J Forensic Sci, Oct. 1975, Vol. 20, No. 4

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Prostatic Acid Phosphatase Activity in the Postcoital Vagina

The criminal offense of rape has been defined in various ways by both legal and medical authorities [*1–4*]. There appears to be general agreement that two requirements are essential for establishing the crime of rape: (1) the lack of consent of the alleged victim and (2) carnal knowledge (constituted by even the slightest penetration by the male organ). Because circumstantial evidence alone may be inadequate for proof, the physician and the forensic scientist are required to assume major roles in supplying objective information in cases of this kind. They can be called upon to furnish evidence pertaining to the above requirements. The physician assesses and describes whatever injuries and medical evidence there may be. The forensic scientist is typically concerned with demonstrating the presence of seminal fluid in locations that would indicate recent sexual activity.

The microscopic identification of spermatozoa in the vagina is ordinarily considered reasonable proof of recent intercourse. However, the failure to demonstrate spermatozoa does not preclude the possibility of intercourse. Forms of aspermia and oligospermia, resulting from pathological and surgical processes, can limit the reliability of this approach. These conditions are not rare among the population of normal adult males [*5,6*]. In addition, it has been suggested that the incidence of abnormal concentrations and morphology of spermatozoa tends to be greater among those males often involved in sexual offenses [*6*].

Several other components of seminal fluid have been measured in the attempt to determine the possibility of recent coitus. Of these, the assay for the enzyme prostatic acid phosphatase (AP) has been used extensively. The rationale for the use of AP as a chemical marker for seminal fluid stems from the fact that this enzyme is present with a very high activity in semen, whereas little or none is present in the normal female urogenital system. In addition, AP has proven to be relatively resistant to many factors often encountered in criminal investigations (for example, desiccation or contamination) [*7,8*].

Most of the literature on the use of AP in forensic investigations has dealt either with the identification of dried seminal stains or with reports of its detection in actual cases of

Research for this study was conducted at the U.S. Army Hospital, Fort Leonard Wood, Mo. The opinions or assertions contained herein are the personal views of the authors and are not to be construed as official or as reflecting the views of the Department of the Army or the Department of Defense.

Received for publication 9 April 1975; accepted for publication 10 April 1975.

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assault. Relatively few studies have been done using large groups of subjects under conditions designed to reduce the possible interfering variables [*9–11*].

This lack of data and the availability of a large population of suitable volunteers prompted the authors to conduct a study of the retention of prostatic acid phosphatase activity in the postcoital vaginal pool. The relationship between the time since last intercourse and the activity of this enzyme under various vaginal conditions was investigated.

Materials and Methods

Vaginal fluid specimens were collected from women who presented themselves at the obstetrics and gynecology clinic of a large hospital either for routine examination or with specific medical complaints. The subjects were informed as to the nature of the test and the possible uses of the data. Consent was obtained before the specimen was taken. Each patient was asked (1) to estimate the number of days since last intercourse, (2) whether she had douched or used any vaginal creams or suppositories since that time, and (3) the date of her last menses. In addition, any pertinent gynecological conditions present in the subject were noted.

At the beginning of the pelvic examination a vaginal fluid specimen was collected from the posterior fornix and external os with a dry, sterile, cotton-tipped applicator. The applicator was immediately placed in a tube containing 1.0 ml of physiological saline (0.9%). The tube was then sealed, marked with the patient's identification, and frozen until the assay was conducted.

The analytical procedure followed that described by Babson and co-workers [*12,13*] using the General Diagnostics Phosphastrate-Acid® reagents. A number of workers have expressed satisfaction with the enzyme specificity and simplicity of this assay, which measures prostatic acid phosphatase activity by determining the amount of alpha-naphthol phosphate hydrolyzed by the enzyme [*14,15*].

