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# Dirty secrets: blood protein and VFA analysis of soil from execution and grave site in the former Yugoslavia

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DIRTY SECRETS:  
BLOOD PROTEIN AND VFA ANALYSIS OF SOIL FROM  
EXECUTION AND GRAVE SITES IN THE FORMER YUGOSLAVIA

A Thesis

Submitted to the Graduate Faculty of the  
Louisiana State University and  
Agricultural and Mechanical College  
in partial fulfillment of the  
requirements for the degree of  
Master of Arts

In

The Department of Geography  
and Anthropology

By  
Hugh Tuller  
B.A. Michigan State University  
August 1991

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## Table of Contents

Acknowledgements .....	ii
List of Tables .....	v
List of Figures .....	vi
Abstract .....	vii
Introduction .....	1
Blood Protein Analysis .....	6
Stutiča Execution Site Materials and Methods .....	6
Immunological Background and Analysis .....	9
Stutiča CIEP Analysis Results .....	12
Volatile Fatty Acid Analysis .....	15
Duz Cemetery Materials and Methods .....	15
Knin Mass Grave Materials and Methods .....	19
Volatile Fatty Acid Background and Analysis.....	20
Duz VFA Analysis Results .....	23
Knin VFA Analysis Results .....	24
Discussion .....	26
Stutiča CIEP Samples.....	26
Duz VFA Samples.....	30
Knin VFA Samples.....	35
Conclusion.....	39
References Cited .....	41
Vita .....	45

## List of Tables

1. Sample Test Pit Association .....	8
2. CIEP Human Antiserum Analysis .....	12
3. Artifact vs Non-Artifact Positive Result Difference of Proportion Test .....	13
4. Duz Cemetery Burial Conditions and Grave Samples .....	17
5. Duz Cemetery Sample Weights, Final Volumes and pH Levels .....	23
6. Knin Mass Grave Sample Weights, Final Volumes and pH Levels .....	23
7. Duz microFAST GC <sup>2</sup> Results .....	24
8. Knin microFAST GC <sup>2</sup> Results .....	24
9. Stutiča Site CIEP % Ratios .....	26
10. Stutiča Site Soil Profile .....	28
11. Duz Cemetery Soil Profile .....	34
12. Knin Mass Grave Soil Profile .....	38

## List of Figures

1. Site Locations in Croatia and Kosovo .....	3
2. Stutiča Execution Site .....	7
3. Bottom of Coffin Style Burial with Spoil Dirt and Coffin Base .....	16

## **Abstract**

Blood proteins and volatile fatty acids (VFAs) deposited in crime scene soil may remain biologically active and detectable after a considerable lapse of time. Soils were sampled from three sites of known criminal activity in the former Yugoslavia. From an execution site near Stutica, Kosovo, soil samples were analyzed for year-and-a-half-old blood proteins using an immunological test, cross-over immunoelectrophoresis. From a total of 72 samples, 44 returned positive results for human blood proteins.

Soil solution from grave soils sampled at a cemetery in Duz, Kosovo, and a mass grave in Knin, Croatia were analyzed for VFAs using gas chromatography (GC) and mass spectrometry (MS). Six samples were removed from four graves in the Duz cemetery for testing. Prior to exhumation and soil sampling, bodies laid in the graves approximately one-and-a-half-years. GC results from the cemetery revealed the presence of iso-butyric and valeric water soluble VFAs in one sample. MS examination of the samples was inconclusive. From the six-year-old mass grave in Knin, four samples were taken. However, one sample was discarded due to mold contamination. GC and MS analysis revealed the presence of iso-butyric and iso-valeric water soluble VFAs in two of the three remaining samples. In addition, MS analysis revealed the following non-water soluble VFAs in all three samples: capric, lauric, myristic, palmitic, stearic, and oleic. Possible causes for inconsistent results between the cemetery graves and the mass grave may be attributed to the differences in burial styles, differences in soil moisture and clay content, and sampling handling.

The positive findings of blood proteins and VFAs in the Kosovar and Croatian soils validate CIEP, GS, and MS analysis of older site soils. These methods may be used to successfully identify crime scenes, and especially suspected scenes more than a year old, in the absence of bodies or other physical evidence.

## **Introduction**

An individual convicted of murder, the most heinous of crimes, can presumably expect the harshest of punishments. With this in mind, those who commit murder regularly attempt to hide or destroy evidence to avoid prosecution. Murder is also unique because it always results in at least one very large, important piece of evidence: a body. As bodies are the most obvious and important piece of evidence at a murder scene, they are often removed from the scene and concealed by the guilty party. After hiding the body, the murderer, rethinking his disposal plan, may even retrieve the body and attempt to hide it in a different, more secretive, location. The murderer may even attempt to destroy the body in some manner, further confounding investigations. This activity is evident in the former Yugoslavia where investigations by the International Criminal Tribunal for the former Yugoslavia (ICTY) have demonstrated an evolution of concealment methods attempted by perpetrators of human rights abuses.

After ICTY investigations demonstrated the ability to detect and extract evidence from mass graves and execution sites in Bosnia-Herzegovina and Croatia, Serbian forces in Kosovo decided on alternative murder concealment tactics. No mass graves containing bodies were discovered by ICTY in Kosovo, and locations described by witnesses as execution sites were often devoid of evidence. Serbian forces had removed shell casings, and bodies. While ICTY investigations in Kosovo were still able to document the removal of bodies and other evidence from many graves and execution sites, the scarcity of evidence at some locations prohibited investigators from making a definitive conclusion about the nature of the site. Similar situations exist in contemporary domestic murder investigations.

The absence of evidence that a body can provide, while an obstacle to investigators, is not necessarily an end to the investigation. Despite removal, the body may have left behind microbiological evidence that can be collected and analyzed. Human blood proteins and volatile fatty acids (VFAs) deposited in soil at murder scenes and concealment/burial sites may remain stable over long periods of time; sophisticated soils analyses should be able to detect them (Allen et al. 1995; Borja et al. 1997; Culliford 1964; Gurfinkel and Franklin 1988; Jahren et al. 1997; Kooyman et al. 1992; Loy 1983; Nolin et al. 1994; Vass et al. 1992; Yohe et al. 1991; Zimmerman 1973).

Drawing on immunological identification techniques used by criminologists, archaeologists have been able to find minute traces of blood proteins on the edges of ancient stone tools (Hyland et al. 1990; Kooyman et al. 1992; Newman et al. 1996; Petraglia et al. 1996). Although these stone tools have been buried for thousands of years, some still retain biologically active blood proteins (Allen et al. 1995; Loy 1983; Newman and Julig 1989). Using immunological tests, these proteins can be identified to the taxonomic level of family—human blood can be discriminated from animal blood (Hayland et al. 1990; Kooyman et al. 1996; Newman et al. 1997). VFAs are short-chained organic acids produced from lipid tissue during body decomposition. Lipids, like proteins, are known to survive on ancient tools and pots, and in soil (Evershed 1993; Evershed and Tuross 1996; Thomas 1993;). Archaeologists have been successful in identifying VFA over a thousand years old (Evershed and Tuross 1996; Nolin et al. 1994).

The same detection methods used by archaeologists for ancient artifact examination and criminologists in contemporary murder cases were used in this study to

examine soils sampled from three sites investigated by ICTY in the former Yugoslavia: an execution site, four individual graves in a cemetery, and a mass grave (Figure 1). An immunological test, cross-over immunoelectrophoresis (CIEP), was used to identify blood proteins as independent evidence of an execution at the Stutiča site in Kosovo. VFAs in soils collected from cemetery graves in Duz, Kosovo and a mass grave in Knin, Croatia, were identified using a microFAST GC<sup>2</sup> (a field portable gas chromatograph) and a mass spectrometer.



**Figure 1: Site Locations in Croatia and Kosovo**

ICTY investigations at the execution site revealed human bone, bone fragments, bullets, and shell casings, suggesting that blood would have been spilled on the ground.

