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Forensic value of the Lugol's staining method: further studies on glycogenated epithelium in the male urinary tract

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Abstract This study presents findings from a series of investigations on the presence of glycogenated epithelium in the male urinary tract and on the penile surface in order to assess the forensic value of the Lugol's method for the identification of vaginal cells. Direct smears obtained from the urethral opening, glans penis, and penile shaft, along with post-mortem samples of the fossa navicularis, and histological sections of the penis were examined. The presence of polygonal, glycogenated, Lugol-positive epithelium cells in the male urinary tract was found to be common. Our results suggest that these cells originate from the fossa navicularis. Because of the possibility of exfoliation of glycogenated male cells and transfer to the penile surface a Lugol-positive reaction in epithelial cells on penile swabs can no longer be assumed to prove the presence of vaginal cells.

Key words Lugol's iodine · Value-Forensic investigations · Glycogenated epithelium cells · Male urinary tract

Zusammenfassung Wir untersuchten das Vorkommen glykogenhaltiger Epithelzellen im männlichen Harntrakt und an der Penisoberfläche, um die forensische Bedeutung der Lugol'schen Färbmethode für den Scheidenzellnachweis zu überprüfen. Das Untersuchungsmaterial bestand aus Abklatschpräparaten von Harnröhrenmündung, Glans Penis und Penisschaft sowie aus postmortalen Abstrichen aus der Fossa navicularis und histologischen Serienschnitten vom Penis. Polygonale, glykogenhaltige, Lugol-positive Epithelzellen waren regelmäßig im männlichen Harntrakt nachweisbar. Unsere Ergebnisse weisen darauf hin, daß diese Zellen aus der Fossa navicularis der Harnröhre stammen. Aufgrund einer möglichen Verschleppung abgeschilferter männlicher Epithelien an die Penisoberfläche, ist die Lugol'sche Färbemethode bei Penisabklatschpräparaten für den Scheidenzellnachweis ungeeignet.

Schlüsselwörter Lugol'sche Färbemethode Forensische Bedeutung · Glykogenhaltige Epithelien Männlicher Harntrakt

Introduction

Glycogenated Lugol-positive epithelial cells found on the male glans penis, have long served as indicators of recent sexual intercourse in forensic investigations [1, 6, 13, 15]. The glycogen content of vaginal epithelial cells was thought to be higher than in all other epithelial cells in the body, hence the Lugol's method, an iodine staining technique used to detect glycogenated cells, was deemed acceptable as evidence in court [2]. It has, however been shown that glycogenated cells can also be detected in the oral mucosa of infants [5] and in the urethral secretion of males with urethritis [2, 7, 11, 14]. In more recent studies glycogen-containing cells were found at the apex of the urethra in healthy males who abstained from sexual intercourse for several days prior to examination [8, 10]. These findings suggest that glycogenated epithelial cells found on the glans penis may not provide definitive evidence of recent vaginal intercourse [8]. Our previous systematic investigation of penile swabs concurs with these findings [3]. Polygonal, Lugol-positive epithelial cells were detected on the glans of the penis in more than 50% of the males examined, even in healthy young volunteers with no urinary infections. In addition, glycogenated epithelial cells were found in urinary sediments, as well as in the oral mucosa of adults.

This paper proposes to assess the questionable value of the Lugol's staining technique and to further investigate the presence of glycogenated epithelium in distinct anatomical regions of the male urinary tract.

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Materials and methods

The study comprised 3 parts. In the first part, direct smears on glass slides were obtained from the external urethral meatus, glans penis, and penile shaft, of 20 healthy volunteers between 21 and 61 years old. The volunteers abstained from sexual intercourse at least 5 days prior to sampling. Staining of air-dried preparations was carried out using diluted Lugol's solution according to Wiegmann [15] and Merkel [6]. Only cells that stained dark brown and contained a clearly recognizable unstained nucleus were defined as Lugol-positive.

In the secod part, 22 post-mortem samples from the fossa navicularis were extracted using a cotton-tipped swab. The material was transferred onto a glass slide and stained in the same manner.

In 2 cases, a series of sections taken from the formalinfixed penis were examined histologically. Glycogen was detected using Best's staining technique described by Romeis [9]. A further histological examination of the vaginal mucous membrane, obtained from a 20-year-old female, was carried out for comparison.

Results

Penile swabs

The frequency and distribution of Lugol-positive epithelial cells found on penile swabs obtained from the urethral opening, glans, and penile shaft are presented in Table 1. In 15 of the 20 males examined, at least several glycogenated polygonal squamous epithelial cells were detected at the urethral opening. In these cases, the percentage of Lugol-positive epithelial cells varied between 2–47% (average 21%).

