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L. T. Lytle,[1] *Ph.D. and D. G. Hedgecock,*[1] *B.A.*

Chemiluminescence in the Visualization of Forensic Bloodstains

The peroxidase-like activity of hemoglobin forms the basis for those tests most commonly employed for the preliminary identification of blood: the benzidine, phenolphthalein, and luminol tests. Among these the luminol reagent is unique in that its reaction with blood results in the production of light rather than color. This distinction makes it use somewhat inconvenient because of the need for darkness when the test is performed but also gives it its unique value. Since the luminol is applied as a spray, large areas may be quickly screened for blood; furthermore, luminol is relatively nondestructive to the surroundings (it is noncorrosive and nonstaining) and to the blood (it does not prevent subsequent identification tests or ABO blood grouping analysis although it does interfere with electrophoretic analysis of those systems thus far tested, erythrocyte acid phosphatase and phosphoglucomutase). Although often used in this manner to locate unnoticed blood for collection and further testing, a large part of the luminol test's value comes from the observation of patterns of blood residue which it makes visible. Traditionally, the only means of recording this information has been through written description. Although luminol photography has been previously reported [*1*] most people still believe that successful photography of the luminol test is impossible or at least very difficult. Since the previous publication we have continued testing and refining the luminol test and its photography, and it is now used and photographed whenever a crime scene is investigated in which it might be of value.

Optimizing the Luminol Reaction

Taking the classical recipe for the luminol reagent (0.5 g luminol,[2] 25 g sodium carbonate, and 3.5 g sodium perborate in 500 ml water) as a starting point we investigated the sensitivity of the luminol test by making a series of six aqueous solutions of whole blood ranging in concentration from 10^4 ppm to 10^{-1} ppm, soaking filter paper strips in each, and air drying and then spraying the strips with luminol solution in a darkened room. As shown in Fig. 1, we were sometimes able to get a visible reaction over the full concentration range and always down to 100 ppm.

The effect of varying the concentration of luminol in the reagent mixture was next evaluated, and we found that luminol concentrations of 0.5 g/500 ml, 1 g/500 ml, and 2 g/500 ml gave the same results both visually and on film.

One of the accepted facts of life has been that the luminol solution is unstable and must be mixed immediately before use. Since this mixing is often inconvenient or dif-

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[1] Chemist and serologist, respectively, N.C. State Bureau of Investigation, Raleigh, N.C. 27601.

[2] 3-Aminophthalhydrazide, Aldrich Chemical Co., or 5-amino-2,3 dihydro-1,4-phthalazinedione, Eastman Kodak Co.

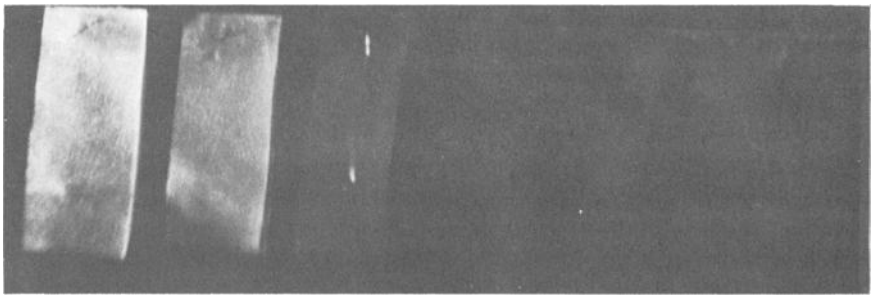

FIG. 1—*Sensitivity of the luminol test when luminol reagent is applied to strips of filter paper stained with solutions of blood ranging in concentration from* 10^4 *to* 10^{-1} *ppm.*

ficult (because of lack of water at the scene, low temperature resulting in poor solubility, and so forth) we investigated and found that luminol/carbonate/water and sodium perborate/water solutions could be stored separately for at least eight weeks and retain full activity when combined.