At the two gravesites, bodies in different stages of decomposition were exhumed. VFAs should be present in the graves having leached into the surrounding soils. As the events surrounding each of the three sites are known, examination of the site soils may thus be viewed as controlled experiments of the testing methods. Testing of soils from sites where the past events are unknown may lead to criticism of the method's reliability, accuracy, and ultimate validity, especially where results are mixed (Eisele 1995). Conversely, objective examination of the soils from known sites, as this study, may establish the dependability of the testing methods (Newman 1996).

The passage of time between residue deposit and testing is an important element in this study. Forensic investigators rarely test soils for blood residue if the event in question is more than a few months old on the assumption that microorganisms would degrade deposited residue beyond our ability to identify them. The Stutiča execution site soils were sampled approximately one-and-a-half-years after the date of the execution. These soil samples were stored for an additional six months prior to being tested for blood proteins. The time period of accumulation of VFAs in the Duz and Knin soils is more difficult to resolve. VFAs are formed during active decomposition of bodies. In the Kosovar cemetery, the bodies were buried approximately one-and-a-half-years before exhumation, while bodies in the Croatian mass grave lay buried for about six years prior to exhumation. However, the burial styles and conditions within the cemetery and mass grave affected the decomposition rates independently of time, in some instances allowing for rapid decomposition, while others prolonging the process. For example, in the mass grave some individuals were so well preserved that fingerprints could still be viewed after six years of burial. For the mass grave as a whole, it is possible that VFAs were

being deposited in the soils during the entire six-year period. However, VFA deposition from the individual graves in the cemetery may have leached more than a year prior to sampling. Soils collected from the cemetery were stored for almost one year, and soils from the mass were grave stored six months before testing.

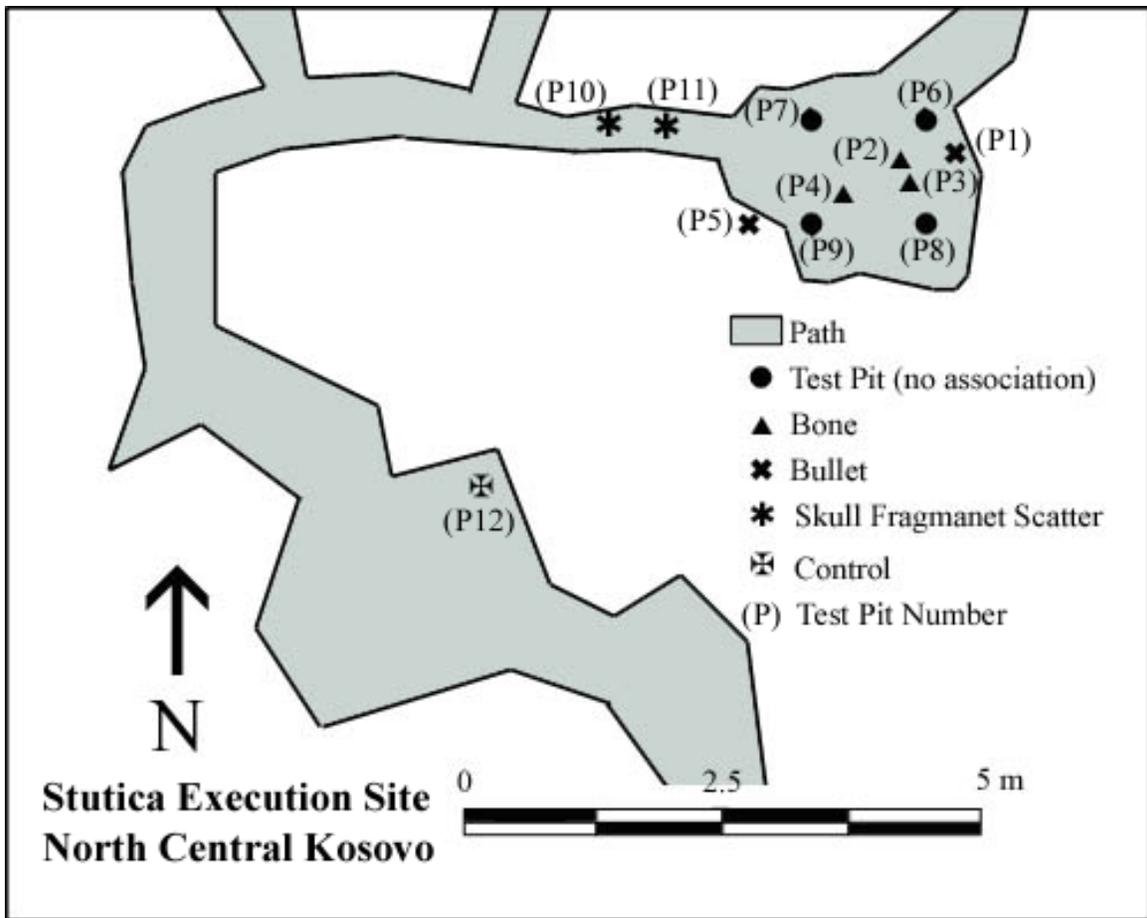
This study presents findings validating CIEP and VFA examination of older crime scene site soils. Positive test results from murder and burial/dump site soils, corroborated with witness statements and other possible physical evidence, provide powerful documentation to support an investigator's evaluation of an aged crime scene. Demonstrating that murder scenes, execution sites, and mass graves can never be completely cleaned—that evidence of past abuses can never be completely erased—may help to deter murder and human rights abuses in the future.

## **Blood Protein Analysis**

### **Stutiča Execution Site Materials and Methods**

According to ICTY investigations, in April, 1999, near the village of Stutiča in Central Kosovo, just north of the city of Glogovac, armed Serbs surprised a small group of unarmed Kosovar Albanian civilians hiding out on a wooded hillside. The Serb gunmen opened fire, killing six to seven of the Albanians and wounding an unknown number of others. The remaining Albanians were forced to strip off their outer layer of clothes and to turn over any valuables they had to the Serbs. They were then marched out of the area; the dead remained where they had fallen. Local Albanians who were informed of the killings by a survivor later buried the bodies in shallow graves at the site. After Serbian forces were driven out of Kosovo in June of 1999, Albanian families returned to the area, and the bodies were exhumed and relocated to cemeteries.

An ICTY field team comprised of archaeologists and forensic anthropologists investigated the Stutiča site on July 11 and 12, 2000. As noted, clothing, hair, bone fragments, shell casings, and bullets were found and collected at the site. It was noted during site investigation, that the site and surrounding area were heavily wooded, providing cover for the Albanians, but also limiting space where they could have physically occupied. A trail through the wooded area became wide enough at points to allow several people to gather together to sit, eat, and sleep (Figure 2). One of these wider areas, a small depressed area approximately three by three meters, appeared to be a spot where at least one body had laid. In this depression two human skull fragments, an intact first cervical vertebrae, and two bullets were found. On the path next to the depression an additional scatter of skull fragments and hair was discovered.



**Figure 2: Stutiča Execution Site**

The depression area and adjacent trail, with high concentrations of bone fragments, hair, and bullets, were regarded as the best locations to sample soils for possible blood proteins. Soils were sampled from the Stutiča site on October 21, 2000. A total of 11 test pits were dug in the depression and adjacent trail, with an additional pit dug as a control approximately five meters away from the center of the depression (Table 1, Figure 2). Each test pit was 5 x 10 cm wide and approximately 10 cm deep. The pits were excavated in six levels, each 1.5 cm thick. Soils from each of these levels were placed in plastic evidence bags. The shovel and trowel used to remove the samples were washed with water after each successive pit and layer dug. Ultimately, nine test pits were

dug in the depression, five directly beneath locations where either bone or bullets were found. The remaining four test pit locations in the depression were chosen to cover the depression area evenly. Along the adjacent path where the skull fragment scatter was discovered, an additional two test pits were dug. A total of 72 layers were sampled from the twelve test pits.

Three days later, each evidence bag was opened, the samples were poured out on clean plastic sheets, and were allowed to air dry indoors over a 12-hour period. Ziploc bags were used as the sample drying surface. Each Ziploc bag was cut open and spread out, with the untouched surface inside of the bags face up. The soil samples were poured onto and spread over the untouched surface areas. The dried samples were then repackaged in new plastic evidence bags and stored out of direct sunlight until analyzed.

