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INTRODUCTION

Coliform bacilli are the most frequent cause of human pyelonephritis. Numerous attempts to produce experimental pyelonephritis by the intravenous injection of this organism into normal animals have failed. Ureteral obstruction has long been recognized as a factor which would render these experimental animals susceptible to a coliform pyelonephritis induced by intravenous inoculation of the organisms. Intrarenal tubular obstruction produced by staphylococcal infection (1,2) or by medullary burning with an electric cautery (3) is another method by which animals have been made susceptible to a subsequent coliform pyelonephritis.

The exact mechanism responsible for this increase in suseptibity is not known. For many years workers had thought slowing of the urinary stream or stagnation to be the major etiologic factor in infections secondary to obstruction. However, it has been shown that partial ureteral obstruction (which would certainly cause some stagnation) does not produce the increased incidence of experimental pyelonephritis that would be expected if this were a major cause. (4) As a result of this evidence stagnation was felt to be of little importance as an etiologic factor. It was then postulated by the same workers that chemical alterations occuring in kidney substance in the presence of sudden and complete ureteral

obstruction may either favor bacterial growth or hinder antibacterial action.

It is possible that excretion of urine which is a better medium for bacterial growth is a mechanism by which the urinary tract is predisposed to infection. Perhaps chemical alterations do take place in the presence of sudden and complete urinary obstruction which make this urine a better location for bacterial growth than normal urine. The continued excretion of such a urine would render the urinary tract more susceptible to chronic infection and make it more difficult to erradicate the infection. This work was designed to see if several types of bacteria would grow better in urine obtained from patients with a history of renal infection than in urine obtained form normal persons.

MATERIALS AND METHODS

Urine specimens: The normal urines were obtained from persons with no history of urinary tract infection and were used only if a routine urinalysis showed no evidence of abnormality. The pyelonephritis urines were obtained from patients being seen in the Pyelonephritis Clinic at the Grace-New Haven Hospital who had a clinical and laboratory history of pyelonephritis. The urines were kept frozen until they were to be used. Before each experiment the urines were sterilized by passage through a Selas bacteriological filter. In the initial set of experiments the ph of the urine was determined with

nitrazine paper and the urine specimens were used as they were obtained except for the filtration. In the second set of experiments the urine was titrated to ph 6.5 with hydrochloric acid and sodium hydroxide. The ph determinations were made with a Beckman ph meter which was checked against a standard buffer each time determinations were to be made.

Organisms: Six organisms were used in the initial experiments; E. coli, enterococcus, proteus, staphylococcus, alpha and beta streptococcus. These were obtained from hospital cultures of pathologic material. The alpha and beta strep were maintained on slants of blood agar. The other organisms were kept on nutrient agar. All organisms were frozen between individuals experiments and were thawed out and subcultured prior to use. In the second set of experiments only the E. coli, enterococcus and proteus were used.

In the initial group of experiments each specimen was innoculated with a loopful of organisms and the actual number determined by colony counts on pour plates. The plates were incubated for at least 24 hours before recording the number of colonies. In the second group of experiments each specimen was innoculated with approximatly 10^2 organisms predetermined by dilution of a 24 hour broth culture.

One c.c. of each specimen was cultured in nutrient

agar after filtration as a control on the sterilization. Any specimen that was not sterile was discarded.

EXPERIMENTAL

In the initial set of experiments from two to six 10 c.c. samples were taken from each sterile urine specimen and placed in test tubes. Each sample was innoculated with a loopful of organisms. Serial hundred fold dilutions in nutrient broth were done at 0 and 8 hours and pour plates were made according to the expected number of organisms. The plates were incubated for 24 hours and the colonies were counted. The results are summarized in tables I-V.

It can be readily seen that the ph of the urine was the most important factor in determining the amount of bacterial growth in the various specimens. The E. coli (table I) and the proteus (table III) grew abundantly at all but the lowest of ph levels (4.5). The alpha strep (table V) would not grow below a ph level of 6.5 and in ten specimens the beta strep would not grow at ph levels below 7. One sample at ph 7 supported thousandfold growth of beta strep. The staphylococci in most cases did not grow much below a neutral ph but growth was obtained at ph 6. The enterococci grew at a ph of 5.5 but only to maximal levels (10^8) when near neutral values.

