Gentamicin and Gram-negative Bacteremia

A Synergism for the Development of Experimental Nephrotoxic Acute Renal Failure

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Abstract

To explore whether bacteremia potentiates gentamicin nephrotoxicity, we injected rats with either 1×10^9 *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa*, or *Staphylococcus aureus*, and then gave them gentamicin, 100 mg/kg. Renal injury was assessed over the next 24–48 h. *Staphylococcus*/gentamicin or gentamicin alone induced no renal injury. However, *E. coli*/gentamicin and *Pseudomonas*/gentamicin caused acute renal failure (severe azotemia; tubular necrosis; cast formation). This effect was not due to acute reductions in arterial blood pressure or renal blood flow, it could be reproduced by substituting nonviable for viable gram-negative organisms, and it was associated with increased renal gentamicin uptake. *E. coli* without gentamicin induced only mild azotemia and no tubular necrosis. Endotoxin-tolerant rats were significantly protected against the *E. coli*/gentamicin nephrotoxic interaction. We conclude that gram-negative bacteremia and gentamicin exert synergistic nephrotoxicities; and that this effect is mediated, at least in part, by endotoxin and in part by increased renal gentamicin uptake.

Introduction

Acute renal failure (ARF)[1] frequently develops in bacteremic patients (1–3). However, the role of bacteremia in the induction of this syndrome remains to be defined. Bacteremia can theoretically induce ischemic ARF by causing septic shock. It may also cause cortical necrosis due to disseminated intravascular coagulation (DIC). Alternatively, bacteremia might cause direct tubular cell injury or enhance renal susceptibility to nephrotoxic agents. This latter possibility has great potential clinical relevance since aminoglycosides, a nephrotoxic class of antibiotics, are a mainstay in the treatment of bacteremic patients. Therefore, the goals of this investigation were to evaluate whether bacteremia induces acute tubular injury and to determine whether it alters renal susceptibility to gentamicin, a prototype of the aminoglycoside antibiotics.

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1. *Abbreviations used in this paper:* ANOVA, analysis of variance; ARF, acute renal failure; BUN, blood urea nitrogen; C_{ioth}, mean clearance of iothalamate; Cr, serum creatinine; DIC, disseminated intravascular coagulation; GFR, glomerular filtration rate; MAP, mean arterial blood pressure; RBF, renal blood flow; uv, ultraviolet.

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Methods

Stock cultures of *Escherichia coli* (ATCC 25922; *E. coli*), *Pseudomonas aeruginosa* (ATCC 27853; *P. aeruginosa*), and *Staphylococcus aureus* (ATCC 25923; *S. aureus*) were subcultured on sheep blood agar plates and incubated at 37°C for 18 h. Bacterial suspensions were prepared by gently removing the growth and suspending them in nonpyrogenic normal saline (Travenol Laboratories, Deerfield, IL). The bacterial density was adjusted by adding saline to an optical density equal to that of a McFardland Barium Sulfate Standard No. 4 (approximates 1×10^9 organisms). Samples were divided into 1-ml aliquots and stored at −70°C until use. This allowed for a large batch of organisms to be prepared at one time, and after thawing a constant number of organisms could be delivered to each rat. Some aliquots of *E. coli* were sterilized by exposing them to 30 min of ultraviolet (uv) light before freezing. The endotoxin content of the bacterial suspensions were: *E. coli*, 10 μg/ml; *P. aeruginosa*, 10 μg/ml; *S. aureus*, 0 μg/ml (Limulus amoebocyte lysate assay; Mallinckrodt, Inc., St. Louis, MO). Approximate colony forming units per milliliter of thawed samples were: *E. coli*, 1×10^8; *E. coli*-uv light treated, 0; *S. aureus*, 1×10^9; *P. aeruginosa*, 2×10^7. Once thawed, some viable *E. coli* suspensions were placed in a boiling water bath for 20 min to sterilize them and denature bacterial enzymes.

Animal experiments: overview

Three basic sets of experiments were performed, denoted below as set I, set II, and set III experiments. In set I experiments, the renal effects of bacterial injections/antibiotic treatment on renal function and morphology were assessed. Groups A through I are depicted in Fig. 1. Additional set I experiments included assessment of the effect of *E. coli* bacteremia on renal gentamicin uptake. In set II experiments, the renal functional effects of purified *E. coli* endotoxin and *E. coli* endotoxin when combined with gentamicin were assessed. In set III experiments, the effects of *E. coli*/gentamicin on normal rats and endotoxin tolerant rats were compared, as depicted in Fig. 2. The effects of endotoxin tolerance on renal gentamicin uptake were also assessed. Finally, the acute effects of *E. coli* injections on renal blood flow (RBF) and mean arterial blood pressure (MAP) under conditions of the set I and set III experiments were determined (*acute hemodynamic assessments*). Female Sprague Dawley rats (160–220 g; Laboratory Supply, Indianapolis, IN; Tyler Labs, Bellevue, WA), housed under standard laboratory conditions were used in all of these experiments.

Set I experiments

Renal functional, morphologic assessments (see Fig. 1). Rats were anesthetized with pentobarbital (20–30 mg/kg) and a polyethylene 50 (PE50) catheter was inserted into the left jugular vein. 1 ml of the *E. coli, Pseudomonas,* or the *Staphylococcus* suspension was injected intravenously. In the case of *E. coli,* either viable or nonviable (uv light; boiling) suspensions were injected. Control rats received 1 ml of saline instead of bacteria (group A; see Fig. 1). 2 h after bacterial or saline injection the rats received either gentamicin, 50 mg/kg, or ampicillin, 150 mg/kg i.v. over 30 min. 1 h after completing the first antibiotic injection, a second dose of the same antibiotic in the same dosage was administered intramuscularly (total dose, 100 mg/kg gentamicin; 300 mg/kg ampicillin). The rats were then allowed to recover from anesthesia. They were also allowed free access to food and water.