**Table 1: Sample Test Pit Association**

<b>Test Pit</b>	<b>Association</b>
1	Bullet
2	Skull Fragment
3	Skull Fragment
4	Vertebrae
5	Bullet
6	N/A
7	N/A
8	N/A
9	N/A
10	Skull Fragment Scatter
11	Skull Fragment Scatter
12	N/A (Control)

## **Immunological Background and Analysis**

Throughout the twentieth century, immunological tests have been used in both the medical and legal professions to identify blood and bloodstains (Lee and Deforest 1976; Sensabaugh et al. 1971). Hospitals routinely use immunological tests to check patients for hepatitis and other diseases. In the past, police forensic experts regularly performed immunological tests at crime scenes and laboratories to detect blood and identify its origin. Today many police departments are now equipped for DNA testing of bloodstains, yet, some departments still rely on immunological techniques as their primary investigative method, demonstrating the availability, speed, and reliability of the technique.

Several immunological techniques have been developed since the initial discovery of precipitin reactions in the late nineteenth century. However, all the techniques use the same basic principle: blood protein is brought into close-reacting contact with a suitable antiserum (Lee and DeForest 1976). Antiserum is a serum of antibodies raised against specific antigens. A precipitate line will form between an antiserum (antibodies) created for a specific antigen (blood protein) when they are brought close enough together. For example, antibodies specific to a particular animal, say deer, can be raised in a different animal (e.g., rabbits—although any animal will do). If deer blood is introduced to the serum created in the rabbit, a reaction will occur. Cross reactivity can occur with related species, for example, deer antisera will also react to elk and moose blood. By controlling the immunoreaction, positive identification to the taxonomic family level of antigen origin can be established (Hayland et al. 1990; Kooyman et al. 1996; Newman et al. 1997).

Many immunological techniques have been used in forensic and archaeological analysis including Ouchterlony double-diffusion (Ouchterlony 1968), cross-over immunoelectrophoresis (CIEP) (Culliford 1964), enzyme-linked immunosorbent assay (ELISA) (Cattaneo et al. 1992; Hyland et al. 1990), and radioimmunoassay (RIA) (Lowenstien 1985). The difference between the techniques is one of sensitivity, with Ouchterlony being the least sensitive and RIA the most. RIA also allows immunoreactions to be numerically quantified (Lowenstien 1985). However, RIA is expensive and uses radioactive tracers as indicators in the technique, something few laboratories and researchers have at their disposal or are qualified to use.

CIEP was chosen as the technique to analyze the Stutiča soil samples. The technique is very sensitive, detecting up to  $10^{-8}$  g of protein (Allen et al. 1995), it is relatively inexpensive compared to RIA, and it lends itself well to multiple examinations (Culliford 1964). Only one gram (1 g) of soil is needed to run a single CIEP test.

Four grams (4 g) of soil were measured out from each of the 72 Stutiča samples and submitted to Dr. Margaret Newman at the University of Calgary, Canada, for analysis. The methodology described here is taken from her final report (Newman 2001). To extract any surviving blood proteins from the soil samples, 1 mL of 5% ammonium hydroxide was added to approximately 1 g of each sample. This mixture was vortexed and then placed in a rotating mixer for 24 hours at 4<sup>0</sup> C. Next, the samples were centrifuged, and the resultant solution was placed in sterile Eppendorf tubes. Initial screening of the samples for non-specific protein reactions not based on the immunological specificity of the antibody was carried out against pre-immune serum (i.e., serum from a non-immunized animal). The pre-immune serum was developed at

the University of Calgary while all the antisera were sourced from Cappel in Aurora, Ohio, and rigorously tested for purity and specificity for forensic laboratories (Newman, personal communication 2001). All samples returned negative against the pre-immune serum. Testing of the samples was then continued against bovine, sheep, and human antisera.

In CIEP, interaction between antigen and antibody is enhanced through the use of an electrophoretic force. The antigen and antibody are placed into paired wells punctured in agarose gel approximately 1.5 mm in diameter and 5 mm apart. The gel has a pH of 8.5. The gel platform is placed over an electrophoresis tank containing two basins of a barbital buffer with a pH of 8.6. Two triple thickness filter papers are used as wicks by placing one end of the paper into one side of the tank and touching the other side of the paper to the agarose gel. Electric current set at a constant 100 v is applied through the tanks, one tank positive and one negative. The wicks act as electric contacts between the buffer and the gel, supplying the antigen (unknown extract) with positive current and the antibody with negative. A precipitin reaction will occur only when the known antibody, raised against a specific type of animal, is tested against an antigen from that type of animal.

Positive and negative controls, also prepared in 5% ammonia hydroxide, were run with each gel to insure procedure quality. Positive controls are antigens that will react with the specific antisera tested against, while negative controls are antigens that will not react with the antisera. If opposite results occur, then something, perhaps contaminants, has adversely affected the test and a new test needs to be run. No opposite reactions were observed during testing. Duplicate testing was carried out on all positive samples.

## Stutiča CIEP Analysis Results

A total of 72 soil samples (11 test pits and a control pit, each pit with 6 levels) were taken from the execution site near Stutiča, Kosovo, and tested against bovine, sheep, and human antisera. The bovine and sheep antisera returned negative results for all 72 samples. Only human antiserum produced positive reactions. The results of the human antiserum analysis are in Table 2.

**Table 2: CIEP Human Antiserum Analysis**

Test Pit 1	Result	Test Pit 2	Result	Test Pit 3	Result	Test Pit 4	Result
Level 1	+	Level 1	+	Level 1	+	Level 1	+
Level 2	-	Level 2	+	Level 2	+	Level 2	+
Level 3	-	Level 3	-	Level 3	+	Level 3	+
Level 4	+	Level 4	-	Level 4	-	Level 4	+
Level 5	+	Level 5	+	Level 5	-	Level 5	+
Level 6	+	Level 6	+	Level 6	-	Level 6	+
Test Pit 5		Test Pit 6		Test Pit 7		Test Pit 8	
Level 1	+	Level 1	+	Level 1	+	Level 1	+
Level 2	+	Level 2	+	Level 2	+	Level 2	+
Level 3	+	Level 3	-	Level 3	-	Level 3	-
Level 4	-	Level 4	-	Level 4	-	Level 4	-
Level 5	weak +	Level 5	weak +	Level 5	-	Level 5	-
Level 6	-	Level 6	weak +	Level 6	-	Level 6	-
Test Pit 9		Test Pit 10		Test Pit 11		Test Pit 12	
Level 1	+	Level 1	+	Level 1	+	Level 1	+
Level 2	+	Level 2	+	Level 2	+	Level 2	+
Level 3	-	Level 3	+	Level 3	+	Level 3	+
Level 4	-	Level 4	+	Level 4	+	Level 4	-
Level 5	-	Level 5	-	Level 5	+	Level 5	-
Level 6	-	Level 6	-	Level 6	+	Level 6	-

In Table 2, each sample reaction result is shown in a column to the right of the level from which it came. Positive results to human antiserum were obtained in 44 of the samples (three of these were weak-positive reactions). The remaining 28 samples

returned negative results. It should be noted that those pits associated with an artifact (skull fragment, bone, or bullet) produced the largest proportion of positive results. A two-sample difference of proportions test was used to compare the results from pits with associated artifacts to pits without associated artifacts for a significant difference (Table 3). The test indicates that pits with associated artifacts did indeed have a significantly larger proportion positive result. The absence of identifiable blood proteins in all samples, especially samples between levels of positive findings, may be due to poor preservation of blood proteins, bioturbation, or sampling error (e.g., if blood proteins were present in most of the soil from a level sample but absent or rare in the 1 g subsample).

