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Quantitative Glucose Measurement in Urine as Screening Test for Urinary Tract Infections

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Summary. Subject: In urological patients the value of quantitative glucose measurement in urine as a screening test for urinary tract infections is examined.

Method: Glucose content, species and concentration of microorganisms in the urine of 334 urological patients were analysed and the bacteriological findings compared with the result of chemical measurement.

In vitro, the dependence of bacterial growth on different properties of urine was examined.

Results: A diagnostic decrease in glucose concentration was found in only about 48% of the infected urines. The result of the glucose test is influenced unfavourably by numerous factors which cannot be standardized.

Conclusions: In its present form, this method is not yet suitable for use as a reliable screening test for urological patients.

Key words: Urinary tract infection, screening test for bacteriuria, glucose content.

Introduction

To make a correct diagnosis of urinary tract infections it is necessary to demonstrate a significant number of pathogenic bacteria. The most reliable results are obtained by direct bacteriological cultures. Standard bacteriological techniques, however, are time consuming and labour intensive which makes them less suitable for screening purposes. Therefore, it is easier to use the so-called indirect chemical methods in which urinary bacteria are identified by metabolic products and processes of the bacteria. Unfortunately, in the indirect chemical methods employed up to now, such as the Griess-test, triphenyltetracolium chloride (TTC)-test and catalase reaction the number of false negative findings of about 50% is too high for them to be used as the sole screening test.

A new test developed by Scherstén and Fritz in 1967 is based upon the observation that the urine of healthy men contains more than 2.0 mg glucose % and the fact that many bacteria have the ability to metabolize glucose (13). A urinary glucose concentration of less than 2.0 mg% indicates a urinary tract infection.

Scherstén and Fritz tested this method in 324 first morning urines obtained from clinically healthy patients. They found such a small number of false positive and false negative results that the method was simplified by the use of a test paper. Their results have been confirmed by other authors (5, 6, 8, 12).

Statement of the Problem

It was the aim of our investigations to examine the diagnostic value of quantitative glucose measurement in the infected urine of urological patients. At the same time it was decided to investigate to what extent pH-value and glucose concentration can influence the results.

Material and Methods

a) Clinical Investigations. Urine was collected from 334 urological patients who had not been treated with antibiotics after careful cleaning and disinfection of the periurethral region. In male patients this was done as a rule by the midstream technique and in females exclusively by catheter. One half of each specimen was investigated by standard bacteriological techniques. Glucose concentration was determined spectrophotometrically by the hexokinase reaction(3) in the remaining portion of the specimen.

When the micturition interval was 6 h and more, the bacteriological investigations and determina-

tion of glucose concentration were carried out immediately. When the interval was shorter, urine of both groups was reincubated in a 37° C water bath under sterile conditions up to the six hours limit.

For the purpose of evaluating the results fasting and non-fasting groups were differentiated. When the glucose content in urine was above 30 mg % the urine was rejected as potentially diabetic urine.

b) Investigations in vitro. Urine was obtained from healthy people, pooled and filtered. In the first experimental series pH value was adjusted to pH 6.9, glucose concentration to 13.7 mg %. The urine was inoculated with a strain of Escherichia coli isolated from an infected urine, in a concentration of 3×10^6/ml and incubated at 37° C for 8 h. Glucose concentration and bacterial count were determined at one hour intervals.

In the second series the urinary pH-value was 5.0 and the initial concentration of viable organisms 7×10^6/ml E. coli. All other conditions were the same. The concentration of glucose and microorganisms was determined every hour as before.

During the third experimental series the urine pH-value was adjusted to 6.9 and the glucose concentration to 6.7 mg %. Different concentrations of E. coli between 10^1/ml and 10^9/ml were added to several specimens and the period of time for the complete destruction of glucose at 37° C was determined.

The glucose concentration was measured spectrophotometrically using the hexokinase reaction. For the enumeration of viable counts during the clinical investigations and the in vitro experiments we employed the Koch pour plate method using Brom-Thymolblau-Lactose-Cystinagar (E. Merck, Darmstadt) and the Placon-counter. For the evaluation of the in vitro experiments we considered the average of 3 parallel measurements.

