MECHANISM OF ASCORBIC ACID ENHANCEMENT OF THE BIOAVAILABILITY AND DIURETIC EFFECT OF FUROSEMIDE

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ABSTRACT:

The following possible explanations for the significant increases in the oral bioavailability and the diuretic and natriuretic effects of orally administered furosemide observed when ascorbic acid was coadministered to dogs were investigated: ascorbic acid might enhance the gastrointestinal (GI) absorption of furosemide, might inhibit GI wall metabolism of furosemide, might enhance the reabsorption of furosemide from the renal tubules, and might increase the unionized fraction of furosemide at the receptor sites. The significant increase in the oral bioavailability with coadministration of ascorbic acid seemed to result from reduced gastric first-pass metabolism of furosemide and not enhanced GI absorption of furosemide. This might be supported by rat studies; the percentages of the oral doses of furosemide recovered from the GI tract at 8 hr after oral administration were similar (p < 0.583) without (39.5%) and with (44.7%) coadministration of ascorbic acid, and the amounts of furosemide remaining per gram of stomach after 30-min incubations of 50 μg of furosemide with 9000g supernatant fractions of stomach homogenates were increased significantly (48.5 vs. 42.4 μg) by the addition of 100 μg of ascorbic acid. The significant increases in the diuretic and natriuretic effects of furosemide with ascorbic acid could be the result of increases in the reabsorption of furosemide from renal tubules and increases in the unionized fraction of furosemide at the renal tubular receptor sites. This was supported by 1.5–4.2-fold increases in urine output and approximately 20% decreases in the time-averaged renal clearance of furosemide when the urine pH was decreased by 1.5–2.5 units by oral administration of ammonium chloride.

Furosemide, a widely used loop diuretic, has been known to be incompletely absorbed after oral administration to healthy subjects or patients with various diseases (Benet, 1979; Hammerlund-Udenaes and Benet, 1989). A mean \( F^{1} \) value of 40% has been reported (Kelly et al., 1974; Waller et al., 1982), independently of the dosage form (solution or tablet) administered. However, \( F \) values as low as 10% were found for some healthy subjects or patients with congestive heart failure (Brater et al., 1982), and values of <40% were found in four of nine healthy subjects (Smith et al., 1981). The reasons for incomplete oral absorption of furosemide in rats have been elucidated (Lee and Chiou, 1983); the \( F \) value in that study was 30.2%, whereas 39% of an oral dose was not absorbed and GI first-pass metabolism involved 20–30% of the oral dose. However, possible methods to enhance \( F \) values and the diuretic and natriuretic effects of furosemide have never been published. In view of the strong interest in and concern about the dissolution and bioavailability of furosemide (Lee and Chiou, 1983), as well as therapeutic problems or failures reported in the literature (Odlind, 1980; Prasad et al., 1982; Brater, 1983), efforts were made to increase the \( F \) value and pharmacological effects of furosemide by coadministration of ascorbic acid.

The rationale for using ascorbic acid to increase the \( F \) value and the diuretic and natriuretic effects of orally administered furosemide in the present study is based on the following reports. First, it was reported (Chungi et al., 1979) that, in an in situ rat GI tract study, the absorption rate for furosemide varied greatly among the stomach, duodenum, and jejunum, with the stomach showing the fastest rate in either the same or different pH environments. Furosemide was found (Lee and Chiou, 1983) to be rapidly absorbed, probably largely from the stomach, in rats; approximately 70% of the oral dose eventually disappearing (presumably because of absorption and first-pass metabolism) in 8 hr was estimated to disappear within 20 min. Because furosemide is a weakly acidic drug with a \( \text{pK}_a \) of 3.80 (Chungi et al., 1979), more unionized furosemide could exist in the stomach if ascorbic acid could increase the acidity of the gastric fluid. Therefore, absorption of furosemide from the stomach might be enhanced, assuming that only the unionized fraction is absorbed, according to the pH-partition hypothesis (Shore et al., 1957). It was reported (Domingo et al., 1994) that ascorbic acid enhanced the GI absorption of aluminum in uremic rats. Second, furosemide is known (Michell et al., 1976) to be metabolized by a mixed-function oxidase system, and approximately 20–30% of an oral dose was reported (Lee and Chiou, 1983) to be metabolized in the GI wall (mainly by gastric first-pass effects) in rats. The insignificant role of the liver in the metabolism of furosemide was reported for humans (Fuller et al., 1981; Lee and Chiou, 1983), dogs (Verbeeck et al., 1981), and rats and rabbits (Lee and Chiou, 1983). It was reported (Rogers et al., 1987; Gonzalez et al., 1995) that ascorbic acid inhibited the conjugation of some drugs in the intestinal wall, and furosemide glucuronide formation in dogs was reported (Yakatan et al., 1976). Therefore, the \( F \) value of furosemide could be increased if ascorbic acid, an antioxidant, could