The following procedure was used for determining the prostatic acid phosphatase activity in the vaginal fluid specimens. For each specimen, one alpha-naphthol phosphate tablet was dissolved in 1.0 ml of distilled water and preincubated in a 37 °C water bath for approximately five minutes. The specimen was thawed and the cotton-tipped applicator was discarded after squeezing its contents into its specimen tube. Exactly 0.2 ml of the specimen solution was added to the alpha-naphthol phosphate solution and the mixture incubated for 30 minutes. At the end of the incubation period, 1.0 ml of diazotized 4-nitro-*o*-anisidine solution (0.2 mg per ml of 0.1*N* HCl) and 5.0 ml of 0.1*N* NaOH were added to the incubated substrate-specimen mixture. The optical density at 590 nm was then determined. Negative controls were taken from 1.0-ml saline samples that had been frozen with unused cotton-tipped applicators. A standard curve for conversion of absorbance units to enzyme activity units was established using known concentrations of alpha-naphthol, as described by Babson and Phillips [*12*]. To facilitate comparison of the findings with previous reports, the enzyme activity is expressed in King-Armstrong units [*16*].

Results and Discussion

One hundred specimens were collected from women visiting the clinic. No attempt was made to preselect the subjects. The ages ranged from 16 to 56 years. The history and specimen were taken only after informed consent had been received. The range of estimates of elapsed time since last sexual intercourse extended from two hours to over three months.

Sixteen percent of the subjects from whom specimens were taken presented some form of urogenital pathology. Of these, simple vaginitis was the predominant finding. All subjects had Pap smears taken at the time of the examination. Most of these were

diagnosed as Class I; the remainder were Class II. Twenty-six percent of the specimens were collected from women who had douched or used a vaginal suppository preparation or both since intercourse. Thirteen percent of the women sampled were menstruating at the time the specimen was taken. Nine percent of those sampled represented a combination of two factors, either pathology and douche/suppository, pathology and menstruation or douche/suppository and menstruation, but none combined all three categories. In total, 54% of the subjects had none of these factors present and 46% had one or more. For the purpose of discussion, the 54% will be called Group A and that segment of the population having some pathology present, having douched or used suppositories, or who were actively menstruating at the time the specimen was taken will be termed Group B.

Figure 1 shows the findings from the total number of specimens collected (Group A + Group B). Each point on the graph represents one specimen. The vertical axis gives the acid phosphatase activity in King-Armstrong units (KAu). The horizontal axis denotes the elapsed time in days since last sexual intercourse. Inspection of this figure shows seven specimens above 30 KAu. None of these had a time lapse since coitus of greater than 48 hours and most were within 24 hours. The majority of specimens were below 20 KAu and after 48 hours none were above this value. Several investigators have recommended minimum AP levels that can only be found in the presence of seminal fluid. Kind [*17*] suggests a minimum of 30 KAu and Kaye [*7*] states that activity greater than 25 KAu is positive for seminal fluid AP. Other authors have shown that various possible contaminants can produce apparent AP activity from 5 to 20 KAu [*8,18*]. A reasonable dividing line for a positive AP test probably lies somewhere between 20 and 30 KAu.

The data of Willott [*11*] and Davies and Wilson [*10*] suggest that by three days post coitus, the level of AP activity found in the vagina can no longer be ascribed to the presence of semen. As another means to establish the minimal AP activity that could be called positive for the presence of seminal fluid in the vagina, we determined the mean and standard deviation of the values for those specimens that could be assured of having

