J Forensic Sci, Apr. 1976, Vol. 21, No. 2

B. W. Given,[1] *M.S.F.S.*

Sex-Chromatin Bodies in Penile Washings as an Indicator of Recent Coitus

Within the past decade several sociologic factors have contributed to a relaxation and greater candor in all spheres of sexual concern, including an increased awareness of the crime of rape. As this crime has burgeoned, so too has the literature of the forensic sciences regarding techniques for the analysis and evaluation of medicolegal evidence of alleged rape.

Despite the growing array of sophisticated procedures, one aspect has received but scant mention—evidence of recent sexual activity of the part of the suspect. Trace evidence, such as fingernail scrapings, hair and fiber transfer, and bloodstain analysis, is obvious as a source of potential evidence from the suspect, but indicia of his recent sexual activity are rarely mentioned.

This study presents a procedure for the possible determination of recent coitus on the part of a man suspected of rape.

In the only study found in the literature with a similar objective, Thomas and Van Hecke [*1*] sought to identify by glycogen content vaginal cells collected from the suspect's penis by use of the Lugol stain. Since glycogen content is dependent on the growth and secretory activity of the epithelium, this measurement may yield equivocal results. Atrophic epithelium of the postmenopausal and amenorrheic female is classified as aglycogenic by Papanicolaou [*2*], and the concentration of this starch in glycogenic-type cells is subject to great variation because of factors such as gravidity, menses, and hormonal therapy. Papanicolaou [*2*, p. 39] also mentions the possibility of prostatic cells being rich in glycogen. The present study concentrates on the morphologic characteristics of cells found and the possible presence of nuclear sex-chromatin bodies.

The collection of cellular material in the Thomas and Van Hecke technique was accomplished by a direct smear of the glans penis on a microscope slide. The transfer of male epithelial cells to the specimen for analysis is minimized in the present study through a washing technique.

Materials and Methods

Penile washings used in this study were obtained from seven adult male volunteers, two of whom were circumcised. Twenty specimens were obtained. The period of time between intercourse and specimen collection was left to the convenience of the volunteer

Received for publication 28 May 1975; accepted for publication 3 July 1975.

The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of the Navy or the Department of Defense.

[1] Fellow, Armed Forces Institute of Pathology, Wash., D.C., and special agent, Naval Investigative Service Headquarters, Alexandria Va.

and varied between 30 min and 12 h. The subjects were instructed, however, not to bathe prior to specimen collection but to merely wipe the glans with tissue.

The principal components of the apparatus used in this technique are illustrated in Fig. 1. Collection of specimens for analysis was accomplished as follows:

1. The prepuce of the penis was retracted to expose the corona and collum of the glans and the frenulum of the prepuce.
2. The glans (primarily the area of corona and collum) and convolutions of skin around the frenulum were irrigated with a jet of physiologic saline solution directed from a squeeze-type wash bottle. The washings (8 to 10 ml is adequate) were collected in a suitable wide-mouth container.
3. Washings were then transferred to a graduated storage tube, and an equal amount of 50% ethyl alcohol was added immediately. (Because of the possible delay between collection and analysis, a fixative must be added to preserve and initially fix any cells collected. The solution of alcohol has been found to be an excellent universal fixative for fluid specimens [*3*].)

