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Effects of glucose, time and temperature on bacterial growth in urine*

by

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Urinary tract infections are very important from the standpoint of human pathology, as their frequency is second only to infections of the respiratory tract. The incidence of pathogens in unselected patients with urinary tract infections reveals *Escherichia coli* as the most common organism, followed by species of *Proteus* (2). According to KASS (4) and SANFORD *et al.* (7), quantitative studies of urine specimens from patients with suspected urinary infections have become a routine examination in clinical laboratories. A urine specimen containing more than 100,000 bacteria per ml is strongly suggestive of bacteria within the urinary tract and not contamination (6). On the other hand, urine specimens from persons without urinary tract infections contain less than 10,000 bacteria per ml (8).

It is known that pH, temperature, composition of the medium, and time, have a marked influence upon bacterial growth (5). AURELIUS (1) studied the effects of pH, temperature, and time on bacterial multiplication in urine samples from an individual by inoculating them with approximately 800 organisms, using a single strain of four different species of urinary tract pathogens. In these studies he demostrated that urine specimens held at room temperature could not be kept longer than four hours before culturing, whereas those refrigerated at 4 C could be held a maximum of six hours. Factors such as glucose content in

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the urine were not determined nor was the inoculum used one which could be considered of clinical significance. The influence of glucose content should be studied, since urinary tract infections occur four times as often in diabetics than in non-diabetics (3). The purpose of this work was to study the effect of time, temperature, and glucose content, upon the growth of two initial populations of either *E. coli* or *P. vulgaris* in sterile urine samples.

MATERIAL AND METHODS

1.—URINE COLLECTION AND PREPARATION: A representative sample of urine was assured by placing a two-liter flask in one of the bathrooms of our laboratory. All the men were requested to void urine in the flask. The urine thus collected was sterilized by Seitz filtration, not more than three hours after the flask was left in the bathroom and divided into portions. One of the samples was used for determination of glucose, albumin, and pH. The remaining samples were stored at approximately 10 C until used.

2.—BACTERIAL STRAINS: Two strains of *E. coli,* HN-81 and HN-82, and two of *P. vulgaris,* HN-83 and HN-84, were isolated from patients with urinary tract infections. Stocks of these cultures were maintained at 4 C on nutrient agar slants.

3.—NUMBER OF BACTERIA: Determination of the number of viable organisms was done by the surface plate inoculation technique. Hundred-fold dilutions of the specimen were made by adding 0.1 ml of the next lower dilution to 9.9 ml of sterile physiological saline; 0.01 ml of the appropiate dilutions was taken with a calibrated loop and used to streak agar plates. The plates were counted after 24 hours at 37 C. The average number of colonies on plates from dilutions showing more than 30 colonies per plate was used to calculate the number of organisms per ml in the original sample.

4.—EFFECT OF TIME AND TEMPERATURE ON BACTERIAL CONTENT: (a) Urine with "high" bacterial content: 0.1 ml of a culture prepared as described under 3 was used to inoculate each of three tubes containing 9.9 ml of urine, so as to obtain between 5.0×10^5 1.0×10^6 bacteria per ml. A urine thus inoculated was refered to as "high". One of the tubes was left at room temperature (25 C), another at 4 C, and the third was kept at −10 C. Viable counts were made 2, 4, and 8 hours after inoculation. These counts were compared to those of the control tubes which were prepared by adding 0.1 ml of the culture to 9.9 ml of sterile saline solution instead of urine.

(b) Urine with "low" bacterial content: the same method described under 4a was followed, with the exception that the samples and controls were inoculated with approximately 10^4 organisms per ml. A urine or control thus inoculated was refered to as as "low".

5.—EFFECT OF GLUCOSE CONTENT, AND TEMPERATURE: The effects of 300 mg of glucose per ml, temperature, and time, were studied by the procedure outlined under 4.

RESULTS

EFFECT OF TIME AND TEMPERATURE ON THE GROWTH OF BACTERIA IN URINE CONTAINING NO GLUCOSE: The growth of approximately 10^4 bacteria per milliliter of four different strains in sterile urine samples is given in Table 1 a, b, c, and d. These results indicate that the original number of bacteria both in the urines and controls showed little or no change during the eight-hour incubation period. Populations of the two strains of *P. vulgaris* used remained practically unchanged with time at the three incubation temperatures, while those of the corresponding buffer showed a strong tendency to decrease as time progressed. The tendency of *E. coli* was to a slight increase with time.