In 6 of the these cases, Lugol-positive epithelial cells were also found on the smear preparations from the glans penis. In the cases in which Lugol-positive epithelial cells were detected in penile swabs from all 3 anatomical regions ($n = 2$), the largest percentage of glycogenated epithelial cells was found at the urethral opening.

In the smear preparations from one volunteer, several Lugol-positive epithelial cells were found on the penile shaft, but were absent in the other swab preparations. In only 4 of the 20 males examined, no glycogenated epithelial cells were found in the 3 anatomical regions.

Table 1 Percentage of Lugol-positive epithelial cells (%) in smear preparations ($n = 20$) obtained from the urethral opening (U), glans penis (G) and penile shaft (S). Numbers of cases with Lugol-positive cells in the 3 different anatomical regions are represented by marginal figures

n	U [%]	G [%]	S [%]
9	2	–	–
	3	–	–
	5	–	–
	17	–	–
	20	–	–
	20	–	–
	22	–	–
	22	–	–
	35	–	–
4	14	19	–
	18	1	–
	27	9	–
	47	21	–
1	–	–	3
2	18	14	6
	43	29	36
4	–	–	–
	–	–	–
	–	–	–
	–	–	–
20	15	6	3

Post-mortem swab investigations

Lugol-positive epithelial cells were found in all 25 post-mortem urethral swabs obtained from the fossa navicularis and in some cases were as high as 98% (average 30%).

Histological examinations

Anterior urethra

The external meatus urethra consists of multilayered, partly cornified, squamous epithelium, similar to that from surface of the glans penis. Intracellular glycogen was not detected in the urethral meatus. The fossa navicularis, a fusiform dilation in the glanular urethra extending from the meatus to the level of the corona of the glans (about 20–25 mm), is lined almost entirely with multilayered, uncornified, squamous epithelium [4]. At the dorsum penis this epithelium comprises 20–25 cell layers. The majority of the polygonal cells found in these layers and up to the basal cell layer exhibited abundant intracellular glycogen (Fig. 1 a). Conversely, opposite the dorsum penis, the mucous membrane was found to be flatter, in some instances cuboidal, and lacking in detectable epithelial glycogen.

The proximal end of the fossa navicularis forms a boundary between the glanular urethra lined with squamous epithelium, and the remaining urethra (pars cavernosa) which is lined for the most part with multilayered, columnal epithelium. Glycogen was absent in each of the 4–6 cell layers of epithelium along this section of the anterior urethra (Fig. 1 b).

Posterior urethra

The membranous urethra is the most rigid portion of the urethra as it passes through the musculomembranous pelvic diaphram opening. This section, and most of the prostatic urethra, is lined almost entirely with multilayered, columnal epithelium containing no glycogen (Fig. 1 c). Though there is individual variation, the proximal end of the pro-