Although these experiments emphasize the importance of the urinary hydrogen ion concentration in determining

TABLE I. E. COLI

ph	NORMAL URINES 0	8		CLINIC URINES 0	8	
4.5				3	2	J
5	4	6	A	4	8	K
	4	7	B	4	y	L
5.5	5	5	C	4	8	M
	4	y	D	4	8	N
	4	y	E	4	6	O
				4	6	P
				5	7	Q
6	3	5	F	4	8	R
	4	6	G			
6.5				4	8	S
				44	8	T
				4	8	U
				5	8	V
				4	8	W
				4	8	X
7	4	8	H	4	8	Y
	3	8	I	4	8	Z
7.5				5	8	Z'

Table showing the number of colonies of E. coli per milliliter of urine expressed in exponents of 10 at 0 and 8 hours. The results are grouped according to the ph value of the urine.

Key to this and subsequent tables:

x indicates no growth at a 10^{-3} dilution
y indicates no growth at a 10^{-5} dilution
capital letters label individual urine specimens

TABLE II. ENTEROCOCCUS

	NORMAL URINES			CLINIC URINES		
ph	0	8		0	8	
4.5				4	1	J
5	4	x	A	4	5	K
	4	y	B	4	y	L
5.5	5	5	C	3	3	M
	4	6	D	5	4	N
	4	6	E	3	y	O
				4	Y	P
				4	Y	Q
6	3	3	F	4	7	R
	4	5	G			
6.5				4	y	S
				4	6	T
				4	7	U
				5	5	V
				4	6	W
				5	y	X
7	4	8	H	4	8	Y
	3	8	I	4	8	Z
7.5				4	8	Z'

Table showing the number of colonies of enterococci per milliliter of urine expressed in exponents of 10 at 0 and 8 hours.

TABLE III. PROTEUS

	NORMAL URINES			CLINIC URINES		
ph	0	8		0	8	
4.5				3	3	J
5	5	3	A	4	8	K
	4	y	B	4	8	L
5.5	5	8	C	3	7	M
	4	8	D	4	8	N
	4	8	E	4	6	O
				4	y	P
				5	y	Q
6	4	7	F	4	8	R
	4	7	G			
6.5				4	8	S
				4	8	T
				4	8	U
				5	5	V
				5	8	W
				5	y	X
7	4	8	H	4	8	Y
	5	8	I	4	8	Z
7.5				4	8	Z'

Table showing the number of colonies of proteus per milliliter of urine expressed in exponents of 10 at 0 and 8 hours.

TABLE IV. STAPHYLOCOCCUS

	NORMAL URINES			CLINIC URINES		
ph	0	8		0	8	
4.5				4	x	J
5	4	x	A	4	3	K
	4	5	B	3	y	L
5.5	5	5	C	3	x	M
	4	y	D	4	3	N
	4	y	E	4	5	O
				3	y	P
				3	y	Q
6	4	3	F	4	7	R
	4	5	G			
6.5				4	y	S
				4	y	T
				4	7	U
				4	y	V
				4	y	W
				4	y	X
7	4	7	H	3	6	Y
	4	7	I	4	8	Z
7.5				4	x	Z'

Table showing the number of colonies of staphylococci per milliliter of urine expressed in exponents of 10 at 0 and 8 hours.

TABLE V. ALPHA STREPTOCOCCUS

	NORMAL URINES			CLINIC URINES		
ph	0	8		0	8	
4.5				3	x	J
5						
5.5	5	x	C	3	2	M
				5	4	N
				4	y	O
				3	y	Q
6	4	4	J	3	y	R
6.5				4	6	T
				4	6	U
				4	y	V
				5	5	W
				5	y	X
7	4	8	H	4	8	Y
	4	6	I			
7.5						

Table showing the number of colonies of alpha streptococci per milliliter of urine expressed in exponents of 10 at 0 and 8 hours.

the amount of growth they were not of much help in comparing the growth in normal urine with that in clinic urine. It was impossible to make a valid comparison of growth due to the lack of a significant number of specimens at any one ph value. Because of this a second set of experiments was done. This time the ph of the urine was titrated to a standard value (6.5) so as to have comparable sets of specimens. A more accurate measure of the important ph value was used. Plate counts were done at 3, 6, and 24 hours so as to better compare the rate of growth as well as the total amount of growth. The results are summarized in tables VI-VIII. There appears to be no significant difference either in the rate of growth or the total growth of bacteria between the two groups.

