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SEMINAL STAIN EXAMINATION: A REAGENT FOR DESTRUCTION OF SUPPORTING FABRIC.

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(The authors of this article are Criminologists in the Technical Laboratory of the Division of Criminal Identification and Investigation of the California State Department of Justice. Both are graduates of the University of California, Mr. Greene receiving his B.S. degree in Chemistry in 1931, and Mr. Burd his A.B. degree in Technical Criminology in 1941. In this article they discuss a method for dissolving cellulose in examination of spermatozoa on cotton fabrics. As the authors point out the cuprammonium solution was tested as a dissolving means and was found to be very effective and a special aid when photomicrographs are to be taken. The method is one of particular interest to those interested in seminal stain examination.—EDITOR.)

One of the very common types of examinations made in police laboratories is the testing of clothing and other objects for seminal stains. This type of examination often presents difficulties, especially when the stains are old and difficult to soften, and also when suitable photomicrographs must be taken for use in court.

The literature suggests many methods for the separation of spermatozoa from fibers and the identification of the cells on fibers without separation. (1) (2) (3) (4) (5) (6). If satisfactory photomicrographs are to be taken which will show the cells with sufficient clarity so that they will not be confused with other material, it is usually advisable and frequently essential that the spermatozoa be separated from the supporting material. Among the separation methods is one based on complete destruction of the supporting fibers with concentrated sulphuric acid. (3) (4). Our attempts to use this method indicated some difficulty in developing a technique to give consistent results. It did, however, suggest the use of other fabric solvents.

The most frequent problem to law enforcement laboratories is the examination of the clothing of victims and suspects in rape or other sex offenses. The commonest fabric encountered is cotton, and at the same time, it is usually more difficult to separate spermatozoa from cotton than from rayon, silk, and wool. A solvent for cellulose must, therefore, be used in these cases, and will find more extensive use than others. Cuprammonium solution was selected for testing since it is a well-known cellulose solvent, and was found to show marked promise. After trying a number of variations, the following procedure seemed to give consistently good to excellent results with both fresh and very old stains, (a few days to over ten years).

Preparation of cuprammonium reagent:

While there are several cuprammonium preparations used as cellulose solvents, their relative activity is unknown to us. A readily prepared reagent is highly desirable in police work, and therefore the following is suggested.

Copper hydroxide is first prepared by precipitation from cupric sulphate solution with slight excess of ammonium hydroxide. This is filtered and the precipitate on the filter paper washed several times with distilled water. The precipitate is then removed from the filter paper and added to ammonium hydroxide in sufficient quantity so that an undissolved excess of cupric hydroxide settles to the bottom.

(This reagent deteriorates with age and is most effective when prepared immediately before use. Usually its solvent action is satisfactory for several days but its action is unreliable after this period.)

Preparation of specimen:

The spot from which the sample is to be obtained is moistened with a little distilled water. A very small sample is then cut out with scissors diagonally across the weave so as to give only short fibers in the specimen. These fibers are teased out completely on a microscope slide in a small drop of distilled water and then dried without heat.

To the dried specimen is added one or two drops of cuprammonium solution and mixed briefly with a needle. Then a small drop of safranin stain solution is added and covered with a cover slip.

Examination of Specimen:

The slide should be examined immediately, or in any case, before it has dried. The prepared slides are not permanent, and we have not developed any simple method of preserving them for future examination. The examination is best made with a magnification between 350 and 500 diameters since this permits a larger area to be in the field of view at any one time and because the depth of focus is greater. The slides tend to be rather thick which makes the use of oil immersion objectives impractical.

Conclusion:

While we have used this method on a number of criminal cases over a period of some months in addition to experimentation on known samples, it is quite possible that improvements might result from the use of other cuprammonium preparations with greater solvent action. Safranin was the only stain tried which was found to work successfully with the cuprammonium reagent, but it is probable that other more satisfactory stains could be found.

Since this method is effective only on cotton and other cellulose fabrics, it is not applicable to all semen stains. Attempts to use organic solvents on rayon, while dissolving the fiber, have not been too promising, perhaps because they fail to dissolve or soften the other seminal stain components present with the spermatozoa. An additional advantage of cuprammonium solution over other solvents is that it is an alkaline aqueous solvent which would be expected to readily dissolve the proteins in seminal plasma.

