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Urinary Excretion of Zinc and Iron following Acute Injection of Dead Bacteria in Dog (41143)

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Abstract. The major goal of this study was to determine whether injection of heat-killed bacteria alters urinary excretion of Zn and Fe. Pentobarbital-anesthetized dogs were prepared for standard renal clearance experiments; following a 1-hr control period, the animals received either heat-killed *Pasteurella multocida* or vehicle. Rectal temperature in bacteria-treated animals increased significantly by 2 hr following bacterial injection. Excretion rates of Zn and Fe were also significantly elevated by this time; by the end of 4 hr they had approximately doubled. The changes in Zn and Fe excretion correlated significantly with changes in rectal temperature. K excretion and urine flow over the 4 hr tended to increase, but the rises were not statistically significant. Plasma concentrations of Zn, Fe, and K did not change, nor did GFR. We conclude that, in the anesthetized dog, injection of heat-killed bacteria can acutely increase urinary trace-element excretion. However, the maximal contribution of the increased urinary losses to a reduction in plasma zinc and iron was calculated to be only a few percentages.

These experiments were designed to determine whether injection of heat-killed bacteria alters the acute urinary excretion of zinc and iron in anesthetized dog. Renal hemodynamics and electrolyte excretion undergo a variety of changes during the elevation in body temperature associated with infection or the administration of pyrogen (1, 2), and a major rationale for the present experiments was to expand this body of information to include trace elements, which have received very little attention. In order to avoid possible secondary effects (such as dehydration) on renal function, our study was limited to the early febrile period, the first 4 hr after injection of the dead bacteria.

The second rationale for these experiments was to determine the relationship of changes in urinary excretion of iron and zinc to changes in plasma concentrations of these trace metals. Decreases in the plasma concentrations of zinc and iron have been reported during both infection (3–12) and endotoxinemia (13–21). One mechanism accounting for at least part of the acute lowering of the plasma concentrations of both trace elements is sequestration by the liver (22–24), an effect induced by leukocytic endogenous mediator (LEM) (25). Another possible mechanism could be increased urinary excretion of the metals, but very few studies have been done to test this hypothesis. In people suffering from acute hepatitis, urinary excretion of zinc was found to be higher than normal (9); unfortunately, preinfection control levels were not available in this study because the disease had been contracted naturally. During experimentally induced sandfly fever in man, daily urinary excretion of zinc was found to be significantly decreased during Days 2 and 3 of infection (7); plasma values were significantly decreased during this same time period. With the exception of this study, to our knowledge, no studies have been reported dealing with the very early effects of infection on the urinary excretion of zinc and iron, despite the fact that significant decreases in the serum concentrations of these metals occur within 4 hr of administration of endotoxin or induction of experimental infection, at least in rat (13–15, 17) and rabbit (11).

Materials and methods. The study was performed on 11 male, anesthetized, mongrel dogs weighing 14 to 20 kg. In several experiments, to test for the effect of

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anesthesia on plasma zinc concentration, a 5-ml blood sample was taken from a cephalic vein prior to anesthesia. To induce anesthesia, sodium pentobarbital, 30 mg/kg, was administered intravenously, followed by 50-mg supplements as needed. A 5-ml postanesthesia blood sample was taken immediately following anesthetization in several experiments. All blood samples were collected in heparin, 10 units/ml, and the plasma was separated by centrifugation for 15 min at 2500 rpm on a Sorvall Model GLC-2B rotary centrifuge. Clean, but not rigorously sterile, surgery for standard renal clearance technique included catheterization of the femoral artery for blood sampling, femoral vein for infusion of creatinine, and the cephalic vein for administration of additional sodium pentobarbital, and injection of either bacteria or vehicle. A right lateral flank incision exposed the right ureter for catheterization. All catheters were gas-sterilized polyethylene tubing. When all surgical manipulations were completed a 5-ml postsurgery blood sample was taken in several experiments to determine whether surgery resulted in changes in plasma zinc and iron levels, and like all further blood samples, this was replaced with an equivalent volume of sterile isotonic saline. A creatinine prime consisting of 600 mg creatinine dissolved in 20 ml sterile saline was administered into the femoral vein over 3 min following completion of surgery. This was followed by a constant infusion at 0.2 ml/min of 3.0 g creatinine/100 ml sterile saline. Both creatinine solutions had been passed through a Millex Millipore filter (pore size 0.22 μm) to minimize microbial contamination of the nonsterilized creatinine.