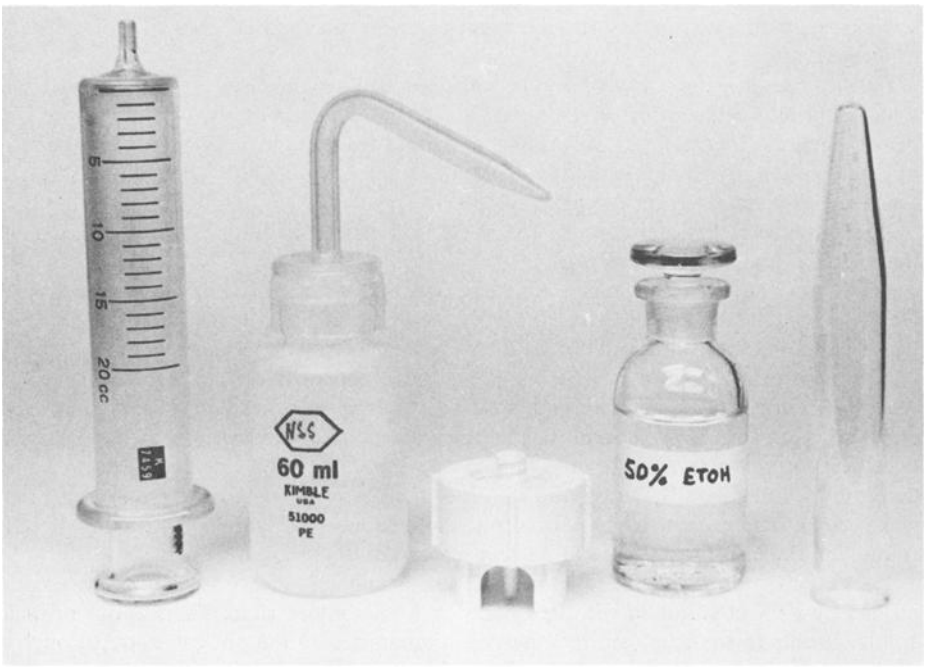

FIG. 1—*Principal components of apparatus used for the collection of cellular material from the penile surface. The glans penis is irrigated with physiologic saline solution from the 60-ml wash bottle. Washings are retained in the centrifuge tube after an equal amount of 50% ethyl alcohol is added. The washings are forced through the 25-mm Nuclepore filter apparatus (center item) with a syringe.*

Care must be exercised to retain as many cells as possible throughout subsequent processing because of the paucity of cells often collected in the washing. Numerous methods were tried, but by far the best technique for cell recovery and concentration entails the use of the Nuclepore® filter membrane [*4*]. The filter, a thin polycarbonate film having pores with a diameter of 5 μm is inserted in a filter holder (Fig. 1), and the penile washings are forced through the membrane by means of a syringe. The membrane is then fastened to a glass slide with stainless steel clips, and normal fixation and staining

procedures are done. Since the cells are already fixed by storage in 50% ethyl alcohol, fixation in 95% alcohol for only 15 min is all that is necessary to fix cells to the filter. The filtrate is stained by a modification of the classic Papanicolaou method [5] and a permanent mount made.

The entire filter-membrane mount was then examined microscopically under high-dry and oil-immersion lenses, the magnification being 450 and 1000, respectively.

Results and Discussion

The collection and staining techniques used yielded uniformly excellent results; the study was therefore expanded to investigate several important areas as they apply to forensic sciences. These areas will be discussed individually.

Source of Cells Found

The first question on initial viewing of a stained slide seems to be, "What are these cells and from where did they come?" In the medicolegal investigation, some obvious sources to be considered for epithelial cells include the vagina, glans penis, and the urethra. The occasional histiocyte or polymorphonuclear leukocyte found in penile washings may be disregarded.

To evaluate the relative contribution of cells from several of these sources, the following procedures were conducted.

Glans Penis—Several washings were taken from circumcised and uncircumcised penes at times later than 48 hours since last coitus and after at least two normal bathings. Most cells found were of the almost fully keratinized, anucleated squamous type and deeply orange in color. Since complete keratinization of cells is extremely rare in the vagina [2, p. 23], these cells were not considered significant for potential confounding of post-coital penile washings. The few superficial cells found were predominately strongly acidophilic with extremely small pyknotic nuclei. A few basophilic superficial cells were seen, but they had smaller nuclei than are normally found in basophilic intermediate vaginal cells. For these reasons, especially in view of the relative paucity of cells in comparison to washings from a penis recently involved in coitus, it does not seem that penile cells should confound forensic investigations.

