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PAPER

ANTHROPOLOGY; PATHOLOGY AND BIOLOGY; GENERAL

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Preliminary Studies into the Characterization of Chemical Markers of Decomposition for Geoforensics*

ABSTRACT: In this paper, we report the results of our preliminary studies into chemical characterization of the fluids produced during decomposition in the absence of a soil matrix. Pig (*Sus domestica*) carcasses were used to model the human decomposition process in two separate locations, Western Australia (Perth) and Canada (Oshawa). Analysis involved simple dilution and filtration of the decomposition fluids followed by gas chromatography–mass spectrometry. Several previously unreported compounds were detected in the decomposition fluid samples during the trials, including benzeneacetic acid, benzenepropionic acid, 2-piperidone, and isocaproic acid. Possible biosynthetic pathways for some of the compounds produced are proposed. Further research trials are required, particularly in the presence of soil matrices.

KEYWORDS: forensic science, geoforensics, decomposition, fatty acids, postmortem interval, gas chromatography–mass spectrometry

A reliable estimation of time since death (TSD), also known as postmortem interval (PMI), is vitally important in forensic investigations to establish the whereabouts and last movements of victims, potentially extending to include victim identification. It is also essential in allowing the possible inclusion or elimination of prospective suspects in a criminal investigation and can be used to corroborate witness testimony (1).

Current methods to estimate PMI include the extent of mortis (2,3), DNA degradation (4), and potassium levels in vitreous humor (5,6). These are usually limited to the early stages of decomposition. They are often subjective, relying primarily on the level of expertise and experience of the examiner. As decomposition progresses into the advanced stages, the use of these and other methods significantly increases the uncertainty and error in estimating PMI (2,3).

Vass et al. introduced a method based on analysis within a soil matrix of the fluids released during decomposition (7). It was suggested that certain short chain volatile fatty acids (VFAs) acted as time dependent biomarkers and could be utilized as an alternative method in estimating TSD. The potential advantages of this method were the application to both early and latter stages of decomposition and its objectivity compared to other techniques (2,3). To date, this approach has only been studied in detail in Tennessee, although there is a report of a pilot study in Queensland, Australia (8). However, no results for this study have been published, and are unlikely to be in the future.

Volatile fatty acids comprise a variety of low molecular weight carboxylic acids, primarily C_2 to C_7 (9). These short chain fatty acids are produced by the aerobic degradation of soft tissues, are water-soluble, and leach from the corpse earlier in decomposition than long chain hydrophobic hydrocarbons (7). Current knowledge of the exact nature of the compounds produced is limited. To gain a better understanding of the chemical components of decomposition fluid, experiments were conducted in the absence of a soil matrix using pork rashers (belly pork) and whole stillborn piglet carcasses in Western Australia and whole adult pig carcasses in southern Ontario, Canada.

Because of the ethical issues involved in the use of human cadavers, pig (*Sus domestica*) carcasses have been used to model the human decomposition process (10). They are considered to be an acceptable substitute because of their similarity to human torsos in weight, fat to muscle ratio, hair coverage, biochemistry, and physiology (11,12).

In this paper, we report the results of our preliminary studies into chemical characterization of the fluids produced during decomposition in the absence of a soil matrix. We have previously reported a procedure for the determination of VFAs by capillary gas chromatography with flame ionization detection (13). In this study, we have modified the earlier procedure to carry out gas chromatography with mass spectrometric detection of the free fatty acids following a simple aqueous dilution and filtration of the decomposition fluid samples. Possible biosynthetic pathways for some of the compounds produced are proposed. The opportunity to conduct a similar experiment using whole pig carcasses allowed for a comparison of the components of decomposition fluid to be made between two distinct locations, Western Australia (Perth) and

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southern Canada (Oshawa). This enabled the study to be carried out under significantly different climatic conditions, which has previously been established as a key factor in the decomposition process (14).

Materials and Methods

Research Facilities

Preliminary decomposition studies were carried out at two distinct locations: Perth, Western Australia and southern Ontario, Canada to provide a comparison between compounds detected in decomposition fluid. Temperature loggers (Thermodata Pty Ltd, Australia) were used to record ambient temperature every 30 min in both locations.

