LAWRENCE R. FREEDMAN*

Department of Internal Medicine, Yale University School of Medicine

EXPERIMENTAL PYELONEPHRITIS. XIII. ON THE ABILITY OF WATER DIURESIS TO INDUCE SUSCEPTIBILITY TO *E. coli* BACTERIURIA IN THE NORMAL RAT†

There is considerable evidence in man to indicate that bacteria ascend from the bladder to the kidney within the lumen of the ureter, thereby accounting for the principal pathway by which bacteria reach the kidney to produce pyelonephritis.[1-3] Although much is known about factors that permit the establishment of bacteria infection in the kidney, very little is known about the circumstances that permit bacteria to persist in the bladder urine in the absence of underlying kidney infection. The disappearance of large numbers of bacteria instilled within the normal bladder is rapid and efficient.[4-6]

During the course of experiments designed to test the effect of water diuresis on unilateral pyelonephritis, it was noticed that bacteria were commonly found in the opposite kidney of animals drinking large amounts of fluid and only rarely in animals on a normal water intake. It appeared that water diuresis facilitated the spread of bacteria within the urinary system. Studies were undertaken, therefore, to investigate the effect of water diuresis on bacteria placed within the bladder lumen. The present report describes the outcome of these experiments.

MATERIALS AND METHODS

The experimental animals were male Sprague-Dawley rats weighing 250-350 grams. The test micro-organism has been used in this laboratory for many years and the culture techniques and quantitative bacteriological techniques have all been described previously.[7] In brief, the inoculum employed was an appropriate dilution in saline of a four and one-half hour broth culture of *E. coli*. Bacterial viable units were enumerated by serial dilution and pour plate techniques.

Inoculation of bacteria into the lumen of the dome of the bladder was carried out after Nembutal® anesthesia, through a mid-line abdominal incision. The bladder was punctured with a 27-gauge needle and its contents aspirated prior

---

* Associate Professor of Medicine.

† Supported by U. S. Public Health Service Development Award No. 6K3-HD 22, 587, National Institute of Child Health and Human Development and by Research Grant No. 4767 from the Institute of Allergy and Infectious Disease, U. S. Public Health Service.

Received for publication 22 November 1966.

to the inoculation of the bacterial culture. If the bladder were not first emptied, the animal was seen to void immediately after injecting the culture fluid. The volume of the inoculum was always 0.1 ml.

Water diuresis was accomplished by allowing the individually housed rats free access to 5% glucose solution made up with tap water. Rats were permitted to drink this solution 3-5 days prior to receiving the test bacterial inoculum. After 2-3 days, the animals drinking glucose consumed approximately 100 to 150 ml. per day. Only in unusual circumstances was a drinking bottle permitted to be emptied. Control animals were given free access to tap water and drank about 20-30 ml. per day. All animals were permitted free access to Purina lab chow pellets.

A separate group of animals drinking 20% glucose solution (made up with tap water) was carefully observed in the laboratory for a period of up to one year. Litter mate controls were housed in a similar fashion but were given tap water to drink. The amounts of drinking water consumed daily were similar to those of the rats drinking 5% glucose.

Glucose tolerance tests were performed in rats drinking 20% glucose and their controls. Blood samples were obtained from scalpel punctures of the foot in calibrated pipettes after an overnight fast (no food or drinking solution or water). After obtaining a fasting blood sample, the animals were injected with 1.0 cc. of 5% glucose per 100 grams body weight. A second blood sample was taken from the foot 90 minutes after the intraperitoneal injection of glucose.

RESULTS

Features of water diuresis resulting from drinking 5% glucose

The consequences of drinking 5% glucose on the chemical composition of the urine and kidney have been described previously.[8] During the present experiments, as in those of Andriole and Epstein,[8] tests for glucose in the urine were negative. Urine specific gravities in urine samples taken at the time of operation and bacteriological study were usually less than 1.010 in rats drinking glucose and varied from 1.020 to 1.045 in animals drinking water. The urine pH varied between 6.0 and 7.4 in both groups. It was generally possible to aspirate 1.0 to 2.0 ml. of urine from the bladders of animals drinking glucose, whereas less than 0.5 ml. was usually obtained from animals drinking water. Occasionally, however, urine samples as large as 1.5 ml. were obtained from normal rats drinking water.

To evaluate the long term consequences of drinking large volumes of water and large quantities of glucose, 15 rats were permitted free access to 20% glucose as drinking water and 15 litter-mate controls were given plain water. All animals were given Purina lab chow pellets *ad libitum*. The animals were weighed weekly and had glucose tolerance tests performed after they had been observed for about eight months.

