Arpad A. Vass,[1] *Ph.D., William M. Bass,*[2] *Ph.D., Jeffery D. Wolt,*[3] *Ph.D., John E. Foss,*[4] *Ph.D., and John T. Ammons,*[5] *Ph.D.*

Time Since Death Determinations of Human Cadavers Using Soil Solution

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ABSTRACT: This study was conducted to collect data on specific volatile fatty acids (produced from soft tissue decomposition) and various anions and cations (liberated from soft tissue and bone), deposited in soil solution underneath decomposing human cadavers as an aid in determining the "time since death." Seven nude subjects (two black males, a white female and four white males) were placed within a decay research facility at various times of the year and allowed to decompose naturally. Data were amassed every three days in the spring and summer, and weekly in the fall and winter. Analyses of the data reveal distinct patterns in the soil solution for volatile fatty acids during soft tissue decomposition and for specific anions and cations once skeletonized, when based on accumulated degree days. Decompositional rates were also obtained, providing valuable information for estimating the "maximum time since death." Melanin concentrations observed in soil solution during this study also yields information directed at discerning racial affinities. Application of these data can significantly enhance "time since death" determinations currently in use.

KEYWORDS: physical anthropology, time since death determination, volatile fatty acids, soil solution

Research and investigations into determining the time interval since death (TSD) have only recently begun to be properly addressed. A method that is reliable, can be applied worldwide under a variety of conditions and circumstances, and uses the one parameter that is *always* present when TSD needs to be determined—the corpse—must be determined.

Realistically there are only two major stages of decomposition, preskeletonization and postskeletonization, the latter being the most difficult to assess. Preskeletonization can be further subdivided into various stages as outlined by Reed [*1*] and even though many

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[1]Forensic anthropologist, University of Tennessee, Department of Anthropology, Knoxville, TN.

[2]Tennessee state forensic anthropologist, professor and head, Department of Anthropology, University of Tennessee, Knoxville, TN.

[3]Environmental Fate Laboratory, DowElanco, Midland, MI.

[4]Professor and head, Department of Plant and Soil Science, University of Tennessee, Knoxville, TN.

[5]Associate Professor, Department of Plant and Soil Science, University of Tennessee, Knoxville, TN.

factors can affect the onset of each stage (temperature, moisture, carnivores, trauma, etc.) reasonable determinations of TSD can be made solely by observation if one has the expertise and experience [*2*]. Several nonanthropologic studies have been undertaken that have not focused on the corpse itself and therefore can only be used in very limited and special circumstances [*3–7*], but since determining the TSD is one of the most difficult questions addressed by the forensic anthropologist, assistance from any discipline is invaluable.

Studies focusing on the corpse itself include studies such as the one by Perry et al. [*8*], who measured the constant decay rate of DNA in ribs. Although promising, this study needs to be expanded to field testing and suffers from complicated procedures that most laboratories cannot easily perform.

Forensic entomology is a growing discipline and has recently been used extensively in TSD estimations by Rodriquez and Bass [*9*], Rulshrestha and Chandra [*10*], and Keh [*11*], among others. These studies are based on patterns or successions of insects associated with the specific stages of decomposition and has been used with great success in certain instances.

Finally, Castellano et al., [*12*] performed an extensive study of bone lipid, cholesterol, and protein forming regression equations that can help estimate the date of bone remains.

Many factors affect the decompositional rate of a corpse, above or below ground. Above ground decomposition is typically faster than burials [*9*], because the corpse is exposed to carnivores, insects, rainfall, and varying temperatures [*13*]. Burials, in many forms, slow decomposition by decreasing gaseous diffusion, limiting both macro and micro-organisms and increasing the amount of carbon dioxide leading to anaerobic conditions [*14–17*].

Because bodies, not skeletons, are typically buried, soft-tissue decomposition must be addressed because the byproducts produced can affect the rate of decay. Decay begins with autolysis, primarily by intra and extracellular enzymes. Putrefaction follows autolysis and through microbial and enzymatic action tends to liquify tissue [*18–22*].

Diagenesis is a natural process that serves to alter the proportions of organic and inorganic components of bone exposed to environmental conditions. This is accomplished by the exchange of natural bone constituents, deposition in voids or defects and adsorption onto the bone surface [*23–32*] and can alter the composition of soils on which bones reside.

In forensics, perimortem weight estimations can be of value to the criminal investigator attempting to identify human remains, and to the forensic anthropologist attempting to understand the origin of stress fractures, bone remodeling due to excessive weight gain and TSD estimations based on decompositional products [*33,34*]. Weight estimations in TSD studies, based on decompositional by-products, is important because individual's weight will be proportional to the amount of by-products produced during decomposition. For this reason, weight estimations have gained considerable importance in this study and the development of weight standards have been undertaken that cover varying ranges of estimated weights in light of the lack of studies and knowledge in this area of forensic anthropology.

By far the most accurate method of determining perimortem weight is by the use of clothing found at the death scene. This procedure is outlined by Morse et al. [*4*] in the *Handbook of Forensic Archaeology and Anthropology* and uses belts, pants, blouses, bras, dresses, etc.

Decomposition of soft tissues not only involves internal organs, but also encompasses structures such as epidermal tissue, which typically is quite resistant to decomposition and which contains a pigment called melanin. This pigment is composed of mixtures of macromolecules formed from the copolymerization of various precursors of macromolecules, the primary one being 5,6-indolequinone [*35*]. Due to the complex nature of the

pigment's structure (numerous phenolic rings), it has a slow decompositional rate (as shown in this study) and can withstand temperatures up to 600° (F) without being decomposed [*35*] and therefore may be useful in determining racial affinities in cases where skeletal information is incomplete or conflicting.

As described by Corry [*36*], microbiology plays a major role in decomposition. According to Jacob [*37*], muscle tissue comprises 40 to 50% of the body's weight and it follows that microbially induced fermentative reactions that serve to decompose proteins are of great significance. These decompositional by-products are governed, in part, by the Strickland reaction, as summarized by Brock [*38*]. By means of oxidative and reductive processes this reaction can result in short chain carboxylic acids, called fatty acids, since they come primarily from fat and protein depots. These short chain fatty acids are water soluble and would leach from the corpse earlier in decomposition than relatively hydrophobic long chain hydrocarbons. Therefore, it should theoretically be possible to arrive at an accurate determination of TSD by monitoring microbially produced degradation products. When dealing with mummified or skeletal material one must rely on inorganic parameters that are not extensively modified by soil microbes and which can continuously leach out of bone over long periods of time.