Perth, Western Australia (March–April, June–August, 2006)

The field trial area for this project was located at Curtin University of Technology, Bentley, Western Australia (32°01′ S, 115°53′ E), 6 km southeast of Perth. It is a partially cleared scrub area, *c.* 4m × 6m.

Data from the three closest official weather recording stations (Perth metro, Perth airport, and Jandakot airport) were obtained on a daily basis from the Bureau of Meteorology for comparative purposes (15). The pork rasher (belly pork) trial was conducted over late summer (March–April 2006) and the stillborn piglet experiment was conducted during winter (June–August 2006).

Ontario, Canada (July 2007)

The field trial was conducted in temperate woodland in southern Ontario, Canada. The study area was located *c.* 51 km northeast of Toronto (43°56′ N, 78°54′ W) across a slight (5°) gradient, with some drainage/depression areas. Dominant vegetation at the site included eastern white cedar (*Thuja occidentalis*), maples (*Acer* spp.), and trembling aspen (*Populus tremuloides*).

The pig trial utilizing adult pig carcasses was conducted over the Canadian summer (July–August 2007).

Decomposition Trials

Initial studies were performed with pork rashers (belly pork, *Sus scrofa*) to aid in method development. Further trials were carried out with stillborn piglets and adult pig carcasses (*S. scrofa*) to create a more realistic model of decomposition and to gain a more accurate understanding of the composition of decomposition fluid.

Pork Rashers (Belly Pork)—A total of eight polystyrene cups, each with the base partially removed and replaced with fine wire mesh, contained *c.* 100 g of cubed pork rashers (belly pork). The rashers were obtained from a commercial retail outlet, so exact levels of fat and protein are not known. Each cup was placed inside a second polystyrene cup for easy access to and collection of the decomposition fluid.

Purpose built, open mesh, metal cages were used to prevent predation by feral cats and other scavengers (ravens) but allowed flies and other insects access to the decomposing rashers. Four of the cups were covered with muslin, to prevent insect activity, and placed in one of the metal cages. The remaining four cups were left uncovered and placed in a second metal cage.

Collection vessels were monitored daily and fluid samples collected at random intervals, depending on the volume of fluid produced. Variable fluid volumes were recovered and recorded at each sampling session. Fluid was collected using a 5 mL sterile syringe and placed in 20 mL preweighed polypropylene vials for transport back to the laboratory. Syringes were discarded after each use. Samples were stored at −4°C until analysis.

After each sampling session, spraying with *c.* 2–5 mL of deionized water using a standard garden water spray gun lightly moistened the collection vessels. In the event of rain, both cages were covered with tarpaulins to prevent damage to the samples. The uncovered meat samples were disposed of on day 17, by which time they had become dry and insect activity had ceased.

On day 24, the muslin cover was removed from the remaining collection vessels. These samples were left uncovered until day 30, when insect activity had ceased and no further fluid was produced.

Stillborn Piglets—Four stillborn piglets (*c.* 2–2.5 kg) (supplied by PPC Linley Valley Fresh) were placed onto a custom made rack fitted into individual 15 L plastic storage boxes. Two of the piglets were completely enclosed with fine wire mesh and the other two were left exposed. The 15 L storage box was contained within a custom made wire cage (80 × 70 × 60 cm) to prevent access by feral animals.

The piglets were monitored daily, and fluid samples were collected from the uncovered piglets three times a week, beginning at day 14, when the first decomposition fluid was evident. Fluid collection procedures were the same as described previously for the pork rasher (belly pork) experiment. Fluid was collected from the protected piglets beginning at day 42. Sampling for all piglets continued until day 60.

Adult Pig Carcasses—This study utilized two adult pig carcasses as a model for human decomposition. The pigs (∼55 lb, 25 kg) were euthanized by a veterinarian with an overdose of anesthetic at a local pig farm prior to experimental treatment. Both carcasses were wrapped in plastic and transferred to the experimental site shortly after death.

The carcasses were placed on wire racks and covered with mesh wire to prevent scavenging by large carnivores. The mesh was sufficiently large to allow normal invertebrate activity to assist with decomposition. Each rack was placed over a small pit dug into the soil and lined with plastic. The wire racks allowed drainage of the decomposition fluids into the pit and aided sampling of the fluids without disturbing the site.