An equilibration period of 45–60 min followed completion of all surgical procedures, after which a 1-hr control period was conducted in all dogs. Consecutive 30-min urine clearances were collected throughout this hour and the ensuing 4 hours with 10-ml blood samples collected at the midpoint of each urine collection. After the control hour, six of the dogs were chosen randomly to receive 2 ml of heat-killed *Pasteurella multocida* in saline, while the other five dogs received a sham injection of 2 ml of sterile saline. The *Pasteurella multocida* (American Type Culture Collection No. 7228) were prepared to a concentration of 3×10^9 bacteria per milliliter in saline (26), and this solution was then heat killed by autoclaving at 118° for 30 min.

Temperatures were recorded either rectally, with a standard glass thermometer, or orally with a Yellow Springs Instrument Company, Inc. telethermometer. Hematocrits were taken occasionally to assure proper replacement of blood with saline. Glomerular filtration rate (GFR) was measured as the creatinine clearance rate (27). The concentrations of zinc and iron in plasma and urine were determined using a Varion Techtron flame atomic absorption spectrophotometer, Model AA375. For urine, the samples were diluted 1:1 with glass-distilled water and read against water standards. Plasma samples for both zinc and iron were diluted 1:1 with 20% trichloroacetic acid (TCA) to precipitate serum protein; plasma standards also contained 10% TCA. For the iron samples, this plasma dilution was also incubated in a water bath at 90° for 15 min to release loosely bound iron. All diluted and centrifuged plasma samples were then centrifuged at 2500 rpm for 15 min in a Sorvall centrifuge to remove protein precipitate, and the supernatant was drawn off. Standards were linear over the range of sample values. Urine sodium and potassium concentrations and plasma potassium concentrations were measured on a National Instrument Laboratories flame photometer, Model 4-7000 after a 1:200 dilution with water containing 14.41 meq/liter lithium chloride. Urinary protein was measured by the Lowry method (28).

All grouped data are presented as mean $\pm$ 1 SE. Student's t test for two group means was used for comparing the changes exhibited by the time-control and bacteria-treated dogs during each period bevond the first hour (the baseline period).

Results. Figure 1 illustrates the mean group changes from the control hour (normalized to zero) for each period for temperature, urinary zinc excretion and iron excretion. The experimental group showed a steady increase in temperature (Fig. 1A)

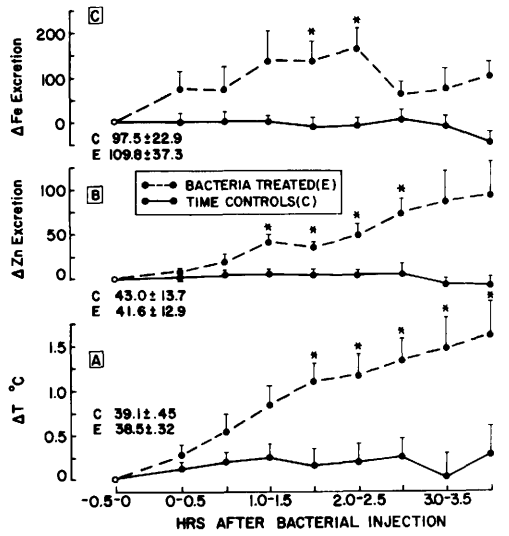

FIG. 1. Effects of injection of heat-killed *Pasturella multocida* on rectal temperature and the excretion of zinc and iron by the right kidney. The values for the baseline period (open circles) have been normalized to zero and all other points are the changes (mean ± SE) from the baseline for the groups (—— = time controls; – – – = bacteria treated). Statistical comparisons are between the groups at each time period; * = $P < 0.05$. Values next to (C) and (E) represent the baseline values for the time-controls (C) and bacteria-treated (E) dogs, respectively.

following administration of bacteria, reaching an average of 1.6° above the control-hour level, whereas the time-control animals showed a rise of approximately 0.3°. Each of the six dogs receiving bacteria had a rise in temperature of at least 0.6°, whereas none of the time controls did so. The rise in temperature for the experimental animals was significantly ($P < 0.05$) greater than the change in temperature for the time-control animals by the fourth clearance period (1.75 hr after administration of the bacteria) and remained significantly higher throughout the remainder of the experiment.