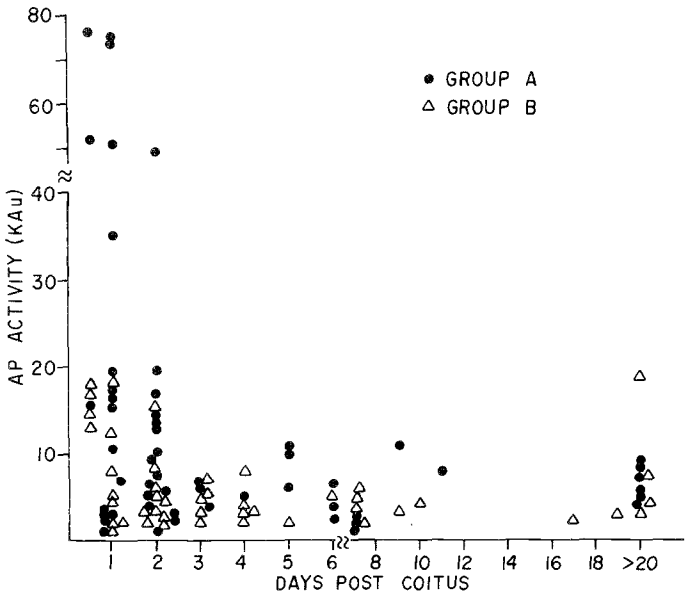

FIG. 1—*Prostatic acid phosphatase activity of all specimens.*

no remaining seminal AP (that is, those taken at three days post coitus and longer). These means and standard deviations are listed in Table 1. An analysis of variance of all specimens taken three days or more post coitus showed no significant difference between Group A specimens and the categories within Group B. In order to estimate the maximal apparent enzyme activity which could be measured in the absence of seminal fluid AP, the 99% tolerance limit ($p < 0.01$) was calculated using the mean and standard deviation of Group A in Table [*19*]. This calculated tolerance limit is 17.7 KAu; that is, 99% of the vaginal specimens with no seminal AP present will have less than 17.7 KAu activity. This statistically determined value is reasonably close to the minimal levels recommended by other authors [*7,17*]. It is, however, near the reported upper limit of values for apparent AP activity that can be obtained from sources other than seminal fluid [*18*]. Considering the potential critical importance of this minimal level and in light of the findings of other investigators, we suggest that when using the techniques described in this report a secure margin of error will be allowed if values above 25 KAu are considered positive for AP.

TABLE 1—*AP activity of specimens taken three or more days post coitus.*

Category	Number	Mean[a]	Standard Deviation
Group A	19	6.13	2.88
Group B			
menstruating	7	3.50	1.48
pathology	8	4.69	1.13
douche and/or suppository	15	4.53	4.34

[a]Means are not statistically different.

From Fig. 1 it is apparent that no positive values were obtained after 48 hours if the value of 25 KAu is considered positive. Of the positive samples, six out of seven were obtained within 24 hours. Using a semiquantitative method for AP in actual cases of sexual assault, Rupp [*20*] found positive reactions for up to 36 hours following intercourse. Davies and Wilson [*10*] reported positive reacting samples for at least 30 hours and perhaps as long as 48 hours. Willott [*11*] appears to have found no significantly elevated AP levels beyond 24 hours post coitus.

In the present study a great deal of variability was found in AP activity of the specimens obtained at 24 and 48 hours. Values as high as 76 KAu and as low as 1.0 KAu were measured. Other investigators have also demonstrated this variability [*10,11*]. Most of the specimens at these time periods are below 25 KAu. Part of this variability can be accounted for by separating the specimens into the two groups (Figs. 2 and 3). Of the 34 specimens in Group A taken within 48 hours of intercourse, 7 were positive for AP activity, (>25 KAu), whereas none of the 22 specimens taken from women in Group B had AP activity above 25 KAu. The apparent negative influence of those factors in Group B is statistically significant at the $p < 0.05$ level, as determined by a one-tail chi-squared test [*19*]. In other words, the chances of being able to demonstrate AP activity are much reduced in women who are menstruating, who have douched or used vaginal suppositories, or who have certain vaginal pathologies present. However, there is still a predominance of negative results among those specimens not presenting these factors (Fig. 2). At this time no explanation can be offered for these results. As pointed out earlier, other authors have reported similar findings.

It has been suggested that simple mechanical drainage can account for much of the

