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May 1989

THE EFFECTS OF ENVIRONMENTAL CONDITIONS ON BLOOD DETERIORATION AND BLOOD DETECTION IN TENNESSEE

A Thesis

Presented for the

Master of Arts

Degree

The University of Tennessee, Knoxville

Frederick J. Snow

May 1989

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ABSTRACT

While blood is frequently present at the scene of a violent crime, little is known of its ability to survive in an outdoor environment. Most studies have been conducted in a laboratory, which is free of precipitation, severe temperature fluctuations and other environmental factors. It is the intent of this thesis to examine the cumulative effects of environmental conditions on blood deterioration and blood detection.

Three units of whole human blood were distributed on six different surfaces commonly found in an outdoor forensic situation. Three catalytic tests and one confirmatory test were used to determine the length of time blood was detectable on each surface. Gross physical changes in blood appearance were noted.

It was found that blood which is completely dry prior to the first rainfall is often long-lived even when only slightly protected from the elements. Differing lengths of time in which blood was detectable is attributed to surface texture, length and amount of rainfall, inclination of the surface and protection from the elements.

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CHAPTER I

INTRODUCTION

A problem for forensic investigators is the ability to detect blood at a crime scene if the body has been moved or if the scene has been subjected to numerous environmental factors. Unlike bones or teeth, which often remain recognizable for years, blood frequently retains its characteristic bright red color for no more than a few hours. In time, it begins to turn jelly-like indicating the formation of fibrin (Cunliffe and Piazza 1980). As it dries, blood turns dark red to reddish-brown and even black. This drying and subsequent color change often causes it to blend into the substrate where it was deposited or to be mistaken for some other substance such as paint or rust (Glaister 1966). This thesis examines the effects of environmental conditions on the detectability of blood in an outdoor setting and formulates some guidelines which will assist in its recovery.

Importance of Blood

While a variety of biological materials, such as hair, blood and saliva, are often present at the scene of a violent crime, blood is, perhaps, the most common and hence has been studied extensively for forensic

applications (Krishnan 1978). An adult has approximately nine pints of blood, and this blood may reveal much about the victim, the perpetrator and the manner in which the crime took place (Svensson and Wendel 1965).

One of the primary uses of blood in forensic science is for identification. Forensic identification establishes that a particular sample is blood, saliva or some other body fluid (Gaensslen and Camp 1988). It also determines the species of origin (Schleyer 1962, Coetzee 1958) and may isolate the part of the body from which the blood came (Whitehead and Divall 1974).

Second, blood may contribute to the reconstruction of a crime scene. The estimation of the age of dried bloodstains by spectrophotometric examination of the absorption spectra (Kind et al. 1972, Kind and Watson 1973) or by immunoelectrophoresis (Rajamannar 1977) may narrow the time frame in which the crime could have been committed. Blood droplet patterns may aid in the location of the victim at the scene, the direction of blows, evasive maneuvers taken by the victim to avoid the assault, and the number of blows struck (Svensson and Wendel 1965, MacDonell 1971). In addition, when the actual volume of a blood drop is known and its diameter on the impact surface can be measured, an indication of the height the blood drop may have fallen can be obtained (Pizzola et al. 1986).

The third major use of blood is for individualization. Individualization means that blood or some other body fluid can be identified with a particular individual. While current methods are not yet this precise (Grunbaum 1976), specific characteristics of blood or some other body fluid may limit the individual to a particular group from which the sample could have come. Some non-genetic markers include the presence of drugs or alcohol (Camps et al. 1976), venereal disease (Leister et al. 1964), rheumatoid arthritis (Leister and Kirk 1963) or carbon monoxide (Gettler and Freimuth 1943). Genetic markers, such as blood group antigens, hemoglobin variants, serum or plasma proteins, red cell isoenzymes and HLA system antigens, may further limit the group to which the individual may belong (Gaensslen et al. 1986). In addition, bloodstains may establish the sex of the individual (Brown 1981, Culliford 1959, Ishizu 1973), race (Blanc et al. 1971) and whether the victim was a child or an adult (King and Whitehead 1975). Differing densities and density distributions of dry blood samples may further individualize blood (Sylvia and Kirk 1961).

Survivability of Blood

Blood and other proteinaceous material have been shown to have extreme biological longevity. Ducos et al. (1962) report that biologically active proteins exposed to

the atmosphere for ten years have survived, and Keilin and Wang (1947) have shown that hemoglobin kept for 44 years at room temperature retained its oxygen-binding capacity, spectroscopic capacity and crystallization property. They found that hemoglobin in the blood had not changed appreciably and predict that since the preservation conditions of their samples were imperfect, these proteins probably have a life span far in excess of 44 years. More recently, Sensabaugh et al. (1971) have shown that in dried human blood kept at room temperature for eight years, eight of eleven globular proteins tested survived although they were modified somewhat. With modification, dried blood proteins become less soluble and more resistant to removal by water thus increasing their longevity.

Loy (1987) has recovered blood residues from prehistoric stone tools dating between 1000 and 6000 years old. By crystallizing the hemoglobin, he has been able, in some cases, to identify the species. D.C. Hyland (personal communication 1989) feels that blood residues are detectable for at least 10,000 years.

Of particular interest to forensic investigators are genetic markers that define the blood groups. While fresh blood is generally easy to type, dried blood is more difficult because of erythocyte disintegration. The ABO blood group, however, persists for as long as 50 years

(Holzer 1931) while MN antigens survive for at least 26 weeks (Denault et al. 1980). Certain antigens in the Kidd, Duffy and Kell systems are less stable (Gaensslen and Lee 1984). The determination of the blood group is particularly important in identifying the victim or the perpetrator of a violent crime.

Previous Studies

While individualizing characteristics such as genetic and non-genetic markers have been shown to survive for varying lengths of time, most of these studies have been conducted in a controlled laboratory environment (Keilin and Wang 1947, Sensabaugh et al. 1971, Kanter et al. 1986, Lincoln and Dodd 1968), Marsters and Schlein 1958). Less is known about the survival of these characteristics when subjected to the vagaries of nature. Olbrycht (1950) states:

> Our knowledge on this subject in spite of many investigations, is not yet too accurate, and the observations of many authors are different, sometimes very much so which is understandable considering different experimental conditions in investigating the influence of these harmful factors; the data obtained in laboratory experiments are the more unlike the real conditions that it is impossible to create in the laboratory the conditions similar to those influencing the bloodstains in nature.

It is known, however, that air, light, high temperatures and humidity have a deleterious effect on blood components (Cunliffe and Piazza 1980, O'Hara and Osterburg 1972).

Scheller (1937) conducted a series of experiments to determine the influence of weathering on blood. Blood was placed in glass dishes and on various wooden and metal surfaces which were than placed in the open, in a dry shed, in a basement and in a room. The stains were periodically examined microscopically, chemically, spectroscopically and by fluorescence analysis. Blood exposed to the weather in glass dishes was successfully detected after six months. Blood on smooth beech bark was completely washed away, but blood which collected in small rough areas was still detectable after eight months.

Blood is useful to forensic investigators although little is known of its ability to survive a variety of environmental conditions. A better understanding of gross physical changes blood undergoes in an outdoor environment and of its ability to survive is needed. This thesis examines some of these changes in blood characteristics and detectability.

CHAPTER II

BACKGROUND

Catalytic Tests

Catalytic tests have long been used by forensic experts as a preliminary sorting procedure for the detection of blood. Often referred to as presumptive tests, they rely on the peroxidase activity of hemoglobin or its derivatives, and are used primarily to locate invisible bloodstains or to verify that a suspected bloodstain is, in fact, blood (Gaensslen 1983). In the presence of an oxidizing agent, often hydrogen peroxide or sodium perborate, a colorless compound such as phenolphthalein or leucomalachite will slowly become colored (Figure 1) or, in the case of luminol, will luminesce (Figure 2). Heme, however, acts as a catalyst, and in its presence oxidation takes place rapidly (Kirk 1974).

Though highly sensitive to the presence of heme, catalytic tests are not entirely specific for blood (Gaensslen 1983). The two primary sources of false positive results with catalytic tests are strong oxidants and plant peroxidases. Oxidants may be detected by adding the test reagent before the test oxidant. If a positive result is observed it is generally considered evidence of an oxidant. Plant peroxidases, unlike heme, are heat

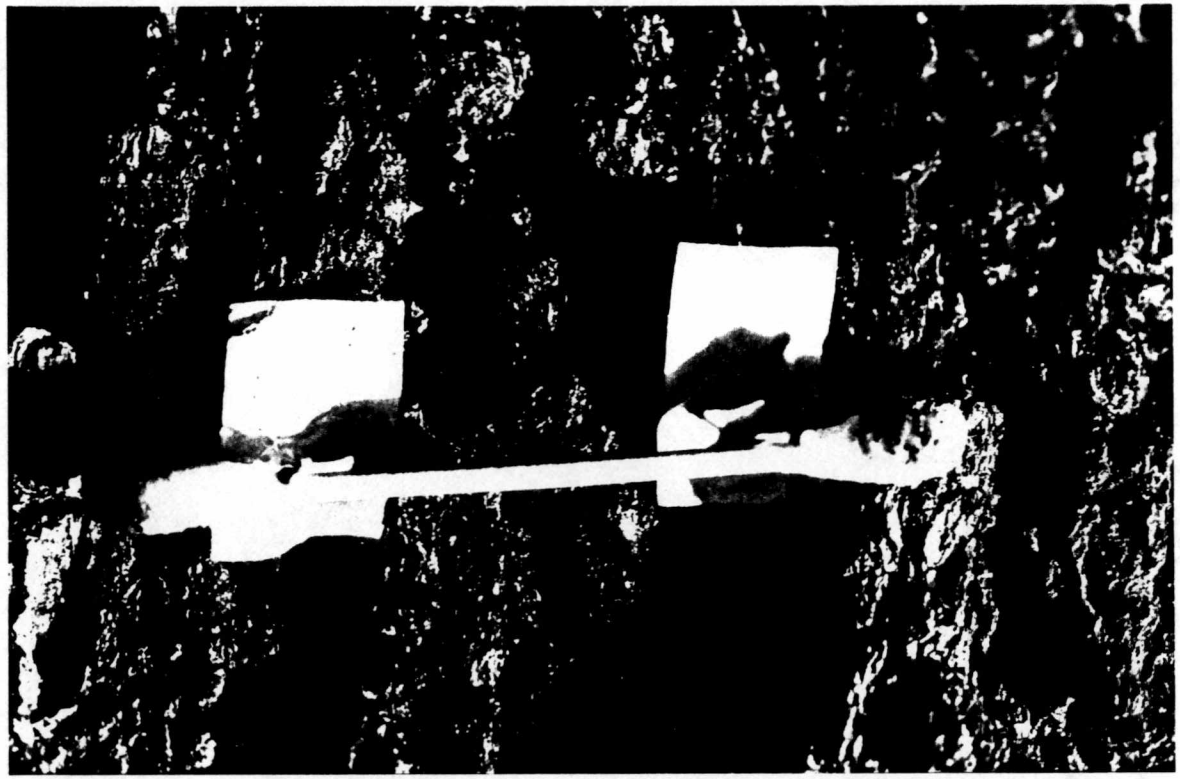

Figure 1. Cotton swab and filter paper showing positive leucomalachite test (left) and positive phenolphthalein test (right).

Figure 2. Oak tree showing positive luminol test.

labile and may be destroyed by heating to 100 degrees Centigrade for about 30 seconds (Fiori 1962). Samples testing positive after heating are generally considered positive for blood (Pinker 1934).

Pinker's study of false positive reactions with the phenolphthalein, leucomalachite and benzidine reagents failed to find any substance except blood that reacted with all three color tests. Grodsky et al. (1951) confirmed this by stating:

> ....there is no case on record in which any substance other than blood has given positive reactions with the four color and chemiluminescence tests described, nor is there any significant probability that this will ever occur if the tests are made by experienced personnel, by proper technique, and with the application of both alkaline and acid reagents.

Blake and Dillon (1973), however, state that certain microorganisms produce positive results with the benzidine, phenolphthalein and luminol tests and that they were often resistant to prolonged heating and drying. They feel that positive results with the presumptive tests should be interpreted with caution particularly with trace amounts and that immuno-chemical tests should be employed as a confirmatory method. Gaensslen (1983) feels that these conditions would seldom be encountered in routine forensic casework.

Leucomalachite test. Since its introduction in 1904 (Adler and Adler) the use of the leuco base of malachite green for the detection of blood has achieved widespread use by forensic investigators. The leucomalachite test compares favorably with the benzidine test in sensitivity ($1:10^5$ dilution of blood) although it is less sensitive than both the phenolphthalein and luminol tests (Grodsky et al. 1951).

Like all catalytic tests, however, the leucomalachite test is not specific for blood. Medinger (1933) reported that his tests on various plant extracts and physiological fluids gave no false positives when peroxide was added after the initial reagent. Two years later Alvarez de Toledo y Valero (1935) found that there are many chemical oxidants which will yield false positive reactions without peroxide, some of which are quite similar to blood. Hunt et al. (1960) found that leucomalachite gave strong reaction with potassium permanganate and other oxidants and weak positives with tomato juice and wet potatoes. Pinker (1934) found that leucomalachite reacts with both clean and rusty iron, cobaltous nitrate, ammonium carbonate, ammonium persulphate as well as a number of uncommon chemical substances. A number of these chemical substances may be eliminated by successive application of the reagent followed by hydrogen peroxide. The remaining chemical substances may be removed by chemical

manipulation. Vegetable peroxidases, Pinker contends, are easily destroyed by boiling for a few minutes and should not present a problem for the experienced investigator.

The leucomalachite reagent is prepared by mixing 1 gram of leucomalachite green in a solution of 100 cc of glacial acetic acid and 150 cc of distilled water. The test is conducted by applying the reagent to the suspected bloodstain followed by a drop of hydrogen peroxide using any of the test methods. A bluish-green color indicates the presence of peroxidase (Figure 3).

Phenolphthalein test. The phenolphthalein or Kastle-Meyer test (Kastle and Shedd 1901, Meyer 1903) is also a non-specific test for peroxidase (Smith and Simpson 1956). In the presence of peroxidase, phenolphthalein, which is colorless in alkaline solution, is oxidized by sodium perborate or hydrogen peroxide and turns pink or red.

The sensitivity of the phenolphthalein test has variously been reported as 1:10,000,000 (Hunt et al. 1960), 1:5,000,000 (Grodsky et al. 1951) and 1:100,000 (Sirchie Finger Print Laboratories, Incorporated 1985). The sensitivity of the phenolphthalein test is exceeded only by the luminol test, and of the three tests employed in this thesis, the phenolphthalein test is intermediate

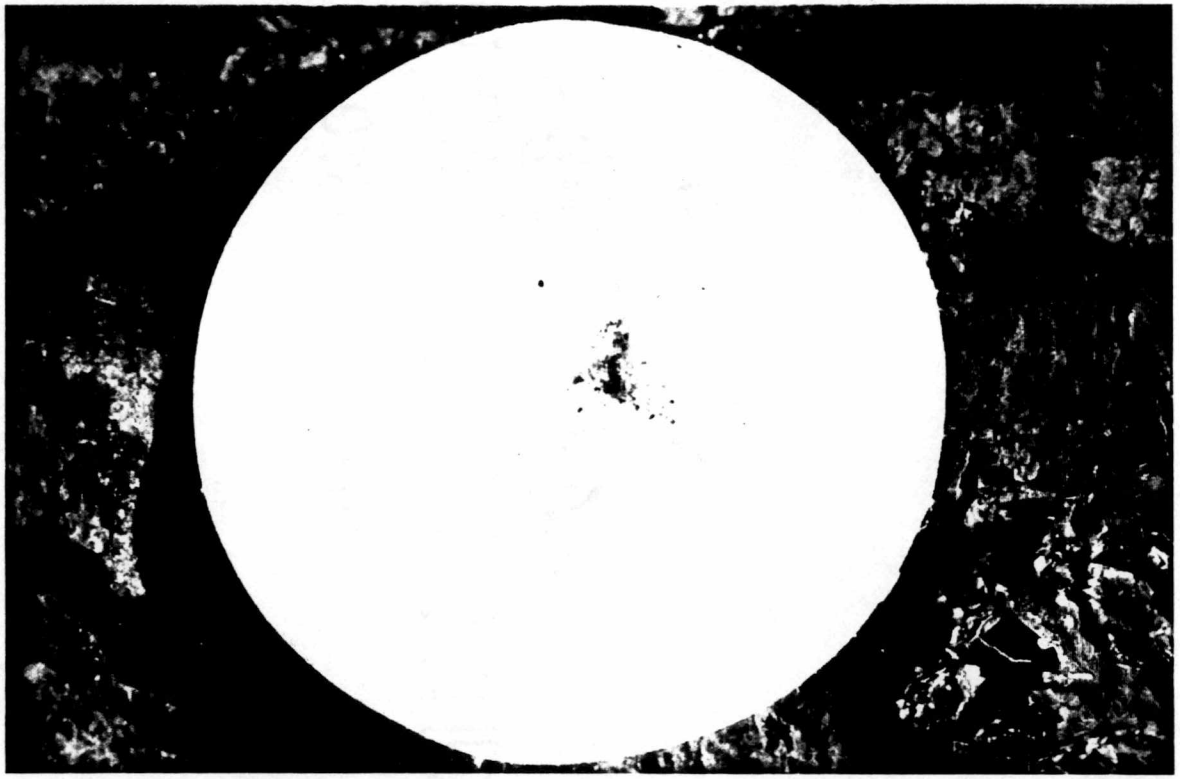

Figure 3. Filter paper showing bluish-green color indicating positive leucomalachite test.

in sensitivity between the leucomalachite test and the luminol test.