Pigs were monitored daily and fluid was collected from both pigs beginning on day 5. Fluid collection procedures were the same as described for the pork rasher (belly pork) trial. Sampling for both pigs continued until day 14 at which time the carcasses were mummified and skeletal.

Sample Preparation and Analysis

Reagents—A stock solution (80 mM of each of the five target short chain VFAs: propionic (Ajax Chemicals, Australia; Sigma-Aldrich, Canada), n-butyric (Chem Supply, Australia; Sigma-Aldrich), isobutyric (Aldrich, Australia; Sigma-Aldrich), n-valeric (Sigma, Australia; Sigma-Aldrich), and isovaleric acid (Aldrich; Sigma-Aldrich), was prepared in deionized water. A set of standard calibration solutions in the concentration range of 0.2–10 mM was prepared by serial dilution of the stock solution. All acids used were of analytical grade (>99%). The internal standard, trimethylacetic acid ([TMA] 2.5 mM) (Sigma; Sigma-Aldrich), was added to each of the calibration standards in a 1:1 ratio immediately prior to analysis. Calibration and stock solutions were stored at 4°C before

and after analysis. All calibration standards were analyzed in triplicate by gas chromatography–mass spectrometry (GC–MS).

A 20 mM formic acid (Sigma) solution was prepared and added (1:1) to TMA (2.5 mM). This mixture was then added to a set of calibration solutions (1:1) and analyzed in triplicate by GC–MS.

A mixture containing TMA (2.5 mM) and deionized water (1:1) was prepared and added to a set of calibration solutions (1:1). The solutions were analyzed in triplicate by GC–MS.

Sample Preparation—The recovered fluid samples were allowed to equilibrate at room temperature. A 1:2 dilution was prepared of the fluid with deionized water. The mixture was filtered (0.8/0.2 μm Acrodisc® filter, PALL Life Sciences, Australia) and 0.5 mL filtrate and 0.5 mL TMA (2.5 mM) solution was prepared immediately prior to analysis by GC–MS.

Chemical Analysis: Pork Rashers (Belly Pork) and Stillborn Piglets (Perth, Western Australia)—VFA calibration standards and samples were analyzed by gas chromatography (Hewlett Packard 5890 Series II) interfaced with a mass selective detector (MSD) (Hewlett Packard HP5971). A 1 μL aliquot was introduced into the split/splitless injector by means of a Hewlett Packard 7673A autosampler. The gas chromatograph was fitted with a 50 m × 0.22 mm ID × 0.25 μm (df) BP20 SGE column. GC conditions are described in section GC conditions. Data acquisition and analysis was performed using Chemstation software.

Chemical Analysis: Adult Pig Carcasses (Ontario, Canada)—Chromatographic analysis was performed on a Thermo-Finnigan Trace GC Ultra coupled with a Thermo-Finnigan Polaris Q mass spectrometer. A 1 μL aliquot was introduced onto a HP-INNOWAX (J&W Scientific 19091N-133) capillary column (30 m × 0.25 mm ID × 0.25 μm df) by means of a Thermo Triplus autosampler. GC conditions are described in section GC conditions.

GC Conditions—The GC oven was programmed from 100°C to 121°C at 3°C/min, then 15°C/min to 265°C and held for 7 min. The injector was operated at 240°C and a split ratio of 10:1. Helium was used as the carrier gas at a constant pressure of 20 psi (1.1 mL/min). Typical MSD conditions were: ionization energy 70 eV, source temperature 160°C, and electron multiplier voltage 2000 V.

Results and Discussion

In 2005, studies were initiated in Victoria (South Eastern Australia) to investigate the potential of soil solution analysis as a means of identifying patterns in decomposition fluid (16,17). The individual acids were identified using GC–MS. The results from these preliminary experiments indicated that while interesting patterns in the production of the VFAs emerged, further studies are required. These include complete characterization of decomposition fluids produced under different environmental conditions as well as investigations of factors that may affect the fluid production.

Method Development

Method development to target short chain VFAs using gas chromatography was based on previous work conducted at Deakin University (16,17), where the soil under whole pig carcasses was sampled at regular intervals and analyzed for C_2–C_5 acids.

