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Experimental *Candida albicans*, *Staphylococcus aureus*, and *Streptococcus faecalis* Pyelonephritis in Diabetic Rats

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Pyelonephritis was studied after an intravenous injection of *Candida albicans*, *Staphylococcus aureus*, or enterococcus in alloxan-diabetic rats and in water-diuresing or non-diuresing nondiabetic rats. The renal microbial populations of *C. albicans* or *S. aureus* were found to be $>10^5$ colony-forming units per g for up to 42 days in diabetic rats, whereas the kidneys tended to become sterile in nondiabetic rats. No significant difference was found in the course of enterococcal pyelonephritis in diabetic versus control rats. The difference in the 50% infective dose for each microorganism between diabetic and control rats was ≤ 1 $\log_{10}$. Neither duration of diabetes nor weight loss contributed to the greater and more sustained renal populations of *C. albicans* and *S. aureus* in diabetic rats. The inflammatory reaction in kidneys infected with *S. aureus* or *C. albicans* was greater in diabetic rats. Fungus balls associated with ureteral obstruction and gross multiple renal abscesses occurred in diabetic, but not in nondiabetic, rats infected with *Candida*. Growth of *C. albicans* and *S. aureus* in vitro in urine from diabetic rats was significantly greater than it was in urine from control rats. Addition of water or glucose to the urine of non-diuresing, nondiabetic rats significantly increased in vitro growth of *S. aureus* and *C. albicans*. These studies demonstrate greater severity of infection in the diabetic kidney due to *S. aureus* and *C. albicans*, which can be partially explained by decreased inhibitory activity of urine for these organisms in diabetic rats.

Experimental models of infections in rabbits, rats, and mice with chemically induced diabetes have shown increased susceptibility to various bacterial and fungal infections (3, 5, 8, 15, 17-19). Some studies to the contrary have shown no increased susceptibility to infection (7, 16). The present investigation was undertaken to determine the course of experimental pyelonephritis due to *Candida albicans*, *Staphylococcus aureus*, and enterococci in alloxan-diabetic rats and to study the effects of chronicity of diabetes, nutritional status of the animal, and the local factors within the urinary tract on the course of renal infection.

MATERIALS AND METHODS

Animals. White male Sprague-Dawley rats (Blue Spruce Farms, Altamont, N.Y.), weighing between 175 and 250 g, were used for all experiments. The rats were fed Purina laboratory rat chow (St. Louis, Mo.), free of antimicrobial drugs.

Organisms. One strain each of *C. albicans* B-311 (kindly supplied by V. T. Andriole) (3), *S. aureus* 502A (9), and an enterococcus, *Streptococcus faecalis* (9, 10), were used in all experiments. The course of pyelonephritis in normal animals after inoculation with these organisms has been characterized in detail (3, 9, 10). Stock cultures of *C. albicans* were maintained on Sabouraud dextrose agar slants at room temperature. Inocula for each experiment were prepared by scraping colonies from stock slants and subculturing in Sabouraud dextrose broth at 37°C for 18 h. Stock cultures of *S. aureus* or enterococci were maintained by storing samples of an 18-h culture in heart infusion broth (BBL Microbiology Systems, Cockeysville, Md.) at −20°C. Inocula for each experiment were prepared by subculturing a sample of the stock culture in heart infusion broth and incubating at 37°C for 18 h.

Enumeration of organisms. The number of organisms in broth was determined by plating 0.1 ml of each specimen on 5% sheep blood-Trypticase (BBL Microbiology Systems) soy agar plates, making serial 100-fold dilutions in heart infusion broth, and plating 0.1-ml samples of each dilution.

The number of organisms per gram of kidney was detetermined in the same manner, after homogenizing the kidney in 1 ml of heart infusion broth with Teflon tissue grinders (Tri-R Instruments, Inc., Rockville Center, N.Y.). The total number of viable organisms in tissue or in broth was calculated from colony counts after incubation of the plates for 24 h at 37°C. This technique does not detect less than 10 colony-forming units (CFU) per g of kidney; therefore, all "sterile" kidneys were recorded as containing 10 ($\log_{10}$ 1.0)

CFU/g. In each experiment, representative colonies were identified to assure identity with the organism originally inoculated.

