Yeasts in Urine and Saliva of Diabetic and Nondiabetic Patients

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In recent years an increased incidence of fungus disease has been reported throughout the world.[1] It is likely that this development is particularly due to the introduction of broad spectrum antibiotics and is apparently a result of a temporary destruction of the harmless and useful bacterial flora which normally hold the fungus flora in check. However, certain primary diseases as diabetes mellitus seem able to favor per se the growth of various fungi, particularly in patients whose diabetes is poorly controlled.[2,3] In rabbits and mice in the acutely toxic state of alloxan diabetes, fulminating mucormycosis can be produced.[4,5] Moreover, it is a well-known fact that yeasts are seen frequently in urine sediments of diabetic patients and that there are sometimes diabetic patients with vaginitis due to Candida albicans ("Monilia"). Even a pyelitis due to Candida was recently seen in a diabetic patient in this clinic (case 2,568, female, aged forty-five, diabetes for thirty-six years). Finally, the skin of the diabetic appears to be more easily infected with Candida than that of the nondiabetic. After a primary incidence on the vulvar mucous membranes a possible later extension to the skin can occur.[6]

The present study was undertaken in an attempt to ascertain the incidence and types of living, potentially pathogenic yeasts in the urine and saliva of diabetic patients as compared with those of nondiabetics and to relate the incidence to the age and sex of patients, as well as to the presence or absence of glucosuria. It was hoped further to determine whether glucose utilization by yeasts could be of sufficient degree to produce a clinically significant decrease in the amount of sugar in the urine.

MATERIAL AND METHODS

Fresh urine specimens of 200 diabetic and 100 nondiabetic fasting patients were examined for the incidence of yeasts. Since there were fifty patients without glucosuria among the diabetics, a comparison was possible between 150 patients with glucosuria and 150 patients without glucosuria and between diabetics with and without glucosuria. In addition, the saliva of 160 diabetic and eighty nondiabetic fasting patients was examined for yeasts.

The culture of the yeasts was done by a recently described new method[7] enabling a selection of Candida species that may be pathogenic in man. By means of a relatively high acidity of the culture medium and cultivation at a temperature of 37° C., one can exclude the growth of nonpathogenic yeasts, occasionally occurring in the mouth, vagina, intestines and on the skin. Since the nonpathogenic yeasts are harmless saprophytes and are unable or only slightly able to grow within the human body, they are without importance for our investigation. Moreover, with this simple method one can avoid the usual time-consuming procedure of yeast identification which involves the use of up to thirty-eight different sugars and other compounds.

Using raw potatoes, boiled cold water, concentrated hydrochloric acid and concentrated lactic acid, two culture media were prepared for the differentiation of various Candida species. In one sterile Erlenmeyer flask were placed 100 cc. of water and 3 cc. of hydrochloric acid (assay: 37.2 per cent HCl). In the others were placed 100 cc. of water and 10 cc. of lactic acid (assay: 85 to 90 per cent $C_3H_6O_3$). To each flask were added raw potatoes, freshly peeled, washed and diced, in such quantity that the diced potatoes were covered by the liquid. The diced potatoes were kept in the flasks for two to three hours. The fluid was then decanted and the potatoes distributed to sterile test tubes filling up approximately one-third of each. To one-half of the test tubes, water and hydrochloric acid (*p*H 1-2), and to the other half, water and lactic acid (*p*H 3), were added so that fluid stood about 1 cm. over the potato substrate. Usually these test tubes, plugged with sterile cotton, can be used immediately. The high acid concentration prevents growth of bacteria and also avoids significant *p*H changes before

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the inoculation as well as during the cultivation at 37° C. However, to exclude any possibility of contamination, the test tubes were placed in an incubator at 37° C. before the inoculation in order to detect and eliminate any whose fluid showed turbidity in forty-eight hours.