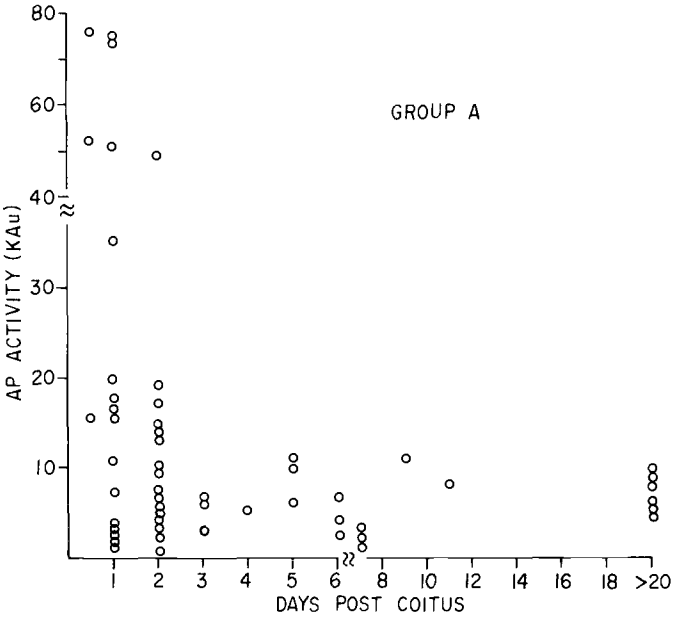

FIG. 2—*Prostatic acid phosphatase activity of specimens in Group A.*

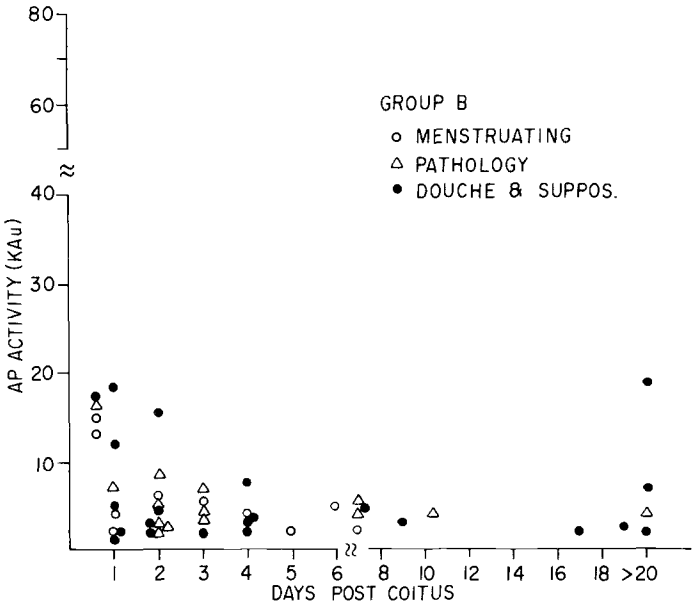

FIG. 3—*Prostatic acid phosphatase activity of specimens in Group B.*

decline in the vaginal concentration of AP and other semen constituents [*10*]. If the subject is actively menstruating at the time the specimen is taken, dilution of the remaining semen may also be a factor in reducing the ability to demonstrate AP. However, in the actual investigation of alleged rape cases, Rupp [*20*] reported being able to find AP activity in four out of four women who were menstruating, if the samples were taken within six hours. At the beginning of this study it was feared that interference from erythrocyte acid phosphatase might cause false high values, but even those specimens that were noticeably tinted with blood gave negative results. Douches and suppositories have the potential for giving false positive AP levels [*8,10*]. In this study all subjects who douched or used a suppository preparation after intercourse had enzyme activity levels below 25 KAu. Here the effect of cleansing the vagina and the resulting dilution of the semen increased the likelihood of obtaining false negative results. We have no opinion as to why those subjects having various pathologies present should uniformly have low enzyme levels.

None of the vaginal specimens had zero enzyme activity. Even one specimen from a subject who indicated that a condom had been used showed some apparent AP activity. It is possible that the particular enzyme method used in this study is not totally specific for prostatic acid phosphatase. Nevertheless, other investigators using different methods have also reported this residual activity [*11,16*]. Therefore, a small amount of AP-like activity appears to be present in the vagina at all times.

Summary

The present results are in general agreement with previous reports on the minimal level of AP activity that should be designated positive and on the period after intercourse in which elevated levels can be demonstrated. In this study 100% of the specimens above 25 KAu were taken within 48 hours of intercourse and 85.7% of these were within 24 hours. For values above 50 KAu, all were within 24 hours of intercourse. A low AP level does not rule out the possibility of recent coitus, particularly if the woman was menstruating or had douched or used some suppository preparation. These findings also point to the possible interference of certain pathologies in correlating AP activity and time since coitus. As other authors have shown and this study has confirmed, some reactive substance seems to be present in the vagina at all times. For the use of AP assays in the forensic investigation of alleged rape cases, it is suggested that a thorough, accurate history be obtained with regard to douches and suppositories and that the presence of vaginal pathologies be noted.

Further research on the techniques used in the forensic investigation of sexually related crimes is proceeding. The large proportion of negative samples within 24 hours of intercourse in this and other studies needs clarification. Other sampling techniques and the quantitative measurement of several different constituents of semen may offer accurate estimates of the time of last intercourse. Large studies with stringent control of the possible variables will further refine this important area of forensic science.

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