Experimental Pyelonephritis in Rats with Alloxan Diabetes.* (28905)

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It is an accepted clinical dictum that pyelonephritis is more common in patients with diabetes mellitus. Whether this is due to the fact that these patients are subjected to more frequent catheterization and instrumentation permitting introduction of bacteria into the urinary tract or whether the kidney of the diabetic is inherently more susceptible to infection has not been determined(1). In the experiments to be described the susceptibility of rats with alloxan diabetes to experimental pyelonephritis with *Escherichia coli* was studied.

Material and methods. All experiments were carried out in female Sprague-Dawley rats weighing 150-200 g. The experimental design is summarized in Fig. 1. Animals were injected intravenously with alloxan in dosage of 0.04 mg per g body weight. At the end of one week the majority of animals had blood sugar levels between 200 and 700 mg%. On the eighth day, left ureteral ligation was performed resulting in development of hydronephrosis. The hydronephrotic kidney is susceptible to hematogenous infection with *E. coli*, an organism which rarely produces renal infection when injected intravenously into normal rats(2). A known number of *E. coli* were injected intravenously on the 15th day, and the animals were sacrificed one week later. Both ligated and unligated kidneys were removed aseptically, weighed and hemisected. Histologic studies were performed on one-half and the remainder was homogenized for quantitative bacterial counts of serial 10-fold dilutions of renal homogenate. Each colony was equated with one viable unit. Infection was defined as the presence of more than 1000 bacteria per g renal tissue. In some animals liver and spleen cultures were also obtained; the organs were handled in similar fashion.

Usually the number of organisms in kidneys either exceeded 10,000 bacteria/g or the kidney was sterile and contained fewer than 10 organisms. For this reason 1000 bacteria/g was considered a reasonable criterion for infection. Thorough homogenization minimized sampling errors. In a number of animals 2 samples from each kidney were counted and the results were always in good agreement (less than 0.5 log). Contamination with other bacteria occurred in less than 5% of animals and these were omitted in calculation of the results.

A strain of *E. coli*, serologic type 0-28, was used in all experiments. The organism was maintained on agar slants and passed through rats at monthly intervals to maintain virulence. On the day before inoculation organisms were transferred to tryptose phos-

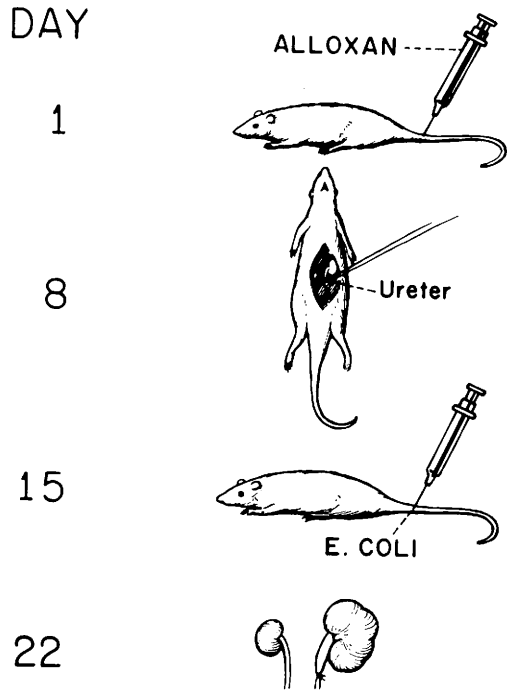

FIG. 1. Experimental design for production of hematogenous *E. coli* pyelonephritis in alloxanized rats.