In the examination of saliva for yeasts, a large drop of each sample was placed into each of two potato-containing test tubes, one containing hydrochloric acid, the other lactic acid. In the urine studies, 1.0 cc. and 0.1 cc. respectively of each specimen were in the same way put in the potato-containing test tubes, one with hydrochloric acid and the other with lactic acid. A microscopic examination of the sediment was done with all urine specimens for later comparison with the culture results. The incubated test tubes were checked macroscopically and microscopically after two days' incubation at 37° C. Growth of yeasts is macroscopically shown by a homogenous turbidity of the liquid medium as opposed to occasional flocculation due to saliva particles or lactic acid. Particular points regarding the identification of the yeasts can be seen in table 1. In cases of positive urine cultures, a "clean" voided specimen was obtained a few days later. With those specimens the same examination for yeasts was done.

To determine the glucose consumption of yeasts in urine containing glucose, 4 cc. of diabetic urine or normal urine (to which glucose was added in different concentrations) were inoculated with 1 cc. of a culture suspension (*p*H 3) of Candida albicans (density 97:3) in water and lactic acid. After twenty-four hours at room temperature, Benedict's test[8] and the di-nitro-salicylate method[9] were carried out both with these urine specimens and with control urine specimens, not inoculated with yeast.

RESULTS

No significant difference was found in the incidence of yeasts in the saliva of diabetics and that of nondiabetics. In almost one-half of all patients, yeasts—mostly Candida albicans—were found independent of the age and sex of the patients and the type or degree of diabetes (figure 1). Similar results in nondiabetics have been obtained by other workers.[10,11,12,13]

However, there was a clear-cut difference in the incidence of yeasts in the urine of diabetic and nondiabetic patients. On the other hand, this difference held only between nondiabetics and those diabetics with glucosuria (figures 2, 3 and 4). Patients with well-controlled dia-

TABLE 1

Growth of yeasts at 37° C.

Species		Evaluation of Growth: In potato substrate with hydrochloric acid (*p*H 1-2)	Evaluation of Growth: In potato substrate with lactic acid (*p*H 3)
Candida albicans*	Macroscopically:	Positive No film on the surface.	Positive No film on the surface. Only a ring appears.
	Microscopically:	Round or short oval budding cells occurring singly, in pairs or clusters. Usually a pseudomycelium is produced.	
Candida krusei	Macroscopically:	No growth	Positive A thin, dull, creeping film is formed on the surface.
	Microscopically:	———	Usually elongate or less commonly, short oval cells occur singly, in pairs or chains. Occasionally pseudomycelium is produced.
Candida tropicalis	Macroscopically:	No growth	Positive
Candida pseudotropicalis			No film on the surface. Occasionally a ring is observed.
Candida utilis			
Candida parapsilosis			
	Microscopically:	———	Short oval to oval budding cells, mostly in clusters. Sometimes pseudomycelium.

* Torulopsis glabrata also grows in HCl. However, it does not form a pseudomycelium and shows a smaller size of cells. This species was not found in the present study.

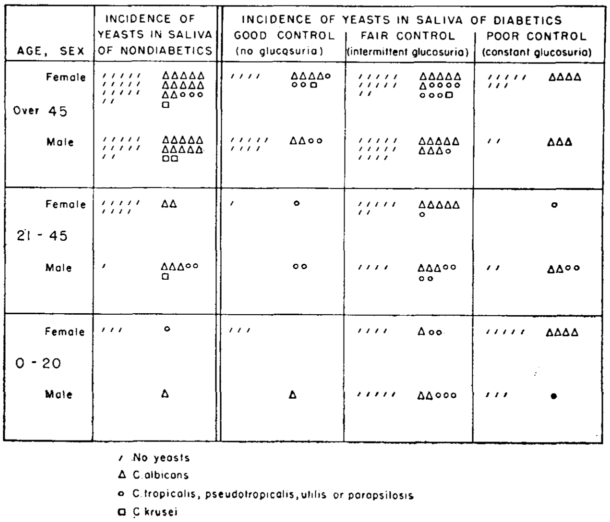

FIG. 1. Incidence of yeasts in saliva.