It has been reported [*2,3*] that old blood reacts better with luminol than fresh blood and that better sensitivity can be obtained with fresh blood by first spraying the area to be tested with 2% hydrochloric acid. We have found no evidence to support these contentions and in fact found that prespraying with hydrochloric acid gave diminished sensitivity and a greatly increased level of background illumination (Fig. 2). The latter possibly explains the previous reports of increased sensitivity, particularly if testing was carried out under actual crime scene conditions.

FIG. 2—*Effect on luminol of prespraying bloodstained strips of filter paper with dilute hydrochloric acid.*

Although the addition of fluorescein to the luminol solutions did not give the reported [*4*] significant increase in light output, the shift in color of the luminescence from the pale blue of luminol to the green of fluorescein may be useful when background illumination cannot be eliminated and occurs in the same region of the spectrum as the luminol chemiluminescence, making the successful use of filters to subdue the background impossible. One drawback to the use of fluorescein is its intense yellow color, making its use impractical where staining would be a problem.

We had originally intended to investigate the possibilities of substituting higher yield chemiluminescent compounds such as benzo(ghi)perylene-1,2-dicarboxylic acid hydrazide [*5*] and the 7-dialkylaminonaphthalene, 1,2-dicarboxylic hydrazides [*6*]. Consideration of the possible benefits versus the probable carcinogenicity of these compounds, however, led us to abandon this aspect of our research.

Reagent Applications

We have experimented with electropneumatic, Freon®, and hand-pump sprayers, and although all have their advantages, the most satisfactory overall has been the simple hand-pump sprayer (Fig. 3). It is readily available from a variety of sources, inexpensive, easily portable, requires no power supply, is usable at any temperature above the freezing point of the luminol reagent, and perhaps most important, gives precise control over the amount of reagent applied. The latter is very important when the luminol reaction is being photographed because of the frequent need to sustain the reaction at full intensity for some time without overloading the area with reagent to the point where the reagent and bloodstain run, thus altering the disclosed pattern.

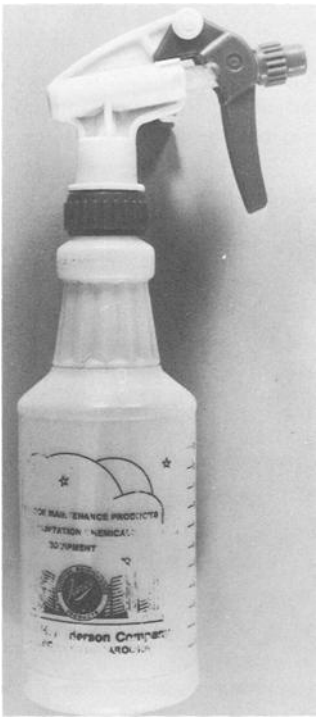

FIG. 3—*Hand-pump sprayer used in applying luminol reagent.*

Effect of Cleaning on Several Bloodstained Surfaces

Placing blood on a variety of surfaces and removing half of the bloodstain after 30 min and the other half after 18 h with a mild soap and water solution showed that porous surfaces and surfaces which were not cleaned quickly retained a relatively large quantity of blood and gave fairly intense reactions with the luminol. Irregular surfaces such as wood-finish paneling proved very resistant to cleaning, with blood remaining in the grooves in the surface even after vigorous scrubbing (Fig. 4). Smooth nonporous surfaces such as nontextured linoleum or vinyl tile were fairly easy to clean completely, although a quick wipe such as might be encountered at a crime scene left a detectable residue.

Additional washing procedures using several different cleaning agents such as Lysol®, Phenola® (an industrial detergent-germicide), and bleach were employed on the pre-

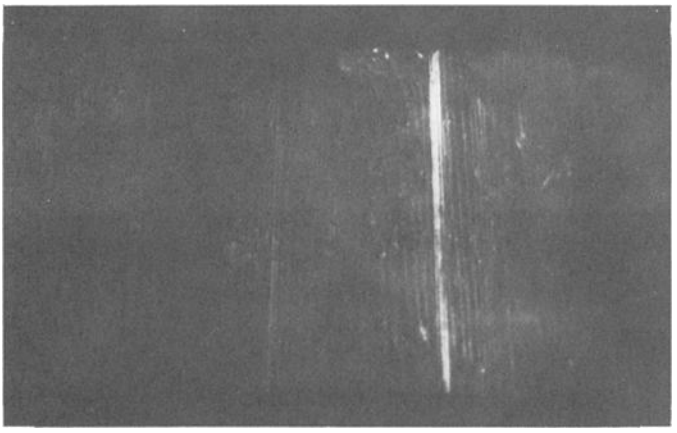

FIG. 4—*Result of spraying luminol on bloodstained piece of paneling after it was thoroughly cleaned with soap and water.*