DISCUSSION

The effect of ph on bacterial growth that is illustrated in the initial experiments has been the subject of much investigation in the past. Regulation of urinary ph was one of the first methods of treating urinary tract infection. An excellent study by Yeuw (5) in 1940 begins by reviewing the work previously done in this field. He then attempted to define experimentally the lowest ph levels compatible with growth and survival of various types of bacteria in the urine. He found that it was possible to obtain levels of urinary

TABLE VI. E. COLI

NORMAL URINES				CLINIC URINES			
3	6	24		3	6	24	
4	6	9	A	4	5	7	L
4	6	9	B	3	5	8	M
4	6	8	C	4	6	8	O
3	5	8	D	3	6	9	P
3	5	8	E	3	5	8	Q
3	5	8	F	4	6	8	R
3	4	8	G				
3	6	8	H				

Table showing the number of colonies of E. cole per milliliter of urine expressed in exponents of 10 at 3, 6, and 24 hours.

TABLE VII. ENTERCOCCUS

NORMAL URINES				CLINIC URINES			
3	6	24		3	6	24	
3	5	8	A	2	3	2	L
3	4	7	B	3	4	8	N
3	4	7	C	3	x	x	O
				3	4	7	P
				2	3	7	Q
				1	x	x	R

Table showing the number of colonies of enterococci per milliliter of urine expressed in exponents of 10 at 3, 6, and 24 hours.

TABLE VIII. PROTEUS

NORMAL URINES				CLINIC URINES			
3	6	24		3	6	24	
4	5	8	A	2	4	7	L
4	6	7	B	4	6	7	M
4	7	7	C	4	6	7	O
4	5	7	D	4	6	8	Q
4	6	7	E	4	6	8	R
4	6	7	F				
4	5	5	G				
4	6	7	H				

Table showing the number of colonies of proteus per milliliter of urine expressed in exponents of 10 at 3, 6, and 24 hours.

acidity in humans that were bactericidal for the alpha and beta streptococcus and bacteriostatic for most other organisms. The results obtained in this experiment are similar to the results obtained by Yeaw. Another interesting observation made by Yeaw was the fact that with the same organism, at a given ph value, there was considerable variation in different urine specimens as to the actual amounts of growth. It was felt that this was due to variations in the nutritive property of individual urine specimens. This is the most probable explanation for the variations in growth at equal ph levels in the present experiment.

The second set of experiments showed that the urine excreted by patients with a history of renal infection was not a better medium for bacterial growth than the urine from normal patients. This would indicate that the excretion of a urine more suitable for bacterial growth is not a mechanism by which the urinary tract is made more susceptible to infection. These findings are not in complete agreement with those of a somewhat similar study by Jackson and Grieble. (6) They compared bacterial growth in urine obtained from normal persons with growth in pooled urine obtained from 22 persons with urinary tract infections. The rate of growth was increased in urine obtained from the patients with

urinary tract infections. The differences in total amount of growth were slight and of doubtful significance. The explanation for this inconsistency in regard to the rate of growth is not clear. Perhaps the urine used by Jackson and Grieble was obtained from patients with acute or active infections. The patients in the present study did not necessarily have active disease at the time the specimens were collected. If this were the case the difference in nutrient property which causes an increased growth rate in their samples would be one of short duration and not present in the urine obtained from patients with inactive or chronic disease. It is also possible that the number of specimens tested was not large enough to rule out the physiologic variation in the nutritive properties of urine as the reason for the difference.

It was mentioned that in the presence of sudden obstruction of urinary outflow there may be a chemical change in the kidney which predisposes it to infection. (4) If this actually does occur and if this is a factor in the pathogenisis of pyelonephritis it would appear that the site of action of this chemical change is in the tissue itself rather than in the urine since there is no increase of bacterial growth in the urine from injured kidneys as compared to urine from normal kidneys.

However, if this substance makes only a transient appearance in the urine its effect would not be shown in this experiment. Future investigation should perhaps include study of bacterial growth in urine obtained from patients with acute infections.

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