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LABORATORY INVESTIGATION

Cortical and papillary absorptive defects in gentamicin nephrotoxicity

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Cortical and papillary absorptive defects in gentamicin nephrotoxicity. Renal function was examined in rats given daily injections of gentamicin (100 to 150 mg/kg) for 10 to 14 days. Whole kidney inulin clearance fell and urine volume increased. Single nephron GFR of surface nephrons varied. Some nephrons had no filtration, some had low rates, and some had high rates. Abnormal renal tubular epithelial inulin permeability was demonstrated by microinjection. Micropuncture of individual nephrons early and later in their course demonstrated reduced fluid reabsorption along the proximal convoluted tubule of superficial nephrons. Rates of fluid delivery to the late proximal and distal tubule were elevated. The rate of fluid reabsorption in the superficial loop of Henle was increased. Maximal urine osmolality and papillary tissue content of urea was reduced. The polyuria, therefore, results from decreased fluid reabsorption by proximal tubules and, probably, by papillary collecting ducts. The decrease in proximal fluid reabsorption is probably secondary to impaired solute reabsorption. A decrease in collecting duct fluid absorption can be attributed to the observed decrease in papillary solute concentration.

Anomalies d'absorption corticale et papillaire dans la néphrotoxicité par la gentamicine. La fonction rénale a été examinée chez des rats recevant des injections journalières de gentamicine (100 à 150 mg/kg) pendant 10 à 14 jours. La clearance de l'inuline du rein entier a chuté, et le volume urinaire a augmenté. Le débit de filtration glomérulaire néphronique individuel des néphrons superficiels a varié. Certains néphrons n'avaient pas de filtration, certains avaient des débits faibles et certains avaient des débits élevés. Une perméabilité anormale de l'épithélium tubulaire rénal à l'inuline a été démontrée par microinjection. Des microponctions du début et de la fin de néphrons individuels ont démontré une diminution de réabsorption de fluide le long du tubule contourné proximal des néphrons superficiels. Les débits de fluide délivré au tubules proximal tardif et distal étaient élevés. Le débit de réabsorption de fluide dans l'anse de Henlé superficielle était augmenté. L'osmolalité urinaire maximale et le contenu tissulaire papillaire en urée étaient diminués. De la sorte, la polyurie est dûe à une diminution de la réabsorption de fluide par les tubules proximaux, et probablement par les canaux collecteurs papillaires. La diminution de la réabsorption proximale de fluide est probablement secondaire à une anomalie de la réabsorption des solutés. Une diminution de l'absorption de fluide dans le canal collecteur peut être attribuée à la diminution observée de la concentration papillaire en solutés.

Drugs that damage the kidney and reduce the rate of glomerular filtration may do so without reducing the rate of urine excretion. In some types of acute renal failure, particularly in some experimental situations in which drug dosage and fluid balance can be controlled, urine volume can increase when filtration rate falls [1–4]. Gentamicin, an aminoglycoside antibiotic frequently used in patients with serious infections, is a drug that can produce this syndrome of polyuric acute renal failure [5–8].

The degree to which gentamicin affects the kidney varies with the dose given and the time of exposure. In addition there are definite strain and species differences in the doses which achieve a given response. In the Sprague-Dawley strain of rat, polyuria may occur with a dose as small as 10 mg/kg/day [9]. A dose of 40 mg/kg/day in one study [9] and as much as 60 mg/kg/day [8] in another did not affect GFR. When 80 to 100 mg/kg/day is given for 10 to 14 days, acute renal failure (reduced GFR and azotemia) and polyuria occur together with extensive necrosis of the proximal convoluted epithelial cells [10, 11]. In Fischer 344 rats a dose as low as 40 mg/kg/day causes the syndrome of polyuric acute renal failure [7, 12, 13]. In dogs 30 mg/kg/day produces such changes [14]. In each of these animals there is a close temporal relationship between the severity of the renal failure and the polyuria [9, 10, 12–14]. In humans, gentamicin appears to produce acute renal failure in much the same manner at doses of 3 to 5 mg/kg [6, 8]. Even the occurrence of frank polyuria has been noted [15]. Thus, although the species sensitivity to gentamicin nephrotoxicity differs, the expression of that renal toxicity both morphologically and functionally is similar in all species.

Yet there is controversy about the timing of the polyuria with the fall in GFR. Cuppage et al [9] report polyuria without reduced GFR, while Avasthi et al [16] report reduced GFR without polyuria and both use 40 mg/kg in Sprague-Dawley rats. In another study in which the Munich-Wistar rat was studied, a modest reduction in GFR was observed before urine flow was clearly increased [17]. A defect in maximum concentrating ability may precede the polyuria, as well [10, 11]. Polyuria, therefore, seems to be most consistently present after prolonged exposure to higher doses of gentamicin when GFR is definitely reduced and azotemia clearly established [6, 11, 18].