viously washed surfaces with the expected results: essentially complete removal of the bloodstains yielding weak to nonexistent reactions with luminol. An exception was the bleached bloodstain on carpet, which gave a vigorous reaction. Not surprisingly, this reaction proved to be attributable to the bleach itself because of its powerful oxidizing properties (Fig. 5).

Photographing the Luminol Reaction

We have tried a number of film/developer combinations for photographing the luminol reaction and have obtained the best results with Kodak Tri-X developed in HC110 developer (dilution B) to which has been added Factor 8 (10 ml/litre of developer).[3] Development for 22½ min at 20°C (68°F) with 5 s of gentle agitation every minute gives an exposure index of 3200 while retaining most of the grain, resolution, and contrast characteristics of normally processed Tri-X. In addition, the film is physically much easier to handle than the polyester-based recording films we had previously tried.

All of our photography has been done with Nikon and Pentax 35-mm single-lens reflex cameras using 35- and 50-mm f/1.4 lenses. All exposures have been made at the maximum aperture of f/1.4 at exposure times ranging from 15 s to 2 min. Although the luminol chemiluminescence is faint, 30 s most often sufficed and often shorter exposures were

FIG. 5—*Reaction obtained when bleached piece of carpet was sprayed with luminol.*

[3] Min Max, 3615 Hayden Ave., Culver City, Calif. 90230.

necessary to avoid overexposure with resulting loss of detail. Long exposures often result not only in overexposure of the luminol but under field conditions also in the swamping of the luminescence by ambient light (Fig. 6). Obviously the optimum exposure length will depend on the intensity of the luminescence. Fortunately the luminol reactions can usually be sustained long enough to allow more than one exposure to be taken and thus different exposure times can be tried.

The photographs obtained under these conditions have been of sufficient quality to require no special printing. They have most often been printed on normal contrast-grade paper from both Kodak and Ilford and on both normal and resin-coated papers.

Often, in photographing luminol results at crime scenes, it is difficult to completely darken the area as can be done in the laboratory. We have tried using a variety of blue filters to transmit only the luminescence but have generally been unsuccessful because almost always the background illumination is from a mercury vapor or fluorescent source whose wavelengths fall in the same region as the luminol chemiluminescence. Perhaps here the addition of fluorescein could be used to shift the wavelength of the luminescence away from that of the background sufficiently to allow filtration to be used to advantage, a technique we have not yet tried.

Interpretation of the Luminol Test

Luminol is unfortunately not completely specific for blood since it reacts quite well with vegetable peroxidase and some metals such as copper and copper alloys. The former limits the usefulness of luminol outdoors, but any confusion which might arise over a stain can usually be resolved by further testing and intelligent observations. Contrary to some expressed opinions we find, as we expected, no reaction with such body fluids as perspiration, saliva, semen, and urine. The false reaction with metal is almost never a problem because such luminescence can usually be anticipated or resolved by careful examination of the scene and in fact often helps establish the locations of the luminescence relative to