Therefore the purpose of this investigation was to determine the types and amounts of microbially produced volatile fatty acids (VFAs) as well as anions and cations (sodium, chloride, ammonium, potassium, calcium, mangesium and sulfate), which could be detected in soil solution (the liquid phase between soil particles) underneath a decomposing corpse in an environmental setting as an aid in TSD determinations.

Materials and Methods

In this study, a total of seven, unembalmed, unautopsied cadavers were placed at the University of Tennessee's decay research facility at various times of the year. This facility is located in a secluded open-wooded area in Knoxville. The subjects were placed at the research facility within 60 h of their death. The subjects were stored in morgue coolers prior to the beginning of the decay study. Information concerning the race, age, weight, stature, time and cause of death was recorded for all individuals (Table 1). All articles of clothing were removed from the subjects and they were placed face down, arms positioned to the side. The soil was unprepared to simulate natural conditions. Leaves and rocks were removed from underneath the bodies only to allow ease of sampling. The subjects were not protected from the environment in any way. Carnivores were, however, restricted from the site by a chain-link fence that surrounds the research area. Site selection for the placement of the corpses was random with the only stipulation being that no other corpse at the decay facility had ever been within 10 meters of the site.

Data concerning climatic conditions, body decomposition and insect activity were recorded each sampling period. Maximum and minimum temperatures at the decay facility were recorded daily. Data concerning the decay of each cadaver were recorded using decompositional stages as described by Reed [*1*].

During the spring and summer months, the soil underneath the subjects was sampled every three days. Once the corpses became skeletonized, soil samples were taken weekly. The soil was sampled weekly during the fall and winter, due to the slower decay rate during the colder months.

As expected in a wooded area, the soil is highly organic. As body fluids flow into the soil, organic matter becomes bound and is difficult to separate from soil. Use of chemical extractants would result in partial dissolution of soil organic matter, which would tend to obscure VFA measurements. Therefore, the analytical technique used in this study focused on the soil solution (the liquid phase between soil particles) and provides a time-dependant measure of VFA intensity in the soil that resulted from decomposition. Soil

TABLE 1—*Comparison of test subjects.*

Subject	Sex	Race	Age (yrs)	Height (inches)	Weight (lbs)	Cause of Death	Date of Death
1	M	N	54	67.5	117	n.c.	08/18/88
2	F	C	59	63.0	98	cancer	11/28/88
3	M	C	51	72.0	138	n.c.	02/25/89
4	M	C	66	71.0	~195	n.c.	05/31/89
5	M	N	56	70.0	~175	n.c.	08/22/89
6	M	C	58	66.0	~130	n.c.	08/21/89
7	M	C	69	68.0	~150	n.c.	01/14/89

M = male, F = female, N = Negroid, C = Caucasian, n.c. = natural causes.

solution was obtained by centrifugation as described by Davies and Davies [*39*] of a 2:1 (water:soil) saturation extract.

The soil type for the sampling area has been classified as a fine, mixed, thermic Typic Paleudalf according to U.S. Soil Taxonomy (Soil Survey Staff) [*40*]. This soil type was sampled at a depth of 3 to 5 cm since this depth maintained the highest pH during the decomposition cycle.

Soil sampling was restricted to encompass only the region from the pelvis to the shoulders. This region under each corpse was divided into equal areas so that at a depth of 3 to 5 cm, each area yielded 10 grams of soil. Soil from three randomly selected areas was collected each sampling period from underneath each corpse. Different sampling areas were chosen each sampling period with no area being sampled more than once.

In order to obtain these samples, the corpses had to be tilted sideways for a brief time. A flat spatula and a soil corer were used to obtain the samples. As soon as the samples were removed, the subjects were placed back on the soil in their original positions. Soil controls were taken each sampling session from unaffected areas as close to the subjects as possible. Control samples were generally taken above seepage zones and the pH was measured to ensure that they were not contaminated by insect migrations or by permeation since the soil pH indicates the presence of decompositional by-products.

The effect of moisture was minimized by determining the gram dry weight (gdw) of the soil rather than by using wet weights. Separate soil samples were collected each sampling session to determine the amount of moisture present in the soil at the time of collection. The samples were weighed and placed in preweighed glass scintillation vials, frozen and then lyophilized for 24 h. After the water was completely removed, the vials were again weighed using a Sartorius balance (Baxter, Model PT600), and the dry weights recorded.

All samples were processed directly in the field. Each soil sample was filtered through a #8 stainless steel sieve (Baxter, Stone Mountain, GA) to remove insects and plant material. The filtered soil samples were weighed using a battery operated Sartorius balance, to obtain uniformity. Once weighed, the samples were placed in 50 mL polypropylene centrifuge tubes (Baxter) and nanopure, deionized water (>18 mohm) was added to form a ratio of 2:1 (water:soil) to extract the soil solution. The samples were placed on ice and returned to the laboratory for further processing.

All samples were vortexed for 1 min before centrifugation at 10 000 X g for 40 min using a Sorvall RC2-B Ultracentrifuge (Newport, Connecticut) with the temperature maintained at 5°C. After centrifugation the soil solution samples were filtered through 0.2 μm Acrodisc low protein binding filters (Gelman Sciences, Inc.) to remove all microorganisms. These filtered soil solution samples were then frozen at −50°C and stored for later analysis.

Statistical analyses of the data, using the SAS statistical package [*41*], included analysis

of variance and analysis of covariance with multiple comparisons to test for significant differences.

Volatile Fatty Acids

A 500 μL aliquot of filtered sample was vortexed for 15 s and measured for pH, using an American H3701-3 pH meter with a gel-filled epoxy body combination pH probe (Baxter). VFAs were analyzed as described by Baumgardt [*42*], by acidifying 0.2 mL of sample with 2.0 μL of 10% formic acid and injecting each sample on a Shimadzu 9A Gas Chromatograph equipped with a Flame Ionization Detector (FID). The carrier gas was Nitrogen. The packed column consisted of Chromosorb-W and was maintained at 120°C. The injector temperature was 160°C. Peak heights were analyzed using a Nelson 3000 Series GC interface, Model 2600 (Nelson Analytical, Inc.). VFA standards were purchased from Supelco, Inc. (Bellefonte, PA).