Renal function was assessed over the next 24–48 h by measuring the blood urea nitrogen (BUN) and serum creatinine concentrations as de-

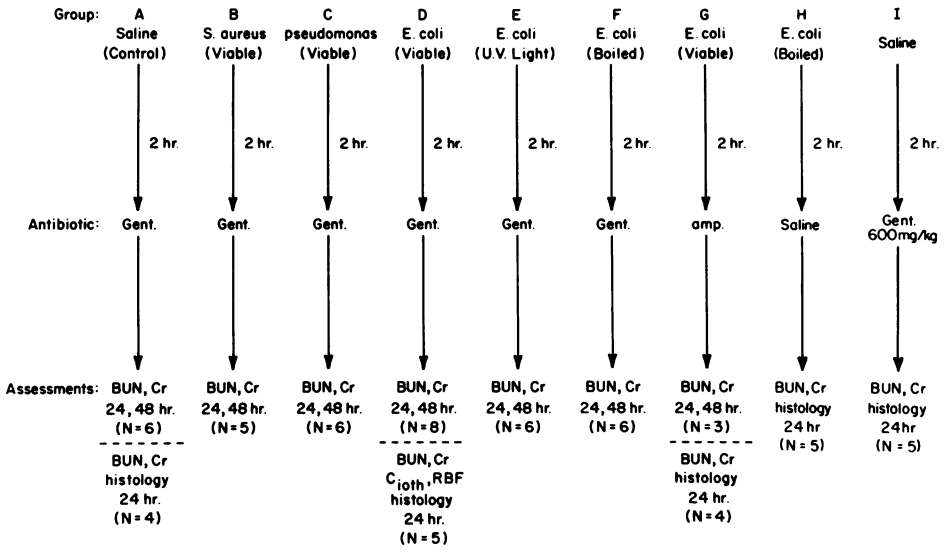

Figure 1. Protocols involving bacteria/antibiotic administration followed by renal functional/histologic assessments (set I experiments). Gent, gentamicin 100 mg/kg; Amp, ampicillin 300 mg/kg; C_{ioth}, clearance of iothalamate; RBF, renal blood flow; BUN, blood urea nitrogen; Cr, serum creatinine.

lineated in Fig. 1. In addition, five group D rats (*E. coli*/gentamicin) had glomerular filtration rate (GFR), RBF, and MAP measured 24 h after bacterial injection as previously described (4, 5). In brief, these rats were anesthetized with pentobarbital and subjected to tracheostomy, jugular vein and carotid artery catheterization. A 2% body weight prime of normal saline containing sodium iothalamate ^{125}I as a marker of GFR (6) was administered i.v. over 30 min. The infusion was then maintained at 2.34 ml/h. Four 30-min urine collection periods were completed. The mean clearance of iothalamate (C_{ioth}) for the last three collection periods was taken as the C_{ioth} for each rat. MAP was continuously monitored via the carotid artery catheter. After completing the C_{ioth} determination, left RBF was determined for 15 min by electromagnetic flow probe technology (4, 5). Then both kidneys were fixed in vivo by retrograde aortic perfusion (140 mmHg) with 2% glutaraldehyde in sodium phosphate buffer (4, 5). In addition, four group A rats and four group G rats had their kidneys perfused fixed in vivo 24 h after saline/bacterial injection. Frontal kidney sections were stored in buffered formalin until processing for light microscopy. The kidneys were embedded in paraffin and 4-μm frontal sections were cut and stained with hematoxylin and eosin.

Two additional groups (H and I) depicted in Fig. 1 were established. Group H (n = 5) received 1 ml of intravenously boiled *E. coli*. 2 h later 0.3 ml of saline, rather than an antibiotic, was administered intravenously. BUNs and creatinines were measured before and 24 h after the *E. coli* injection and then the kidneys were fixed for histology. Group I is described below (see Renal gentamicin uptake).

Renal gentamicin uptake: effects of bacteremia. Six rats received 1 ml of the viable *E. coli* suspension intravenously and six rats received 1 ml of saline intravenously (controls). 2 h later both groups received gentamicin to a total dose of 100 mg/kg administered in divided doses as described above. One-half hour after completing the gentamicin administration, the kidneys were removed. Each was homogenized in 2 ml of saline. One part homogenate was incubated at room temperature with nine parts of Triton X-100 (0.15%) for 10 min. Then the samples were diluted 1:2 with distilled water, centrifuged at 1,000 *g* (4°C), and the supernatants were assayed for gentamicin by fluorescence polarization (TDX; Abbott Laboratories, Irving, TX). Controls included: kidney homogenate from a non–gentamicin-treated rat and the same homogenate to which gentamicin was added in concentrations of 2, 4, and 8 μg/ml. As a further control, five kidneys from gentamicin-treated rats (two bacteremic; three nonbacteremic) were sent frozen to the Schering Corporation (Bloomfield, NJ) where they were assayed for gentamicin (by radioimmunoassay).

To obtain very high renal tissue gentamicin concentrations (comparable to those seen in the *E. coli*/gentamicin treated rats), eight rats were infused with gentamicin, 600 mg/kg i.v. over 1½ h. The kidneys from three rats were removed and assayed for gentamicin ½ h after completing the gentamicin treatment. The remaining five rats had BUNs and serum creatinines measured 24 h later and then their kidneys were perfused fixed in vivo for histologic evaluation (group I, Table I).

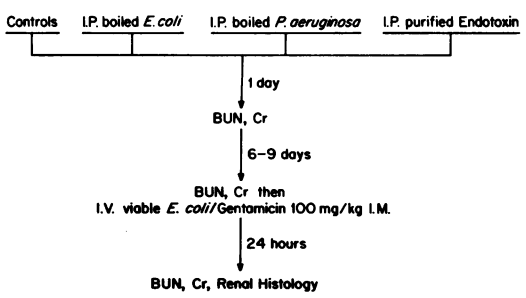

Figure 2. Protocols used for the endotoxin tolerance (set III) experiments. I.P., intraperitoneal injection.

Set II experiments: purified endotoxin/gentamicin injections

Anesthetized rats (n = 11) had baseline BUN and serum creatinine concentrations determined and then they received 10 μg i.v. of purified *E. coli* endotoxin (Mallinckrodt, Inc., St. Louis, MO). 2 h later, six of the rats received gentamicin, 100 mg/kg, as previously described. The remaining five rats received an equal volume of normal saline. BUN and serum creatinine concentrations were redetermined 24 and 48 h later. The results of these studies were compared to those of group A rats (see Fig. 1) which had received only gentamicin (100 mg/kg) treatment.