Zinc excretion (Fig. 1B) remained relatively stable in all time-control animals; in contrast, five of the six bacteria-treated dogs had progressive increases in zinc excretion over the entire 4 hr. The rise in zinc excretion relative to time controls became significant 1.25 hr after administration of bacteria, and this difference remained significant for the following three urine collections. Although the mean change in zinc excretion continued to rise after that point to reach an average change of approximately 90 ng/min per kidney above baseline levels, this rise relative to the time-control dogs was not significant because of the large variation among animals. The total increase in zinc excretion above baseline during the 4 hr after administration of bacteria amounted to approximately 13 μg (Fig. 2) and was significantly different from that exhibited by the time-control dogs.

The total change in iron excretion induced by the bacteria was also greater than that of the time-control animals (Fig. 2). Figure 1C illustrates the time course of the effect; the differences between the two groups were significant ($P < 0.05$) only during the fourth and fifth experimental periods. The differences were not significant beyond this time despite the fact that large increases were manifested by all bacteria-treated animals; this was due to the great variability of the increase and to the small sample size. In contrast to the continuous rise in zinc excretion, iron excretion reached its maximal value at 2.25 hr following administration of the bacteria, and then fell from this zenith by approximately 37%.

Figure 3 presents the mean group changes from the baseline for each period

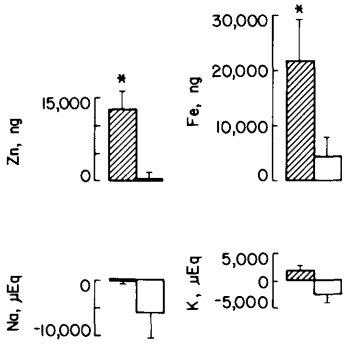

FIG. 2. Changes in total urinary excretion of minerals ascribable to injection of heat-killed bacteria. Each bar (mean ± SE) equals total excretion/4 hr after vehicle or bacteria minus the following product ((baseline excretion per minute) × 240 min). The hatched bars are bacteria injected; the open bars are time controls.

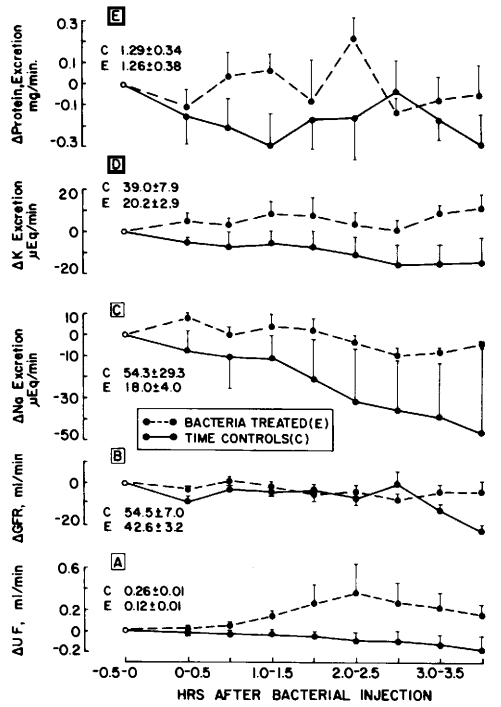

FIG. 3. Effects of heat-killed *P. multocida* on glomerular filtration rate (GFR) and the renal excretion of protein, potassium, sodium, and water. All data are for the right kidney. See text and legend for Fig. 1 for description of data presentation and analysis.

for other parameters of renal function. A large variation was found in the bacteria-induced changes in urine flow (Fig. 3A), mainly due to a single animal which had an unusually large increase in urine flow following administration of the bacteria. No statistical significance was obtained for the differences between groups, although the trend toward larger urine flows in the bacteria-treated group is evident. Five of the six bacteria-treated animals showed substantial increases, whereas three of the four time-control dogs showed decreases in urine flow rate.