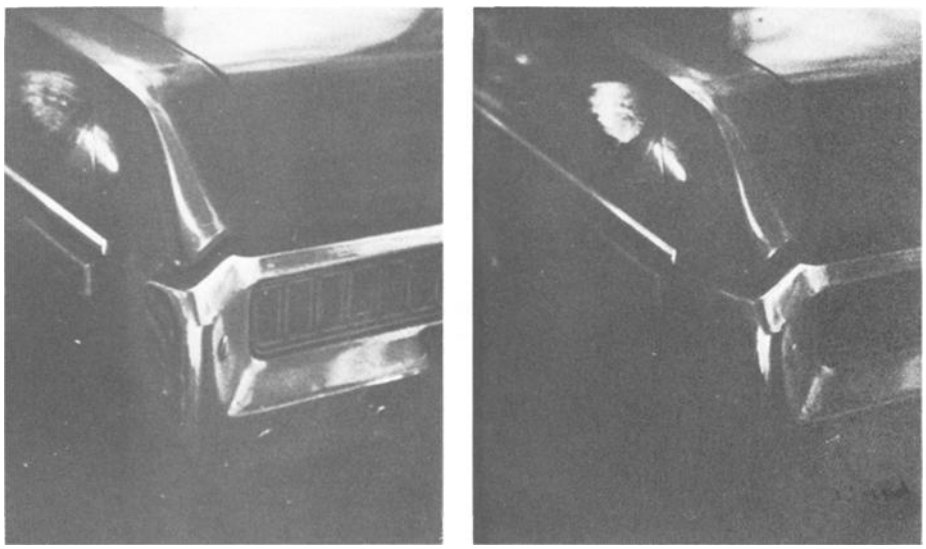

FIG. 6—*Two- and one-second exposures of luminol reaction with a high level of background light present.*

the scene. Although none of these observed false reactions should provide serious confusion, they do call for the use of trained personnel to conduct the luminol test.

Since luminol photographs usually show a pattern of light against a dark background, it is often difficult to determine the size of the luminescence and its relationship to the scene. One somewhat crude technique for establishing scale uses the false reaction of luminol with copper by including in the area sprayed and photographed a 15-cm (6-in.) ruler with two pennies taped to the ends giving two luminescent spots at a known separation. A more elegant technique is the inclusion of a scale marked with luminescent tape or paint.

Occasionally the inability to completely darken a crime scene establishes the relationship of the luminescence to the scene by accidentally providing a balance between background light and luminescence that results in a photograph showing both (Fig. 7). This is chancy, however, and our attempts to produce this balance deliberately by adding faint light, filtration, and double exposure have not been very successful. The most satisfactory solution to this problem has been two separate exposures employing two cameras and different films from the same location, one with flash of the background and one a time exposure of the luminol reaction (Figs. 8 and 9).

Luminol in the Field

Our laboratory has used the luminol test in the field for many years to assist investigations by establishing the presence and location of minor or hidden bloodstains at a particular scene and occasionally by enabling the investigator to reconstruct some of the

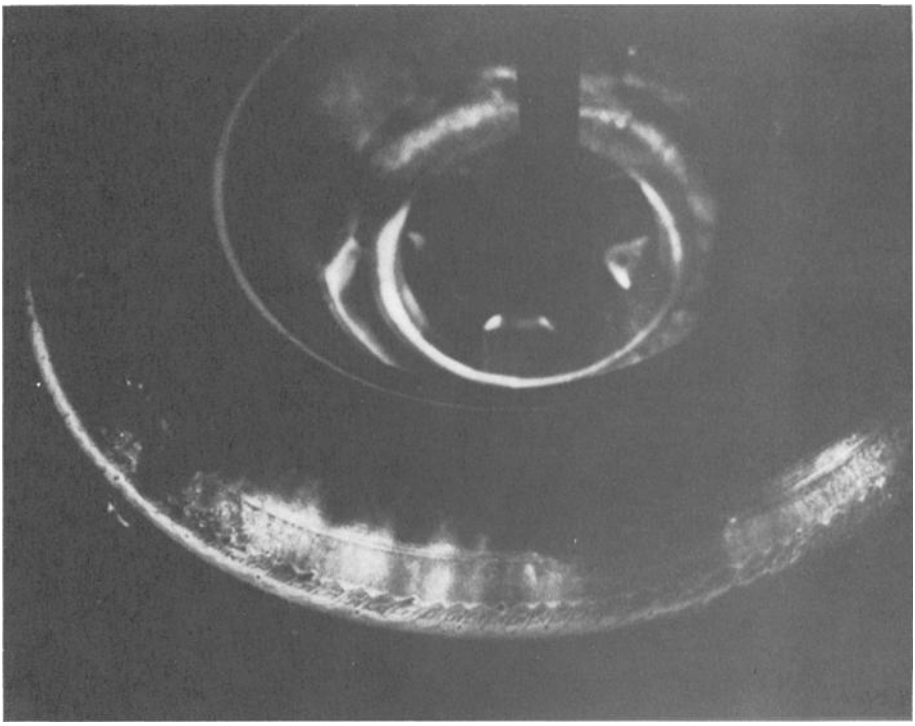

FIG. 7—*Front right tire, visualized with luminol, of a vehicle allegedly involved in a homicide.*

