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A Scanning Electron Microscope Study of Dried Blood

The identification of a suspected stain or crust as being blood can be a difficult task for the forensic scientist. To date, either the benzidine test, discovered in 1904, or the phenolphthalein test [*1*] has been employed with either the Takayama crystal test, discovered in 1912, or the Teichmann crystal test [*2*] in confirming the presence of blood.

The benzidine test is a preliminary color test indicating the presence of substances with peroxidase-like activity (such as heme, which is found in blood and the peroxidases), whereas the Takayama test is a confirmatory crystal test identifying the presence of heme found in blood. Both tests have drawbacks. The benzidine test, although quite sensitive, is not totally specific for the presence of blood; and the Takayama test, although a positive test, requires a sufficient sample of stain or crust which is not always available. The Takayama test will also give negative results on old stains and crusts.

Scanning electron microscopy (SEM) has been used successfully by forensic scientists in recent years [*3*] for the analysis of trace evidence. The present study was aimed at using SEM as an aid in the identification of blood stains by studying the morphology of the stain and analyzing the stain for chemical composition with an energy dispersive X-ray analyzer. The high resolution and depth of field of the SEM coupled with the minute sample size needed for X-ray analysis makes SEM an ideal tool for the study of small or dilute stains.

Experimental Procedures

The morphological studies were carried out in an SEM operated between 10 and 15 kV, and the X-ray analyses were performed at 15 kV and 3 to 4 $\times 10^{-10}$ A beam current. Fresh blood stains and smears, and crusts and stains on cloth collected during the past 7 years, were examined in the SEM. The X-ray intensities were collected for 500 s. The peaks were analyzed by setting X-ray energy "windows" of appropriate width for each element present, with the intensity obtained by subtracting background counts from the total counts. X-ray analysis was done at least three times, after the specimen was displaced by random motion.

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The samples analyzed were prepared by spreading the whole blood over graphite stubs or by mounting the cloth pieces on carbon stubs with Duco cement. All samples were coated with vapor-deposited carbon to render them electrically conducting.

Samples of blood smears, crusts, and dried blood on cloth were analyzed. Stains from coffee, catsup, Tabasco® sauce, milk, and soft drinks were also analyzed, as were red paint and rust. Those materials resemble dried blood and can possibly give false positive reactions to the benzidine test.

Discussion

The study showed that blood cell-like structure could be observed in the dried stains and crusts. These cell-like structures shown in the fresh blood smear (Fig. 1*a*) or the crust (Fig. 1*b*) resemble the cup-shaped cells described by Bessis and Weed [*4*] which result from improper sample preparation. The cup-shaped cells are especially noticeable in the cracks that occur during the drying of the crust (Fig. 1*c*). In very old stains, these cup-shaped cells could also be observed if the old crust or stain could be broken open to expose new surfaces (Fig. 1*d*). The crusts, in general, were more readily broken open