The phenolphthalein test, in addition, is more specific than either the more familiar benzidene test or the leucomalachite test. Glaister (1926), Hunt et al. (1960) and Gettler and Kaye (1943) obtained false positive results with copper salts. Pinker (1934) found that of all the materials which interfere with the benzidine test only prepared horseradish reacted with the phenolphthalein test. Even this substance can be easily distinguished from blood by a transient color. Certain vegetables may contain peroxidases that produce slight reactions, however they may be destroyed by heating or drying. Chemical substances such as cobaltous nitrate, ammonium nitrate and ammonium carbonate may produce false positive results, but they may be eliminated by chemical manipulation. As with luminol, phenolphthalein is an alkaline reagent and is less affected by enzymatic materials that affect the acid reagents such as leucomalachite and benzidine (Higaki and Philip 1976).

Despite the sensitivity and specificity of the phenolphthalein test, the reagent is difficult to prepare and to apply. The solution must be boiled two to three hours in a reflux condenser until colorless and kept in a dark bottle containing zinc dust (Kirk 1953). Hunt et al. (1960) found that the test is only satisfactory on

bloodstain extracts and that it failed to produce a positive result on smaller spots and with filter paper rubbings. Culliford (1971) states that the reagent becomes rapidly oxidized by air and will turn pink. The difficulty of preparation led to the purchase of commercially prepared ampules of the phenolphthalein reagent. One ampule contains 100 percent sodium perborate. The second ampule contains .82 percent phenolphthalein, .82 percent zinc, 16.39 percent sodium hydroxide and 81.97 percent distilled water (Sirchie Finger Print Laboratories, Incorporated 1985). The test is conducted by mixing the contents of the two ampules and applying the solution to bloodstain extracts, filter paper rubbings or by direct application. A bright pink or red color usually appears in three to five seconds in the presence of peroxidase.

Luminol test. The luminol test is used primarily to locate invisible blood and is characterized by chemiluminescence rather than a color change as the other catalytic tests. This luminescence requires its use in the dark, and it is frequently used where a rapid search of a large area for blood is necessary. The bluish-white glow emitted by luminol in the presence of hemoglobin or its derivatives allows the detection of otherwise

invisible bloodstains as well as the identification of their sizes and shapes.

Although it was first synthesized in 1902, it was not until Specht's (1937) work that luminol began to be used in a medico-legal application for the detection of blood. Five years later McGrath (1942) also recommended luminol for both clinical and forensic applications. Luminol is widely used today by most law enforcement agencies.

One of the primary advantages of luminol is its sensitivity. Proescher and Moody (1939) report a sensitivity of 1:100,000,000 and Grodsky et al. (1951) report a sensitivity of 1:5,000,000 depending upon the condition of the stain and the composition of the reagent. Luminol has the added advantage of reacting more strongly to dried and decomposed blood than fresh blood (Proescher and Moody 1939).

Luminol is one of the most specific presumptive tests. Grodsky et al. (1951) found that luminol is quite specific for blood with false positive results being obtained only with materials containing copper salts. Kirk (1974), however, states that luminol will react with both dandelion and poinsettia root exudate. Steigmann (1941) recommended that luminol be used for the investigation of iron and cobalt complexes, and Shevlin and Neufeld (1970) report that luminol reacts readily with potassium ferricyanide. Proescher and Moody (1939) state

that while luminol will react with manganese peroxide, gold chloride, coloidal platinum and several other inorganic substances, these are not commonly encountered in forensic casework.

It has often been claimed that luminol does not interfere with subsequent catalytic, confirmatory or serological tests (Specht 1937, Proescher and Moody 1939, Grodsky et al. 1951). Sirchie Finger Print Laboratories, Incorporated (1985) report that luminol is non-destructive for subsequent testing using either leucomalachite or phenolphthalein. Srch (1971) states that luminol interferes with neither the benzidine nor the precipitin test but may interfere with the Takayama test, with the inhibition method of ABO groupings and with ABH agglutinins using the method of Lattes. Judicious use of the luminol test is recommended.

Luminol was mixed according to the method given by Grodsky et al. (1951). The components are 0.5 gram of luminol (3-aminophthalhydrazide), 25 grams of sodium carbonate and 3.5 grams of sodium perborate dissolved in 500 ml of distilled water. The ingredients are kept in separate capsules and mixed immediately before use. As had been noted earlier by Grodsky et al. autoluminescence will develop if all ingredients are mixed simultaneously and the sodium carbonate dissolves slowly in water resulting in solubility problems. Following the

recommendations of Lytle and Hedgecock (1978), a more satisfactory solution was obtained by using sodium perborate/water (250 ml) and luminol/sodium carbonate/water (250 ml) that were kept in amber bottles at room temperature. This solution eliminated solubility problems inherent in mixing dry ingredients and was found to be stable for at least two months. Nevertheless, a fresh solution was prepared once every two weeks and the old solution discarded. The solution was applied directly to the suspected bloodstain.

Confirmatory Test

Upon receiving a positive result from one or more of the catalytic tests, the investigator usually employs a confirmatory test for the detection of hemoglobin. The Takayama test, devised by Masao Takayama (1912), is one of the better tests and depends upon the microscopical examination of pyridine hemochromogen crystals. Unlike the older Teichmann test (Teichmann 1853), the Takayama test does not require heating and is less sensitive to the presence of rust or tannins. When the blood is old and weak or insoluble, the Takayama test will sometimes fail in the presence of known blood (Olbrycht 1950). A negative Takayama test, therefore, does not necessarily indicate the absence of blood.

Takayama test. The Takayama test (Takayama 1912) is based upon the microscopic examination of hemochromogen, a derivative of hemoglobin. Though less sensitive than the catalytic tests, the Takayama test is more specific, and most authors (Glaister 1966, Lucas 1945, Schleyer 1949, Greaves 1932) feel that a positive Takayama test offers proof of the presence of blood. Hemochromogen crystals have been obtained from rusty knives 23 years old and from 22-year-old bloodstained linen (Mahler 1923). Kerr and Mason (1926) obtained positive results from blood on a razor 45 years old, from blood that had decomposed for four months and from a week-old stain on wood. Kerr and Mason, in recommending the Takayama test over the earlier Teichmann test, state that when the presence of blood had been proven by other means that the test never failed to give them a positive result.

Hemoglobin or its derivatives become increasingly insoluble with aging or when exposed to heat or adverse weathering conditions (Hammerl 1892, Sutherland 1907, Minett 1928, Haseeb 1972). Insoluble hemoglobin or its derivatives will not produce the characteristic salmon-pink crystals when subjected to the Takayama test even though blood may be present.

Blake and Dillon (1973) showed that pure peroxidase may yield crystals similar to blood while pure catalase yields crystals indistinguishable from blood. In

addition, certain microorganisms rich in both catalase and peroxidase were found to test positive using the luminol, phenolphthalein and benzidine tests. It was also found that unlike vegetable peroxidase, these bacteria are resistant to both heat and drying. None of the bacteria, however, contained concentrations of catalase or peroxidase high enough to produce a positive Takayama test. Gaensslen (1983) feels that bacteria which produce catalase in quantities capable of producing a positive Takayama test would not be encountered in routine casework.

Although both alkaline and acid solutions may be used to obtain hemochromogen crystals, alkaline solution use is more common (Olbrycht 1950, Hunt et al. 1960, Kirk 1953). Of the two solutions proposed by Takayama in 1912, the Takayama II solution is generally preferred (Greaves 1932, Kerr and Mason 1926). The Takayama II reagent is the confirmatory test used for this thesis and is prepared by mixing 3 ml of saturated glucose solution, 3 ml of pyridine and 3 ml of 10 percent sodium hydroxide in 7 ml of distilled water (Glaister 1966). The reagent is unstable so it should be prepared daily and kept in a dark bottle (Spalding and Cronin 1976).

To use the Takayama reagent, a small fragment of the suspected bloodstain is placed on a microscope slide and covered with a cover slip. A drop of the reagent is

placed at the edge of the cover slip and allowed to flow under it, coming into contact with the sample. Microscopic examination reveals characteristic pink to red feathery crystals if the test is positive (Figure 4). Greaves (1932) notes that older stains may take longer to form hemochromogen crystals than fresher stains. The formation of crystals may be hastened by gentle heating on a slide warmer (Kerr and Mason 1926).

Testing Procedures

Three broad categories of testing were employed--direct application of reagents, testing of extracts, and testing of filter paper or cotton swabs rubbed or pressed on the bloodstain. The method of testing depends on the particular circumstances of each forensic case as well as the nature of the test itself.

Direct application of reagents. The direct application of reagents applies primarily to the luminol test. Because one of the primary uses of luminol is to locate hidden blood for further testing, it is generally applied with a hand pump sprayer to areas suspected of having blood (Lytle and Hedgecock 1978). For this thesis, however, luminol was applied with a hypodermic syringe with a plastic pipette tip. This method allowed precise control of both the placement of the reagent and amount, and prevented possible contamination of untested areas.

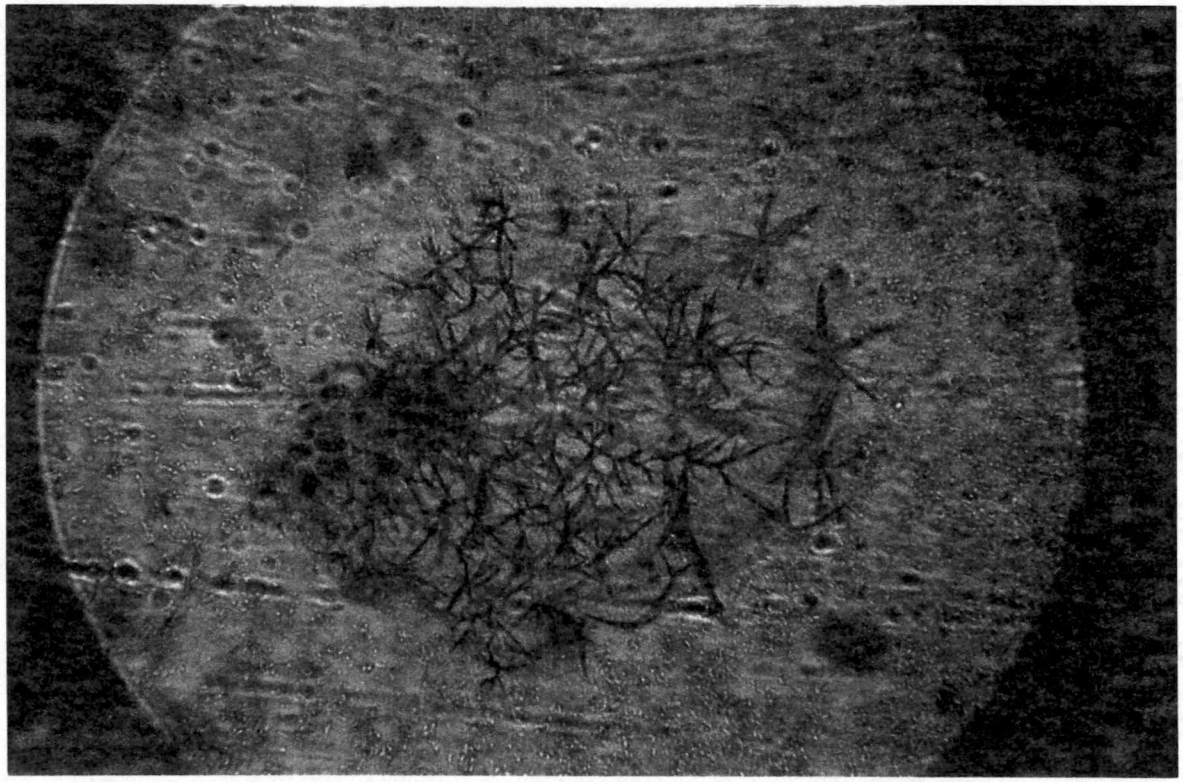

Figure 4. Pyridine hemochromogen crystals indicating positive Takayama test. Magnification X200.

Areas tested with luminol were marked with a felt tip pen and were excluded from further testing. The luminol test is normally not effective with filter paper that is rubbed or pressed on the stain (Hunt et al. 1960, Kirk 1953). The direct application method has been advocated by Pinker (1934), who removed small specimens of the bloodstain and added them to the depressions of a porcelain spot plate containing the phenolphthalein, leucomalachite and benzidine reagents (Figure 5). This method was used with the luminol test (Figure 6).

Testing of bloodstain extracts. Although testing of blood in solution usually is impractical at the typical crime scene (Higaki and Philip 1976), nevertheless it is favored by some for use with the phenolphthalein test. Hunt et al. (1960) state that the phenolphthalein test reacted only with bloodstain extracts and not with filter paper rubbings or by direct application on smaller stains. Higaki and Philip (1976) state that in a one-stage alcohol/phenolphthalein/sodium perborate test the sensitivity of blood in solution increased five times.

Testing blood extracts is conducted by removing a small amount of the bloodstained material and placing it in a test tube with a few drops of physiological saline solution (0.9 percent sodium chloride in distilled water) for several hours. The resulting extract is then boiled

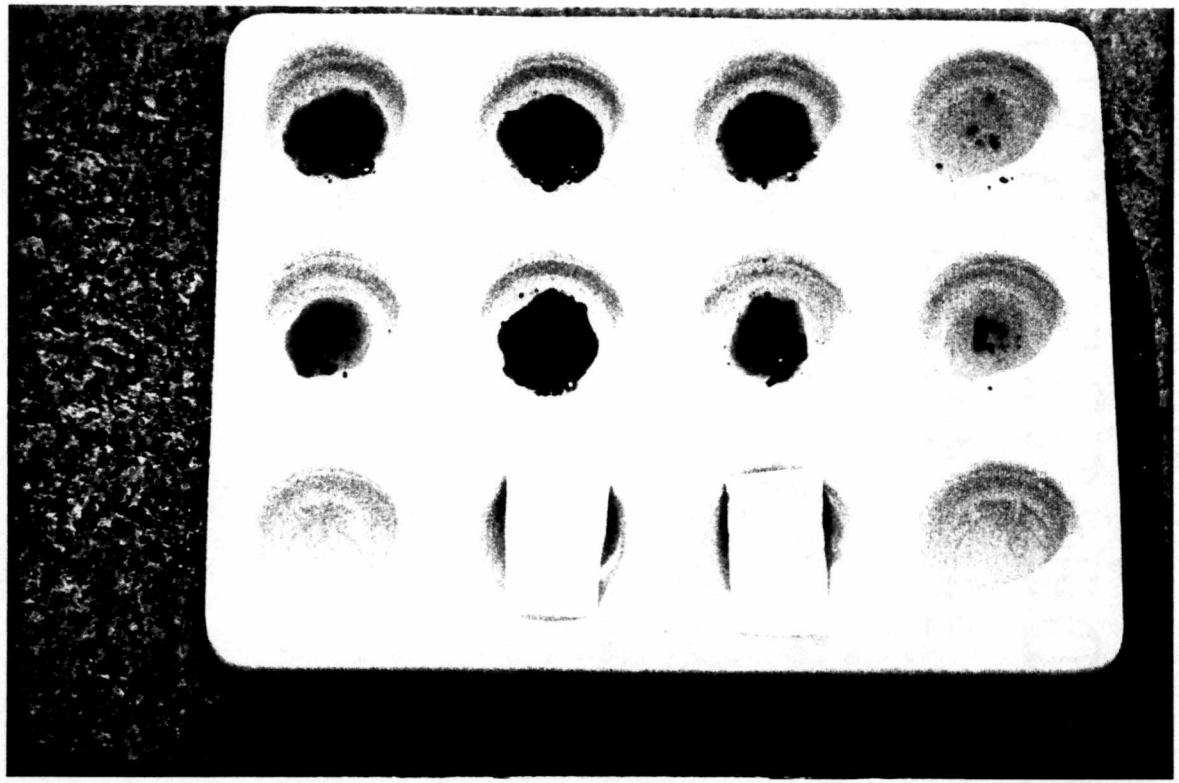

Figure 5. Positive leucomalachite tests in porcelain spot plate using direct application of reagent.