Method of producing diabetes. Alloxan monohydrate (J. T. Baker Chemical Co., Phillipsburg, N.J.) was dissolved in sterile distilled water immediately before use, and 40 mg/kg was injected via the lateral tail vein of rats in a volume of 0.2 ml. Ten days after alloxan injection, 2-ml blood samples for glucose determinations were collected from all alloxan-treated and control animals by amputation of the tip of the animal's tail. Serum glucose determinations were performed by the ortho-toluidine method (6). Only those alloxan-treated rats with a serum glucose of ≥250 mg/dl (range 250 to 900 mg/dl) were used in experiments. The mean serum glucose for control rats was 102 ± 2 mg/dl.

Method of producing diuresis. Because glycosuria produces an osmotic diuresis and diuresis has been implicated in reducing susceptibility of the renal kidney to infection (2), both diuresing and non-diuresing rats were used as controls. Rats having access to 5% dextrose in tap water drink more than rats offered tap water and undergo water diuresis without glycosuria (2, 10). Control rats were begun on 5% glucose water 5 to 7 days before infection to insure a well-established water diuresis at the time of infection. Their urine was free of glucose when tested by a method using a glucose oxidase system (Keto-Diastix; Ames Co., Elkhart, Ind.).

Determination of the ID_{50}. Diabetic and age-matched control rats were divided into groups, and each group was given inocula of various sizes intravenously of either *C. albicans*, *S. aureus*, or the enterococcus. Rats were killed 5 days after infection. Rats were considered to be infected if the kidney grew $>10^2$ CFU/g of kidney. The 50% infective dose (ID_{50}) was estimated by the Reed-Muench method (4).

Course of pyelonephritis in short-term diabetic rats. Diabetic rats, 10 to 15 days after alloxan injection, and control nondiabetic litter mates were inoculated intravenously via the lateral tail vein with 1×10^5 *C. albicans*, 5×10^7 *S. aureus*, or 7×10^8 enterococci. Alloxan-diabetic rats were permitted free access to tap water, and the nondiabetic control rats were divided into two groups: one group drank tap water, and the other group drank 5% dextrose water (except enterotocci-infected control rats, who were allowed tap water only). Animals were killed at 18 h to 42 days after infection. Just before autopsy, all rats were placed in individual siliconized metabolic cages, and urine was collected for detection of glycosuria by Keto-Diastix.

After the urine collection, the rats were anesthetized by intrathoracic injection of pentobarbital sodium. The abdominal cavity was opened by sterile technique, blood was aspirated from the inferior vena cava, and serum was separated for glucose determinations. The left kidney was removed for determination of the microbial count, and the right kidney was placed in Bouin's solution for histological examination.

Course of pyelonephritis in long-term diabetic rats. Eleven months after alloxan treatment, 25 diabetic rats and 25 nondiabetic control rats were inoculated intravenously with 2×10^4 *C. albicans*. The diabetic rats were permitted free access to tap water, whereas control rats drank 5% glucose water ad libitum. Urine and blood were collected, and animals were handled as described above. Animals were killed at 18 h, 4 days, 8 days, 14 days, and 21 days after infection.

Pair feeding. Alloxan-diabetic and age-matched control rats were used in each experiment. Thirteen days after receiving alloxan, pairs of rats were inoculated intravenously with 1×10^5 *C. albicans* or 6×10^7 *S. aureus*. Animals were weighed daily. Alloxan-diabetic rats had unlimited access to tap water and food. Control animals were allowed to drink tap water ad libitum, but food intake was limited to maintain weights comparable to those of their alloxan-treated litter mates.

In vitro growth of *S. aureus*, *C. albicans*, and an enterococcus in urine. Nine pair-fed rats (three alloxan-diabetic, three water-diuresing control, and three non-diuresing control rats) were used in all experiments (Table 1). To collect urine, rats were placed in individual siliconized metabolic cages with unlimited access to water or 5% glucose water. Urine was collected from each rat under oil in iced containers and stored at −4°C. The samples from each rat were pooled. Samples were sterilized by filtration through a 0.45-μm filter (Nalgene), and osmolality and urea and glucose concentrations were determined (Table 1). *S. aureus*, *C albicans*, or enterococci (10^2 CFU) were inoculated into 1-ml samples of filtered, pooled

TABLE 1. *Osmolality, glucose and urea concentrations, and microbial populations of rat urine*