Time and temperature of incubation affected the growth of a higher initial number of organisms, i.e., approximately 10^6 organisms per ml in a similar fashion as that observed with 10^4 organims.

TABLE 1

Effect of time and temperature on the growth of four organisms in urines and buffer of pH 6.5, in the absence of glucose*

Temperature (C)	Time after inoculation 2 hours		4 hours		8 hours	
	Urine	Buffer	Urine	Buffer	Urine	Buffer
	a. *Proteus vulgaris* (HN-83)					
−10	4.34	4.47	4.36	4.17	4.30	3.70
4	4.32	4.66	4.30	4.41	4.30	4.46
25	4.52	4.69	4.53	4.53	4.20	4.40
	b. *Proteus vulgaris* (HN-84)					
−10	4.08	4.17	4.40	4.29	4.04	4.36
4	4.41	4.39	4.67	4.50	4.11	4.38
25	4.56	4.59	4.37	4.41	4.71	4.14
	c. *Escherichia coli* (HN-81)					
−10	4.18	4.30	4.25	4.79	4.41	4.46
4	4.25	4.25	4.36	4.00	4.45	4.49
25	4.32	4.18	4.41	4.18	4.49	4.55
	d. *Escherichia coli* (HN-82)					
−10	4.11	4.95	4.55	3.52	4.23	4.25
4	3.95	4.00	3.87	3.97	4.25	4.32
25	4.08	4.11	4.11	4.00	4.32	4.40

* The initial number of bacteria, expressed as $\log_{10}$, were 4.50, 4.42, 4.41, and 4.38, for *Proteus vulgaris* HN-83, *P. vulgaris* HN-84, and *Escherichia coli* HN-81, and *E. coli* HN-82, respectively.

EFFECT OF TIME AND TEMPERATURE ON THE GROWTH OF URINE CONTAINING GLUCOSE: Glucose affected the populations of organisms depending upon the temperature of incubation and the pH. The results of these experiments are presented in Table 2, a, b, c, and d.

In general, all the bacterial strains studied showed reductions in their populations after two hours of incubation at −10 C. The effect was very similar for both urine and buffer. On the other hand, there was a steady increase in bacterial numbers with time in those samples incubated at room temperature (25 C), reaching populations of at least 10^5 organisms per ml after four hours. In the latter, only the two strains of *P. vulgaris* had reached 10^5 organims per ml after two hours of incubation.

The bacterial populations showed almost no change when the incubation temperature was 4 C, regardless of bacterial strain.

TABLE 2

Effect of time and temperature on the growth of four organisms in urines and buffer of pH 6.5, containing 3 grams per liter of glucose*

Temperature (C)	Time after inoculation: 2 hours		4 hours		8 hours	
	Urine	Buffer	Urine	Buffer	Urine	Buffer
	a. *Proteus vulgaris* (HN-83)					
−10	4.40	4.61	4.02	4.00	3.88	3.75
4	4.76	4.73	4.85	4.80	4.76	4.69
25	4.88	4.88	5.13	4.85	5.33	4.77
	b. *Proteus vulgaris* (HN-84)					
−10	4.48	4.12	3.96	4.09	3.20	3.70
4	4.87	4.69	4.76	4.76	4.68	4.72
25	4.86	4.72	5.02	4.79	5.35	4.78
	c. *Escherichia coli* (HN-81)					
−10	4.39	4.56	4.32	4.53	4.26	4.55
4	4.39	4.71	4.53	4.78	4.73	4.74
25	4.70	4.77	5.01	4.73	5.12	4.80
	d. *Escherichia coli* (HN-82)					
−10	4.10	4.31	4.37	4.43	4.18	4.25
4	4.69	4.68	4.64	4.68	4.67	4.61
25	4.70	4.62	5.30	4.84	5.20	4.77

* The initial number of bacteria, expressed as $\log_{10}$, were 4.50, 4.42, 4.41, and 4.38, for *Proteus vulgaris* HN-83, *P. vulgaris* HN-84, and *Escherichia coli* HN-81, and *E. coli* HN-82, respectively.