Several recent studies in Sprague-Dawley rats have examined the polyuria during gentamicin-induced renal failure. In one study neither water deprivation nor vasopressin administration restored urine volume to normal [18]. In clearance studies, solute free water reabsorption [10] and free water clearance [11] were normal during the polyuria, suggesting normal thick ascending limb solute transport. These studies did not identify

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the sites of impaired fluid reabsorption. We undertook the present study to more closely examine nephron function during gentamicin-induced polyuric renal failure. To be certain that polyuria was a consistent feature, we administered 100 to 150 mg/kg gentamicin per day to the Sprague-Dawley strain of rat. These animals were studied only after urine flow was clearly increased.

Methods

Induction of polyuric acute renal failure

A total of 53 Sprague-Dawley rats were studied using three protocols. All rats were placed in metabolic cages and were given free access to water. They were fed 15 g of a standard rat pellet diet (Mouse, Rat, and Hamster Formula, Charles River, North Wilmington, Massachusetts) daily. After a 5-day period of adjusting to their new environment, urine samples were collected under oil in graduated plastic cylinders for 24-hr periods; daily urine volume and osmolality were also determined. After a 3-day period, the rats were divided into two groups. One group received 100 to 150 mg/kg gentamicin intramuscularly daily in a single dose; the other group received an equal volume of isotonic saline. When daily urine volume in the gentamicin-treated group exceeded that in the control group by 50% or more for 3 consecutive days, rats were studied using one of three protocols described below. This induction period usually lasted 10 to 14 days.

Preparation for micropuncture and microinjection

Polyuric and normal rats were anesthetized with Inactin (BYK Gulden, Konstanz, West Germany) 100 mg/kg i.p. and placed on a heated surgical table. Body temperature was monitored by a rectal temperature probe (Yellow Springs Instruments Co., Inc., Yellow Springs, Ohio) and maintained at 37 to 38°C. Polyethylene catheters were placed in the right external jugular vein and in the left internal carotid artery to permit monitoring of arterial pressure continuously with a strain-gauge transducer (Ailtech, City of Industry, California) and recorder (Gould Inc., Cleveland, Ohio). A short polyethylene tube was placed in the trachea. The left kidney was exposed through a flank incision and supported in a plastic cup after carefully freeing it from the surrounding perirenal fat. The kidney surface was bathed continuously with mineral oil warmed to 37°C (YSI, Yellow Springs, Ohio), and the surface was illuminated with a fiber optic illuminator fitted with an infrared filter to minimize heat. The left ureter was cannulated with a polyethylene catheter (0.28 mm I.D.); urine samples were collected under oil into preweighed plastic tubes. Following cannulation of the external jugular vein, isotonic NaCl-$NaHCO_3$ solution was infused intravenously at either 0.8 ml/hr and per 100 g of body weight (free flow micropuncture studies) or 2.0 ml/hr/100 g of body weight (microinjection studies). No attempt was made to replace surgical losses of fluid. In experiments in which the recovery of microinjected inulin was analyzed, the urinary bladder was exposed by a low midline abdominal incision; the bladder was cannulated by a polyethylene catheter inserted through an incision at the dome of the bladder.

Free-flow micropuncture. A total of 27 (11 control and 16 gentamicin-treated) rats were studied. In these experiments the infusion solution contained enough (methoxy-^{3}H)-inulin (New England Nuclear, Boston, Massachusetts) to deliver 150 μCi/hr. The first of two to four clearance periods was begun 100 min after starting the infusion. Blood was collected in heparinized microhematocrit tubes at the start of the first clearance period and thereafter at 30-min intervals throughout the course of the experiments. The tubes were spun in a microhematocrit centrifuge (International Equipment Co., Needham Heights, Massachusetts), and the plasma was separated for later analysis. Clearance periods lasted 60 to 90 min. Because tubule fluid flow rate varied from one nephron to another, fluid samples were obtained from two sites in the same nephron. These sites were identified by inserting a searching micropipet (tip O.D. 3 to 4 μm) into a proximal tubule and injecting small volumes of a solution of isotonic NaCl-$NaHCO_3$ lightly colored with a green dye (FD&C Green no. 3, Keystone Aniline and Chemical Co., Chicago, Illinois) (final concentration of 1 g/liter). By observing the passage of the green fluid, the last proximal segment on the surface was identified. This segment was then punctured with a micropipet (tip O.D. 10 to 12 μm) filled with Sudan black stained oil and a complete, timed collection of fluid was made. The searching pipet remained within the lumen throughout the collection. At the conclusion of the collection the searching pipet was withdrawn, and earlier segments of the same nephron were sought using the same pipet. Only nephrons having five surface segments were used. During the collection of fluid from these proximal tubules, special care was taken not to distend or collapse the tubule. Surface distal tubules and late proximal tubules belonging to the same nephron were punctured using the same microperfusion technique described above. Distal tubules were punctured with pipets having tip diameters of 8 to 10 μm.