The intake of fluid in rats drinking 20% glucose was similar to the animals drinking 5% glucose, approximately 100 to 150 ml. per day. Glucose was not detected in the urine. Peak weights in the glucose animals were slightly higher (569 gm.) than in water-drinking controls (521 gm.). The average weight of both groups at the start was 178 gm. Over the course of one year, three rats in each group died.

Glucose tolerance tests performed at the end of the period of observation showed that none of the animals drinking glucose had become diabetic. The only abnormally high blood sugar value (greater than 140 mg/100 ml.) 90 minutes after glucose injection was in an animal drinking water.

The kidneys of glucose and water-drinking rats, examined eight months to one year after observation in the laboratory, differed grossly in one important aspect. None of the animals drinking glucose had stones, whereas small gravel in the renal pelvis was encountered in 4 of 15 rats drinking water. Stones were never encountered in other normal rats except when they had been kept under observation in the laboratory for about one year.

The ratio of the combined weights of both kidneys to total body weight was less in glucose drinkers than in water drinkers; 0.54 as compared with 0.69. The explanation for this difference is not apparent. Slight hydronephrosis, as is occasionally seen in normal rats of all ages, was found in two animals of each group.

To test susceptibility of the kidney of animals drinking glucose to infection, 10^8 *E. coli* were inoculated intravenously in rats drinking 5% glucose for 4-5 days (five animals) and 20% glucose for one year (14 animals). Cultures of urine, blood and kidneys 4-5 days later were sterile in all instances.

Thus, rats drinking glucose solutions, excreting large volumes of urine, were not susceptible to infection when challenged with bacteria intravenously. Although the kidneys of animals drinking large volumes of 20% glucose for one year were somewhat smaller than those of normal animals, slight hydronephrosis was no more common in one group than the other, and renal pelvic stones were not seen in animals drinking glucose whereas they were found in about 25% of normal animals.

The effect of water diuresis on renal cortical infections

Initially, experiments were designed to determine the effect of water diuresis on renal cortical infection in a single kidney. Rats drinking 5% glucose and control animals drinking water were given injections of 10^4 *E. coli* into the renal cortex. After 3 to 5 days the kidneys were removed along with samples of heart blood and bladder urine. The results of such

experiments are shown in Table 1. Bacterial injections were made into the cortex of the left kidney. Gross abscesses were usually identified and large numbers of bacteria were recovered from the left kidney and urine.

Directing attention to the right kidney, it was noticed that in the animals drinking water, 2 of 11 kidneys contained bacteria, and in those two instances, only 10^2 viable units were recovered. On the other hand, in the animals drinking glucose, 7 out of 13 right kidneys contained bacteria and they were present in large numbers.

TABLE 1. INJECTION OF 10^4 *E. Coli* INTO THE LEFT KIDNEY CORTEX OF RATS; BACTERIOLOGICAL STUDY 4-5 DAYS LATER

Rats drinking water			*Rats drinking 5% glucose*		
*Lt. kidney**	*Rt. kidney**	*Urine***	*Lt. kidney**	*Rt. kidney**	*Urine***
10^4	0	10^3	10^5	10^5	10^4
10^4	0	0	10^6	10^6	10^4
10^4	0	10^2	10^4	0	0
10^4	10^2	—	10^5	0	10^2
0	0	0	10^5	0	10^4
10^4	10^2	10^5	10^5	10^5	10^5
10^4	0	10^5	10^5	0	0
10^5	0	10^4	10^5	10^5	10^5
10^5	0	10^3	10^3	10^4	—
10^5	0	0	10^5	10^5	10^5
10^5	0	10^4	10^4	0	0
			0	0	0
			10^4	10^3	10^5

* Colonies per kidney.
** Colonies per ml.

It appeared from this experiment that water diuresis had no effect on the renal cortical infection but did have an effect on the ability of bacteria to reach the uninjected kidney. Since blood cultures in all instances were sterile, it was reasoned that the bladder urine represented the most likely source of the bacteria. Experiments were, therefore, undertaken to determine the effect of water diuresis on bacteria injected into the bladder lumen.

Injection of E. coli into the bladder lumen of rats drinking water as compared with rats drinking 5% glucose

The injection of large numbers of *E. coli* into the bladder of rats drinking water demonstrated the remarkable efficiency with which the animal

rids itself of the bacteria (Table 2). Within 4 to 5 days of receiving 10^5 to 10^7 viable bacterial units, only 3 of 19 animals had any bacteria remaining in the bladder urine and in two of these animals there were only 10^2 microorganisms recovered.

