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Experimental Study of Postmortem Change Under Field Conditions: Effects of Freezing, Thawing, and Mechanical Injury

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ABSTRACT: Understanding the processes of postmortem change in biologic systems is important to the forensic sciences. Previous experimental studies of postmortem change in animals under field conditions made use of animal carcasses that had been incidentally exposed to the effects of freezing and thawing or mechanical damage, or both, and were limited to gross observations. The current study was designed to document intrinsic processes of postmortem change, and the effects of freezing-thawing and mechanical injury, under controlled conditions in the field, using histologic and microbiologic techniques, as well as gross observation. Insect and microbiologic succession sequences, and patterns of decomposition and disarticulation, were observable over time. Previously frozen-thawed animals showed predominantly decay (aerobic decomposition) in the field, while freshly killed animals showed predominantly putrefaction (anaerobic decomposition). Previously frozen animals showed the same sequence, but accelerated rates, of disarticulation. Mechanically injured tissues showed accelerated rates of decomposition. These findings have implications for the interpretation of results of previous studies, as well as the interpretation of human and animal remains subjected to freezing and thawing.

KEYWORDS: pathology and biology, decomposition, putrefaction, disarticulation, decay, postmortem change, freezing, thawing, mechanical injury, histology, microbiology

At the end of life, the law of entropy finally prevails. Understanding the entropic processes of postmortem change is important to forensic science in determining differential survival of organic remains and organic lesions, detecting "pseudo-pathology," distinguishing antemortem from postmortem features, and estimating the season of death and the interval between death and discovery of organic remains. Much research concerning postmortem decomposition rates has used an entomological approach to answer the latter concerns [*1-6*].

Previous experimental studies on postmortem decomposition in animal models have been performed using baby pigs (*Sus scrofa*), either stillborn or crushed by the mother shortly after birth [*3*]. These animals were frozen at undefined temperatures for variable intervals, and thawed before field placement for gross observation of postmortem decomposition and insect succession patterns. No control animals were employed in the experiment, and no comparisons were reported in decay patterns between animals with fatal "crush" injuries and stillborn animals. In another study, primarily squirrels (*Sciurus carolinensis* and *S.*

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niger) and rabbits (*Sylvilagus floridanus*) were live-trapped, killed, and placed in the field immediately, or frozen and thawed under variable conditions for future placement [5]. In either study, no control procedures were employed for comparison of decay patterns in freshly killed, versus frozen-thawed, animals.

Recently, Rodriguez and Bass [6] have reported findings on decay rates of four human cadavers in East Tennessee. No histologic or microbiologic techniques were applied in any of these experimental field studies.

The present study was conducted in an experimental animal model under controlled field conditions. The objectives of this research were to (1) observe the gross, histologic, and microbiological features associated with immediate postmortem change in soft tissue and skeletal remains; (2) determine the effects of freezing and thawing on patterns of postmortem change; and (3) observe the effects of mechanical injury on postmortem change.

Background

Immediate postmortem change may be viewed essentially as a competition between decomposition (decay and putrefaction) and desiccation. External factors, such as temperature, humidity, and sunlight, acting with internal factors such as surface area-to-volume ratio and body temperature, largely determine the outcome of this contest [7]. Environmental conditions which favor desiccation, such as hot, dry climates, may result in mummification and partial preservation of soft tissue [8]. Climatological conditions favor desiccation and preservation of soft tissue in many parts of the world, including the canyons of the Southwestern United States and Northern Mexico [8], the coastal zones of Chile and Peru [9], and especially the deserts of North Africa [10] and Australia [11]. It may be inferred that the "secret" of Egyptian mummification, for example, was environmental desiccation, since "natural mummies" left in the desert to dry without artificial preparation in Egypt and elsewhere are among the best preserved of any specimens [7].