FIG. 8—*Linoleum floor in the kitchen at the residence of a missing person.*

FIG. 9—*Circular pattern revealed when the same section of the floor shown in Fig. 8 was sprayed with luminol.*

events of a crime by visualizing the patterns of bloodstains that otherwise would not be seen. Successful photography of crime scene luminol reactions encountered by several of the serologists in our laboratory in the past few years demonstrates the value of this method of permanently recording such reactions as opposed to the awkward, inadequate, written descriptions which otherwise must suffice.

Figures 10 and 11 illustrate a night table photographed with normal lighting and when sprayed with luminol. In addition to the reaction of the luminol with the metal drawer pulls, a large number of luminescent specks and streaks can be seen in the lower half of the photograph. These reactions were obtained while a luminol examination was conducted of a bedroom in which a shooting victim was found. Subsequent visual inspection of this area of the night table revealed visible bloodstains sufficient for collection and ABO blood grouping analysis.

Figures 12 and 13 illustrate a portion of a linoleum floor at the foot of a metal bed as seen under normal lighting and when sprayed with luminol during an examination of a residence where an individual was alleged to have been beaten to death. No visible bloodstains were noted in this area (the dark spots seen surrounding the bedpost in Fig. 12 are rust stains), but Fig. 13 dramatically illustrates a smeared pattern as would be created by wiping or mopping up blood in an attempt to remove blood from the floor. These smears extended to a brown recliner chair; visual examination disclosed a rather heavy bloodstain along its bottom edge. The subsequent analysis of this bloodstain in addition to the results of the luminol examination itself played a major role in obtaining a guilty plea from the suspect in this case.

Figure 7 illustrates the front right tire removed from a vehicle suspected of having been involved in a homicide in which the victim was beaten, shot, and finally run over. The intense luminol reaction in the tread region of this tire was consistent with other findings in the case such as the previous discovery of human blood in the wheel well above the tire.

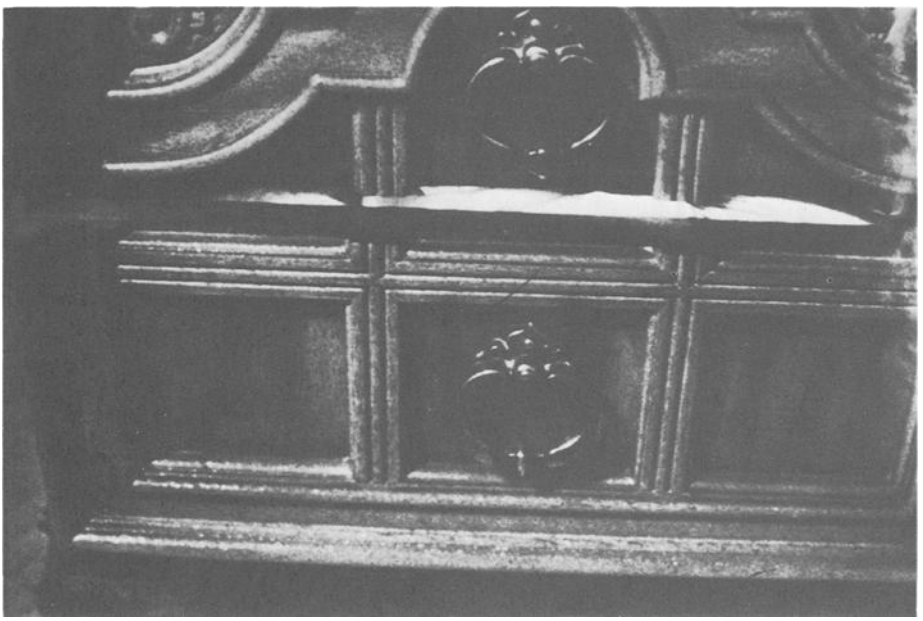

FIG. 10—*Night table in a bedroom at the residence of a shooting victim.*

FIG. 11—*Various luminescent reactions exhibited when the night table shown in Fig. 10 was sprayed with luminol.*