At this point a distinction must be made between physiologic ("normal") and traumatic exfoliation. The previously mentioned penile washings were obtained from what must be considered relatively untraumatized penes; as stated, the cell counts were relatively quite low. Any vigorous rubbing of the glans as experienced during coitus should produce a traumatic exfoliation, and washings would consequently evidence an elevated cell count.

Interestingly, it was the uncircumcised penes that contributed the greatest number of basophilic cells, and from washings of separate areas on the penis it was found that they probably originated from the area of the collum and frenulum.

Urine—Normal voided urine contains only a scanty amount of squamous cells. Urine samples voided at least 48 hours after the last act of coitus were taken from the volunteers. The samples showed a few superficial cells with an occasional polymorphonuclear leukocyte. More common were clusters or sheets of exfoliated transitional cells from the urinary tract. These findings were consistent with other cytologic examinations [6]. The amount of urine residue obtained from penile washings is not sufficient to cause problems.

Semen—The mucosae of the prostate and seminal vesicles are composed of cylindrical columnar cells and small basal cells easily distinguished from intermediate or superficial epithelial cells. Semen was washed from the traumatized glans and showed transitional urethral cells (resembling intermediate squamous vaginal cells) and karyorrhectic squames from the glans itself.

Vagina—Penile washings were undertaken after recent coitus. The washings were rich in intermediate and superficial vaginal cells and, less commonly, parabasal cells. Depending on the donor's health, varying numbers of histiocytes, erythrocytes, polymorphonuclear cells, and monilial and trichomonal organisms, along with other debris, were also found.

The results of this portion of the study are summarized in Table 1.

TABLE 1—*Relative amount of nuclear squamous epithelial cells from various sources.*

Source of Cells	Relative Amount Found
Postcoital penile washings	+ + + +
Postejaculation penile washings (no coitus)	+ +
"Clean" glans washings	+ to + +
Semen	+
Urine	+

Sex of Cell Donor

One of the most critical aspects of this procedure is determining by cytologic examination the sex of the person from whom the cells came. This study explored the potential of sex-chromatin bodies for this purpose.

The normal male cell contains one X chromosome and one Y chromosome; the genetically normal female cell carries two X chromosomes. The first X chromosome in the interphase nucleus uncoils and is twined throughout the desoxyribonucleic acid of the nucleus. Any additional X chromosomes present remain tightly condensed and adherent to the inside of the nuclear membrane. This mass, the sex-chromatin body or Barr body [*7*], is virtually absent in cells of the male. A smaller chromatin mass, the male chromocentrum, may be seen, however, in a few nuclei (about 5%) of squamous cells from the normal male [*6,* p. 131]. While it is believed by some [*8*] that Barr bodies exist in all female cells except those with pyknotic nuclei, a 100% finding is never possible, probably because the cell may fall in an improper orientation for visualization of the Barr body as adherent to the nuclear membrane, and it may appear instead to be situated somewhere within the body of the nucleus.

Renard [*9*], in an article on the significance of epithelial cells in forensic sciences, used a counting criterion for determination of sex of cells from oral mucosa, wherein if 10 consecutive nuclei of 30 consecutive cells examined showed Barr bodies, the smear was recorded as from a female. This does not seem to be a reasonable approach for vaginal cells in this study, a high percentage of which are superficial with pyknotic nuclei, and the specimen used is of necessity a commingling to some degree of two cell sources. In the present study, only those cells with vesicular nuclei were considered, and, if greater than 5% of those cells contained Barr bodies, the specimen was classified as "chromatin positive consistent with female morphology." No errors were made with either oral or vaginal cells in an informal blind screening of 14 specimens.

Several staining techniques for the visualization of Barr bodies were explored, including the aceto-orcein procedure [*4*]. The author's personal preference is the Papanicolaou method, which has already been mentioned.