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1 Abbreviations used are: \( F \), extent of absolute oral bioavailability; GI, gastrointestinal; \( C_{\text{GRC}} \), time-averaged renal clearance.

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inhibit the metabolism of furosemide by enzyme systems in the GI wall. Third, furosemide has been known to be reabsorbed from the renal tubules in rats (Green and Mirkin, 1981) and rabbits (Lee, 1982), and the distal tubules and collecting ducts were the proposed sites for reabsorption of furosemide in rats (Green and Mirkin, 1981). Therefore, reabsorption of furosemide from the renal tubules could be increased if ascorbic acid renders the urine more acidic, assuming that only the unionized fraction is reabsorbed, according to the pH-partition hypothesis (Shore et al., 1957). Fourth, the site of action of furosemide is believed to be on the luminal surface of the thick ascending limb of the loop of Henle (Benet, 1979; Hammerlund-Udenaes and Benet, 1989). If ascorbic acid renders the urine more acidic, diuretic effects could be enhanced because more unionized drug could be available at the receptor sites. The main purpose of the present study was to test the aforementioned hypotheses, using dogs as model animals. Rats were used for some preliminary studies.

Materials and Methods

**Chemicals.** Furosemide (10 mg/ml iv solution of Lasix, as well as powder) and one of its metabolites (4-chloro-5-sulfamoylanthranilic acid) were obtained from Höcht-Roussel (Sommerville, NJ) and United States Pharmacopoeia (Rockville, MD), respectively. UDP-glucuronidic acid, Tris buffer, glucose-6-phosphate, MgCl₂, glucose-6-phosphate dehydrogenase, and NAD were obtained from Sigma Chemical Co. (St. Louis, MO). Lactated Ringer’s and 0.9% NaCl injectable solutions were purchased from Travenol (Deerfield, IL). Ascorbic acid and Flo-Cillin suspension (penicillin G, 300,000 units/ml) were obtained from Merck (Rahway, NJ) and Bristol-Meyers (Syracuse, NJ), respectively. Other chemicals were of reagent grade or HPLC grade and were used without further purification.

**Pretreatment of Animals.** Seven male Sprague-Dawley rats (250–300 g; Biological Resources Laboratories, University of Illinois at Chicago, Chicago, IL) were fasted overnight and up to 4 hr after commencement of the experiment, with water available ad libitum. Rats were kept individually in metabolism cages (Maryland Plastic Inc., Federalsburg, MD) with mesh floors to minimize coprophagy during the experiment. A minimum washout period of 1 week elapsed between experiments (by crossover design).

Six conditioned, male, beagle-mongrel hybrid dogs (dogs A–F, 7.3–16.0 kg; Biological Resources Laboratories, University of Illinois at Chicago) were fasted overnight, with water available ad libitum, and were restrained by means of a dog sling (Alice King Cathamm Medical Arts, Los Angeles, CA) during the experiments. An iv cannula (2 inches, 22 gauge; Sovereign, St. Louis, MO) with a three-way stopcock (Pharmaseal K75; Pharmaseal Inc., Toa Alto, Puerto Rico) was placed in the cephalic vein of one (for the oral administration study) or both (for the iv infusion study) forelegs for blood sampling or for infusion of furosemide and lactated Ringer’s solutions. Urine was collected via an indwelling polypropylene urinary catheter (5 French, 22 inches; Sovereign) introduced into the urinary bladder. At the end of the experiment, 1 ml of Flo-Cillin suspension was administered im for prophylactic purposes. A minimum washout period of 1 week elapsed between experiments (by crossover design).