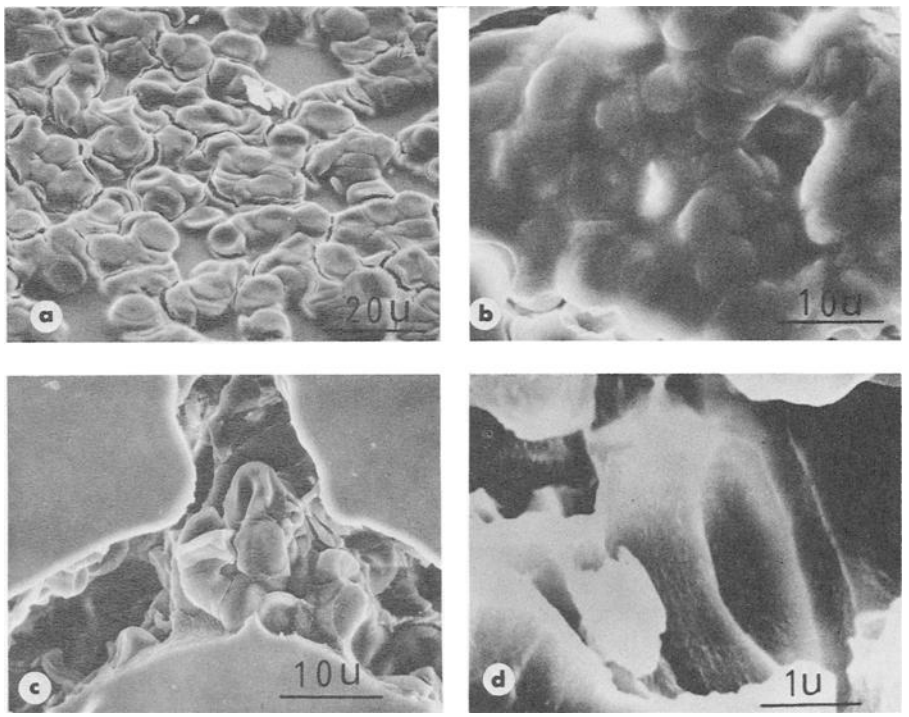

FIG. 1—*Morphologies of blood samples as revealed by SEM;* (a) *blood smear;* (b) *area from a crust;* (c) *exposed cells within crack in a blood crust; and* (d) *cell in a 7-year-old crust obtained by breaking the crust before mounting on stub.*

to expose new surfaces than were the stains. However, it is not always obvious that the cell-like material is blood, as is demonstrated by the photomicrographs of Figs. 2*a, b,* and *c*. Careful examination of these structures reveals differences from the dried blood cells (Fig. 2*d*), but X-ray analysis is required to positively eliminate these materials. Table 1,

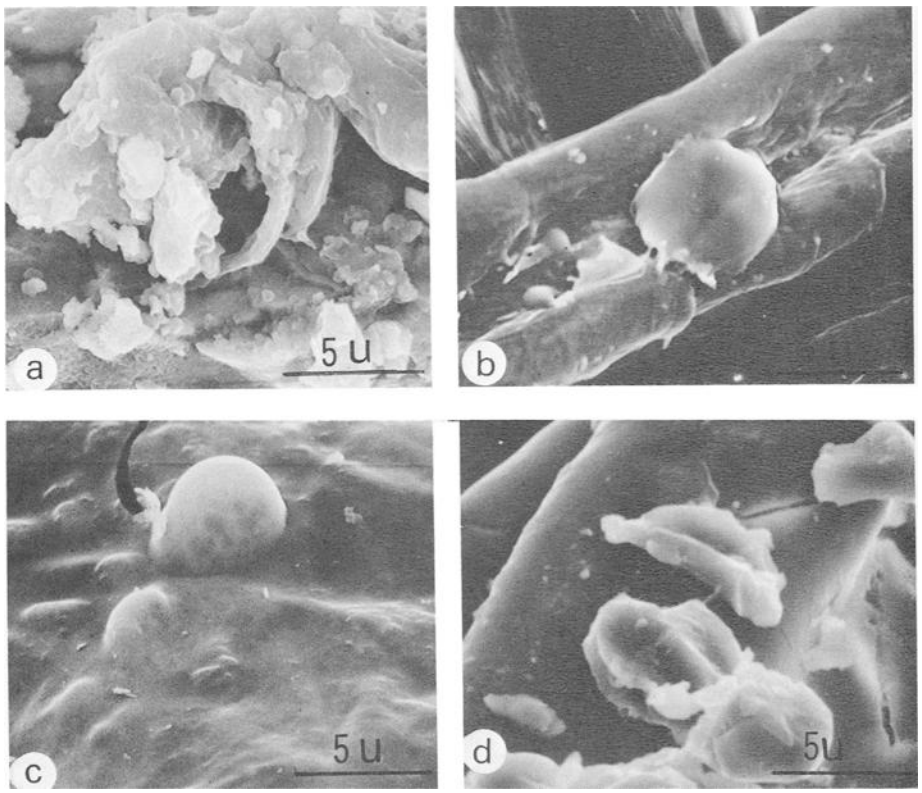

FIG. 2—*Morphologies of various samples as revealed by SEM;* (a) *rust;* (b) *clean cloth (the particle shown is a dirt particle);* (c) *catsup; and* (d) *dried blood on cloth.*

which gives the results of the X-ray analysis of the samples, shows the inorganic composition of all samples analyzed to be different.