Set III experiments: endotoxin-tolerant rats (see Fig. 2)

Prior endotoxin exposure can produce endotoxin tolerance (7). To assess the effect of such tolerance on the renal response to gram-negative bacteria/gentamicin injections, we performed the following experiments: Rats were inoculated intraperitoneally (i.p.) with one of three sources of endotoxin: 1 ml of boiled *E. coli* (n = 9), 1 ml of boiled *P. aeruginosa* (n = 8), or 100 μg of purified *E. coli* endotoxin (n = 6). The BUN and serum creatinines were measured before and 24 h after these immunizations. 6–9 d later, these rats and 10 control rats were anesthetized with pentobarbital, the BUN and serum creatinines were measured, and then the rats received a 1-ml i.v. injection of the viable *E. coli* suspension. As an index of endotoxin tolerance, rectal temperatures were monitored with a digital thermometer for 15 min post-injection. The rats then received gentamicin, 100 mg/kg i.m. (Note: subsequent studies to those

Table I. Degree of Azotemia In Response to the Bacteremia Protocols

Group	n	Baseline		24-h		48-h	
		BUN	Cr	BUN	Cr	BUN	Cr
A Saline/gent controls (100 mg/kg)	6	17±1	0.55±0.03	17±1	0.58±0.02	19±1	0.52±0.02
				NS	NS	NS	NS
B *S. aureus*/gent (100 mg/kg)	5	16±1	0.52±0.02	17±1	0.56±0.02	16±1	0.52±0.02
				NS	NS	NS	NS
C *P. aeruginosa*/gent	5	17±1	0.54±0.02	55±12	0.93±0.08	32±6	0.65±0.03
				<0.05	<0.01	<0.05	NS
D *E. coli*/gent (100 mg/kg)	8	19±1	0.58±0.02	91±11	1.14±0.09	37±5	0.84±0.04
				<0.001	<0.001	<0.01	<0.001
E *E. coli*-uv light/gent (100 mg/kg)	6	17±1	0.57±0.03	79±12	1.25±0.18	42±8	0.71±0.06
				<0.01	<0.02	<0.05	NS
F *E. coli*-boiled/gent (100 mg/kg)	6	18±1	0.52±0.02	119±28	1.43±0.25	62±14	0.90±0.10
				<0.02	<0.02	<0.05	<0.01
G *E. coli*/ampicillin (300 mg/kg)	6	17±1	0.56±0.03	32±9	0.77±0.04	17±2	0.60±0.06
				<0.02	<0.02	NS	NS
H *E. coli*-boiled/saline	5	16±1	0.52±0.02	28±4	0.68±0.03	ND	ND
				<0.05	<0.05		
I Saline/gent (600 mg/kg)	5	16±1	0.48±0.02	29±3	0.58±0.04	ND	ND
				<0.05	<0.01		

The groups (A–I) correspond to those in Fig. 1. BUN and serum creatinine (Cr) concentrations (mg/dl; mean±SEM) were obtained before, 24 h after, and 48 h after the particular intervention (*P* by paired Student's *t* test compared to baseline values). Groups D, E, and F did not differ in their degree of azotemia (ANOVA), groups G and H had significantly less azotemia than groups D, E, and F ($P < 0.05$, ANOVA). ND = not done.

performed in the sets I and II experiments showed no difference in the severity of renal injury whether gentamicin was given as a single intramuscular dose 15 min after inducing bacteremia or whether it was given intravenously/intramuscularly starting 2 h after bacterial injection. Therefore, for simplicity, the gentamicin was given as a single intramuscular dose.)

24 h after these *E. coli*/gentamicin injections the BUN and serum creatinine concentrations were remeasured and the kidneys from the control group and the endotoxin immunized groups were perfused fixed for histologic evaluation. Four rats in the control group and four rats in the purified *E. coli* endotoxin immunized group had MAP and RBF measured 24 h after the *E. coli*/gentamicin injections as described above.

To assess the influence of endotoxin tolerance on renal gentamicin uptake in the setting of gram-negative bacteremia, three control rats and three purified *E. coli* endotoxin inoculated rats (7 d previously) received the intravenous viable *E. coli* injection and 15 min later they received gentamicin (100 mg/kg i.m.). 2 h later their kidneys were removed and assayed for gentamicin as described above. For comparison, three normal rats were injected intramuscularly with 100 mg/kg of gentamicin (no *E. coli* injection) and 2 h later their kidneys were removed for gentamicin assay.

Acute effects of bacteremia on MAP and RBF

Five rats were anesthesized with pentobarbital, a tracheostomy was performed, and PE50 catheters were inserted into the left carotid artery and jugular vein. Then 1 ml of the viable *E. coli* suspension was injected intravenously. MAP was monitored for 2 h. Then the abdomen was opened and left RBF was measured for 15 min by electromagnetic flow probe methodology.

Three additional rats were surgically prepared as noted above and the flow probe was positioned. After obtaining baseline MAP and RBF measurements, 1 ml of viable *E. coli* was injected intravenously, followed 15 min later by 100 mg/kg gentamicin i.m. These conditions reproduced those described in the set III experiments. MAP and RBF were then continuously monitored for 2 h.

Statistics

All values given are means±SEM. Comparisons of data within a single group of rats were made by paired Student's *t* test. Comparisons between two sets of data were made by unpaired Student's *t* test. Comparisons between multiple groups of data were made by one-way analysis of variance (ANOVA) with aftertesting performed by the Newman-Keule's test. The temperature responses to intravenous *E. coli* injection were nonGaussian in distribution so comparisons between the immunized and nonimmunized rats were made by Wilcoxon rank sum test.