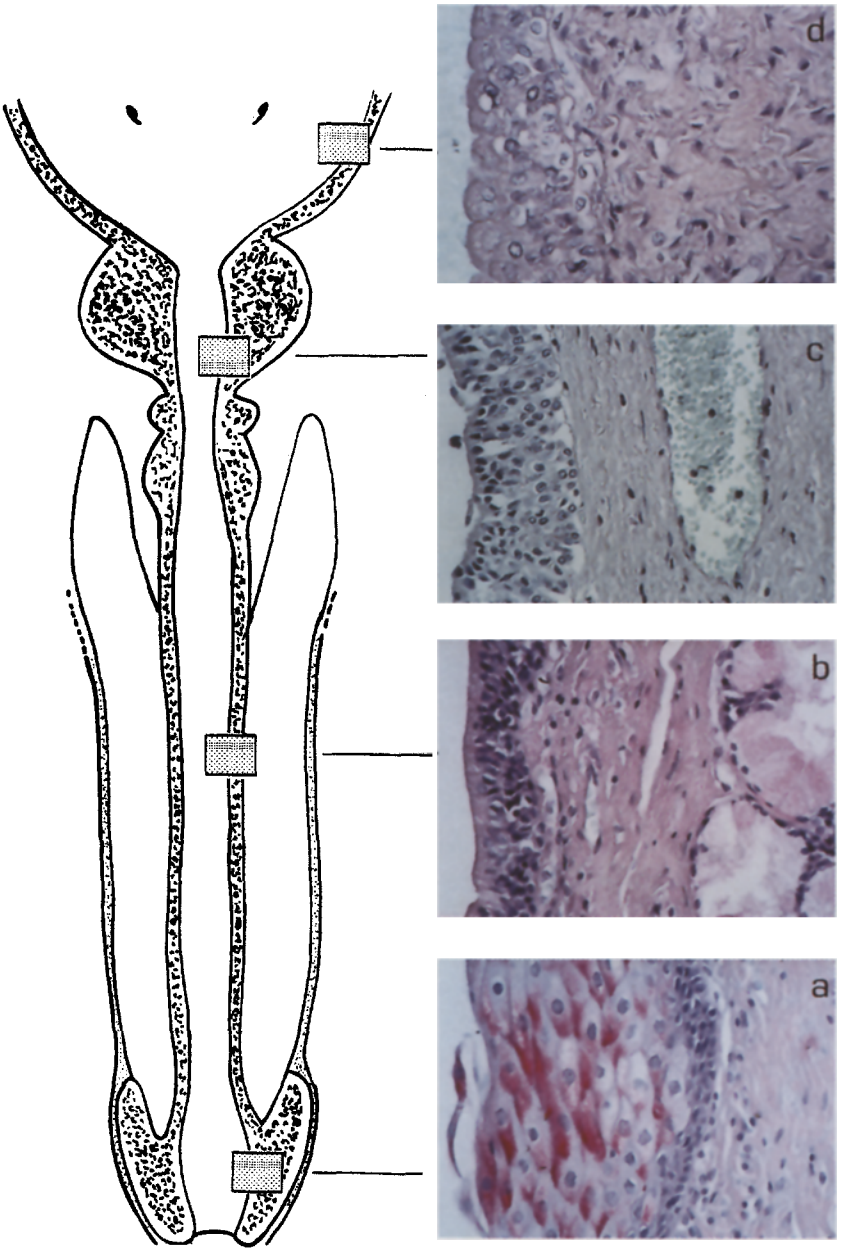

Fig. 1 Histological sections of the urethra from a 20-year-old male (no urological diseases). Multilayered squamous epithelium with abundant intracellular glycogen in the fossa navicularis (**a**). Columnar epithelium and submucous urethral glands of the pars cavernosa containing no glycogen (**b**). Prostatic urethra (**c**) and transitional epithelium of urinary bladder (**d**) both lacking intracellular glycogen (Best's stain × 150)

static urethral mucous membrane is frequently comprised of transitional epithelium, as found in the urinary bladder [4]. Glycogenated intracellular epithelium was not present in these sections (Fig. 1 d).

The submucous urethral glands and glandular ducts in the pars cavernosa, as well as the glands of Cowper in the bulbous urethra contain typical high, columnal epithelial cells with nuclei located at the base. Glycogen was not detected in these cells, however, granular intracellular glycogen was detected in many columnal epithelial cells of the glandulae prostaticae. These cells along with the amorphic secretion of the prostatic gland lumina were stained by Best's method.

The penile epidermal surface, covered with multilayered, cornified, squamous epithelium, was found to be free of glycogen, as was the inner surface of the prepuce, which like the glans penis, consists for the most part of multilayered, but less cornified, squamous epithelium.

Discussion

Our findings suggest that glycogenated, Lugol-positive epithelial cells are commonly found in the male urinary tract. The post-mortem penile swabs in which glycogenated epithelial cells were detected in every smear, offer particularly strong evidence for this conclusion.

Furthermore, the histological findings indicate that glycogen-containing cells may originate from the fossa navicularis, the majority of which is lined with multilayered non-cornified, squamous epithelium. A high concentration of glycogen-containing epithelium was found in