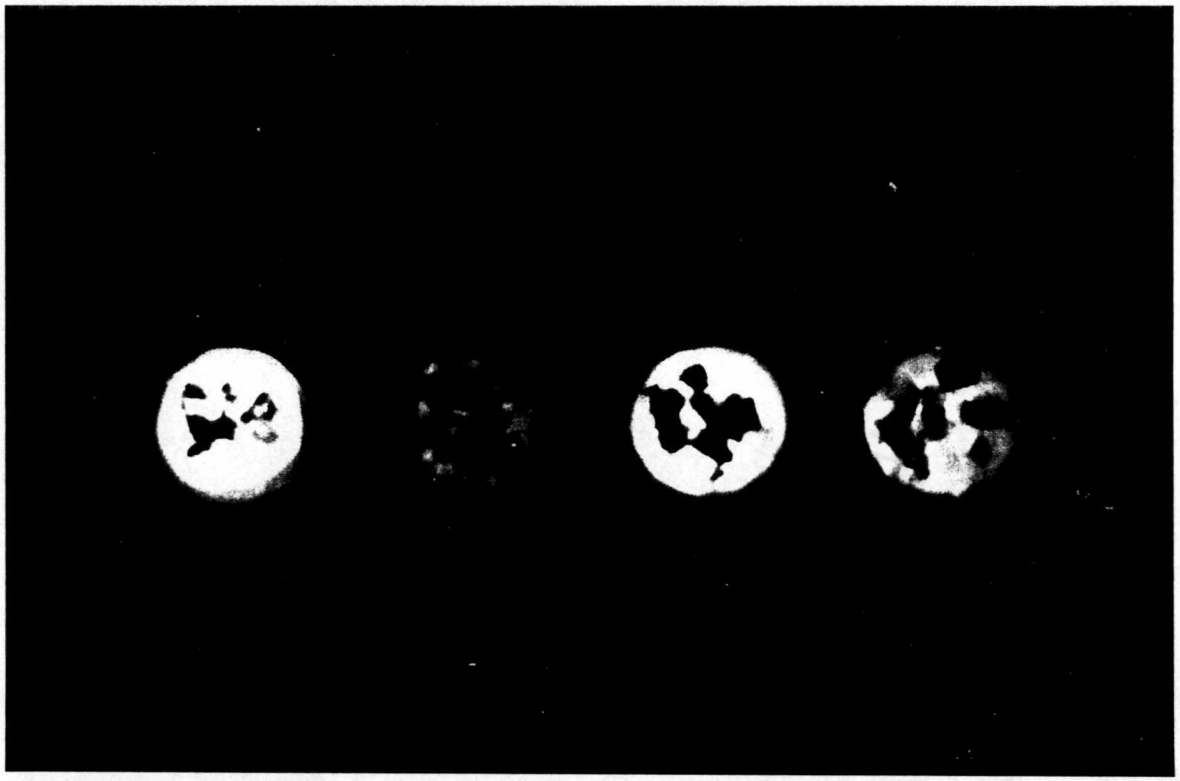

Figure 6. Positive luminol test in four depressions of porcelain spot plate using direct application of reagent.

for 30 seconds to remove any vegetable peroxidase (Fiori 1962). After cooling, the extract is poured into a depression in a porcelain spot plate, the appropriate reagent added and the color changes observed (Figure 7).

A modification of this method was devised by Kirk et al. (1951) that allows blood to be separated from a mixture of debris by capillary action. With this method, the mixture of blood and debris is placed in a shallow dish containing distilled water. One end of a strip of filter paper is placed in the water while the top end is clipped to a rack overhead. In time, the blood solution ascends the filter paper strip by capillary action and precipitates in a narrow band just below the highest point reached by the solution. This band of concentrated blood is cut perpendicular to its axis and tested directly (Kirk 1953) or may be reconstituted in extract form by a decending run with a few drops of saline solution (Fiori 1962).

Rubbing with filter paper or cotton swabs. Blood may also be tested by gently rubbing or pressing the stain with either wet or dry filter paper or cotton swabs. A small amount of blood is transferred to the filter paper or swab and is then tested using any of the color tests (Figure 8). This testing is particularly advantageous in

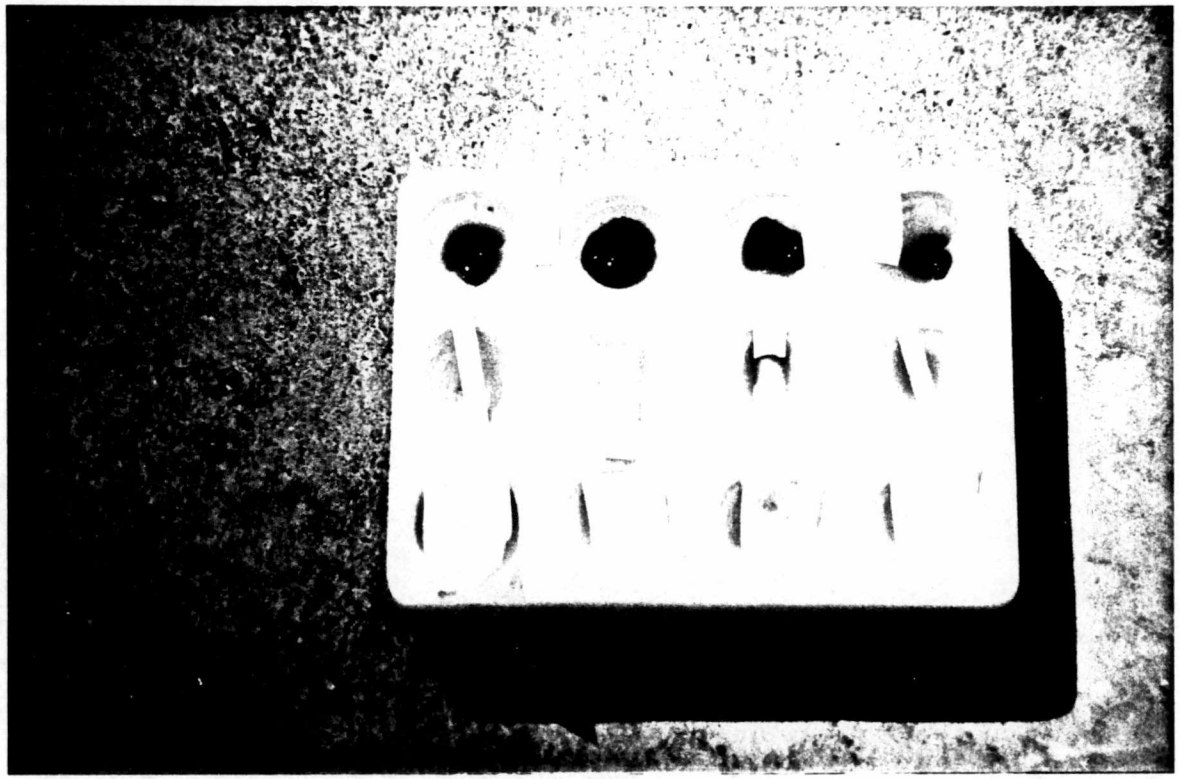

Figure 7. Testing of bloodstain extracts (top row) with phenolphthalein reagent.

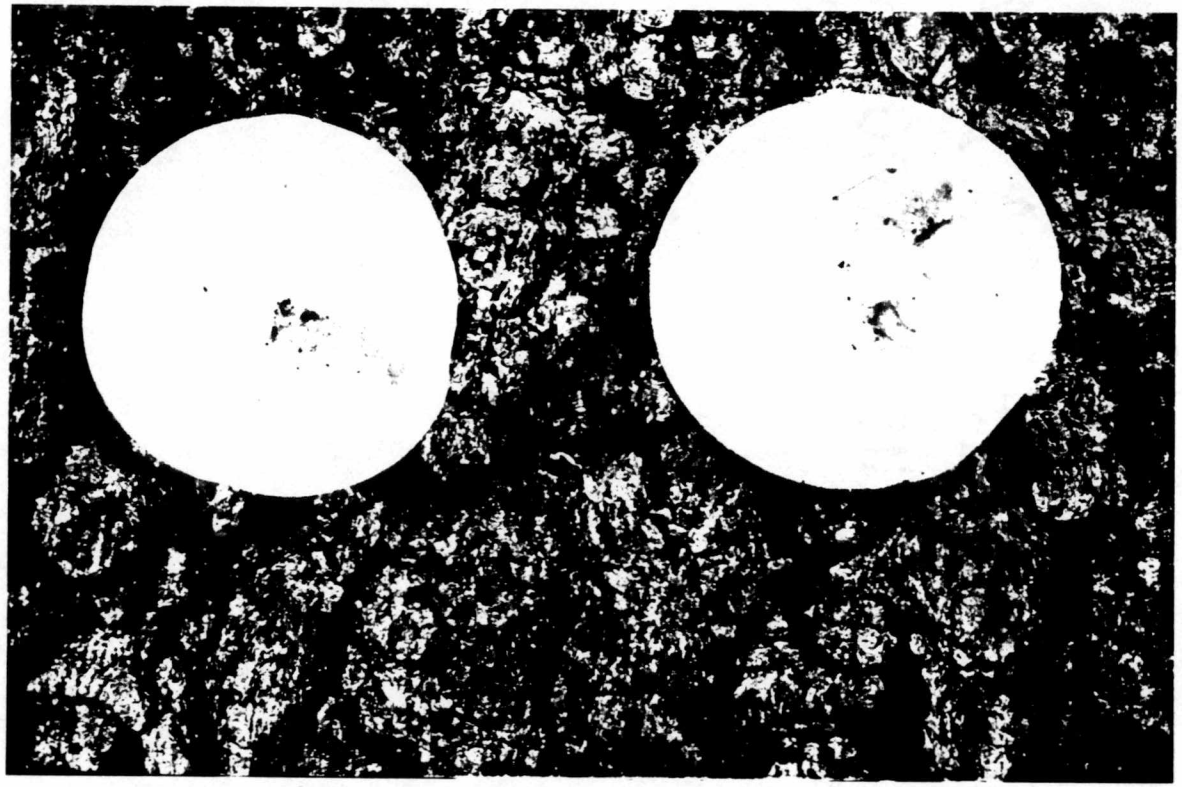

Figure 8. Testing of bloodstain with filter paper rubbings (phenolphthalein left, leuco-malachite right).

the field because it is easy to perform and does not harm the stain for further testing.

Controls

Regardless of the method of testing, a series of controls should be employed to minimize false positive results. With all color tests, the reagent was first applied to a clean piece of filter paper and observed for possible color change. The reagent was also applied to filter paper strips containing known blood. Only after these two tests were conducted was the test performed on the suspected bloodstain. If the suspected bloodstain tested positive, it was checked with a binocular microscope for insect parts (Kirk 1953) and for plant tissue (Nicholls 1956), both of which may yield false positive results. The luminol reagent, which becomes faintly luminescent when the components are combined, was similarly tested with both stained and unstained filter paper before testing. No case was found where material known to be blood-free caused a color change in the catalytic tests or increased the intensity of chemiluminescence in the luminol reagent.

CHAPTER III

MATERIALS AND METHODS

On September 1, 1988 three units (450 ml each) of whole human blood were obtained from the University of Tennessee Medical Center and transported to the research site in Blount County, Tennessee. The blood was normally used for transfusion purposes, but was out of date and could no longer be used. Of the three units obtained, none was out of date by more than three days. Although kept under refrigeration at the hospital, the blood was slowly warmed to 99 degrees Fahrenheit to approximate normal human body temperature.

Locations

The blood was then divided into six equal volumes of approximately 225 milliliters each and distributed at six different locations in a mixed hardwood forest and an adjacent field. Each location covered approximately one square foot of surface area. The locations and corresponding identification numbers are described below:

Location 1 (Figure 9). A concrete pad measuring 12 inches by 12 inches placed in a field.

Location 2 (Figure 10). The trunk of a living Northern Red Oak tree (Quercus rubra). The tree had a trunk diameter of approximately 20 inches and a height of

Figure 9. Concrete pad (Location 1) 30 minutes after blood distribution.

Figure 10. Oak tree (Location 2) 30 minutes after blood distribution.

approximately 30 feet. The bark is nearly black in color and is broken by shallow fissures.

Location 3 (Figure 11). The stump of a Northern Red Oak tree which had been cut in December 1987. The stump measures 34 inches in diameter. The heartwood is dense and firm though insect holes are present close to the bark.

Location 4 (Figure 12). A horizontal oak log (species indeterminate) measuring approximately 8 inches in diameter. The bark is intermittently broken exposing the underlying heartwood which is friable to a depth of approximately one inch.

Location 5 (Figure 13). A firm Northern Red Oak log measuring 24 inches by 6 inches. The bark is shallowly fissured and is permeated with wood beetle holes.

Location 6 (Figure 14). Ground. A 12 inch by 12 inch area on shallow loam soil underlain by dense red clay.

In addition to the locations described above a series of control locations was established to monitor the effects of rainfall on blood. Each control location was located adjacent to its corresponding test location. Unlike the test locations, which were completely exposed to the elements, these controls were protected from precipitation by covering them with transparent plastic sheeting. This sheeting was supported by small wooden

Figure 11. Oak stump (Location 3) 30 minutes after blood distribution.

Figure 12. Decayed oak log (Location 4) 30 minutes after blood distribution.

Figure 13. Oak log (Location 5) 30 minutes after blood distribution.

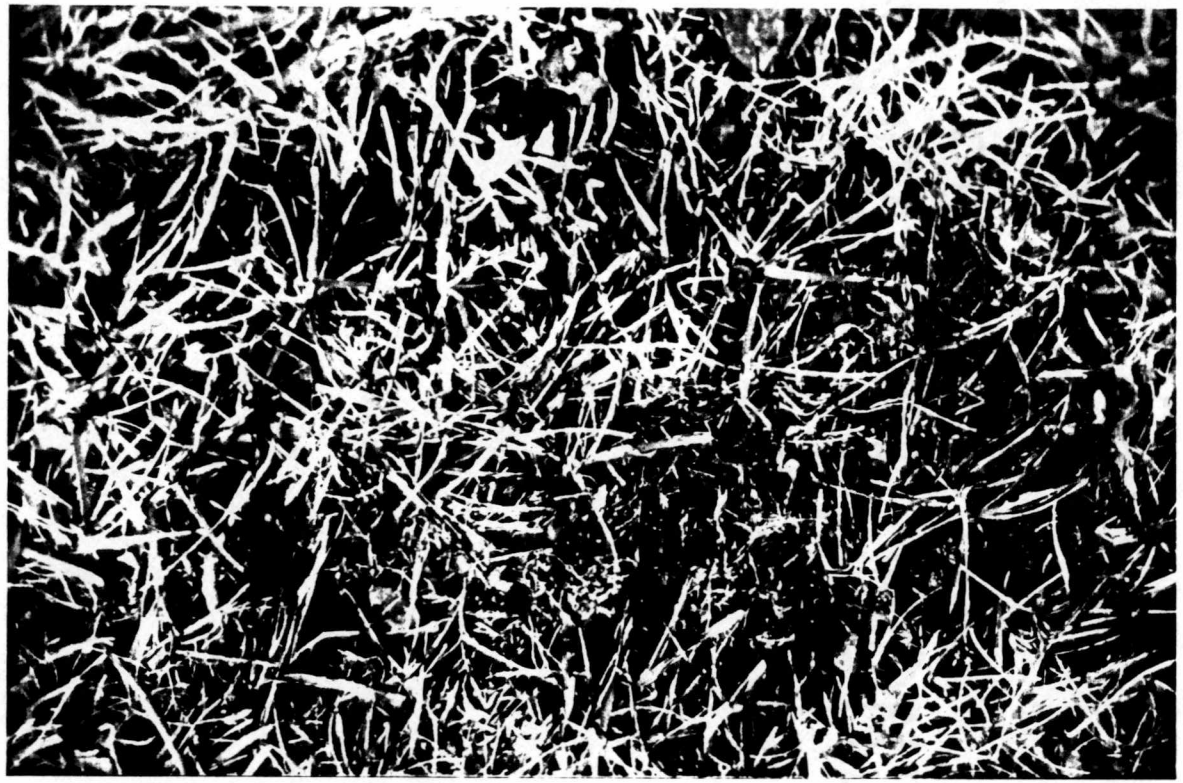

Figure 14. Ground (Location 6) 30 minutes after blood distribution.

frames allowing sunlight, temperature and humidity fluctuations, and air circulation but not precipitation.

