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The influence of pH and urine composition on urease enzymatic activity in human urine

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Summary. It is reasonable to assume that the rate of pH increase in urine induced by urease-producing microorganisms is one of the factors which determine whether crystallisation with subsequent stone formation will occur or not. To evaluate how the time needed to increase urine pH varies between different urine samples and how it depends on urine composition, a standardised amount of urease was added to different human urine samples. The incubations were performed in a pH-stat. This allowed simultaneous study of how urease enzymatic activity depends on urine pH and how it varies between different urines. The enzymatic activity was found to be negatively correlated to urine pH and to vary between different urines. The rate of the pH increase varied markedly between different urines. Small pH increases depended on the native urine pH and urease enzymatic activity. Higher pH increases up to the levels of phosphate crystallisation depended more on urine phosphate, the major urine buffer. The results presented show that urine composition influences the urease-induced pH increase. This might have clinical implications.

Key words: Urease activity – Urine pH – Urine crystallisation – pH-stat

There are many causes behind urinary tract stone formation. Endogenous variations in urine composition are one, and exogenous factors such as urinary tract infection are another. Stones can, however, also form in sterile urine of, as far as can be determined, normal composition. Stones composed of magnesium ammonium phosphate (MAP) form secondary to a urinary tract infection with urease-producing microorganisms but such stones also occur without any detectable infection [9]. An infection with unrease-producing microorganisms can, on the other hand, be present in the urinary tract for long periods of time without stone formation. This implies that urine from different individuals varies in susceptibility to the impact of urease. The results of previous experiments support this clinical observation [7]. Urease-induced crystallisation and stone formation involve several consecutive but interrelated steps. One important factor is time. It appears reasonable to assume that urine outflow, at least locally, must be delayed for urease to increase the urine pH to a level where crystallisation occurs. The time needed for urease to increase pH depends on the urease activity of the colonising microorganism and on the urine composition. Urine buffer capacity must thus be of importance. Up to now, no data have shown that the enzymatic activity varies between different urines. There are, however, few studies which have addressed this question and urine contains both potential promoters and inhibitors of urease enzymatic activity [8]. This study was undertaken to measure the time it takes for a standardised amount of urease to increase the pH in urines from different individuals, and to determine whether urease enzymatic activity varies in urines from different individuals.

Materials and methods

In a pre-assay, we compared urease enzymatic activity estimated by manual sampling and conventional ammonium ion determination with the results obtained by automatic pH titration using a pH-stat [3, 11].

Pre-assay

To 10-ml aliquots of urea solutions (31.2 g urea $\cdot$ 100 ml^{-1}), with three different buffer capacities [0.01 M tris (hydroxymethyl)aminomethane (TRIS), 0.001 M TRIS and 0.9% NaCl], 0.165 units of urease was added. The activity of urease is such that 1 unit will liberate 1.0 μmol of ammonia from urea per minute at pH 7.0 and 25°C. The urease (E.C. 3.5.1.5) used was a urease buffer reagent from a diagnostic commercial kit (Sigma Chemical Company, St. Louis, Mo., USA, no: 640 A). The test was performed at 37°C. During the incubation, pH was kept constant by the pH-stat (VIT ammonium ion content 90, Radiometer, Copenhagen, Denmark). The titrator solution was 0.01 M HCl. Manual sampling for determination of ammonium ion content was performed from the

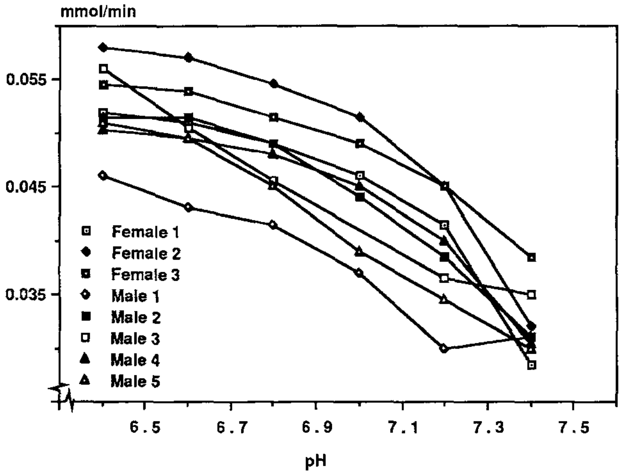

Fig. 1. The urease activity measured in urine from eights individuals at six different pHs (mmol $NH_4^+ \cdot min^{-1}$)

solution in the pH-stat at the start and after 2, 4, 6, 8 and 10 min. Each incubation was performed in duplicate.