The major constituents of blood are chlorine, sulfur, potassium, sodium, and phosphorus. The blood could be distinguished from the other materials analyzed, either in the ratio of the elements or by the presence or absence of certain elements. For example, Tabasco® sauce analyzes much higher in chlorine and sodium but much lower in potassium than blood, while coffee has a considerably higher silicon level than blood. The photographs of Fig. 3, which show the spectrum from the energy dispersive analysis of Tabasco® sauce, catsup, cherry soft drink, and blood illustrate the readily recognizable differences in chemistry that exist between blood and the substances that resemble blood in color or morphology.

The microchemistry of blood is fairly constant, whether from a small or large crust or a blood smear, as shown in Fig. 4; thus, the characteristic energy dispersive spectrum of blood can be an aid in the identification of blood stains, even when no morphological features can be observed. Problems that could arise from the varied composition of the substrate material are removed by working with the net intensity, that is, by subtracting the spectrum generated by the substrate from the total spectrum.

One final comment on the examination of blood standards should be made. Care must be taken to use only stains obtained from whole blood to which no preservatives or anticoagulants have been added, since, as shown in Fig. 5, the potassium chloride crystals will precipitate out of the stain, and these artifacts should not be mistaken as characteristic of dried blood.

TABLE 1—*Composition of samples, given in percentages, as a result of X-ray analyses.*

Elements	Blood on Graphite	Blood on Cloth	Red Paint	Catsup	Tabasco® Sauce	Milk	Coffee	Cherry Soft Drink
Sodium	0.9 - 5.87	0.17- 5.3	...	7.46- 7.75	14.3 -16.02	...	...	...
Magnesium	...	1.26- 3.13	0 - 2.82	...	...	...	...	...
Aluminum	1.58- 8.93	1.22- 8.66	0 -17.49	0 - 1.18	0 - 2.22	4.05	...	7.60
Silicon	0.82- 4.18	7.44-17.19	5.82-11.78	3.35- 4.39	0 - 2.88	25.47	36.83	62.29
Phosphorus	3.23- 5.45	0.42-12.81	0 - 8.06	3.29- 3.51	1.07- 3.17	6.31	6.78	...
Sulfur	19.68-17.51	12.4 -21.51	...	2.06- 2.98	12.32-12.93	11.54	6.62	11.31
Lead	...	...	47.88-65.17	...	...	...	...	...
Chlorine	32.67-38.41	21.67-27.4	...	57.72-61.07	60.16-66.93	30.17	15.74	4.89
Potassium	18.0 -24.79	10.73-22.64	...	21.0 -22.49	1.98- 2.16	...	10.17	2.88
Calcium	...	4.6 -13.75	7.23-11.43	...	0.97- 1.06	14.54	9.06	2.12
Iodine	0 - 0.54	0 - 1.72	...	0 - 1.46	...	...	...	...
Titanium	...	0 - 1.12	0 - 0.74	...	0 - 0.67	2.27	1.11	...
Chromium	0.0 - 0.2	0 - 1.34	3.22- 6.29	...	0 - 0.69	...	4.45	...
Manganese	...	0 - 0.89	2.11- 2.56	...	...	...	2.81	3.24
Iron	0.8 - 2.08	0 - 1.61	0.88- 2.59	0 - 0.25	0 - 0.41	4.01	2.22	3.41
Cobalt	...	0 - 0.77	...	...	...	...	...	...
Nickel	0.36- 1.14	0 - 0.66	0 - 2.29	...	...	...	...	...
Copper	0.0 - 0.57	0 - 2.81	0 - 1.59	...	...	1.62	4.18	2.24
Zinc	0 - 0.48	0 - 0.64	...	...	...	...	...	...