Urine taken from:	Osmolality[a] (mOsm/kg of water)	Glucose concn (mg/dl)	Urea concn (mg/dl)	*C. albicans* (CFU/ml)	*S. aureus* (CFU/ml)
Diabetic rats (3)[b]	1,083 ± 11	10,583 ± 161	2,104 ± 77	6.1 ± 0.5[c]	9.9 ± 0.0
+ Urea	1,265 ± 12	10,583 ± 161	2,967 ± 47	6.6 ± 0.2	9.0 ± 0.1
Water-diuresing rats (3)	85 ± 10	20 ± 5	192 ± 23	4.3 ± 0.5	8.8 ± 0.0
+ Glucose	671 ± 1	12,400 ± 1,382	207 ± 15	7.0 ± 0.0	9.7 ± 0.0
+ Urea	477 ± 3	20 ± 5	2,710 ± 23	2.2 ± 0.5	9.8 ± 0.0
Non-diuresing rats (3)	1,218 ± 64	15 ± 1.4	2,613 ± 143	<1.0	4.4 ± 0.2
+ Glucose	2,343 ± 90	10,617 ± 179	3,367 ± 412	1.0 ± 0.0	5.6 ± 0.4
+ Water	102 ± 5	10 ± 2	344 ± 17	5.2 ± 0.5	9.0 ± 0.0

[a] Values are mean ± standard error.

[b] Number of determinations.

[c] Log_{10}.

urine from each rat or heart infusion broth and incubated at 37°C. The number of organisms per ml was determined by sampling portions immediately after addition of the inoculum and at 24 h.

Glucose was added to samples of urine from diuresing and non-diuresing rats to achieve the same glucose concentration as was present in urine from diabetic rats. Urea was added to samples of urine from diabetic and diuresing rats to achieve the same concentration as was found in urine from non-diuresing rats. Samples of urine from non-diuresing rats were diluted with distilled water to produce an osmolality equal to that of urine from diuresing rats. *S. aureus* or *C. albicans* (10^2 CFU) were inoculated into 1-ml portions of the altered urine samples and handled as described for unaltered urine.

RESULTS

ID_{50}. Table 2 presents the ID_{50} for *C. albicans*, *S. aureus*, and the enterococcus. In both diabetic and control rats, the ID_{50} was least for *C. albicans* and greatest for the enterococcus. With all three organisms, the ID_{50} for diabetic and control rats differed by less than 1 $\log_{10}$. Although statistical significance cannot be determined when the ID_{50} is calculated by the Reed-Muench method (4), there did not appear to be a major difference in the susceptibility of diabetic and control rats to initiation of infection for the three organisms studied. The calculated *C. albicans* ID_{50} for diuresing and non-diuresing control rats also differed by less than 1 $\log_{10}$.

Course of *Candida* pyelonephritis. Figure 1 presents the renal microbial population in short-term diabetic, nondiabetic diuresing control, and nondiabetic non-diuresing control rats. At 2 days after infection, the renal population of *C. albicans* in diabetic rats was significantly greater ($P < 0.05$) than in diuresing controls. Thereafter, renal *Candida* counts were significantly greater in diabetic rats throughout the 42 days of the experiment than in either group of control rats ($P < 0.01$). By 42 days, virtually all control kidneys were sterile. In contrast, at 42 days in diabetic kidneys there were $\log_{10}$ 5.4 CFU/g.

The course of infection with *C. albicans* in-

TABLE 2. *ID_{50} for renal infection in diabetic and control rats*

Subject	ID_{50} (CFU) of:		
	C. albicans	*S. aureus*	Enterococcus
Diabetic rats	1.2×10^3	1.3×10^5	1.1×10^7
Non-diuresing control rats	1.0×10^4	3.2×10^5	5.4×10^6
Diuresing control rats	3.2×10^3	—[a]	—

[a] —, Not done.