Main experiment

Morning urines from eight healthy adults between 29 and 48 years old with sterile urines and with no history of stone disease were collected in sterile bottles. The urines were centrifuged at 1,500 *g* for 15 min at +4°C, according to Lanzalaco et al. [13], and then immediately stored at +4°C until the analysis was performed later the same day. The pH was measured and the urines were further analysed for their phosphate and ammonium ion content. Each urine specimen was divided into twelve 20-ml portions. All portions were incubated in the pH-stat with 46.4 units of urease and pH continuously recorded until the preset pH levels was reached. A highly purified urease preparation (60,000 units/88 mg) from Jackbeans (Sigma no. U0251) was used. A new urease solution was prepared each day (6.8 mg urease $\cdot$ 50 ml^{-1} 0.1 M TRIS). The pH-stat function was then activated, pH was kept constant at the preset pH level and the amount of HCl necessary to keep the pH constant automatically recorded. The titrator solution was 1 M HCl. The urine portions were studied in duplicate at six preset pH levels (set points): 6.4, 6.6, 6.8, 7.0, 7.2 and 7.4. The incubation took place at 37°C and the time to reach each preset pH level was noted. Each urine specimen was studied at all six preset pH levels during the same day. The ammonium ion concentration was analysed at the start of the incubation, when the preset pH level was reached and at the end of the incubation.

Control experiment

To check that the activity of the urease preparation did not alter from one day to another, 20 ml of a synthetic urine (pH 5.7) was incubated with the same concentration of urease as the other urines every day of the study. pH was then followed to 6.8, where it was kept constant and the amount of 1 M HCl necessary recorded.

Assay methods

The ammonium ion-ammonia concentration was measured as a sum using a slightly modified commercial kit (Sigma no. 640 A) [2, 4]. Urine *phosphate* was measured using a colorimetric phosphomolybdate method [14].

Statistical methods

The coefficient of correlation was computed using the Stat View 512$^+$ program (Brain Power, Calif., USA) using provisional means.

Results

The pH-stat allowed accurate assessment of urease enzymatic activity even in solutions with a high buffer capacity, in contrast to the method based on manual sampling from the reaction vessel. The urease activity was 1.3 $\mu mol \cdot min^{-1}$ in all three differently buffered solution measured with the pH-stat. Measured manually, it varied from 1.6 in the TRIS buffers to 1.9 $\mu mol \cdot min^{-1}$ in the unbuffered solution.

The initial (native) pH of the different urines was between 5.3 and 6.2. The phosphate concentration varied between 1.2 and 5.0 $mmol \cdot l^{-1}$, and the ammonium ion concentration between 11 and 37 $mmol \cdot l^{-1}$.

Urease activity

The urease activity decreased in all urines with increasing pH (Fig. 1). The coefficient of variation for the duplicate estimations was 2.2%. In 16 of the 48 duplicate estimations the results were exactly identical, which further highlights the high precision of the method. The interspecimen variation in urease activity was obvious (Fig. 1). The difference between the highest and the lowest activity in the eight urines, at the same pH, was close to 25%. The urease activity was, at all pH levels, negatively correlated to the initial ammonium ion concentrations of the urine specimens. The correlation was significant ($P < 0.05$) at pH 6.8 and 7.2 (Table 1). The ammonium ion concentration reached at different pH levels was negatively correlated to the urease activity at pH below 7.4. The correlation was significant ($P < 0.05$) at set points between pH 6.6 and 7.2 (Table 1). No correlation between urease activity and urine phosphate concentration was observed (Table 1).