Proximal tubule microinjection. In these experiments five control and seven gentamicin-treated rats were studied. (^{3}H) inulin was added to isotonic NaCl/$NaHCO_3$ to achieve a final concentration of 1 mCi/ml. This solution, colored lightly with FD&C green No. 3 dye, was placed between oil columns in a constant bore quartz capillary tube of 100 μm I.D. Four-nanoliter portions of this fluid measured with the aid of an eyepiece micrometer were drawn into oil-filled pipets having diameters of 7 to 10 μm. A small quantity of mineral oil was then aspirated into the tip. Alternately, these samples were either injected into glass vials and used as reference standards for total (^{3}H) inulin injected or were injected into tubules. Urine samples were collected for 15 min before microinjection from the left ureteral and bladder catheters to establish the background radioactivity. The pipet was then inserted into a proximal tubule segment chosen at random and a small volume of tubule fluid was aspirated to clear the tip of oil. The fluid was injected slowly over 2 to 3 min. Care was taken to avoid distention of the tubule, retrograde flow of colored fluid, injection of oil, and leakage of the test solution. Only injections which met these conditions were analyzed. After starting the microinjection, urine samples were collected in scintillation vials during four 15-min intervals at which time counting rates returned to background or added insignificantly to calculated recoveries.

Tissue solute determination. A total of seven gentamicin-treated, and seven control rats were studied. On the day of the study no drinking water was provided and no drug (or vehicle) was injected. Each rat was given 15 g of food and 1 U

Table 1. Weights, blood pressure, and plasma measurements in control and experimental rats[a]

	Body weight *g*	Blood pressure *mm Hg*	Hct *ml/dl*	Na *mEq/liter*	K *mEq/liter*	Urea *mmoles/liter*
Control (N = 11)	225 ± 12	107 ± 2	51 ± 1	141 ± 1	4.4 ± 0.2	7.5 ± 0.7
Gentamicin (N = 16)	211 ± 8	107 ± 8	50 ± 1	144 ± 1	4.0 ± 0.3	22.9 ± 2.5
P	NS	NS	NS	NS	NS	< 0.001

[a] Values are means ± SEM: N = number of animals studied in each group.

vasopressin (pitressin tannate in oil, Parke David & Co., Morris Plains, New Jersey) was injected intramuscularly. Urine samples were collected under oil into graduated cylinders. In five gentamicin and five control rats, the collections were divided into two 12-hr collections. After 24 hr without water, the rats were anesthetized with ether, and the kidneys were exposed via a large midline abdominal incision. The kidneys were dissected free from the surrounding fat and, in rapid sequence, the renal pedicle was clamped and cut, and the kidneys were removed and placed in a mixture of dry ice and acetone. Using a razor blade, the frozen kidneys were then cut longitudinally, and the exposed papilla was cut along its base to free it from the inner medulla. Then tissue samples were taken from cortex, outer medulla, and inner medulla. Mean tissue weights (± SEM in mg) for cortex, outer medulla, inner medulla, and papilla were (range) 61.9 ± 10.3 (21.6 to 163.3), 21.9 ± 1.8 (14.2 to 30.3), 13.7 ± 1.0 (7.8 to 17.2), and 4.81 ± 0.57 (2.8 to 10.5) for controls; and 47.3 ± 8.1 (20.8 to 98), 19.5 ± 2.9 (11.5 to 40.6), 13.2 ± 1.0 (9.1 to 17.9), and 6.01 ± 0.64 (2.5 to 9.7) for gentamicin. Tissue sample weight was not different statistically in the two groups. In two rats each from the control and gentamicin-treated groups, only cortical and papillary tissue were taken. The tissue samples were placed in aluminum foil buckets which had been cleaned and dried to constant weight beforehand. These sealed, light weight buckets have been shown to increase the accuracy of weighing tissue samples as small as 2 mg [19]. The buckets were carefully sealed and weighed on an analytic balance (Model 2502 Sartorius, Gottingen, Germany). The tissue samples were then treated in one of two ways. One set was removed from the buckets and placed in preweighed glass homogenizing tubes (TRI-R Instruments Inc., Rockville Center, New York) containing 2.0 ml of deionized water and homogenized by hand. The urea content of the slices was determined on the same day. The other set was placed in an oven (100°C) and dried to constant weight over 48 hr. The tissue was then placed in preweighed test tubes containing 0.5 ml 16 M nitric acid and digested at 100°C. After the addition of 1.5 ml deionized water the tubes were reweighed and centrifuged. The diluted digests were then analyzed for sodium and potassium.

