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Effect of Diuresis on *Staphylococcus aureus* Kidney Infections in Mice

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In the Cornett strain of mice, water diuresis did not prevent hematogenous production of pyelonephritis by *Staphylococcus aureus*. Increased fluid intake did not affect the numbers of organisms deposited in the kidneys or the rate of growth during the first 4 hr after inoculation. Drinking the glucose solution did not enhance bacterial proliferation within the renal parenchyma. Subcutaneous injection of saline to supplement for interruption of drinking after inoculation reduced the numbers of organisms recovered in the kidneys but not sufficiently to prevent production of pyelonephritis. Incorporating penicillin as a marker indicated that fluids administered by subcutaneous injections were rapidly delivered to the kidneys. Combining diuresis with treatment did not influence the rapidity of delivery of antimicrobial to the kidneys or the length of time that it was present in the renal homogenate.

Water diuresis has been reported by Andriole and Epstein (3) to prevent and to cure hematogenous *Staphylococcus aureus* infections of the kidneys in rats. Furtado and Freedman (6) did not observe any protective effects when mice drinking 5% glucose were intravenously injected with *S. aureus*. However, in response to inoculation, there was a severe interruption in drinking by the mice and it took at least 4 days to reach preinfection levels of fluid intake. In rats, if diuresis was started 4 days after injection of *S. aureus* or *Streptococcus faecalis*, drinking 5% glucose did not prevent the development of pyelonephritis (2, 3).

An enhanced, more rapid inflammatory reaction in response to injury was described by Andriole (1) in kidneys of rats drinking 5% glucose as compared to water controls. Furthermore, Chernew and Braude (4) reported that phagocytosis was impaired in hypertonic, concentrated urine. Therefore, the loss of hypertonicity in the medulla appears to favor cellular defense mechanisms. Interruption of drinking could eliminate this advantage and could account for the differences observed for experimental studies using rats.

The extent of in vivo growth of *S. aureus* in the kidneys of mice during the first 24 hr was described by Gorrill et al. (8) as a reproducible method for predicting the rate of production of pyelonephritis. Furtado and Gorrill (7) observed that antibiotic control of bacterial proliferation in the kidney was readily achieved during the first 6 hr but not by 24 hr after inoculation. Therefore, changes in blood flow and hypertonicity within the renal parenchyma associated with water diuresis could affect the numbers of organisms deposited in the kidneys, the rate of in vivo proliferation, and the production of pyelonephritis.

To determine whether renal hemodynamics during diuresis affected the numbers of organisms deposited in the kidneys, in vivo growth studies were undertaken in mice intravenously inoculated with *S. aureus*. Because interruption of diuresis possibly restored the normal hypertonic gradient within the kidney, the effect of sustained diuresis, subcutaneous administration of saline before and immediately after inoculation, was measured on in vivo growth and production of pyelonephritis by *S. aureus*. To verify that subcutaneously injected saline was rapidly delivered to the kidneys and did exert an effect on microbial proliferation, quantitative studies using different doses of penicillin were included.

MATERIALS AND METHODS

Bacteria. A strain of *S. aureus* recovered from a clinical specimen was used in these studies. Stock cultures were maintained on tryptic soy agar (TSA) slants and stored at 4 C; they were subcultured once a month. The bactericidal concentration for penicillin G (lot 71925) was 0.125 units as measured by the tube sensitivity assay.

Media and preparation of inoculum. The inoculum was prepared by inoculating a tube of tryptic soy broth (TSB), and incubating overnight at 37 C; 1 ml was transferred to a flask containing 45 ml of TSB and placed on a water bath shaker at 37 C for 3 hr. The

culture was diluted with sterile physiological saline for inoculation. Bacterial counts were made by surface plating 0.1 ml of the dilution on TSA plates.

Mice. Cornett strain albino male mice, weighing 20 to 25 g, were used throughout. Animals were housed in air-conditioned quarters in groups of six mice per cage and were given laboratory chow ad libitum.

Water diuresis and supplementary saline injections. The drinking solution of 5% glucose (w/v) in distilled water was changed daily. Control mice were given tap water. Average fluid intake per mouse was calculated on the basis of total fluid intake for mice grouped in a single cage.

Supplementary saline (1.0-ml volumes) was given subcutaneously under the skin on the back at hourly intervals beginning 3 hr before and then immediately after inoculation.