Since normal animals are well known to be resistant to bacterial infection of the kidney, it was decided to test animals whose susceptibility to renal infection had been increased by inserting a 27-gauge needle three

TABLE 2. INJECTION OF *E. coli* INTO THE BLADDER LUMEN OF NORMAL RATS DRINKING WATER; BACTERIOLOGICAL STUDY 4-5 DAYS LATER.

No. bacteria injected	*Lt. kidney**	*Rt. kidney**	*Urine***
10^7	0	0	0
	0	0	0
	0	0	0
	0	0	0
	0	0	10^2
	0	0	10^2
10^6	0	0	0
	0	0	0
	0	0	0
	0	0	0
	0	0	10^5
	0	0	0
10^5	0	0	0
	0	0	0
	0	0	0
	0	0	0
	0	0	0
	0	0	0
	0	0	0

* Colonies per kidney.
** Colonies per ml.

times into the medulla of the left kidney. The left kidney was traumatized in animals drinking water and in those drinking 5% glucose at the time of operation and small bacterial inocula were placed in the bladder. The results are shown in Table 3. After 3-5 days, large numbers of bacteria were recovered from animals undergoing water diuresis but not from animals drinking water. It was remarkable that as few as ten viable bacterial units were capable of persistence and multiplication within the urinary tract under these circumstances (Table 3). A few animals in-

jected with 10^3 bacteria into the bladder were examined after 7 to 14 days and the results were similar to those seen after 3 to 5 days.

Since bacteria were recovered as often from the normal kidney as from the traumatized one, these studies were repeated in normal rats whose kidneys were not traumatized. Once again, as few as 10 viable bacterial units were found capable of multiplying and animals examined 14 to 21

TABLE 3. INJECTION OF *E. coli* INTO THE BLADDER LUMEN OF RATS WITH TRAUMATIZED LEFT KIDNEYS; BACTERIOLOGICAL STUDY 4-5 DAYS LATER

No. bacteria injected	*Rats drinking 5% glucose*			*Rats drinking water*		
	*Lt. kidney**	*Rt. kidney**	*Urine***	*Lt. kidney**	*Rt. kidney**	*Urine***
10^3	10^5	10^5	10^5	0	0	0
	10^5	10^5	10^5	0	0	10^2
	10^4	10^4	10^5	0	0	0
	10^5	10^5	10^5	0	0	0
	10^4	10^4	10^5	0	0	0
	0	0	10^3	0	0	0
10^2	10^5	10^3	10^5	0	0	0
	10^5	10^5	10^5	0	0	0
	10^5	10^5	10^5	0	0	0
	10^3	10^2	0	0	0	0
	10^5	10^5	——	0	0	0
	10^3	10^3	10^5	0	0	0
10^1	10^4	10^5	10^5	0	0	0
	0	0	0	0	0	0
	10^4	10^5	10^5	0	0	0
	0	0	0	0	0	0
	0	0	0			
	0	0	0			

* Colonies per kidney.
** Colonies per ml.

days after the injection of 10^3 or 10^4 bacteria into the bladder were frequently found to have large numbers of bacteria in their kidneys and bladder urine (Table 4).

To test any possible effect of the operative procedure on the production of bacteriuria, 12 animals drinking 5% glucose had 0.1 ml. sterile saline injected into the bladder lumen. At examination 4-5 days later, cultures of urine, blood, and kidneys were sterile. These control studies are also pertinent to the question of what role bacterial contamination of the glucose drinking solutions may be playing in these infections. Culture of

the 5% glucose solutions which were in place in the animal room showed large numbers of a variety of gram-negative rods. These ingested bacteria are considered not to be playing a role in these experiments since contaminating micro-organisms were not recovered from the blood, urine, or kidneys of control or experimental animals. The role of ingested bacteria was also studied by contaminating the glucose drinking solutions with