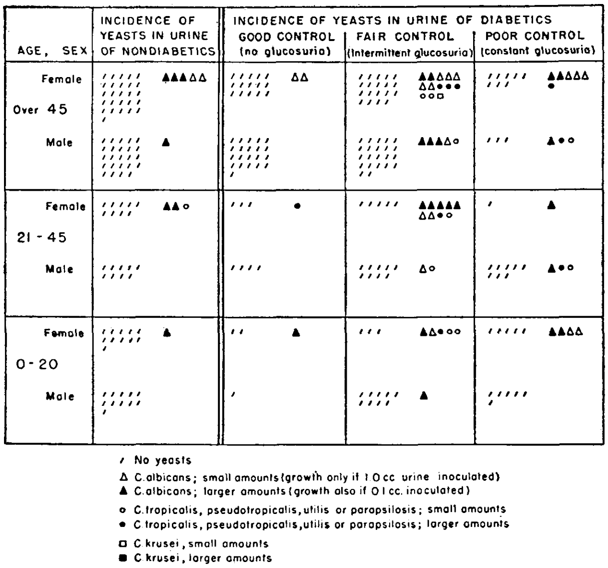

FIG. 2. Incidence of yeasts in urine.

betes and without glucosuria showed the same incidence of yeasts as nondiabetics and only one-fourth that of patients with glucosuria (figure 4). All yeasts isolated were species of Candida ("Monilia"), preponderantly Candida albicans (figure 2). There was no difference in the incidence of yeasts in diabetics with "fair" control (intermittent glucosuria) and those with "poor" control (constant glucosuria) (figure 2). Apparently the deciding factor favoring the growth of yeasts in urine is glucose alone, independent of its amount. There was also no significant difference in the various age groups. However, female diabetic and nondiabetic patients

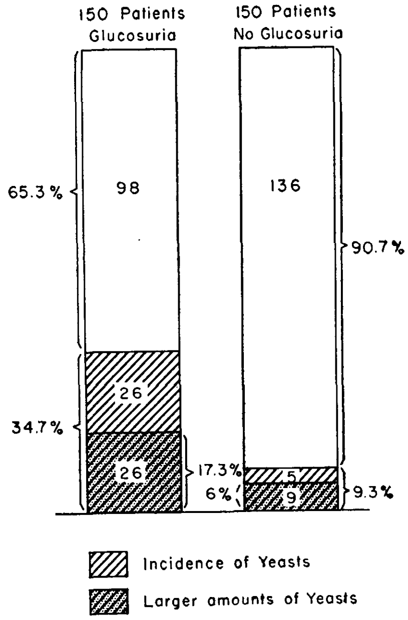

FIG. 3. Incidence of yeasts in urine of patients with glucosuria and without glucosuria.

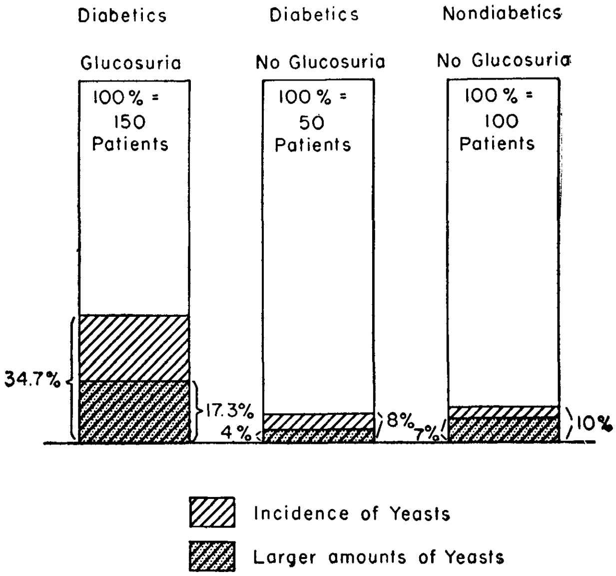

FIG. 4. Incidence of yeasts in urine of diabetics with glucosuria and diabetics and nondiabetics without glucosuria.

