

The value of the Lugol's iodine staining technique for the identification of vaginal epithelial cells

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Summary. This paper reports on the specificity of the Lugol's iodine staining technique for the detection of vaginal epithelial cells on penile swabs. Air-dried swabs taken from the glans of the penis of 153 hospital patients and from 50 healthy volunteers, whose last sexual intercourse had taken place at least 5 days previously, were stained with Lugol's solution. Glycogenated cells were found in more than 50% of the cases studied, even in healthy volunteers without urethritis. In almost all of these cases the smear contained at least a few polygonal nucleated epithelial cells showing an unequivocal positive Lugol reaction. These cells cannot be distinguished from superficial or intermediate vaginal cells, by cytomorphology or staining. Urinary tract infections had no influence on the glycogen content of male squamous epithelial cells. On the basis of these results the Lugol's method can no longer be assumed to prove the presence of vaginal cells in penile swabs.

Key words: Lugol's iodine staining technique – Identification of vaginal cells – Penile swabs

Zusammenfassung. Wir untersuchten die Spezifität der Lugol'schen Färbetechnik für den Nachweis von Scheidenzellen in Penisabklatschpräparaten. Dazu wurde das luftgetrocknete Material von der Glans Penis von insgesamt 153 Klinikpatienten und 50 freiwilligen Probanden mit Lugol'scher Lösung gefärbt. Der letzte Geschlechtsverkehr lag jeweils mindestens fünf Tage zurück. Glykogenhaltige Plattenepithelien waren in mehr als 50% aller untersuchten Fälle nachweisbar, auch bei gesunden Probanden ohne Urethritis. In nahezu allen dieser Fällen waren im Penisabklatsch zumindest einzelne polygonale Epithelzellen mit eindeutig positiver Lugol-Reaktion nachweisbar. Diese Zellen lassen sich morphologisch nicht von superficialen oder intermediären Vaginalzellen unterscheiden. Harnwegsinfekte hatten keinen Einfluß auf den Glykogengehalt männlicher Epithelzellen. Unsere Ergebnisse zeigen, daß die Lugol'sche Färbetechnik für den

Nachweis von Scheidenzellen in Penisabklatschpräparaten nicht mehr als geeignet angesehen werden kann.

Schlüsselwörter: Lugol'sche Färbetechnik – Scheidenzellnachweis – Penisabklatsch

Introduction

Ever since the publications by Wiegmann [5] and Merkel [2], the Lugol's iodine staining technique has been used in forensic medicine as a simple and reliable test for the identification of vaginal epithelial cells, for example in seminal stains or smears prepared from penile swabs. The method is based on the fact that iodine reacts with intracellular glycogen to produce a dark brown colour (Fig. 1). Since the glycogen content of vaginal epithelial cells was thought to be higher than in all other epithelial cells in the body, the Lugol's method has been considered specific and acceptable as evidence in court [1]. It has, however, since been shown that glycogen-containing cells can also be found in the oral mucosa of infants, and in the urethral secretion of males with urethritis. Other authors found glycogenated cells in swabs obtained from the tip of the male urethra in men who had had their last sexual intercourse several days previously [3, 4]. To our knowledge no systematic studies on possible errors of the Lugol's method have so far been carried out. It is against this background that we now present a discussion of our own results.

Materials and methods

The material investigated was air-dried swabs taken from the glans of the penis in a total of 153 patients aged between 19 and 87 years (average age 54 years) hospitalised in the Urological Department of the University Hospital, Erlangen, and from 50 healthy volunteers aged between 15 and 60 years (average age 31.7 years).

In all cases, at least 5 days had elapsed since the last sexual intercourse. In addition, urinary sediments obtained from the patients