In cold climates, soft tissue preservation may result from freezing in permafrost zones. Several frozen Siberian woolly mammoths (*Mammuthus primigenius*) have been well preserved, well studied, and are well known. Scythian tombs in the Altai Mountains of Siberia were found to contain well-preserved human remains [12]. Human soft tissues have also been preserved through freezing in circumpolar areas of North America during ancient and historical periods [13]. Soft tissue preservation under frozen conditions may be potentially ideal, but the practical limitations of discovery conditions often preclude examination prior to the onset of postrecovery deterioration [8]. Further, exposure of soft tissues to alternate freeze-thaw cycles over time may introduce confounding variables [7]. Depending upon latitude, season of death, and stratigraphy of placement in the ground, animal and human remains may be subjected to freezing with or without subsequent thawing and refreezing. Wood and Johnson [14] have illustrated zones of continuous permafrost worldwide, and maximum depths of frost penetration in seasonally frozen ground in the United States.

Decay (aerobic decomposition) and putrefaction (anaerobic decomposition) in other climatic regions generally result in tissue destruction and information losses. Where tissue loss does occur, however, patterns of decomposition and disarticulation will ultimately determine the final deposition and survival of skeletal remains. Decomposition and disarticulation are actually a constellation of interrelated postmortem processes which are dependent upon several factors including: morphology and physical condition of the organism before death [15]; the location of remains in soil [16], or standing [17,18], or running [19] water; the temperature, humidity, soil pH, ground cover, season, and a host of other vegetational and climatic factors [7]; and, often, the actions of insects [1-6] and carnivore scavengers [20]. Existing studies of postmortem change have focused on observations of naturally occurring sequences of decay and disarticulation [18,21-23]; or experimental observation and documentation of decay and disarticulation sequences [3,5,15]. Different taxonomic se-

quences of decomposition have been proposed by these different workers. Some results have been difficult to reconcile, and it is difficult to formulate meaningful generalizations from them. Also, the extent to which postmortem processes in previously frozen tissues duplicates processes in freshly killed tissues is questionable and requires experimental validation.

Materials and Methods

Materials for the study included eight female Wistar rats (mean weight 316 ± 40 g); three 5- by 5- by 20-in. (127- by 127- by 508-mm) paired tandem cages constructed of pinewood and ½-in. (12.7-mm) square heavy-gauge wire mesh; and a field exposure site in a shaded, mixed deciduous woodland. Standard necropsy, histological, and microbiological equipment were utilized in the laboratory component of the study. Field placement cages were constructed with the dual objective of protecting animal specimens from gross damage by rodents or large predators in the field, while allowing access of arthropods. Varying mesh sizes in such cages could conceivably be used to selectively admit or exclude certain subpopulations of microfauna for isolated observation of effects.

Pregnant female Wistar rats were housed and fed identically before death, and were killed immediately postpartum by cervical dislocation or by pentobarbital injection. These animals had been used only for breeding purposes and were scheduled for sacrifice as part of another experiment. Four of these rats were frozen at −7°C for four weeks and thawed at room temperature (22°C) for 8 h before field placement. Four other rats were killed 2 h before field placement. All animals were tail-tagged for easy identification in the field. One pair each of "freshly killed" and "frozen-thawed" animals were necropsied at baseline. The remaining pairs were placed in three tandem cages. The cages were secured within a secluded area of Wissahickon Park, Philadelphia, PA, during late summer 1980. The immediate area was observed for soil conditions, humidity, and ground cover. Soil analysis was performed and ambient insects were collected.

Each animal was placed lying on its right side; in effect, creating two separate microenvironments, one in contact with the ground and the other exposed to the ambient air. The design of the cages otherwise ensured that each pair of animals was exposed to identical conditions over time (Fig. 1). One pair each of animals was removed at two days (48 h), four days (96 h), and six days (144 h). All remaining animal pairs were examined in the field at each time interval. Environmental conditions during the six-day observation period were constant, as shown in Table 1. The animals showed no evidence of external disturbance at any time during the observation period. After retrieval, necropsies were performed on each animal including complete evisceration and sterile bacterial culture of heart, lungs, spleen, and abdominal viscera (where possible). Tissues and cultures were prepared and examined according to standard laboratory procedures.

