TECHNICAL NOTE

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A Simple Histochemical Technique for the Identification of Gunshot Residue

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ABSTRACT: Alizarin red S (ARS) is a commonly used organic dye useful in the histologic identification of calcium deposits. ARS also forms colored reaction products with other metal ions, including barium and lead, which are present in primer residue. In histochemical studies, ARS is shown to identify primer residues from several manufacturers as well as primer residue deposited in tissue, either experimentally or in close-range gunshot wounds. This can be easily accomplished with routine histologic techniques. ARS does not stain gunpowder residue, tattoo pigment, melanin, graphite, india ink, or anthracotic pigment.

KEYWORDS: pathology and biology, gunshot residues, alizarin red S (ARS), wound ballistics, histology

In the United States each year there are more than 30 000 deaths from firearms injury, the large majority of which are suicides and homicides [*1*]. All suicides and most homicides occur at close range, making the detection of deposited gunshot residue an important clue in the forensic investigation. Many techniques are available to accomplish this task, including neutron activation analysis (NAA), atomic absorption spectrophotometry (AAS), and scanning electron microscopy with energy dispersive analysis (SEM-EDA) [*2–4*]. Although these methods are all relatively sensitive and specific, they are costly and require highly specialized equipment. A method that is simple, readily available, and inexpensive may be useful in situations where other techniques are not cost-effective or applicable.

Alizarin red S (ARS) is an organic dye capable of forming a red-orange insoluble precipitate when combined with calcium ions. This is the basis for a simple and elegant histochemical stain for calcium deposition in tissue [*5*]. The authors demonstrate that ARS also identifies several metal ions contained within primer residue and that this provides a simple and inexpensive method of identifying gunshot residue in routine paraffin embedded-tissue sections.

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Materials and Methods

A working solution of ARS was prepared as described by Luna [6]. One gram of ARS (National Analine and Chemical Co., New York) was dissolved in 100 mL of distilled water. To this was added 10 mL of 0.1% ammonium hydroxide. This solution was used undiluted for all subsequent experiments.

The ability of ARS to form an insoluble complex with metal ions commonly found in primer formulations was tested after the method of Dahl [5]. To 2.0 mL of 0.2*M* acetate buffer, adjusted to pHs of 4.0, 5.0, 6.0, and 7.0, was added 0.5 mL of a 1% (weight/volume) solution of each of the following: calcium chloride, barium chloride, lead acetate, antimony trichloride, and water as a negative control. Each tube was then vortexed, a drop of ARS solution was added, and the tube was revortexed. The tubes were examined for the presence of a precipitate, the color of which was recorded.

Positive control samples were deposited on glass microscope slides by shooting them with a specially prepared primer-only cartridge. Clean .357 magnum cartridge cases (Federal Cartridge Corp., Minneapolis, Minnesota) were loaded with primers from Federal, Remington Arms Co. (Bridgeport, Connecticut), CCI-Omark (Lewiston, Indiana), and Winchester-Western-Olin (East Alton, Illinois). These were discharged in turn onto glass microscope slides at a distance of 5 cm from a stainless steel .357 magnum revolver with a 4-in. (10.2 cm) barrel (Smith & Wesson, Springfield, Massachusetts). These slides with adherent primer residue were then treated like tissue sections for the purpose of staining.

Positive control samples of gunpowder residue were deposited on glass slides by igniting a small amount of powder on a microscope slide. Gunpowder from Winchester-Western-Olin, Hercules (Wilmington, Delaware), and Dupont (Wilmington, Delaware) was placed on a glass microscope slide and ignited with a match. These slides with adherent gunpowder residue were then treated like tissue sections for the purpose of staining.

Experimental gunshot wounds were made in detached skin specimens, and the resulting tissue was sectioned into blocks, routinely fixed in 10% neutral buffered formalin, paraffin embedded, and sectioned at 6 to 8 μm thicknesses on a microtome. A primer-only cartridge, as described above, was discharged onto incised skin at a distance of 5 cm. Another specimen was shot at a similar distance with a cartridge loaded with a Federal 100 primer, a reduced amount of Hercules Unique, and a 158-grain lead bullet. All shooting tests were conducted with the previously described revolver at the University of Southern California's Wound Ballistics Research Laboratory. Paraffin embedded negative control tissue was selected for use from the archives of surgical pathology and autopsy at Los Angeles County–University of Southern California Medical Center.

Residue-containing slides were stained by routine histochemical methods. Primer residue was stained with hematoxylin and eosin, periodic acid–Schiff, Ziehl-Neelsen, oil red O, Perl's iron (Prussian blue), Brown and Hopps, von Kossa, and ARS. ARS staining was accomplished as has been described [6], with the exception of the omission of the counterstain. Gunpowder residue slides were stained with ARS in a similar fashion. Tissue sections were stained routinely with hematoxylin and eosin and with ARS.

Results

ARS formed an insoluble colored precipitate with salts of calcium, barium, and lead, but not with the antimony salt. A red-orange (pH 4.0) to red (pH 7.0) precipitate was formed with calcium chloride. A red precipitate was formed at all pHs with barium chloride. A dark purple precipitate was formed at all pHs with lead acetate. Antimony trichloride failed to precipitate with ARS at any tested pH.