The initial procedures employed involved the addition of formic acid to sample solutions and standards prior to analysis. This has been suggested to increase reproducibility (18) and improve the volatility of short chain acids by increasing the acidity of the mixture (19). According to Vass et al. (7), VFAs become more volatile at pH <7.0. Formic acid has also been reported to prevent peak tailing and ghosting in gas chromatography of VFAs (18). The use of formic acid in this study was questioned, when separations of a calibration series involving mixtures of VFA standards, performed in the absence of formic acid, showed no obvious, immediate difference in peak area or height. Peak tailing or ghosting was also not evident.

To determine the significance of formic acid in sample preparation, the nonparametric test, the Wilcoxon's signed-rank test, was performed accounting for the possibility that the data was not normally distributed (20). The outcome of the Wilcoxon's signed-rank test ($\alpha = 0.05$, $z_{crit} = 1.960$, $z_{calc} = 2.324$, $W = 28$, $n = 7$) showed that there was no significant difference in acid peak area between standards analyzed with and without formic acid. There was also no significant difference in the ratio between acid peak area and internal standard. The calibration procedure with formic acid did, however, produce steeper calibration curves. This suggested that calibration with formic acid was slightly more sensitive, however, although not sufficient to produce a significant difference. Formic acid was eliminated from further sample preparation and analysis as it was deemed to have no effect on the volatility or reproducibility of the acid standards.

The method for the preliminary study in 2005 used a final column temperature of 200°C (16), however, unresolved and unidentified compounds eluting at the latter stages of the analysis run time were observed. To improve the detection and resolution of the unknown compounds in this study, the final column temperature was increased from 200°C to 265°C. The improved resolution at the higher final temperature resulted in the detection of several previously unidentified components (Fig. 1). The identity, relative abundance, and the possibility of trends in the newly resolved components were considered to be of importance as they had previously not been described and their validity in the estimation of PMI was unknown. The retention time for the short chain acids remained within the limits of instrumental precision with the increased final column temperature.

The upper temperature limit for the SGE BP20 (polyethylene glycol) column, as suggested by the manufacturer, is 270°C. Increasing the column temperature to 265°C can cause degradation of the column phase which in turn led to a significant amount of column bleed observed in the total ion chromatogram (TIC). The

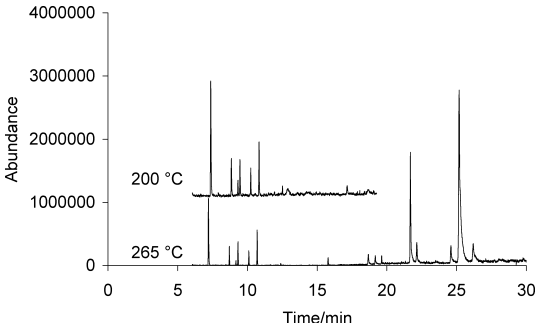

FIG. 1—*Typical chromatograms from pork rasher (belly pork) trial illustrating differences in compounds detected with an increase in column temperature for an individual sample.*

limited final temperature and polar nature of the BP20 column phase, combined with the increased carbon content of the long chain fatty acids may affect the peak shape and resolution of the long chain fatty acids. This can be evident by peak broadening and tailing of the higher molecular weight long chain fatty acids. These factors can lead to significant issues if quantification is required.

Field Trials

Two distinct locations are utilized in the field trials to provide a comparison between compounds detected during the analysis of decomposition fluid.

Megyesi et al. suggest that decomposition rates and a more accurate estimation of PMI can be achieved when accumulated temperature is taken into account rather than individual daily temperatures (1). Accumulated degree days (ADD) are calculated by taking the sum of the average daily temperature (average of the maximum and minimum) for the length of time the corpse has been decomposing (1). Results for all trials are presented using calculated ADD.

As the study was conducted in the absence of soil, pH measurements of the decomposition fluids were not taken, as the only pH effect would have been internal to the body, which is part of the decomposition process and not something that could be controlled.