Results

The baseline necropsies at 0 h revealed differences between the "fresh" and "frozen" animals. The freshly killed animal had normal gross and microscopic examinations, and the internal organs were distinct in color and consistency. The frozen-thawed animal had a normal external examination, but internal examination revealed gross blood loss from oral and nasal mucosa, grossly indistinct internal organs and cortico-medullary junctions of the kidneys, and hemolysis of blood. Microscopic examination revealed loss of nuclear detail in tissue cells.

All three remaining pairs of animals were observed undisturbed in the field after two days (48 h). Insects (Hymenoptera, Coleoptera) and insect larvae were concentrated around carcasses and the surrounding areas. All frozen-thawed animals manifested greater external aerobic decomposition (decay) than the fresh-killed controls; assessed by greater percent

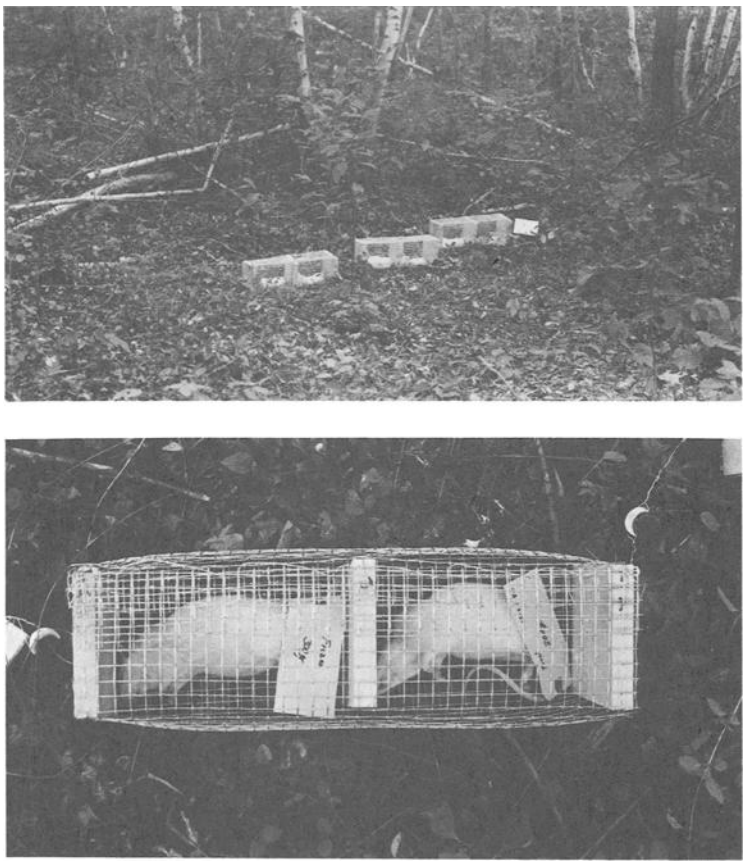

FIG. 1—*Initial experimental field conditions in a mixed deciduous woodland showing placement of animal carcasses. The close-up shows the ground cover. The paired tandem cages were designed to facilitate identical exposure of carcasses to arthropod populations, while preventing carnivore damage.*

TABLE 1—*Environmental conditions: temperature, precipitation, and wind.*

Condition	Day						Average
	1	2	3	4	5	6	
Average temperature, °F	81	82	84	84	81	81	82
Precipitation, in.	0	0	0	0	0	0	0
Sunshine, %	80	98	90	76	47	55	74
Wind, mph	5	6	6	6	8	7	6
Humidity, %	59	70	65	67	72	69	67

"t°C=(t°F − 32)/1.8, 1 in. = 25.4 mm, and 1 mph = 1.609 kph.