The bacteria clearly produced no effect on GFR during the entire experiment (Fig. 3B); the basal value of 42.6 ± 3.2 ml/min kidney for the experimental animals was not significantly different from 54.6 ± 7.0 ml/min kidney for the time-control animals.

The difference (54.3 meq/min vs 18.0) in baseline sodium excretion (Fig. 3C) between the two groups was due to a very large baseline sodium excretion in a single dog. Time-control dogs had a gradually decreasing sodium excretion rate, whereas the experimental animals showed very little change in their sodium excretion rates. The differences between the groups for the entire period were not significant (Fig. 2). The potassium excretion of time-control dogs steadily declined, while the potassium excretion rate of the experimental animals increased slightly (Fig. 3D). This overall change in potassium excretion rate manifested by the experimental group, relative to the time-control group, was of borderline statistical significance (Fig. 2) ($P = 0.07$).

Protein excretion (Fig. 3E) was unaffected by the administration of the bacteria (note the magnitude of the vertical-axis scale); the maximal increase from basal levels in no animal was greater than 20%.

Figure 4A illustrates the changes in plasma zinc concentrations. Both groups of animals showed significant within-group decreases (paired t test) ($P < 0.05$) in plasma zinc concentrations from the control hour to the last experimental hour; however, the decreases manifested by the two groups were similar, i.e., there were no significant decreases when the responses of the two groups were compared. Decreases in plasma zinc of this magnitude have been consistently observed for anesthetized dogs in all experiments in our laboratory; the mechanism is unknown. Plasma iron levels also fell throughout the

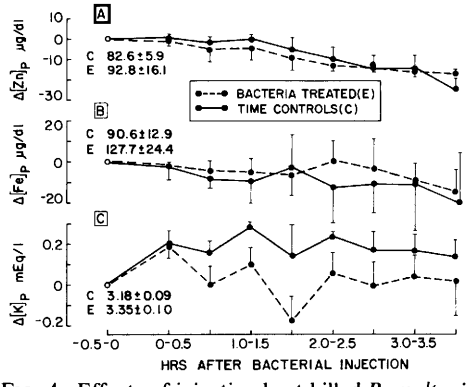

FIG. 4. Effects of injecting heat-killed *P. multocida* on the plasma concentrations of zinc, iron, and potassium. See text and legend for Fig. 1 for description of data presentation and analysis.

experiment (Fig. 4B). However this fall (approximately 15%), was not significant, and was virtually identical for the two groups. Plasma potassium concentrations (Fig. 4C) tended to decrease following injection of the bacteria, as compared to the time-control animals. However, the difference was not significant.

Figure 5 summarizes the effects of anesthesia and surgery on plasma zinc concentration. There is considerable variation among animals, but neither the anesthetic nor the surgical procedures produced any consistent change in plasma zinc concentration.

Discussion. These experiments demonstrate that injection of heat-killed *P. multocida* into anesthetized dogs results in a significant elevation of the urinary excretion of both zinc and iron. The absolute magnitude of these changes is small (see below) but the peak percentage change is very large relative to other physiological inducers of increased trace element excretion; for example, we have found that glucagon induces only a 30% increase in zinc excretion (personal observation).

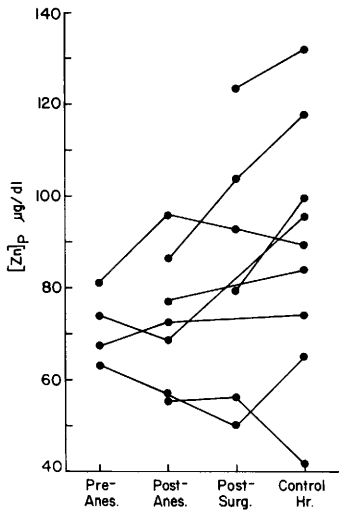

FIG. 5. Effects of anesthesia and surgery on plasma zinc concentration. Each point is a single value for one dog; lines connect points obtained for the same dog. Preanes. = samples obtained immediately prior to delivery of the anesthetic; post-anes. = samples obtained 15–30 min after delivery of the anesthetic; post-surg. = samples obtained immediately upon completion of all surgical procedures (1–2 hr after the anesthetic); control = samples obtained approximately 1 hr after completion of surgery.