**Table 3: Artifact vs Non-Artifact Positive Result Difference of Proportion Test**

	<b>Total</b>	<b>Positive</b>	<b>Negative</b>
<b>Associated Artifact</b>	42	31(0.7381)	11(0.2619)
<b>Non-Associated Artifact</b>	30	12(0.4333)	17(0.5666)
<b>p-value</b>	0.0035		

The fact that human blood protein was found in the upper three layers of the control sample needs to be addressed. The control sample test pit was located approximately five meters from the central point of the main sample cluster. The close location of the control pit to the other sample pits was chosen for a number of reasons; the main one was safety. Kosovo has approximately 50,000 land mines, 30,000 unexploded cluster bombs, and thousands of other unexploded ordinance buried and scattered around the countryside. All ICTY sites are checked for landmines, however, this is limited to a path leading to the site and the immediate area of the site. Although a few locations outside the Stutiča site area were nervously examined for possible control pit locations resembling the sample area topography, the safest area to stick a shovel in the

ground for a control was deemed to be within the actual site area checked by the French Army de-miners who assisted ICTY field teams with the site.

To select a location for the control within the limits of the cleared area, I tried to envision a spot where no blood would have been shed. Shell casings were scattered randomly along the length of the trail through the site. However, in one area near the sampled basin a high concentration of shell casings was found. I believe that this was the spot where the executioners stood as they shot most of their victims. The type of shell casings found were representative of the type used in AK-47 assault rifles. AK-47s, when fired, eject their shell casings about a meter to two meters forward and to the right. Based on this knowledge, the direction of fire from this spot was north-northeast, toward the location where skull fragments and other bone were found. Thus, the control pit was dug on the spot where the executioners were believed to have been standing. At the time of sampling, it was assumed no one was shot in the immediate area where the executioners probably stood. However, this may not be the case.

In addition to someone being shot in the immediate area of the control pit, there are two other explanations for the presence of human blood proteins in the control samples. First, a wounded, bleeding person may have passed over the area and deposited blood into the soil as he was being led along the trail down the hill. The second possibility is that the control samples were contaminated by either the shovel or the trowel used to take them. As discussed in the methods section, both the shovel and trowel were washed between each sample. However, contamination cannot be ruled out. Nevertheless, the possibility that human blood may indeed be present in the control pit sample has convinced me to view the control as another sample of the execution site.

## **Volatile Fatty Acid Analysis**

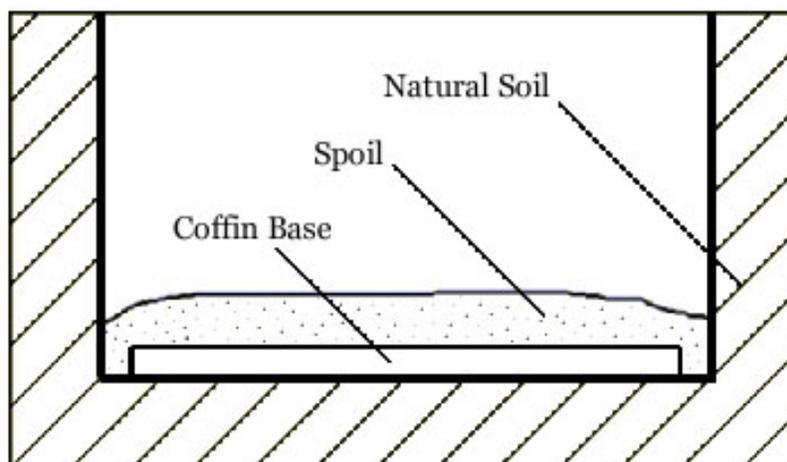
### **Duz Cemetery Materials and Methods**

Soils were sampled from four individual graves in the Duz village cemetery near Podujevo in northeast Kosovo. ICTY field teams exhumed the majority of the bodies, alleged victims of war crimes, on September 25 and 26, 2000. On October 10, 2000, a total of six soil samples from the bottom of four graves, and one control sample from surface soil in the cemetery, were gathered. Individual graves were chosen for sampling based on the burial style and the state of preservation of the body within the grave. The Duz cemetery had two types of burials—traditional Albanian style and coffin style. In both styles, either commercial body bags or cloth or plastic wrappings were placed around the bodies. A wide range of body preservation related to the burial style and body wrappings was noted in the Duz cemetery, with extremes running from almost completely skeletonized to partially mummified bodies, and to saponified bodies.

To understand the context of the soil samples from Duz, some information must be given on burial and ICTY exhumation methods. In traditional Kosovar Albanian burial practices, the dead are placed in the grave on a type of low wooden pallet, called a *tabut*, if one is available. A series of boards, or *drassa*, are laid at an angle from the top of one edge of the side of the grave, near the surface, down to the opposite bottom of the grave, forming a triangular chamber in which the body lies. As long as the walls of triangular chamber remained intact and did not collapse on the body, the traditional burial usually offered a larger compartment for the body to lay in. Coffins uncovered by ICTY field teams in the Duz cemetery, as in most of Kosovo, were simple pine constructions. The lids of the coffins were often found to have warped or collapsed in on the body

during the year to two-year of use. Because ICTY provided new coffins to families when bodies were returned after examination, the old coffins, *tabut*, and *drassa* were removed from the grave at the time of exhumation. However, due to poor construction quality, many coffins fell apart as they were removed. The base of the coffins often remained wedged in the bottom of the grave. These bases were left in the bottom of the grave on the assumption that new coffins would simply be placed on top of the old coffin bases.

In burials where body bags were used, if the bag had not decomposed over time or been punctured, decomposition fluids from the body would gather within the body bag, causing the bag to balloon. Decomposition fluid had to be drained from the body bag as the body was exhumed. This fluid pooled at the bottom of the grave in the coffin. To keep odor to a minimum and allow removal of the coffin sides without slipping on the decomposition fluid, ICTY field team members covered the pooled fluids with a layer of spoil left over from digging the grave. As a result of throwing spoil onto the bottom of the coffin to soak up the decomposition fluid, the bottom of graves that used coffins and contained un-punctured body bags had three base layers: spoil on top, the coffin base, and then the natural soil base (Figure 3).



**Figure 3: Bottom of Coffin Style Burial with Spoil and Coffin Base**

In the Duz cemetery, soil was sampled from four graves—one traditional style and three coffin style burials. Table 4 illustrates the burial style, conditions, and number of samples (and sample designation number) taken from each grave. Natural soil bases from all the graves sampled were handled in the same way. The shovel and trowel were washed with water between each sampling. Spoil handling is discussed below.

**Table 4: Duz Cemetery Burial Conditions and Grave Samples**

Grave	# Samples (ID)	Burial Style	Body Wrap	Burial Condition
1	2 (samples 1a, 1b)	coffin	body bag	wet, body bag unpunctured
2	2 (samples 2a, 2b)	coffin	body bag	wet, body bag unpunctured
3	1 (sample 3)	coffin	body bag	dry, body bag punctured
4	1 (sample 4)	traditional	blanket	dry, no body bag

The traditional style burial had *drassa* but the *tabut* was absent. The body was fully wrapped in a blanket and placed on the ground at the bottom of the grave. During exhumation it was noted that the grave was dry and the body was light in weight, indicating it was either mummified or partially skeletonized. Most bodily fluids had already leached out and into the surrounding soil. As per standard procedure, if nothing appeared to be out of place within the burial, the body was not inspected in the field, but placed in a body bag and transported back to the mortuary for examination. Soil was sampled from the center of the grave using a clean shovel and trowel. An area approximately 3 cm deep and 8 cm wide was removed and placed in a plastic evidence bag.

The three coffin style burials contained bodies in body bags. One of these coffin burials had a punctured body bag. The grave was dry, as decomposition fluid had previously leached out and the body was likewise noted to be very light. The two other coffin burials had un-punctured body bags that required draining and spoil to soak up the

decomposition fluid. Two soil samples were gathered from each of the un-punctured body bag graves, one from the spoil above the coffin base and one from the natural soil bottom, under the coffin base. The spoil was sampled in a slightly different manner than the natural soil bases. A soil depth of 5 cm was measured from the coffin base up. Soil above 5 cm was discarded and a 5 x 5 x 5 cm amount of the remaining spoil was gathered and placed into plastic evidence bags. Spoil samples from Graves 1 and 2 are designated as 1a and 2a, respectively. Samples from below the coffin base of Graves 1 and 2 are designated as 1b and 2b (Table 4).