**Oral Administration Study in Rats.** Lasix (6 mg) was administered orally (total oral volume, 0.6 ml), using feeding tubes (Fopper & Sons Inc., New Hyde Park, NJ), without (to serve as a control) or with 1 ml of aqueous solution containing 2.5 (pH 3.20), 5 (pH 3.01), 10 (pH 2.58), 50 (pH 2.36), or 100 (pH 2.29) mg of ascorbic acid, to rats (N = 7, in crossover design). Each ascorbic acid solution was given 1–2 min before the administration of Lasix. Urine was collected for up to 24 hr, and 50 ml of distilled water was used to rinse the metabolism cage. The rinsings were combined with the 24-hr urine samples. After measurement of the exact volume of urine output and combined urine samples, two 0.1-ml aliquots of each combined urine sample were stored in the freezer until HPLC analysis for furosemide (Lee and Chiou, 1983).

**Disappearance of Furosemide in Homogenates of Rat Stomach and Liver.** The procedures were similar (Lee and Chiou, 1983; Kim et al., 1993) to the reported method (Litterer et al., 1975). Five rats were exsanguinated and sacrificed by cervical dislocation. Approximately 1 g of each stomach and liver was excised, rinsed with 50 mM Tris-HCl buffer (pH 7.4), blotted dry with paper tissue, and weighed. All subsequent procedures were conducted at 4°C. Each tissue was cut into small pieces using scissors and then homogenized with 4 volumes of cold 0.25 M sucrose, in a tissue homogenizer (Tissuemizer model SDT-1800; Tekmar, Cincinnati, OH). Each homogenate was then centrifuged, using a Beckman (Palo Alto, CA) model J2–21 centrifuge, at 9000g for 20 min. After the floating fat layer was discarded, the supernatant fraction was collected for incubation.

Metabolic activity was initiated by adding 1 ml of the aforementioned supernatant to a glass test tube containing 0.05 ml of Lasix (50 µg of furosemide), 2.045 ml of an NADPH-generating system (1 mmol of NAD, 10 mmol of glucose-6-phosphate, 5 mmol of magnesium chloride, and 2 units of glucose-6-phosphate dehydrogenase), 3.3 mmol of UDP-glucuronic acid, and 100 mmol of Tris-HCl buffer (pH 7.4), with or without 100 µg of ascorbic acid. The mixture was thoroughly mixed by hand and then shaken, in a water-bath shaker maintained at 37°C, at a rate of 50 oscillations/min. After 30 min of incubation, 1 ml of 1 M NaOH was added to terminate the enzyme activity; an aliquot was stored in the freezer until HPLC analysis for furosemide (Lee and Chiou, 1983).

**Furosemide Recovered from the GI Tract after Oral Administration to Rats.** The procedures were similar to those reported previously (Lee and Chiou, 1983; Kim et al., 1993). Food (but not water) was withdrawn overnight and during the study. Lasix (6 mg) was administered orally (total oral volume, 0.6 ml), with or without 1 ml of an aqueous solution of 100 mg of ascorbic acid, to rats (N = 6). The ascorbic acid solution was administered 1–2 min before the administration of Lasix. Approximately 8 hr later, each rat was sacrificed by cervical dislocation and the abdomen was opened. The entire GI tract (including its contents and feces) was removed, cut into small pieces using scissors, and transferred into a beaker containing 0.01 M NaOH (to facilitate the extraction of furosemide), to adjust the volume to a total of 200 ml. After stirring with a glass rod for 10 min, two 0.1-ml aliquots of the supernatant were collected from each beaker and stored in the freezer until HPLC analysis for furosemide (Lee and Chiou, 1983).