FIG. 12—*Linoleum floor at the foot of a metal bed inside a residence where an individual was allegedly beaten to death.*

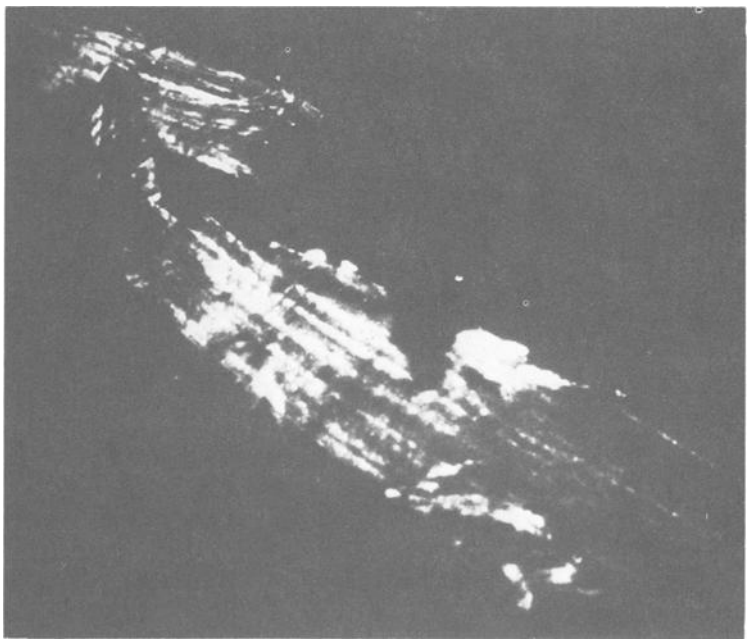

FIG. 13—*Smeared patterns disclosed when the same portion of the floor shown in Fig. 12 was sprayed with luminol.*

Very distinct patterns are sometimes obtained during luminol examinations. In addition to a small area of visible bloodstains that had initially been discovered in one room of a residence from which an individual was reported missing a subsequent luminol examination of the residence indicated that a relatively large amount of blood had been shed and then cleaned up. It was concluded that the circular pattern shown in Fig. 9 was left by the bottom of a bucket carried about during the cleanup. A small clump of sponge, blood, and hair was found near where this photograph was taken. Figure 8 illustrates this same section of floor under normal lighting.

Other outstanding luminol patterns were revealed throughout the residence at this same crime scene. Figure 14 illustrates only one of the many shoe tracks found at this scene. Figure 15, *left*, illustrates the print of a bare left foot found at this scene, and Fig. 15, *right*, shows an inked impression of the left foot of a suspect in the case. Although no positive identification could be made on the basis of this photograph many points of similarity such as the length of the second toe, the instep feature, and the overall length of the footprints can be seen, and this photograph continues to be one of the few pieces of physical evidence linking this individual to this homicide case in which the victim's remains have just recently been discovered, almost two years since his disappearance.

A handprint on the wooden floor at the scene of a brutal stabbing (Fig. 16) and the print of a bare right foot on the carpet outside the bedroom of a shooting victim (Fig. 17) even further illustrate some of the types of patterns we have experienced in field situations and the variety of background materials encountered from one scene to another.

