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Postmortem interval of skeletal remains through the detection of intraosseal hemin traces. A comparison of UV-fluorescence, luminol, Hexagon-OBTI®, and Combur® tests

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ABSTRACT

With the goal of obtaining additional practically applicable methods for estimating the PMI of skeletal remains, 39 samples of human and 5 samples of domestic animal long bones with known PMI (PMI = 1 to approximately 2000 years) were tested with two established methods (UV-fluorescence of a freshly sawn cross-section and the luminol test) and two screening tests (Hexagon-OBTI® test and Combur® test) that were being tried out in this context for the first time.

The hypothesis underlying this experiment was the supposition that the PMI-related chemiluminescence of the luminol reaction for bone is based on the presence of persisting hemin from hemoglobin molecules in bone.

Our results showed that lack of luminescence and reduced UV-fluorescence were more meaningful results for estimating PMI and excluding forensic relevance than a positive luminol reaction or strong UV-fluorescence, as both of the latter findings revealed the limitations of these methods in this particular context. Particularly for cases showing a positive luminol reaction, the use of additional absolute dating methods may be indicated. Against our expectations, both the Combur® test strips and the Hexagon-OBTI® test, which were both devised to demonstrate blood, delivered negative results for all samples. They are thus not suitable for estimating the PMI of skeletal remains. Future research will be necessary to elucidate whether the negative results obtained for these tests may be due to the poor solubility of potentially present hemoglobin or hemoglobin breakdown products in the Tris buffer used in this experiment.

1. Introduction

Determining the postmortem interval (PMI) of skeletal remains has always been one of the greatest challenges in forensic osteology. When human remains are found out in the open, it is almost impossible to reach a sound conclusion about the time since death. Even bones that have always lain in earth burial sites are affected by numerous environmental factors that have an influence on the bones and their decomposition and make dating difficult [1,2]. Estimating the PMI with any certainty is thus very difficult, even if the goal is only to distinguish between remains that are recent (PMI smaller than 50 years) or historic [2,3].

At present only absolute dating methods, primarily radionuclide measurements, provide estimates of the PMI which are not influenced by external, e.g. climatic, factors [4,5]. These methods are, however, not only elaborate and costly, they are also not available everywhere.

Despite all methodological limitations, every estimate of the PMI in a forensic osteology context begins with the macroscopic examination of the remains. Some authors recommend assessing the UV-reflection on a freshly cut cross surface of one of the long bones [2]. A second step that usually follows is the microscopic examination of cut or ground bone sections [6], but these, in turn, take time to prepare, and, in practice, their informational value is limited. A further approach to estimating the PMI is the use of chemical analyses, such as testing if the remains can be stained with indophenol and Nile blue [7], or testing for fluorescence of the bone [8]. As early as 1937, Specht [9] described the luminol method for detecting blood traces. The luminol test was later successfully used to estimate the PMI of bone samples [10,11]. Bone powder that accumulates when a fresh cross surface is being cut on the bone is used for the test. This method is fast and inexpensive. In addition, for a random sample of $n = 80$ bones [11] it allows a positive predictive value of 80% for luminol-positive samples and a negative–predictive value of 96% for chemiluminescence-negative samples. Since the validity of the negative samples seems more reliable, elaborate radionucleotide measurements could in future

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be restricted to cases in which a positive luminol test indicates a shorter, therefore forensically relevant, PMI.

The question arises whether other tests originally devised to detect blood can also be used to determine the PMI. For a long time, the Sangur® test [12,13] was used to test forensic blood trace evidence. However, for several years now, this test has only been available as a combination test strip, named Combur®, that also tests for protein, glucose, nitrite, etc. A further rapid test used for forensic trace evidence that was also originally devised for clinical purposes (detection of occult blood in stool) is the Hexagon-OBTI® test.

The Hexagon-OBTI® test is based on an antibody reaction to human hemoglobin. According to the manufacturer, the detection limit is as low as 0.88 mg Hb/g of stool. The Combur® test strips, by contrast, contain hydrogen peroxide that together with any present hemoglobin catalyzes the oxidation of the indicator. This test has an even lower detection limit (0.03 mg Hb/dl test substance) than the Hexagon-OBTI® test. The aim of this study was not only to test bones with known PMI from earth burial sites with the UV-reflection method, the luminol test, the Hexagon-OBTI® test, and the Combur® test, but also to compare the results in view of validity requirements in a forensic setting.