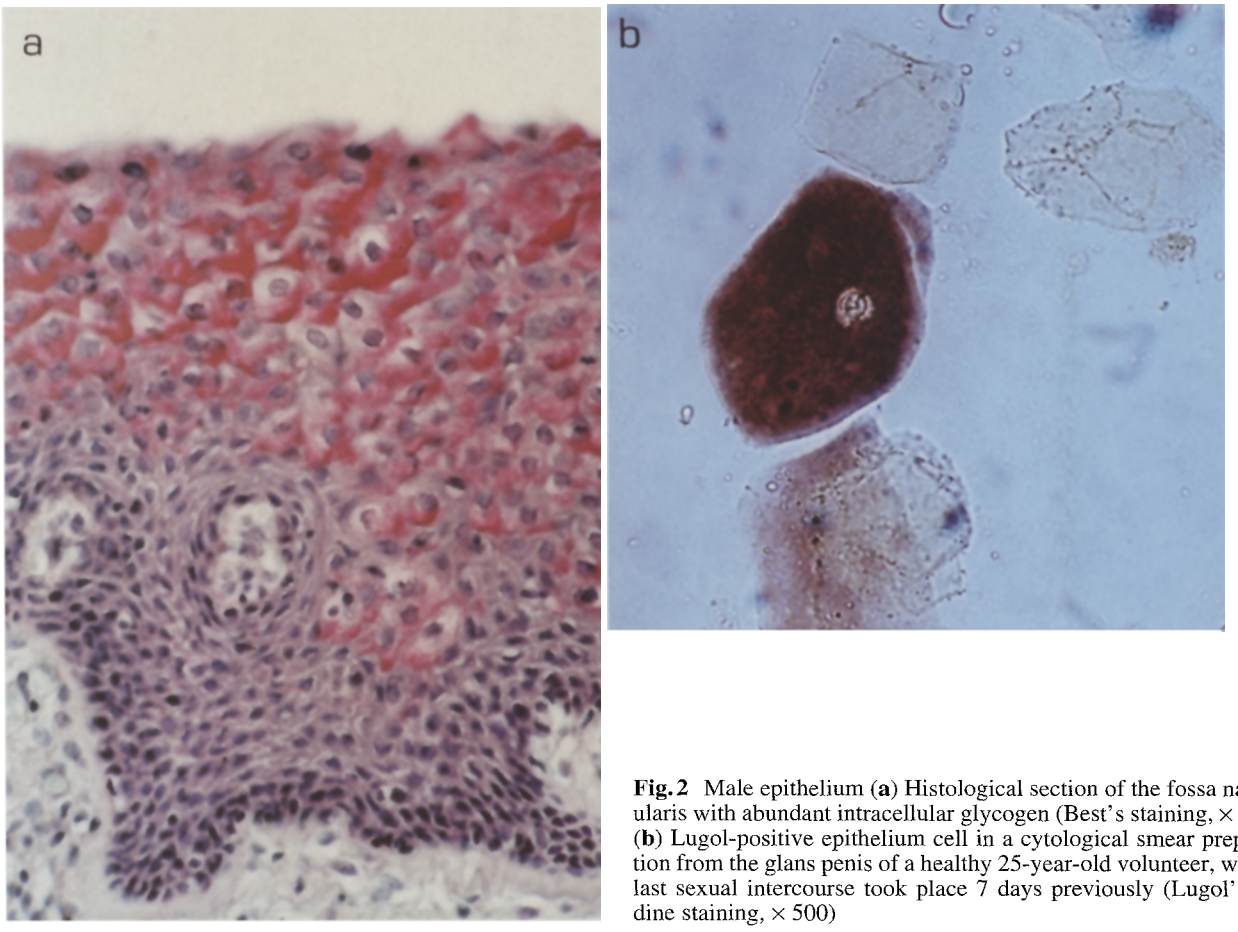

Fig. 2 Male epithelium (**a**) Histological section of the fossa navicularis with abundant intracellular glycogen (Best's staining, × 250) (**b**) Lugol-positive epithelium cell in a cytological smear preparation from the glans penis of a healthy 25-year-old volunteer, whose last sexual intercourse took place 7 days previously (Lugol's iodine staining, × 500)