showed yeasts in a distinctly higher percentage than male patients, both diabetic and nondiabetic (figure 5). Examination of "clean" voided specimens (figure 6) yielded confirmation of our results in approximately 80 per cent. Therefore, one may suppose in these cases that yeasts occurred in the bladder as well as in the external urogenital tract. However, using the common

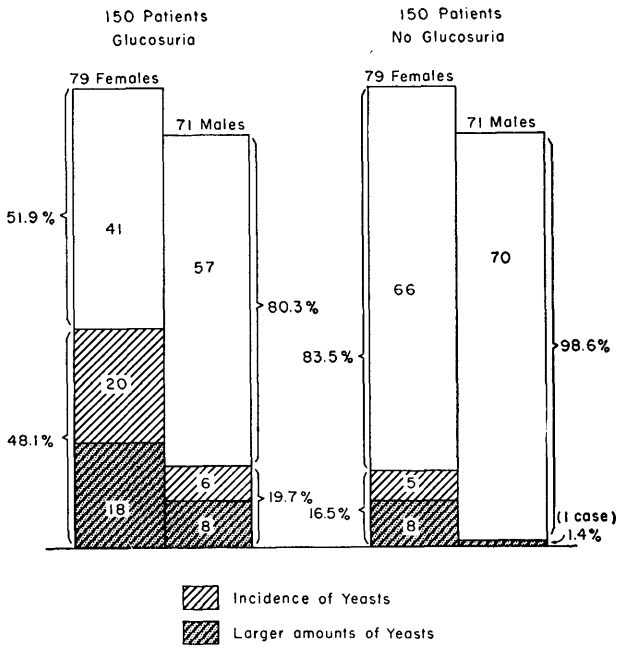

FIG. 5. Incidence of yeasts in the urine of female and male patients with and without glucosuria.

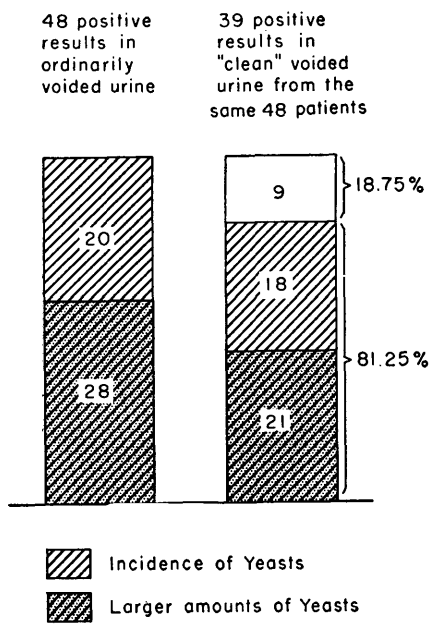

FIG. 6. Diabetic and nondiabetic urine (ordinarily voided) containing yeasts compared with "clean" voided specimens of the same patients.

microscopical examination of urine sediment, yeasts were recognized in only 10 per cent of the urine specimens which were later shown on culture to contain yeasts. This finding shows the possibility of overlooking yeasts by microscopic examination alone and demonstrates the sensitivity of the culture method used. Among patients in whom no growth of yeasts occurred in the potato substrate, there was no instance in which yeasts were seen in the urine sediment on microscopic examination.

TABLE 2

Effect of 24-hour growth of Candida albicans on concentration of glucose in urine

Case	Control urine not inoculated Glucose, per cent	Urine inoculated with Candida Glucose, per cent
1	1.0	0.6
2	1.9	1.4
3	3.4	2.8
4	3.0	2.6
5	0.6	0.5

The di-nitro-salicylate method (Sumner and Exton) was used. In each case the urine originally contained no yeasts. Starting the test, yeasts were inoculated into one-half of every specimen. These results were confirmed by the Benedict's test (no change was shown in the glucose concentration in Case 5).