Soil was sampled from the spoil in Grave 1 (Sample 1a) and Grave 2 (Sample 2a) with the expectation of finding VFAs. The other samples from the cemetery were viewed as the unknown elements of the study. The spoil as well as the natural base of the grave was sampled, assuming that the spoil would have soaked up decomposition fluids, and thus VFAs, before they had the chance to seep through the coffin bases and into the natural soil. If this was the case, and the natural soil did not have enough exposure to the decomposition fluid, then the spoil would at least demonstrate the ability to detect VFAs in soil.

All samples were air dried indoors over a 12-hour period. The untouched, inside surface of Ziploc bags were again used as the sample-drying surface. After repacking the dried samples in new plastic evidence bags, they were stored out of direct sunlight until analyzed.

Approximately ten grams (10 g) of soil from each of the six samples were analyzed for detectable amounts of volatile fatty acids (VFAs) using a microFAST GC<sup>2</sup>

(a fast, portable gas chromatograph, developed at Louisiana State University's Institute for Environmental Studies), and a mass spectrometer (MS).

### **Knin Mass Grave Materials and Methods**

During April and May, 2001, the ICTY field team located and exhumed a small mass grave in a cemetery near Knin, Croatia. The mass grave was approximately three meters deep and contained 19 bodies. Upon completion of the exhumation on May 2, four soil samples from the base of the grave were gathered. The removal of adjacent soil directly to the north and east of the mass grave during the excavation of the grave allowed for sampling of controls from the same level as the base of the grave. One control was sampled approximately 30 cm north of the grave, the other control approximately 30 cm from the east.

Four new, sterilized trowels were used to sample soils from the grave, one trowel for each sample. A single trowel was used to gather the control samples and was washed with water prior to each sampling. All samples were placed in plastic evidence bags and stored in a refrigeration unit for three days until they were removed and air-dried.

Air-drying took place out of doors. New plastic garbage bags were cut open and placed, untouched surface side up, in the bottom of six new buckets, one bucket for each soil sample. The buckets were used to help eliminate any foreign material from being blown onto the samples. The samples were poured into the buckets onto the plastic sheets, allowed to dry for one hour in the hot summer sun, and were repacked into new evidence bags and stored out of direct sunlight until analyzed. Unfortunately, one of the samples had not dried completely and green mold was discovered in the evidence bag

when it was opened to measure out a sample. The moldy sample was not analyzed, reducing the number of samples from the mass grave to three.

Approximately ten grams (10 g) of each soil sample were measured and analyzed for detectable amounts of VFAs using a microFAST GC<sup>2</sup> and mass spectrometer (MS).

### **Volatile Fatty Acid Background and Analysis**

Lipids are inherently more resistant to the degradation effects of time, temperature, and groundwater infiltration than are proteins (Evershed and Tuross 1996). The superior preservation of lipids over proteins in ancient artifacts and soils may reflect the greater vulnerability of protein to the effects of heat during cooking and to microbial attack, and the greater hydrophobicity of lipids. A number of methods, including thin layer chromatography, infrared spectroscopy, and gas chromatography (GC) have been used to detect lipids in archaeological ceramics and soil (Evershed 1993; Evershed and Tuross 1996; Nolin et al. 1994). Given that the human body produces lipids, lipids or their by-products, volatile fatty acids (VFAs), leached out of a cadaver could potentially be used as evidence in criminal investigations.

VFAs are short-chained carboxylic acids produced by a series of microbial-induced oxidative and reductive reactions of lipids; reactions that are catalyzed by aerobic and/or anaerobic bacteria during the decomposition of a body. The successful use of gas chromatographs to identify the presence of VFAs has led to the development of sampling methods for locating buried bodies (Ashton 2000). In addition, quantification of VFAs can be used in time since death determinations (Vass et al. 1992).

Vass et al. (1992) established a method for determining time since death of an individual by investigating the presence and concentration of certain VFAs in soil

associated with test cadavers placed on the surface of the ground. VFAs leached from the bodies during decomposition were assumed to amass in the soil under the bodies. Soil solution, the liquid phase between soil particles, was the focus of the research. Soil samples were weighed and deionized water added in a 2:1 (water:soil) ratio. The researchers then vortexed and centrifuged the samples, after which the samples were left to settle. The resulting solution was extracted and injected into a Shimadzu 9A GC equipped with a flame ionization detector (FID). Analysis of the data demonstrated patterns of VFAs in the soil solution that could be used to estimate the maximum time since death.

Ashton (2000) modified Vass et al.'s technique while researching a sampling method to locate clandestine burials. Using VFA-spiked soil, soils from forensic exhumations, and soils involved in a ground-surface decomposition experiment, Ashton examined soil solution with a microFAST GC<sup>2</sup> equipped with two FIDs, a Hewlett Packard 5890 Series II GC with FID, and a HP 5890 Series II GC with a 5971 Mass Selective Detector (MSD). The soil solution extraction method differed from that of Vass et al. in that after water was added to the soil samples, the solution was pH adjusted until ionization equilibrium was met. Unionized VFAs will separate from ionized particles in the solution allowing for easier selectivity, extraction, and analysis.

There are 41 types of VFAs. However, only seven VFAs are water soluble and thus detectable in soil solution. Two of the seven water soluble acids, formic and acetic, are too abundant in nature to be useful for this kind of research. Of the remaining five acids, caproic and heptanoic are only found in significant amounts during winter months

when the temperature is below 10° C. This limited the focus of the Ashton and Vass et al. studies, as well as this study, to three specific VFAs: propionic, butyric, and valeric.

GC analysis of pH adjusted soil solution was conducted on the Duz cemetery and Knin mass grave soils in the following manner. All soil samples were weighed individually into 40 mL volatile organics vials (VOA vials). After 15 mL of deionized water were added to each vial, they were shaken by hand to mix the solution. The vials were then placed in a sonic bath for 15 minutes to settle the larger soil particles. The water phase was then transferred to another vial using a disposable pipette and 10 mL of dichloromethane (DCM) was added. A dropper was used to add a solution of 37% hydrochloric acid to each vial until a pH level of 3 or lower was obtained. The vials were sonicated again for 15 minutes. Using disposable pipettes, the DCM phase was removed from the vials and filtered through sodium sulfate as the phase was transferred to smaller vials. The samples were concentrated to approximately 1 mL using a gentle stream of nitrogen gas. The samples were then capped with PTFE lined caps and stored in a refrigerator until analysis. Table 5 and Table 6 demonstrate Munsell colors, soil weights, gas volumes, and adjusted pH levels of each sample from the Duz cemetery and Knin mass grave, respectively. The Munsell colors were added on this chart only for descriptive purposes and are not assumed to at this time to have influenced the results.

Calibration standards were prepared from stock solutions of acetic, propionic, butyric, iso-butyric, valeric, and iso-valeric VFAs. The concentration range was 3, 6, 12, 25, 50, and 100 µg/mL. The calibration standards were used to establish a detection limit and identify the retention times of the acids during instrumental analysis.

**Table 5: Duz Cemetery Sample Weights, Final Volumes, and pH Levels**

Sample ID	Munsell Color	Weight of Soil (g)	Final Volume (mL)	Adjusted pH
1a	10YR 4/6	9.76	1.06	3
1b	2.5Y 5/4	9.92	1.09	2
2a	10YR 5/4	9.95	0.96	3
2b	2.5Y 5/4	10	1.04	3
3	10YR 5/6	10	1	3
4	10YR 5/4	10.04	0.88	2
Control	10YR 4/4	9.89	0.98	2

**Table 6: Knin Mass Grave Sample Weights, Final Volumes and pH Levels**

Sample ID	Munsell Color	Weight of Soil (g)	Final Volume (mL)	Adjusted pH
1	10YR 7/2	9.89	0.94	1
2	10YR 7/1	9.93	1.12	1
3	10YR 7/2	9.83	1.1	1
Control 1	10YR 6/4	9.74	0.92	1
Control 2	10YR 6/4	10.87	0.96	1

Initially, the calibration standards and sample solutions were analyzed using a microFAST GC<sup>2</sup> with two FIDs. Any samples producing a “hit,” as well as the controls from each site, were re-analyzed on a HP 5890 II GC coupled with a HP 5971 MSD for confirmation. In addition, unidentifiable peaks in any of the samples were also scrutinized using the HP MSD.