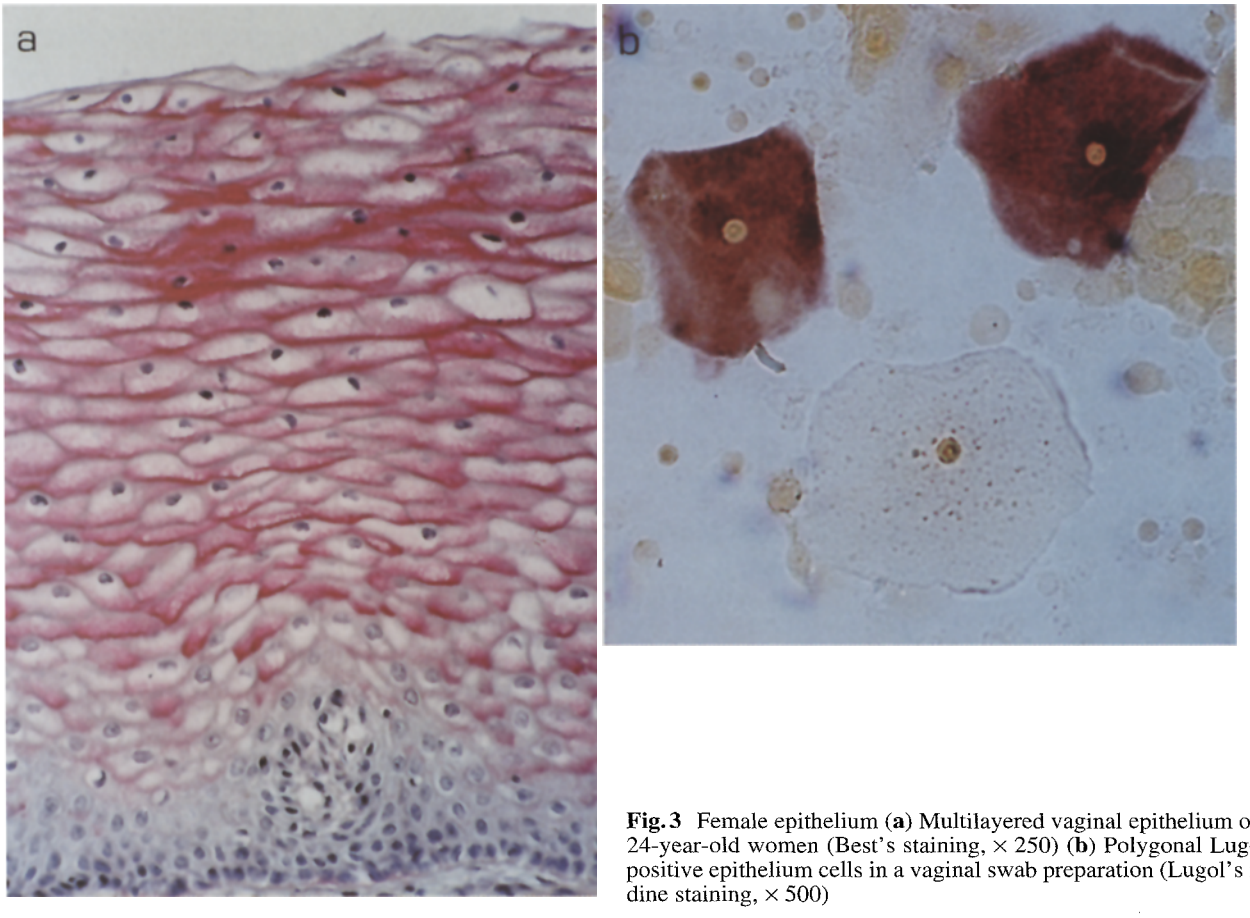

Fig. 3 Female epithelium (**a**) Multilayered vaginal epithelium of a 24-year-old women (Best's staining, × 250) (**b**) Polygonal Lugol-positive epithelium cells in a vaginal swab preparation (Lugol's iodine staining, × 500)

this area and up to the basal cell layers; the remaining urethral regions were for the most part entirely free of glycogen. As the histological examinations indicate, glycogenated epithelium was not evident on the penile shaft surface nor the glans penis, although traces of granular glycogen substances were detected in the amorphic secretion and columnal epithelium of the glandulae prostaticae.

Based on these findings, we conclude that the Lugol-positive cells found in the cytological smears obtained from the different anatomical regions of the penile surface, are superficially exfoliated and transferred male urethral cells most probably originating from the fossa navicularis. Accordingly the largest number of glycogen-containing cells were detected on the area around the urethral-opening and on the glans penis. In contrary to other authors [8] we found Lugol-positive epithelial cells even on the penis shaft. This findings demonstrate, that the presence of glycogenated squamous cells on the penile surface does not prove the origin of vaginal epithelium. In our opinion the fossa navicularis represents a definite source of potential false positivity.

The glycogenated epithelial cell found in the samples investigated could not have originated from vaginal epithelium as the males in our study abstained from sexual intercourse at least 5 days prior to sampling.

Furthermore, we can assume that at least several of the polygonal, glycogenated, squamous epithelial cells found in the urinary sediments examined in our previous study [3], also originated from this urethral region.

We found it difficult to distinguish between the glycogenated cells from male urethral mucosa and the superficial and intermediate vaginal epithelium cells (Figs. 2, 3). Although superficial vaginal cells typically contain pyknotic nuclei smaller than those in most normal male urethral cells, we find nucleus size cannot serve as cytomorphologic criteria in differentiating between male and female cells in forensic examinations. The nucleus/plasma ratio is dependent on hormonal and environmental factors (i.e. swelling of nucleus due to dehydration [12]). Similarly, the intermediate cells of the epithelium lining the female vagina, which typically appear similar to male urethral cells, cannot be distinguished either cytomorphologically or by staining techniques.

In conclusion, the high percentage of glycogenated cells in the fossa navicularis, given the possibility of exfoliation and transfer, means that a Lugol-positive reaction in epithelial cells obtained from penile swab preparations can no longer be assumed to prove the presence of vaginal cells. The positive test cannot serve as an indication of recent vaginal intercourse.

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