Results

a) Clinical Investigations. Glucose concentration in the urine of fasting and non-fasting groups showed no significant differences. The urine of fasting patients showed a higher average glucose concentration of 8.6 mg % than the urine of non-fasting patients with an average of 7.4 mg %. Therefore, the results of both groups could be summarized.

270 of the 334 investigated urines were sterile (Table 1). We called those urines sterile which showed either no bacteria or bacteria which by virtue of species and concentration were considered to be contaminants.
259 sterile urines had a normal glucose concentration of more than 2.0 mg %. In 11 cases subnormal values of less than 2.0 mg % were measured. Approximately 96 % were therefore correctly designated sterile by the criterion of Scherstén and Fritz. About 4 % of the results were false positives (reduced glucose concentration in sterile urine).

In the 64 infected urines the glucose concentration was much less reliable as an indicator of the presence of infection. It must, however, be taken into account that for reasons which shall later be explained, viable counts of 10^3/ml were considered as significant bacteriuria.

About 52 % of the infected urines had normal glucose concentrations above 2.0 mg % (Table 2). Thus, the result would have been false negative

Table 1. Bacteriological findings in 334 urines

Pathogenicity of microorganisms	Concentration of microorganisms/ml	Number of urines
No bacteria	-	242
Non pathogenic bacteria	$< 10^3$	13
Non pathogenic bacteria	$> 10^3$	13
Pathogenic bacteria	$< 10^3$	2
	Total	270
Pathogenic bacteria	$10^3 - 10^4$	22
Pathogenic bacteria	$10^4 - 10^5$	11
Pathogenic bacteria	$> 10^5$	31
	Total	64

Table 2. Correlation between concentration of microorganisms and content of glucose in 64 infected urines

Microorganism/ml	Number	Urines investigated: Content of glucose < 2 mg%	Content of glucose > 2 mg%	% True positive	% False negative
10^3 - 10^4	22	7	15	31.8	68.2
10^4 - 10^5	11	6	5	54.5	45.5
> 10^5	31	18	13	58.1	41.9
> 10^3	64	31	33	48.4	51.6

Table 3. Correlation between species and concentration of microorganisms and the content of glucose in 64 infected urines

Species	Content of glucose mg %	Concentration of microorganisms/ml in urines investigated: 10^3-10^4	10^4-10^5	> 10^5	Total
Escherichia coli	< 2	3	2	9	14
	> 2	4	1	4	9
Proteus species	< 2	3	3	3	9
	> 2	5	1	2	8
Pseudomonas aerug.	< 2	-	-	-	-
	> 2	3	2	4	9
Streptococcus faecalis	< 2	1	-	2	3
	> 2	3	-	1	4
Enterob. aerogenes	< 2	-	1	3	4
	> 2	-	-	-	-
Staphyloc. aureus	< 2	-	-	-	-
	> 2	-	1	-	1
Mixed flora	> 2	-	-	1	1
	< 2	-	-	2	2

in 52% of the cases. As shown in Table 2, a significant relation between viable counts and the number of true positive findings can be recognised. Whilst in the range of 10^3 - 10^4 microorganisms/ml only 32% of the cases had a true positive result, in the so-called significant bacteriurias with more than 10^5 microorganisms/ml about 58% showed true positive values.

The evaluation of the results according to species, viable count and glucose concentration (Table 3) shows that E. coli was the most frequent cause of the urinary tract infections observed in our patients. For this species quantitative glucose measurement was the best method of evaluation. The results showed 14 true positive and 9 false negative measurements. For most of the other species found - as far as can be concluded from this small number - the number of false negative findings was even higher. In infections with Pseudomonas aeruginosa no subnormal glucose concentration, i.e. no positive result could be found.

b) In Vitro Investigations. In the first group of these investigations multiplication rate and glucose consumption of E. coli was examined at a favourable urinary pH. After a two hours lag phase the logarithmic growth of bacteria could be observed. (Fig. 1). The glucose concentration in urine fell correspondingly and reached zero after 7 hours. The greatest utilisation of glucose took place between the 6th and 7th hours. As a result of the complete

glucose consumption after the 7th hour at least one growth factor fell to below the minimum level required for multiplication, or a toxic waste product accumulated to inhibitory levels. Thus, the organisms could no longer generate and changed to the stationary phase. Under the test conditions chosen the mean generation time of the test strain was about 50 min.