In vivo growth studies. A 0.5-ml volume of the appropriate dilution was inoculated into a lateral tail vein. Mice were sacrificed at intervals at various times after inoculation up to 7 days. All mice were sacrificed by cervical dislocation, the urine was aspirated, and the kidneys were removed. The kidneys were examined for gross abscesses and then homogenized in glass grinding tubes containing 0.9 ml of sterile saline. Samples of the homogenate were quantitatively analyzed for bacteria by the surface plating technique. The counts were recorded as numbers of organisms per pair of kidneys or per milliliter of urine.

Antibiotic studies. Potassium penicillin G lot 71925 (Pfizer and Co., Inc., New York, N.Y.) was diluted in physiologic saline and given by subcutaneous injection in the back at the times indicated in the experiments.

RESULTS

Fluid intake and the rate of production of pyelonephritis. To assess the effect of diuresis on hematogenous *S. aureus* infections of the kidneys and on subsequent fluid intake for the Cornett strain of mice, groups of 12 animals on 5% glucose or tap water were intravenously inoculated with 10^6 or 10^7 *S. aureus*. Figure 1 shows that interruption of drinking followed inoculation in all groups, the degree of interruption being greatest with the larger inoculum. Mice drinking 5% glucose had a fluid intake at least double that of controls throughout the 7-day observation period. Increased fluid intake appeared to prevent deaths after inoculation of 10^7 organisms (Table 1), for there were five fewer deaths recorded in the group of mice undergoing diuresis than in the control group. The rate of production of gross abscesses after injection of 10^7 *S. aureus* was 80% in the diuresis group and 85% in the controls. Diuresis did not prevent infection since $>10^5$ organisms were recovered from all urines and from all the kidneys.

When the inoculum was reduced to 10^6 organisms, diuresis appeared to protect slightly. Whereas all kidneys from control animals were infected, diuresis prevented death, abscess production, or infection in one or two mice. The lowest fluid intake, 17 ml, occurred in the interval 24 to 48 hr after injection and within 3 days was back to fluid intake levels characteristic of diuresis before challenge.

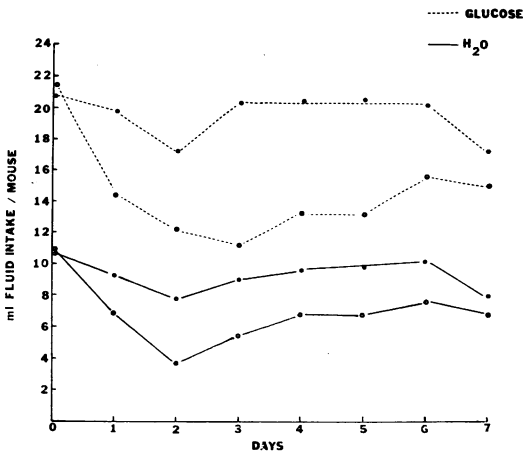

FIG. 1. *Effect of intravenous injection of S. aureus on fluid intake. Symbols: ●, 10^6 inoculum; ○, 10^7 inoculum.*

TABLE 1. *Production of kidney infections in mice[a] after intravenous injection of Staphyloccus aureus*

Dose	Fluid	Deaths	Abscessed kidneys	Infected[b] kidneys	Infected urine
10^7	Water	7	8/10	8/10	5/5
	5% Glucose	2	17/20	20/20	10/10
10^6	Water	2	15/20	20/20	10/10
	5% Glucose	1	12/22	17/22	8/11

[a] Twelve mice in each group.
[b] Recovery of $>10^5$ organisms at sacrifice on day 7.

Apparently, the effect of diuresis on the infectious process in the Cornett strain of mice was similar to that reported with the Yale strain of Swiss-Webster mice (6).

Effect of fluid intake on in vivo proliferation of S. aureus in mouse kidneys. Groups of 24 mice on 5% glucose or water were intravenously injected with 10^6 or 10^7 *S. aureus*. Four mice were sacrificed at times 0, 0.5, 1, 2, 3, and 4 hr after inoculation; the kidneys were removed, homogenized, and quantitatively analyzed for bacteria. As shown in Table 2, the numbers of organisms deposited in the kidneys of mice drinking water were 1.5×10^5 as compared to 1.6×10^6 *S. aureus* recovered from kidneys of animals drinking

5% glucose. In the control group, the numbers of organisms increased to 1.2×10^6 at 2 hr and decreased to 4.0×10^5 after 4 hr. For mice drinking 5% glucose, a 10^6 level was maintained throughout the 4 hr.