external hair loss, greater percent skin mummification, and greater percent loss of facial features. For the pair examined, the upper surface of the fresh-killed animal was intact (left eye present) and fully articulated. The undersurface (right) showed massive abdominal distention. The upper surface of the frozen-thawed animal showed focally denuded fur (left eye absent), partially mummified skin, and numerous ectoparasites. The undersurface was completely denuded, with skin focally decayed and flat abdominal wall. Internally, the freshly killed animal showed greater anaerobic decomposition (putrefaction) of abdominal fat and viscera, massive gastrointestinal distension, and "moth-eaten" lungs (right more than left). Insect larvae were present only in the anus and vagina. By contrast, in the frozen-thawed animal internally, the abdominal organs were preserved, lungs were intact, and gastrointestinal tract was nondistended. However, there were insect larvae in the oral cavity, tracheobronchial tree, and abdominal wall, as well as anal and vaginal external orifices. Microscopically, the fresh animal showed histologic preservation of the skin, superficial insect larvae, marked autolysis of liver and other viscera, and bacterial overgrowth in the gastrointestinal tract. The frozen animal showed decay of skin and muscle, larvae in deep tissues, partial preservation of viscera, intact pulmonary and biliary trees, and bacterial overgrowth in the upper airway. Weight change in the fresh animal was 2% loss, and in the frozen, 8% loss (including biomass).

After four days (96 h), both remaining pairs of animals displayed advanced decomposition with partial mummification and skeletonization, and insect larvae in abdominal cavities. Internally, abdominal and thoracic viscera were grossly absent in the fresh animal necropsied at this interval. The skeleton was fully articulated, except for the temporo-mandibular joint (TMJ). Insect larvae were present only in the right (ground contact) posterior thoracic region. In the frozen animal, internal organs were still partially preserved, but insect larvae were present throughout. The skeleton had undergone partial disarticulation (at TMJ, cervical vertebrae, symphysis pubis, and right knee), with forelimbs and thoracic cage completely articulated. Microscopic examination revealed extensive decay, large gram-positive rods, and multiple insect larvae. Weight loss was 83% of baseline in each animal at this stage.

After six days (144 h), the remaining pair of animals showed no insects or insect larvae on the carcasses or in the surrounding areas. There was mummification and partial skeletonization. Partial disarticulation processes in the fresh animal (TMJ, atlanto-occipital, cervical and lumbar vertebrae, sterno-costal junctions, symphysis pubis) continued to be less advanced than in the frozen animal (TMJ, cervical and lumbar vertebrae, costo-vertebral, sterno-costal, lumbo-sacral, sacro-iliac, pelvis-hip). The fresh animal had forelimbs and hindlimbs articulated, but no remaining viscera. The frozen animal had only forelimbs fully articulated, but remnants of viscera were still present. Weight loss in the fresh animal was 72%, and in the frozen 87%. These animals are shown in Fig. 2 after six days (undersurface).

A microbiologic succession sequence was observable from enteric to soil organisms over six days (Table 2). The sequences in fresh and frozen organisms were similar in order, but it is evident from the gross, histologic, and microbiologic evidence that enteric microorganism growth proceeds more rapidly in the fresh animals despite some initial contamination in the frozen animals.

In comparing the upper surfaces (exposed to air) to the lower surfaces (in ground contact), decomposition proceeded more rapidly on the side in contact with the ground in all animals, as compared to the other side in each. The animals that were killed by cervical dislocation at the start of the experiment showed rapid decay in the cervical tissue subjected to mechanical stress and injury (Fig. 3).

Discussion and Conclusions

A sequence of arthropod and microbiological succession, as well as patterns of decomposition and disarticulation, were discernable over the time interval. Using observations on all