The mechanisms responsible for the increase in zinc and iron excretion cannot be deduced from these experiments. It is possible that the increased excretion of these trace metals is part of a relatively nonspecific solute diuresis. Various changes in renal function have been reported by others to occur during infection or endotoxinemia, including increases in renal plasma flow and the excretion of water, potassium, and soium. A tendency toward increased urinary excretion of water, sodium, and potassium (relative to time controls) was also present in our experiments, and would probably have reached the level of statistical significance if more animals had been used. We did not pursue this question because evaluation of the excretion of these substances was not a primary aim of these experiments; these data do indicate, however, that the effect on the trace metals is larger and more consistent than those on water and the major monovalent cations. The percentage increase in trace metal excretion by every dog was much greater than that for water excretion, and there were no significant correlations between zinc or iron excretion, on the one hand, and the excretion of water, sodium, or potassium.

In contrast, as illustrated in Fig. 6, there were moderately strong correlations between temperature and the excretion of the trace elements; both correlations were statistically significant ($P < 0.05$). Of course, this does not prove that the increased temperature was the actual cause of the increased excretion. An equally likely hypothesis is that LEM induces both the fever and the changes in excretion. Alternatively, a hormone other than LEM might be involved.

Finally, we do not know whether, regardless of the mediator inducing it, the increased zinc and iron excretion results from an increased filtration of these trace metals or an alteration in their tubular handling. The plasma concentrations of both trace metals did not increase, but increases in the very small plasma functions which are ultrafilterable could have occurred.

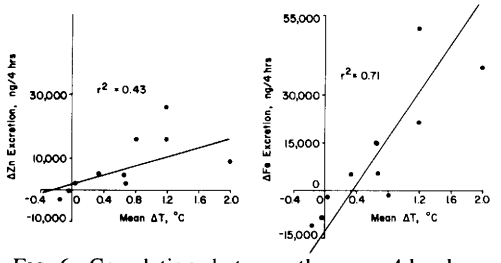

FIG. 6. Correlations between the mean 4-hr change in body temperature for all dogs and the total change in zinc and iron excretion over this same period. Changes from the baseline values were calculated as described in the text.

The second aim of these experiments was to determine how much altered urinary excretion of iron and zinc could contribute to changes in the plasma concentrations of these trace elements. Figure 2 quantifies the total magnitude of the increase in urinary excretion; each cross-hatched bar represents the "excess" excretion of the element by the right kidney during the 4 hr after bacterial administration. Multiplying this value by 2 gives the total excess excretion for both kidneys. Thus, the excretion of approximately 26 μg of zinc during the 4 hr could be attributed to the effects of the bacteria. In a 20-kg dog with 1 liter of plasma and a plasma zinc concentration of 100 μg/100 ml, this 26 μg could account for a 2.6% decline in plasma zinc levels. Similarly, excess iron excretion which could be attributed to the bacterial injection would account for a 4.2% decrease in plasma iron concentrations. Thus, for neither element would urinary excretion constitute a major reason for any fall in the plasma concentrations of these elements.

In fact, no effect of the bacteria on plasma zinc and iron concentrations was demonstrated despite the fact that the rise in temperature documented that leukocytic endogenous mediator (LEM), the presumed inducer of the decrease in plasma trace metals, was definitely being released. This was surprising since, in rats (13–15, 17), the single species most extensively studied, and in rabbits (11) the plasma concentrations of both zinc and iron were significantly reduced within 4 hr after the administration of endotoxin or bacteria (although the maximal decreases occurred some hours later). In contrast, in human beings treated with attenuated Venezuelan equine encephalomyelitis virus (6) or sandfly fever virus (7) the decreases in plasma concentration were delayed for several days. The only relevant previous study in dogs is that of Fisher (29), who reported that the plasma zinc concentration of Beagles with one form of infection (chronic interdigital cyst) was slightly (10%) but statistically significantly lower than that of control dogs; Beagles suffering from two other types of infection (chronic dermatitis or chronic otitis) did not manifest a lowered plasma zinc. Clearly, more work is required to define the time course and magnitude of changes (if any) in plasma trace metals induced by pyrogenic stimuli in dogs.

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