Staining of the primer residue slides (Federal 100) revealed no or minimal staining

with any of the stains utilized except ARS, which stained the residue bright red-orange. ARS staining of primer residue from other manufacturers revealed similar results. Staining of gunpowder residue slides with ARS revealed no detectable staining.

The tissue sections showed positive ARS staining of gunshot residue in the primer-deposited skin, in the experimental gunshot wound (Fig. 1), and in a close-range suicidal wound to the head. ARS failed to stain the pigment in tissue sections with tattoo pigment, melanin, pencil lead (graphite), india ink, or anthracosis.

Discussion

Alizarin red S (ARS) is the basis of a simple and sensitive histochemical stain for calcium [5]. In contrast to the older and more commonly used von Kossa technique, ARS reacts directly with the calcium ion rather the associated phosphate ion. The stain has a sensitivity of 0.02 to 0.002 μg/mm2, comparing favorably with the von Kossa stain. However, ARS is not specific in its staining for calcium. Dahl shows that ARS forms insoluble colored precipitates with many metal ions including iron, barium, strontium, beryllium, cadmium, lanthanum, lead, and uranium. He demonstrates nonreactivity with magnesium, mercury, and zinc [5]. Our results confirm precipitate formation with calcium, barium, and lead, adding a negative result for antimony. Despite this nonspecificity, ARS is a biologically useful stain because of its relative specificity between calcium and magnesium, the only two of the above metal ions found in tissue in significant amounts. Dahl demonstrates a nonstaining of iron in tissue sections, presumably due to protein complexation [5].

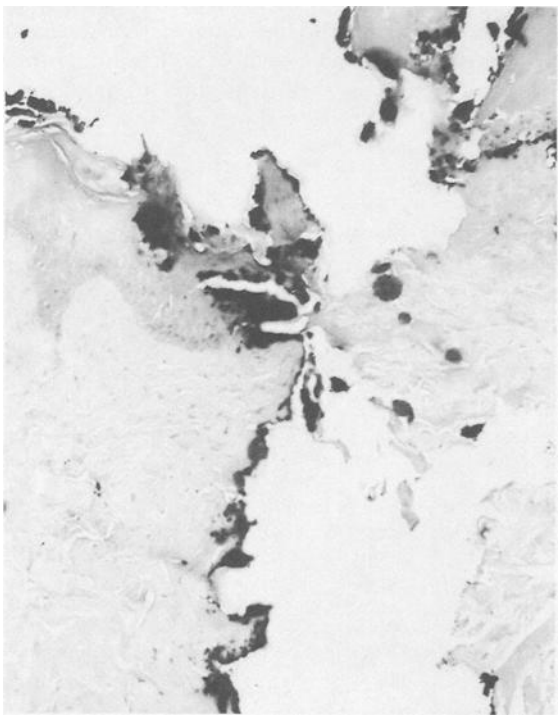

FIG. 1—*A section of an experimental gunshot wound through skin. The lacerated edges are covered with primer residue which stains red-orange with alizarin red S (ARS, ×200).*

Primer compounds in the United States most commonly contain lead styphnate, barium nitrate, antimony sulfide or some combination of these [7]. ARS forms a colored precipitate with barium and lead, but not with antimony. Our studies demonstrate that the residue from primer compounds from several major U.S. manufacturers are brightly stained by ARS, confirming this observation. In the limited tissue samples examined, ARS unambiguously demonstrated the gunshot residue by staining it bright red-orange and failed to stain the commonly found dark pigments that may be confused histologically with gunshot residue, including tattoo, pencil (graphite), melanin, ink, and anthracosis. Calcifications, of course, stain and may be a problem when fragmented bone is present. A routine hematoxylin and eosin stain should make the distinction clear, but if not, a von Kossa stain can be used. This stains the biologic calcium complexed with phosphate in hydroxyapatite, but will not stain gunshot residue.

Gunshot residue is composed of many additional components other than primer residue. Modern gunpowder is composed of nitrocellulose, nitroglycerin, and other organic compounds, the residue of which can be detected histologically as described by Rolfe [8]. Although not difficult, this method involves incubation in hot sodium cyanide for 30 min, making the procedure somewhat unattractive. Stains of this kind have been called nonspecific [9]. Other materials that may be present in gunshot residue include lubricants, copper, zinc, lead, nickel, or aluminum from the cartridge case or bullet. These substances can be detected by the more sophisticated and expensive techniques such as SEM-EDA, NAA, or AAS.

The value of identifying amorphous black material in tissue sections as gunshot residue is challenged by Di Maio [9]. He recommends, instead, gross identification confirmed by chemical analysis. While this is preferable in all situations in which the identification of gunshot residue is used as criminal evidence, there are situations in which histologic identification may be interesting and useful: for example, when a seemingly obvious gross diagnosis is questioned after the fact and a suitable sample for more sophisticated tests is not available, for surgical specimens where cost prohibits more sophisticated tests, or in locations where more sophisticated tests are not available.

Summary

Alizarin red S is a readily available and simple histochemical test that can identify primer residue in routinely prepared paraffin-embedded tissue sections with good specificity. This technique may be useful in situations where more sophisticated tests are not possible, available, or cost-effective.

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