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The legal proof of rape is not a simple matter, and any and all bits of information that may be of material interest and medico-legal importance must be considered.

From the legal aspect, the identification of a given specimen as semen may provide objective evidence strongly indicative and corroborating charges of rape, seduction, and unnatural practices such as sodomy or the like. The fact that such specimens retain their identity over long periods of time without marked decomposition or loss in reliability further enhance their value and use as evidence at some future date.

There is no doubt or argument that the isolation of spermatozoa, properly stained and photographed, are "par excellence" for the demonstration of ejaculate. However, chemical identification of the presence of seminal fluid serves to strengthen the proof. In some instances this may be of even more importance since it is not always possible to demonstrate the presence of whole spermatozoa consisting of both head and tail or sometimes even to identify microscopically with absolute accuracy the separate parts. In an old specimen, especially, some of the tails may be missing or abnormally twisted, and the disjointed sperm heads may be confused with spores, pus cells, or artifacts if casually examined. For just such situations, in addition to the possibility of the condition of aspermia in which the ejaculate lacks spermatozoa, reliable tests for seminal fluid become very significant.

There is no need for a detailed discussion on the extraction and staining of spermatozoa at this point. While the writer has found Giemsa or Wright staining especially good many varying techniques are available and are described in detail in the literature. (1) (2) (3) (4) (5) (6)

MICRO-CHEMICAL TESTS FOR SEMINAL STAINS

By way of summary of the available means of identifying seminal stains the following procedures are briefly noted.

Florence Test. A minute fragment of the stained garment is cut away, transferred to a slide, and treated with a drop of distilled water. It is then allowed to soak for two to three minutes after which a small drop of the reagent[1] is added along the edges and the slide is covered with a watch glass. Examination is made microscopically for seminal crystals which have the appearance of hemin crystals.

Peltzer Test. Peltzer uses a modification of the Florence test. (7) The suspected spots are moistened with hydrogen peroxide. If semen is present extensive foaming occurs. The sample is centrifuged, placed on a slide, and stained with two percent aqueous eosin. If a positive reaction is obtained, long lance-like crystals appear. The addition of iodine-potassium iodide solution to the slide colors the crystals brown. These crystals may disappear but can be recrystallized by the addition of more iodine-potassium iodide solution.

Puramen Test. Puramen (8) suggests using naphthol yellow sulfur in aqueous solution which reacts differently with human semen at neutral and slightly alkaline reactions. A positive reaction is indicated by formation of micro-crystals which are characteristically large, have a double refraction, and are of a definite orange hue.

Niederland Test. This test employs dilute sulphuric acid. (9) When added to a suspected specimen, the weak sulphuric acid produces a white crystaline precipitate in the presence of semen. These crystals are probably sulphates of calcium and other minerals found in semen and body fluids.

Gold Chloride Test. Villami (10) has shown that solutions of gold chloride give characteristic crystals with dilute semen as well as with solutions of spots and stains produced by semen.

The Florence test and these various modifications of crystal formation are not specific, positive indication of seminal fluid since other body fluids may give similar results with these tests. A negative test, however, informs the investigator that the suspected stain is not semen.

Test for Hydrogen Ion Concentration. If a drop of distilled water is added to suspected semen and well mixed, the hydrogen ion concentration of the solution when tested with appropriate indicator paper will give evidence of definite alkalinity.

1 Florence reagent is prepared in accordance with the following formula: Potassium iodide, 1.65 grams; iodine, 2.54 grams; distilled water, 30 ml.

Fluorescence or Ultra-Violet Test. Ito first demonstrated that dried seminal stains give a characteristic color effect under ultra-violet light. However, this same brilliant characteristic fluorescence may be also obtained by many other substances and is therefore not specific for semen. It is of great value in locating suspected stains over large areas of a garment. Seminal spots irrespective of spermatozoa, azoo-spermia, or age of the spot show a characteristic fluorescence in ultra-violet rays between 4200 and 4900 A°.

Recommended Test for Micro-Chemical Identification of Seminal Fluid

The determination of acid phosphatase activity affords an excellent means for a specific micro-chemical identification for seminal fluid and stains. This procedure employs a new idea postulated by Ova Riisfelt of Denmark in 1946 (11) and promises to be of definite medico-legal value in the identification of seminal fluid either as an adjunct to the demonstration of spermatozoa or of particular value where spermatozoa cannot be demonstrated.