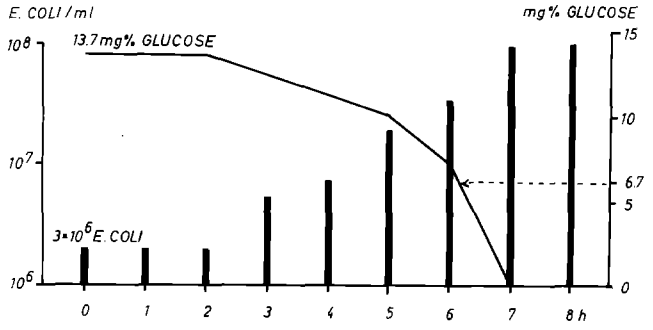

Fig. 1. Glucose consumption by E. coli at pH of 6.9

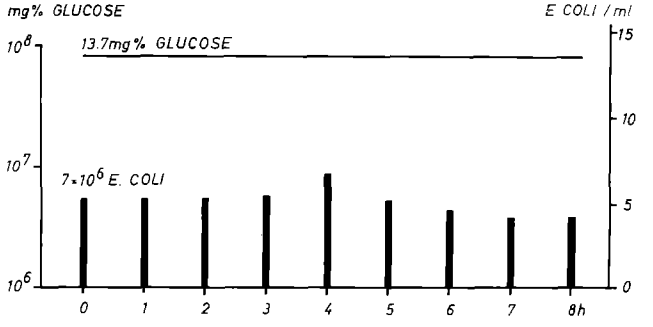

Fig. 2. Glucose consumption by E. coli at pH of 5.0

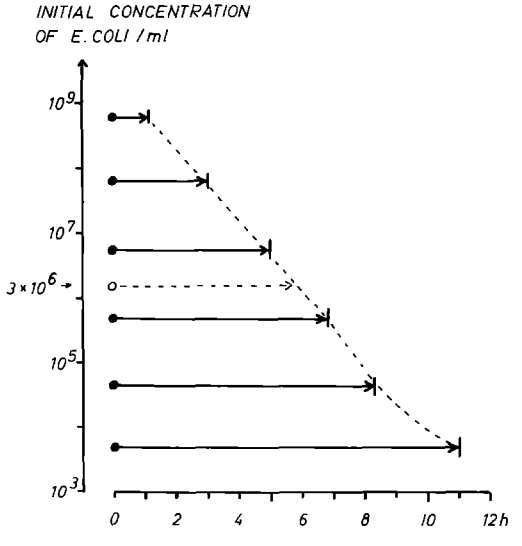

Fig. 3. Time needed for the complete utilisation of glucose by varying concentrations of bacterias (initial glucose concentration 6.7 mg %, pH 6.9).

In the second group, at urinary pH 5.0 for the growth of E. coli, the mean generation time increased to more than 240 min. The death rate balanced the growth rate (Fig. 2), so that the concentration of bacteria and glucose remained nearly unchanged during the total observation time of 8 h.

The results of the third group in which the influence of initial bacterial concentration and incubation time on glucose consumption was examined showed clearly that glucose utilization becomes more rapid with rising initial bacterial concentration. Complete consumption of glucose within the 6 hours of the clinical studies was reached under optimal conditions if the initial bacterial concentration was at least 10^6/ml. With a bacterial concentration of 10^5/ml this value could be achieved only after 8 h. In the interpretation of results the lag phase must be considered.

Discussion

Quantitative glucose measurement in infected urines showed that this method may indicate infections in urological patients. The decrease of urinary glucose concentration to less than 2.0 mg % served as the criterion of infection. This limit was reached in 48% of infected urines, but in only 4% of sterile urines.