Anions/Cations

Water extracts from soil ("soil solution") were used to measure the "intensity" of inorganic ions in soil at any time of sampling. This measure is more indicative of the process of decomposition than would be a chemical extraction procedure which measures soil ion "capacity" and which would be more indicative of soil-related rather than cadaver-related properties.

Anions (Cl^-, HCO_3^-, Br^-, F^-, NO_2^-, NO_3^-, HPO_4^{2-}, SO_4^{2-}) were analyzed on a Waters 431 conductivity detector equipped with a Waters WISP 710B autosampler, a Waters Model 510 HPLC pump with noise suppression and a Waters Anion IC-Pak A column with an anion guard column (Waters, Milford, MS). Cations (Li^+, Na^+, NH_4^+, K^+, Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}) were analyzed similarly, but used a Waters Cation IC-Pak C column with a cation guard column.

Filtered soil solution was placed through a Hamilton Chromatography Preparatory C18 cartridge and 200 μL was placed in the Waters Autosampler. To prepare 1L of eluent for anion analysis, 20 mL of buffer concentrate, (851.5 mL distilled deionized water, 23.5 mL gluconic acid solution (50% wt/wt), 8.6 g lithium hydroxide, 34 g Boric acid, 250 mL glycerin) is added to 20 mL n-butanol, 120 mL acetonitrile and 840 mL distilled deionized water. This eluent was then filtered through a Magna, Nylon 66 filter (Micronsep, Honeoye Falls, NY). A 50 μL sample was injected with a flow rate of 1.2 mL/min. Peak heights and areas were measured using a Nelson 3000 Series GC interface, Model 2600 (Nelson Analytical, Inc.). Anion standards were prepared in the laboratory.

Monovalent cations (Li^+, Na^+, NH_4^+, K^+) required an eluent composed of 2 mM HNO_3 and 0.05 mM EDTA per L of distilled, deionized (>18 mohm) water. Divalent cations (Mg^{2+}, Ca^{2+}, Sr^{2+}, Ba^{2+}) required an eluent composed of 35 μl anhydrous ethylenediamine (EDA) per liter of distilled, deionized (>18 mohm) water, adjusted to pH 5.8 to 6.2 with HNO_3 (nitric acid) and/or 0.1 N NaOH. All cation eluents were degassed and filtered with 0.2 μm Anapore Membrane Discs (Alltech Associates, Inc., Deerfield, Ill.). A 50 μL sample was injected at a flow rate of 1.2 mL/min. Peak heights and areas were measured using a Nelson 3000 Series GC interface, Model 2600 (Nelson Analytical, Inc.). Cation standards were also prepared in the laboratory.

Melanin

Melanin was neither extracted nor detected in the typical fashion [*43*]. A 1 mL sample was placed in a quartz cuvette (1 cm lightpath) and the contents scanned at 240 nm to 800 nm using a Shimadzu 2000 Scanning UV-Visible Spectrometer (Shimadzu Corp.)

Maximum absorbance, usually detected at 280 nm, was quantitated using an internal integrator and the concentration of melanin in mg/L soil solution was calculated.

Confirmation that the peak integrated was actually melanin (since the peak tended to shift slightly depending on the soil type and race of the individual) was accomplished using Thin Layer Chromatography. The mobile phase consisted of 60 mL n-butanol, 25 mL water and 15 mL acetic acid. The solid phase consisted of cellulose 100 plates prepared in the laboratory. Standards were purchased from Sigma Chemical. Samples were run simultaneously along with the controls to determine their Rf values and to confirm their identity. Rf values for tyrosine, DOPA and Melanin were respectively, 0.61, 0.38, and 0.00.

Soil Characterization

A determination of the soil profile of the University of Tennessee's decay facility was conducted to establish baseline data for this study and to allow comparisons between this soil type and those found worldwide.

The site was sampled with a Giddings Hydraulic Probe with a three inch tube to a depth of 232 cm. Short cores obtained from a hand auger were used to select an uncontaminated, representative site in a wooded area of the decay facility. The soil formed in colluvium over old alluvium. Slope on the site ranged from 9 to 13% with a heavy forest canopy.

Laboratory analysis included both chemical and physical characterization. Chemical analyses performed were pH, cation exchange capacity (CEC), exchangeable bases, percent base saturation and free iron oxides. Physical analysis included percent sand, silt and clay as well as sand fractionation. The pipette method was used for particle-size analysis [*40*].

Results

The initial results of this study demonstrated that two variables became important determinants of the concentration of VFAs in soil solution. These are the amount of moisture already present in the soil and the weight of the body prior to decomposition.

The second variable, pre-death weight, was standardized through the use of a weight correction, since every individual has a different ratio of fat and muscle tissue, producing different concentrations of VFAs. This standard is based on the average adult weighing 150 lbs. and was calculated to allow for varying levels of precision in the use of this factor (Table 2).

TABLE 2—*Weight standards based on increments of 50, 100, and 150 lbs.*

Range	Increment (lbs)	Standard
50	0– 49	0.1667
	50– 99	0.5000
	100–149	0.8333
	150–199	1.1677
	200–249	1.5000
	250–300	1.8333
100	0– 99	0.3333
	100–199	1.0000
	200–299	1.6667
150	0–149	0.5000
	150–299	1.5000

Figure 1 shows the averaged VFA data for all seven subjects in millimolar (mmol) concentrations of VFAs. Each VFA is expressed as undiluted mmol concentrations of VFA per g dry weight of soil per weight standard values, which are based on the pre-death weights of the subjects. Once moisture and an individual's weight are taken into account, the VFA concentrations, regardless of the subject or season in which the subject began to decompose, are the same for any given total of accumulated degree days (ADD). Control values for the VFA examined in this study were insignificant and never exceeded 0.2 mmol/gdw soil. ADD, as described by Edwards et al. [*45*], are determined by taking the sum of the average daily temperatures (C) for however long the corpse has been decomposing. For Subject 1, it may require 4 days (assuming an average daily temperature of 25°C) to attain an ADD score of 100, while Subject 2, decomposing in the winter months, may also have an ADD of 100, but which would require 20 days (assuming a daily average temperature of 5°C) to attain the same decompositional status and hence the same concentration of VFAs in soil solution.