Calculations

Single nephron inulin clearance (SNC_{IN}) was calculated by multiplying the ratio of tubule fluid to plasma radioactivity by the measured flow rate of tubule fluid. Whole kidney clearance of inulin was calculated by multiplying the ratio of urine to plasma radioactivity by the measured flow rate of urine. Absolute reabsorptive rates between late proximal tubule puncture sites and distal puncture sites were calculated by subtracting the measured flow rates at the distal puncture sites from the measured flow rates of the late proximal tubule puncture site within the same nephron. Absolute urinary excretion rates of sodium, potassium, and urea were calculated by multiplying the solute concentrations in urine by the volume flow rate of urine. The recovery of microinjected ^{3}H-inulin was calculated after correcting the observed cpm for quench using the external standards method. The dpm recovered in the urine were divided by the dpm in the reference standards and expressed as percent recovery. Tissue solute content was determined as the sum of the measured sodium, potassium, and urea contents in the digest divided either by the tissue water content or the tissue dry weight. The water content of tissue was determined from the difference between the wet and dry weights of the dessicated tissue samples. Osmotic concentration of measured solutes in tissue water was estimated as the sum of urea concentration plus 1.84 times the sum of sodium and potassium concentrations [20].

Analytical methods

Total volumes of the tubular fluid collections were determined in a calibrated quartz-glass constant bore capillary (100 μm I.D.) tube with the use of an eyepiece micrometer. ^{3}H activity in urine, tubule fluid, and plasma was determined in a liquid scintillation spectrometer (Packard Tri-Carb Model 2425, Downers Grove, Illinois). Only samples in which count rates were three times above background were accepted. Sodium and potassium in blood, urine, and tissue digests were determined on a flame photometer (IL Model 143, Instrumentation Laboratory, Lexington, Massachusetts) which was standardized daily. Appropriate standards bracketed the unknowns. Osmolality of urine was determined by freezing-point depression (Fiske Associates, Oxbridge, Massachusetts). Urea in plasma, urine, and tissue was determined by the diacetyl monoxime as modified by DiGiorgio [21] with standard curves drawn daily. Homogenates were diluted to achieve urea nitrogen concentrations below 50 μg/ml (O.D. = 0.450) where the method is nearly linear. Recovery of urea in tissue samples by this method was 98.6 ± 1.7% (N = 16) from control kidneys and 103.3 ± 4.4% (N = 8) from gentamicin-treated kidneys.

Statistical analysis

The statistical significance of the results was assessed by the Student t test with paired and unpaired data as indicated. Equality among rat variance for SNGFR in control and gentamicin-treated rats was tested by the F-test [22]. Significance was indicated by a P value of 0.05 or less.

Results

Table 1 summarizes the general features of the control and gentamicin rats studied by free-flow micropuncture. Body

Table 2. Function of micropunctured kidney in control and gentamicin-treated rats

	V *μl/min*	U/P_{In}	C_{In} *μl/min*	[Na]u *mEq/liter*	[K]u *mEq/liter*	$U_{Na}V$ *nEq/min*	U_KV *nEq/min*
Control (N = 11)	2.9 ± 0.9	416 ± 79	890 ± 56	80 ± 16	299 ± 39	310 ± 128	630 ± 66
Gentamicin (N = 16)	9.7 ± 1.4	30 ± 4	265 ± 44	89 ± 10	88 ± 8	953 ± 205	763 ± 80
	< 0.001	< 0.001	< 0.001	NS	< 0.001	< 0.025	NS

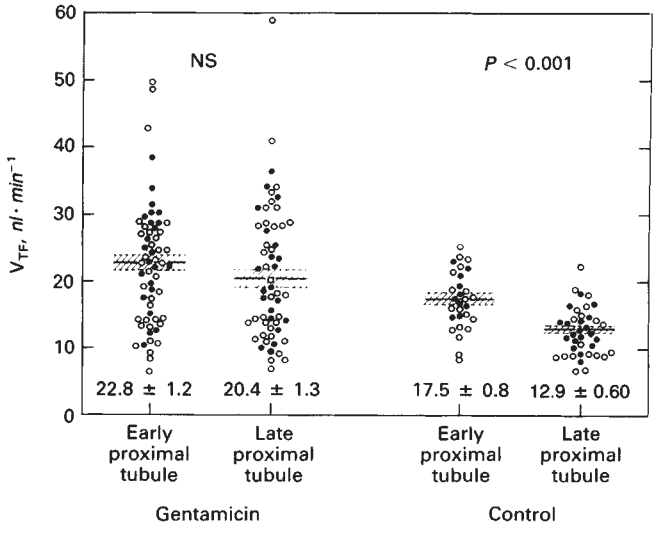

Fig. 1. *Individual values for flow rate of tubule fluid* (V_{TF}) *in early and late segments of proximal tubules in gentamicin-treated and control rats.* Measurements performed in the same nephron are indicated by *open circles*, those performed in different tubules are indicated by *closed circles*. *Solid lines* indicate the mean values; *hatched areas* indicate ± 1 SEM.