The low incidence of false positive results (4%) in sterile urines is probably acceptable for a simple screening test but the large number of false negative results in infected urine (52%) are unacceptable and would suggest that this technique is unsuitable as the sole screening test for urinary infection. Obviously, the high number of false negative results has in part been caused by the fact that we have considered a bacterial concentration of 10^3/ml as a significant urinary infection. We believe, however, that this criterion is acceptable and is indeed necessary as the urines could be examined and cultivated immediately. Urine from patients with chronic urinary infections in which the bacterial concentration was 10^3/ml would usually have shown a bacterial concentration of 10^5/ml if cultured after a delay.

The real reason for the great number of false negative findings is undoubtedly the effect of the environmental conditions on bacterial glucose consumption in urine. The details of the clinical and experimental results indicate that this phenomenon is modified by several factors: 1. the ability of microorganisms to utilise glucose; 2. the metabolic activity of bacterial population; 3. the initial concentration of glucose; 4. the initial concentration of organisms and 5. the incubation time.

It is apparent that there is species variation in the ability of bacteria to utilise glucose. Pseudomonas aeruginosa metabolises glucose so slow-

ly that the incubation time of 6 h is not long enough to observe significant glucose consumption.

As was shown by the experimental investigations at different pH-values urinary glucose is rapidly utilised by E. coli during the logarithmic phase of growth. Can the bacterial growth rate be depressed by physico-chemical conditions, as for example by an unfavourable pH-value, high osmolarity or low temperature, or inhibited by antibacterial substances? Glucose would then not be utilised or metabolised more slowly and thus false negative results would be recorded.

The fermentation of high glucose concentrations needs, of course, more time than fermentation of lower glucose concentrations under the same conditions. Comparison of figures 1 and 3 shows, however, that the difference up to a glucose concentration of about 14 mg % is not very great. Higher glucose concentrations are, however, bound to lead to false negative results. It is thus not possible to use this method as a screening test for diabetics who are especially predisposed to pyelonephritis.

Our results suggest that the request of Scherstén and Fritz to investigate fasting patients only seems to be unnecessary. Other investigations (1, 4, 14) found, as we did, on the average higher glucose values in the urine of fasting patients than in postprandial urine.

Regarding the initial concentration of microorganisms it was shown that the period of time needed for the fermentation of glucose is shorter with increasing numbers of viable and glucose utilising organisms. Accordingly, true positive results were more often observed in patients with significant bacteriuria than in patients with a non-significant concentration of microorganisms. Higher concentrations of microorganisms in urine, i.e. more than 2×10^5/ml can, however, be observed by microscopy without recourse to complicated testing methods.

In principle, subnormal glucose concentrations can be achieved by the ability of each species to ferment glucose if urine was incubated for a sufficiently long period of time. Under optimal working conditions it is of no importance whether urine is kept in or outside the bladder during incubation time. If, however, aseptic conditions cannot be guaranteed for the in vitro incubation of the urine and if bacterial contamination is possible, one must confine the test to urine which has been kept in the bladder for 6 h. For the glucose test this demand excludes, however, a great number of non-hospitalised urological patients, especially those who are consulting the doctor for increased frequency of micturition.

Moreover, two further reasons for mistakes must be considered. If the urine has to be re-incubated because it was not kept in the bladder for 6 h and if it is necessary to keep it at first in the refrigerator, an estimated lag phase of bacterial growth must be added to the additional incubation period. Moreover, additional glucose utilisation by the leucocytes present in urine must be taken into account as has been demonstrated in pyogenic meningitis (9).

This glucose consumption can hardly be calculated exactly since it depends on the number and activity of the phagocytic leucocytes present.

Of all the factors influencing the glucose content of urine, only the incubation time of urine depends on the mode of investigation and can thus be influenced by the investigator. After an incubation period of 6 hours subnormal glucose values, which depend on the bacterial metabolism, can be found only under optimal conditions as is shown by the experimental results. The great number of false negative results observed during the clinical investigations indicates that the favourable conditions necessary for a reliable result from the glucose test are quite rare. Thus, with this method the determination of glucose content in urine hardly seems to provide the urologist with a reliable new screening test for urinary tract infections.

Theoretically, better results could be achieved with longer incubation periods. This problem is still being examined in current investigations.

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