Time to reach set point

A clear and strong interspecimen difference in the time to reach each preset pH level was noted. The time to reach pH 7.4 varied, for example, from 7.5 to 25 min and the time to reach set point 6.4 varied between 1.8 and 6.6 min (Fig. 2). The coefficient of variation between the duplicate estimations was low, 2.5%. The time to reach a certain pH may be anticipated to depend on the native urine pH, the buffer capacity and the urease activity in the particular urine. To investigate the importance of these three parameters, they were correlated to the time necessary to reach each preset pH level. The correlation coefficients of these relationships were then plotted as a function of the preset pH (Fig. 3–5). The *native pH* was of major importance for the time necessary to reach the lower pH levels 6.4 and 6.6. The correlation at these pH levels was significant ($P < 0.05$). The time to reach higher pHs was not correlat-

Table 1. The correlation between urine phosphate concentration ($[PO_4^{3-}]$), the ammonium ion concentration ($[NH_4^+]$) at the start and at the predetermined pH levels (set points) and the urease activity at each pH level

pH	Correlation of urease activity in urine with:					
	$[NH_4^+]$ at start	*P* value	$[NH_4^+]$ at set point	*P* value	$[PO_4^{3-}]$	*P* value
6.4	-0.37	NS	-0.51	NS	-0.14	NS
6.6	-0.58	NS	-0.71	<0.05	-0.19	NS
6.8	-0.71	<0.05	-0.78	<0.05	-0.43	NS
7.0	-0.61	NS	-0.59	NS	-0.28	NS
7.2	-0.73	<0.05	-0.69	<0.05	-0.37	NS
7.4	-0.38	NS	0.19	NS	0.28	NS

NS, Not significant

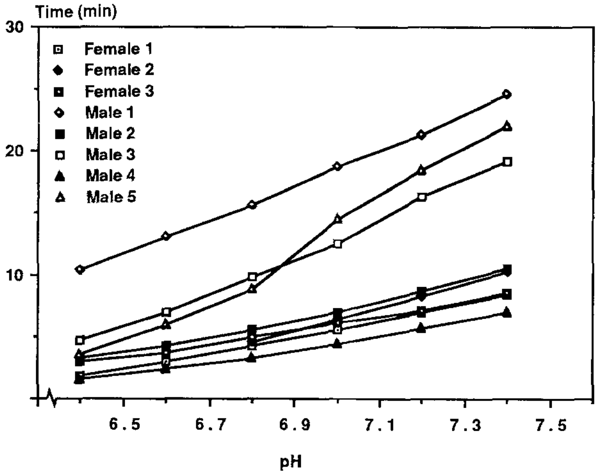

Fig. 2. The time taken to reach six different predetermined pH levels in urine from eight individuals after addition of a certain amount of urease

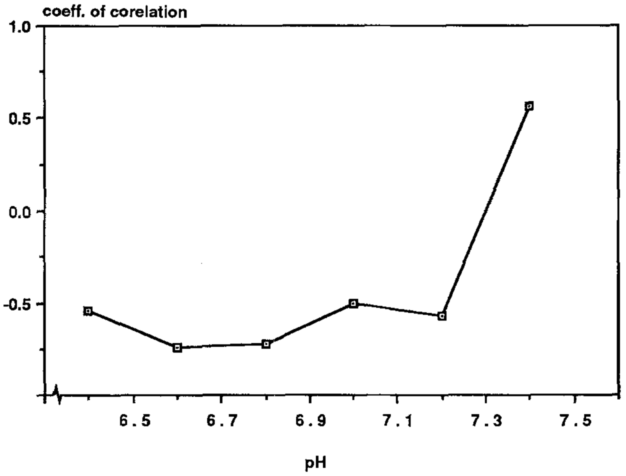

Fig. 4. The correlation between the *urease activity* and time necessary to reach six predetermined pH levels after inoculation of urine from eights individuals with the same amount of urease, plotted as a function of the preset pH