At each location the blood was poured over approximately one square foot of surface area. The accuracy of restricting blood distribution to the designated areas was strongly influenced by both the texture of the surface as well as its inclination. Blood poured on the concrete pad (Location 1), for example, was easily controlled because of its relatively smooth surface and horizontality. Blood poured on the oak tree (Location 2), however, splashed laterally due to the rough texture of the bark and dripped vertically due to its inclination. Maintaining even blood distribution was hampered by the absorbency of the surface. Blood poured on the ground (Location 6) and on the decayed log (Location 4) was quickly absorbed while blood poured on the concrete pad and on the oak stump ran off quickly because of their less permeable surfaces. Blood distribution was found to vary considerably not only between locations but within the same location. Blood tended to run off elevated surfaces and collect in depressions, grooves and in insect holes. Each of these factors will be shown to have a profound effect on blood detection.

Each location was visited daily beginning September 1, 1988, and the project was concluded February 28, 1989. During each visit climatic conditions including local sky

conditions were recorded. Temperature, relative humidity, rainfall, wind speed and total minutes of sunshine were obtained from the National Weather Service at McGhee Tyson Airport, which is located approximately seven miles from the study area. The maximum daily temperature and precipitation are presented in the Appendix. Three catalytic tests and one confirmatory test were employed to determine the presence or absence of blood.

Photographs

Photographs were made frequently during the initial hours of the study since this period was when the greatest change in blood characteristics took place. After the first day, photographs were taken every fourth day. Photographs were made with a Minolta XG-7 35 mm camera using both normal and macro lenses. During daylight hours, both Kodachrome 64 and Ektachrome 200 slide film were used.

Photographing the luminol test. The luminol test requires darkness to observe the chemiluminescence and presented special problems in recording the results photographically. A number of films were used including Kodak 2485 High Speed Recording film (Pierce 1972), Kodak Tri-X Panchromatic film (Zweidinger et al. 1973) and Kodak TMAX P3200 Professional film. Of these, TMAX P3200

Professional film consistently produced the clearest photographs with the least grain. While photographing the luminol reaction at night a 50 mm lens was used exclusively with the maximum aperature of f 1.7. Exposure times varied between five seconds and two minutes.

Photographs obtained using Kodak TMAX film varied in quality from varying exposure times and the amount of ambient light. On moonless nights, even exposures of up to two minutes failed to bring out details of the various surfaces although it was particularly effective in defining blood distribution patterns (Figure 15). Photographs made during the full moon, however, clearly depicted the surface where the blood was deposited (Figure 16). During the full moon, exposure times of 30 to 45 seconds usually sufficed. Whenever possible, multiple exposures were made because the chemiluminiencence can be reactivated several times by the addition of more luminol (Kirk 1953).

Hemochromogen crystals formed during the Takayama test were photographed through a Zeiss microscope at 200 power with both Kodachrome 64 and Ektachrome 200 slide film.

Each location was tested every fourth day using the leucomalachite, phenolphthalein and luminol reagents. The three broad categories of testing-- direct application, testing of extracts and filter paper and cotton swab

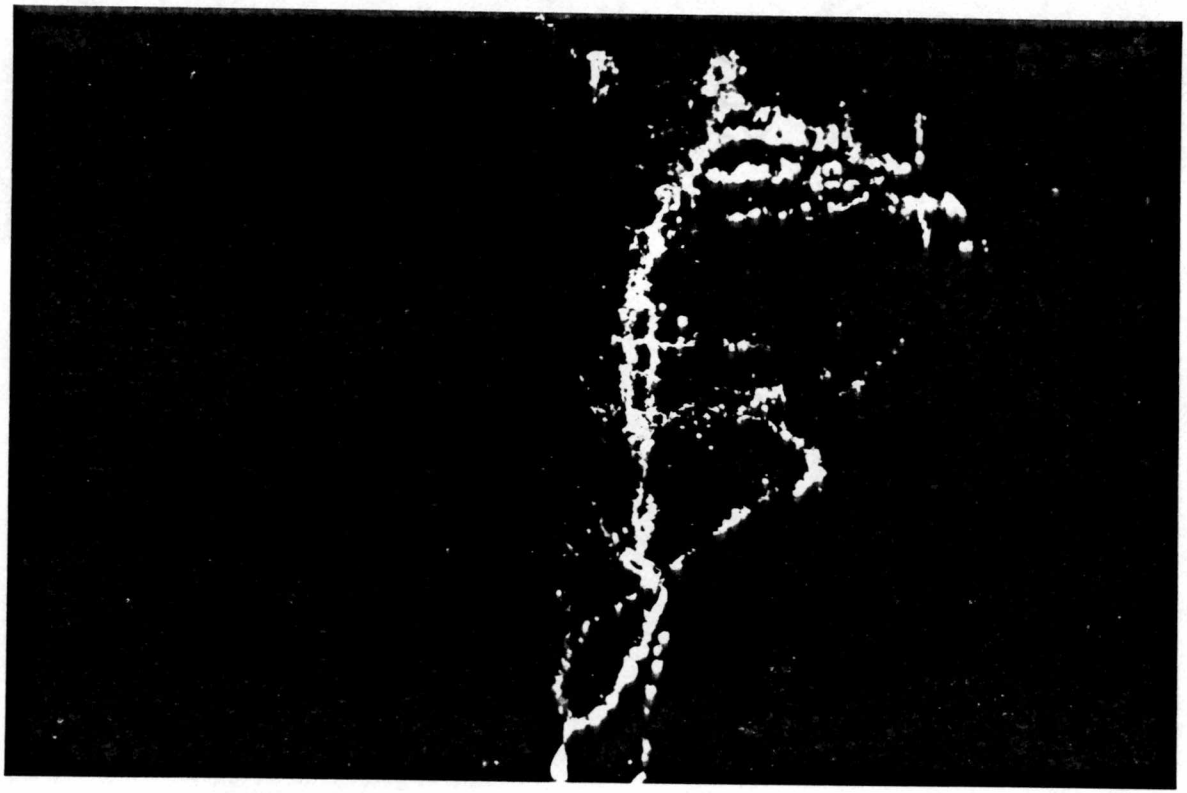

Figure 15. Luminol test showing blood distribution pattern on oak tree.

Figure 16. Luminol test showing collection of blood in depressions of oak log bark.

rubbings were employed. When a location produced negative results with all three reagents it was excluded from further testing.

CHAPTER IV

RESULTS

Location 1

Though thoroughly dry by the end of the second day, much of the blood on the concrete pad was removed by 1.58 inches of rainfall which occurred on days three and four. Before this precipitaion, the blood was dark red in color and displayed a glossy appearance. Immediately after the rain, however, the surface had lost both its red color and its luster (Figure 17), yet all three catalytic tests were positive. With each rainfall the blood appeared to diminish in quantity and the glossy finish was reduced. In addition, reaction times with the leucomalachite test lengthened, probably from dilution of the blood.

The leucomalachite, phenolphthalein and luminol tests were positive until October 4, 1988 (day 34). On this date, both the leucomalachite and phenolphthalein tests were negative following .67 inch of rainfall on October 1-3. A positive luminol test occurred for an additional 12 days.

The concrete pad was excluded from further testing on October 16, 1988 (day 46) when all three catalytic tests proved negative. At this time, it was broken and retested using cotton swabs probed into crevices beneath the surface of the pad. Again, all tests were negative.

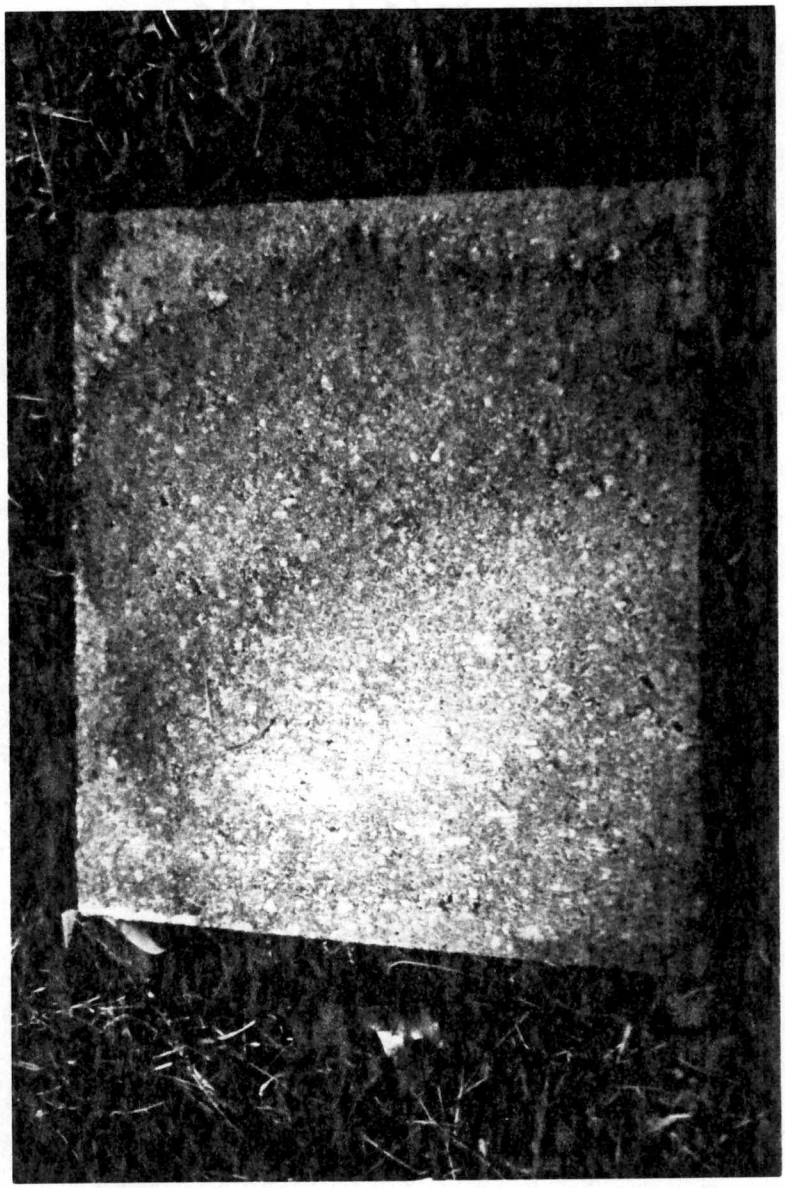

Figure 17. Concrete pad (Location 1) immediately after rain showing loss of color and luster.

The control location, which was protected from rainfall by plastic sheeting, continued to test positive with all tests, including the Takayama test, until the conclusion of the study. Blood on the control location was uniformly dark in color and exhibited a high gloss where it had pooled in the depressions.

A small amount of blood was accidentally spilled on a broken concrete block during the first day of the study. Although not considered a part of the project, the concrete block, which was rougher and more permeable than the concrete pad, was tested at irregular intervals. It was found that the concrete block tested positive after six months even with leucomalachite, the least sensitive of the catalytic tests employed. It was also noticed that the surface where the blood was spilled tested negative with all tests at approximately the same time as the concrete pad. When the concrete block was broken and tested at the conclusion of the study, however, it was found that blood had penetrated at least 5 mm and positive leucomalachite, phenolphthalein and luminol tests were obtained (Figure 18).

Location 2

The oak tree continued to test positive with all three catalytic tests until the conclusion of the study despite cumulative rainfall of 24.23 inches during the six

Figure 18. Concrete block after six months showing positive phenolphthalein (top) and leucomalachite (bottom) tests on cotton swab.

month period. This was the only vertical surface in the study, and the test results are markedly different than the horizontal surfaces of Locations 1,3,4,5,and 6.

At the end of six months blood which had collected in depressions was found to have maintained a high gloss when viewed at an oblique angle. Although the dark color of the blood resembled the color of the bark, the lustrous appearance of the blood made it discernable to the naked eye (Figure 19). Blood on more elevated surfaces of the bark maintained less of a shiny finish than the more protected blood in the depressions.

During the distribution of blood, much of the blood dripped down the bark and fell to the ground, but a surprising amount collected in small grooves and depressions in the bark. These depressions did not collect as great a quantity of blood as the horizontal surfaces of the other locations. After a rain, however, they did not hold water as the horizontal surfaces. The vertical inclination of the oak tree permitted rapid drainage of water. This location was frequently dry to the touch when locations 1,3,4,5,and 6 had water standing in their depressions. The absence of prolonged contact with water is believed to have contributed significantly to the longevity of the blood.

The control location continued to test positive throughout the six month period. While blood in the

Figure 19. Blood (arrows) on oak tree (Location 2) after six months.

depressions of the control location closely resembled that of the test location, blood on the more elevated surfaces was more lustrous than that found on the elevated surfaces of the test location. At both locations, blood adhered tenaciously to the bark with little flaking and could not be separated from it without difficulty.

Location 3

The oak stump tested positive with all catalytic tests until November 5, 1988 (day 66). On this date, the leucomalachite and phenolphthalein tests were negative following 1.27 inches of rainfall on November 4 and 5. The luminol test was positive for an additional 16 days (Figure 20). On day 82 the luminol test was also negative following 1.3 inches of rain on November 19 and 20.

The heartwood of the oak stump was still firm and relatively impermeable, the tree having been cut only one year previously. During the initial hours of the study, little blood actually penetrated the heartwood. Rather, it tended to follow the natural inclination of the cut and run off the side of the stump. The smooth cut offered the blood little protection from the elements and few catchment areas were found. The lack of protection combined with the relatively smooth surface is probably responsible for the relatively rapid disappearance of the blood.

Figure 20. Positive luminol test on oak stump (Location 3).

Location 4

The decayed oak log tested positive with all catalytic tests until January 4, 1989 (day 126). Then none of the three tests produced a positive result on any of the exposed surfaces. On December 30 and 31 the area received .72 and 1.16 inches of rainfall respectively and an additional 1.74 inches between December 21 and December 30.

One area continued to test positive for an additional 12 days. This was a small area where blood had seeped beneath the bark and thus had received some protection from the elements (Figure 21). The bark could be lifted gently, the area tested and the bark carefully replaced without disturbing the blood. On day 140, two days after the last tests were conducted, the bark had slipped from the log, exposing the previously protected blood. Due to the friable nature of the log, the surface where the tests were being conducted was disturbed as well and subsequent tests proved negative.