Therefore, it is possible to estimate the TSD for any individual, given these specific VFA ratios, a gross description of the corpse and National Weather Service data concerning the environmental temperature where the corpse was found.

The ratios of VFAs hold true for any season and any amount of precipitation yet studied. Areas subjected to constant flooding or extreme moisture may require sampling from greater depths or may be unusable. This procedure could also be unreliable for estimating TSD intervals when dealing with mummified or burned remains.

Decompositional rates were based on the number of ADD required for VFA production to fall below detectable limits (Fig. 2). This coincided with either complete skeletonization or mummification of any remaining soft tissue.

Decomposition occurs in four stages as described by Reed [*1*] in 1958. These include: fresh, bloating, decay and dry. Tables 3*a* and 3*b* compare the seven subjects in this study and their relationship between these four stages. Decompositional stages were not always apparent since the subjects were exposed to widely different temperatures, and therefore were, by themselves, a poor indicator of the decompositional process.

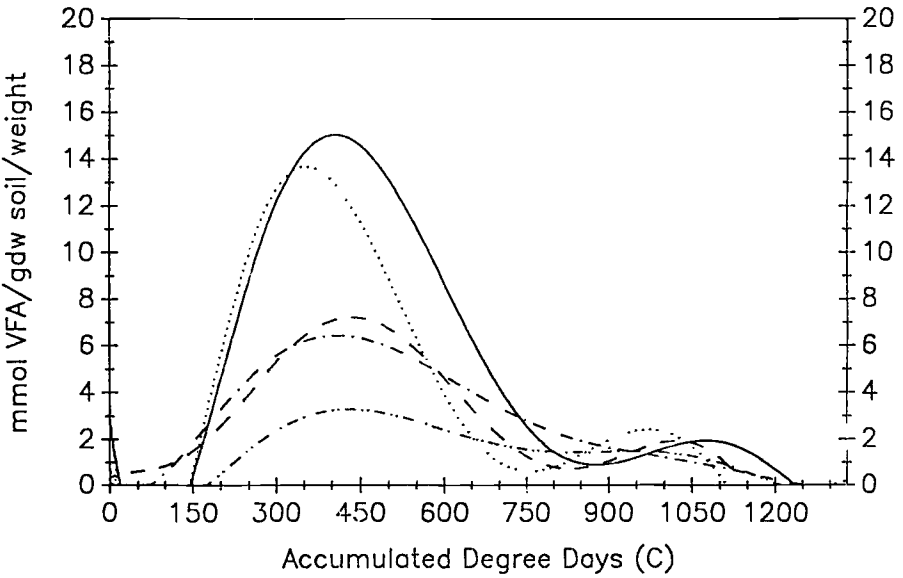

FIG. 1—*Ratios of volatile fatty acids, over time, in soil solution underneath a decomposing corpse. Propionic (——), iso-butyric (— — —), n-butyric (····), iso-valeric (— · —), n-valeric (·· — ··). Results are best fit regression models.*

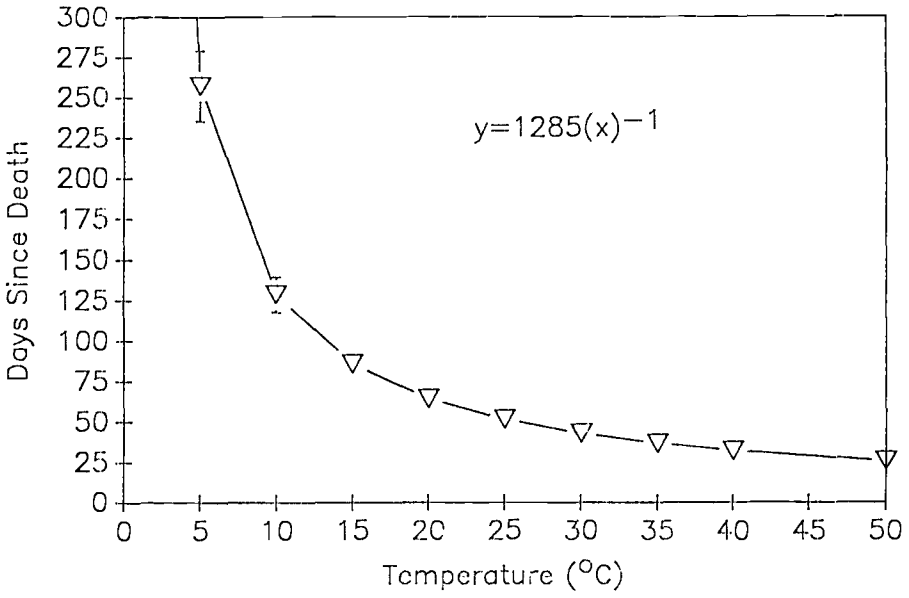

FIG. 2—*Representation of maximum time since death estimates based on the presence of volatile fatty acids.*

TABLE 3*a*—*Appearance of decompositional stages in test subjects.*

Subject	Day	Stage	Observations
1	3	1	Skin slippage apparent, slight insect activity
1	6	2	Heavy drainage from the head region, heavy maggot infestation
1	9	3	Abdominal drainage, beetles present
1	12		Maggots massing, heavy drainage, muscle collapse
1	15		Thick mucoid drainage from abdomen, maggots migrating
1	18		Beetles predominate, drainage complete
1	21	4	Partially skeletonized, heavy mold
1	27		Remaining skin becoming leathery
2	42	1	Skin slippage, rare insects
2	—	2	Not observed
2	98	3	Heavy abdominal drainage, slt insect activity, beetles present
2	>170	4	Remaining skin becoming leathery
3	14	1	Skin slippage, few insects present
3	28	2	Slight bloating, moderate drainage from abdomen
3	70	3	Muscle collapse, head skeletonized, moderate insect activity
3	91	4	Remaining skin becoming mummified, adipocere present

Stage 1—Fresh, Stage 2—Bloat, Stage 3—Decay, Stage 4—Dry:
Subject 1—(summer), Subject 2—(fall/winter), Subject 3—(winter/spring).