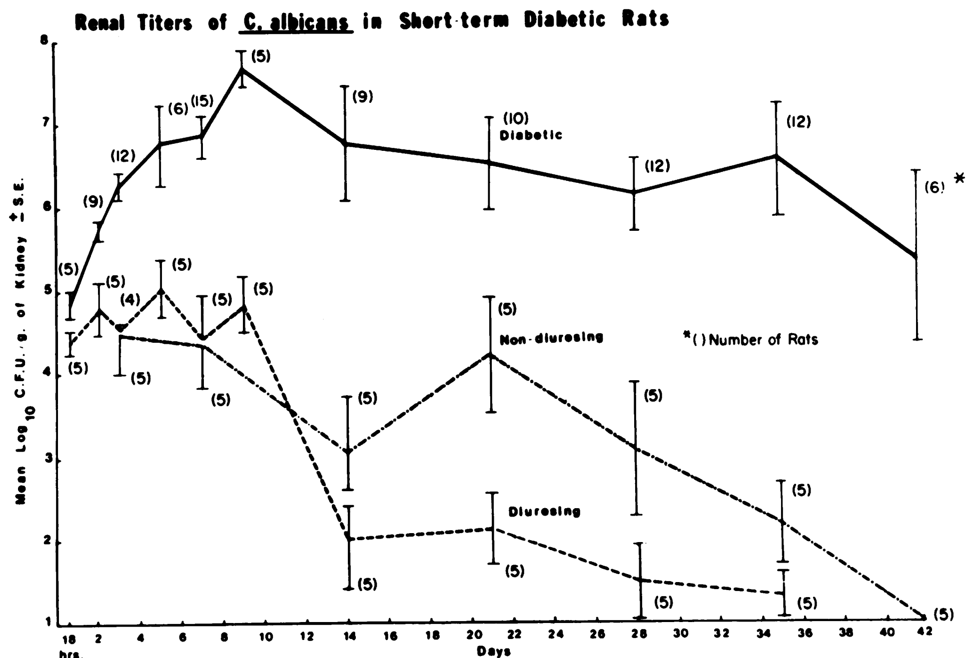

FIG. 1. *Microbial count of C. albicans (mean $\log_{10}$ CFU/g of kidney ± standard error) after intravenous injection of C. albicans in short-term alloxan rats and in nondiabetic rats drinking tap water or 5% glucose in water.*

diuresing controls was similar to non-diuresing controls ($P > 0.05$), except the renal *Candida* counts in non-diuresing rats were significantly greater ($P < 0.05$) than in diuresing control rats at 21 days.

The results of *Candida* infection in long-term diabetic rats are illustrated in Fig. 2. At 4 days after infection, renal *Candida* counts were significantly greater in diabetic rats as compared to diuresing control rats ($P < 0.01$). The difference in titers became even greater at 8 days and 14 days after infection. By 21 days after infection, four out of five control kidneys had counts of less than 10^1 CFU/g, whereas the mean count in the diabetic kidneys was greater than 10^7 CFU/g.

The mean renal weight (± standard error) in diabetic infected rats almost doubled from 1.4 ± 0.1 g at 1 to 3 days to 2.7 ± 0.4 g at 3 weeks and 2.2 ± 0.1 g at 7 weeks, whereas the mean renal weights in nondiabetic rats remained stable at 1.4 g. Multiple renal abscesses and ureteral dilatation, frequently associated with ureteral obstruction by fungus balls, were seen in diabetic, but not in nondiabetic, rats.

Course of staphylococcal pyelonephritis. Figure 3 presents the course of infection in diabetic, nondiabetic diuresing control, and nondiabetic non-diuresing control rats. The mean renal bacterial counts in non-diuresing control rats and diabetic rats were similar during the first week after infection. However, at 14 and 21 days after infection, the mean renal counts in the diabetic rats were significantly greater ($P < 0.01$) than the mean counts in non-diuresing controls. The diabetic rats maintained high counts throughout the 42 days of the experiment, with no indication of clearing the infection. Throughout the experiment, the mean renal counts in diuresing control rats were significantly lower than the counts in diabetic rats ($P < 0.01$). At 42 days after infection in diuresing control rats, the mean renal count was 1.9, and three out of five animals had sterile kidneys ($\log_{10} \leq 1.0$ CFU/g). The non-diuresing control rats had mean counts similar to diuresing controls at 18 h, 4 days, and 14 days ($P > 0.05$), but counts in non-diuresing controls were significantly higher than in diuresing controls at 2 days ($P < 0.05$), 7 days ($P < 0.01$), and 21 days ($P < 0.05$).