Pork Rasher (Belly Pork) Trials—Collection cups (numbered 1–8) containing pork rashers (belly pork) were placed in the ambient environment on 28th March 2006 (early autumn). Polystyrene cups were utilized for ease of use. There is the potential for leaching of organic compounds from the polystyrene; however, an extensive literature review found no mention of issues with aqueous extractions, just with extractions involving alcohols (21,22). We thus do not consider it to be a significant issue for this preliminary study; however, with hindsight it may have been advisable to use glass containers to eliminate this possible source of error. The warm weather (average maximum temperature 27.5°C) facilitated a fast rate of decomposition for the rashers exposed to insects (cups 5–8) as expected, with the first fluid samples collected on day 3 (ADD 85). It was noted that insect activity was at a premium from days 5 to 7 (ADD 121–212) and that as insect activity increased, the production of fluid also increased. Visible insect activity ceased for collection vessels 5–8 on day 17 (ADD 387) at which time fluid production significantly decreased. Day 24 (ADD 510) was the last sample collection day. The experiment was allowed to proceed for a further 6 days (day 30—ADD 595), but no further fluid was apparent. Figure 2 illustrates a typical chromatogram from the analysis of the decomposition fluid. No fluid was produced from the samples covered with muslin (cups 1–4) so a direct chemical comparison of fluid composition, taking into account the effects of insect activity, could not be made.

Piglet Trial—Stillborn piglets were utilized to represent a more realistic model of decomposition. Four piglets were placed in the field trial area on 22nd June 2006 (winter). Decomposition fluid first appeared on day 14 (ADD 189) and samples were collected from piglets 1 and 3. In contrast, the calculated ADD for the pork rashers (belly pork) on day 14 was 339. The difference in daily temperature compared with the pork rasher (belly pork) experiment accounted for the delay in fluid production. It was evident that fly and maggot activity was much greater in the warmer months compared with the cooler months. This allows the inference that increased activity leads to greater fluid production at an increased rate. Sampling continued for the piglets exposed to insect activity

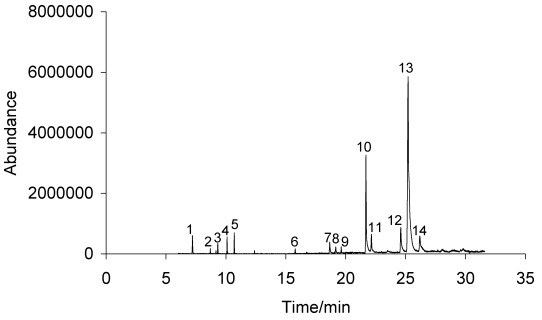

FIG. 2—*Chromatogram showing compounds produced during the pork rasher trial (day 24 – ADD 510) with identified peaks: acetic acid (1), propionic acid (2), TMA (3), butyric acid (4), isovaleric acid (5), 2-piperidone (6), phenylacetic acid (7), phenylpropanoic acid (8), myristic acid (9), palmitic acid (10), palmitoleic acid (11), stearic acid (12), oleic acid (13), linoleic acid (14).*

until day 47 (609 ADD). The protected piglets did not produce fluid until day 42 (525 ADD) and it was only produced for a very limited period of time, until day 54 (696 ADD). Piglet 2 was seen to have limited insect activity. Once decomposition had advanced, flies were able to lodge underneath the piglet, where the stomach had burst and this activity may account for the fluid production in this particular case. Piglet 4 was not seen to have any activity and still produced a limited amount of fluid. These results highlight the importance of the need for a much larger sample set because of the complex and variable nature of the decomposition process. A sample chromatogram of the piglet trial is shown in Fig. 3. Other chromatograms from this trial indicated similar traces for the production of short chain acids. Chromatograms illustrating the variation in compounds produced and their relative abundance for piglet 1 are shown in Fig. 4. The variety of compounds observed in the pork rasher (belly pork) trial, however, was not seen in the piglet trial. Compounds such as isocaproic acid, piperidone, and long chain fatty acids were only occasionally detected, and this sporadic appearance eliminates their usefulness in establishing any trends in production. Widdowson (23) found that the fat content of stillborn piglets is *c.* 1% by mass of their total body weight, measured as gm/100 gm fresh weight. The lack of long chain acids in the piglet trials can be related to their fat content as long chain acids are produced by the cleavage of triglycerides during aerobic degradation of fats and soft tissues (12).