TABLE 3*b*—*Appearance of decompositional stages in test subjects.*

Subject	Day	Stage	Observations
4	7	1	Skin slippage apparent, slight insect activity
4	12	2	Heavy drainage from orifices, moderate bloating, insects present
4	19	3	Heavy abdominal drainage, maggots and beetles present
4	23		Muscle collapse, extremities beginning to skeletonize
4	38		Partially skeletonized, adipocere forming
4	50	4	Remaining skin becoming mummified
5,6	3–4	1	Skin slippage, rare insects
5,6	7–10	2	Heavy drainage from head regions, maggots predominate
5,6	15–22	3	Heavy abdominal drainage, maggot migrations, beetles present, muscle collapse
5,6	25–42	4	Partially skeletonized, remaining skin becoming leathery
7	35	1	Skin slippage, rare insects present
7	—	2	Not observed
7	>70	3	Not observed

Stage 1—Fresh, Stage 2—Bloat, Stage 3—Decay, Stage 4—Dry:
Subject 4—(spring), Subjects 5,6—(summer), Subject 7—(fall/winter).

Of the 16 ions investigated in this study, only seven (Na^+, Cl^-, NH_4^+, K^+, Ca^{2+}, Mg^{2+}, and SO_4^{2-}) proved useful due to their stability in the environment, their reproducibility from subject to subject and in the amount detectable using this procedure.

Figure 3 shows the relevant anion/cation data for all seven subjects and are expressed as undiluted part per million (ppm) concentrations of ions per gram dry weight of soil

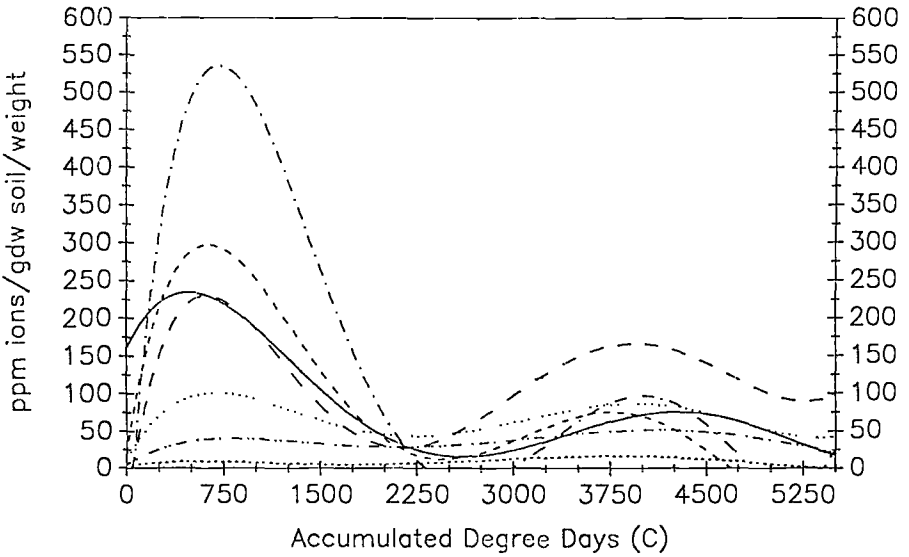

FIG. 3—*Ratios of anions/cations, over time, in soil solution underneath a decomposing corpse. chloride (——), sulfate (— —), sodium (····), ammonium (— · — ·), potassium (— — —), calcium (— ·· —), magnesium (— — — —). Results are best fit regression models.*

per weight standard values that are based on the perimortem weights of the subjects. Once moisture and an individual's weight are taken into account, the anion/cation concentrations in ppm or (mg/L), regardless of the subject or season in which the subject began to decompose, are the same for any given total of ADD. Control values for the relevant ions examined in this study never exceeded 22 ppm/gdw soil.

Discussion

Volatile Fatty Acids (VFAs) include a large assortment of organic compounds as outlined by Morrison and Boyd [*44*]. Of these 41 compounds only formic, acetic, propionic, butyric, valeric, caproic, and heptanoic acids are readily detectable in soil solution because they are soluble in water. Formic and acetic acids are both abundant in nature and proved much too variable to be used successfully in this study. Caproic and heptanoic acids, although always present in small amounts in soil solution during decomposition, are only found in significant amounts during the colder months when the temperature drops below 10°C. Of the remaining VFAs present during decomposition, propionic, butyric and valeric acids are formed and deposited in soil solution in specific ratios. These are formed and released from a decomposing corpse in a temperature dependent pattern, and can be used in TSD determinations.

VFAs are primarily breakdown products of both muscle and fat, which every human possesses in various concentrations. Muscle, composed of protein, which in turn is composed of amino acids, readily yields to the formation of VFAs through bacterial action. This process is temperature dependent and because decomposition involves both aerobic and anaerobic bacteria, VFAs can be formed by both processes [*38*]. Butyric acid and propionic acid are formed by anaerobic bacteria, primarily in the gut, which produce, in part, a majority of the gases seen in the bloating stage of decomposition.

There appears to be a direct correlation between the decompositional stages and VFA production. This is due in part to the sequential decomposition of proteins and carbohydrates. Very little change in VFAs are associated with Stage 1 of decomposition. Bloating (Stage 2), the result of anaerobic fermentation primarily in the gut, causes skin breakage and leakage of fermentation by-products rich in butyric acids. Active decay (Stage 3) causes a surge in aerobic as well as anaerobic bacterial by-products that rapidly disappear by the onset of Stage 4. Values for all volatile fatty acids are highest just after maggot migrations for pupation (~400 ADD) because the presence of these insects tends to restrict the flow of body fluids into the soil. The ratios of VFAs, in addition to visual inspection of a corpse, can be used to ascertain which decompositional stage exists, thereby determining which area of the figure to use (Fig. 1). This may be definitive in terms of pinpointing the TSD of an individual. This is especially valuable when it is noted that rainfall does not appear to affect these findings. The soil underneath a corpse is protected from precipitation and the heavy, mucoidlike secretions produced from anaerobic fermentation seem to bind the soil together making dilutional factors from rainfall insignificant.