After inoculation of 10^7 *S. aureus*, the numbers recovered from the kidneys at time zero were comparable in both groups (Table 2). This was followed by an almost identical rate of in vivo proliferation in the kidneys during the 4-hr observation period. Prior drinking of 5% glucose did not enhance the rate of in vivo proliferation of this strain of *S. aureus*.

Effect of supplementary saline diuresis on in vivo growth of S. aureus. Because actual fluid intake at the time of inoculation could not be accurately quantitated, the effect of a sustained diuresis on the numbers of *S. aureus* deposited in the kidneys and the rate of in vivo proliferation were next examined. Supplementary diuresis was started by giving 1 ml of saline subcutaneously under the skin on the back beginning 3 hr before inoculation and immediately after inoculation. Mice intravenously inoculated with 1.9×10^7 *S. aureus* were sacrificed in groups of seven at 0, 0.5, 1, 2, 3, and 4 hr; the kidneys were removed, homogenized, and analyzed for recovered bacteria. As shown on Table 3, fewer organisms were deposited in the kidneys of mice given saline than in control mice (*see* Table 2). This was followed by a continued decrease during the next 30 min to 9.7×10^5 *S. aureus*, a level which was maintained during the subsequent 3.5 hr. Although there appeared to be an early decrease in the numbers deposited, the kinetics of proliferation apparently did not change after diuresis maintained by supplementary injection of saline.

TABLE 2. *In vivo proliferation of Staphylococcus aureus in the kidneys of mice[a] drinking 5% glucose or water*

Dose[b]	Time (hr)	No. of bacteria recovered per pair of kidneys[c]	
		Water	Glucose
10^6	0	$1.5 \times 10^5 \pm 0.1$	$1.6 \times 10^6 \pm 0.6$
	0.5	$8.5 \times 10^5 \pm 3.1$	$8.8 \times 10^5 \pm 1.7$
	1	$1.8 \times 10^6 \pm 1.1$	$9.6 \times 10^5 \pm 1.8$
	2	$1.2 \times 10^6 \pm 0.7$	$1.3 \times 10^6 \pm 0.5$
	3	$7.0 \times 10^5 \pm 2.0$	$7.8 \times 10^5 \pm 1.4$
	4	$4.1 \times 10^5 \pm 1.7$	$1.0 \times 10^6 \pm 0.3$
10^7	0	$2.7 \times 10^6 \pm 0.1$	$3.2 \times 10^6 \pm 0.9$
	0.5	$1.3 \times 10^6 \pm 0.3$	$1.5 \times 10^6 \pm 0.2$
	1	$1.5 \times 10^6 \pm 0.4$	$1.4 \times 10^6 \pm 0.4$
	2	$1.9 \times 10^6 \pm 2.2$	$1.3 \times 10^6 \pm 0.3$
	3	$8.0 \times 10^5 \pm 2.5$	$5.2 \times 10^6 \pm 5.6$
	4	$1.2 \times 10^6 \pm 0.3$	$1.0 \times 10^6 \pm 0.3$

[a] Twenty-four mice in each group.
[b] Intravenous injection of 0.5 ml of *S. aureus*.
[c] For each interval, mean value of four pairs of kidneys ± standard deviation.

TABLE 3. *Effect of supplementary diuresis[a] on in vivo proliferation and disease production by Staphylococcus aureus[b] in mouse kidneys*

Time (hr)	No. of bacteria recovered per pair of kidneys[c]
0	$2.4 \times 10^6 \pm 0.7$
0.5	$9.7 \times 10^5 \pm 2.5$
1	$1.1 \times 10^6 \pm 0.6$
2	$6.6 \times 10^5 \pm 3.0$
3	$1.8 \times 10^6 \pm 2.2$
4	$8.6 \times 10^5 \pm 4.6$
Day 7	Infected kidneys[d] 20/22
	Abscessed kidneys 15/22

[a] Subcutaneous injection of 1 ml of saline at hourly intervals beginning 3 hr before and immediately after injection.
[b] Intravenous injection of 1.9×10^7 *S. aureus* in 0.5 ml.
[c] For each interval, mean value of seven pairs of kidneys ± standard deviation.
[d] Recovery of $>10^5$ organisms.

The rate of production of pyelonephritis for 11 mice given saline at hourly intervals, starting 4 hr before and immediately after inoculation, are presented in Table 3. Fifteen of the 22 kidneys had gross abscesses and 90% were infected (rates quite similar to those shown on Table 1 for mice undergoing water diuresis or drinking water).