DISCUSSION

Quantitative studies of urine specimes have become a routine test in clinical laboratories. It is considered that urine from "normal" persons is either sterile or contains less than 10,000 bacteria per ml (8), while a "pathological" specimen, indicative of urinary tract infection, is that which contains more than 100,000 organisms per ml (6). Depending upon the work load, it is a common practice in clinical laboratories to store the specimens in the refrigerator before making the bacterial counts.

Temperature, pH, glucose content and composition of the medium are factors that influence bacterial growth (6). AURELIUS (1) studied these factors by collecting urine from a healthy volunteer subjected to various administrations of ammonium chloride or sodium bicarbonate in order to obtain specimens with different pH values He obtained similar results in the growth of *E. coli* and *P. mirabilis* at pH 6.5 in urine samples incubated at 4 C and 22 C. In general, both organisms remained relatively constant or even decreased during the firt six hours of incubation.

At comparable pH and temperature values, the results of the present work are very similar to those of Aurelius, although we observed a tendency of *E coli* to increase with time. However, the bacterial populations of either *E. coli* or *P. mirabilis* never reached 100,000 organisms per ml when the initial inoculum was 10,000 organims per ml, regardless of the time of observation and the temperature of incubation. In other words, a "normal" urine, from the standpoint of bacterial content, was never transformed into a "pathological" specimen. The lack of reduced substrates as energy yielding sources in normal urines, might explain the fact that addition of glucose to these specimens resulted in bacterial growth, when such samples were inoculated with 10^4 organims per ml. Under these circumstances, populations higher than 10^5 organisms per ml were observed in all of these specimens of pH 6.5 incubated at room temperature.

SUMMARY

Sterile urine and buffer samples of pH 6.5, with or without glucose, were inoculated with either 10^4 or 10^6 organims (two strains of *Escherichia coli* and two of *Proteus vulgaris)* per ml and incubated at −10, 4, and 25 C. The subsequent growth was determined at 0, 2, 4 and 8 hours. In the absence of glucose, bacterial populations of these organisms never reached 10^5 bacteria per ml when the initial inoculum was 10^4 organisms, regardless of time and temperature of incubation. In presence of glucose, populations higher than 10^5 were observed in all of the specimens at room temperature.

RESUMEN

Se inoculó con 10^6 y 10^4 organismos (dos cepas de *Proteus vulgaris* y dos de *Escherichia coli)* por mililitro a muestras de solución amortiguadora y de

orina estéril, con y sin glucosa, regulándose el pH a 6.5 y se les incubó a temperaturas de - 10, 4 y 25 C. El crecimiento bacteriano se determinó a las 0, 2, 4 y 8 horas. Los resultados indican que el número de bacterias muestra poco o ningún cambio durante las ocho horas de incubación y a las tres temperaturas cuando la glucosa no está presente.

La adición de glucosa y la temperatura ambiente, se constituyeron en factores importantes en el incremento del número de bacterias en la muestra.

LITERATURE CITED

1. AURELIUS, G.
1968. Bacterial growth in urine. *Acta Path. Microbiol. Scandinav.*, 55: 203-208.

2. COLEMAN, P. N., & S. TAYLOR
1959. Coliform infections of the urinary tract. *J. Clinc. Path.*, 2: 134-138.

3. EDMONSON, H. A., H. E. MARTIN, & N. EVANS
1947. Necrosis of the renal papillae and acute pyelonephritis. *Arch. Intern. Med.*, 79: 148-154.

4. KASS, E. H.
1955. Chemotherapeutic and antibiotic drugs in the management of infections of the urinary tract. *Amer. J. Med.*, 18: 764-781.

5. LAMANNA, C., & M. F. MALLETTE
1965. *Basic bacteriology. Its biological and chemical background.*. The Williams & Wilkins Co., Baltimore, 1001 pp.

6. MERRIT, A. D., & J. P. SANFORD
1958. Sterile-voided urine culture. An evaluation on 100 consecutive hospital women. *J. Lab. Clinc. Med.*, 52: 463-470.

7. SANFORD, J. P., C. R. FAVOUR, & F. H. MAO
1956. Evaluation of the "positive" urine culture. An approach to the differentiation of significant bacteria from contaminants. *Amer. J. Med.*, 20: 88-93.

8. VIRTANEN, S., & A. KASANEN
1962. "Asymptomatic" bacteriuria in female patients in the medical ward. A comparison between mid-stream voided and catheterized urine specimens. *Acta Path. Microbiol. Scandinav.*, 55: 365-371.