2. Materials and methods

In our study, long bones from 39 individuals with known PMI were tested at the Institutes for Legal Medicine at the Universities of Frankfurt Main and Gießen (Table 1). For most of the cases, the date of death was determined from investigation and identification records. In a few cases – for all historic bones – the PMI was either determined or verified by radiocarbon dating. The results of radiocarbon dating were given as a time-interval within the standard 2-sigma limits. The known PMI data were transformed into ordinally scaled data, and the cases were assigned to one of the PMI classes 1–5 (Table 1).

For comparison, five non-human mammalian bones (cow, horse, pig, and dog) were also included in the testing process to verify the human specificity of the tests (HumanOpti®) on postmortem bone.

The bones used in the experiment had been stored in a special drying room at temperatures of 16–18 °C for 1–10 years after being found. This latency time was added to the known PMI (Table 1) but did not lead to a change in PMI-class in any case.

The bones were masked by randomly being assigned consecutive numbers. For the experiment, a fresh cross surface was cut across the shaft of each bone with a medical saw. The bone powder from the cut was used for the luminol-, the Hexagon-OBTI® test, and the Combur® test.

Before being cut, the shaft of each bone was cleaned with fresh sandpaper. The bone powder from each cut was collected on a fresh piece of clean, white paper and then transferred to a test tube. UV-reflection on the fresh cross section was assessed in a dark room with an UV-lamp at wave lengths of 254 nm and 366 nm. The assessment of UV-reflectivity was initially descriptive. For the inter-observer test

Table 1
Results for all bone samples (#) for the luminol test and the UV-reflection test. The "(R)" behind the PMI value indicates that radiocarbon dating was performed. Sample a1 is from a pig; sample a2 from a horse, samples a3–a4 from a cow, and sample a5 from a dog. PMI-classes 1–5: (1) ($>1 \leq 10$), (2) ($>10 \leq 50$), (3) ($>50 \leq 100$), (4) ($>100 \leq 1000$), (5) (>1000).

#	Sample	PMI-class	PMI [y]	Luminol	UV-reflection
4	Compl. skeleton	1	4	++	Entire surface
9	Humerus	1	0.2	++	Entire surface
12	Humerus	1	3	++	Entire surface
21	Tibia	1	5	+	Entire surface
23	Radius	1	7	+++	Entire surface
27	Radius	1	8	++	Patchy areas of decreased fluorescence
28	Femur	1	8	+++	Entire surface
29	Femur	1	4	++	Entire surface
37	Tibia	1	4	+++	Entire surface
a1	Humerus	1	2–5	+++	Entire surface
a2	Femur	1	1–3	++	Entire surface
a3	Tibia	1	0	+++	Entire surface
a4	Femur	1	0	++	Entire surface
a5	Humerus	1	1	++	Entire surface
2	tibia	2	ca. 50	+	Sandwich effect
10	Various	2	40	+	Patchy areas of decreased fluorescence
11	Femur	2	5–20	+++	Entire surface
14	Ulna	2	37	++	Entire surface
18	Femur	2	25	–	Entire surface
19	Femur	2	17	+	Patchy areas of decreased fluorescence
26	Femur	2	33	+	Patchy areas of decreased fluorescence
33	Ulna	2	50	++	Entire surface
38	Tibia	2	30	+++	Entire surface
7	Femur	3	50–64	++	Patchy traces
8	Femur	3	50–70	+++	Almost entire surface
16	Humerus	3	>50	++	Reduced, slight sandwich effect
17	Femur	3	88	++	Sandwich effect
22	Femur	3	55	+++	Entire surface
34	Tibia	3	88	–	Patchy areas of decreased fluorescence
39	Femur	3	75	+	Sandwich effect
1	Tibia	4	ca. 200 (R)	+	Sandwich effect
3	Tibia	4	ca. 1000 (R)	–	Sandwich effect
6	Femur	4	ca. 150	+	Sandwich effect
13	Femur	4	145–160	++	Only small traces left
15	Humerus	4	ca. 1000 (R)	–	Only small traces left
20	Tibia	4	267 (R)	–	None
24	Femur	4	466 (R)	–	Remaining sandwich effect
25	Femur	4	123	–	Sandwich effect
32	Humerus	4	178 (R)	+	None
35	Femur	4	384 (R)	–	Sandwich effect
36	Tibia	4	120	–	Almost entire surface
5	Tibia	5	ca. 2000 (R)	–	Still minimal traces
30	Femur	5	2500 (R)	–	None
31	Femur	5	2200 (R)	+	None