These crime scene photographs represent exceptional results. More often than not the patterns obtained with luminol in crime scene situations are general and obscure. Because of the need to interpret the cause and meaning of a given luminol reaction as it occurs, the need to collect and analyze samples when possible, and testimony that might be required with regard to the luminol examination and any subsequent analyses,

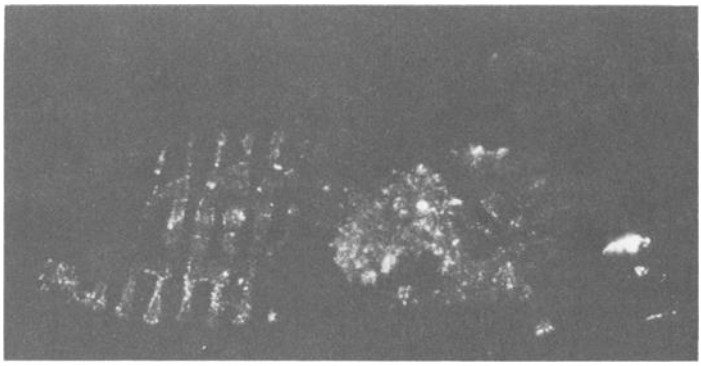

FIG. 14—*Shoe track pattern revealed by luminol at the residence of a missing person.*

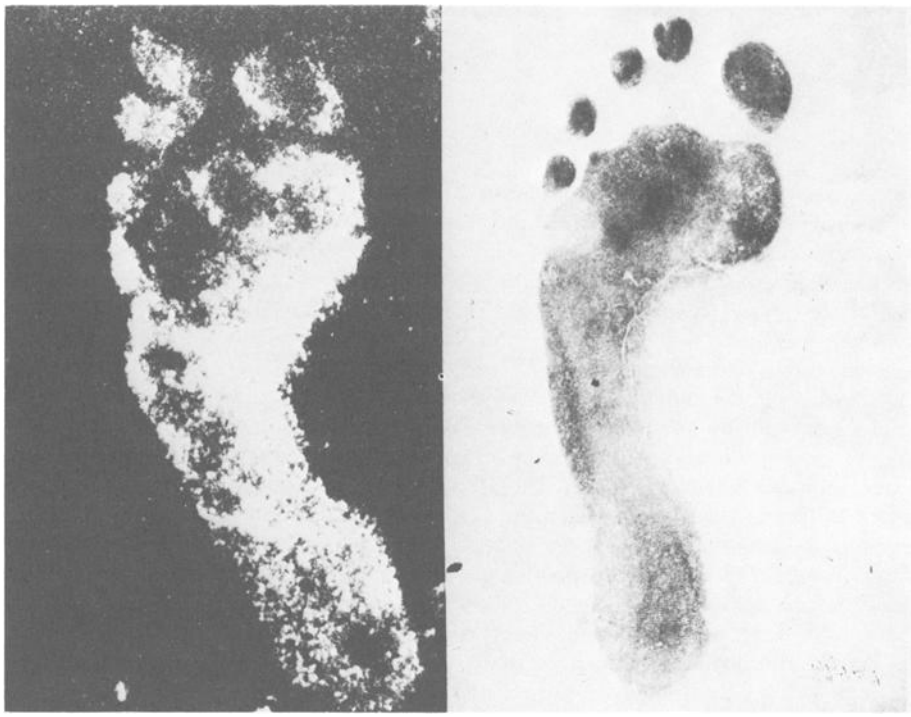

FIG. 15—*Comparison of the pattern of a bare left foot disclosed by luminol at the residence of a missing person and the inked impression of the left foot of a suspect.*

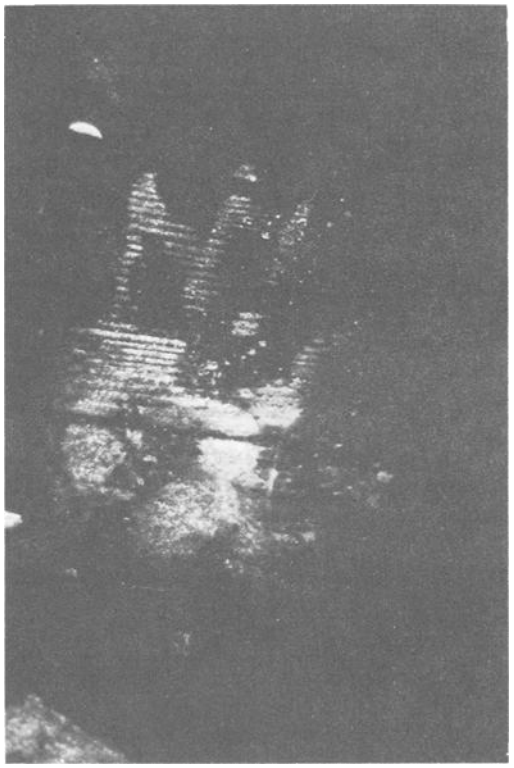

FIG. 16—*Pattern revealed by luminol of a left hand on a wooden floor at the scene of a stabbing.*