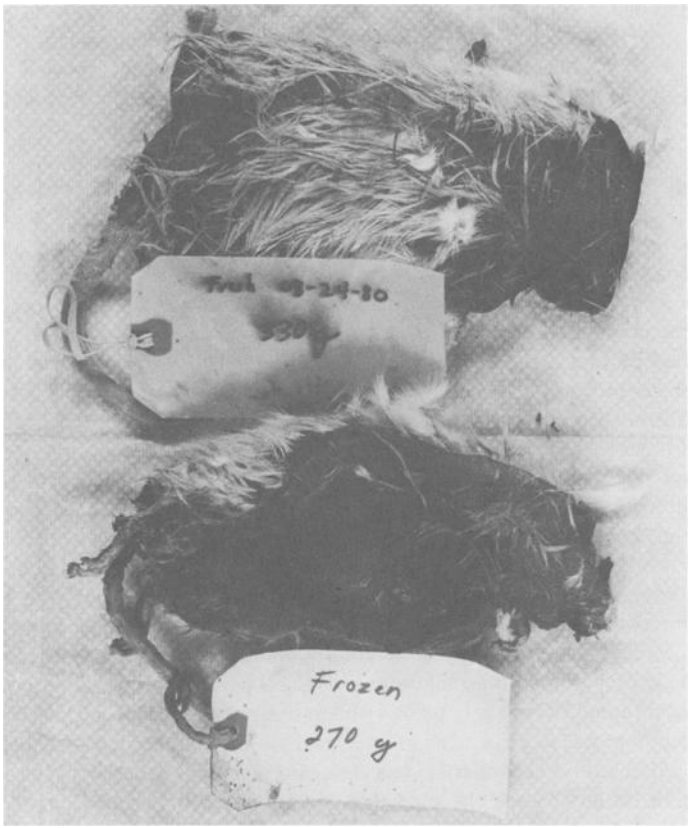

FIG. 2—*Lower surfaces (ground contact) of a freshly killed ("fresh") and of a frozen-thawed ("frozen") animal after six days (114 h) in the field. The frozen animal displays a greater degree of external decomposition (aerobic decay). Insects and insect larvae are absent from both animals at this stage.*

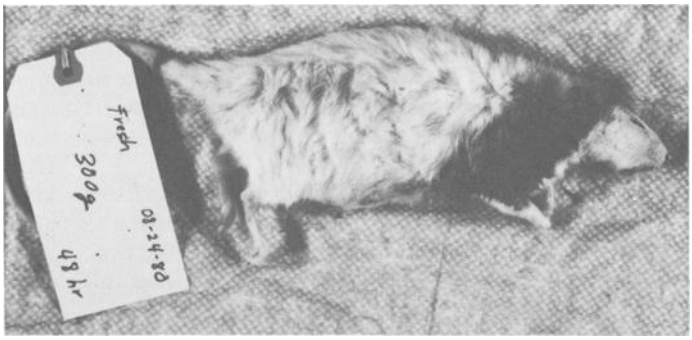

FIG. 3—*After two days (48 h), an animal sacrificed by cervical dislocation demonstrates a characteristic zone of advanced decomposition around the cervical tissues subjected to mechanical stress and injury. The lower (ground contact) surface of this fresh-killed animal is otherwise externally intact.*

TABLE 2—*Comparison of postmortem changes in bacteriology with time.*

Fresh	Frozen
DAY 0	DAY 0
Heart: sterile	Heart: Enterococcus, Staph aureus
Right lung: sterile	Right lung: sterile
Left lung: sterile	Left lung: sterile
Spleen: sterile	Spleen: sterile
Bowel: Strep × 2 (+/++)	Bowel: Strep × 2 (++)
Gram neg rods × 2 (++)	Staph (++)
Diphtheroids (+++)	Bacillus (++)
DAY 2	DAY 2
Heart: E.coli, Enterococcus	Heart: E.coli, Enterococcus
Clostridium	Proteus, Clostridium
Right lung: Proteus (+++)	Right lung: Proteus (++)
Strep × 2 (++)	
Gram neg rods × 2	
Left lung: Proteus (+++)	Left lung: Proteus (++)
Staph (++)	Strep (++)
Spleen: Gram neg rods × 2 (++)	Spleen: Gram neg rods × 2 (++)
Bowel: Strep (++)	Bowel: Strep (++)
Diphtheroids (++)	Diphtheroids (++)
Gram neg rods (++)	Gram neg rods (++)
DAY 4	DAY 4
Abdomen: Strep (++), Staph (++)	Abdomen: Strep (++), Staph (++)
Gram neg rods × 2 (+/++)	Proteus (++)
Bacillus (++), Clostridium	Clostridium
DAY 6	DAY 6
Abdomen: Enterococcus (++++)	Abdomen: Enterococcus (++++)
Strep (+++)	Staph (+++)
Proteus (+++/++++)	Proteus (++)
Gram neg rods (++++)	Bacillus (++)
Clostridium × 2 (++++)	Clostridium × 2 (++++)

