TECHNICAL NOTE

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Glycogenated Squamous Epithelial Cells as a Marker of Foreign Body Penetration in Sexual Assault

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ABSTRACT: Nonconsensual insertion of a foreign object into the vagina, anus, or mouth in some judicial jurisdictions is synonymous with rape, and elsewhere may constitute some degree of sexual assault or battery. Few techniques, however, are available to assist the criminalist in determining whether an object has been criminally inserted. Glycogenated epithelial cells have been used as a marker for vaginal epithelium, and as such, may indicate vaginal insertion if recovered from an object. This hypothesis was tested by studying orally and vaginally inserted objects from 42 volunteers and 20 rectally inserted objects recovered from cadavers. Glycogen positivity was assayed from smears of object swabbings stained with the periodic acid-Schiff (PAS) technique.

More than 75 glycogen positive cells were recovered from 39 of 42 vaginally inserted objects. Glycogenated cells were recovered from 8 of 20 rectally inserted objects (5 with more than 100 positive cells). Of 42 orally inserted objects, 32 also contained glycogen positive cells, but none with more than 28 positive cells. No glycogen positive cells were seen in skin exposed but not inserted objects. Large numbers of glycogen cells were seen in dried saliva drops. Amylase activity was not seen on 5 of 20 orally inserted shields, and thus the possibility of noninsertional saliva contamination could not be ruled out with shields yielding only small numbers of positive cells. Recovery of large numbers of glycogenated cells from foreign objects is strongly suggestive of either vaginal or anal insertion assuming amylase negativity. Glycogen positive cells are not seen secondary to glabrous skin exposure.

KEYWORDS: criminalistics, criminal sex offenses, glycogenated epithelial cells, rape, sodomy, vagina

Nonconsensual vaginal penetration by a foreign object in most U.S. judicial jurisdictions is considered synonymous with rape. Rape, some form of sexual abuse charge, or battery also usually applies to nonconsensual anal or oral penetration by a foreign object. The criminalist examining evidence from such a crime may well be asked to determine whether a specific foreign object had been inserted into a body orifice and which orifice was penetrated.

Victim-specific blood group substances recovered from a foreign object would suggest that the object had been in contact with the victim's secretions, but would not indicate that the

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object actually penetrated a body orifice. Similarly, demonstrable fecal material and amylase recovery from an object may suggest the anal or oral origin of deposited secretions, but not necessarily whether the object was inserted.

Well established vaginal secretion markers are not well recognized. Glycogenated epithelial cells have been proposed as a vaginal epithelial marker for many years [*1*]. Glycogenated epithelial cells can be recovered from the vagina from menarche to menopause [*2*] and often postmenopausally [*3*]. Randall and Riis [*4*] showed that the recovery of glycogenated squamous epithelial cells from the penile shaft was an excellent marker of recent intercourse.

Oral epithelium also contains glycogenated epithelial cells, although usually in significantly smaller numbers than those recovered from the vagina [*5,6*].

The recovery of glycogenated epithelial cells from a foreign object will be used to determine whether the laboratory can differentiate vaginally, orally, anally, and noninserted skin exposed foreign objects. The potential for saliva to produce false positive indications of insertion will also be explored.

Method

Forty-two paid female volunteers were recruited according to guidelines established by the Human Subjects Committee of the University of South Dakota School of Medicine. Informed consent was obtained from all volunteers. All of the subjects were menarcheal. Volunteers ranged in age from 20 to 43 years with a mean age of 29 years. The subjects were asked to vaginally and orally insert and remove separate plastic tampon inserter shields, discard the tampon, allow the shield to dry, and return the shields in supplied cardboard mailing tubes. Subjects were asked not to collect the vaginal specimens within five days of their period.

Anally inserted tampon shield were recovered from 20 cadavers of random sex, age, postmortem interval (not exceeding 24 h), and embalmed/unembalmed status.

Ten additional volunteers (male and female) were asked to rub vigorously separate tampon inserted shields on glabrous skin anywhere above the waist. These shields were returned to the laboratory in cardboard mailing tubes. These volunteers also placed a free fallen drop of saliva on a glass microscopic slide, allowed the drop to dry completely, and returned the slide with the shield.

All of the shields were identically processed. The entire shield was swabbed with a moistened (tap water) cotton swab. Special attention was given to removal of any obvious dried secretions. The swabs were then immediately smeared onto a 1-in.^{2} (6.45-cm^2) area of a standard glass microscopic slide. The slides were allowed to air-dry at least overnight. The slides were then fixed in absolute methanol for 1 min, dried, and then stained with the standard periodic acid-Schiff (PAS) technique [*7*].

Positive and negative standard slides for glycogenated squamous epithelial cells utilized previously obtained vaginal and precoital penile swabs, respectively [*4*]. PAS glycogen positivity was arbitrarily rated 0 to 4+. Cells were considered glycogen positive only if the PAS staining was 3+ or 4+, a definite nucleus could be identified, and the cells were not stacked one on top of another (Fig. 1). The number of PAS positive (3+ or 4+) cells in each 1-in.^{2} (6.45-cm^2) smear area was obtained from each object smear.

Twenty of the orally inserted shields following the above routine processing were randomly selected, eluted with distilled water, and the eluate tested for amylase activity using standard techniques [*8*].