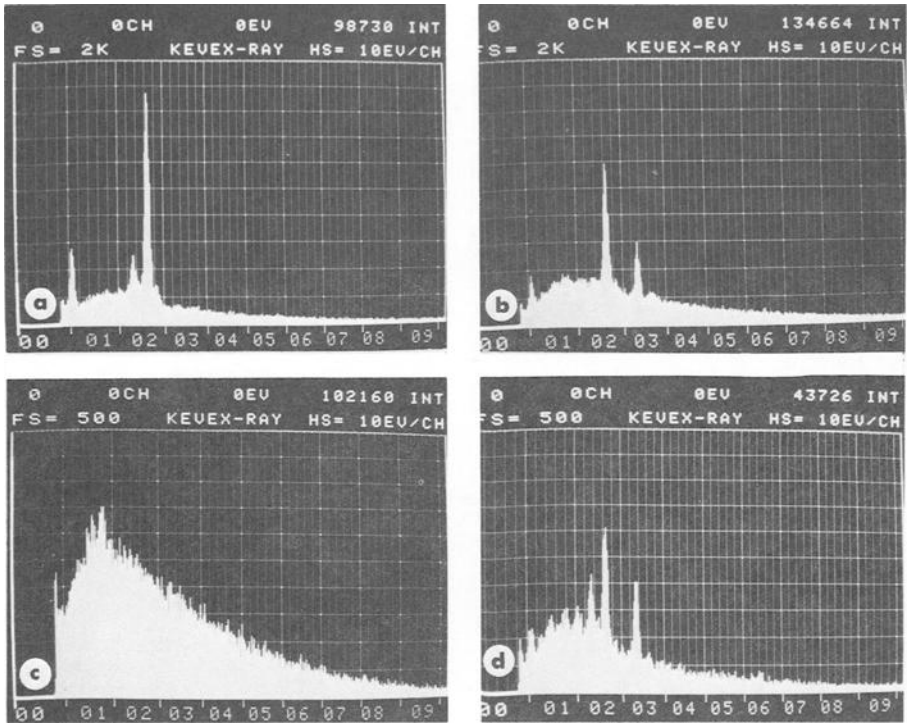

FIG. 3—*X-ray microanalyses of various samples;* (a) *Tabasco® sauce;* (b) *catsup;* (c) *cherry soft drink; and* (d) *dried blood crust.*