Results

Set I experiments

Renal functional assessments after induction of bacteremia (see Table I). The dose of gentamicin used in the bacteremia protocols (100 mg/kg) had no independent effect on renal function, as assessed by serial BUN and serum creatinine determinations (group A). *S. aureus* injection followed by gentamicin caused no renal insufficiency (group B). However, both *P. aeruginosa* (group C) and *E. coli* (group D) bacteremia followed by gentamicin caused significant azotemia at both 24 and 48 h. The ability of the *E. coli*/gentamicin combination to induce azotemia was not diminished by rendering the *E. coli* suspension nonviable by exposing it to uv light (group E) or by boiling (group F). However, if the *E. coli* infected rats were treated with ampicillin (group G) instead of gentamicin, mild and significantly less ($P < 0.05$) azotemia resulted (BUN 24 h post-bacteremia, 32±9) and this azotemia totally resolved by 48 h post-bacterial injection. Boiled *E. coli* injections without antibiotic treatment (group H) gave comparable results to the viable *E. coli*/ampicillin injected rats (group G).

The five additional group D rats (not shown in Table I) studied 24 h post-bacteremia had a normal MAP (119±9 mmHg). C_{ioth} was 0.18±0.07 ml/min per 100 g body weight, approximately ⅓ of normal values (0.61±0.04) (4, 5). The degree of azotemia in these five rats at 24 h (BUN, 90±8; Cr, 0.93±0.08) was comparable to that observed in the eight group D rats presented in Table I. RBF was 2.2±0.3 ml/min, approximately ⅓ of normal values (6.1±0.3) (5).

Renal histology (see Figs. 3–6). Kidneys harvested 24 h after 100 mg/kg of gentamicin (group A) or 600 mg/kg gentamicin (group I) showed no tubular cell necrosis or cast formation. The proximal tubular brush border was normal. Occasional tubules showed mild proximal tubular cell vacuolization. However, the great majority of tubules in both groups looked totally normal (Fig. 3).

Kidneys harvested 24 h after *E. coli*/ampicillin injection (group G) or after boiled *E. coli* alone (group H) showed no tubular cell necrosis or cast formation. Some proximal tubular cells were slightly swollen and the brush border, although intact, appeared flattened in some areas. Some proximal tubules showed mild cellular vacuolization. Otherwise these kidneys appeared normal (Fig. 4). No vascular thrombosis was noted.

The kidneys harvested 24 h after *E. coli*/gentamicin injection (group D) showed major histologic changes. Focal areas of proximal tubular cell necrosis were seen in the cortex, medullary rays, and in the outer medulla (Fig. 5). Cellular necrosis was observed in approximately 1 of every 10 high powered (400 ×) light microscopic fields. Intraluminal casts were seen scattered throughout the cortex and in the outer/inner medullary stripe (Fig. 6), being observed in ~50% of low powered (100 ×) fields. In selected areas cast formation was observed in multiple adjacent tubular cross sections. Severe tubular cell vacuolization was widely apparent in both the cortex and outer medulla. >50% of lumina were closed, apparently due to this vacuolization. Mild medullary vascular congestion and edema were seen. The glomeruli were normal. There was no evidence of clot formation in blood vessels which appeared normal. There was no evidence of bacterial infection.

Tissue gentamicin concentrations for the set I experiments/high dose gentamicin infusion experiments. Gentamicin standards added to tissue homogenate had ≤10% error from predicted values. The five samples assayed by the Schering Corporation were: *E. coli*/gentamicin-treated rats, 525±79 (μg/gm tissue wet weight; $n = 2$); control gentamicin-treated rats, 285±77; $n = 3$). Tissue samples assayed in our laboratory were: *E. coli*/gentamicin-treated rats, 407±56; control gentamicin-treated rats, 255±44. Combining both sets of assay results, the values were: *E. coli*/gentamicin-treated rats, 446±58; control gentamicin rats, 263±37 ($P < 0.02$, unpaired t test; $n = 12$, each group).

The tissue gentamicin value achieved by infusing 600 mg/kg i.v. was 485±17 (NS vs. the above *E. coli*/gentamicin-treated group). This degree of gentamicin uptake induced significant azotemia 24 h post-injection (BUN, 29±3; Cr, 0.58±0.04; group I, Table I), but it was significantly less than that seen in the Gram negative bacteria/gentamicin treated groups. The high dose gentamicin infusion caused no tubular cell necrosis or cast formation.

Set II experiments: purified endotoxin/gentamicin injections (see Table II)

10 μg of endotoxin caused no significant change in renal function as assessed by BUN and serum creatinine concentrations at 24 and 48 h post-injection. As previously shown (Table I, group A), gentamicin, 100 mg/kg, caused no increase in the BUN and serum creatinine concentrations. However, when gentamicin was given after endotoxin, significant azotemia resulted (BUN, 24 h post-injection = 35±2 mg/dl).

Set III experiments: endotoxin-tolerant rats (see Table III)

The immunization procedures (boiled *E. coli*, boiled *P. aeruginosa*, or purified *E. coli* endotoxin) did not change the BUN