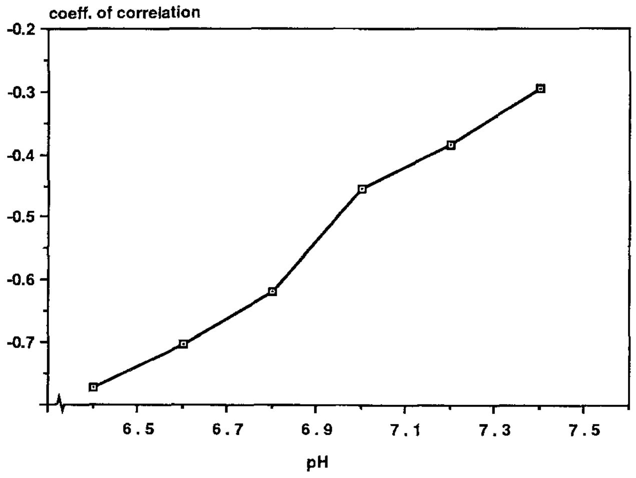

Fig. 3. The correlation between *native urine pH* and time necessary to reach six predetermined pH levels after inoculation of urine from eight individuals with the same amount of urease, plotted as a function of the preset pH

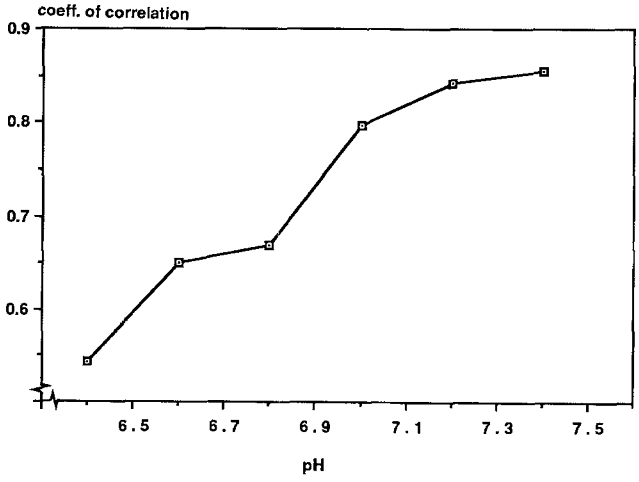

Fig. 5. The correlation between the *concentration of phosphate* and the time necessary to reach 6 predetermined pH levels after inoculation of urine from 8 individuals with the same amount of urease, plotted as a function of the preset pH

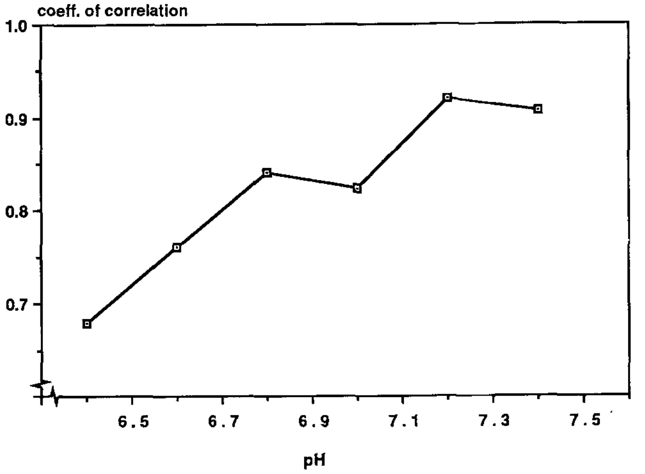

Fig. 6. The correlation between the *ammonium ion concentration* at each pH level and the time necessary to reach 6 predetermined pH levels after inoculation of urine from 8 individuals with the same amount of urease, plotted as a function of the preset pH

ed to the native pH (Fig. 3). The *urease activity* in each urine was also of importance for the time necessary to reach lower pH levels. The correlation between urease activity and the time necessary was thus significant for pH 6.6 and 6.8 ($P < 0.05$) (Fig. 4).

Urine phosphate concentration was related to the time it took to reach a certain pH, a relationship which was stronger at higher pH levels (Fig. 5). The correlation was thus significant at both pH 6.8 and 7.2 ($P < 0.05$), and at pH 7.4 it was even stronger ($P < 0.001$).