It is interesting to note that all four piglets, whether covered or exposed, decomposed at a different rate, and showed variation in the compounds produced, even though they were in close proximity

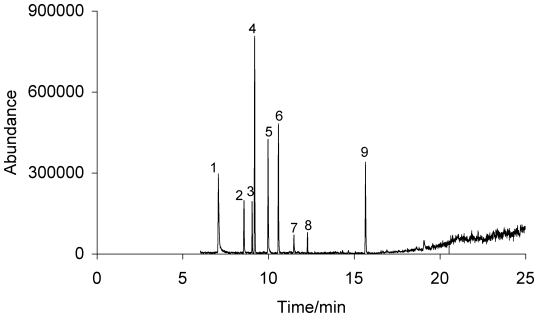

FIG. 3—*Chromatogram from piglet 3 (day 26—ADD 342) with identified peaks—acetic acid (1), propanoic acid (2), isobutyric acid (3), TMA (4), butyric acid (5), isovaleric acid (6), valeric acid (7), isocaproic acid (8), 2-piperidone (9).*

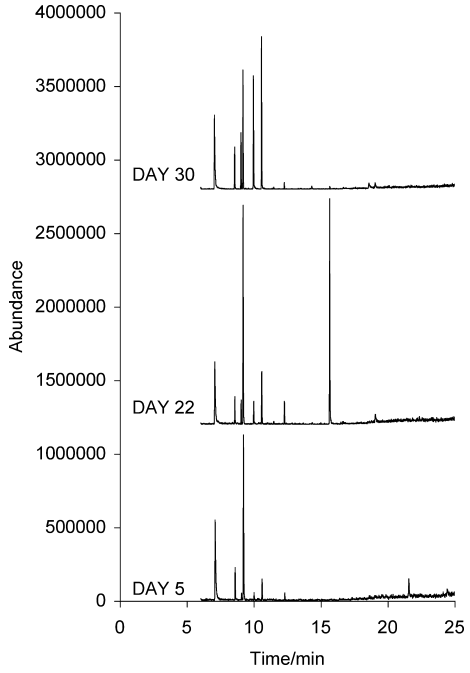

FIG. 4—*Chromatograms representing changes in compounds detected in decomposition fluid for piglet 1 on day 5 (i), 22 (ii), and 30 (iii).*

to each other and subjected to the same environmental conditions and surroundings. This is another indication that a larger subject group is vitally important. A clear distinction can be made, however, for the rate of decomposition of the piglets exposed to maggots and those that were not. The exposed piglets (1 and 3) reached a state of advanced decomposition considerably faster than the two in which maggot activity was excluded (2 and 4).

Pig Trial—Two whole pig carcasses were placed in the field trial area in July 2007 (summer) in southern Canada in a dedicated research facility. Fly activity was immediately apparent and maggot activity was evident from day 2 (ADD 43). Decomposition proceeded at a fast rate with an average maximum daily temperature of 26.45°C. The need for a larger sample set was yet again emphasized by the differing rate of decomposition for both adult pigs which were placed within meters of each other and exposed to comparable levels of sunlight and shade.

Decomposition fluid first appeared on day 5 (ADD 104) and this was the first sampling day for both pigs. The main issue encountered during this trial was the large number of maggots present in the collection pit making sampling of fluid difficult. This occurred predominantly on days 7–8 (ADD 147–168) when insect activity was deemed to be at a maximum. Samples were collected until day 14 (ADD 310), when insect and maggot activity had ceased and only mummified skin and skeletal remains were evident. A sample chromatogram from the pig trial is shown in Fig. 5.

The variety of compounds detected in the pig trial was similar to that found in the pork rasher (belly pork) trial run in Western Australia, ranging from short chain acids to long chain acids and cyclic compounds. The detection of long chain acids was expected due to the fat content in an adult pig. Additional compounds not previously detected in other trials included phenol and indole. Further investigation into the presence of these two compounds is required to assess their value in estimating PMI.