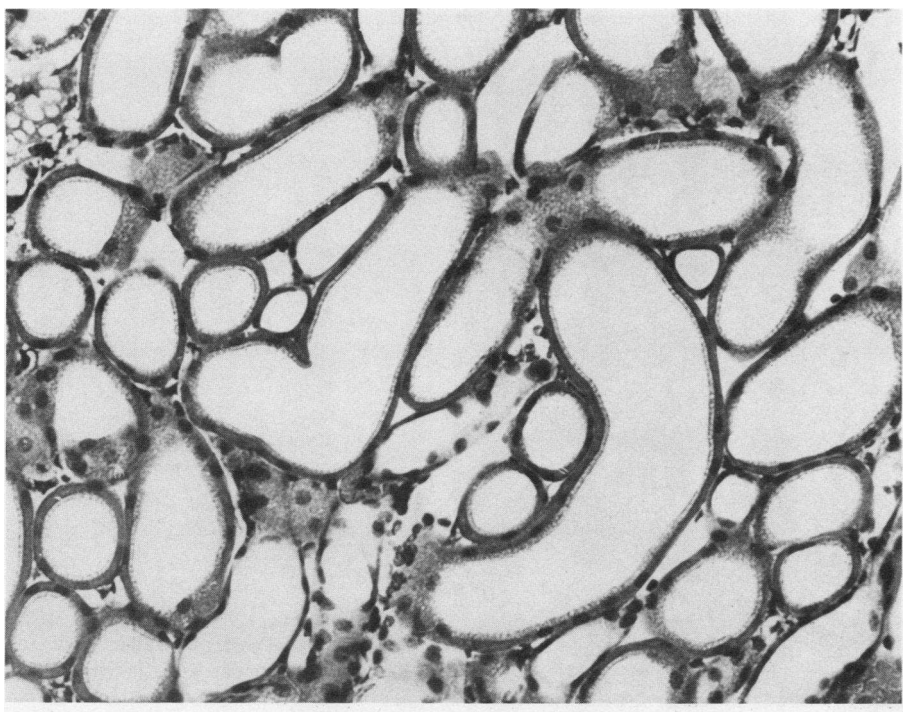

Figure 3. Renal cortex 24 h after gentamicin (600 mg/kg) treatment. The tubules demonstrate normal histology (× 252).

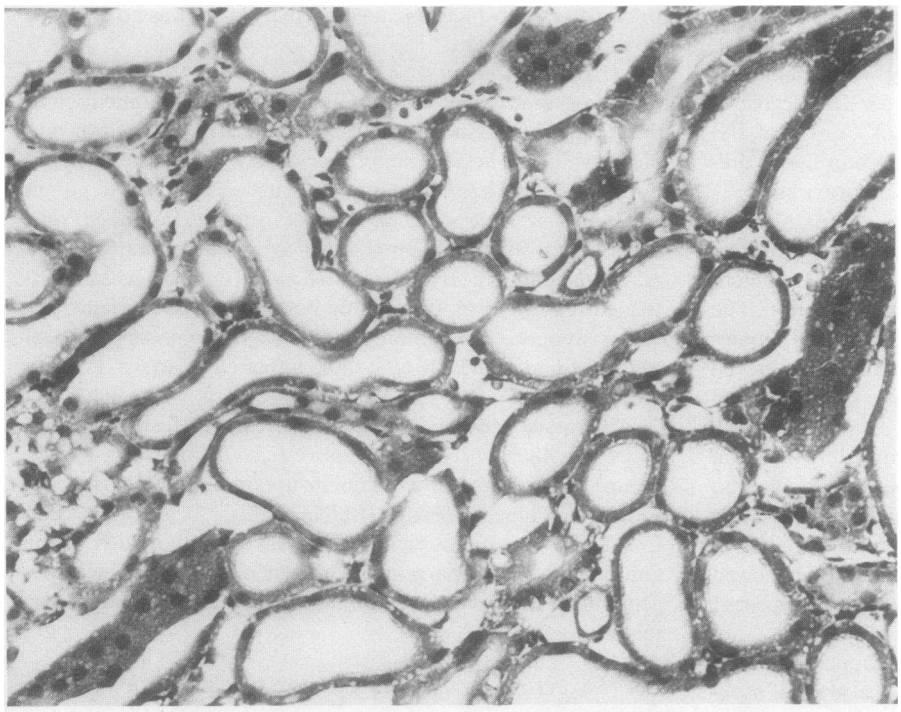

Figure 4. Renal cortex 24 h after *E. coli*/ ampicillin treatment. The tubules are normal except for some mild flattening of the brush border and occasional tubular cell vacuoles (× 252).

or serum creatinine concentrations (preimmunization: BUN, 16±1 mg/dl; Cr, 0.55±0.02; 24 h post-immunization: BUN, 16±1; Cr, 0.54±0.03) (all rats pooled). 6–9 d later, just before the viable *E. coli* injection, the BUNs and serum creatinines of the immunized and control rats were not significantly different (immunized: BUN, 15±1; Cr, 0.42±0.02; controls: BUN, 15±1; Cr, 0.41±0.01).

Rats typically have a hypothermic, not a febrile, response to endotoxin injection (7). After intravenous *E. coli* injection the body temperature of the control rats fell 2.5±0.7°F, whereas the immunized rats (all three groups combined) had a temperature drop of 0.8±0.3°F ($P < 0.05$), indicating endotoxin tolerance in the previously immunized groups.

24 h after intravenous viable *E. coli*/i.m. gentamicin injections, all immunized and nonimmunized rats had significant increments in the BUN and serum creatinine concentrations (see Table III). However, the degree of azotemia was significantly reduced by prior endotoxin immunization. The same degree of functional protection was afforded by all three types of endotoxin immunizations (no significant differences in BUNs, creatinines;

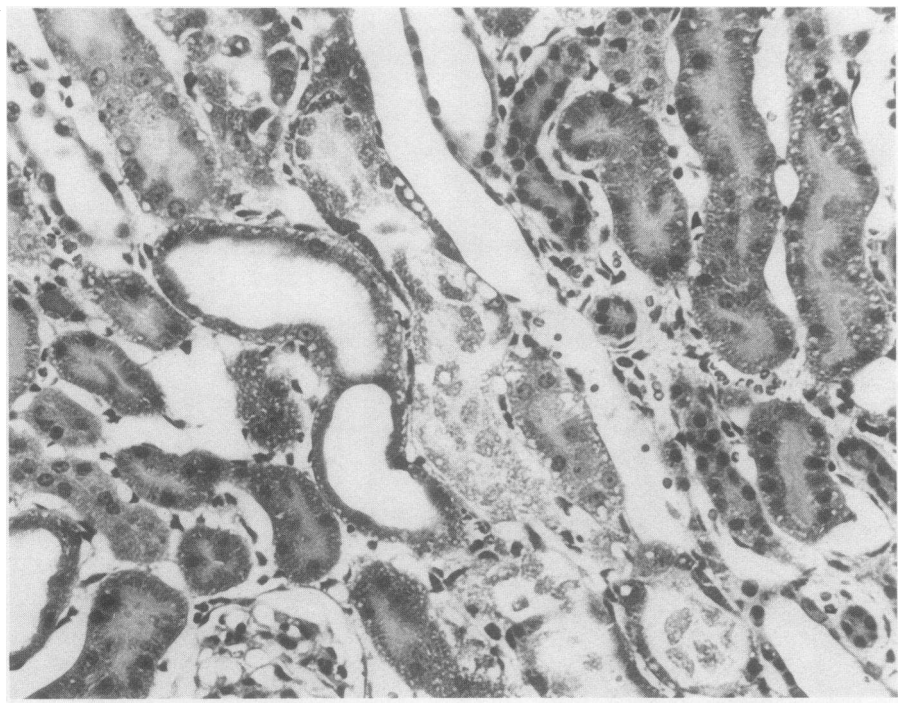

Figure 5. Renal cortex 24 h after *E. coli*/ gentamicin treatment. Tubular cell necrosis is evident (× 252).