It has been found that male ejaculate produces a very high acid phosphatase activity in comparison to the very low acid phosphatase activity of the various body fluids (saliva, perspiration, urine, etc.) and the common vegetable and fruit juice stains. The differences are so marked and definite that it leaves little room for doubtful interpretation of results. The average seminal fluid contains approximately 2000 King units per ml., ranging from an upper limit of 3000 to a lower limit of 300 units. Ejaculate in cases of aspermia gives comparable high results. Semen is characteristic in these high values while the other body fluids (urine, saliva, perspiration, etc.) have values of much less than 5 King units per ml.

The enzyme is resistant to all known body fluids including vaginal secretion but might be inhibited by disinfectants and contraseptives. Seminal fluid aged two weeks in a condom type contraseptive showed no appreciable loss of acid phosphatase activity. Experiments were performed on varying aged known seminal stains, and they were found to be strongly positive even after six months.

Procedure. Any accepted technique or modification of the Gutman acid phosphatase method (12) (13) (14) (15) is satisfac-

tory so long as a blank and control are first performed for technique and reliability.[2]

The method of estimating activity of a stain on clothing is to extract a small unit area of sample with 2 ml. of water and to perform an acid phosphatase determination on the filtered extract. In positive cases color intensity is too great to read directly in a colorimeter or spectrophotometer. Consequently, dilution of the final color with distilled water is made in order to approximate the color intensity of the standards similarly prepared, and the dilution factor must be taken into consideration when making the final calculations.

For increased accuracy the following suggestions are offered. Sufficient time should be allowed for complete extraction of the stain. An estimation ought to be made of the unit size of the stain and approximate thickness of the garment extracted so that comparison of results can be made with a graph of performed control experiments on known seminal stains of various unit size and thickness of garment.

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2 The writer prefers the Gutman method as modified by Benotti, Rosenberg, and Dewey (15). Three solutions are used a citrate buffer, a substrate, and a dilute phenol reagent solution. They are prepared as follows.

Citrate Buffer. (pH 4.9) Dissolve 18.9 g. of citric acid in 500 ml. of water; add 180 ml. of normal sodium hydroxide and 100 ml. of 0.1 normal hydrochloric acid. Make up to 1 liter. (pH of buffer should be checked.)

Substrate. This consists of 1% disodium phenyl phosphate which will keep approximately two weeks.

Dilute Phenol Reagent (Folin and Ciocalteau). Dilute 1 volume of stock phenol reagent with 2 volumes of water. While stock phenol can be prepared in the laboratory it is tedious, and the writer suggests that the solution be purchased commercially.

Sodium Carbonate used is 20% strength.

Procedure. Three tests are run: An extract blank, a substrate blank, and the specimen test. For the final value the results of the first two are substracted from the specimen test. The incubator is set at 37° C for these tests.

1. Extract Blank	2. Substrate Blank	3. Specimen Test
.....	9 ml. buffer	9 ml. buffer
10 ml. water	0.5 ml. water	.....
.....	1 ml. substrate	1 ml. substrate
0.5 ml. extract	.....	0.5 ml. extract
Immediately add 4.5 ml. phenol reagent	Incubate 1 hour at 37° C Add 4.5 ml. phenol reagent	Incubate 1 hour at 37° C Add 4.5 ml. phenol reagent
Filter	*Filter*	*Filter*
10 ml. filtrate	10 ml. filtrate	10 ml. filtrate
2.5 ml. Na_2CO_3	2.5 ml. Na_2CO_3	2.5 ml. Na_2CO_3

Incubate all at 37°C for 5 minutes and read immediately

A standard solution may or may not be necessary, depending on whether a calibrated photometer is available. If it is not, phenol solutions must be used to establish a curve. These replace the 10 ml. filtrate with the 2.5 ml. sodium carbonate in the final steps.

For a more detailed account of procedures the reader is referred to the various references on the Gutman method at the end of this paper.

An activity of 30 King units of acid phosphatase should be considered positive for seminal stains.

If precipitin tests are desirable for differentiation between human or animal origin, reference to Gradwohl (16) and Pollak (17) are suggested.

Conclusion

This method is particularly good in that differences between negative and positive are wide and easily interpreted. Reaction is specific for seminal fluid. The sample can be originally extracted and treated for spermatozoa identification, but in such procedure instead of discarding the supernatant fluid it is used for an acid phosphatase test.

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