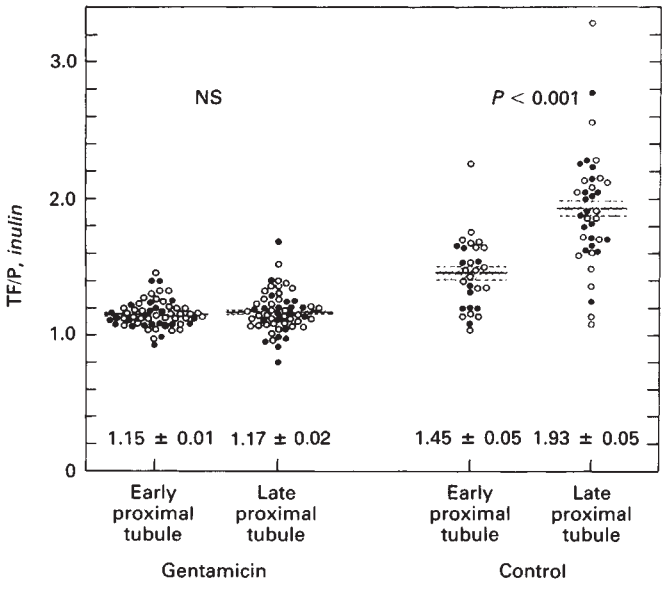

Fig. 2. *Individual values for* (TF/P) *inulin in early and late segments of proximal tubules in gentamicin-treated and control rats.* Symbols are the same as in Figure 1.

weights, blood pressures, and hematocrits were not different. Plasma urea nitrogen concentration was significantly greater in the gentamicin-treated group, while plasma sodium and potassium were not significantly different from control.

Table 2 shows several manifestations of gentamicin nephrotoxicity: increased urine flow, decreased $(U/P)_{In}$, decreased inulin clearance, and increased sodium excretion.

The appearance of the kidney surface in gentamicin-treated animals was distinctly abnormal. The cell boundaries of the tubular segments were ragged, and many nephrons had white casts, presumably composed of cellular debris, within their lumens. The luminal space was closed in some nephrons and widely dilated in others. Occasionally, in nephrons with patent lumens, debris could be seen flowing along the segments under observation. In general, rats with higher plasma urea concentrations had a greater number of closed, debris-filled nephrons. In addition it was observed that microinjected fluid moved slowly in some nephrons and rapidly in others. It was apparent that with such variation from one tubule to another, random sampling of nephrons alone would not be meaningful. For this reason early and later segments of the same nephron were studied. Many more nephrons were sampled where only an early or later site was punctured.

In Figure 1 individual values are plotted for early and late proximal tubule flow in gentamicin and control animals. In 16 gentamicin-treated animals 58 early and 59 later proximal tubules were sampled. On the average, no fluid reabsorption could be demonstrated between the two points of puncture. In contrast, the expected fall in fluid flow rate was demonstrated along control proximal tubules punctured in the same way. Similarly, as shown in Figure 2, $(TF/P)_{In}$ failed to increase between these two proximal sites in the gentamicin-treated rats. The expected increase in $(TF/P)_{In}$ between the two proximal sites was demonstrated in the control group. The delivery of fluid out of the proximal convoluted tubule was significantly higher in gentamicin-treated rats than that in control animals ($P > 0.001$). The absence of fluid reabsorption is also noted when only paired samples are compared (Table 3).

Calculated single nephron inulin clearance rates from all early proximal tubule punctures in the gentamicin-treated animals was 25.8 ± 1.3 nl/min, a value not significantly different from the mean SNC_{In} measured in the control group (24.9 ± 1.2 nl/min). A wide range of single nephron inulin clearance was observed in the gentamicin group (7.1 to 63.4 nl/min). The variance of SNC_{In} in the gentamicin-treated animals was significantly greater than that observed in the control animals ($P < 0.005$).

In Figure 3 individual values are shown for flow rates in distal tubules from 8 control and 11 gentamicin-treated rats. In 23 punctures from the gentamicin-treated animals mean distal tubular flow rate was 8.2 ± 0.7 nl/min, significantly higher than 5.1 ± 0.5 nl/min measured in 23 punctures from the control

Table 3. Paired measurements from early and late segments of single proximal tubules in control and gentamicin-treated rats

	Number rats/tubules	V_{TF}, *nl/min*		(TF/P)In	
		EPT	LPT	EPT	LPT
Control	9/19	16.9 ± 1.1	12.9 ± 1.1	1.49 ± 0.06	1.90 ± 0.12
P (early vs. late)		< 0.001		< 0.001	
Gentamicin	12/36	21.5 ± 1.7	20.5 ± 1.8	1.15 ± 0.02	1.19 ± 0.02
P (early vs. late)		NS		NS	
P (control vs. gentamicin)		NS	< 0.01	< 0.001	< 0.001