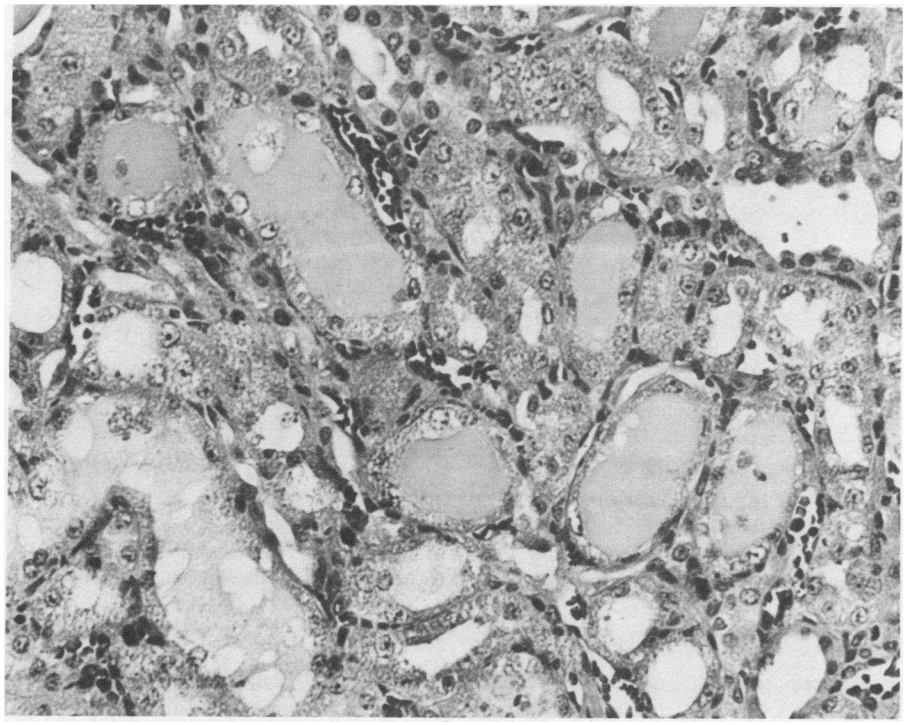

Figure 6. Outer medulla 24 h after *E. coli*/gentamicin treatment. Extensive cast formation and tubular cell vacuolization are apparent (× 252).

Table III). RBF was twice as high in the immunized compared with the nonimmunized controls despite comparable MAPs for the two groups (Table III).

Renal histology in the nonimmunized controls 24 h after intravenous *E. coli*/intramuscular gentamicin injections was comparable to that described above for the *E. coli*/gentamicin treated rats (group D), demonstrating focal tubular cell necrosis and cast formation. The immunized rats demonstrated a virtual absence of both tubular necrosis and cast formation.

Renal gentamicin uptake 2 h after viable *E. coli* injection was significantly less in the immunized (223±10 μg/g) vs. the nonimmunized group (383±41; $P < 0.01$). The degree of gentamicin uptake for the immunized group did not significantly differ from that observed in normal rats injected with gentamicin, 100 mg/kg i.m. (203±7).

Acute hemodynamic monitoring after E. coli bacteremia

The five rats subjected to MAP monitoring for 2 h after viable *E. coli* injection without gentamicin treatment had a baseline MAP of 132±6 mmHg. At 15 min, 1 h, and 2 h after *E. coli* injection, MAP was 115±12, 112±6, and 128±7 mmHg, respectively. None of these values significantly differed from the basal values. RBF 2 h after *E. coli* injection was 6.0±0.6 ml/min, a normal value (5).

The three rats subjected to continuous hemodynamic monitoring for 2 h after *E. coli*/gentamicin injection never developed a RBF value of <6.2 ml/min. One rat had a transient (30-min) decline in MAP to 101 mmHg which occurred 30 min after *E. coli* injection. However, the other two rats showed no decline in MAP from their normal basal values.

Overall animal survival rates

All rats injected with *S. aureus* and *Pseudomonas* survived the experimental protocols. Two of 13 rats injected with viable *E. coli* followed 2 h later by gentamicin treatment died. However, all rats injected with a sterile *E. coli* preparation (boiled; uv light) survived. In the set III experiments all rats, both immunized and nonimmunized, survived. Thus, the overall survival rate for gram-negative bacteria/gentamicin-treated rats, not counting the rats protected by endotoxin immunization, was 95%. Con-

Table II. Renal Function After Endotoxin, Gentamicin, or Endotoxin plus Gentamicin

		Baseline		24-h		48-h	
Group	*n*	BUN	Cr	BUN	Cr	BUN	Cr
Endotoxin, 10 μg	5	14±1	0.48±0.02	16±1	0.60±0.04	17±1	0.58±0.04
				NS	NS	NS	NS
Gentamicin, 100 mg/kg	6	17±1	0.55±0.03	17±1	0.58±0.02	19±1	0.52±0.02
Endotoxin/gentamicin	6	17±1	0.50±0.03	35±2*	0.70±0.03*	20±1	0.53±0.02
100 mg/kg				<0.001	<0.02	<0.05	NS

Endotoxin alone and gentamicin alone caused no significant rise in the BUN or serum creatinine (Cr) concentrations (by paired *t* test). However, the combination of endotoxin plus gentamicin induced significant azotemia ($P < 0.05$ compared with baseline values; paired *t* test). The gentamicin group is the same as group A in Table I and is presented here for the sake of comparison. Values are in mg/dl and are given as means±SEM.