As may be seen in table 2, even at room temperature, Candida may cause a significant loss of glucose from urine. Although the addition of toluene (5 cc. to 2 liters of urine) suppresses the growth of bacteria, it does not prevent the growth of yeasts in urine collected in a large bottle over a twenty-four-hour period at room temperature.

DISCUSSION

The present study has shown that yeasts are found much more frequently in urine containing glucose, especially in female patients. It is well known that bacterial urogenital infection is also more common in women than in men, due chiefly to Escherichia coli from the feces. The gastrointestinal tract is a reservoir for Candida as well.[14] Since yeasts prefer to grow on mucous membranes (e.g., those of the vagina), there seem to be two reasons for the greater incidence of yeasts in the urine of female persons: (1) easier infection of the urogenital tract with yeasts from feces, and (2) favorable conditions for growth of yeasts in the vagina. In diabetic women with glucosuria a third and probably most important factor for better growth is present: glucose in the urine.

With certain types of Candida found in the human body, the degree of fungus concentration is related to pathogenicity.[15] Therefore, in diabetic patients the treatment of vaginitis or other urogenital infections due to Candida requires a rigid control with complete lack of

glucose in the urine as the primary feature of therapy. One cannot expect success from local treatment of vaginitis as long as growth of yeasts continues in the bladder urine. Furthermore, a logical assumption would be to expect an increased incidence of Candida and greater danger of fungus infection in patients with glucosuria who are receiving antibiotics. Therefore, strict attention should be given to the attainment of a sugar-free urine in these patients as well.

Occasionally the glucose consumption by Candida in diabetic urines can amount to several grams within twenty-four hours and can lead to an undervaluation of the real glucose excretion in urine collected by the patient for one day. A report regarding substances able to prevent growth of yeasts in such urine will be made later.

SUMMARY

1. Urine specimens of 200 diabetic and 100 nondiabetic persons as well as the saliva of 160 diabetic and 80 nondiabetic individuals were examined for the incidence of those yeasts which are potentially pathogenic in men. A new method of identification was used.

2. Almost half of the diabetic and nondiabetic patients showed yeasts in the saliva without any significant differences between the two groups.

3. The incidence of yeasts in the urine of patients with glucosuria was almost four times that in patients without glucosuria without regard to whether the patients without glucosuria were nondiabetics or patients with well-controlled diabetes. Furthermore, yeasts occurred in the urine of diabetic and nondiabetic female patients much more commonly than in male patients.

4. The growth of Candida species in glucose-containing urine kept at room temperature reduced the concentration of glucose.

SUMMARIO IN INTERLINGUA

Saccharomycetes In Le Urina De Patientes Diabetic E Nondiabetic

1. Specimens de urina ab 200 diabeticos e 100 nondiabeticos e specimens de saliva ab 160 diabeticos e ab octanta nondiabeticos esseva examinate pro le incidentia del typos de saccharomycete que es potentialmente pathogene in humanos. Un nove methodo de identification esseva usate.

2. Quasi un medietate del diabeticos e del nondiabeticos habeva saccharomycetes in lor saliva. Le differentia inter le duo gruppos non esseva significative.

3. Le incidentia de saccharomycetes in le urina de patientes con glucosuria esseva quasi quatro vices illo in le urina de patientes sin glucosuria, sin reguardo a si le patientes sin glucosuria esseva nondiabeticos o patientes con diabete de forma ben regulate. In plus, saccharomycetes occurreva in le urina de patientes diabetic e nondiabetic de sexo feminin multo plus communmente que in patientes de sexo mascule.

4. Le crescentia de species de Candida in urina a contento de glucosa, quando mantenite al temperatura normal de interior, reduceva le concentration de glucosa.

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