Throughout the testing period, that blood distributed on the bark had consistently faster reaction times with the leucomalachite and phenolphthalein reagents than blood on the crumbling heartwood. In addition, bloodstains on the heartwood faded more quickly and tested positive for approximately two weeks less than bloodstains on the bark. This disappearance of blood is believed to

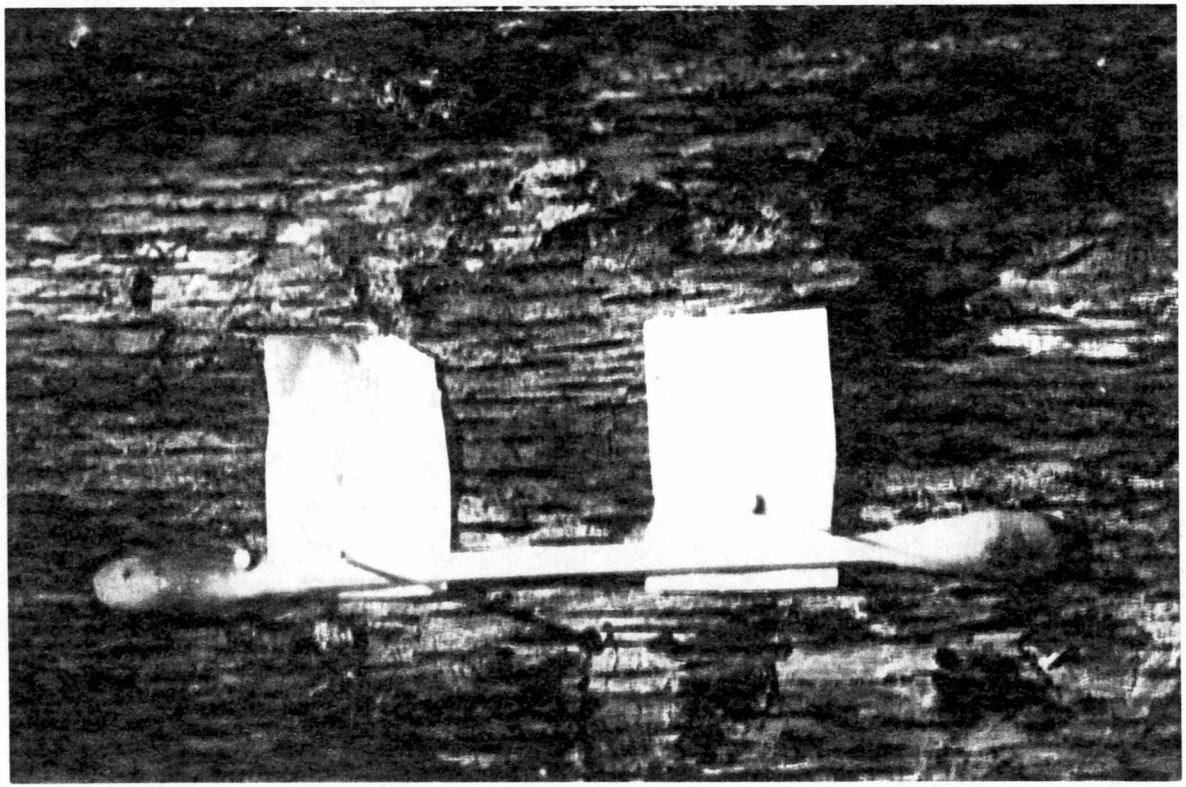

Figure 21. Positive phenolphthalein test (left) and leucomalachite test (right) from beneath bark of Location 4.

be from the relatively permeable heartwood absorbing and dispersing the blood. The heartwood was damp for days after the last rainfall. Prolonged contact with water may have contributed to an increased in blood solubility and removal by subsequent rainfall.

Location 5

The oak log continued to test positive until the conclusion of the study. As other locations, Location 5 had a variety of blood collection areas that tested negative at different times. The more elevated surfaces ceased testing positive first, with the last positive tests on December 27, 1988 (day 118). Tests conducted on December 31 after 1.88 inches of rainfall were negative on the elevated bark surfaces. Depressions in the bark tested positive until January 20 (day 142).

Two areas continued to test positive until the conclusion of the study. Although the bark and heartwood were firm, both contained small oval-shaped wood beetle holes (Family Cerambycidae) measuring approximately 3 mm by 5 mm in diameter (Figure 22). Though no conscious effort was made to introduce blood into the holes, it was unavoidable. Probes inserted into the holes in at attempt to extract blood were not successful because the tunnels were convoluted. When the log was sawed open at the conclusion of the project, however, the tunnel could be

Figure 22. Wood beetle hole (arrow) in bark of Location 5.

easily followed. Catalytic tests conducted near the opening of the beetle holes were all negative. At a distance of approximately 5 mm from the opening, however, positive leucomalachite, phenolphthalein and luminol tests were obtained. Realizing that insect parts may be responsible for positive results, material tested was scraped from the tunnel walls and carefully checked with a binocular microscope. No insect parts were found.

The second area testing positive after six months was a lichen attached to the bark. Two rivulets of blood dripped onto the lichen measuring approximately 12 cm by 7 cm when the blood was being distributed (Figure 23). Crenulations served as natural catchment areas, and the relatively impermeable surface prevented absorption into the lichen. Blood was distinctly identifiable by the naked eye at the conclusion of the study and continues to be visible at the time of this writing (April 8, 1989). All catalytic tests continue to be positive. The blood is reddish-brown in color and is cracked extensively revealing the lichen beneath (Figure 24). Although cracked, no evidence of flaking is apparent. As Location 2, the blood on the lichen adheres tightly to its substrate though it is less glossy than blood on the oak tree. The control location continued to test positive with all catalytic tests and the Takayama test at the

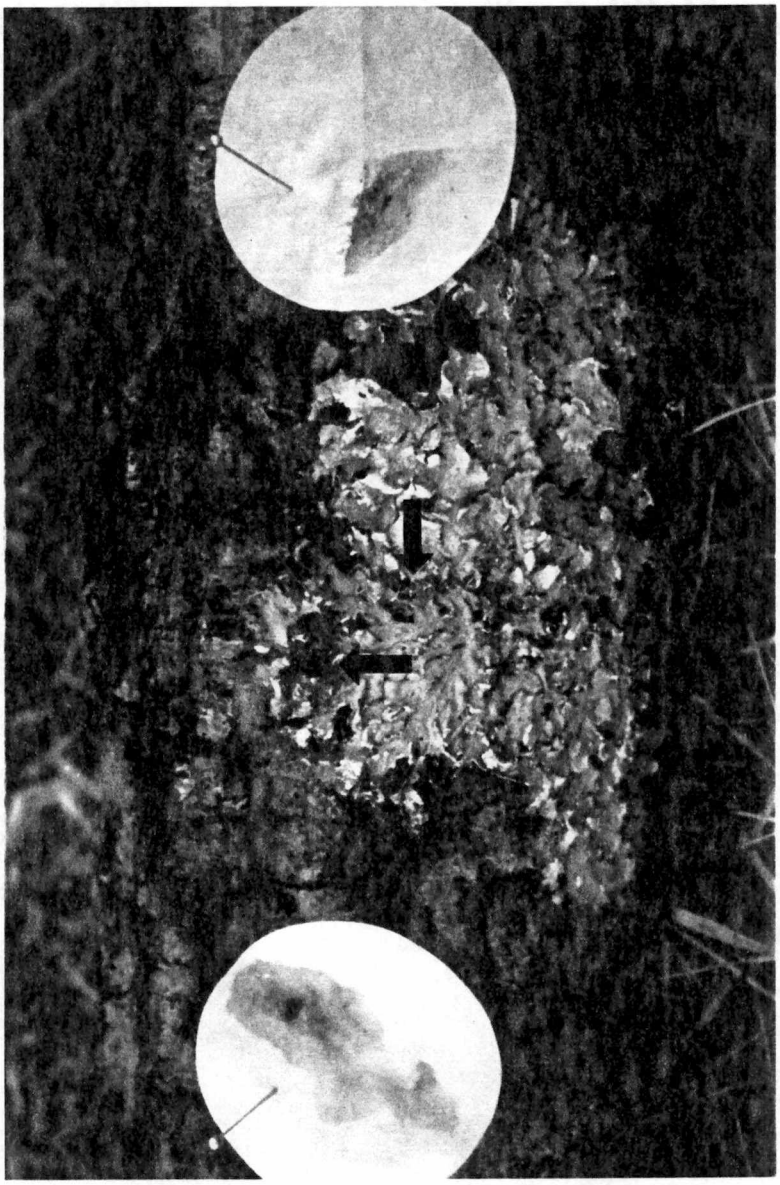

Figure 23. Rivulets of blood (arrows) on lichen of Location 5.

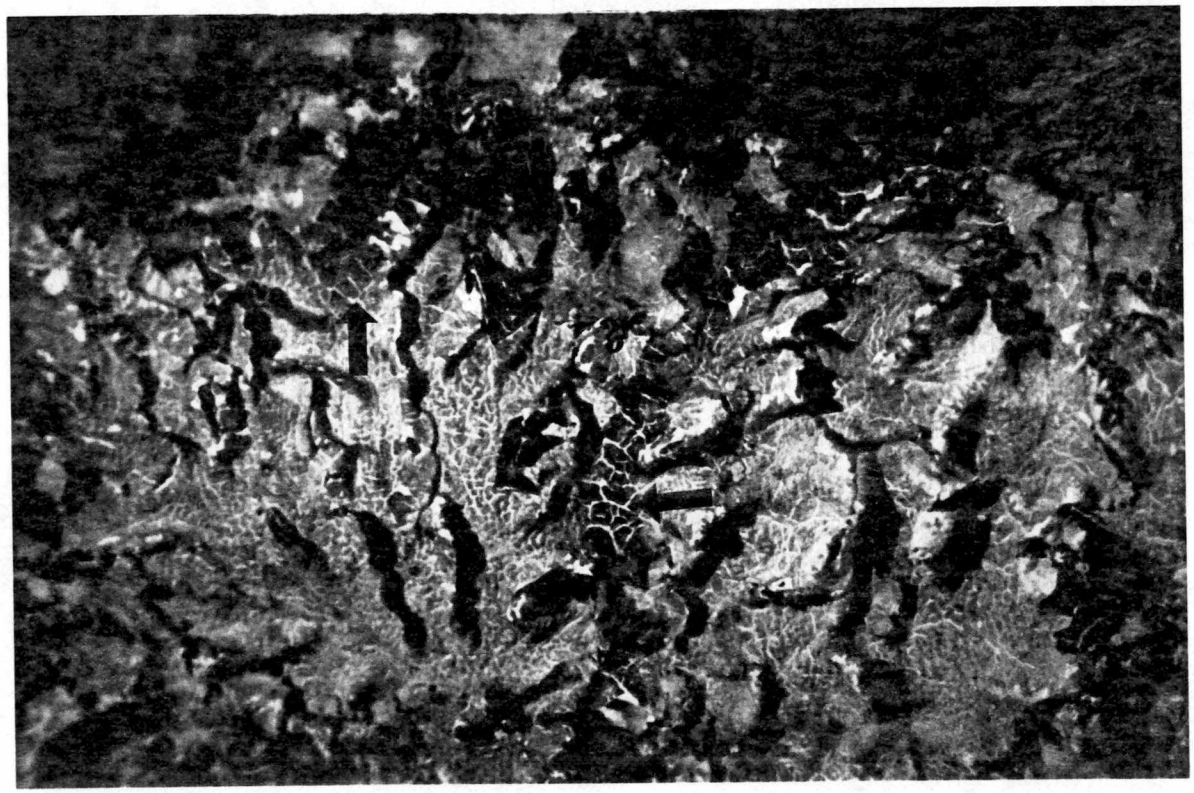

Figure 24. Closeup of blood rivulets (arrows) shown in Figure 23.

conclusion of this study. The blood is black and glossy in appearance.

Location 6

Of the six locations tested, the blood deposited on the ground tested positive for a much shorter time than any of the others. Repeated attempts failed to detect blood beyond the fourth day using any of the catalytic or confirmatory tests. This is not surprising as Hirose (1971a) found that only 22 percent of hematin in a sandy granite soil at 22 degrees Centigrade was recoverable after two days. This disappearance is attributed to the absorbant property of the soil (Hirose 1971b). Hirose (1966) found that the higher the soil temperature and the greater the water content, the shorter the time human hemoglobin was identifiable by the precipitin test. Although soil temperature and water content were not measured, the high temperatures during the first two days were 88 degrees Fahrenheit. During days three and four the high temperatures were 77 and 71 degrees, respectively. No precipitation was recorded during the first two days. On the third day, the study area received .56 inches of rainfall followed by 1.02 inches on day four. This combination of high temperature and moderate rainfall combined with an absorbent soil is probably

responsible for the short period of time in which positive catalytic tests results were obtained.

The control location also tested negative after day four although it was protected from direct rainfall by covering it with a piece of transparent plastic sheeting. Lateral movement of percolating groundwater from unprotected areas may account for the negative test results.

CHAPTER V

DISCUSSION

The idea for this thesis resulted from a forensic case in Knoxville, Tennessee where a white female was nearly decapitated and left in a field. Although the body was found the next day, the scarcity of blood led investigators to question whether the victim had been killed in the field or whether she had been killed elsewhere and the body dragged to the field. During the trial nearly a year later, catalytic tests were conducted at the scene failed to detect blood. Ensuing research revealed that few studies have been done on blood longevity.

This project began during a severe drought, and the drought continued for approximately four of the six months of this study. Although the scarcity of rainfall contributed significantly to blood survival, other factors such as protection from the elements and textured surfaces that permit blood to bond to the substrate are also important. It is anticipated that additional tests on different surfaces may reveal blood detection limits that differ significantly from the surfaces used in this thesis.

Studies conducted in other geographical areas will undoubtedly differ somewhat from these results. In more

humid areas, blood may decompose at a faster rate because of bacterial action. In colder climates, repeated freezing and thawing may increase the rate of blood flaking from its substrate. Desert areas may be more conducive to preservation because blood dessication reduces bacterial activity and because of infrequent rainfall.

This thesis is limited to the detection of blood with catalytic tests commonly used by law enforcement agencies. The fact that blood is detectable does not necessarily mean that genetic and non-genetic markers allowing individualization survived. Many of these markers are short-lived and are easily damaged by adverse envoronmental conditions. Additional research should focus on the genetic and non-genetic markers and the environmental factors that are deleterious to their survival.

CHAPTER VI

CONCLUSIONS

When allowed to dry completely, blood is detectable on certain surfaces for at least six months even when subjected to adverse environmental conditions. The ability to detect blood depends on a number of factors such as the texture of the surface, the inclination of the surface and its permeability and the amount and duration of rainfall. Of these, rainfall probably has the greatest effect. Olbrycht (1950) states:

> It seems also that the importance of the water as a noxious factor is not properly emphasized. And yet the water may have a harmful influence for many reasons. As a solvent of many substances it facilitates the noxious action of the many chemical and biological factors of the substratum, and on the other hand, as a solvent of both the blood pigment and protein and of the isoagglutinogens and isoagglutinins, it renders their demonstration difficult or impossible because of their strong dissolution or their being washed out.

Rainfall appears to have had a pronounced effect on blood deposited on the ground (Location 6) where all tests were negative after the fourth day. The decayed oak log (Location 4) was similar, where blood on the crumbling heartwood appears to have been quickly dispersed by rainfall. In most cases, negative catalytic tests followed an extended rainfall. Light precipitation or

rainfall of short duration did not have a marked effect on test results.

The inclination of the surface also appears to affect blood detectability. The oak tree (Location 2) was the only vertical surface used in the study, and it tested positive with all catalytic tests at the end of the six month study. It is speculated that horizontal surfaces allow water to remain in contact with blood longer than vertical ones. Prolonged contact with water may increase blood solubility making it more subject to washing away by subsequent rainfall.

The lichen on the oak log (Location 5) represents an anomalous situation where a horizontal and relatively impermeable surface continued to test positive after six months. Unlike the other locations, blood which collected on the lichen retained both a slightly glossy finish and reddish color. Microscopic examination showed that the blood had not penetrated deeply into the lichen; it had bonded firmly to the surface and could not be separated from it except by soaking in a physiological saline solution.

Surface texture likewise plays an important part in the detection of blood. Smoother surfaces, such as the oak stump (Location 3) and the concrete pad (Location 1), permit relatively rapid removal of blood by rainfall. Conversely, rougher surfaces, such as the oak tree bark

(Location 2), permit blood to be detected for longer periods of time. Textured surfaces allow greater adhesion of blood, increase surface area and often form small natural blood catchment basins. On numerous occasions, a cotton swab probed into minute pockets tested positive while a piece of filter paper rubbed over the more exposed surfaces failed to detect blood.

Blood may survive considerably longer when afforded even the slightest protection from direct rainfall. Blood that accumulated beneath the bark of the decayed oak log (Location 4) was detected for two weeks longer than blood deposited on the exposed heartwood less than three centimeters away. Blood on the oak log (Location 5) was detected inside the relatively protected wood beetle holes while only a few millimeters away on the exterior surface of the log tests proved negative.

Other climatic variables, such as temperature, wind, humidity and sunlight, did not appear to have a major effect on blood detection. Except for the ground control location (Location 6), all control surfaces, which were protected only from direct rainfall, tested positive with each of the catalytic tests at the end of six months. The blood at control locations had turned black, and both elevated and depressed surfaces were glossy. After approximately six weeks, the appearance of the controls changed little until the conclusion of the study.

Low temperatures combined with rainfall may affect blood detection, although not greatly. On several occasions small blood crusts spalled from the larger blood crusts following a rain and freezing temperatures. This flaking may be caused by water either entering the dried blood or by water coming between blood and its substrate. With subsequent freezing, the water expands and the blood crust may flake off. This phenomenon was noticed on the control locations and the exposed locations . As blood dries, it cracks and curls around the edges and loosens from the surface where it was deposited. This removal of blood crusts, therefore, may be due solely to loose crusts being removed from the exposed locations by rainfall and from the control locations by wind.