TABLE 4. INJECTION OF *E. coli* INTO THE BLADDER LUMEN OF NORMAL RATS

No. bacteria injected	*Days between injection and culture*	*Rats drinking 5% glucose*			*Rats drinking water*		
		*Lt. kidney**	*Rt. kidney**	*Urine***	*Lt. kidney**	*Rt. kidney**	*Urine***
10^1	3-5	0	0	0	0	0	0
		0	0	0	0	0	0
		10^5	10^5	10^5	0	0	0
					0	0	0
					0	0	0
					0	0	0
10^3	14-21	0	0	0	0	0	0
		10^4	10^2	10^4	0	0	0
		10^4	10^5	10^5	0	0	0
		10^5	10^5	10^5	0	0	0
		0	0	0	0	0	0
		10^5	10^5	10^5			
		0	0	0			
		10^4		10^5			
		10^2	10^2	10^1			
		0	0	0			

* Colonies per kidney.
** Colonies per ml.

large numbers of the experimental *E. coli* and injecting 0.1 ml. sterile saline into the bladder cavity while the animals were drinking the contaminated 5% glucose solutions. In the eight rats used for these experiments, all cultures of urine, kidneys, and blood were sterile 4-5 days following the injection of saline.

Thus, it was evident that the ability of bacteria to reach the kidney after injection into the bladder lumen depended not on whether the kidney had been made susceptible to infection (by injury) but only on the animals' ability to clear the bladder urine of bacteria. *In the presence of*

water diuresis there was a serious impairment of the host's ability to clear a bacterial inoculum placed in the bladder lumen.

Bacteria in the kidney vs. pyelonephritis

It was remarkable that large numbers of bacteria were recovered from kidneys without detecting gross evidence of infection. Histological sections were not taken during the present experiments in order to obtain complete bacteriological data. It remains to be determined whether persistent bacteriuria in normal rats leads to the development of pyelonephritis. The presence of large numbers of bacteria in the kidney may in part reflect the large numbers of bacteria in the urine, but for the moment, judgment on this matter must be withheld.

DISCUSSION

Experiments have been presented which demonstrated that rats drinking plain water are able, within 3-4 days, to rid the urinary tract of as many as 10,000,000 bacteria injected into the bladder lumen. On the other hand, rats undergoing water diuresis (drinking 5% glucose) may not be able to clear as few as 10 viable bacterial units from their urine. Large numbers of bacteria were recovered from the urine and kidneys of water diuresis animals up to three weeks later, the longest interval studied. Looked at another way, *rats undergoing water diuresis were at least one million times more susceptible to the establishment of bacteriuria than rats drinking normal quantities of water.*

Although a positive explanation for these findings is not at hand, a certain number of factors can be eliminated. Data were presented which rule out kidney damage as a possible factor since animals drinking large volumes of glucose solutions were, as normal animals, not infected after intravenous challenge with 10^8 *E. coli.* The production of acute kidney damage which is known to induce renal susceptibility to infection did not impair the host defense mechanisms in the bladder of rats drinking normal amounts of fluid. Urinary obstruction does not seem to be the explanation since hydronephrosis was not observed any more frequently in rats drinking large amounts of fluid than in controls. In addition, if urinary obstruction were involved, it might be anticipated that renal susceptibility would be demonstrable after intravenous challenge with bacteria, but this was not the case.

It is unlikely that the ingestion of carbohydrate as glucose plays a role since the blood sugars of animals drinking 5% glucose remain normal[8] and carbohydrate tolerance, even after prolonged ingestion of large amounts

of glucose, is not impaired. Also, glucose is not excreted in the urine under conditions of the experiments. Nevertheless, subtle metabolic changes may exist which might impair host defenses. This, too, is unlikely, however, since the kidneys of animals drinking 5% glucose have been shown to be *less* susceptible to infection by staphylococci given intravenously.[8]

Bacterial contamination of the 5% glucose drinking solutions with a variety of gram-negative rods was a regular occurrence. These ingested bacteria were not considered to be playing a role in these experiments since they were never recovered from the blood, urine, or kidneys of experimental or control animals. Furthermore, intentional contamination of the glucose drinking solutions with the test *E. coli* did not cause these organisms to appear in the urinary tract of control-operated animals.

There are factors that do influence bacterial multiplication in urine and host defense mechanisms that are known to be altered during water diuresis. Solutions containing concentrations of sodium below 100 mm/L may inhibit phagocytosis and lowered urine concentrations support bacterial multiplication.[9-11] In addition, the greater the distention of the bladder the less the opportunity for surface phagocytosis[12] and the less the blood flow to the bladder mucosa.[13] It is possible, also, that high rates of urine production act by providing a constant pool of urine in the bladder, in much the same manner as residual urine in patients with partial obstruction to urine flow. Frequent bladder emptying, however, tends to decrease bacterial multiplication in the bladder.[4, 14]

Thus, increased urine flow might lower host resistance to bladder infection in many ways that are well known to apply within normal physiological limits. In addition, there may be other factors of importance that have not yet been studied; e.g. opsonic substances in urine which are diluted to the point of ineffectiveness during water diuresis. There must obviously be a delicate balance of mechanisms which determine the outcome of bacterial multiplication within the lumen of the bladder. The remarkable finding in the present studies was the extent to which it was possible to tip the balance in favor of the bacteria by merely increasing water intake.