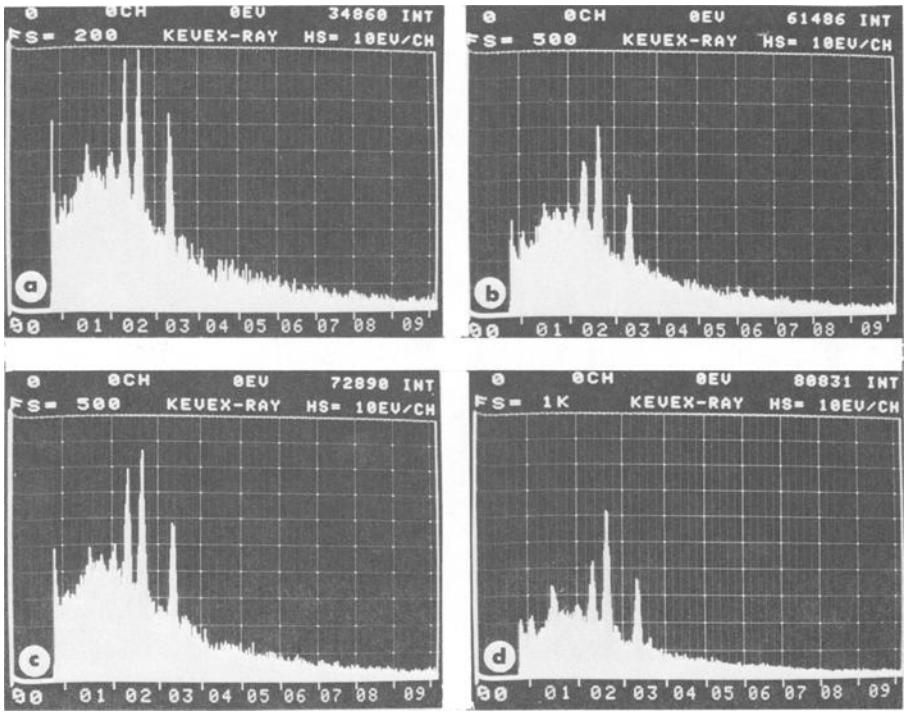

FIG. 4—*X-ray microanalyses of fresh blood prepared in several ways;* (a) *blood smear on a graphite stub;* (b) *large piece from a blood crust; and* (c) *and* (d) *two different small pieces from a blood crust. Chlorine, sulfur, and potassium peaks predominate in nearly the same ratio.*

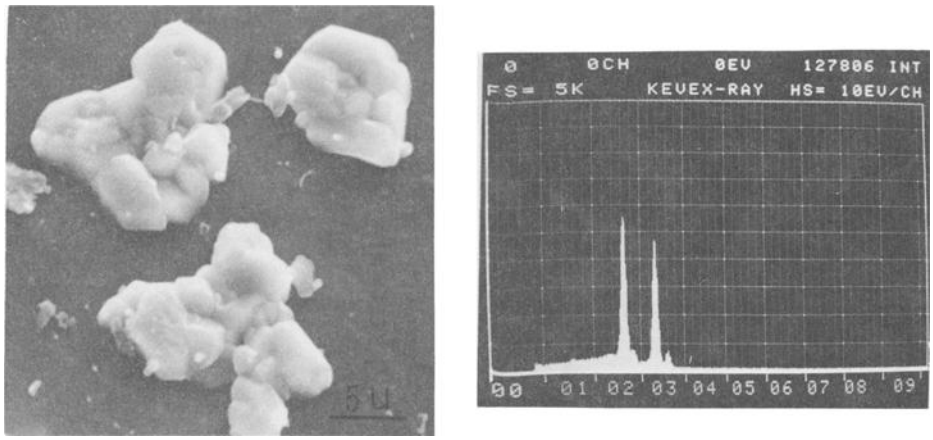

FIG. 5—*Crystals of potassium chloride from the anticoagulant added to the whole blood which precipitated out of the dried crust.*

Conclusion

This preliminary study has shown the SEM equipped with an energy dispersive analyzer to be an aid, when coupled with preliminary tests such as the benzidine test, in the identification of blood stains. The SEM is particularly suited for the analysis of small stains and crusts and of old crusts where weak or no reactions occur with the confirmatory tests.

Acknowledgments

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References

[*1*] Culliford, B. J., "The Examination and Typing of Blood Stains in the Crime Laboratory," U.S. Government Printing Office, stock no. 2700-0083, Washington, D.C., 1971.

[*2*] Kerr, D. and Mason, V., "The Haemochromogen Crystal Test for Blood," *British Medical Journal,* Vol. 1, 1962, pp. 134–139.

[*3*] Stewart, W. D., *Bibliography on Forensic Applications of the SEM,* Illinois Institute of Technology, publication IITRI/SEM/1976, Chicago, 1976, pp. 739–743.

[*4*] Bessis, M. and Weed, R. I., *Preparation of Red Blood Cells (RBC) for SEM. A Survey of Various Artifacts,* Illinois Institute of Technology, publication IITRI/SEM/1972, Chicago, 1972, pp. 589–592.

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