Results

Of the 42 vaginally inserted shields, 38 were positive for more than 100 glycogenated cells. The remaining cases were positive for 78, 44, 12, and 4 cells, respectively. No spermatozoa were identified on any smear.

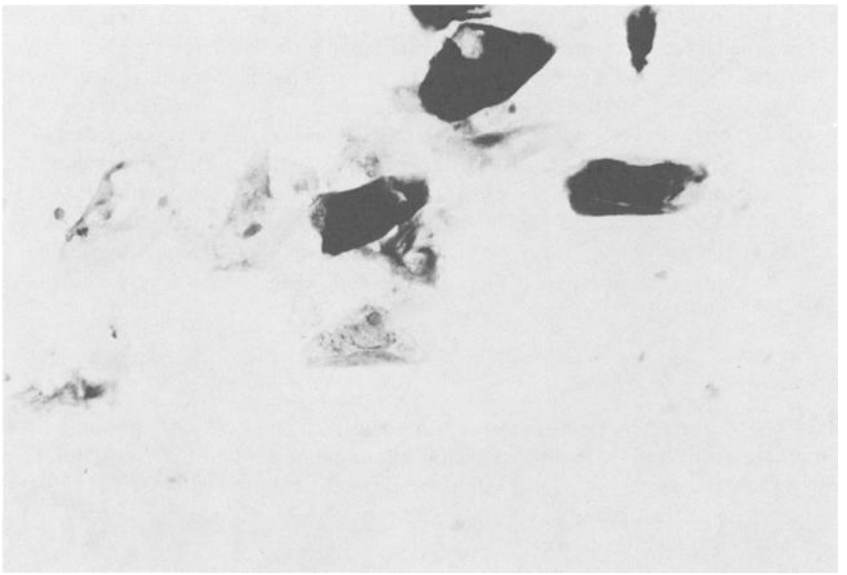

FIG. 1—*Glycogenated* (darkly stained) *and nonglycogenated* (lightly stained) *cells recovered from a vaginally inserted shield, PAS stain, original magnification* ×*400.*

Of the 42 orally inserted shields, 10 were negative for glycogenated cells. Among the remaining 32 shields, no more than 28 glycogenated cells were identified per smear. The 32 positive shields had an average (mean) of 5.5 positive cells per shield.

Of the 20 anally inserted shields, 5 contained more than 100 glycogenated cells, and 3 shields were positive for 52, 26, and 8 cells, respectively. The remaining 12 shields were negative for glycogenated cells. The positive and negative shields were equally divided between embalmed and unembalmed cases and within the postmortem interval distribution.

None of the handled (skin exposed) shields were positive for glycogenated cells. The dried saliva pools were quite cellular and averaged 4.7% (range 1 to 9%) of the squamous epithelial cells positive for glycogen.

Of the 20 shields tested for amylase, 15 were positive (9 of the 15 positive were negative for glycogenated cells).

Discussion

The recovery of glycogenated epithelial cells from foreign objects represents a simple and technically easy technique potentially to demonstrate that an object had been either anally or vaginally inserted. Objects manually handled or rubbed or both on glabrous skin were all uniformly negative for glycogenated epithelial cells. Similarly, 45 precoital direct penile glans swabs were negative for glycogenated cells in a previous study [*4*]. Assuming amylase negativity and the recovery of large numbers of glycogenated cells, the technique appears to be 100% specific for vaginal or anal insertion and relatively sensitive for vaginal insertion (39 of 42—93%), but poorly sensitive for anal insertion (8 of 20—40%). The poor anal sensitivity may reflect the limited squamous epithelial exposure in anal insertion as compared to the vagina. The tight nonglycogenated anal external sphincter may also have physically wiped the glycogenated cells off of the shields.

Despite the good sensitivity of amylase testing in saliva detection [*8*], only 15 of 20 oral shields were positive for amylase. The incomplete amylase positivity and few recovered oral

glycogen positive cells suggest that in this study much of the saliva was removed from the shields by the lips as the shield was pulled from the mouth. The large number of glycogenated cells seen in dried saliva pools indicates that direct deposition of saliva on an object could produce a false positive indication of bodily insertion using the presence of large numbers of glycogenated epithelial cells alone. Amylase testing, however, should be expected to indicate the presence of saliva in those cases. Amylase positivity with or without glycogenated epithelial cells may indicate the presence of saliva but precludes any statement of oral insertion. Of the 20 orally inserted shields, 5 were amylase negative, yet all contained glycogenated epithelial cells. Therefore, the possibility of noninsertional saliva contamination cannot be excluded when only small numbers of glycogenated cells are recovered from an object which is negative for amylase.

Conclusion

Recovery of numerous glycogenated epithelial cells alone does not appear to be able to differentiate anally and vaginally inserted objects either on a quantitative or qualitative basis assuming amylase negativity. The inability to differentiate between anal and vaginal penetration, however, probably is not overly significant since in most judicial jurisdictions both have similar legal ramifications.

Both the male and female urethral orifices are known to contain glycogenated epithelium [4, 9]. The possibility of urethral insertion of small objects, therefore, should not be excluded when large numbers of glycogenated epithelial cells are recovered.

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