Effectiveness of the subcutaneous route of injection in delivering fluids to the kidneys. To assess whether subcutaneously administered fluids reached the kidneys promptly and influenced early in vivo proliferation of bacteria within the renal parenchyma, an assay incorporating antibiotic in the fluid was made by injecting six mice intravenously with 2.9×10^7 *S. aureus* followed by a subcutaneous infection of 750 units of penicillin G. The results in Table 4 indicate that subcutaneous administration of penicillin did effectively reduce the rate of disease production to 1 out of 12 kidneys. Only two kidneys were infected with more than 10^5 *S. aureus*.

To define the amount of time needed for delivery of penicillin to the kidneys, a growth study was carried out with 48 mice intravenously given 2.9×10^7 *S. aureus* and 750 units of penicillin by the subcutaneous route. Groups of eight mice were sacrificed at times 0, 0.5, 1, 2, 3, and 4 hr. As shown in Table 4, simultaneous injection of antibiotic did not affect the numbers of organisms deposited (3.1×10^6) or the numbers recovered up to 3 hr after inoculation. However, at 4 hr, less than 10% of the inoculum was present

as compared to 50% in the groups not given antimicrobial. Therefore, apparently the effects of this concentration of penicillin were detectable within 3 to 4 hr.

Effects of subcutaneous injection of large doses of penicillin on bacterial growth. To determine the rapidity with which subcutaneously administered antibiotic was delivered to the kidneys, the effect of 30,000 units of penicillin on in vivo proliferation of *S. aureus* was studied. It was also necessary to determine if the indicator substance should be given in a large volume to detect the effects more promptly. Therefore, groups of 24 mice were intravenously inoculated with 1.2×10^7 *S. aureus* followed by subcutaneous injection of 30,000 units of penicillin in either a 0.3-ml volume or in a 3.0-ml volume. Four mice from each group were sacrificed at times 0, 0.5, 1, 2, 3, and 4 hr. The number of *S. aureus* deposited in the kidneys were 6.8×10^5 in the group given penicillin in the smaller volume as compared to 2.0×10^6 in the group which received 30,000 units in 3.0 ml (Table 5). Within 30 min the effect of penicillin was detected by observing that fewer organisms were recovered in plating the homogenate directly than in a serial 1:10 dilution of the same homogenate. Furthermore, the morphology of the individual colonies on the plates with homogenate was typical of bacterial growth in the presence of excess antibiotic. These kinds of observations were recorded in both groups for animals sacrificed between 30 min and 3 hr after injection. At 4 hr, no penicillin effect (i.e., on colonial morphology or quantitative distribution on serial dilution) was evident although the numbers of organisms recovered were substantially reduced. Dispensing the 30,000 units of penicillin in a 3.0-ml volume did not affect the rapidity with which the antibiotic was delivered to the kidneys or its effectiveness in influencing in vivo proliferation.

This dose of penicillin was effective in preventing persistent infection and abcess production as indicated by the failure to recover *S. aureus* from the kidneys of mice sacrificed on day 7 (Table 5).

TABLE 4. *Influence of subcutaneous administration of penicillin G[a] on in vivo proliferation and production of pyelonephritis by Staphylococcus aureus[b]*

Time (hr)	No. of bacteria recovered per pair of kidneys[c]
0	$3.1 \times 10^6 \pm 2.9$
0.5	$1.7 \times 10^6 \pm 1.1$
1	$1.0 \times 10^6 \pm 0.7$
2	$7.8 \times 10^5 \pm 4.4$
3	$3.7 \times 10^5 \pm 1.7$
4	$2.3 \times 10^5 \pm 1.0$
Day 7	Infected kidneys[d] 2/12 Abscessed kidneys 1/12

[a] At 250 units per ml given at hourly intervals beginning 2 hr before and immediately after injection for a total of 750 units in 3.0 ml.

[b] Intravenous injection of 2.9×10^7 *S. aureus* in 0.5 ml.

[c] For each interval, mean value of eight pairs of kidneys ± standard deviation.

[d] Recovery of $>10^5$ organisms.