### **Duz VFA Analysis Results**

Soil solution from the seven soil samples was extracted and examined for VFAs using the microFAST GC<sup>2</sup>. Only one sample out of the seven contained detectable levels of VFAs. This was sample 1a from the spoil in Grave 1. VFA concentrations, as well as the peak area (the measurable area of “hit” represented by a peak in the results), final volume, volume injected and weight extracted, for this single sample are given in Table 7. As noted on the table, two types of VFAs were found in this single sample, iso-butyric

and valeric acids. When Sample 1 was analyzed, however, the HP MSD analysis of Sample 1a was unable to separate any VFAs from the DCM solution.

**Table 7: Duz mircoFAST GC<sup>2</sup> results**

Sample ID	Peak Area	F.V. (µL)	Vol Inject (µL)	Wt. Extracted (g)	Concentration (µg/kg)	VFA
1a	61724	1060	10	9.76	412	Iso-Butyric
1a	25060	1060	10	9.76	159	Valeric

### Knin VFA Analysis Results

In the Knin mass grave, four soil samples and two controls were taken. One grave sample developed mold on the inside of the evidence bag during transit and was not used in this study. Soil solution was extracted from the remaining soils and examined with a microFAST GC<sup>2</sup> for VFAs. Results of the examination are listed in Table 8. Two of the three samples contained detectable levels of VFAs: Sample 2 and 3. Sample 3 contained two types of VFAs, iso-butyric and iso-valeric. Sample 2 contained only iso-valeric.

**Table 8: Knin microFAST GC<sup>2</sup> Results**

Sample ID	Peak Area	F.V. (µL)	Vol Inject (µL)	Wt. Extracted (g)	Concentration (µg/kg)	VFA
2	1668	1120	10	9.93	11.5	Iso-Valeric
3	1352	1100	10	9.83	9.3	Iso-Butyric
3	1067	1100	10	9.83	7.3	Iso-Valeric

Interesting HP MSD results were obtained from the Knin samples. Similar to the Duz sample, the targeted fatty acids (propionic, butyric, and valeric) could not be detected due to co-elution with the solvent (ie., the VFAs could not be separated from the DCM). However, in Samples 1, 2, and 3 from Knin several other fatty acids were identified: capric, lauric, myristic, palmitic, stearic, and oleic. Of these six fatty acids, myristic, palmitic, stearic, and oleic acid are common in animal fats. It is likely that these

fatty acids were associated with decomposing bodies given the circumstances under which the soils were collected. These acids were not detected in the soil controls.

## Discussion

### Stutiča CIEP Samples

CIEP results of the one-and-a-half-year-old human blood deposits in soil from Stutiča, Kosovo, were better than expected. CIEP blood protein analysis of ancient stone tools from archaeological finds regularly produces 25-30% positive results (Kooyman et al. 1992; Newman and Julig 1989; Petragila et al. 1996). The Stutiča soil CIEP tests returned a 61% positive ratio (Table 9). As noted in the results section, three of the control pit samples returned positive readings. The results of the control pit samples have been included in Table 8 as if they were normal test pit samples.

**Table 9: Stutiča Site CIEP % Ratios**

Result	# of Samples	Percentile
Positive	44	61%
Negative	28	39%
Total	72	100%

The positive Stutiča findings should be encouraging to crime scene investigators. Police departments rarely test soils suspected of containing blood after a few months time, assuming microorganism activity would consume deposited protein in a short amount of time (personal communication, Louisiana State Crime Laboratory personnel 2001). Similarly, critics of immunological testing of archaeological artifacts have challenged the idea that blood protein survives over long periods of time (Cattaneo et al. 1993; Eisele et al. 1995; Fiedel 1996; Leach and Mauldin 1995).

In defense of immunological testing of ancient blood proteins, a number of other studies demonstrate that these proteins can endure for long periods of time (Allen 1995; Hyland et al. 1990; Kooyman et al. 1992; Newman and Julig 1989; Yohe et al. 1991;

Zimmerman 1973). The present analysis of the Stutiča soils demonstrated that enough protein microstructure remains intact in soils of high clay content after at least a year-and-a-half, and quite probably longer, and that these proteins can be identified if carefully examined. The survival mechanics of blood proteins in the Stutiča samples are not fully understood but may have to do with the process of protein degradation, soil matrix, and microstructure of the proteins themselves.

In thermal experiments with amino acids, the building blocks of proteins, Dungworth et al. (1975) demonstrated rapid degradation of the acids followed by stability, suggesting that only the most stable components survive the hostile environment outside the body. Similarly, Loy and Hardy (1992) suggested that degradation of protein occurs rapidly as blood dries but then becomes more stable, degrading at a substantially slower rate from then on. In a study of surgical tools from the American Civil War, Newman et al. (1998) demonstrated that biologically active protein and DNA are still detectable after more than 130 years of exposure to the atmosphere. Tests on denatured protein and extensively washed bloodstains provide evidence of the resilience of blood proteins, even when attempts are made to conceal them (Lee and DeForest 1976). Studying buried stone tools, Cattaneo et al. (1993) postulated that the amount of blood on the artifacts, good drying condition prior to burial, and the type of matrix in which the artifacts are buried are all critical elements in preservation.

Soils high in sand and clay appear to help preserve protein from microbial attack better than other matrixes (Cattaneo et al. 1993; Ensminger and Giesecking 1942; Loy 1983; Pinck and Allison 1951). Loy (1983) suggested that positively charged blood proteins bind to negatively charged silica particles in clay and that this action helps to

protect proteins from microorganisms that would feed upon them. Other studies indicate that the types of clays that provide the best protection for proteins are clays with a high-base exchange, such as illites and smectites (Ensminger and Gieseking 1942; Pinck and Allison 1951; Rice 1987). In addition, clay morphology may protect protein molecules upon absorption by orienting the molecules in a manner that makes them inaccessible to microorganisms (Ensminger and Gieseking 1942).

The Louisiana State University Soil Characterization Laboratory carried out soil profiles of Test Pit 2, levels 3 and 6, from the Stutiča site (Table 10). Mineralogy of the soils was characterized by approximately equal proportions of smectite and chlorite clays with a small amount of mica. An unusual phenomenon is the absence of kaolinite in the soils. However this absence is not viewed as having an effect upon the preservation of blood proteins in the soil. Table 10 indicates that the phosphorus levels of the samples are low. Such low levels are typical of forest soils such as the Stutica site. Soil class for Level 3 and 6 are clay and silty clay loam, respectively.

**Table 10: Stutiča Site Soil Profile**

<b>Test Pit 2 Sample</b>	<b>pH</b>	<b>Phosphorus mg/kg soil</b>	<b>Clay %</b>	<b>Silt %</b>	<b>Sand %</b>
<b>Level 3</b>	6.9	3.7	46.5%	34.5%	19.0%
<b>Level 6</b>	6.8	1.6	39.0%	40.8%	20.2%

As blood proteins are water soluble, proteins in soil are potentially susceptible to dissolution and degradation by groundwater. However, evidence exists that protein aggregation may be taking place, helping proteins survive. By binding together, proteins form higher molecular weight and are therefore less soluble (Benjamin et al. 1984; Lowenstein 1993; Prager et al. 1980; Sensabaugh et al. 1971;). Proteins may also bind

with insoluble fatty acids, thus protecting them against groundwater dissolution (Hayland et al. 1990).

