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The Enhancement of Blood Prints by Chemical Methods and Laser-Induced Fluorescence

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Numerous papers have been written concerning the detection of latent fingerprints through their inherent fluorescence, when illuminated by the blue-green light of the argon ion laser.[1-4] The detection of latent fingerprints is further extended by the use of an ever increasing variety of chemical methods ranging from dusting with fluorescent powders to the enhancement of ninhydrin-developed prints with zinc chloride.[5-12]

The detection of blood and the enhancement of blood prints have been discussed in detail in a number of articles.[13-17] Conventional techniques currently employed for the detection and screening of blood are generally considered catalytic tests; that is, they test for the catalytic peroxidase activity of heme in blood. These tests are not specific for blood; they have, however, proven to be quite sensitive and have been well adapted as a means of searching for and screening suspected blood stains.[18] These methods have been further applied to the development and enhancement of visible and invisible blood prints with success. Several other tests rely on the formation of hematoporphyrins and their subsequent fluorescence when excited by long wave ultraviolet light.[19,20] These test are very sensitive, but they tend not to lend themselves well to the enhancement of blood prints.

The use of the laser for the detection of blood and/or the enhancement of blood prints has not been widely written about.[17] In this paper, the potential of chemically treating blood prints followed by argon laser illumination and examination is considered.

We initiated this investigation after a blood print, which had been treated with ortho-tolidine reagent and a hydrogen peroxide solution (see below), followed by an examination with an argon laser. The blood print had yielded its characteristic blue oxidation product, but when examined with the laser and viewed through anti-laser goggles (for the argon laser), there were oberved predominant areas of orange florescence where the red blood had been most concentrated. To one of the authors (J.F.), the intense orange fluorescence appeared characteristic of hematoporphyrin fluorescence after blood has been treated with concentrated sulfuric acid.[21]

At this point, a series of experiments were conducted to determine which reagent or combination of reagents had been responsible for the observed fluorescence under the argon laser.

These examinations were carried out using a Laser Ionic four-watt argon laser Model 552, at four watts. The fluorescence was viewed through Laser Guard LG-A anti-laser goggles, and photographed with a Nikon FE 35MM camera using a Micro-NIKKOR lens with an orange Pro YA-2 filter.

Initially, the blood print area which was located on a sheet of plastic, was lightly sprayed with an ortho-tolidine solution prepared as follows:

One gram of ortho-tolidine dissolved in 90 milliters of absolute methanol, followed by the addition of 10 milliters of 90% formic acid. It had been found that this solution tended to prevent leaching and stabilized the print.

When the treated area had dried, it was lightly sprayed with a hydrogen peroxide solution prepared as follows:

Thirty-five milliters of 3% hydrogen peroxide in 65 milliters of absolute methanol.

An appropriate indication for the presence of blood was observed; in as much, as the blood print area turned blue in color. Very little, if any, enchancement was observed. We then examined the blood print area using the laser. We, again, found little, if any, of the print. It was noted, however, that where there had been an accumulation of liquid blood prior to drying, an intense fluorescence was observed.

As previously mentioned, it was the author's belief that the hemoglobin itself might be involved in forming a fluorscent hemoglobin derivative. Subsequently, several dried flakes were treated with concentrated sulfuric acid and then illuminated by the laser. It was found that the blood flakes fluoresced intensely. This suggested to the authors that the fluorescence might be due to the formation of hematoporphyrins when excited by the blue region of the argon laser.

A series of experiments was set up in an effort to determine which reagent or combination of reagents

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t be responsible for initiating the observed fluorescence. A series of test tubes was filled with reagents and combinations of reagents. The results of their fluorescence or lack of fluorescence are given in Table No. 1. →

It was found that when blood was treated with a solution of formic acid, followed by a weak hydrogen peroxide solution, an intense orange fluorescence was obtained. The two reagents (A and B) were prepared as follows: Solution A - five milliter of 90% formic acid in 95 milliters of absolute methanol. Solution B - thirty-five milliters of 3% hydrogen peroxide in 65 milliters of absolute methanol.

After the above working solutions were obtained, a second experiment was set up. This involved applying a blood print to a small plastic sheet. The prints were allowed to dry for several hours, and then photographed in room light in an effort to il- ate what could be visualized prior to treatment with the reagents (see Figure 1). The blood was then lightly sprayed with Solution A (formic acid solution). After the print had dried, it was lightly sprayed with Solution B (hydrogen peroxide solution). The print was then illuminated by the laser, examined, and photographed (see Fig. 2 on page 15).

Figure 2 shows that where large amounts of liquid blood were deposited, these areas exhibited the most intense orange fluorescence. Preliminary indications are that the fluorescence is possibly due to hematoporphyrin formatin, or the formation of a hematin derivatives.

Though no serological examinations were conducted, it is believed that the reagents would be deleterious to the stain for any future serological examinations. It is, therefore, recommended that a sample of a visible stain be recovered to the application of the ents.

Further applications, of this laser technique beyond the enhancement of blood prints, would be utilizing it as a search technique, or as a method to visualize wipe marks indicating an effort to clean up. Efforts to visualize blood stains or prints will always be dependent on the inherent fluorescence of the substrate on which the stain or print is left. The laser and laser techniques, such as this one, will become more beneficial when the laser itself becomes more portable.□

TABLE NO. 1: Results of individual reagents, combined reagents, and reagents combined with blood.

GROUP	REAGENT(S)	RESULTS
A	formic acid (solution)	slight yellow green fluorescence of solution
B	hydrogen peroxide (solution)	very little fluorescence of solution
C	A and B combined	slight yellow fluorescence
D	A and liquid blood	very weak oronge fluorescence
E	B and liquid blood	very weak orange fluorescence
F	A and B combined with liquid blood	intense orange fluorescence

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PHLOXINE B

SOLUTION A:

0.1 g phloxine B
200 ml methanol
0.2 ml formic acid

SOLUTION B:

0.15 g phloxine B
245 ml methanol
3-5 ml formic acid

Does not create background