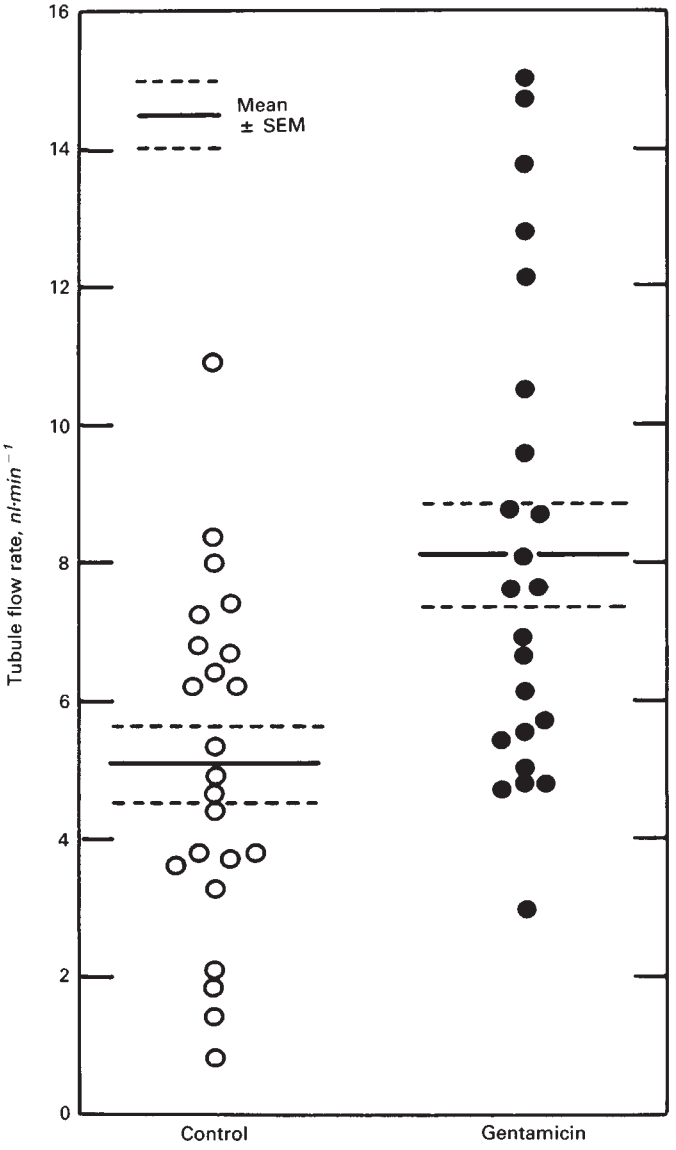

Fig. 3. *Individual values for distal tubule fluid flow rates measured in control* (○) *and gentamicin-treated* (●) *rats.*

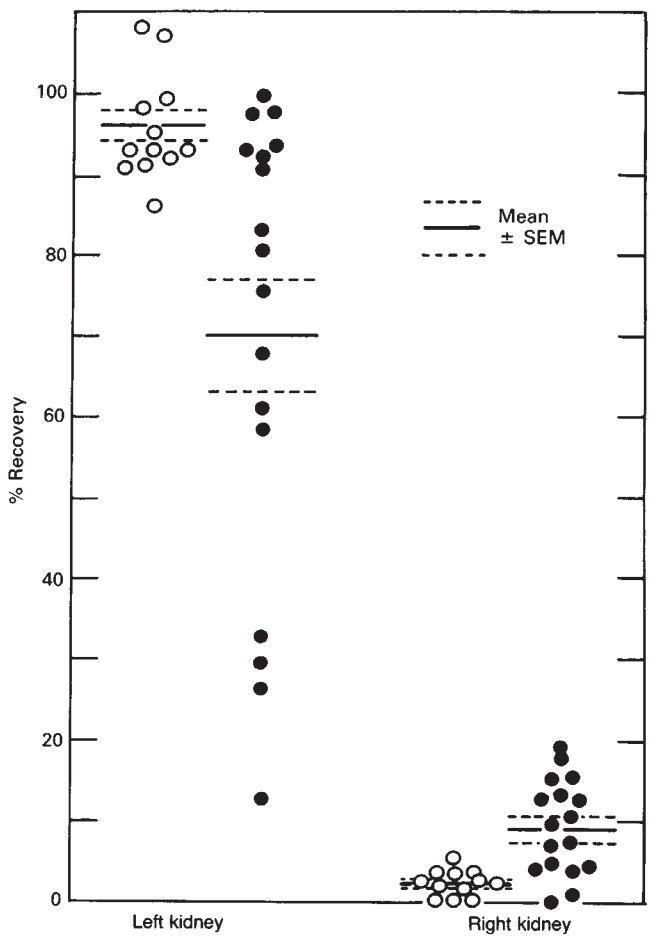

Fig. 4. *Individual values for recovery of inulin from left (microinjected) and right (non-injected) kidney in control* (○) *and gentamicin-treated* (●) *rats.*

animals ($P < 0.005$). It was possible to sample fluid from the late proximal tubule of the same nephron in 13 distal punctures in the gentamicin-treated group and calculate the absolute rate of reabsorption of fluid between these sites. The associated proximal tubule flow rate in these distal tubules was 20.9 ± 2.2 nl/min, a value not significantly different from the proximal flow rates measured in nephrons without demonstrable distal tubules, indicating that these nephrons were representative of the functioning nephrons sampled in the study. Mean reabsorption of fluid between these two sites was 13.3 ± 1.6 nl/min in gentamicin-treated rats and was significantly higher than the 7.5 ± 0.9 nl/min reabsorbed along the loop in control animals, ($N = 8$, $P < 0.025$). Calculated SNC_{In} in the distal tubule in these paired punctures was significantly lower than that measured in the proximal tubule in the gentamicin-treated animals (17.9 ± 2.2 vs. 23.7 ± 2.3 nl/min, respectively, $P < 0.025$). This