An excellent schedule of criteria and limitation for sex-chromatin body evaluation is presented by Naib's volume on exfoliative cytopathology [*10*]. In summary, a Barr body is

(1) seen tightly adherent to the inner surface of the nuclear membrane,
(2) about 1 μm in diameter,
(3) well defined, with sharp borders, and
(4) at the same focusing plane as the nucleus.

Chromosomal abnormalities can influence the presence or absence of sex chromatin, but these anomalies are rather easily minimized in an alleged rape investigation, since both victim and suspect are known and can be evaluated. The two abnormalities that have potential for confounding results are Turner's and Klinefelter's syndromes.

In Turner's syndrome females have only one X chromosome and therefore exhibit no Barr bodies. These persons usually have obvious abnormalities, such as short stature, webbed neck, small mandible, cubitus valgus, and sometimes slight mental retardation. This anomaly accounts for only 0.5 female per 1000 in an institutionalized population [*11*].

In Klinefelter's syndrome, males have an extra X chromosome (XXY) and therefore exhibit cellular Barr bodies. Obvious abnormalities in this syndrome frequently include retardation (sometimes severe), apathy, poor socialization, long legs, and atrophic testes. In at least one study the XXY abnormality was found in 10.7 males per 1000 population in institutions for the mentally retarded [*11*].

Duration of Evidence

The duration of evidence is so variable that little can be said regarding the effects of time on this type of procedure. Obviously, bathing will usually eradicate all evidence. It was found that cursory washing, however, often failed to remove some vaginal cells from the folds of the frenulum.

The warm, moist environment around the collum and frenulum possibly acts as a partial preservative against gross morphologic cell damage, since even after 12 hours Barr bodies are discernible. Drying may account for the greater cytoplasmic eosinophilia seen in the intermediate and parabasal cells exposed for a longer time. This is in agreement with the findings of Naib [*10*, p. 21].

An attempt was made to estimate roughly the time of extravaginal exposure of the cells by viability tests by use of the trypan blue staining technique [*12*]; there was no success. Cells used from specimens of even the shortest postcoital period, 30 min, were negative for viability. It is believed that morphologic indicia of exposure would also have little practical significance because of the variety of uncontrolled factors, such as precoital viability and postcoital environment.

Class Characteristics

While there is presently no method of establishing a connection between cells found by this procedure and an alleged victim, various cytologic characteristics have the potential for grouping the donors in certain broad categories that might be of evidentiary value. For example, a large percentage of parabasal cells would indicate a possible physiologic absence of epithelial maturation consistent with premenarchal or postmenopausal females. Clumping of superficial cells may indicate imminent onset of menses. Endometrial cells could give supportive evidence of a donor in her menstrual period. In addition to possible evidence of pathologic conditions, some indicators of severe bacterial, protozoan, or fungal infection may also be seen.

Limitations of Technique

The technique described is a potential aid in the determination of recent coitus, though not necessarily rape. Only through adept investigative and interrogative techniques can it be determined that no voluntary coitus occurred during the period of the validity of this technique, thereby giving an innocent explanation for positive findings.

Further research is needed to assess more accurately the length of time after coitus

vaginal cells may be found in penile washings of sufficient morphologic integrity to permit identification of Barr bodies. Habits of personal hygiene are known to contribute heavily to this factor, but to what degree was not pursued in this study. An ancillary study of environmental effects on configuration of cells is currently being conducted with artificially injured cells in a manner similar to that employed by Rubio et al [*12*]. To what extent these results can be applied to in vivo situations is not known.

Summary

A technique for the collection and examination of penile washings for the possible presence of vaginal cells is presented. Various characteristics of cellular form and structure are discussed insofar as they pertain to the primary goal of this study—the detection of evidence of recent sexual intercourse on the part of a male by the examination of cellular content of penile washings.

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Armed Forces Institute of Pathology
Washington, D.C. 20306