Test results vary depending on the method used. With the color tests, blood extracts tested in a porcelain spot plate were generally found to be more consistent than any other method. The direct application of reagents was likewise found to be effective when using the spot plate method of Pinker (1934) or the luminol test. Of the three methods employed, filter paper or cotton swab rubbings were the easiest to apply as well as the least effective. Cotton swabs were more efficient in blood detection than filter paper primarily because they can be probed into small blood catchment areas unreachable with filter paper. The performance of both methods is enhanced by wetting the

swab or filter paper in distilled water and leaving it in contact with the blood for a short period of time. A physiological saline solution produced better results than distilled water because it is a better solvent. The Takayama test was successful only during the first four weeks of the study presumably because of the increasing insolubility or dilution of the blood. On control locations the Takayama test was positive at the conclusion of the study.

CHAPTER VII

SUMMARY

This study has shown that under certain conditions blood which dries is remarkably durable. The blood was subjected to temperatures as high as 89 degrees Fahrenheit and as low as 14 degrees Fahrenheit. In addition, it survived three inches of snow, sleet, torrential rain, high humidity and wind speeds of up to 40 miles per hour. It cannot be tacitly assumed that adverse weather conditions preclude the possibility of detecting blood.

The study has also shown that while blood on certain surfaces is often long-lived, blood on other surfaces is transitory. This fact emphasizes the need to locate blood evidence as quickly as possible.

It has also pointed out the necessity of conducting more than a cursory search for blood. Numerous times this research has shown that blood on exposed surfaces was removed or diluted to the point where it was no longer detectable with any of the catalytic tests. Nearby, often within a few centimeters, however, blood survived when only slightest protected from the elements. It is imperative that the forensic investigator search for these protected locations so that valuable evidence will not be overlooked.

It is obvious that much more research is needed in this area. Studies using different surfaces under different climatic conditions will undoubtedly reveal various weathering patterns of blood.

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APPENDIX

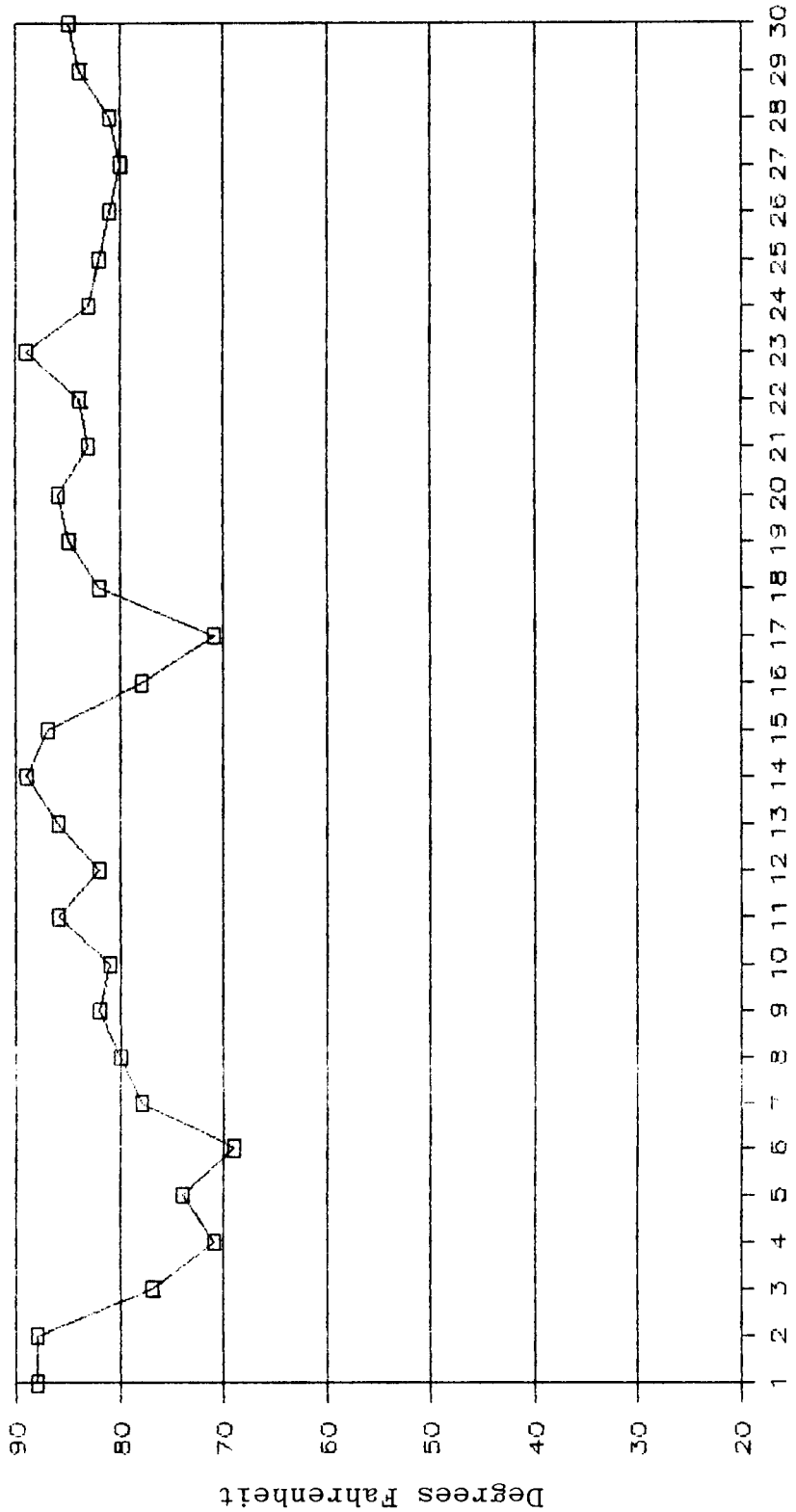

Figure 25. Maximum daily temperature for the Knoxville, Tennessee area for September 1988.

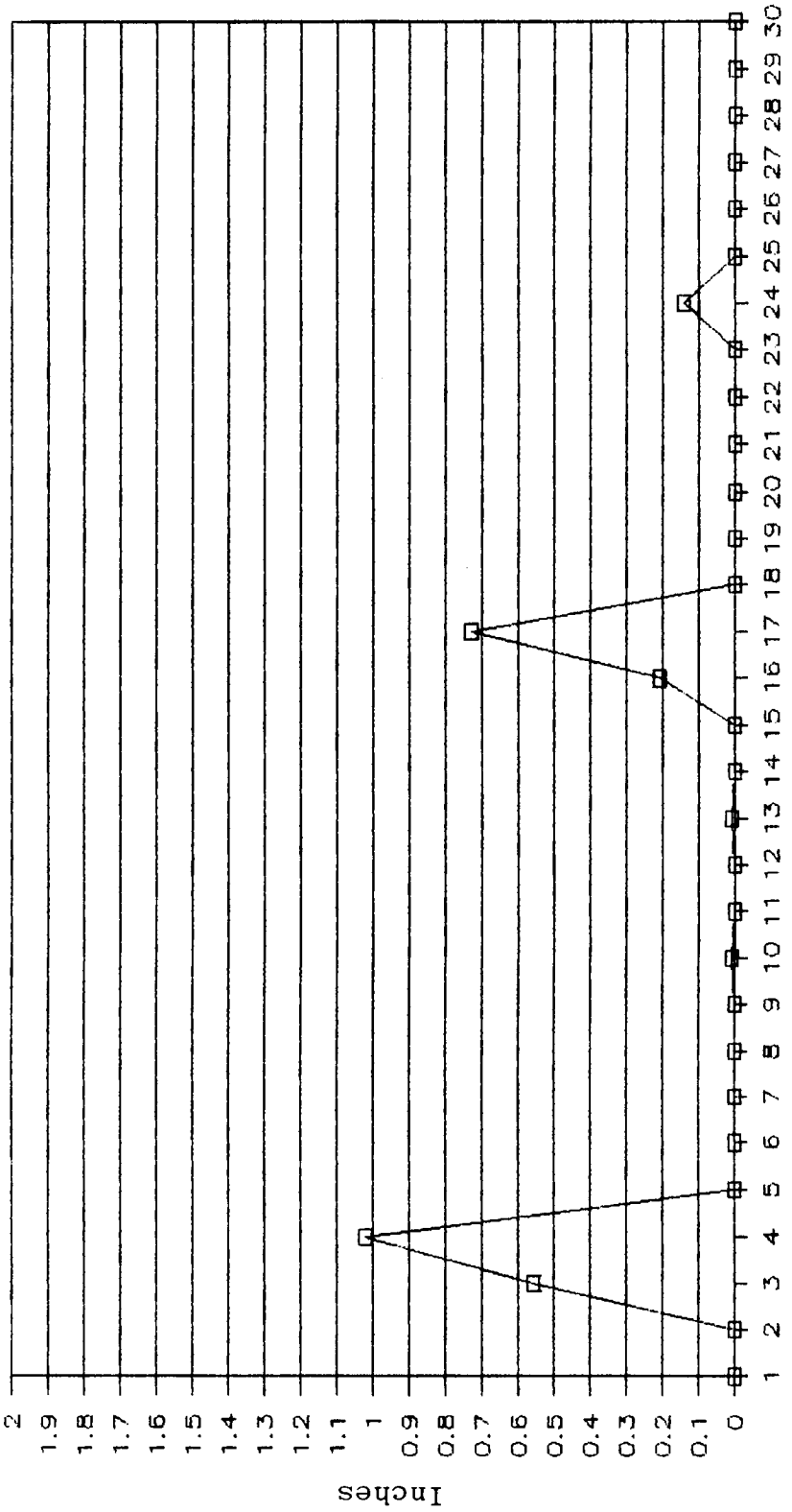

Figure 26. Daily precipitation for the Knoxville, Tennessee area for September 1988.

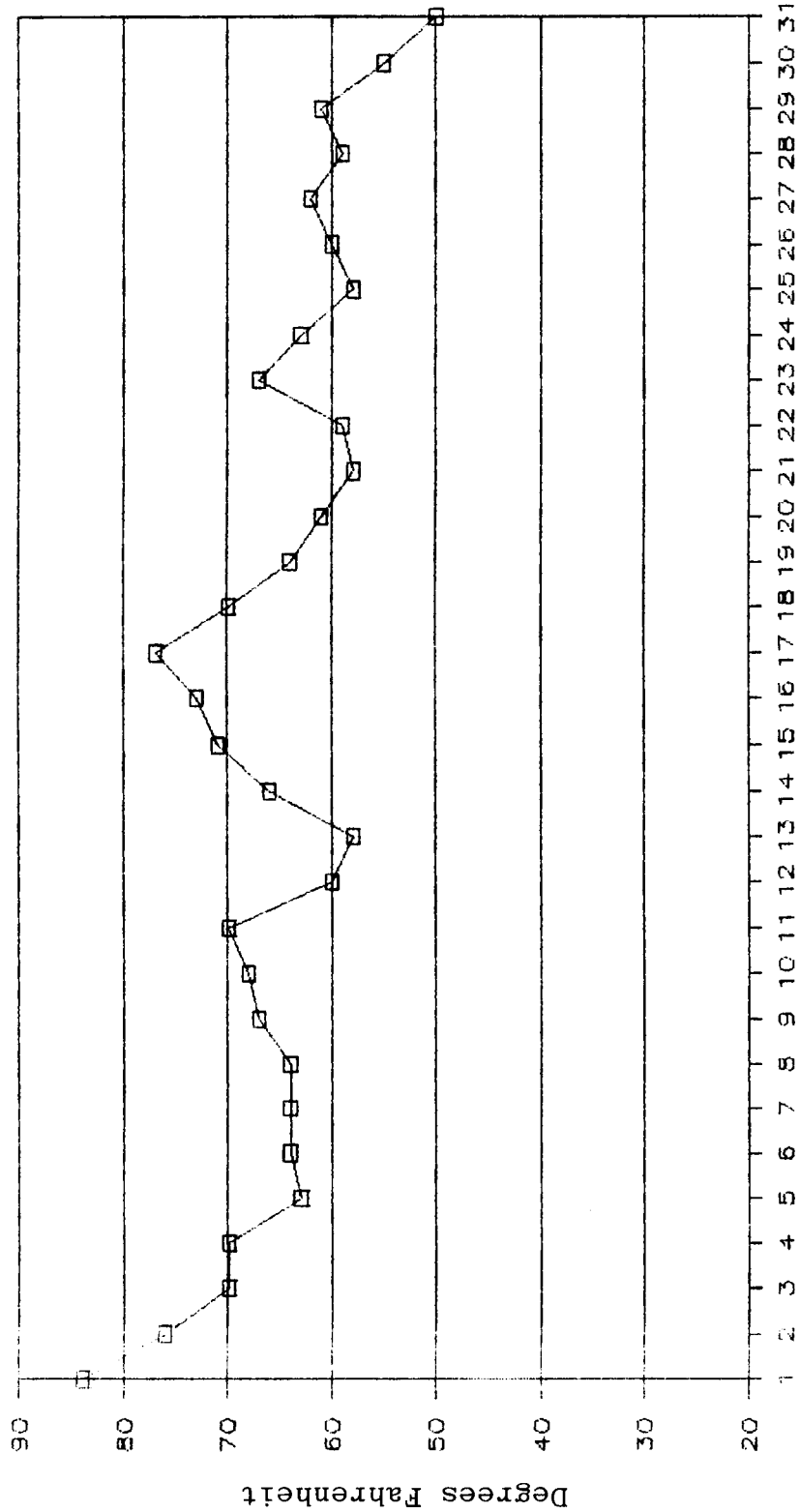

Figure 27. Maximum daily temperature for the Knoxville, Tennessee area for October 1988.

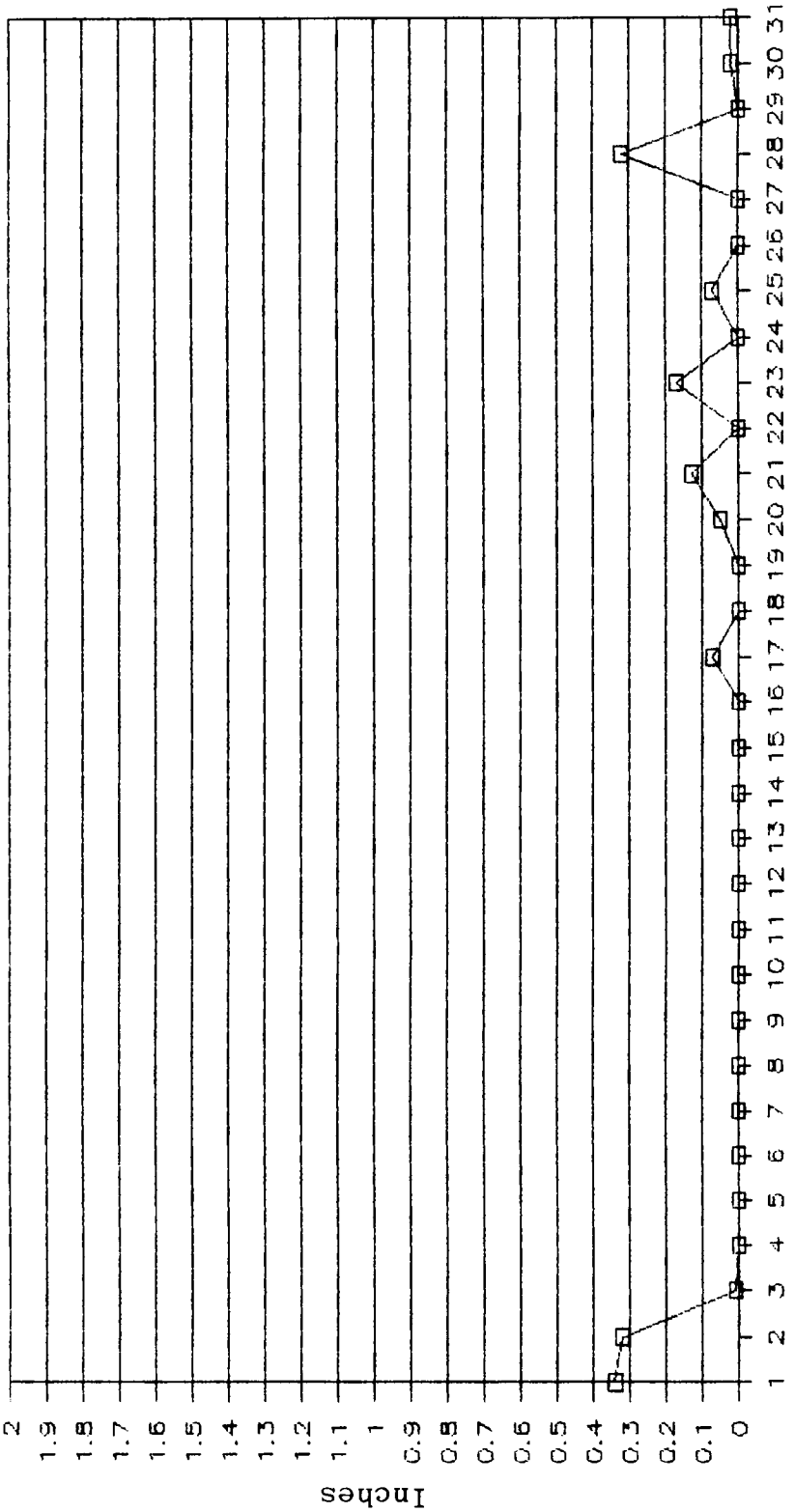

Figure 28. Daily precipitation for the Knoxville, Tennessee area for October 1988.