Table 4. Urine flow and solute excretion during water restriction and vasopressin administration

	V		U_{osm}		$U_{Na}V$		U_KV		$U_{urea}V$	
	ul/min		*mOsm/kg H_2O*		*uM/min*					
Collection period	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
Control	5.6	1.5[b]	2251	2926	1.8	0.5[b]	1.9	0.6[b]	4.8	2.0[b]
(N = 5)	±0.7	±0.4	±117	±291	±0.2	±0.1	±0.5	±0.2	±0.5	±0.4
Gentamicin	12.5[a]	6.4[a,b]	748[a]	1232[a,b]	1.2[a]	0.8	1.4[a]	0.9[b]	4.8	4.4[a]
(N = 5)	±1.3	±0.7	±50	±173	±0.2	±0.1	±0.2	±0.03	±0.7	±0.3

Abbreviations: 1st and 2nd refer to 12-hr urine collection periods during 24 hr of water restriction; N, number of rats in each group.
[a] The mean value is significantly different from control.
[b] The mean value is significantly different from the 1st 12-hr values.

disparity was not as large in the control animals (20.7 ± 2.1 distal, vs. 23.9 ± 1.9 proximal, $P > 0.1$).

The results of the microinjection studies are shown in Figure 4. Mean recovery of microinjected inulin (left kidney) in the gentamicin group (seven animals), while quite variable, was significantly lower than that observed in control animals (five animals) (70 ± 7 vs. 96 = 2%, $P < 0.025$). Recovery of inulin from the contralateral (right) ureter in the gentamicin group was significantly higher than in control animals ($P < 0.005$).

Table 4 gives urinary data collected during the period of dehydration in control and gentamicin-treated rats. The gentamicin rats remained polyuric throughout the period of dehydration, although the extent of the polyuria diminished as dehydration proceeded. The increased urine flow was associated with a greater weight loss in the gentamicin-treated rats than that experienced by controls (22.4 vs. 8.0 g, $P < 0.001$). Urinary osmolality was significantly lower in both collection periods compared to control, but rose between the first and second collection periods. The gentamicin-treated rats were unable to lower the rate of excretion of urea and sodium in the second 12-hr period of dehydration. In marked contrast, urea and sodium excretion decreased in the control rats.

Figure 5 depicts the tissue sodium and urea content from cortex, outer medulla, inner medulla, and papilla at the conclusion of the dehydration period. Although the cortical to inner medullary gradients for sodium and urea were intact in the gentamicin-treated animals, papillary urea content was reduced profoundly. Papillary sodium content fell modestly. Calculated papillary osmolality fell from 1446 ± 104 in controls to 833 ± 97 mOsm/kg H_2O in gentamicin-treated rats ($P < 0.005$). Tissue water content in the gentamicin-treated group exceeded that in the control in cortex (82 ± 1 in gentamicin vs. 78 ± 1 in control, $P < 0.01$) and outer medullary tissue (88 ± 0.3 vs. 86 ± 1, $P < 0.025$). Water content of the inner medulla and papilla was the same for the two groups.

Discussion

The present studies were designed to examine whole kidney and single nephron function when reduced inulin clearance, azotemia, and polyuria coincide following prolonged exposure to gentamicin. A previous study has examined factors contributing to the fall in glomerular filtration seen after a shorter period of gentamicin exposure and before the appearance of polyuria and azotemia [17]. In that study as little as 4 mg/kg of body weight of gentamicin was given for 10 days; SNGFR was found to be reduced uniformly. In the present studies, however, SNGFR was not uniformly reduced. Reduced whole kidney inulin clearance was a consequence of both reduced numbers of filtering nephrons and leakage of inulin across tubule walls. It is likely that the differences between these two studies are real and reflect the different degrees of gentamicin nephrotoxicity studied. Azotemia, polyuria, and cell necrosis seen in the present study are more typical of the later stages of gentamicin nephrotoxicity in rats [7, 9, 12, 13]. Thus, while the initial event in gentamicin-induced renal failure seems to be reduced filtration in all nephrons later on, when proximal cell necrosis accompanies the fall in glomerular filtration, filtration may cease altogether in some nephrons while in others filtration may continue even at supernormal rates. The factors which sustain filtration in some nephrons but not others are unknown.