animals, a reproducible sequence of disarticulation can be reconstructed (Table 3). Although the *rates* of disarticulation were slower in fresh-killed animals than in frozen-thawed animals, the *sequence* in the two was the same, as determined by morphologic and biomechanical factors. The tempero-mandibular joint (not a "true joint") is the first to disarticulate in all animals. The atlanto-occipital joint and cervical vertebrae follow in close succession. The cervical vertebrae disarticulated at this early interval even in animals that had not been sacrificed by cervical dislocation. Thus, the mandible and skull would have the first opportunity to become mechanically separated from the less-identifiable postcranial portions of the skeleton. The limbs have a tendency to remain intact during the immediate postmortem period. This pattern is not inconsistent with the observed behavior of mammalian skeletal remains in archaeologic context. Mammalian mandibles and skulls are often found in disassociation from postcranial skeletal remains [*7,15,19,20,23*]. However, few definitive studies of natural mammalian disarticulation have been performed. Although sequences of natural disarticulation have been proposed by Hill [*22,23*], these studies are ambiguous in that the activity of decay organisms and intrinsic processes was not differentiated from that of predator-carnivores. It has been suggested that various biologic agents and physical agents may accelerate the process of disarticulation, but do not alter its sequence

TABLE 3—*Disarticulation sequences reconstructed from observations on all animals.*

Order of Joint Disarticulation	Time Intervals, days	
	Frozen-Thawed Animals	Fresh-Killed Animals
Tempero-mandibular	>2<4	>2<4
Atlanto-occipital	>2<4	>4<6
Cervical vertebrae	>2<4	>4<6
Symphysis pubis	>2<4	>4<6
Lumbar vertebrae	>4<6	>4<6
Sterno-costal	>4<6	>4<6
Costo-vertebral	>4<6	>6
Lumbo-sacral	>4<6	>6
Sacro-iliac	>4<6	>6
Pelvis-hip	>4<6	>6
Knee (ground contact)	>2<6	>6
Hindlimbs (other)	>6	>6
Forelimbs	>6	>6

[*24*]. Freezing-thawing appears to be one such physical agent which accelerates rates of disarticulation, but does not alter the sequence, compared to fresh-killed controls.

In general, the frozen-thawed animals were more susceptible to invasion by insects and microorganisms from the outside, and aerobic decay of the skin and external surfaces. The fresh-killed animals were less susceptible to external decay, but putrefaction (anaerobic decomposition) proceeded more rapidly from within. It appears that the freeze-thaw cycle diminished the capability of enteric organisms to grow and participate in postmortem putrefaction. The mechanical disruption of the tissues caused by freezing also weakens the skin, connective tissue, and joints, thus facilitating aerobic decay and skeletal disarticulation, and making internal organs more susceptible to invasion by foreign organisms and insects.

In summary, it appears that in the frozen-thawed animals, postmortem decomposition proceeds from the "outside-in" (predominantly decay); while in the fresh-killed animals, it proceeds from "inside-out" (predominantly putrefaction). In a subsequent study, Aufderheide[2] observed that exposure of mice carcasses to both 4 and 40°C subsequent to death caused a decrease in gross internal putrefaction rates. He commented that the susceptibility of enteric flora to reduced temperatures is well known, and that the equally well-known susceptibility of enteric flora to increased temperatures does not seem to have received the attention it deserves.

It is evident that previous studies on the various processes of postmortem change using frozen-thawed animals [*3,5*] may not have given an accurate picture of the decomposition sequences and timing that would occur in fresh-killed animals for purposes of determining the interval since death. Such studies should be repeated using freshly killed animals. These findings have implications for the interpretation of human remains from individuals who die with, or without, injuries during the frost season in temperate climates. These remains may "winter over" and subsequently be discovered in the spring or summer, following one or more freeze-thaw cycles, and after the onset of postthaw decompositional changes.

Acknowledgments

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[2]A. C. Aufderheide, personal communication, 23 Nov. 1981.

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