Description of photomicrographs:

The illustrations are photomicrographs of spermatozoa mag-

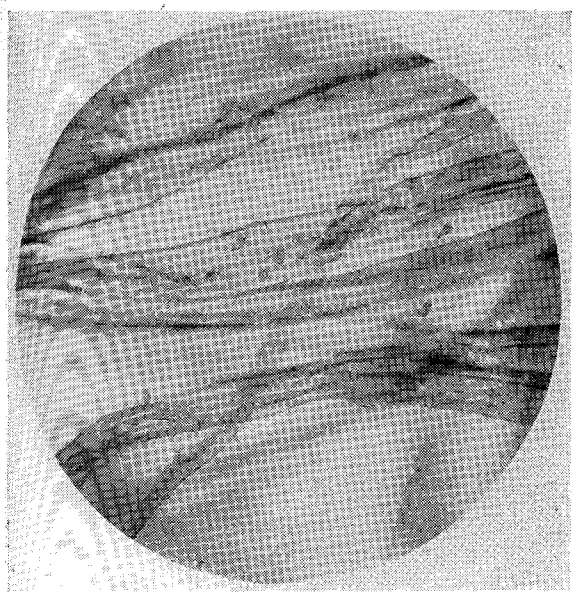

FIGURE 1
Spermatozoa on cotton fibers

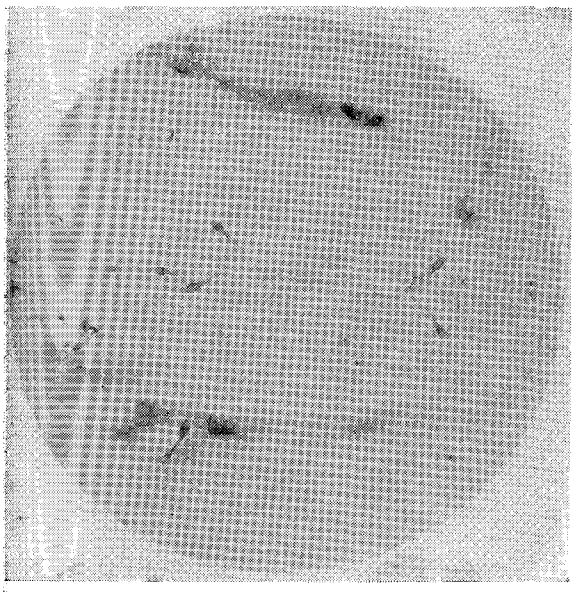

FIGURE 2
Spermatozoa after destruction of cotton fibers with cuprammonium solution and staining with safranin

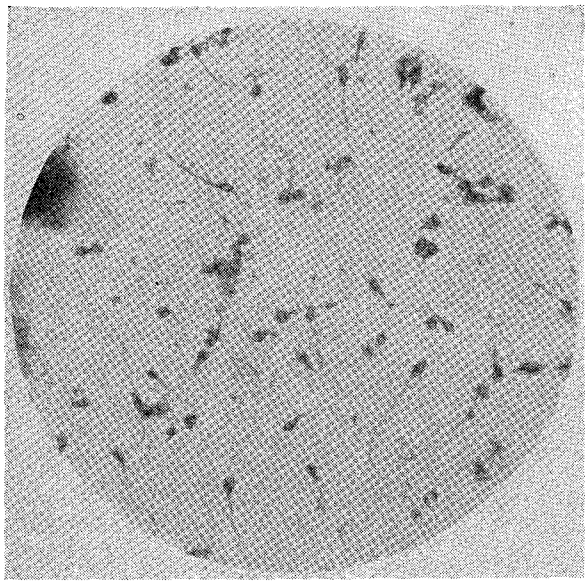

FIGURE 3
Spermatozoa from cotton handkerchief in rape case, after destruction of cotton fibers

nified approximately 390 diameters. Figure 1 shows spermatozoa on cotton fibers. The slide was stained with safranin, but no cuprammonium solution was added, and no attempt was made to separate the cells from the fibers. Figure 2 shows several spermatozoa after destruction of the cotton fibers with cuprammonium solution and staining with safranin. This slide was made from the same stain as the one used in Figure 1. Figure 3 is a photomicrograph of spermatozoa on a cotton handkerchief in a rape case. A large number of cells were obtained by destruction of the fibers with cuprammonium solution and staining with safranin.

Summary:

Cuprammonium solution was tested as a method for dissolving cellulose in examination of spermatozoa on cotton fabrics. It was found to be very effective and of special aid when photomicrographs are to be taken since it not only separates the cells from the fabric, but also removes most of the interfering material which is objectionable in the photomicrographs.

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