**Intravenous Infusion Study in Dogs.** Twenty milligrams (2 ml) of Lasix were diluted with 46 ml of 0.9% NaCl injectable solution and then infused in 30 min (treatment I), with the assistance of an infusion pump (model 975; Harvard Instruments, South Natick, MA). Approximately 0.5 ml of blood was collected at −30 min (to serve as a control), −15 min, 0 min (at the end of infusion), and 5, 15, 30, 60, 90, 180, 240, 300, and 360 min after the dose. Approximately 1 ml of heparinized 0.9% NaCl injectable solution (10 units/ml) was used to flush the cannula after each blood sampling, to prevent blood clotting. Blood samples were centrifuged immediately to minimize the potential "blood storage effect" (the change in the plasma concentration of furosemide resulting from the time elapsed between collection and centrifugation of the blood sample) for furosemide (Lee et al., 1981). Urine samples were collected in the following time intervals: −0.5, 0, −0.1, −1, −2, −3, −4, −3, −4, −8, and −8–24 hr. Approximately 30 ml of air was used to flush the urinary bladder to ensure completion of each urine collection. The pharmacodynamic effects of furosemide were found to be dependent on the rate of fluid replacement in dogs (Li et al., 1986); therefore, volume-for-volume fluid replacement was made as soon as the urine was voided (spontaneously, especially during strong diuresis periods) or collected, with iv infusion of lactated Ringer’s solution for up to 8 hr. Each dog was kept individually in a metabolism cage (Lab Products, Maywood, NJ), with food and water available ad libitum during the last (8–24-hr) urine collection. Plasma and aliquots of urine samples were stored in the freezer until HPLC analysis for furosemide (Lee and Chiou, 1983).

Five milligrams (0.5 ml) of Lasix were diluted with 46 ml of 0.9% NaCl injectable solution and then infused in 8 hr into dogs E and F, with the assistance of an infusion pump (model 975; Harvard Instruments). Four grams of ammonium chloride dissolved in 45 ml of lactated Ringer’s solution were administered orally, using a stomach tube, at 4 and 5 hr during the 8-hr infusion. Approximately 0.5 ml of blood was collected at 0 (to serve as a control), 1, 2, 3, 4, 5, 6, 7, and 8 hr. Urine was collected at 0–1, 1–2, 2–3, 3–4, 4–5, 5–6, 6–7, and 7–8 hr, and the pH of each urine sample was measured. The other procedures were similar to those of the iv infusion study. The concentrations of sodium in urine were also measured.

**Oral Administration Study in Dogs.** Forty milligrams (4 ml) of Lasix were administered orally, without (treatment II) or with (treatment III) 1000 mg of ascorbic acid in water (25 mg/ml), and the mouth was flushed with 10
ml of water. Ascorbic acid solution was given 1–2 min before administration of Lasix. With the assistance of 40 ml of water, the same dose (40 mg) of furosemide powder was administered orally, without (treatment IV) or with 500 mg (treatment V) or 150 mg (treatment VI) of ascorbic acid powder, with 500 mg of citric acid (treatment VII), sodium bicarbonate (treatment VIII), or sodium ascorbate (treatment IX) powder, to dogs (N = 6 by crossover design).

Results

Oral Administration Study in Rats. In the preliminary rat study, a fixed dose (6 mg) of Lasix was administered orally, without or with various amounts of ascorbic acid. The concomitant administration of ascorbic acid produced increases in both cumulative 8-hr urinary excretion of unchanged furosemide and 8-hr urine output, and the enhancements seemed to be dose-dependent up to 10 mg of ascorbic acid (fig. 1). For example, coadministration of 10 mg of ascorbic acid resulted in mean increases of 62% in 8-hr urine output and 147% in 8-hr urinary excretion of unchanged furosemide, whereas the corresponding values were 14% and 46% when 2.5 mg of ascorbic acid was coadministered (fig. 1). However, coadministration of 100 mg of ascorbic acid was found to have effects similar to those of coadministration of 10 mg of ascorbic acid (fig. 1). Note that ascorbic acid (50 mg) alone did not have any effect on urine output in rats (16.0 and 16.3 ml/24 hr with and without ascorbic acid, respectively).