Proposed Biosynthetic Pathways

Decomposition processes are the result of bacterial and enzymatic action under aerobic and anaerobic conditions (12). A large proportion of the decomposition products produced should reflect the amount of fat and protein content of the remains. The major contributors to the products found in decomposition fluid will be

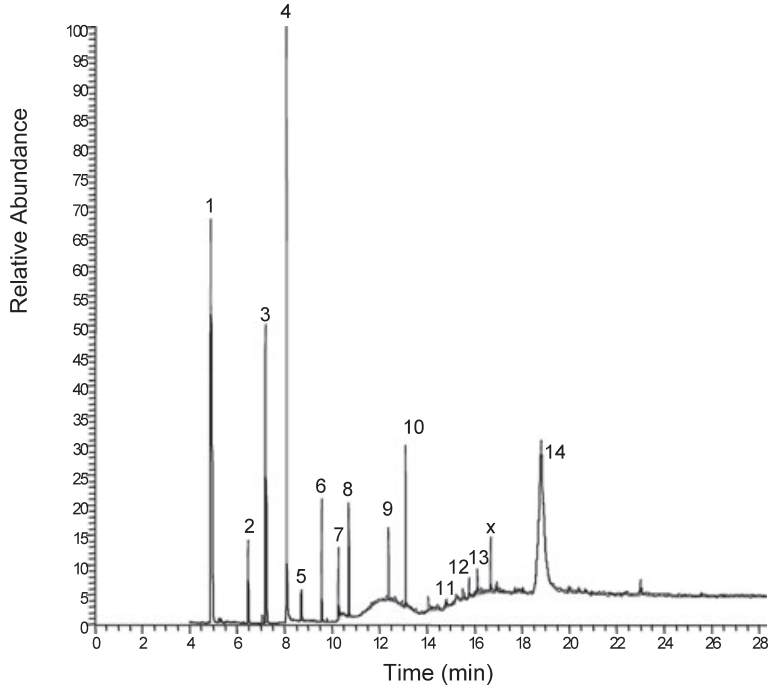

FIG. 5—*Chromatogram showing compounds produced during the Canadian pig trial (day 6—ADD 126) with identified peaks: acetic acid (1), propionic acid (2), TMA (3), butyric acid (4), isovaleric acid (5), valeric acid (6), 4-methylvaleric acid (7), caproic acid (8), phenol (9), 2-piperidone (10), indole (11), phenylacetic acid (12), phenylpropionic acid (13), oleic acid (14), unidentified peak (x).*

protein, carbohydrate, and fats (12), with the majority coming from adipose tissue and amino acid degradation.

Adipose Tissue—Adipose tissue is specialized connective tissue that functions as the major storage site for fat in the form of triglycerides (12). The body's adipose tissue comprises, on average, 5–30% water, 2–3% proteins, and 60–85% lipids (fats), of which 90–99% are triglycerides (12). Triglycerides are composed of one glycerol molecule attached to three fatty acid molecules. During decomposition, triglycerides are cleaved by enzymatic action to produce a glycerol molecule and three separate fatty acid chains.

In Dent's research into adipose tissue, monounsaturated oleic acid was by far the most prominent, followed by linoleic, palmitoleic, and palmitic acids (12). This held true for the oleic acid in both the pork rasher (belly pork) trial and the pig trial but not for the other long chain acids. Although oleic acid is the most abundant in the triglyceride molecules, it is also formed by the hydrogenation of linoleic acid. The second most abundant acid found in the pork rasher (belly pork) and pig trials was palmitic acid. Palmitic acid is formed from hydrogenation of palmitoleic acid. All six of the long chain acids found in the pork rasher (belly pork) trial are considered to be possible components of adipose tissue (12,24).

Amino Acids—Amino acids are carboxylic acids that contain an amino group at the α-carbon atom. Hydrolysis of a protein cleaves the peptide linkage and individual amino acids are released. Amino acids can then either be deaminated or decarboxylated to produce straight and branched chain VFAs (25).

The short chain VFAs have a wide variety of amino acid sources: acetic acid (alanine, glycine, serine), propionic acid (lysine, threonine), butyric acid (alanine, glutamic acid, aspartic acid), isovaleric acid (leucine, isoleucine), valeric acid (proline), and isobutyric acid (valine) (25).