All of the possible preservation mechanisms mentioned—a large quantity of blood, insoluble fatty acids, and high clay soil content—either were present or were potentially present at the Stutiča site. Seven to eight individuals were killed and an unknown number wounded at the site. Areas sampled contained human skull fragments indicating the victims sustained massive trauma to their heads. A lot of blood would have been deposited in the sampled areas as a result of these wounds. It is also suspected that the dead remained where they had fallen for a long period of time. Survivors were marched out of the area by the Serbs and did not return until NATO drove Serbian forces out of Kosovo. An intact, unmarked first cervical vertebra was located at the site, suggesting that it became detached from the body, not through the trauma of a gunshot or carnivore activity, but by the slow process of decomposition. If extended decomposition of the bodies occurred before they were removed, insoluble fatty acids as well as blood would have been deposited in the soil, perhaps allowing the two to bind. A search for insoluble fatty acids was not conducted with the Stutiča samples, although these results suggest that such a search would yield interesting results. And finally, the Stutiča soils have high clay content. It is possible that all of these preservation factors, or a combination of some of these factors, helped the Stutiča blood protein survive over a long period of time. Investigators should take note of the positive results found at Stutiča and be encouraged to test soils from suspected murder/execution sites for blood evidence regardless of the amount of time between the murder event and the sampling.

## **Duz VFA Samples**

Because lipids are known to be better preserved than proteins (Evershed and Tuross 1996), it was assumed GC/MS testing for VFAs in the soil from the four Duz cemetery graves would produce strong, positive results. However, only a single sample had high enough concentrations of VFAs to be detectable. Furthermore, the concentrations from the single sample, Sample 1a, were of VFAs not naturally deposited in soils. That is, Sample 1a came from the spoil thrown into the base of the coffin to soak up decomposition fluid.

Curiously, although VFAs were found in Sample 1a, none was found in the spoil of Grave 2, Sample 2a. Because the situation was analogous to Grave 1—body bags contained fluid, which was released during exhumation and soaked up with spoil—I expected both samples to have the same results. According to Ashton (2000), recovery of water soluble VFAs is low in soils with moisture content higher than 25%. Because all samples in this study were dried, the moisture levels of the site soils are unknown. It is possible, though unlikely given the close proximity of the two graves, that Sample 2a had a higher moisture level than Sample 1a, thereby diluting the water-soluble acids to a point they could not be detected.

Graves 3 and 4 were the real test of naturally deposited VFAs in soils under bodies. Grave 3 was a coffin burial with a body in a body bag. The body bag had previously been punctured or otherwise had decomposed to the extent that all fluids associated with decomposition had leached away—the coffin and the burial area in general were dry. Grave 4 was a traditional Albanian style burial in which the body was wrapped in a blanket and placed on the bare earth at the bottom of the grave. Grave 4

was likewise dry, indicating fluids had previously leached out of the body. Both bodies were light in weight, being partially skeletonized or mummified. Testing of soil solution from these graves did not find VFAs in high enough concentrations to be detected by the instrumentation.

It is unknown precisely why VFAs were absent in the majority of samples at Duz. However, several possible explanations are offered. First, survivability over time could be a factor. Although the literature suggests that lipids such as fatty acids survive better than proteins (Evershed and Tuross 1996), it may be inaccurate to believe this survivability extends to VFAs. The time between death and burial could play a significant roll in VFA preservation. The time between death and burial is unknown. In most cases in Kosovar Albanian families following the NATO drive returned home to find family members and/or neighbors killed in their house, yards, fields or on the village streets. The bodies were often unburied and depending upon when in the conflict they were killed, could have had several weeks time to decompose. VFAs are released in the greatest amounts during early stages of decomposition (Ashton 2000; Galloway et al. 1988). It is possible that much, and perhaps most of the VFAs produced during decomposition had leached out of the bodies prior to their discovery and subsequent burial in the Duz cemetery.

Time between burial, exhumation, and sampling may also have an effect on VFA detection. The dates of interment of the bodies are unknown. Burial dates can only be estimated at about one year to a year and a half prior to exhumation. Despite this relatively tight date range, decomposition in the cemetery was quite variable, with partially skeletonized/mummified remains like those in Graves 3 and 4 and well

preserved remains like those in Graves 1 and 2. This variability of preservation is attributed to burial style and body type wraps; it may have had a profound effect on the recovery of VFAs. Leaching of VFAs from a body occurs in the active stages of decomposition (Galloway et al. 1989). It is quite possible Graves 3 and 4 deposited VFAs early, over a year prior to sampling. This long period of time may have had an effect on the retention and extraction of VFAs from sampled soil solutions.

Connected with timing between death and burial is A. K. Mant's (1987) observations of the effects exposure to air has on bodies during decomposition. Shortly after World War II, Mant examined underground coffin and non-coffin burials. Mant surmised the air around a body contained by the coffin accelerated decomposition. Both Graves 3 and 4 were noted to be dry upon exhumation. Grave 3, being a traditional style grave, offered an even larger chamber for the body to lie in than did coffins. Although the body in Grave 4 was in a body bag, the bag was punctured and allowed more exposure to the air within the coffin than did Graves 1 and 2. Indeed, Graves 1 and 2, with their bloated body bags, offered very little exposure to air. The advanced decomposition of Graves 3 and 4 may have contributed to the negative findings within these graves, as VFAs may have been produced, leached out, and dissipated at a faster rate than those of the first two graves.

A second factor that may have affected the results is the time between exhumation and sampling. The graves were exhumed on September 25 and 26, 2000, but sampling of the grave soils did not take place until October 10. During that two-week period, the graves were left open, uncovered. The open graves may have been exposed to moisture from rain or another unknown source. Water-soluble VFAs were focused on in this study

as previous research indicated that water-soluble VFAs were the only fatty acids likely to be found in soil solution. Because these VFAs are water soluble, water collecting at the bottom of the open graves could have had a negative effect, possibly degrading and/or dissolving them to a point where GC testing could no longer detect them. And as mentioned, if moisture content of the soil is above 25%, recovery of VFAs will be difficult. However, it should be noted that MS analysis failed to identify non-water soluble VFAs which would be more resistant to rain or other sources of water.

A third explanation for the negative findings may be attributed to the difference between my sample and Vass'; the latter's research was used as a model for this study. Vass et al. (1992) were able to recover VFAs from soil under bodies throughout the span of a year. However, the bodies used in the Vass et al. experiment were unclothed and placed on the surface of the ground where decomposition occurred uninhibited and very quickly. In my sample, all bodies were clothed and wrapped in some way prior to burial. The body in Grave 4 was in a heavy wool blanket. Perhaps this blanket and the individual's clothing prevented VFAs from being deposited in the soil. Similarly, the body in Grave 3 was clothed and wrapped in a body bag. While the plastic body bag would not have absorbed decomposition fluid, the clothes and even the wood coffin base could have absorbed VFAs as the body decomposed slowly over time in the grave.

Another difference from the Vass et al. study was the sample handling. While Vass et al. were able to collect samples, add deionized water, and place the solution on ice at the site before transporting them back to their laboratory, where the samples were further processed and frozen, the samples from the Duz cemetery and Knin mass grave were air-dried for storage and transportation. Similarly, Ashton (2000) used "fresh,"

VFA spiked soil and soils from actual forensic cases and decomposition experiments in her research. Although air-drying the Duz and Knin samples was done out of necessity for storage and transportation, as mentioned, it may adversely affect the preservation of VFAs.

Air-drying of the soil samples did add an interesting element to the study. Can water-soluble VFAs be found in dried soils? The answer appears to be yes. One sample from Duz did produce positive findings of VFAs, as did two of the three samples from Knin. Still, drying of the soils may prevent accurate, consistent results of soil solution GC testing and should be used only when necessity demands or after controlled experiments can be done to address the issue of preservation of VFA in dried soils.

The LSU Soil Characterization Laboratory profiled the soils from the Duz control and Sample 1b, the natural soil base in Grave 1 (Table 11). Mineralogy was similar to the Stutiča site; equal proportions of smectite and chlorite with a small amount of mica. Kaolinite was again absent. However, the Duz soil was much more sandy than the Stutiča samples. The control sample was classified as sandy loam while the soil from 1b is loam. The high sand/silt content, and subsequent lower clay content, of the Duz soil may be added as a final possibility for the disappointing GS/MS results. As previously noted, studies of soils with high clay content appear to attribute to blood protein preservation. Perhaps similar mechanisms are applicable to VFA preservation.