If the individual's identity is known, the actual weight of the individual, divided by 150, can be used as the standard. Weight can, at times, be very difficult to estimate, especially if the corpse is severely decomposed or skeletonized. If the individual's actual weight is unknown a weight standard can be used, which is based on increments of 50, 100 or 150 lbs (Table 2). If, for example, a corpse is found and its weight is estimated between 100 to 200 lbs, a weight standard of 1.000 is used in the TSD calculations. The closer one can judge the individual's weight the narrower the TSD range will be. The size of clothing found on the corpse as summarized by Morse et al. [*4*], and the robustness of the skeletal material can at least indicate the frame size of the individual and aid in estimating weight.

When dealing with soil types other than a fine, mixed, thermic Typic Paleudalf, and in order to limit variability imposed by sampling among diverse soil types, the optimum sampling depth is determined as that region with the highest pH. Intact cores can be returned to the laboratory on ice until this determination is completed. Cores, if collected, should be at least six inches in depth. Although this study was restricted to East Tennessee, a temperate region of the United States, possessing primarily broadleaf forests and averaging 40 to 60 inches (100 to 150 cm) of rainfall per year, soils with different compositions in various regions of the United States have been examined and do not appear to appreciably affect the results.

Temperature is by far the most important environmental factor affecting decomposition. This parameter not only affects the breakdown of proteins and carbohydrates, but also affects the insects and bacteria in a similar fashion. When the temperature decreases, so does the rate at which the insects consume the corpse, the rate at which the bacteria break down protein into fatty acids and the way the fatty acids themselves are used. Fewer insects digesting less body material results in a greater percentage of protein and carbohydrates turning into VFAs by bacterial action and more VFAs ending up in the soil solution. Subjects 1, 5, and 6 were exposed to relatively constant temperatures averaging 21.5°C, with minimal rainfall. Subject 2 was subjected to wildly fluctuating temperatures with precipitations ranging from 4 inches of snowfall to near drought conditions. Subjects 3, 4, and 7 were subjected to environmental conditions between the two extremes. While the causes of decomposition are similar in all subjects, the rates were markedly different.

Temperature data supplied by the National Weather Service, when used to determine ADD values, can be adjusted to compensate for differences near the corpse at a crime scene. This is accomplished by taking average temperature measurements at the site for at least a week. These values are compared to those obtained from the National Weather Service for the same time period. The daily differences are then averaged to obtain an adjustment value. National Weather Service temperatures, when used, are corrected prior to establishing the number of days required to attain the ADD value obtained in the VFA procedure. The number of days obtained by this process is the TSD. The range of VFAs will determine the range for the TSD estimation.

If the average temperature drops below 4°C it becomes much more difficult to estimate the time since death based on a single time point. The presence of increased mmol concentrations of longer chained VFAs (that is, caproic, heptanoic) will indicate to the investigator that the corpse has been exposed to cold temperatures as well as decreased insect and bacterial activity. In this case, a very detailed knowledge of past temperature conditions will be crucial in making any assessment as to the time since death. Because of the increased salt concentrations in the human body, decomposition still occurs when the temperature falls to 0°C. For this reason any ADDs below zero degrees Centigrade are counted as zero and not as a negative number in order to avoid subtracting days from the length of time since death estimate.

The finding that the liberation of VFAs cease at 1285 ± 110 Accumulated Degree Days (Fig. 1), also allows one to calculate a decompositional rate. This is expressed in Fig. 2 and presents investigators with a 'rule of thumb.' When a corpse is discovered, and soft tissue is still present, the investigator can divide 1285 (the ADD at which skeletonization occurs—that is, VFA liberation ceases) by the average temperature (C) on the day which the corpse was found and arrive at a maximum TSD. The closer the corpse is to being completely skeletonized, the more likely that the estimated maximum TSD approximates the actual TSD. Although crude, the investigators will at least have an idea of the maximum TSD and can begin their investigation at that point.

Soil pH is also an important parameter to consider when undertaking time since death determinations since this can affect bone solubility as well as the solubility of compounds

such as melanin. Figure 4 depicts the changes in soil solution over time associated with the decomposition process. These changes can be linked to decompositional stages as well as the production and detection of VFAs, since these acids only become volatile at low pHs. The high pH (> 7.0) associated with the early and intermediate stages of decomposition allow for the preservation and hence the detection of VFAs in soil solution. Control values for the decay facility ranged from pH 5.7 to 6.5 at a maximum depth of 6 cm. VFAs become volatile at pHs less than 7.0 and could have resulted in the lack of detectable VFAs in the initial 150 ADD.

The human body contains many different elements [*46*], some of which are found in higher concentrations in specific regions of the body. Electrolytes are the first to leach out of soft tissue and saturate the soil. These electrolytes will initially be in the soil aqueous phase (soil solution), but with time will be largely adsorbed to the solid matrix of soil particles or incorporated into microbial biomass. This happens rapidly and the remaining ions that subsequently inundate the soil are sequestered in the organic matrix formed from the decomposition of proteins and carbohydrates. Bacteria can use a number of the ions in their metabolism, and it quickly becomes apparent that HPO_4^{2-}, HCO_3^-, and NO_3^- are rapidly scavenged by the bacteria. The release of ions from a corpse does not follow the typical stage sequence usually associated with decomposition as described by Reed [*1*]. As seen in Fig. 3, there is a large release of elements quite rapidly, followed by an equally rapid decline when soft tissue is completely decomposed by 1285 ADD (Fig. 1). This decline could, in part, be due to bacteria utilizing alternative metabolic pathways (such as nitrate reduction) when their carbon/nitrogen sources have been depleted. Once fermentation of soft tissue ceases, there is an acidic pH shift in the soil in the vicinity of the decomposing body and new types of bacteria predominate.