These findings are of interest in view of the common clinical practice of forcing fluids, thereby inducing water diuresis, in patients with urinary infections. However, it must be emphasized that there are no clinical data demonstrating the value of this long established practice. There have been no studies, of which the author is aware, on the outcome of therapy in patients undergoing water diuresis compared with patients on a normal fluid intake. When one considers the variable course of urinary infections

in man and the natural tendency toward symptomatic improvement,[8] it is necessary to conclude, for the moment, that the beneficial effect of forcing fluids in the management of urinary infections in man is unproved. Indeed, if these experiments are applicable to man, water diuresis in the presence of urinary infection may be harmful.

There are arguments in favor of establishing water diuresis in the treatment of pyelonephritis which derive from studies in animals.[15, 16] Osmotically fragile forms of bacteria (protoplasts, spheroplasts) can survive, revert to normal forms[17] and persist in the renal medulla.[18] Andriole and Epstein induced a water diuresis in rats and brought about more rapid healing and prevention of the initiation of staphylococcal pyelonephritis.[8] Explanations for this effect include the production of an environment in the renal papilla unfavorable for the survival of osmotically fragile bacterial forms and increasing medullary blood flow, thereby facilitating the inflammatory response and the delivery of blood-borne antibacterial factors.[19] It is important to note, however, that staphylococcal urinary infections are uncommon in man and that the host defense mechanisms effective against hematogenously induced pyelonephritis may not be the same as those effective against ascending infections. Preliminary experiments in the author's laboratory inoculating staphylococci in the bladder of rats undergoing water diuresis suggest that bacteriuria is not established as with *E. coli.*

It is, of course, difficult to say whether the same delicate balance operates in man or whether the same factors are operative. Nevertheless, it is interesting to note that circumstances where there is increased urine flow in man may be associated with increased susceptibility to urinary infection. Clinical states such as diabetes mellitus and any renal disease with a relative decrease in ability to concentrate the urine (hypokalemia, hypercalcemia, hypertension, partial urinary obstruction, renal insufficiency), and therefore high rates of urine flow, might be expected to be associated with increased susceptibility to bladder infection. Although there are suspicions that this may be so, conclusive clinical data are for the most part lacking. It may be very difficult to determine the relative role of any single factor in these complex clinical conditions where there are also many abnormalities of blood vessels and metabolic state.

With the demonstration of the effect of water diuresis on bacterial multiplication in urine, both the renal papilla[8] and the bladder urine have had plausible circumstances defined in experimental animals which bring about dramatic increases in the ability of bacteria to survive and multiply in tissues. This exquisite susceptibility to infection may help explain why

urinary tract infections are among the most common infections encountered in clinical medicine.

It is of interest to speculate that one of the consequences of pyelonephritis, i.e. decreased urinary maximal concentrating ability may be a factor increasing susceptibility to infection: increased susceptibility to bladder urine infection, renal papillary damage due to infection, decreased renal concentrating ability, increased flow of dilute urine, increased susceptibility to bladder urine infection, and so on.

The production of chronic bacteriuria in the normal rat provides an animal model that closely mimics the most common form of urinary infection in man: large numbers of bacteria in the urine, without obvious renal damage. This model may be employed to investigate phenomena of clinical significance: the development of renal disease under circumstances of chronic bacteriuria, the effect of chronic bacteriuria on blood pressure and the course of pregnancy in animals with bacteriuria.

SUMMARY

Whereas 10,000,000 *E. coli* are quickly cleared from the bladder lumen of normal rats drinking water, as few as 10 viable bacterial units are capable of survival and multiplication in the bladder lumen of normal rats undergoing water diuresis. Bacteria have been recovered from the kidneys and bladder urine 21 days after inoculation, the longest interval studied so far. This millionfold increase in susceptibility to chronic bacteriuria permits the establishment of a model in normal animals that closely resembles the most common form of urinary infection in man. If the events described in the rat are applicable to man, it would appear necessary to question the value of the common practice of increasing fluid intake in patients with urinary tract infections.