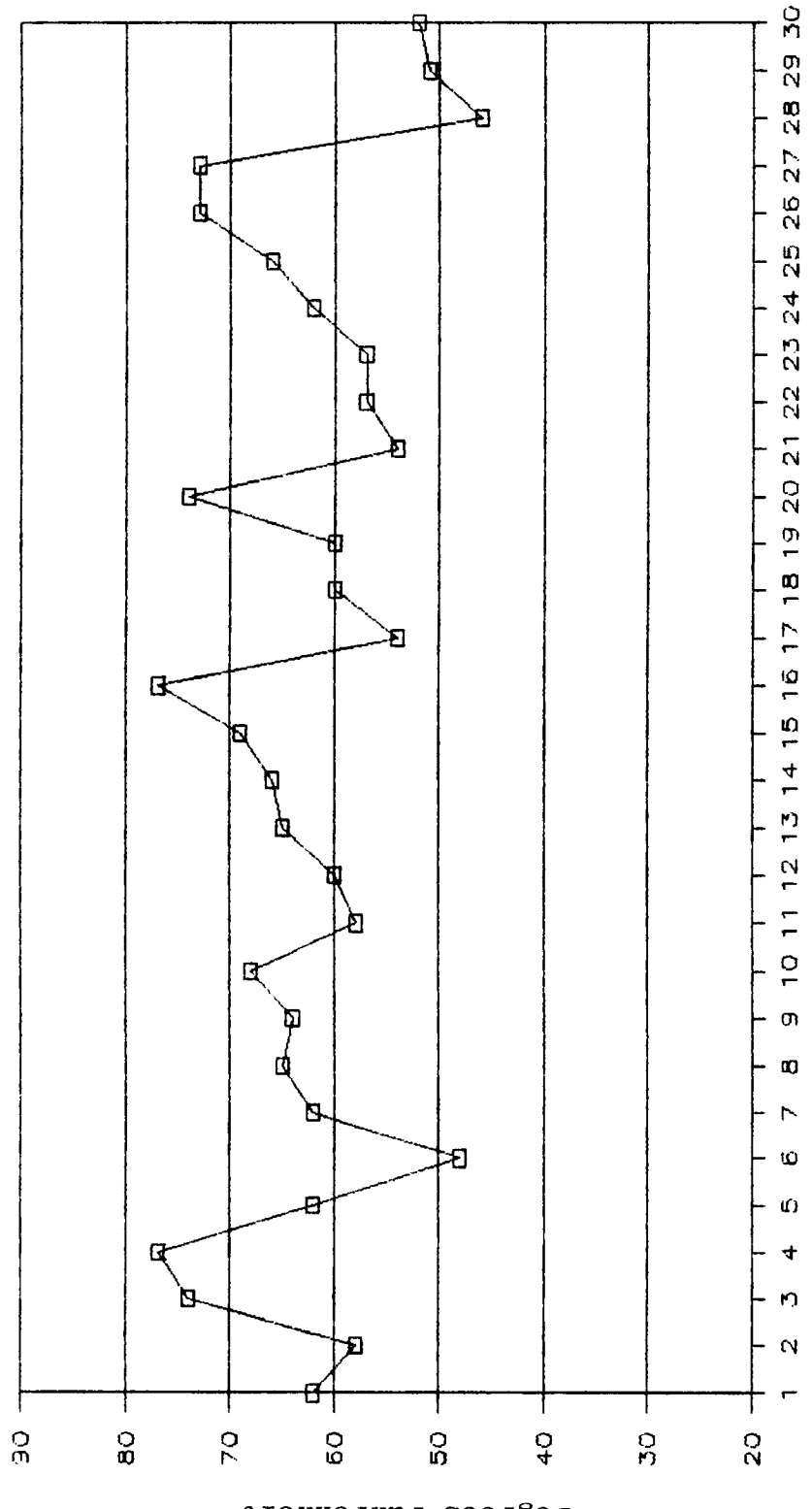

Figure 29. Maximum daily temperature for the Knoxville, Tennessee area for November 1988.

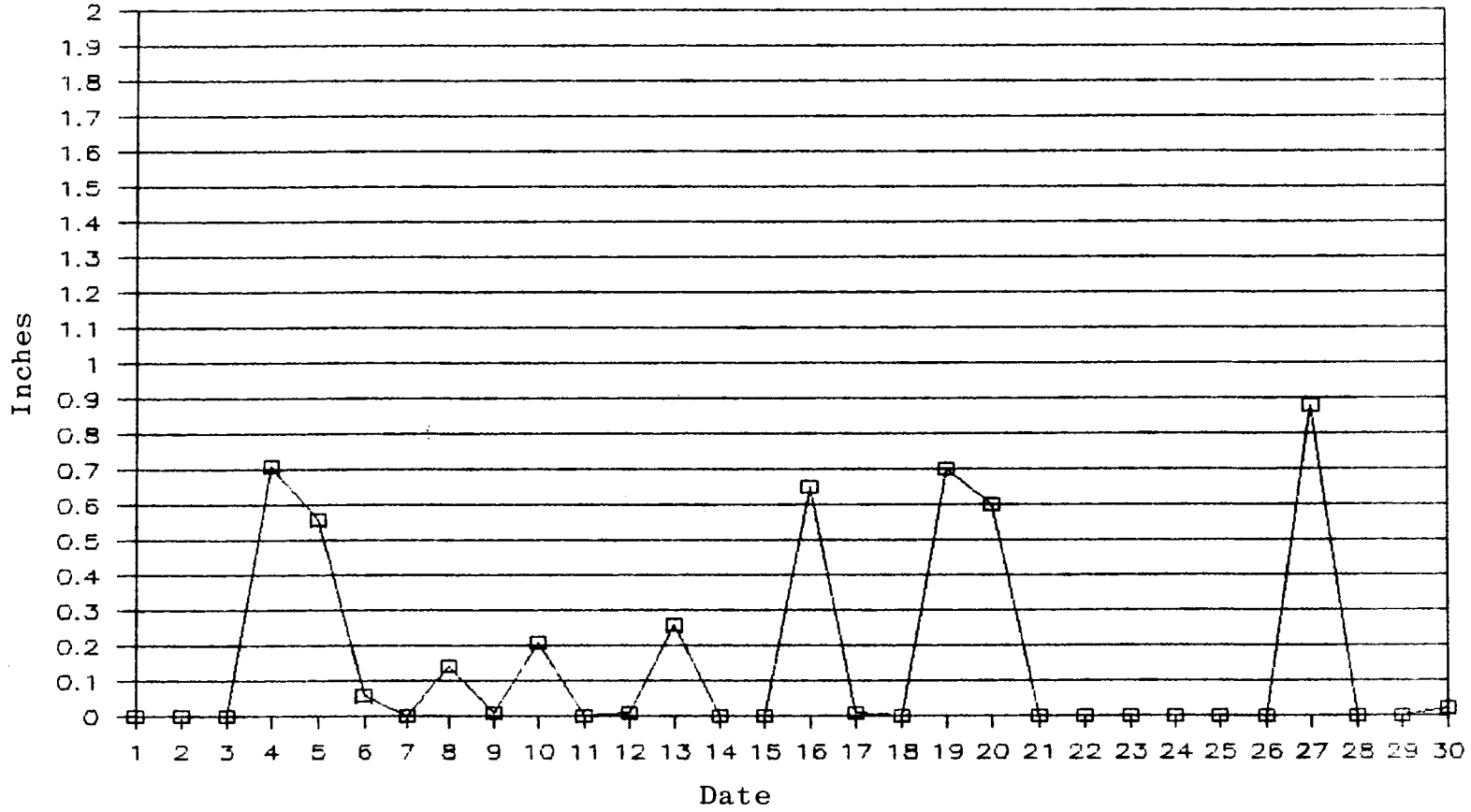

Figure 30. Daily precipitation for the Knoxville, Tennessee area for November 1988.

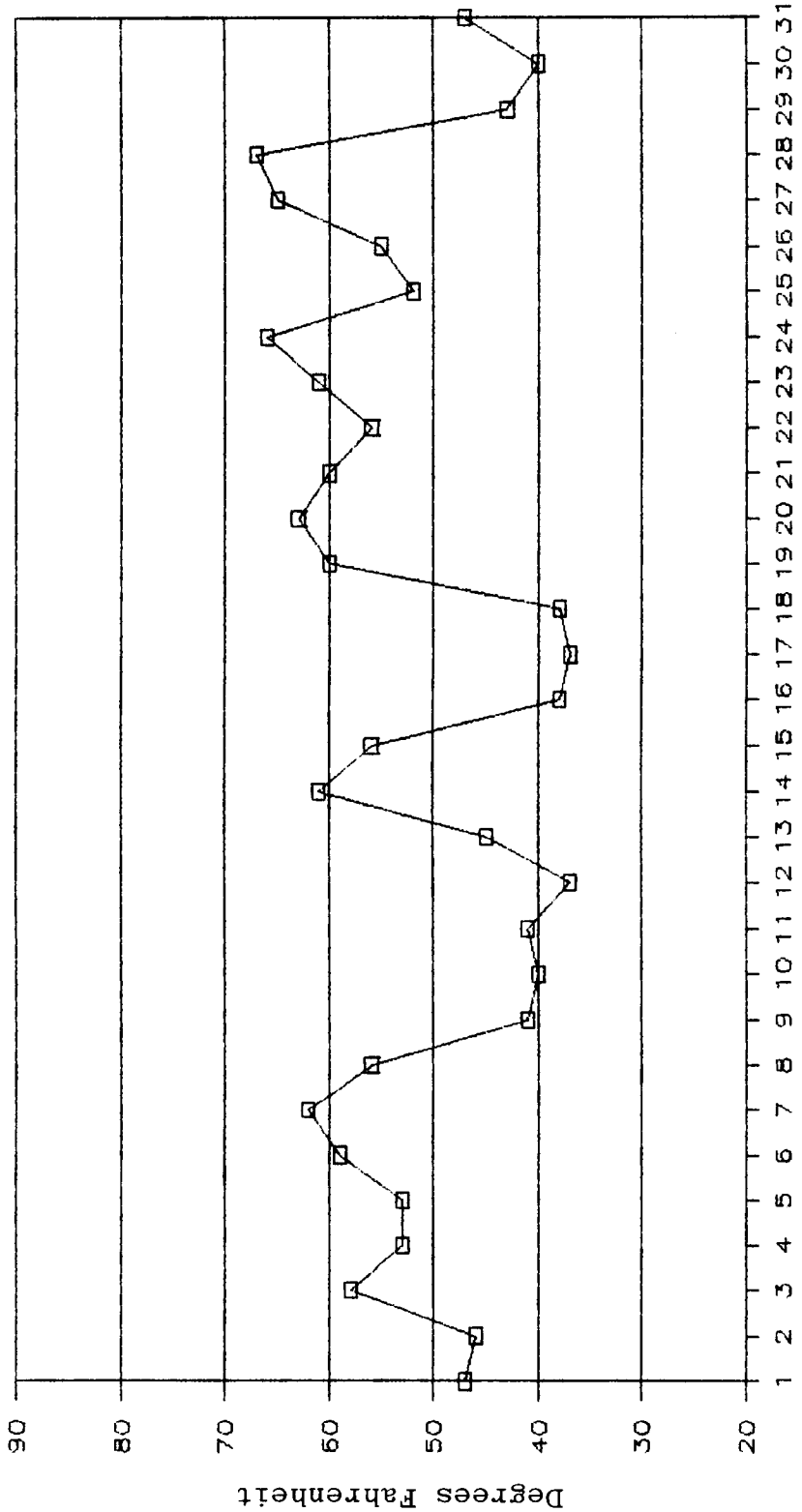

Figure 31. Maximum daily temperature for the Knoxville, Tennessee area for December 1988.

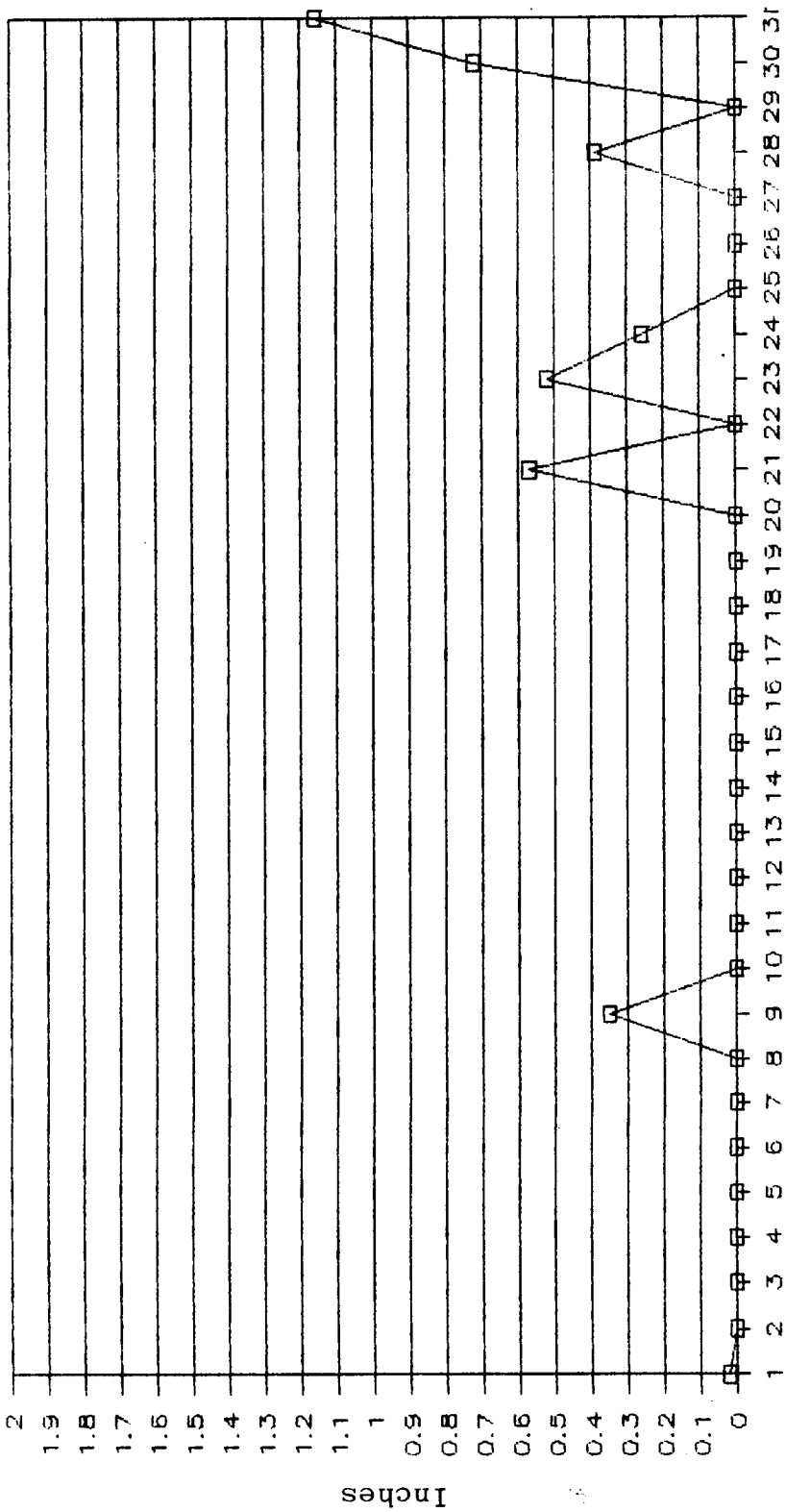

Figure 32. Daily precipitation for the Knoxville, Tennessee area for December 1988.