Amino acid degradation by anaerobic bacteria always involves oxidation–reduction reactions. The ultimate reduction products include a variety of short chain fatty acids, δ-amino valeric acid, and molecular hydrogen (26).

At a neutral pH (pH 6–7), deamination is the major pathway for metabolic degradation of amino acids (27). Deamination results in the production of VFAs, CO_2, H_2, and NH_3 (Fig. 6).

Amino acid decarboxylase is induced at pH 5–6 but is a much less common degradation pathway (27) (Fig. 7).

The amino acid phenylalanine, on putrefaction, yields three different aromatic acids: phenylacetic acid (produced by oxidation), phenylpropionic acid (reduction), and benzoic acid (28) (Fig. 8). Benzoic acid was not detected in any of the fluid samples. Phenylacetic and phenylpropionic acids are also produced by the deamination of tyrosine by anaerobic bacteria (25).

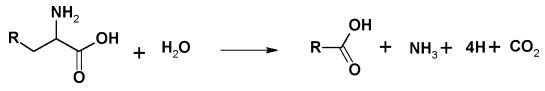

FIG. 6—*Deamination pathway for amino acids.*

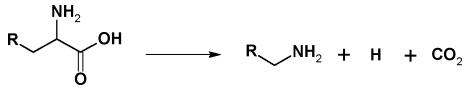

FIG. 7—*Decarboxylation pathway for amino acids.*

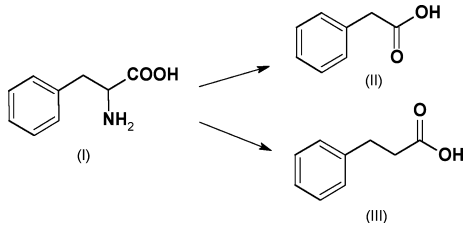

FIG. 8—*Degradation pathway of phenylalanine (I) to produce phenylacetic (II) and phenylpropanoic (III) acids.*

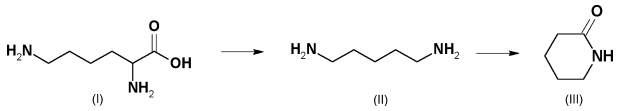

FIG. 9—*Degradation pathway of lysine (I) via cadaverine (II) to produce 2-piperidone (III).*

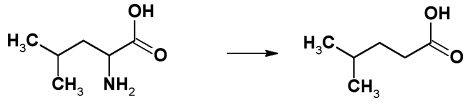

FIG. 10—*Reductive deamination of leucine to produce isocaproic acid.*

2-Piperidone is a lactam (cyclic amide) that was found to be a metabolite of cadaverine, along with 1-piperidine and 5-aminopentanoic acid (29). Cadaverine is a volatile amine produced by the decarboxylation of the amino acid lysine (Fig. 9) (25,27).

Isocaproic acid (4-methylvaleric acid) is formed via the reductive deamination of the amino acid leucine (Fig. 10) (25,30). Another suggested pathway for the origin of isocaproic acid via leucine is the enzymatic cleavage of the cholesterol side chain (31,32).

Conclusions

Several previously unreported compounds were detected in the decomposition fluid samples during the trials, the main ones being benzeneacetic acid, benzenepropionic acid, 2-piperidone, and isocaproic acid. While having previously been reported as breakdown products of amino acids (25,26,28–30), these compounds have not previously been identified in decomposition fluid. Further research trials are required, particularly with carcasses within the soil matrices, as well as continuing analytical method development to ensure valid quantitative results. Confirmation of the presence of these compounds in human decomposition fluids is also required to establish their potential as reliable markers of PMI.

All three trials showed differences with respect to rate of decomposition, both between trials and between subjects in the same trial. This emphasizes the importance of choosing the correct subject size and establishing a larger sample set to ensure accurate models of decomposition are represented. Future work should include greater replication to ensure appropriate significances are detected.

Proposed degradation pathways are taken from literature predominantly concerned with fermentation processes, particularly for the breakdown of amino acids (26,27) as none exist in the forensic literature. Further analytical study is required to confirm these biodegradation pathways for the decomposition process. The majority of studies cited accept the cleavage of triglyceride molecules as the origin of long chain fatty acids (12).

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