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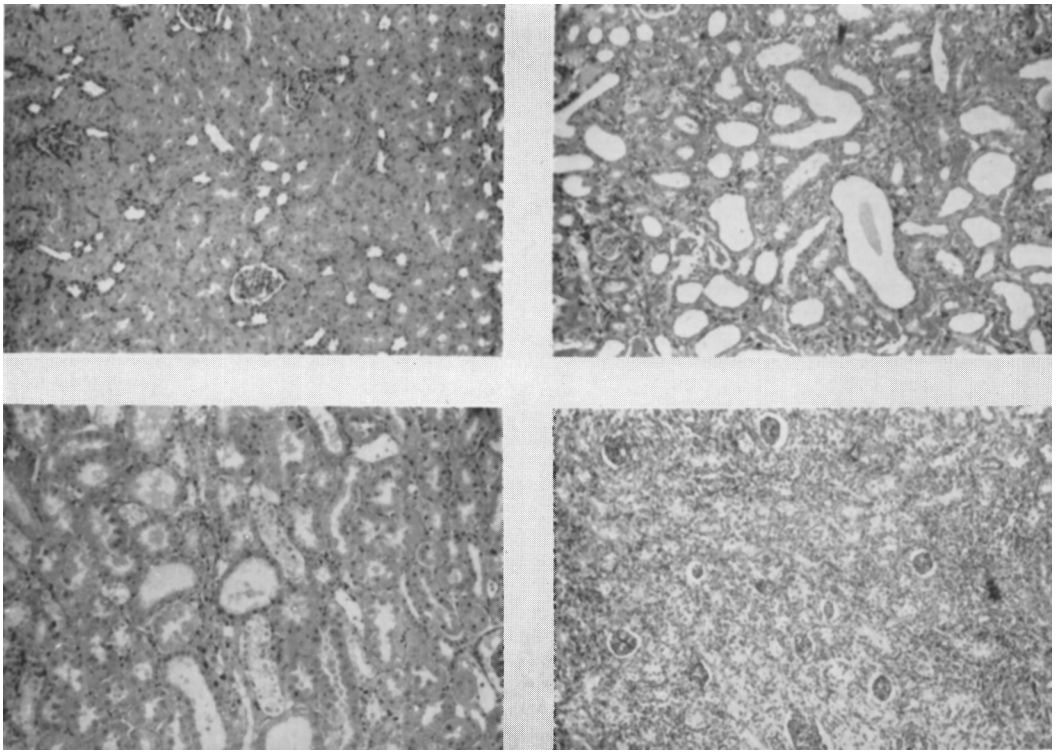

FIG. 2. Histologic features of experimental diabetes and infection: (a) normal kidney (top left); (b) hydronephrosis following ureteral ligation (top right); (c) alloxan diabetes without ureteral ligation (bottom left); (d) pyelonephritis in rats with ligated ureter, following intravenous injection of *E. coli* (bottom right).

phate broth and incubated at 37° for 18 hours. Organisms were then subcultured to tryptose phosphate broth and a fresh 5 hour culture used in all experiments. The number of organisms injected was estimated by nephalometry and was subsequently verified by plate counts of serial 10-fold dilutions. Growth characteristics of the organisms remained constant throughout these studies. Glucose was determined on venous blood on day 8, 15 and 22, using glucose-oxidase(3).

Experimental results. Pathology: On gross examination no detectable abnormalities were noted between the kidneys of normal and alloxanized animals which had not been subjected to ligation of the ureter. Microscopically, however, some of the kidneys from alloxanized animals showed tubular degeneration and dilatation and thinning of tubular epithelium (Fig. 2c), in contrast to normal kidneys in which the tubular epithelium was cuboidal and virtually obliterated the tubular lumen (Fig. 2a). Uninfected hydronephrotic kidneys showed marked tubular dilatation (Fig. 2b); kidneys from ligated control animals could not be distinguished from ligated alloxanized animals. Infection in ligated kidneys of both normal and alloxanized animals was characterized by abscess formation grossly, and a dense inflammatory exudate microscopically (Fig. 2d). Unligated infected kidneys also showed abscesses grossly although the pyonephrosis which characterized ligated infected kidneys was not present. Microscopically these kidneys showed a dense inflammatory exudate with formation of micro-abscesses and some degree of tubular dilatation.

Susceptibility of ligated kidneys in normal and alloxanized animals (Table I): Hydronephrotic kidneys were highly susceptible to infection regardless of pretreatment with alloxan. When the number of bacteria injected was decreased 10-fold to 10^7 viable units,

TABLE I. Infection in Ligated Kidneys of Rats with Alloxan Diabetes and Normal Controls.

No. bacteria inj	No. kidneys infected: Alloxan-diabetes	Control	p value
10^8	29/37 (78%)	21/26 (86%)	$\geq .5$
10^7	17/21 (81%)	9/18 (50%)	$\leq .05$

TABLE II. Infection in Unligated Kidneys of Rats with Alloxan Diabetes and Normal Controls.

No. bacteria inj	No. kidneys infected: Alloxan-diabetes	Control	p value
10^8	24/27 (65%)	12/26 (46%)	$\geq .1$
10^7	16/21 (76%)	3/18 (17%)	$\leq .001$

there was some indication of a higher incidence of infection in hydronephrotic kidneys from alloxanized animals.