Table III. Immunized vs. Nonimmunized Rats: Assessments After Intravenous Viable E. coli/i.m. Gentamicin Injection

Group	2-h post-injection	24-h post-injection			
	Renal gentamicin	BUN	Cr	MAP	RBF
	μg/g wet weight	*mg/dl*	*mg/dl*	*mmHg*	*ml/min*
Nonimmunized	383±41	72±12	1.05±0.16	117±8	2.3±0.3
	(6)	(10)	(10)	(4)	(4)
Immunized	223±10*	33±7*	0.68±0.06‡	127±12	4.8±0.3*
Purified *E. coli* endotoxin	(6)	(6)	(6)	(4)	(4)
Boiled *E. coli*	ND	30±2*	0.66±0.02‡	ND	ND
		(9)	(9)		
Boiled *P. aeruginosa*	ND	42±6‡	0.75±0.04‡	ND	ND
		(8)	(8)		

Numbers in parentheses are number of determinations.
* $P < 0.01$. ‡ $P < 0.05$ (compared with nonimmunized group). No difference in the degree of azotemia existed among the three immunized groups (by ANOVA).
Gentamicin, μg/gm tissue wet weight. For comparison, normal rats injected with 100 mg/kg gentamicin i.m. (without *E. coli* injection) had a renal gentamicin concentration of 203±7 (NS vs. the purified endotoxin immunized group). Thus, endotoxin tolerance restored renal gentamicin uptake to normal levels. ND = not done.

versely, 50% of viable *E. coli*-infected rats treated with ampicillin died. (The same mortality rate was noted in pilot studies when chloramphenicol was used to treat the *E. coli* infection, a protocol which also induced only mild azotemia; BUN, 29±5, 24 h post-infection). These differences in survival rates presumably reflected, at least in part, differences in antibiotic efficacy for treating the viable *E. coli* infection.

Discussion

The results of these studies indicate that: (*a*) when gram-positive bacteremia (*S. aureus*), is treated with a nephrotoxic antibiotic (gentamicin) no renal injury is produced; (*b*) when either nonviable gram-negative organisms or viable gram-negative organisms plus a nontoxic antibiotic (ampicillin) are injected into rats, mild, transient azotemia is induced but no tubular cell necrosis results; and (*c*) when gram-negative bacteremia (*E. coli, P. aeruginosa*) is treated with a nontoxic amount of gentamicin, acute renal injury results which is both functional (increased BUN, creatinine, decreased C_{ioth}) and morphologic (tubular necrosis, cast formation) in nature.

Several mechanisms exist for the development of ARF in the setting of sepsis. First, ischemic ARF can presumably occur as a result of septic shock. However, shock is a most unlikely cause for the ARF which followed the gram-negative bacterial/gentamicin injections. No significant reduction in either MAP or RBF was noted from 0 to 2 h post-*E. coli* injection. Since this was presumably the height of the bacteremia, shock, had it occurred, would have been expected at this time. MAP was also normal at 24 h after bacterial injection. Although it is theoretically possible that septic shock could have developed between 2 and 24 h after bacterial injection, this seems most implausible since the *E. coli* and *Pseudomonas*-injected rats treated with gentamicin had an overall survival rate of 95%. This is comparable to that seen with pentobarbital anesthesia alone (unpublished observations). This high survival rate makes the occurrence of septic shock highly unlikely. Lastly, it remains to be proven that severe shock, even had it occurred, could have caused the ARF. For example, Dobyan et al. (8) and Kreisberg et al. (9) have demonstrated that although severe shock in the rat (MAP ≤ 40 mmHg × 2 h) can induce focal tubular necrosis, ARF as defined by azotemia, does not develop. Thus, for all of these reasons, sepsis-induced hypotension seems excluded as the cause of the ARF which followed the gram-negative bacteria/gentamicin injections.

A second possible mechanism for ARF in the setting of sepsis is disseminated intravascular coagulation (DIC) leading to intrarenal vascular clotting and cortical necrosis. However, the renal histology of this study excludes this possibility. Cortical necrosis was absent and there was no intrarenal vascular thrombosis. Thus, even had DIC occurred, it could not be implicated in the pathogenesis of the ARF which developed in these experiments.

A third possible mechanism for renal insufficiency in the setting of gram-negative bacteremia is an endotoxin-mediated hemodynamic reduction in GFR. Endotoxin injection into rats has been shown to decrease RBF and GFR even in the absence of hypotension (10). This effect may be due to intrarenally generated vasoconstrictor products of both the cyclooxygenase and lipoxygenase pathways of arachidonic acid metabolism (10). However, these hemodynamic changes cannot be the sole explanation for the ARF seen in our experiments. First, although endotoxin can decrease RBF and GFR it does not produce tubular cell necrosis, a feature observed in our experiments. Second, neither boiled *E. coli* (group H) nor purified *E. coli* endotoxin (10 μg; Table II, 100 μg, Fig. 2) reproduced the degree of azotemia seen in the *E. coli*/gentamicin experiments. Note that the quantity of endotoxin in 1×10^9 *E. coli* has been calculated to be 10 μg (11). Third, *E. coli* injection followed by ampicillin resulted in only mild, transient azotemia. Thus, *E. coli* bacteremia in the presence of an antibiotic known to disrupt bacterial cell walls, maximizing endotoxin release (12), did not reproduce the degree of ARF which was seen following *E. coli*/gentamicin injections.

Thus, these considerations indicate that no single factor (shock, DIC, endotoxin) induced the ARF, but rather that it resulted from a gram-negative bacteremia/gentamicin nephrotoxic synergy. That a true synergistic toxicity existed is supported by both the renal functional and morphologic data: the degree

of azotemia observed in the *E. coli*/gentamicin treated rats cannot be accounted for by the sum of the actions of gentamicin alone (group A; no azotemia) plus *E. coli* alone (groups G, H; BUN, 28–32 mg/dl). Furthermore, neither gentamicin alone (group A) nor *E. coli* alone (group G or H) induced tubular cell necrosis. However, when *E. coli* and gentamicin injections were combined, tubular cell necrosis resulted.