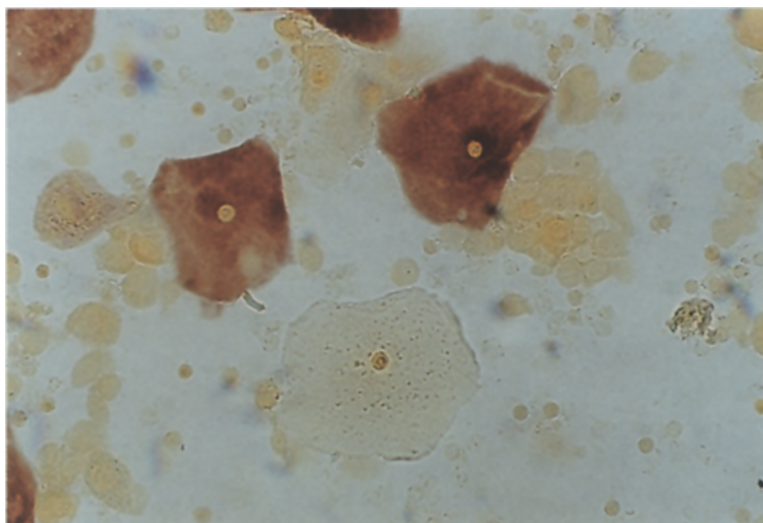


Fig. 1. Typical polygonal Lugol-positive, as well as unstained, superficial cells in a vaginal smear obtained from a healthy 20-year-old woman (Lugol-iodine staining, $\times 200$)

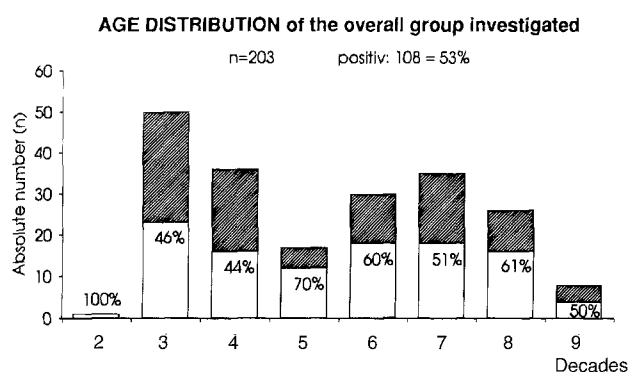


Fig. 2. Number of Lugol-positive cases in each age group investigated. (□) Positiv; (▨) negativ

and swabs of the oral mucosa in the volunteers were also investigated. Staining was carried out with diluted iodine solution as described by Wiegmann [5] and Merkel [2]. Only those cells that stained dark brown and contained a clearly recognizable unstained nucleus were defined as Lugol-positive.

Results and discussion

Glycogenated male squamous epithelial cells were found on the glans of the penis in 53% of the overall group of 203 men aged between 19 and 87 years (Fig. 2). In almost all of these cases (about 98%) the smear made from the penile swab contained at least a few polygonal nucleated epithelial cells measuring $70\mu\text{m}$ in diameter and showed an unequivocal positive Lugol reaction (Fig. 3). These cells could not be distinguished from the superficial or intermediate cells of the epithelium lining the female vagina, either cytomorphologically or by staining techniques (Fig. 1).

The frequency of glycogen-positive cases in patients with various urological diseases and in the healthy controls is shown in Fig. 6. In the group with urinary tract infections the percentage of positive cases (54%) was no higher than in the remaining patients. Since urinary tract

infections were always combined with other diseases, some patients have been counted more than once. The somewhat smaller percentage of positive cases among the volunteers is possibly due to the fact that the number of cells in the smears prepared by the volunteers themselves, was slightly smaller as compared with the urological patients.

In order to investigate more closely the influence of urinary tract infections on the glycogen content, the Lugol-positive hospital patients with urethritis were compared with those with no such infection. Only 13 of the 89 glycogen-positive urological patient had a urinary tract infection, while in 76 of the Lugol-positive cases no such infection was present. Conversely, the presence of urinary tract infection (24 cases) had no significant influence on the finding of Lugol-positive epithelial cells of the glans of the penis (Fig. 8).

In 12 out of a total of 46 volunteers studied, glycogenated cells were found in the urinary sediment (Fig. 9). In addition to a few polygonal squamous epithelial cells presumably stemming from the tip of the urethra, we found mainly ovoid or round nucleated cells similar in shape and size to the transitional cells of the male urinary tract. A number of these cells show an unequivocal of positive reaction to Lugol's iodine staining (Fig. 4).

In our view they can barely be distinguished cytomorphologically from vaginal cells from deeper epithelial layers which may also contain glycogen.

We also found typical polygonal glycogen-containing squamous cells in a number of oral swabs from healthy volunteers (Fig. 5). Lugol-positive findings were obtained in 18 out of the total of 47 oral swabs investigated (Fig. 10).