The kidneys from each group of infected rats appeared grossly similar, and the mean renal weights (± standard error) were similar in diabetic rats at 1 to 3 days (1.3 ± 0.0 g) and at 35 days (1.7 ± 0.2 g) and in nondiabetic controls (1.4 ± 0.1 g) at these times.

Course of staphylococcal pyelonephritis.

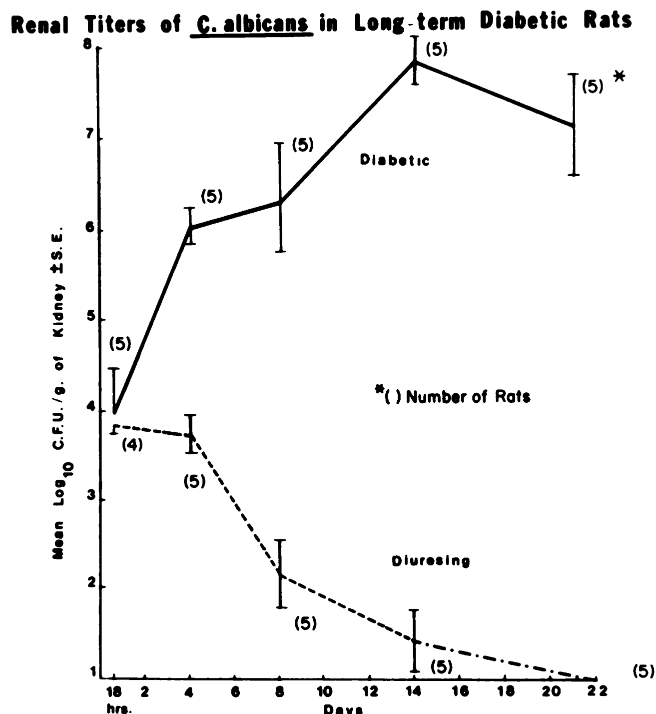

FIG. 2. *Microbial count of C. albicans (mean $\log_{10}$ CFU/g of kidney ± standard error) after intravenous injection of C. albicans in long-term alloxan rats and in nondiabetic rats drinking 5% glucose in water.*

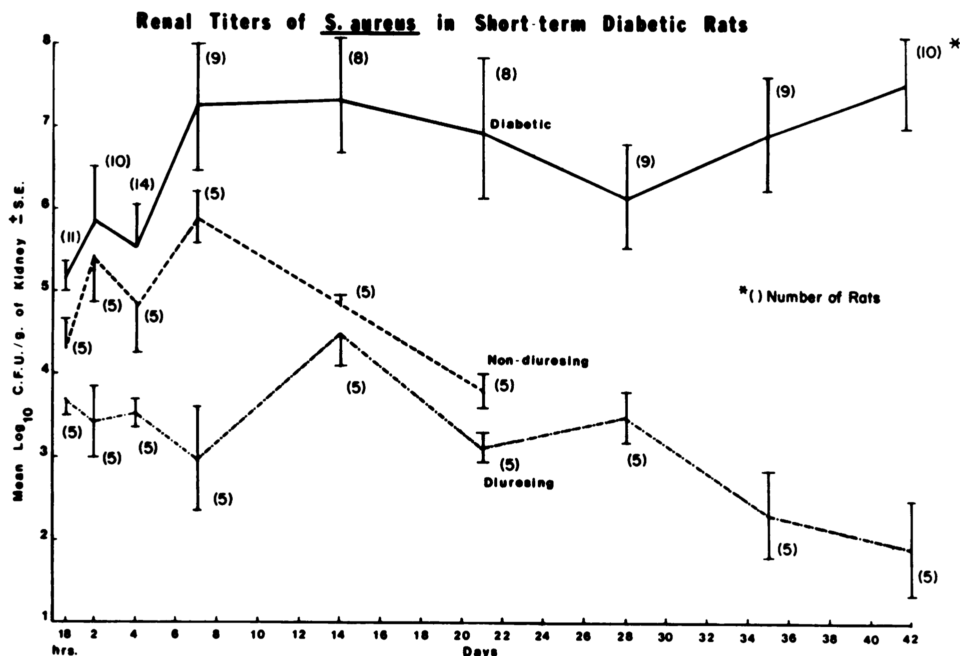

FIG. 3. *Microbial count of S. aureus (mean log_{10} CFU/g of kidney ± standard error) after intravenous injection of S. aureus in short-term alloxan rats and in nondiabetic rats drinking tap water or 5% glucose in water.*