Disappearance of Furosemide in Homogenates of Rat Stomach and Liver. The amounts of furosemide remaining per gram of stomach after 30-min incubations of 50 µg of Lasix with the 9000g supernatant fractions of rat stomach homogenates were increased significantly (48.5 ± 1.24 vs. 42.4 ± 1.60 µg, p < 0.001) by the addition of 100 µg of ascorbic acid. However, the corresponding values for rat liver were not significantly different (41.3 ± 0.80 vs. 43.2 ± 3.03 µg, p < 0.234).

Furosemide Recovered from the GI Tract after Oral Administration to Rats. The absorption of furosemide from the rat GI tract seemed not to be enhanced by coadministration of ascorbic acid. The percentages of oral doses of furosemide recovered from the GI tract at 8 hr after administration of oral doses to six rats were 39.5 ± 13.4 and 44.7 ± 15.3% (p < 0.583) without and with coadministration of 100 mg of ascorbic acid, respectively.

Intravenous Infusion Study in Dogs. After 30-min iv infusion of 20 mg of Lasix (treatment I), the plasma concentrations of furosemide declined quickly (fig. 2), with a mean apparent t½ of 32.0 min (range, 30.0–35.5 min) (table 1). The contribution of CLR to the time-averaged total body clearance of furosemide was 51.4% in dogs (table 1).

Lasix (5 mg) was infused for 8 hr to dogs E and F, and 4 g of ammonium chloride was administered orally at 4 and 5 hr during the 8-hr infusion. The urine pH was reduced by approximately 1.5–2.5 units after oral administration of ammonium chloride (table 2). The hourly urine output and urinary excretion of sodium were increased significantly after oral administration of ammonium chloride; the urinary excretion of sodium was increased from 10.6–12.3 mmol to 30.1–31.6 mmol for dog E and from 9.22–9.38 mmol to 10.3–16.5 mmol for dog F (table...

**TABLE 1**

Mean ± SD pharmacokinetic and pharmacodynamic parameters for furosemide after 30-min iv administration of 20 mg of furosemide iv solution (treatment I), oral administration of 40 mg of furosemide iv solution without (treatment II) or with (treatment III) 1000 mg of ascorbic acid solution, or oral administration of 40 mg of furosemide powder in a capsule without (treatment IV) or with 500 mg (treatment V) or 150 mg (treatment VI) of ascorbic acid powder, with 500 mg of citric acid (treatment VII), sodium bicarbonate (treatment VIII), or sodium ascorbate (treatment IX) powder, to dogs (N = 6 by crossover design).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treatments</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
<th>V</th>
<th>VI</th>
<th>VII</th>
<th>VIII</th>
<th>IX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (kg)</td>
<td>12.1 ± 2.89</td>
<td>12.3 ± 2.64</td>
<td>11.8 ± 2.48</td>
<td>12.2 ± 2.62</td>
<td>12.1 ± 2.59</td>
<td>12.4 ± 2.65</td>
<td>13.0 ± 2.75</td>
<td>12.9 ± 2.79</td>
<td>12.7 ± 2.81</td>
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<tr>
<td>F (%)</td>
<td>30.0 ± 2.38</td>
<td>43.0 ± 16.1</td>
<td>73.0 ± 16.4</td>
<td>38.3 ± 13.2</td>
<td>68.9 ± 14.8</td>
<td>66.5 ± 14.2</td>
<td>38.6 ± 7.57</td>
<td>39.6 ± 9.91</td>
<td>45.2 ± 11.3</td>
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</tr>
<tr>
<td>t½ (min)</td>
<td>11.3 ± 1.49</td>
<td>12.6 ± 3.24</td>
<td>12.8 ± 4.28</td>
<td>9.74 ± 0.95</td>
<td>8.30 ± 3.08</td>
<td>13.5 ± 2.71</td>
<td>7.92 ± 3.12</td>
<td>12.8 ± 3.83</td>
<td>12.4 ± 4.00</td>
<td>7.96 ± 1.77</td>
</tr>
<tr>
<td>CL (ml/min/kg)</td>
<td>22.0 ± 2.59</td>
<td>74.1 ± 34.5</td>
<td>83.5 ± 18.3</td>
<td>20.6 ± 2.59</td>
<td>74.1 ± 34.5</td>
<td>83.5 ± 18.3</td>
<td>22.0 ± 2.59</td>
<td>74.1 ± 34.5</td>
<td>83.5 ± 18.3</td>
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<tr>
<td>AUC 0–8 hr (mg/ml/min)</td>
<td>603 ± 1470</td>
<td>1720 ± 2100</td>
<td>1940 ± 2750</td>
<td>1220 ± 253</td>
<td>1450 ± 377</td>
<td>2750 ± 579</td>
<td>1350 ± 352</td>
<td>2700 ± 863</td>
<td>2920 ± 702</td>
<td>2120 ± 567</td>
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<tr>
<td>AUC 0–24 hr (mg/ml/min)</td>
<td>181 ± 567</td>
<td>2920 ± 1810</td>
<td>3920 ± 2920</td>
<td>210 ± 567</td>
<td>1720 ± 414</td>
<td>3390 ± 711</td>
<td>1510 ± 346</td>
<td>3110 ± 827</td>
<td>3220 ± 680</td>
<td>2410 ± 559</td>
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</table>