Our studies indicate that gentamicin-treated animals were polyuric despite the reduced numbers of filtering nephrons. Two additional defects in renal function were found to accompany the polyuria. First, fluid reabsorption was diminished along the proximal convoluted tubule (Fig. 1, Table 3). This was true whether tubule fluid flow rates were high or low. This defect may be due to the extensive cell damage gentamicin produces at this nephron site [8, 12, 23]. Appearance in the urine of glucose [24], protein, and enzyme markers of proximal tubular epithelial cell damage [8, 23] are other indicators of proximal tubule damage. Extensive cell damage may limit the capacity of the proximal tubule cells to reabsorb solutes and, since this is the basis for fluid absorption in the proximal tubules, thereby lead to diminished fluid reabsorption. Alternatively, the diminished proximal convoluted tubule reabsorption may be due to the uremia [25] which accompanies this phase of the disease. Several pieces of information argue against this explanation. Fractional fluid reabsorption along the proximal convoluted tubule is normal in several other forms of nephrotoxic renal failure at comparable degrees of azotemia [2, 4, 26]. Using micropuncture techniques Biber et al [2] have demonstrated reduced proximal water reabsorption after $K_2Cr_2O_7$, a toxin which damages the proximal convoluted tubule, and normal proximal water reabsorption after $HgCl_2$ administration, a toxin which damages the straight portion of the proximal tubule inaccessible to micropuncture. Thus structure and function correlated perfectly. Therefore, the most likely cause of reduced proximal fluid reabsorption seems to be the severe morphologic damage produced by gentamicin to this portion of the nephron.

Second, the gentamicin-treated rats failed to accumulate solute, particularly urea, in the papillary interstitium (Fig. 5). This defect was not corrected by administration of vasopressin or by prolonged dehydration. The cause for this failure to

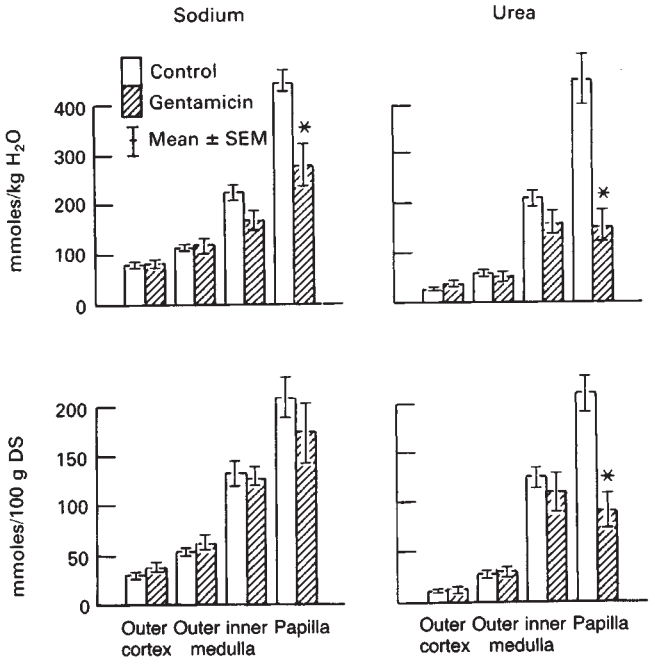

Fig. 5. *Sodium and urea concentrations in tissue samples from control and gentamicin-treated rats.* Tissue solute concentration is expressed in mmoles/kgH_2O in *upper panel* and mmoles/100 g dry solid in *lower panel.* The *asterisk* represents P value < 0.05.

concentrate solute in the papilla is much less clear since gentamicin produces no consistent morphologic damage to this area of the kidney [7, 12, 23]. Although high rates of flow out of functioning superficial nephrons was observed in this study, a similar reduction in papillary solute content was observed in rats treated with cisplatin where the polyuria was associated with a reduced rate of flow in superficial nephrons [4]. Thus changes in superficial nephron fluid flow rates may not be causally linked to the low solute content in the papilla. The ability to reabsorb water, and presumably solute, in Henle's loop of superficial nephrons appears to be intact as evidenced by the reabsorption of fluid in the loop of Henle of the gentamicin-treated animals seen in this study, and the normal rates of free water clearance and reabsorption shown by others [10, 11]. Other explanations for the lower solute concentration in the papilla such as diminished collecting duct responsiveness to vasopressin produced by uremia [27] and increased papillary blood flow are possible. Regardless of the mechanism by which solute in the papilla is reduced, such reduced solute concentration in the renal papilla would limit the capacity of the collecting ducts to reabsorb fluid.

The present results suggest, therefore, that gentamicin-induced polyuria is the result of at least two defects. Proximal tubule fluid reabsorption is markedly reduced. Second, papillary solute content is reduced. This low papillary solute concentration would limit the capacity for water reabsorption from the papillary collecting ducts. So long as filtration is present in enough nephrons these defects in absorptive capacity can produce polyuria. While the cause of reduced proximal reabsorption is most probably related to gentamicin-induced cell necrosis, the cause of reduced papillary solute content in the medulla is not known.

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