Figure 3 presents the course of infection in diabetic, nondiabetic diuresing control, and nondiabetic non-diuresing control rats. The mean renal bacterial counts in non-diuresing control rats and diabetic rats were similar during the first week after infection. However, at 14 and 21 days after infection, the mean renal counts in the diabetic rats were significantly greater ($P < 0.01$) than the mean counts in non-diuresing controls. The diabetic rats maintained high counts throughout the 42 days of the experiment, with no indication of clearing the infection. Throughout the experiment, the mean renal counts in diuresing control rats were significantly lower than the counts in diabetic rats ($P < 0.01$). At 42 days after infection in diuresing control rats, the mean renal count was 1.9, and three out of five animals had sterile kidneys (lot_{10} ±1.0 CFU/g). The non-diuresing control rats had mean counts similar to diuresing controls at 18 h, 4 days, and 14 days ($P > 0.05$), but counts in non-diuresing controls were significantly higher than in diuresing controls at 2 days ($P < 0.05$), 7 days ($P < 0.01$), and 21 days ($P < 0.05$).

The kidneys from each group of infected rats appeared grossly similar, and the mean renal weights (± standard error) were similar in diabetic rats at 1 to 3 days (1.3 ± 0.0 g) and at 35 days (1.7 ± 0.2 g) and in nondiabetic controls (1.4 ± 0.1 g) at these times.

Course of enterococcal pyelonephritis. The renal enterococcal counts in diabetic and control rats infected with 7×10^8 enterococci were not significantly different at either 5 or 14 days after infection (Table 3).

Histology. Changes due to diabetes in both short- and long-term alloxan-treated rats consisted of glomerular basement membrane thickening. In pyelonephritis due to *S. aureus*, microabscesses were distributed in the interstitium of both cortex and medulla. The inflammatory reaction (initially composed of primarily polymorphonuclear leukocytes and later mononuclear cells) occurred earlier (1 to 4 days versus about 7 days), was more severe, and persisted longer in diabetic than in nondiabetic rats. Similarly, a focal distribution of yeast and hyphal forms accompanied by an intense cellular response was observed in the interstitial tissues of the medulla and cortex in both diabetic and nondiabetic rats. Initially, polymorphonuclear reaction surrounded the fungal elements, but by 2 weeks this was replaced by giant cell granulomas and eventually focal cortical scars. The histological changes occurred earlier, were more severe and generalized, and persisted longer (up to 7 weeks) in the diabetic in comparison to nondiabetic rats.

Pair-feeding. Table 4 presents the mean renal microbial counts in pair-fed diabetic and non-diuresing control rats infected with *C. albi-*

cans or *S. aureus*. At both 6 and 14 days, the mean renal *Candida* counts in diabetic rats were significantly higher than in control pair-fed rats ($P < 0.01$). The mean renal *S. aureus* counts at 14 and 21 days in diabetic rats were significantly higher than in pair-fed control rats ($P < 0.05$).

In vitro growth of *S. aureus*, *C. albicans*, and an enterococcus in urine. The number of colony-forming units of enterococci per ml after 24 h of incubation was similar in broth ($\log_{10}$ 8.7 CFU/ml), in urine from non-diuresing rats (mean $\log_{10}$ CFU/ml ± standard error, 8.8 ± 0.2), in urine from water-diuresing rats (8.2 ± 0.1), and in urine from diabetic rats (8.9 ± 0.1).

Growth of *C. albicans* in urine from diabetic rats (6.1 ± 0.5) was similar to that in broth ($\log_{10}$ 6.7 CFU/ml), but was greater than growth of *C. albicans* in urine from non-diuresing rats (<1.0) or in urine from diuresing rats (4.3 ± 0.5). Growth of *C. albicans* was enhanced in diuresing urine (7.0 ± 0.0) and to a lesser extent in non-diuresing urine (1.0 ± 0.0) supplemented with glucose. Dilution of non-diuresing urine with distilled water resulted in markedly increased growth of *C. albicans* (5.2 ± 0.5). Growth of *C. albicans* was reduced in urine from diuresing rats supplemented with urea (2.2 ± 0.5), but growth was unaltered in diabetic urine supplemented with urea (6.6 ± 0.2).