* CL, time-averaged total body clearance; A, total amount excreted in urine.
2). Note that oral administration of 4 g of ammonium chloride alone did not have any diuretic effect in the two dogs. The plasma concentrations at steady state (from 2 hr after infusion) were fairly constant before and after oral administration of ammonium chloride to the two dogs (table 2). However, the $CL_R$ was decreased by approximately 20% (table 2) after oral administration of ammonium chloride to the two dogs.

**Oral Administration Study in Dogs.** The absorption of furosemide from the GI tract of dogs was fast after oral administration of Lasix without (treatment II) and with (treatment III) 1000 mg of ascorbic acid. The mean time to reach the peak concentration of furosemide was 45–60 min (based on experimental data) for both treatments II and III, and then the plasma concentrations declined more slowly than after treatment I (fig. 2), with mean apparent $t_{1/2}$ values of 74.1 and 83.5 min (table 1) for treatments II and III, respectively. Note that coadministration of 1000 mg of ascorbic acid (treatment III) significantly increased the $F$ value (73.0 vs. 43.0%), mean cumulative 8-hr (13.5 vs. 8.30 mg) and 24-hr (14.9 vs. 8.90 mg) urinary excretion of unchanged furosemide, and mean cumulative 8-hr urine output (2750 vs. 1450 ml), compared with values measured without coadministration of ascorbic acid (treatment II), as listed in table 1. The plasma concentrations of furosemide were higher (fig. 2) after coadministration of 1000 mg of ascorbic acid (treatment III) than were those without coadministration (treatment II), and this resulted in a considerable increase in AUC values (93.0 ± 34.0 vs. 51.6 ± 24.1 $\mu$g/min/ml, $p < 0.3866$) for treatment III.

Two control oral administration studies (without coadministration of ascorbic acid) were conducted for each dog, using oral administration of Lasix (treatment II) and furosemide powder in a capsule (treatment IV). There were no significant differences (table 1 and fig. 2) between treatments II and IV with respect to mean cumulative 8-hr and 24-hr urinary excretion of unchanged furosemide, mean cumulative 8-hr urine output, and $F$ values. Similar results have been reported (Kelly et al., 1974; Waller et al., 1982), indicating that $F$ values were similar after oral administration of iv solutions and tablets of furosemide to humans. The mean $F$ values for treatments II and IV were comparable to those measured in humans (Kelly et al., 1974; Waller et al., 1982). Therefore, treatment IV was selected as an oral administration control for the following discussion.

Three oral administration studies using coadministration of ascorbic acid, i.e. 1000 mg in aqueous solution (treatment III) and 500 mg (treatment V) and 150 mg (treatment VI) as powder, were conducted for each dog. There were no significant differences (table 1) among treatments III, V, and VI with respect to mean cumulative 8-hr and 24-hr urinary excretion of unchanged furosemide, mean cumulative 8-hr urine output, and $F$ values. Therefore, treatment V was selected as an ascorbic acid treatment for the following discussion.