TABLE 5. *Effect of diuresis and penicillin treatment[a] on the in vivo proliferation of Staphylococcus aureus[b] in mouse kidneys*

Time (hr)	No. of bacteria recovered per pair of kidneys[c]	
	Vol at 0.1 ml	Vol at 1.0 ml
0	$6.8 \times 10^5 \pm 2.6$	$2.0 \times 10^6 \pm 0.4$
0.5	$2.3 \times 10^5 \pm 0.6$	$9.6 \times 10^5 \pm 7.6$
1	$3.3 \times 10^5 \pm 1.1$	$3.0 \times 10^5 \pm 1.3$
2	$2.8 \times 10^5 \pm 0.7$	$1.6 \times 10^5 \pm 0.7$
3	$2.4 \times 10^5 \pm 1.1$	$1.5 \times 10^5 \pm 0.5$
4	$1.6 \times 10^5 \pm 0.8$	$1.4 \times 10^5 \pm 0.8$
Day 7	Infected kidneys[d] 0/12 Abscessed kidneys 0/12	

[a] Penicillin (10,000 units) given at hourly intervals beginning 2 hr before and immediately after injection for a total of 30,000 units in total volume of 0.3 or 3.0 ml.

[b] Intravenous injection of 1.2×10^7 *S. aureus* in 0.5 ml.

[c] For each interval, mean value of four pairs of kidneys ± standard deviation.

[d] Recovery of $>10^5$ organisms.

DISCUSSION

S. aureus infection of the kidneys was not prevented by water diuresis in the Cornett strain of mice. Interruption of drinking, either 5% glucose or water, was observed after inoculation of 10^6 or 10^7 organisms. Although the Cornett mice drank larger total volumes of fluid, the relative glucose-water intake was 2:1 during the postinoculation observation period as compared to a 2:0 ratio when similar studies were carried out in the Yale strain of Swiss-Webster mice (6). Resumption of drinking to prechallenge levels took 3 to 4 days, depending on the size of the inoculum. Therefore, the effect of infection on drinking as well as the influence of increased fluid intake on the establishment of urinary tract infection in mice remain opposite to that observed when rats are the experimental hosts.

The protective effect observed in rats has been attributed to increased blood flow resulting in delivery of blood borne antibacterial factors including increased inflammatory response (1)

and to loss of hypertonicity aiding the process of phagocytosis (4). The results of in vivo growth studies in mice indicate that the number of bacteria deposited in the kidneys were comparable for mice undergoing water diuresis or drinking water and that the kinetics of in vivo proliferation of the bacteria during the first 4 hr were also quite similar. These observations could well be reflecting a return to normal hypertonic conditions within the renal parenchyma because fluid intake was minimal during this period. It should also be noted that drinking 5% glucose did not enhance growth. Enhanced proliferation was not observed in studies using a strain of *S. aureus* possessing bound coagulase (D. Furtado, Bacteriol. Proc., p. 71, 1971). This indicator strain had been described by Gorrill et al. (8) to grow in mouse kidneys without a lag phase. These combined observations further support that prior drinking of 5% glucose does not provide additional nutrients enhancing bacterial proliferation and therefore the rate of infection. As reported by Freedman (5), glucose was not detected in the urine or at elevated levels in the blood.

The decrease in numbers of organisms deposited in the kidneys of mice given supplementary subcutaneous injections of saline indicated that a briefly sustained diuresis did reduce the numbers of bacteria recovered from the kidneys. Such an effect could possibly be due to wash-out associated with increased blood flow. The reduction in numbers surviving after the first few hours without prolonged diuresis was not adequate for reducing the rate of production of pyelonephritis. It is possible that prolonged supplementary diuresis in mice already drinking 5% glucose would prevent pyelonephritis. Such a schedule of supplementary diuresis would be necessary to achieve levels of fluid intake comparable to those seen in rats. It would then be possible to determine whether degree of diuresis determines if increased fluid intake is protective, especially for extrapolating experimental observations to clinical practice. It is necessary to know if a substantial quantitative increase in fluid intake should be prescribed to realize any beneficial effects ascribed to increased flushing of the urinary tract.

The results of experiments using the antibiotic marker confirmed that administration of fluids subcutaneously under the skin on the back of the mouse did reach the kidneys promptly and did influence early in vivo proliferation as well as disease production. With large doses of penicillin, the effects were evident for 3 hr in the kidney homogenates regardless of the volumes used for the injection. It appeared that the larger volume did not reduce the amount of time needed for delivery of the penicillin to the kidneys or the duration of its detected effects.

Presumably, diuresis could affect treatment of infections of the urine when antimicrobial would be diluted by excretion of larger volumes of urine. Such studies on the effect of supplementary diuresis on treatment of bladder infections in mice are in progress.

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