Journal of Criminal Law and Criminology

Volume 47 | Issue 5 Article 12

1957

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THE USE OF THE ACID PHOSPHATASE TEST IN SEARCHING FOR SEMINAL STAINS

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The purpose of this communication is to report our experience with the use of the acid phosphatase test in searching articles for seminal stains. Prior to two years ago we made use of the test only as a guide to which stains justified microscopic preparations after first locating them by naked eye examination in daylight, artificial light, or under the ultraviolet lamp.

It is our experience that the value of ultraviolet lighting in the location of seminal staining is frequently overestimated in the literature on the subject. The conditions for successful location under ultraviolet light are first, that the background should be substantially nonfluorescent; second, that other fluorescent materials must be absent or present only in small amount; and third, that the seminal stain itself should be heavy enough and superficial enough to give good fluorescence. Because these conditions are rarely attained we decided to adopt the acid phosphatase method for large-scale searching.

Our method is essentially that of Walker (1) and Seligman and Manheimer (2) with minor modifications. Originally we used diazo-1-amino anthaquinone in the technique, but recently, on the suggestion of Mr. Harry Powell of this laboratory, we tried Brentamine Fast Blue B Salt (Imperial Chemical Industries) and have found it equally satisfactory and readily obtainable commercially in Great Britain. Many other diazo salts would doubtless be equally satisfactory (3), and the only criteria to be observed in the selection are that the salt should be stable enough to obviate the necessity of making up fresh batches of reagent each day and that the color given by it and α-naphthol should be quite different from those given by the commonly occurring phenol containing interfering substances. In England the most common non-enzymatic interfering reagents are probably carbolic soap and tea. The colors given by these substances are easily distinguishable from that given by α-naphthol using the technique below.

METHOD

The article to be examined (which may be a garment, rug, blanket, or similar object) is arranged in a flat position and then completely covered with damp blotting or filter paper. We use white esparto blotting paper in 60-cm. by 40-cm. sheets, wetted in batches of 20. All excess water must be removed by blotting or by rolling in order to ensure that the imprint of the soluble acid phosphatase enzyme is a faithful

reproduction of its disposition on the article and to diminish unwanted diffusion. It is a common error to wet the extracting paper more than is necessary in the mistaken belief that better extraction is obtained. Extraction with a heavily wetted paper is doubtless quicker, but the resulting color on spraying with the detecting reagent is in the form of a featureless patch. A further advantage of using paper which is not too wet occurs in the situation when it is of interest to know on which side of a thin garment a seminal stain originated. A pair of simultaneous imprints, one from each side of the garment, will frequently decide the issue. The dampened extracting paper should be firmly pressed on the sample with clean hands and left in position for five minutes, removed and sprayed with detecting reagent of the following constitution:

Calcium-α-Naphthyl Phosphate	2 gm./liter
Brentamine Fast Blue B Salt (or other suitable diazo compound)	4 gm./liter
Glacial Acetic Acid	5 ml./liter
Sodium Acetate (trihydrate)	20 gm./liter
Sodium Chloride	210 gm./liter
Detergent (we use Teepol)	1 ml./liter

All the above constituents are readily soluble with the exception of the calcium-α-naphthyl phosphate which should be triturated thoroughly in a mortar with the detergent and a little water and added to the solution last. The mixture is thoroughly shaken, left to stand for one hour, and filtered.

The resultant solution should be a clear amber color. A precipitate which begins to form after a few days does not affect the reaction. The solution is stable for at least six months in a refrigerator and probably much longer. In practice we keep the solution at room temperature after making up in 2-liter batches inasmuch as we find there is no appreciable diminution in activity during the life of any one batch. Recently, we have found that the use of sodium chloride in such high concentration as above has doubtful virtue and that there would probably be little disadvantage in excluding it altogether. It was included by Seligman and Manheimer to diminish diffusion of the acid phosphatase enzyme over short distances in microscopic preparations. From the point of view of seminal stain imprints its presence slows down the reaction and has little appreciable effect on diffusion if the imprint is properly made.

After spraying the paper imprint with detecting reagent the color resulting from acid phosphatase appears between a few seconds and a few minutes, according to the efficiency of the extraction. The position of the stain on the imprint paper can be measured and microscopic preparations made from corresponding positions on the article.

Examples

The test is now in daily use in the biological department of this laboratory, and the following summaries are given as examples in which examination for semen would quite possibly have given negative results had the acid phosphatase test not been available.

Case 1—Rape: The complainant alleged that the accused raped her and before ejaculating withdrew and discharged the semen on the floor. He then tried to obliterate the semen with his slippered foot. The accused alleged full intercourse with consent.

Examination of a mat submitted showed a small acid phosphatase positive area which yielded spermatozoa. Examination of the soles of the slippers also yielded semen. It is doubtful if the seminal stain on the mat would have been detectable by any method at present available other than the acid phosphatase test. This test obtained useful corroboration of the complainant's story.

Case 2—Bestiality: In a case of alleged bestiality with a sheep a large sample of wool clippings from around the sheep's hind parts was submitted for examination. An acid phosphatase imprint of the wool showed a single tuft of wool to give a positive reaction. The tuft was washed with distilled water and the washings centrifuged. The sediment on mounting showed spermatozoa morphologically similar to human spermatozoa and dissimilar to ram spermatozoa. The supernatant fluid gave a good reaction with anti-human precipitin antiserum and no reaction with anti-sheep goat precipitin antiserum.

Case 3—Indecent Assault: In a case of indecent assault on a young girl the accused was alleged to have ejaculated on the ground in a field. The police submitted a piece of turf from the scene, and the position of the semen on it was easily determined by use of the acid phosphatase test.

The above cases are but three examples of many instances in which the test has been of use.

Interfering Agents

In conclusion, a reference to our experience with interfering agents may be of interest. These can be classed as enzymatic and non-enzymatic.

In the first group, saliva and feces may give faint positive reactions in addition to male urine which can give a fairly marked reaction (see also Burgen (4)).

In the second group are any materials containing free phenolic substances (see above).

The first group are distinguishable on the basis of the weakness of the reactions and the second by the resulting color usually being different. Another distinction between the two classes of false positive reactions is that with enzymatic interfering agents the color increases with time while with the non-enzymatic agents the color appears almost immediately, and then the intensity remains constant.

The following is an interesting case where a non-enzymatic false positive occurred.

Case 4—Indecent Assault: In an alleged indecent assault, the injured party (a 4-year-old girl) gave the impression on questioning that a stick had been introduced into her vagina. A stick found at the scene was submitted for examination and gave a marked false positive which was found to result from the phenol content of the wood. Subsequent questioning showed that the initial impression gained was the result of a misunderstanding of childish enunciation.

Controls

When using the acid phosphatase test we always run a control of a weak seminal stain. Our present series of controls was made up 10 months ago and consists of small filter papers each bearing a single drop (about 25μ liter) of 2 per cent human semen in distilled water. These papers still give a good reaction. It is of interest to note, in

passing, that the acid phosphatase on these papers is now so insoluble that it cannot be extracted fully by a 36-hour extraction into distilled water at room temperature.

Summary and Conclusion

Walker's modification of Seligman and Manheimer's acid phosphatase test has been in use in this laboratory for the past two years as a large-scale screening technique. The results, in the opinion of the author, justify its use in all laboratories concerned in the detection of seminal stains.

Acknowledgments

The author wishes to thank Dr. F. G. Tryhorn for his helpful criticism and for permission to publish this article, and Mr. J. Manning for technical assistance in some of the case examinations.

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