Incidence of infection in contralateral kidneys with unligated ureters (Table II): An appreciable number of animals whose ligated kidneys were infected also harbored infection in the contralateral kidney which had not been subjected to ureteral ligation (henceforth called unligated kidney). When 10^8 *E. coli* were administered 46% of control and 65% of alloxanized animals developed pyelonephritis. This difference was much more marked when the infecting dose was reduced to 10^7 bacteria. With this inoculum kidneys from alloxanized animals were significantly more susceptible to infection than those of controls. Animals which developed infection in the unligated kidney always had infection in its hydronephrotic mate. In general, the number of bacteria in both kidneys was equal; occasionally the unligated kidney contained slightly fewer organisms than its ligated partner.

These results suggested the possibility that kidneys from alloxanized rats not subjected to ureteral ligation might be more vulnerable than those of normal controls. With an inoculum of 10^7 *E. coli* only 1 of 24 kidneys from alloxanized rats and none of 20 from controls harbored infection. When 10^8 organisms were injected into rats whose ureters had not been ligated, 2 of 34 (6%) kidneys of controls were infected while 12 of 104 (21%) of alloxanized kidneys contained more than 1000 *E. coli* per g renal tissue. These results are statistically significant ($p = .05$), and indicate that in some instances alloxanization *per se* renders the kidney susceptible to hematogenous pyelonephritis, although certainly less so than ureteral ligation.

Infection in other organs: Liver and spleen were removed at random from some animals in each experimental group (alloxanized-ligated, normal-ligated, alloxanized-unligated, normal-unligated) and bacterial counts were performed. In no instance was infection present. Blood cultures were not performed.

Relationship of hyperglycemia to development of pyelonephritis: Measurements of blood sugar in rats given alloxan demonstrated great variability in response of different animals; some remained relatively normoglycemic while others developed blood sugars in excess of 500 mg%. Table III summarizes the relationship between degree of hyperglycemia and incidence of infection in ligated and unligated kidneys, as well as kidneys of animals not subjected to ureteral ligation. There was no correlation between level of blood sugar and development of infection, suggesting that the susceptibility of alloxanized animals to infection is a consequence of the renal lesion induced by this chemical.

Discussion. These results indicate that kidneys of rats with alloxan diabetes are somewhat more susceptible to hematogenous pyelonephritis with *E. coli* than kidneys of normal rats. This propensity to infection occurred both in the presence and absence of ureteral obstruction, and was noted only with relatively small inocula (10^7 organisms) in animals with ligated ureters, and with a large number of bacteria (10^8 viable units) in the

TABLE III. Relationship of Blood Sugars to Development of Pyelonephritis in Rats with Alloxan Diabetes.

Blood sugar	% infection: Ligated kidneys	Unligated kidneys	Normal kidneys
	(58)	(58)	(104)
200 mg %	82%	68%	18%
200-500 "	86%	71%	29%
500-750 "	17%	67%	15%

No. of kidneys in parentheses.

absence of gross obstruction. The effect appeared to be specific for renal tissue and infection was not noted in other organs; likewise, the loss of body weight (approximately 20%) which regularly accompanied alloxanization did not predispose animals to renal infection.

The capacity of alloxan for rendering the kidney more vulnerable to infection is not surprising since a number of stimuli which produce injury to renal tubules(4,6), reduce the vascular supply or induce hypertension, both permanently(7,9) and transiently(10), have rendered the kidney more vulnerable to hematogenous infection. The lesions produced by alloxan were primarily tubular; however, animals developing evidence of infection did not always have these tubular lesions in the uninfected contralateral kidney, suggesting that the injury may, at times, be more subtle than overt intrarenal hydronephrosis. Furthermore, it should be emphasized that alloxanization was a much weaker predisposing stimulus to renal infection than ureteral obstruction, and in the absence of gross obstruction large numbers of bacteria were necessary to demonstrate increased susceptibility of the alloxan-treated kidney to hematogenous infection. Other maneuvers including induction of hypertension with DCA and unilateral nephrectomy(7), unilateral artery constriction(8), administration of angiotensin(10), and production of intrarenal hydronephrosis by electrocautery(11) were much more effective in increasing renal susceptibility to infection than administration of alloxan.