Note that, after coadministration of 500 mg of citric acid (treatment VII), the mean value for 8-hr urine output was increased significantly (2120 vs. 1350 ml), compared with that for treatment IV, although the 8-hr and 24-hr urinary excretion of unchanged furosemide and $F$ values were not significantly different between treatments IV and V (table 1). After coadministration of 500 mg of sodium bicarbonate (treatment VII) or sodium ascorbate (treatment IX), 8-hr and 24-hr urinary excretion of unchanged furosemide, 8-hr urine output, and $F$ values were not significantly different, compared with those for treatment IV (table 1).

**Discussion**

The following possible explanations for the significant increases in the $F$ values and the diuretic and natriuretic effects of orally administered furosemide when ascorbic acid was coadministered to dogs (treatment V) were investigated: 1) ascorbic acid might enhance the absorption of furosemide from the GI tract in dogs, 2) ascorbic acid, an antioxidant, might inhibit the metabolism of furosemide in the GI wall in dogs, 3) ascorbic acid might enhance the reabsorption of furosemide from the renal tubules in dogs, and 4) ascorbic acid might
increase the unionized fraction of furosemide at the receptor sites. First, the significant increases in the F values (80% increase) and the 8-hr (62% increase) and 24-hr (79% increase) urinary excretion of unchanged furosemide produced by coadministration of ascorbic acid (treatment V) in dogs (compared with those for treatment IV) might be the result of enhanced absorption of furosemide from the canine GI tract. However, this seemed to be a remote possibility, based on rat studies; the percentages of oral doses of furosemide recovered from the rat GI tract at 8 hr after administration of oral doses to six rats were not significantly different (p < 0.583) without (39.5 ± 13.4%) and with (44.7 ± 15.3%) coadministration of 100 mg of ascorbic acid. It was also reported (Matsuki et al., 1992) that ascorbic acid did not enhance the absorption of iopiridin in rats. The value of 39.5% in the control rats in the present study was very close to the reported values (40.3% (N = 12) and 40.1% (N = 6) in other rat studies (Lee and Chiou, 1983; Kim et al., 1993). Although furosemide is known to be unstable in acidic media (Cruz et al., 1979), it is stable in human gastric and/or duodenal fluids (Beermann et al., 1975; Andreasen et al., 1982; Lee and Chiou, 1983).

Second, the significant increases in the F values and the 8-hr and 24-hr urinary excretion of unchanged furosemide produced by coadministration of ascorbic acid (treatment V) in dogs (compared with those for treatment IV) might also be the result of decreases in GI first-pass effects after coadministration of ascorbic acid. It was reported (Lee and Chiou, 1983) that the metabolic activity of the stomach (9000g supernatant fractions of stomach homogenates) from five rats was found to be much greater (e.g., 5–10.5-fold) than those for treatment IV) might also be the result of decreases in the gastric first-pass metabolism of furosemide, rather than enhanced absorption of furosemide from the GI tract. The exact mechanism for decreases in the gastric first-pass metabolism of furosemide with ascorbic acid remains to be fully explored.

Third, the significant increases in the diuretic (8-hr urine output, 107% increase) and natriuretic (8-hr urinary excretion of sodium, 107% increase) effects of furosemide with coadministration of ascorbic acid (treatment V) in dogs (compared with those for treatment IV) were the result of significant increases in F values and resultant significant increases in the 8-hr urinary excretion of unchanged furosemide with treatment V. Moreover, this could also be the result of increases in the reabsorption of furosemide by the canine renal tubules with coadministration of ascorbic acid. This was supported by the following study. Lasix (5 mg) was infused for 8 hr to dogs E and F, and 4 g of ammonium chloride was administered orally at 4 and 5 hr during the 8-hr infusion. The urine pH was reduced by approximately 1.5–2.5 units after oral administration of ammonium chloride (table 2). The $C_L_R$ of furosemide was decreased by approximately 20% (table 2) after oral administration of ammonium chloride, suggesting that furosemide could be reabsorbed from renal tubules in the two dogs, especially with acidic urine. It was reported (Green and Mirkin, 1981) that the furosemide $C_L_R$/glomerular filtration rate ratio in rats was reduced from 1.07 at a urine pH of 7.15 to 0.14 at a urine pH of 5.67. Based on the aforementioned data, one could estimate that at least 87% [100 × (1.07 − 0.14)/1.07] of the filtered and secreted furosemide could be reabsorbed from rat renal tubules at the lower urine pH.