The bacterial determinants of this toxic synergy have been at least partially defined. Bacterial viability was not a factor since *E. coli* sterilization did not diminish the severity of the gentamicin/gram-negative bacterial interaction. Bacteremia, per se, was not a factor since *S. aureus*/gentamicin injection produced no ARF. However, endotoxin is a major determinant of the toxic synergy. In support of this conclusion are the following: First, both *Pseudomonas*/gentamicin and *E. coli*/gentamicin induced ARF but *S. aureus*/gentamicin did not. *S. aureus,* unlike *Pseudomonas* and *E. coli,* has no endotoxin. Second, boiling of the *E. coli* did not diminish the severity of the *E. coli*/gentamicin nephrotoxic interaction. Endotoxin is 100°C heat stable, whereas bacterial enzymes and other proteins are denatured by the boiling process. Third, induction of endotoxin tolerance significantly attenuated all the major consequences of the gram-negative bacteremia/gentamicin toxic synergy (one-half the azotemia and doubled RBF at 24 h; prevention of increased gentamicin uptake and tubular cell necrosis). These findings strongly implicate endotoxin as a prime mediator of the gram-negative bacteremia/gentamicin toxic interaction. Fourth, a toxic synergy between endotoxin and gentamicin was directly confirmed. Neither purified endotoxin alone nor gentamicin alone induced azotemia. However, when administered together, azotemia resulted (Table II).

The reason that the purified endotoxin/gentamicin combination did not induce the same degree of azotemia as the *E. coli*/gentamicin combination cannot be answered on the basis of the available data. However, several likely explanations exist: First, purified endotoxin has been shown to react with plasma proteins which convert it to a low density form which can have altered biological activity (13, 14). Thus, it would not be surprising that endotoxin sequestered in bacterial cell walls and purified endotoxin would have different capabilities for inducing tissue injury. Second, the process of endotoxin purification can affect its structure, possibly causing a decrease in its ability to induce renal injury. For example, when extracted from bacteria, endotoxin assumes different molecular configurations, including disks, lamillae, ribbons, and vesicles (15). When then exposed to aqueous solutions the molecule can form a bilayer, burying the lipid portion in surrounding polysaccharide (15). Since endotoxin toxicity is probably triggered by its binding to cell membranes these conformational changes could explain why purified endotoxin and bacterial wall-associated endotoxin have different renal effects. Third, it is possible that some heat stable gram-negative bacterial component, in addition to endotoxin, contributes to the gram-negative bacteria/gentamicin interaction. Such a factor would be lost during the endotoxin purification process.

Several pathophysiologic factors in the ARF which followed the gram-negative bacterial/gentamicin injections have been identified. Increased renal gentamicin tissue uptake was one responsible factor since achieving a comparable renal gentamicin level in nonbacteremic rats by infusing 600 mg/kg of gentamicin produced modest renal insufficiency (group I, Table I). Of note in this regard is that gentamicin induces a dose dependent decline in GFR, primarily due to a decrease in the glomerular ultrafiltration coefficient (K_f) (16). How the bacteremia increased renal gentamicin uptake is unknown. Another factor which probably contributed to the decrease in renal function is that endotoxin plus gentamicin, both of which can reduce RBF (10, 17) together induced a late, hemodynamically mediated reduction in GFR. That RBF was doubled and that azotemia was halved by the induction of endotoxin tolerance suggests that part of the renal insufficiency was hemodynamically mediated. The presence of severe renal failure but only mild tubular necrosis, e.g., compared with that which follows ischemic ARF (4, 5), also suggests a hemodynamic component to the renal functional impairment. This notion is further supported by the fact that *E. coli* injections without gentamicin (group H) or 600 mg/kg gentamicin without bacteria (group I) each induced modest azotemia in the absence of tubular necrosis. Tubular obstruction is a third likely pathophysiologic factor in the induction of the renal failure. However, the cellular mechanism for the tubular cell necrosis which produced this obstruction is unclear. Both endotoxin (13) and gentamicin (18) can cause mitochondrial dysfunction, suggesting that together they might exert a synergistic mitochondrial toxicity. However, whether such a mechanism is involved in the cell necrosis noted in these experiments remains to be defined.

The endotoxin tolerance studies not only indicate the importance of endotoxin in the gram-negative bacteremia/gentamicin interaction; they also suggest that prior endotoxin exposure with resulting tolerance may be an important clinical modulator of gentamicin nephrotoxicity. Critically ill patients frequently experience multiple gram-negative infections. Thus, prior endotoxin exposure with resulting tolerance could confer protection against gentamicin when used for a second bout of infection. Since *Pseudomonas* inoculations were shown to protect against the *E. coli*/gentamicin toxic synergy, it appears that one gram-negative infection can confer protection against gentamicin nephrotoxicity when the drug is administered for a different gram-negative organism. Lipid A is believed to be the toxic and a constant moiety of most gram-negative endotoxins (7). This may explain why prior exposure to one gram-negative organism, e.g., *Pseudomonas,* can blunt gentamicin toxicity in the setting of a second and different gram-negative infection, e.g., *E. coli.*

In conclusion, these experiments indicate that: (*a*) gram-negative bacteremia and gentamicin exert a synergistic nephrotoxicity which can produce both acute tubular necrosis and acute renal failure; (*b*) this synergism does not require bacterial viability and it is mediated, at least in part, by endotoxin; (*c*) gram-negative bacteremia can markedly increase renal gentamicin uptake which contributes to the induction of the renal failure; and (*d*) endotoxin tolerance significantly attenuates the gram-negative bacteremia/gentamicin nephrotoxic synergy by normalizing renal gentamicin uptake, by preventing tubular cell necrosis and cast formation, and possibly by improving renal blood flow at the height of the renal functional impairment. In view of these findings, it appears that gram-negative bacteremia, a prime indication for gentamicin treatment, may dramatically predispose to the drug's nephrotoxic potential. Conversely, prior endotoxin exposure with resulting tolerance may protect against gentamicin nephrotoxicity in the setting of gram-negative sepsis.

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