7. Foldes V, Kosa F, Viragos-Kis E, Rengei B, Ferke A. Atomabsorptionsspektrophotometrische Untersuchung des Gehaltes an anorganischen Substanzen von Skelettbefunden zur Ermittlung der Dauer des Begrabenseins in der Erde. Arch Kriminol 1980;166(3–4):105–11.
8. Waldron HA. Postmortem absorption of lead by the skeleton. Am J Phys Anthropol 1981;55(3):395–8.
9. Jaworowski Z, Barbalat F, Blain C, Peyre E. Chronologic course of the content of lead, cadmium and zinc in human bones in France. CR Acad Sci III 1984;299(10):409–12.
10. Hisanaga A, Hirata M, Tanaka A, Ishinishi N, Eguchi Y. Variation of trace metals in ancient and contemporary Japanese bones. Biol Trace Elem Res 1989;22(3):221–31.

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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 puterius 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

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