The rise of anions and cations after 2250 ADD has not been fully explained, but most likely has its origin in the death of large populations of bacteria leaving behind their constituents as well as in bone decay releasing additional leachates into the soil. Both

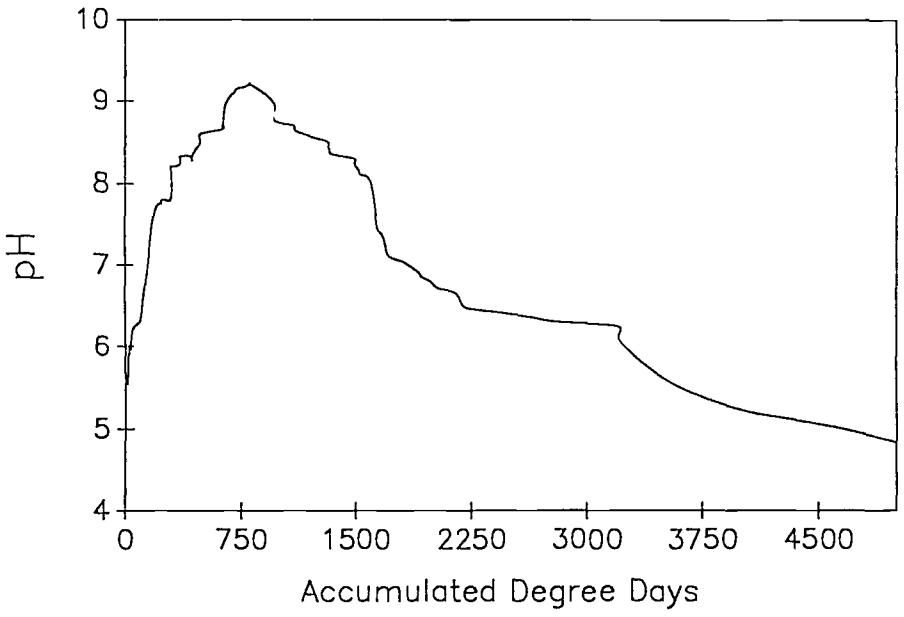

FIG. 4—*pH changes over time in soil solution. Results represent mean values for all seven subjects in this study.*

calcium and magnesium are found in higher concentrations after the 2250 demarcation and indicate bone seepage, although no difference in the bone is visually apparent. With the exception of sulfate, nearly all the anions and cations investigated in this study have almost reached baseline levels after 5250 ADD. Depending on the subjects, this approximates 1.5 to 2 years of decomposition. Preliminary studies have shown that sulfate is still present in large amounts after 4 years and that calcium continues to exhibit a cyclic release from skeletal material and is found in considerable amounts. While elemental analysis of soil solution at greater than two years is still preliminary, it is possible that deeper cores from underneath the corpse, using a 1:1 (water:soil) extraction procedure, or analyzing for different ions could prove useful in determining the time since death in these instances.

When a skeletonized corpse is found, great care must be taken to note any changes in the bone such as bleaching and exfoliation. Special care must be exercised when dealing with skeletonized or mummified corpses to be certain that close attention is given to ratios between the various ions. This is very important in determining whether the corpse has been decomposing greater than 2250 ADDs and which area of the curve to use (Figs. 1 and 3). This may be definitive in terms of pinpointing the time since death of an individual.

Samples obtained in this study were not chemically treated as is typically done when attempting to isolate melanins. This, in part, led to the observations seen in Fig. 5. Melanin becomes soluble in water at high pHs and this is seen typically only in the early stages of decomposition when the pH can range over 9.0 in soil solution (Fig. 4). Additional base was not added to the samples that would have solubilized additional melanin. These lower pH ranges may have resulted in the great degree of similarity between the early and late areas of the curves for both blacks and whites. The primary advantage of this methodology (that is, not adding additional base) is the elimination of contaminating humic acids and tannins, found in soil solution which can, in part, obscure these results. This procedure also highlights the difference between black and white melanin concentrations in the 500 to 1400 ADD range, which corresponds to Stages 2 to 4 of decom-

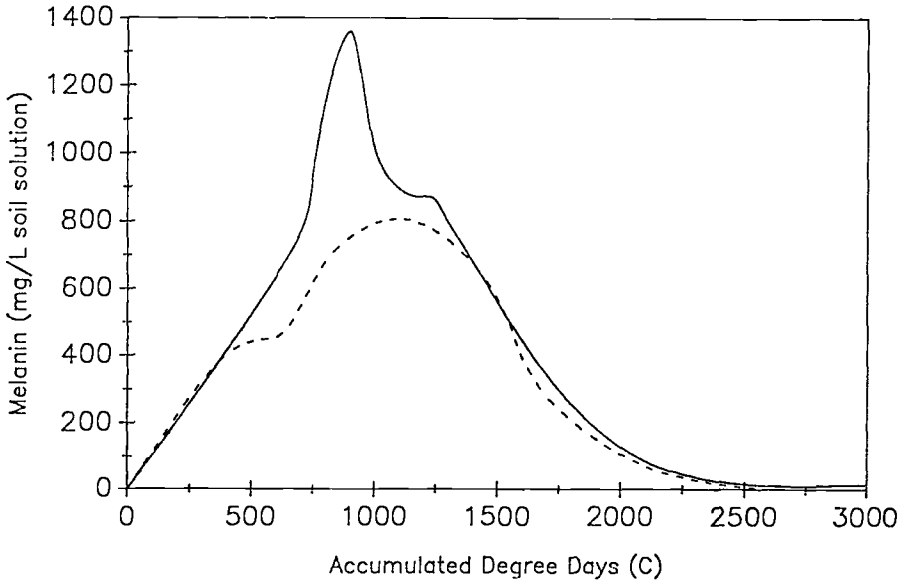

FIG. 5—*Accumulation of melanin in soil solution over time for five Caucasian (— —) and two Negroid (——) corpses. Results represent mean values for all subjects.*

position. While the sample size in this study for melanin research is quite small, application of this procedure to three forensic cases where the victim had been subsequently identified, and when the TSD was determined to be between 650 and 1375 ADD, has proven to accurately determine the correct racial affinity.

It is hoped that the preliminary work carried out in this investigation will lead to the development of more accurate and reliable techniques for the detection of melanin in soil solution, clothing or adhering to other macromolecules.

Summary

This study has shown that VFAs extracted from the water phase between soil particles (soil solution) under a corpse can yield valuable information regarding time since death. Visual inspection of the corpse alone is usually not sufficient to accurately pinpoint the TSD, especially when the corpse is skeletonized or mummified. Trends between specific VFAs were apparent when following Stages 1 to 4 of decomposition. Insect activity, anaerobic fermentation of soft tissues, and the proteolytic activity of microbes can also be followed accurately using VFA analysis.