our laboratory prefers that a serologist conduct or at least be in attendance during luminol examinations.

Summary

The luminol test is a valuable field test for the detection of blood because it is sensitive, can easily and rapidly screen large areas with a simple and inexpensive hand-pump spayer, is relatively nondestructive both to blood and to the scene, and is reasonably specific for blood even though it does react with vegetable peroxidases and with some metals and chemicals. Photography using readily available equipment and materials provides a permanent record of patterns of blood residue made visible by the luminol.

Experiments show that the luminol test is independent of the concentration of luminol in the reagent mixture and is sensitive to at least 100 ppm of blood and occasionally to as little as 10^{-1} ppm of blood. No increase in sensitivity is observed after blood is presprayed with dilute hydrochloric acid nor with the addition of fluorescein to the reagent mixture. The degree of success attained in preventing detection of blood with the luminol test by cleaning bloodstained articles is influenced by the surface texture of the bloodstained material, the length of time between the deposition of the blood and the cleaning effort, the type of cleaner used, and the diligence of the cleaning effort.

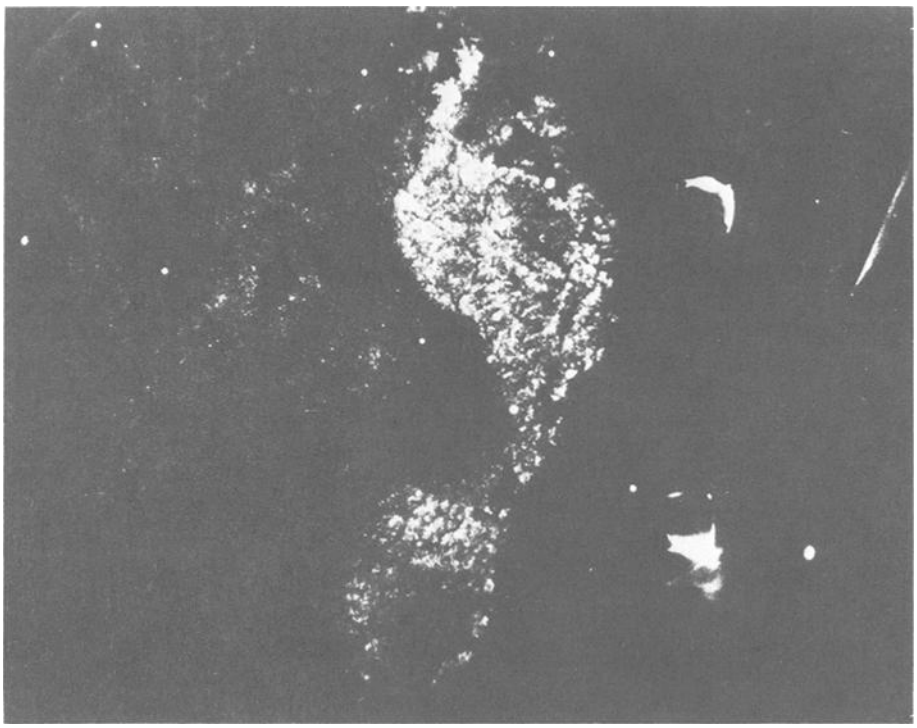

FIG. 17—*Pattern revealed by luminol of a bare right foot on a carpet at the residence of a shooting victim.*

Acknowledgments

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Address requests for reprints or additional information to
L. T. Lytle, Ph.D.
N.C. State Bureau of Investigation
421 N. Blount St.
Raleigh, N.C. 27601