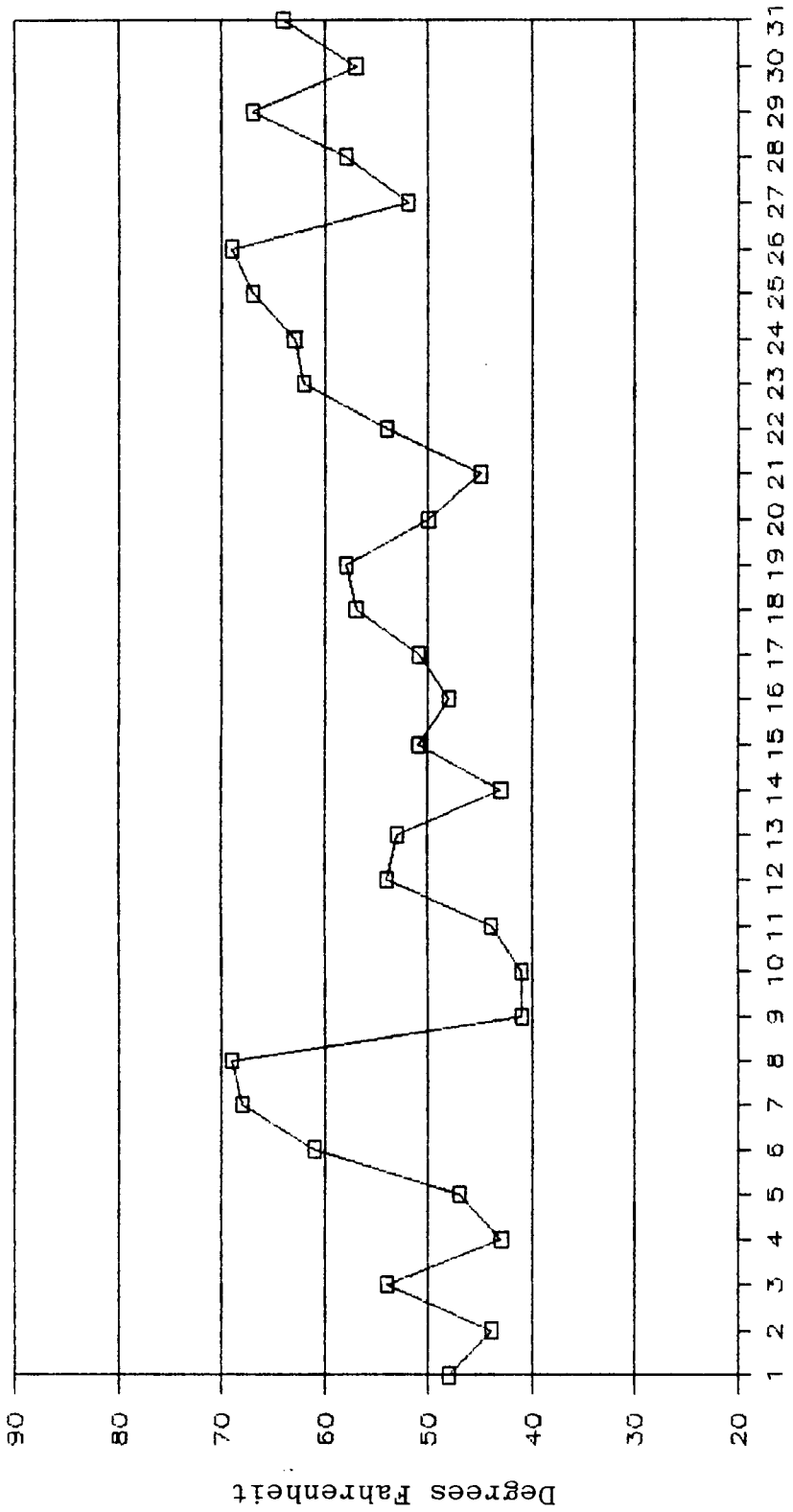

Figure 33. Maximum daily temperature for the Knoxville, Tennessee area for January 1989.

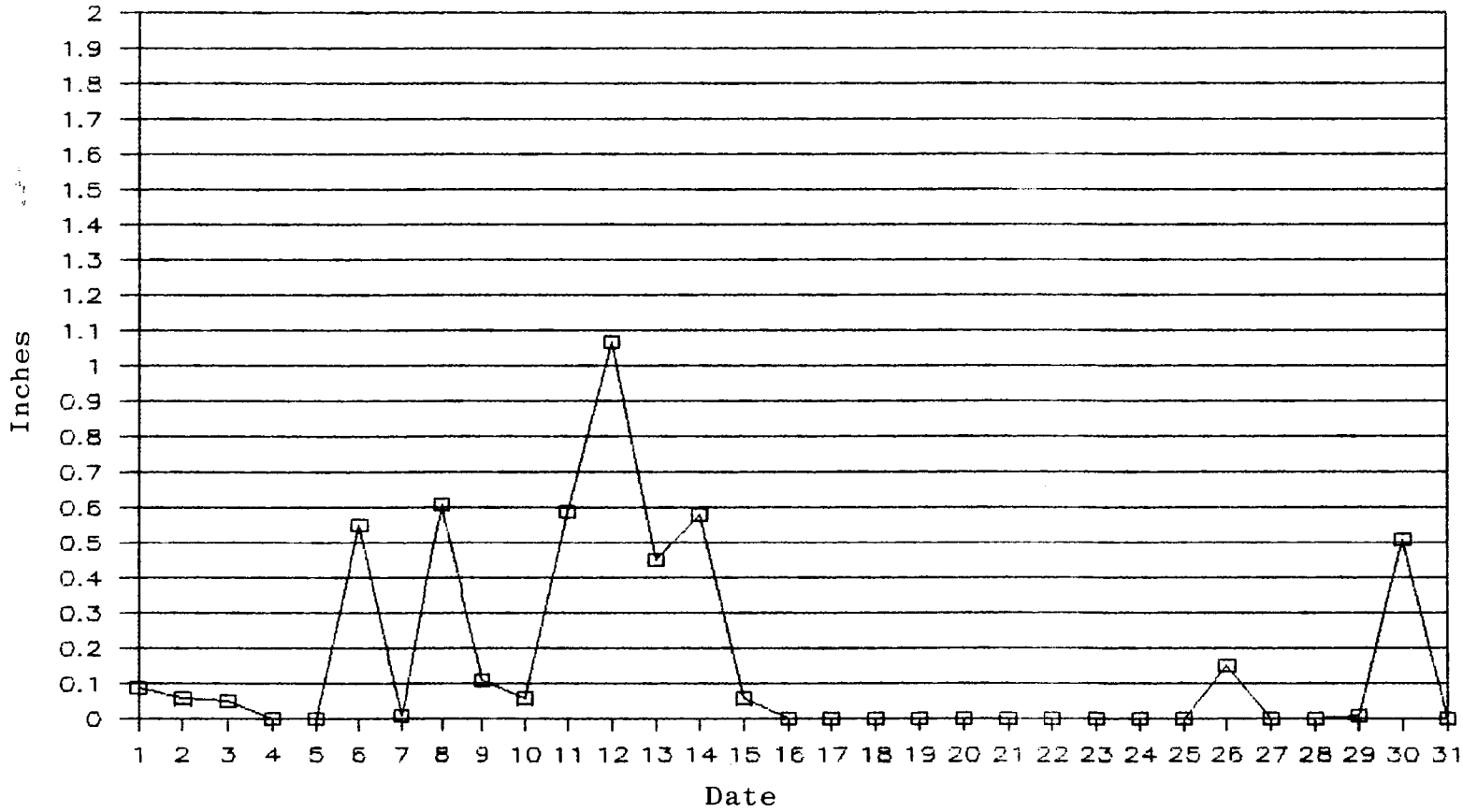

Figure 34. Daily precipitation for Knoxville, Tennessee area for January 1989.

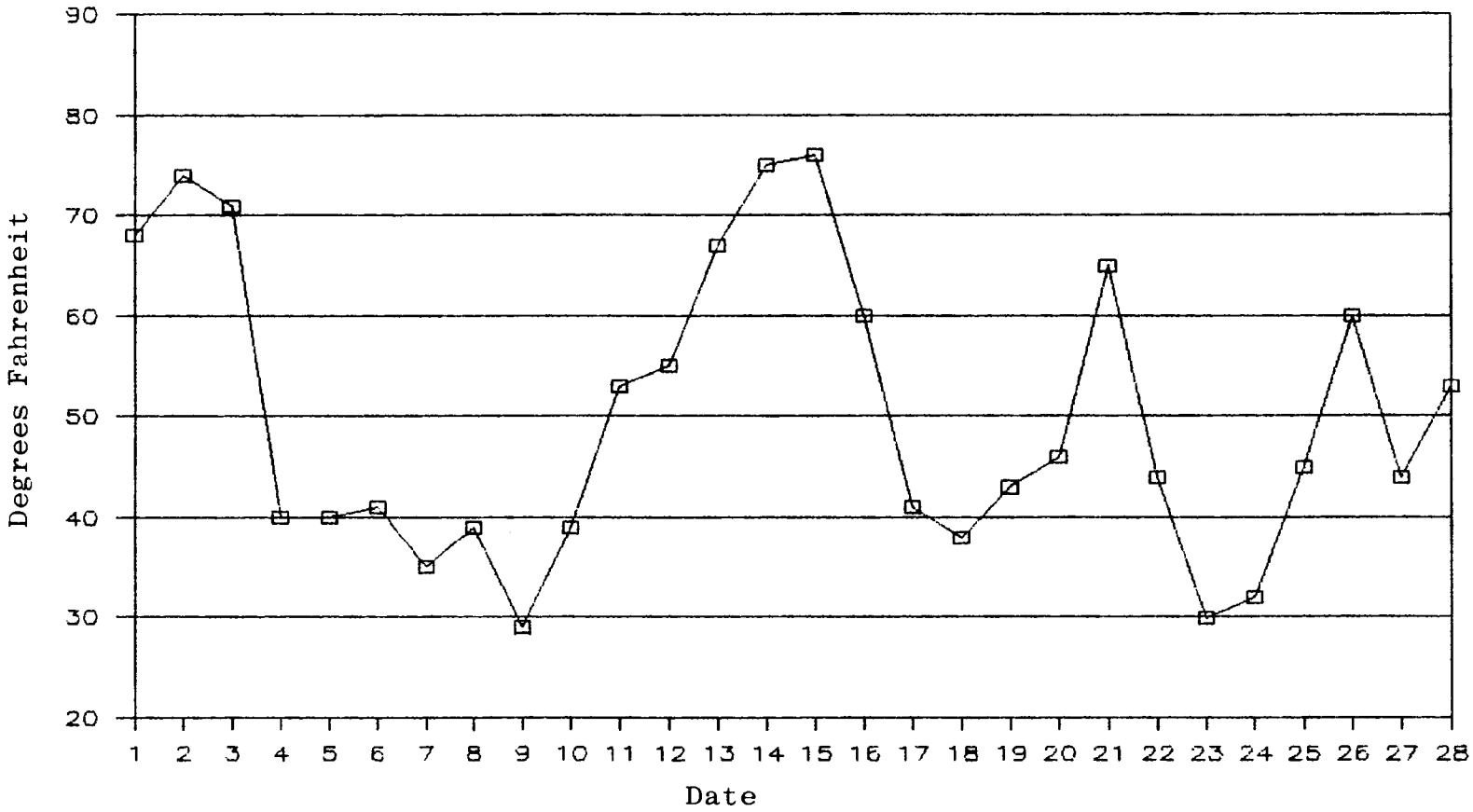

Figure 35. Maximum daily temperature for the Knoxville, Tennessee area for February 1989.

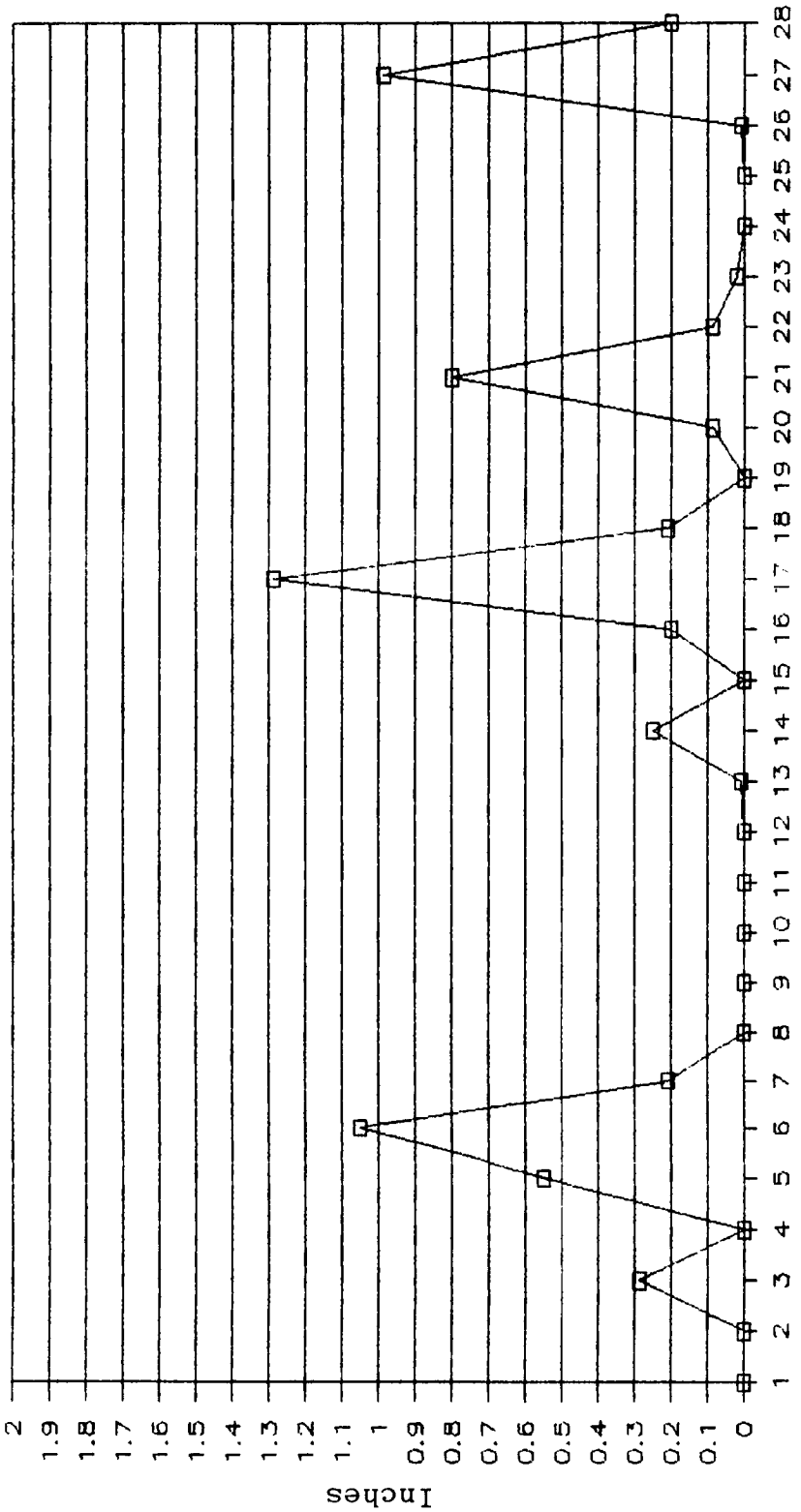

Figure 36. Daily precipitation for the Knoxville, Tennessee area for February 1989.

VITA

Frederick J. Snow was born in Louisville, Kentucky,on April 4, 1948. After graduating from Central High School in Knoxville, Tennessee, in June 1966 he entered Georgia State University and received a Bachelor of Arts degree in English in June 1970.

After graduation he worked for three years as a Geologic Test Technician with the Georgia Geological Survey. In July 1973 he joined the Dekalb County Police Department in Decatur, Georgia, as a patrolman. After leaving police work in early 1980, he became a freelance freefall photographer filming skydiving formations for the advertising and motion picture industries.

In September 1980 he went to work with Federal Express Corporation, where he is currently employed. In September 1985 he entered graduate school at the University of Tennessee, Knoxville, and received a Master of Arts degree in Anthropology in May 1989.

He is married to Cynthia S. Snow.