The ammonium ion concentration at the different pH levels strongly influenced the time to the predetermined pHs (Fig. 6). The relationship was stronger at higher pH levels. At the two highest pHs, 7.2 and 7.4, the correlation was strong and highly significant ($P < 0.01$). This strong correlation was secondary to the association between the phosphate concentration and the ammonium ion concentration at the set points. Phosphate is the major urine buffer and when the urine phosphate content is high more ammonia is necessary to increase the urine pH. The time for urease to increase urine pH will then be long and the ammonium ion concentration will be high.

Control experiment

The urease activity in synthetic urine showed a very small variation between the duplicate samples, with a coefficient of variation of 1.6%. The day-to-day coefficient of variation for the time necessary to reach pH 6.8 was also low, 1.0%.

Discussion

Urease enzymatic activity varied between different urine samples and was influenced by urine pH. The time necessary for a standardised amount of urease to increase urine pH was subject to a distinct variation between different urines. The factor of major importance was urine phosphate but the initial urine pH, urease enzymatic activity and urine ammonium ion concentration also exerted an influence. The pH-stat method has many advantages [3]. The enzyme reaction can be followed continuously with automatic recording, allowing both convenient and accurate determination. The results of the duplicate estimations with low coefficients of variation verified this. The observation that the urease activity determinations were not influenced by variations in the buffer capacity of the test solution was especially advantageous since human urines are subject to vast variations in buffer capacity. Urease activity was correlated to urine pH. It was highest at pH 6.4. At higher pH levels, it decreased and was reduced by almost 50% at pH 7.4. This may not entirely be a pH effect but may partly be due to inhibition by the product of the enzyme, i.e. ammonia, since urease activity is affected by product inhibition [12, 15]. The experimental model used did not separate these two effects. The rather distinct interspecimen variation in urease activity might be due to many factors. The ammonium ion concentration obviously played a role, as can be seen from the results in Table 1. At a physiological urine pH, 99% of urine ammonia is present as ammonium ions. Normally the urine ammonium ion content is said to vary between 14 and 43 $\mathrm{mmol \cdot 24 h^{-1}}$ with high levels after protein intake [5]. The initial ammonium ion concentration had an impact on urease activity according to our results, and may be one of the factors which explain the intersample variation in urease activity. There are also other potential promoters and inhibitors present in urine however [8]. The interspecimen variation is thus probably due to the combined effect of many urine components. Whether this interspecimen variation has any clinical implications remains to be clarified. If urease is present inside the urease-producing microorganism, it is less likely to be of great importance since the reaction then takes place in the intracellular millieu uninfluenced by urine composition. If, on the other hand, the enzyme is situated at the very periphery of the bacteria in their glycocalyx, as claimed by Nickel and McLean's group [16], urine composition may influence urease enzymatic activity. Calcium phosphate will start to precipiate in synthetic urine when the urine pH reaches 6.8 and MAP at a pH above 7.0 [6]. The time to reach these pH levels varied markedly between the different urines in this study. The factor of major importance was phosphate. Phosphate is the major urine buffer and the pH increase induced by urease has also previously been found to be related to the urine phosphate concentration [10]. There are, of course, other urine buffer systems like amino acids, hippurate, citrate and isocitrate [1]. They appear to be of less importance in this context. The phosphate concentration in urine does not alone decide the time it will take for urine to reach the pH of precipitation. The native pH, as well as urease activity and the ammonium ion concentration, is also of importance. The native pH is of importance for smaller pH increases. Urease activity is of importance for the time akten to reach somewhat higher pHs and the phosphate concentration is of increasing importance only at higher pH increases. Urines with a high initial pH and a low phosphate concentration will thus reach the pH of precipitation more rapidly than urines with a low initial pH and a high phosphate concentration. More ammonia is necessary to increase urine pH when urine phosphate is high. Ammonium ions inhibit urease and thus phosphate will also delay the urease-induced pH increase by this mechanism. Whether the variation in the rate of the pH increase is of clinical importance cannot at present be conclusively established. It seems reasonable to assume, however, that the time factor plays a role. If the phosphate concentration is high and the urease activity tends to be low, pH may never reach a level high enough for precipitation to start before urine is flushed out of the kidney.

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