Although there are many possible explanations for the experimental results, the mechanisms that are critical have not as yet been determined. It was possible, however, to exclude factors such as glycosuria, hydronephrosis and kidney damage as having roles of significance.

The establishment of bacteriuria in normal animals provides a means, heretofore not available, for studying the relation of bacteriuria to chronic renal disease, hypertension, and abnormalities of fetal development during pregnancy.

ACKNOWLEDGMENT

The author expresses his gratitude to Mrs. Mary Lou Breitenstein, Mrs. Barbara MacLearn and Miss Andrea Plaskonos for their assistance in carrying out this work.

REFERENCES

1. Beeson, P. B.: Factors in the pathogenesis of pyelonephritis. *Yale J. Biol. Med.*, 1955, *28,* 81-104.
2. Vivaldi, E., Cotran, R., Zangwill, D. P., and Kass, E. H.: Ascending infection as a mechanism in the pathogensis of experimental non-obstructive pyelonephritis. *Proc. Soc. exp. Biol. (N. Y.),* 1959, *102,* 242-244.
3. Freedman, L. R.: Pyelonephritis and urinary tract infection. In: *Diseases of the Kidney,* Ed. by M. B. Strauss and L. G. Welt. Boston, Little, Brown, and Co., 1963. Pp. 469-500.
4. Cox, C. E. and Hinman, F., Jr.: Experiments with induced bacteriuria, vescical emptying and bacterial growth on the mechanism of bladder defense to infection. *J. Urol.,* 1961, *86,* 739-748.
5. Vivaldi, E., Munoz, J., Cotran, R., and Kass, E. H.: Factors affecting the clearance of bacteria within the urinary tract. In: *Progress in Pyelonephritis,* Ed. by E. H. Kass. Philadelphia, F. A. Davis Co. 1965. Pp. 531-535.
6. Cobbs, C. G. and Kaye, D.: Antibacterial activity of the urinary bladder. *J. Clin. Invest.,* 1966, *45,* 996.
7. Guze, L. B. and Beeson, P. B.: Experimental pyelonephritis. I. Effect of ureteral ligation on the course of bacterial infection in the kidney of the rat. *J. exp. Med.,* 1956. *104,* 803-815.
8. Andriole, V. T. and Epstein, F. H.: Prevention of pyelonephritis by water diuresis: Evidence for the role of medullary hypertonicity in promoting renal infection. *J. clin. Invest.,* 1965, *44,* 73-79.
9. Chernew, I. and Braude, A. I.: Depression of phagocytosis by solutes in concentrations found in the kidney and urine. *J. clin. Invest.,* 1962, *41,* 1945-1953.
10. Jackson, G. G. and Grieble, H.: Pathogenesis of renal infection. *Arch. int. Med.,* 1957, *100,* 692-700.
11. Miller, T. E. and North, J. D. K.: Effect of protein intake on bacterial growth in the kidney. *Brit. J. exp. Path.,* 1966, *47,* 105-115.
12. Wood, W. B., Jr.: Studies on the cellular immunology of acute bacterial infections. *Harvey Lectures,* series 47, 72-98, 1951-52.
13. Mehrota, R. M. L.: An experimental study of the vescical circulation during distention and in cystitis. *J. Path. Bact.,* 1953, *66,* 79-89.
14. O'Grady, F. and Pennington, J. H.: Bacterial growth in an *in vitro* system simulating conditions in the urinary bladder. *Brit. J. exp. Path.,* 1966, *47,* 152-157.
15. Mahoney, S. A. and Persky, L.: Observations on experimental ascending pyelonephritis in the rat. *J. Urol.,* 1963, *89,* 779-783.
16. Smith, B. N.: Fluid intake and experimental pyelonephritis. In: *Progress in Pyelonephritis,* Ed. by E. H. Kass. Philadelphia, F. A. Davis Co. 1965. Pp. 239-241.
17. Alderman, M. H. and Freedman, L. R.: Experimental pyelonephritis. X. The direct injection of *E. coli* protoplasts into the medulla of the rabbit kidney. *Yale J. Biol. Med.,* 1963, *36,* 157-164.
18. Guze, L. B. and Kalmanson, G. M.: Persistence of bacteria in "protoplast" form after apparent cure of pyelonephritis in rats. *Science,* 1964, *143,* 1340-1341.
19. Andriole, V. T.: Acceleration of the inflammatory response of the renal medulla by water diuresis. *J. clin. Invest.,* 1966, *45,* 847-854.