It has been shown that a corpse, exposed to conditions outlined in this study, will produce the same ratios of propionic, butyric, and valeric acids for any given ADD. The range between these acids can determine the range by which an estimate of the TSD can be established. Since it has been established that it takes 1285 ± 110 ADD for a corpse to become skeletonized (or for VFA production to cease), a decompositional rate can be established that would enable the investigator to rapidly arrive at a maximum TSD estimate while at the death scene.

This study has also shown that the ions (Na^+, Cl^-, NH_4^+, K^+, Ca^{2+}, Mg^{2+}, and SO_4^{2-}) extracted from the water phase between soil particles under a corpse can yield valuable information regarding TSD of skeletonized human remains.

It has also been shown that a corpse will produce the same ratios of the ions (Na^+, Cl^-, NH_4^+, K^+, Ca^{2+}, Mg^{2+}, and SO_4^{2-}) for any given ADD. Determining the undiluted concentrations of these ions (ppm) per g dry weight of soil and adjusting these values for the known, or estimated, weight of the individual, will result in a range of ADD for which an estimate of the TSD can be established.

Further analyses of soil solutions are also beginning to answer questions concerning the individual's weight and race. VFA concentrations also yield information on the perimortem weight of the individual if soft tissue is still present. Since fat decomposes into known patterns (chain lengths) of fatty acids, it may be possible to quantitate these fatty acids and compare these values to amounts of protein present to (at the very least) determine if someone was extremely heavy or very muscular. Melanin concentrations obtained from soil solution can be used to determine racial affinity in some instances. Anions and cations may also be extractable from clothing and it is possible that analysis of bodily remains in an urban environment will yield similar results to those obtained in this study.

The presence of large concentrations of water insoluble, long chain fatty acids indicate decomposition in a cold environment and can be useful in determining whether a corpse had been recently moved from a cold to a warm environment.

It is hoped that this research will initiate a greater reliance and awareness of the value of collecting soil from underneath a corpse that can be used in TSD, weight and racial determinations.

Applications

CASE #1—The corpse of an adult male was found lying in a field. The average temperature for the day was 18°C. Visual inspection of the corpse indicated that he was

in late Stage 3 (Active decay) of decomposition. Maggot migrations had apparently already taken place. All indications pointed to the fact that he had decomposed where he was found and had not been moved. Soil samples to a depth of 6 inches were taken from under the corpse with 3 in. diameter aluminum tubes. The ends of the tubes were covered with aluminum foil, labeled as to which side was up, and placed in an ice chest making sure that they stayed dry. Once in the laboratory the cores were opened and using pH paper the most basic region of the core was located. This region (6 to 8 cm in depth) was then isolated and 10 to 20 g was used for VFA determinations.

Using a 2:1 (water:soil) extract yielded 0.58 mmol of n-valeric acid, 0.31 mmol of iso-butyric acid, 1.54 mmol of propionic acid, 0.92 mmol of iso-valeric and 0.3 mmol of n-butyric acid. These concentrations were then adjusted to accommodate any dilutions, soil moisture and the weight of the corpse which, based on skeletal robustness, was estimated to be between 100 and 150 lbs.

Example: N-butyric

Weight standard (Table 2) : 0.8333
Gram dry weight of 10 g of soil : 7.38 g
Dilutional Factors : 20 mL of water added to 10 g of soil

$$\frac{(0.3 \text{ mmol/mL})\ (20 \text{ mL}/10 \text{ g soil})}{(7.38 \text{ gdw soil}/10 \text{ g soil})} / 0.8333 = 0.98$$

0.98 mmol n-butyric/gdw/weight std, correlates to approximately 700 ADDs (Fig. 1). This was performed for each VFA with a resulting ADD range of 675 to 775. Since propionic acid was still high, any ADD's greater than 800 could be ruled out. Determining the number of days required to obtain 675 to 775 ADDs resulted in a time since death estimate of 41 to 48 days. Maximum time since death (MTSD) estimates (Fig. 2) predicted the MTSD to be 1285/18 = 71 days.

Although the exact time since death is not yet known, he was subsequently identified and seen alive 52 days prior to his corpse being found.

CASE #2—The corpse of a skeletonized adolescent was found lying in a dry ditch. Visual inspection of the corpse indicated that he was in Stage 4 (Dry) of decomposition. All indications pointed to the fact that he had decomposed where he was found and had not been moved. His right leg had been dragged off by a carnivore. Soil samples to a depth of 8 inches were taken from under the corpse with a soil corer. Once in the laboratory the cores were opened and using pH paper the most basic region of the core was located. This region (11 to 12.5 cm in depth) was then isolated and 10 to 20 g was used for anion/cation determinations.

Using a 2:1 (water:soil) extract yielded 27.9 ppm of sulfate, 1.4 ppm chloride, 10.2 ppm sodium, 6.9 ppm potassium, 7.1 ppm calcium. Ammonium and magnesium were below detectable limits. These concentrations were then adjusted to accommodate any dilutions, soil moisture and the pre-death weight of the corpse which, based on his weight at the time of his disappearance (since he had been identified), was 96 lbs.

Example: Sulfate

Weight Standard (Table 2) : 96 lbs/150 lbs = 0.64
Gram dry weight of 10 g of soil : 8.64 grams
Dilutional Factors : 20 mL of water added to 10 g of soil

$$\frac{(27.9 \text{ ppm})\ (20 \text{ mL}/10 \text{ g soil})}{(8.64 \text{ gdw soil}/10 \text{ g soil})} / 0.64 = 101$$

101 ppm sulfate/gdw/weight std correlates to approximately 3000 ADD (Fig. 3). This was performed for each ion with a resulting ADD range of 2250 to 3000. Since all other ions

were low, any ADDs less than 2000 or greater than 3750 could be ruled out. A lack of appreciable amounts of magnesium present ruled out ADDs greater than 3250. Determining the number of days required to obtain 2250 to 3000 ADDs resulted in a time since death estimate of 168 to 183 days.

This time frame was within two weeks of when the individual was reported missing.

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Address requests for reprints or additional information to
Arpad A. Vass, Ph.D.
Oak Ridge National Laboratory
Y-12 Bldg. 9207 Room 523
MS 8077
Oak Ridge, TN 37831