The observation that the kidneys with unligated ureters in animals subjected to left ureteral ligation became infected in a significant number of instances is interesting for two reasons. First, infection was not noted in these kidneys of non-diabetic controls unless large inocula were used. Second, the incidence of infection was much higher in unligated kidneys of animals with ureteral ligation on the opposite side than in normal animals with unobstructed ureters. The best explanation for these results is that the pyonephrotic kidney served as a reservoir for repeated bouts of bacteremia which resulted in infection of the contralateral kidney made somewhat more susceptible with alloxan. In contrast, animals with unobstructed ureters did not trap a significant number of microorganisms in the kidney following a single injection, and therefore did not possess a nidus for repeated discharge of bacteria into the blood stream.

The renal lesions associated with alloxanization are relatively non-specific and were certainly no more effective in predisposing the kidney to hematogenous infection than a number of other stimuli. Furthermore, the renal injury produced by alloxan is somewhat dissimilar from the pathologic picture seen in the kidney of patients with diabetes in which glomeruli and blood vessels as well as the tubules are usually affected. For these reasons, these data cannot be interpreted to mean that the kidney of the diabetic is inherently more susceptible to infection. However, the observation that there was complete dissociation between the severity of diabetes, as measured by hyperglycemia, and development of pyelonephritis is an important piece of evidence against the argument that the metabolic abnormalities associated with diabetes are important predisposing factors to renal infection. Whether diabetic renal disease *per se* predisposes to infection must await the experimental production of glomerular and vascular lesions in animals more closely resembling those seen in human diabetes.

Summary. Rats with alloxan diabetes were made hydronephrotic and injected with *E. coli* intravenously. When 10^7 bacteria was injected, the incidence of pyelonephritis in hydronephrotic and contralateral kidneys was higher in diabetic than control animals. Alloxanized rats without hydronephrosis were also somewhat more susceptible to infection than controls. There was no relationship between degree of hyperglycemia and development of infection.

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Increased Susceptibility of Burned Rats to *Pseudomonas aeruginosa.** (28906)

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The problem of bacterial infection following extensive, severe burns has been the subject of intensive investigation. In addition to clinical studies this problem has been investigated utilizing laboratory animals. Rosenthal and co-workers(1-3) have long studied the role of infection in extensively burned mice. They have found that septicemia is an important factor in the delayed deaths of burned mice and that certain chemotherapeutic agents are effective in promoting survival. Goshi *et al*(4) reported that thermal injury in rabbits increased their susceptibility to *Staphylococcus aureus* and Liedberg(5) has shown that moderately burned guinea pigs also demonstrate increased susceptibility to *Pseudomonas aeruginosa*. The purpose of this study was to determine whether there was an increased susceptibility in burned rats to *Ps. aeruginosa* and if so, how soon this decrease in innate host resistance occurred following burns.

Materials and methods. Culture. A chloramphenicol resistant, streptomycin sensitive strain of *Ps. aeruginosa,* which had been isolated from a hospital patient, was used for all experiments. This strain was maintained on trypticase soy agar (BBL) and frequently passed through rats.

Burning techniques. Male albino rats of the Sprague-Dawley strain weighing 180 to 190 g were shaved. Burning was accomplished by means of a steam chamber which consisted of a metal cylinder with one side molded in the general shape of the back of the rat and connected at the other side to a steam generator. A rat anesthetized with sodium pentobarbital was placed on the chamber in an inverted position and burned for 30 sec. Full thickness burns of 30-34% of the total body surface were produced. To obtain 80-90% survival over the first 48-hour post-burn period, 0.85% NaCl solution equivalent to 15% of the body weight of a rat was injected intraperitoneally.

LD_{50} determination. The test organism after being passed through a rat was grown on trypticase soy agar for 20 hours at 37°C. Cells were harvested, washed 3 times with 0.1 M phosphate buffer (pH 7.0), and adjusted to a concentration of 3.4×10^9 cells/ml by means of a Klett-Summerson colorimeter (650 mμ filter). The number of organisms in the stock suspension was verified by replicate plates. Serial 10-fold dilutions were made and groups of 5 rats injected intraperitoneally, or subcutaneously with 0.1 or 1.0 ml aliquot of each dilution and observed for 28 days. Liver, spleen, and blood were cultured from all animals which died, and data were included only for those animals from which *Ps. aeruginosa* was recovered. The method of Reed and Muench(3) was used for calculating LD_{50}.

Results. In studying bacterial infection in burned animals it is necessary to distinguish

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