Growth of *S. aureus* in diabetic urine (9.9 ± 0.0) was greater than in broth (8.8 CFU/ml) or in urine from diuresing rats (8.8 ± 0.0). In contrast, growth of *S. aureus* was inhibited in urine from non-diuresing rats (4.4 ± 0.2). Growth of *S. aureus* was enhanced in urine from diuresing rats (9.7 ± 0.0) and to a lesser extent in urine from non-diuresing rats (5.6 ± 0.4) supplemented with glucose. Dilution of urine from non-diuresing rats with distilled water resulted in markedly increased growth of *S. aureus* (9.0 ± 0.0). Growth of *S. aureus* in urine from diuresing rats and from diabetic rats supplemented with urea was unaltered (9.8 ± 0.0 and 9.0 ± 0.1, respectively).

TABLE 3. *Renal counts of an enterococcus*

Day	Renal counts[a] of enterococcus in:	
	Diabetic rats	Non-diuresing control rats
5	4.9 ± 0.8	5.0 ± 0.7 ($P > 0.05$)
14	5.1 ± 0.6	4.5 ± 0.4 ($P > 0.05$)

[a] Mean $\log_{10}$ colony-forming units per gram ± standard error.

DISCUSSION

Despite a similar ID_{50} in diabetic and nondiabetic rats, renal microbial populations of *C. albicans* and *S. aureus* were found to be higher and more persistent in diabetic rats; in contrast, renal enterococcal populations were similarly sustained in both diabetic and control rats. Neither the duration of the diabetic state nor weight loss was found to be a contributing factor. The severity of renal infection was probably not a nonspecific effect of alloxan toxicity since infection was induced at least 10 to 15 days after alloxan treatment when the effects of the acute toxic phase or transient renal damage due to alloxan itself are no longer present (11).

In renal *S. aureus* infection, the renal weight remained stable, and no gross changes in the kidneys were noted in both diabetic and nondiabetic rats, but the inflammatory response was more severe in the diabetic kidney.

Renal *Candida* infection in diabetic rats was associated with a more severe renal inflammatory reaction, increased renal weight, gross renal abscesses, and ureteral obstruction with fungus balls. Findings in this study suggest that the diabetic kidney is more susceptible to reinfection by the ascending route as a result of an increased

TABLE 4. *Renal microbial counts in diabetic and pair-fed non-diuresing control rats*

Day	Renal counts[a] of:			
	C. albicans		*S. aureus*	
	Diabetic rats[b]	Non-diuresing control rats	Diabetic rats	Non-diuresing control rats
3	—[c]	—	6.4 ± 0.5 (5)	5.8 ± 0.3 (5) ($P > 0.05$)
6	6.9 ± 0.3 (5)	4.9 ± 0.5 (4) ($P < 0.01$)	—	—
14	8.0 ± 0.2 (3)	3.4 ± 0.4 (5) ($P < 0.01$)	7.2 ± 0.6 (10)	5.5 ± 0.4 (10) ($P < 0.05$)
21	—	—	6.2 ± 0.8 (6)	3.4 ± 0.5 (7) ($P < 0.05$)

[a] Mean $\log_{10}$ colony-forming units per gram ± standard error.
[b] Number of rats is given in parentheses.
[c] —, Not done.

size or infectivity of the urinary *S. aureus* or *Candida* population possibly due to (i) increased vesico-ureteral reflux, which is known to occur in diuresing rats (1); (ii) the decreased antimicrobial activity in diabetic urine due to the presence of glycosuria or dilution of inhibitory substances; and (iii) ureteral obstruction by masses of fungi in diabetic urine. The first factor per se seems not to be significant since the kidneys of diuresing nondiabetic rats cleared these organisms relatively rapidly. Enterococci which grew equally well in urine from diabetic and nondiabetic rats caused equally sustained renal infection.

Other possible mechanisms responsible for the greater severity of renal infection in diabetes include defects in polymorphonuclear leukocyte function or cellular immunity, which were not specifically addressed in the present study. Suppression of cell-mediated immunological reactivity has been reported in both diabetic mice (12, 13) and in nondiabetic rats with experimental *Escherichia coli* pyelonephritis (14, 20). However, the earlier, more intense and sustained inflammatory response in the renal lesions in diabetic rats suggests that cell reactivity was not suppressed, but may have been relatively defective in clearing microorganisms from the kidney in this model, because of conditions in diabetes which favored urinary proliferation of *S. aureus* and *C. albicans*.