On evaluation of the penile swab preparations, the positive cells were counted to determine the percentage of glycogenated epithelial cells on the microscope slide.

In the large majority of the cases showing a positive Lugol's reaction, the percentage of glycogenated cells was less than 10%. In 16 cases, however, these cells accounted for up to 30%, and in single cases 60–70% of the nucleated epithelial cells clearly stained brown with the Lugol's method (Fig. 7).

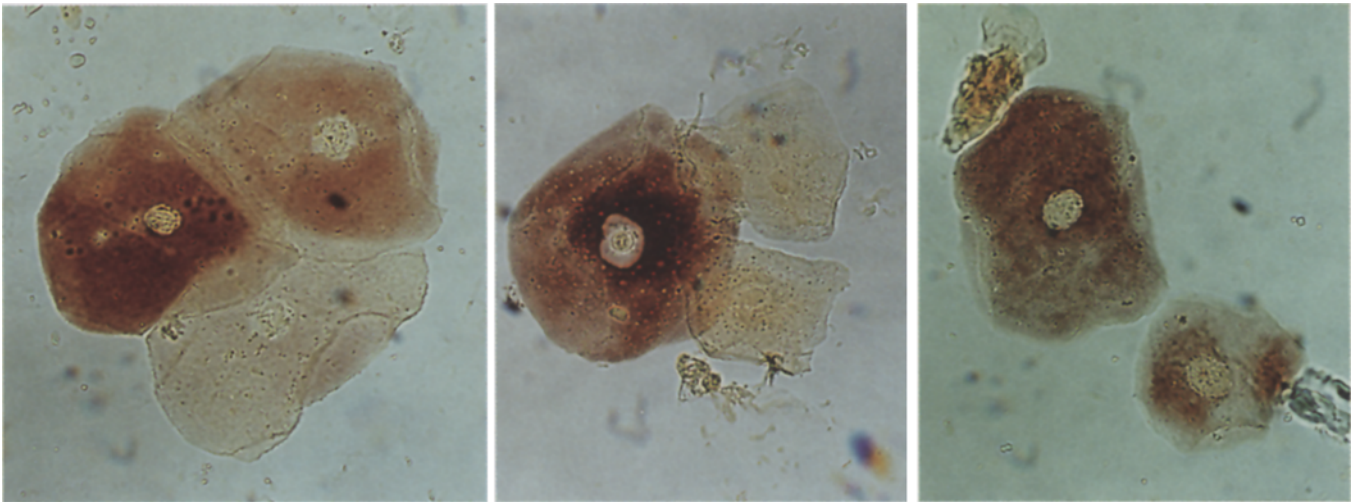


Fig. 3. Lugol-positive, nucleated squamous epithelial cells in penile swabs (glans penis) obtained from volunteers with no urethritis, whose last sexual intercourse took place at least 5 days previously (Lugol-iodine staining, $\times 400$)

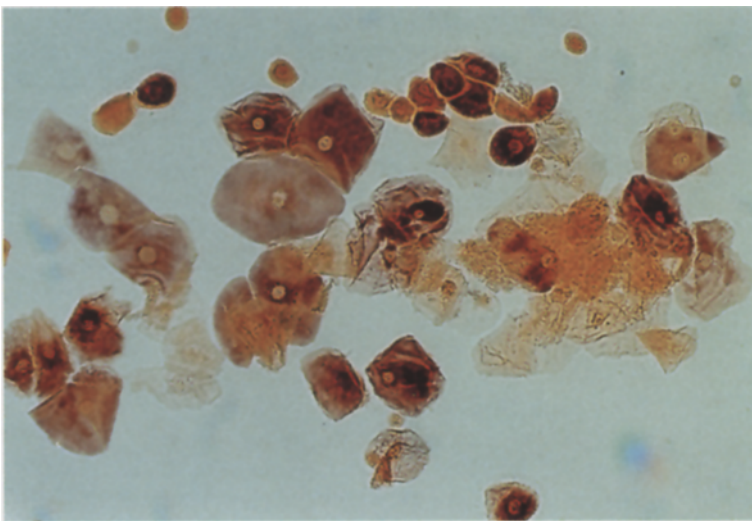


Fig. 4. Urinary sediment (27-year-old healthy male volunteer): mainly ovoid or round nucleated cells in addition to a few polygonal squamous epithelial cells. A number of the cells show a clear positive reaction to Lugol's iodine staining ($\times 150$)