**Table 11: Duz Cemetery Soil Profile**

<b>Sample</b>	<b>pH</b>	<b>Phosphorus mg/kg soil</b>	<b>Clay %</b>	<b>Silt %</b>	<b>Sand %</b>
<b>Control</b>	6.6	4.3	15.5%	29.0%	55.5%
<b>1b</b>	6.3	1.5	20.0%	40.3%	39.7%

In sum, several elements could have contributed to the poor findings from the Duz cemetery, either individually or in combination. Time since death, burial and exhumation, time between exhumation and sampling, possible rain, soil moisture and clay/sand content, burial styles, and air-drying of the samples could all be factors in the negative results. However, it should be noted that two types of VFAs were found in high enough concentrations in one of the air-dried soil samples. This suggests that, with proper controls, testing for VFAs in soils might be a useful forensic technique. It certainly needs more investigation.

### **Knin VFA Samples**

Soil solutions tested from samples at the bottom of the mass grave in Knin, Croatia, produced more positive results than the Duz cemetery samples. Two of the three soil samples contained high enough concentrations of water-soluble VFAs to be detected by gas chromatography. Iso-valeric acid was found in both positive samples and one sample also contained iso-butyric acid. MS testing revealed capric, lauric, myristic, palmitic, stearic, and oleic non-water-soluble VFAs in all the grave samples.

The mass grave at Knin, like all mass graves, was a complex coalescence of many elements. A total of 19 bodies were exhumed from this grave, all in different stages of decomposition. Some of the bodies were in body bags, but most were not. As Mant (1987) noted in his analyses of mass graves from World War II, the bodies at the bottom and in the middle of the Knin mass grave were better preserved than those on top and to the sides of the grave. All bodies were clothed and commingled; elements that Mant also noted arrested decomposition. Despite the age of the six-year-old grave, some of the bodies exhibited remarkable preservation. To give an idea of this preservation, the last

item to be removed was a sizable item wrapped in plastic, assumed to be a body part. Upon opening the plastic a whole, recognizable hock of ham, rather nicely preserved for its age, was discovered. Added to the mixture within the grave were pieces of garbage, turf from the surface of the ground, explosives (hand grenades), and a large quantity of a substance suspected to be lime. In fact, several bags of this lime material were found within the grave.

Visual and olfactory examination of the dried soil samples from Knin indicates that they are associated with a grave. The samples are light gray in color (Munsell Soil Color Chart: 10YR 7/1-2), as opposed to the light yellowish brown color of the control soil samples from outside the grave (10YR 6/4). The grave samples also give off a pungent odor that anyone familiar with exhumations would recognize. However, the olfactory experience of an investigator would be difficult to qualify or quantify for presentation as evidence in a court of law. Hence, a method of testing the soil in a scientific manner to evaluate a crime scene is needed.

GC examination of the site soils indicated that two of the three samples did contain water soluble VFAs (Samples 2 and 3). However, Sample 1, despite looking and smelling of decomposition, did not have high enough concentrations of the targeted water soluble VFAs for detection. Perhaps other items in the grave, like the suspected lime substance, affected the results, or sampling of the soil in that particular spot did not contain enough water soluble VFAs to be detected. Clearly, visual and olfactory indicators of decomposition are not necessarily indicators that VFAs are present. They are only an indication that a body was present at one time on/in the soil.

GC analysis of the grave samples revealed several unidentifiable “hits” in the results. These “hits” were further investigated with the MS revealing them to be non-water soluble fatty acids. It is suspected that these fatty acids were adsorbed on the surface of the soil, most likely the finer clay particles, that remained in suspension during the extraction procedure. There is currently very little research about the full range of fatty acids regarding their detection and use as indicators for clandestine graves and/or past crime scenes.

Of note when comparing the positive Knin sample results with the rather poor results from the Duz cemetery results is the soil similarities. The LSU Soil Characterization Laboratory profile of the Knin Sample 3 soil (Table 12) is relatively comparable to the Duz grave sample that produced negative results. Mineralogy is again very similar, but this time kaolinite is present in amounts approximate equal to the smectite and chlorite. A small amount of mica is also present. The pH and phosphorus values between the Knin and Duz sites are similar. Both soils were classified as loam, and both have higher sand and silt content than clay. In fact, the Knin soil has a sand content greater than 50% (Duz contains 39.7%), suggesting that VFA preservation and detection is not reliant upon high clay content in soil. However, one must keep in mind that the Duz cemetery and Knin mass gravesites are vastly different. Leaching of VFAs may have already stopped or at least vastly slowed in the Duz graves, while in the Knin mass grave, bodies were in a wider range of decomposition. The number of bodies and style of burial probably play such a large roll in the creation and preservation of VFAs that the two sites should not be directly compared. More investigation into soil and burial style variables are needed to fully understand GS/MS VFA detection.

**Table 12: Knin Mass Grave Soil Profile**

<b>Sample</b>	<b>pH</b>	<b>Phosphorus mg/kg soil</b>	<b>Clay %</b>	<b>Silt %</b>	<b>Sand %</b>
<b>3</b>	8.0	1.6	14.5%	33.6%	51.9%

Investigators should be encouraged that most samples from the mass grave did produce positive findings and that combined GC/MS detection of VFAs in soils should be considered when evaluating a possible crime scene. Since GC tests for water soluble VFAs in both the Duz cemetery and Knin mass grave did produce negative results in some of the soils sampled, multiple samples of suspected crime scene soils should be taken and examined to avoid possible sample error. If during GC testing, unidentifiable peaks are found, then the sample soil solution should be tested using MS instrumentation. Special attention should be given to the identification of possible non-water soluble acids within the solution.

## **Conclusion**

Soils from a known execution site were examined for human blood proteins using the immunological test, cross-over immunoelectrophoresis (CIEP). Although blood was deposited in the soil one-and-a-half-years before sampling, 61% of the samples returned positive results. The large amount of blood spilled and the soil matrix are believed to be the two main contributing factors to the high percentage of positive results.

Soils from the base of four individual graves in a cemetery and soils from the bottom of a small mass grave were examined for volatile fatty acids (VFAs) using a miroFAST GC<sup>2</sup> (a fast, portable gas chromatograph) and mass spectrometer. For the individual graves only one sample out of six returned positive results for water soluble VFAs. The low recovery rate of VFAs from the cemetery could be attributed to a combination of time since death, burial, and exhumation, burial styles, soil moisture and clay/sand content, possible rainwater run off, and sample handling. Results from the Croatian mass grave samples were more conclusive, with two of the three samples returning positive results of water soluble VFAs and all samples returning positive results for non-water soluble VFAs. It may be that there is insufficient FVA preservation in individual graves over a year old, but a mass grave, with its large number of bodies/body parts, may provide substantially better VFA retention. Mass graves produce differential body preservation with bodies on the periphery decomposing faster than bodies in the middle and bottom. As decomposition is progressing at a much slower pace than in individual graves, the possibility of VFA preservation and detection may be greater.

Neither CIEP nor GC/MS testing is new to forensic investigations. However, investigators often overlook or ignore murder scene and body concealment/burial site

soils as testable material, especially if potential deposited blood or VFA residue is suspected of being a few months or even sometimes a few weeks old. The success of CIEP testing of older blood deposits in soil and GC/MS testing for deposited VFAs in this study should encourage investigators to examine site soils regardless of the time between deposits and sampling. This is especially true for GS/MS soil analysis where little research has been undertaken for body location using fatty acid identification. Future testing of crime scene soils for older deposits of blood proteins or VFAs, with an eye toward developing information on the variables responsible for preservation or degradation (e.g., soil types, climate, weather conditions, and other variables) eventually could provide us with a practical, working knowledge of where and when to test for these elements.

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## **Vita**

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