The significant increase in urinary excretion of sodium with coadministration of ascorbic acid (treatment V) was also found in dogs; the mean 8-hr value was increased 2.07-fold with treatment V, compared with treatment IV (fig. 3). However, the urinary excretion of potassium was not significantly different (p < 0.166) between treatments IV and V (fig. 3), although the urine output (table 1) and urinary excretion of sodium (fig. 3) were significantly different. Similar results have been reported for humans (Branch et al., 1977), dogs (Lee et al., 1986), and rats (Kahn et al., 1983; Jang et al., 1994). This might be the result of constant rates of potassium secretion in the distal tubule (Giebisch, 1978).

Fourth, the significant increase in the diuretic and natriuretic effects of furosemide with treatment V, compared with treatment IV, might also be the result of increases in the unionized fraction of furosemide at the receptor sites. This could be supported by the results for the citric acid-treated group (treatment VII). The mean value for cumulative 8-hr urinary output with treatment VII was significantly higher than that for the control group (treatment IV), although the mean F values and cumulative 8-hr and 24-hr urinary excretion of unchanged furosemide were not significantly different between control and citric acid-treated groups (table 1). Therefore, the increase in urine output in the citric acid-treated group (treatment VII) might be the result of increases in the unionized fraction of furosemide at receptor sites, because of acidic urine produced by citric acid. This explanation could also be applied to ascorbic acid treatment (treatment V) and oral administration of ammonium chloride during the 8-hr infusion of Lasix to dogs E and F (table 2). Note that the increase in the unionized fraction of furosemide at the receptor sites with coadministration of citric acid (treatment VII) could not be the result of increases in the

**TABLE 2**

<table>
<thead>
<tr>
<th>Urine Collection Interval</th>
<th>Dog E</th>
<th>Dog F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine pH</td>
<td>Urine Output</td>
<td>Sodium</td>
</tr>
<tr>
<td>2–3</td>
<td>8.26</td>
<td>35</td>
</tr>
<tr>
<td>3–4</td>
<td>8.06</td>
<td>37</td>
</tr>
<tr>
<td>4–5</td>
<td>7.44</td>
<td>63</td>
</tr>
<tr>
<td>5–6</td>
<td>5.98</td>
<td>156</td>
</tr>
<tr>
<td>6–7</td>
<td>5.63</td>
<td>151</td>
</tr>
<tr>
<td>7–8</td>
<td>5.63</td>
<td>142</td>
</tr>
</tbody>
</table>
unbound fraction of furosemide in plasma produced by citric acid metabolism of furosemide, increases in the reabsorption of furosemide from renal tubules, and increases in the unionized fraction of furosemide at the receptor sites. The increased diuretic effects of furosemide with coadministration of citric acid could be the result of increases in the reabsorption of furosemide from renal tubules and increases in the unionized fraction of furosemide at the receptor sites. 

In conclusion, the significant increases in the $F$ values and the diuretic and natriuretic effects of furosemide with coadministration of ascorbic acid might be the result of decreased gastric first-pass metabolism of furosemide, increases in the reabsorption of furosemide from renal tubules, and increases in the unionized fraction of furosemide at the receptor sites. The increased diuretic effects of furosemide with coadministration of citric acid could be the result of increases in the reabsorption of furosemide from renal tubules and increases in the unionized fraction of furosemide at the receptor sites.

If the results described above could be extrapolated to humans, they might have important clinical implications. For example, variability in urine pH among normal subjects or patients with different clinical conditions might have, in part, contributed to the marked intersubject and intrapatient variability in the diuretic response observed after the same doses of furosemide (Benet, 1979; Brater, 1983). In addition, acidification of urine might offer an alternative means to increase the clinical efficacy of this drug, especially for some patients with resistant or refractory conditions (Benet, 1979; Brater, 1983). The effect of coadministration of ascorbic acid on furosemide absorption in humans remains to be explored.