LITERATURE CITED

1. **Anderson, B. R., and G. G. Jackson.** 1961. Pyelitis, an important factor in the pathogenesis of retrograde pyelonephritis. J. Exp. Med. 114:375-384.
2. **Andriole, V. T., and F. T. Epstein.** 1965. Prevention of pyelonephritis by water diuresis: evidence for a role of medullary hypertonicity in promoting renal infection. J. Clin. Invest. 44:73-79.
3. **Andriole, V. T., and H. F. Hasenclever.** 1962. Factors influencing experimental candidiasis in mice. I. Alloxan diabetes. Yale J. Biol. Med. 35:96-112.
4. **Bancroft, H.** 1960. Bioassay, p. 198-203. *In* Introduction to biostatistics. Harper and Brothers, New York.
5. **Coil, J. A., and J. H. Davis.** 1967. Altered host response to experimental pyelonephritis in alloxan diabetic rats. J. Surg. Res. 7(S):26-34.
6. **Cooper, G. R., and V. McDaniel.** 1970. The determinations of glucose by the ortho-toluidine method (filtrate and direct procedure), p. 159-170. *In* R. P. MacDonald (ed.), Standard methods of clinical chemistry, vol. 6. Academic Press, Inc., New York.
7. **Cruickshank, A. H.** 1954. Resistance to infection in the alloxan-diabetic rabbit. J. Pathol. Bacteriol. 67:323-334.
8. **Hurley, R.** 1966. Experimental infection with *Candida albicans* in modified hosts. J. Pathol. Bacteriol. 92:57-67.
9. **Kaye, D., and H. Rocha.** 1970. Urinary concentrating ability in early experimental pyelonephritis. J. Clin. Invest. 49:1427-1437.
10. **Levison, S. P., and D. Kaye.** 1972. Influence of water diuresis on antimicrobial treatment of enterococcal pyelonephritis. J. Clin. Invest. 51:2408-2413.
11. **Lukens, F. D. W.** 1948. Alloxan diabetes. Physiol. Rev. 28:304-330.
12. **Mahmoud, A. A. F., A. W. Cheever, and K. S. Warren.** 1975. Streptozotocin-induced diabetes mellitus and the host-parasite relation in murine schistosomiasis mansoni. J. Infect. Dis. 131:634-642.
13. **Mahmoud, A. A. F., H. M. Rodman, M. A. Mandel, and K. S. Warren.** 1976. Induced and spontaneous diabetes mellitus and suppression of cell-mediated immunologic responses. J. Clin. Invest. 57:362-367.
14. **Miller, T., L. Scott, E. Stewart, and D. North.** 1978. Modification by suppressor cells and serum factors of the cell-mediated immune response in experimental pyelonephritis. J. Clin. Invest. 61:964-972.
15. **Mukherji, A. K., and K. C. Basu Mallik.** 1975. *Candida albicans* infection in alloxan induced diabetic mice. Indian J. Med. Res. 63:539-544.
16. **Schofield, R. A., and R. D. Baker.** 1956. Experimental mucormycosis (rhizopus infection) in mice: failure of chronic alloxan diabetes mellitus to modify host susceptibility. Arch. Pathol. 61:407-415.
17. **Sheldon, W. H., and H. Bauer.** 1959. The development of the acute inflammatory response to experimental cutaneous mucormycosis in normal and diabetic rabbits. J. Exp. Med. 110:845-852.
18. **Sood, U., D. S. Agarwal, and A. L. Aurora.** 1975. A study of subcutaneous staphylococcal infection in alloxan diabetic mice. Indian J. Med. Res. 63:1564-1576.
19. **Wertman, K. F., and M. R. Henney.** 1962. The effects of alloxan diabetes on phagocytosis and susceptibility to infection. J. Immunol. 89:314-317.
20. **Williams, T. W., A. M. Friedlander, J. M. Lyons, and A. I. Braude.** 1976. Cellular immunity in pyelonephritis: identification of suppressor cell activity of spleen cells in response to concanavalin A and inhibition of lymphocyte-mediated L cell cytotoxicity. J. Immunol. 116:778-781.