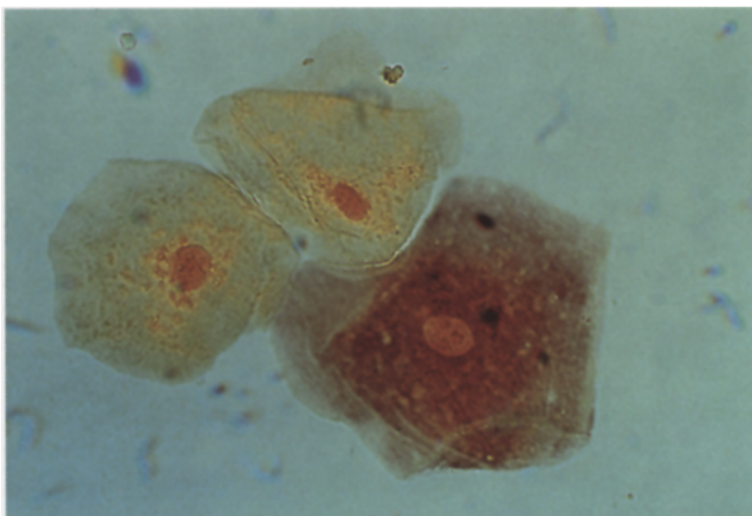


Fig. 5. Polygonal glycogen-containing squamous cell in an oral swab from a 25-year-old healthy volunteer (Lugol-iodine staining, $\times 400$)

	n	P +	
urological diseases	156	89	57 %
concrements	10	6	60 %
tumor	67	38	57 %
urinary tract infections	24	13	54 %
other	55	32	58 %
healthy volunteers	50	19	38 %

Fig. 6. Frequency of glycogen-positive cases (P+) in the patients with various urological diseases and in the healthy controls

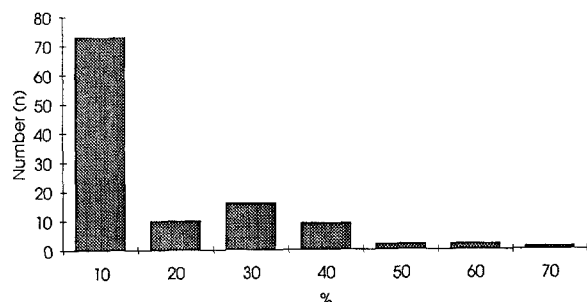


Fig. 7. Percentage of glycogenated cells in the penile swab preparations (overall group investigated, $n = 203$)

Conclusions

In summarizing the results obtained, we found a number of possibilities for error in the interpretation of Lugol-stained cellular material:

1. Glycogenated male squamous epithelial cells were found on the glans of the penis in more than 50% of the cases studied, even in healthy young volunteers with no urinary tract infection.
2. In the majority of cases, differentiation of glycogenated male epithelial cells from vaginal cells was not possible by cytomorphology or by staining.
3. Glycogenated epithelial cells were also found in the oral mucosa of adults, as well as in the mucosa lining the male urinary tract.
4. Our results show that urinary tract infections have no appreciable influence on the glycogen content of the male epithelial cells.

	P +	P -	
I +	13	11	24
I -	76	53	129
	89	64	153

Fig. 8. Lugol-positive penile glans swabs (P+) obtained from hospital patients with urethritis (I+) compared with those with no urinary tract infection (I-)

	P +	P -	
U +	9	3	12
U -	7	27	34
	16	30	46

Fig. 9. Frequency of cases with glycogenated cells in the sediments of male urine (U+) in male volunteers with Lugol-positive (P+) and Lugol-negative (P-) penile swabs

	P +	P -	
O +	13	5	18
O -	4	25	29
	17	30	47

Fig. 10. Frequency of cases with glycogenated epithelial cells in oral smears (O+) in healthy volunteers with Lugol-positive (P+) and Lugol-negative (P-) penile swabs

On the basis of these findings, the Lugol's method can no longer be assumed to prove the presence of vaginal cells in penile swabs. More use will now have to be made of modern methods of DNA investigation, which will certainly become more important in the future for the identification of vaginal epithelial cells.

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