Rate and Pattern of Gastric Emptying in Humans Using ^{99m}Tc-Labeled Triethylenetetraamine–Polystyrene Resin

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Received September 17, 1979, from the **Department of Veterinary Biosciences, Division of Nuclear Medicine, College of Veterinary Medicine, University of Illinois at Urbana–Champaign, Urbana, IL 61801,* and the ‡*Divisions of Medicinal Chemistry and Nuclear Medicine, Colleges of Pharmacy and Medicine, University of Kentucky, Lexington, KY 40506.* Accepted for publication December 26, 1979.

Abstract □ The preparation and application of a new radiopharmaceutical used in the investigation of the rate and pattern of gastric emptying by external scintigraphy are described. Triethylenetetraamine was bound covalently to cross-linked chloromethylated polystyrene. The triethylenetetraamine–polystyrene resin tenaciously and rapidly bound technetium 99*m*. The gastric emptying rate was evaluated in normal adult volunteers and patients by serially recording the gastric radioactivity after ingestion of a test meal mixed with ^{99m}Tc-labeled triethylenetetraamine–polystyrene resin. The data indicated that ^{99m}Tc-labeled triethylenetetraamine–polystyrene resin was an ideal agent for assessing the rate and pattern of gastric emptying in humans. The gastric emptying half-time ($t_{1/2}$GE) in normal subjects ranged from 25 to 75 min.

Keyphrases □ Radiopharmaceuticals—^{99m}Tc-labeled triethylenetetraamine–polystyrene resin, determination of gastric emptying rate and pattern, humans □ GI motility—determination of gastric emptying rate and pattern, radionuclide imaging, humans □ Triethylenetetraamine–polystyrene resin, ^{99m}Tc-labeled—determination of gastric emptying rate and pattern, humans

The introduction of γ-emitting short-lived radionuclides and the development of the scintillation camera connected to a fast-acquisition digital data processor, multimode analyzer, and videotape recorder permit the noninvasive investigation of the rate and pattern of gastric emptying by external scintigraphy. Quantitative dynamic data and excellent scintigraphic images of the stomach, intestines, and colon can be obtained without physical or physiological influences on the GI tract.

BACKGROUND

In several clinical situations, detailed information about abnormalities in the pattern and rate of gastric emptying would be helpful. Such abnormalities already are recognized as being concomitant with certain diseases. For instance, hypermotility has been associated with duodenal ulcers, while diminished gastric peristalsis has been associated with gastric ulcers (1, 2). Furthermore, delayed gastric emptying has been observed in malignant diseases of the stomach and in pyloric stenosis (3, 4).

Measurement of gastric emptying is not only an important aid to the clinician studying gastroduodenal disease, dumping syndrome, and postvagotomy disturbances but also is a major determinant in the absorption rate of drugs. Individual variations in the drug absorption rate from any dosage form may be due largely to differences in the rate of gastric emptying (5, 6).

Since sodium [^{51}Cr]chromate and a rectilinear scanner first were used to measure the gastric emptying rate (7), several other radiopharmaceutical preparations have been used for the same purpose, including: ^{113m}In- and ^{99m}Tc-labeled diethylenetriaminepentaacetic acid (8, 9), a ^{113m}In-labeled microcolloid (10), a ^{99m}Tc-labeled colloid of antimony sulfide (11), ^{99m}Tc-labeled human albumin microspheres (12), cesium 129 adsorbed onto a suspension of zirconium phosphate (13), and their combinations (8, 13).

The disadvantages of these radiopharmaceutical preparations are well known. For instance, ^{113m}In- and ^{99m}Tc-labeled diethylenetriaminepentaacetic acid are water soluble and remain in the liquid phase of the gastric content, which is discharged through the pylorus more rapidly than the solid phase, and thus lower half-time values of gastric emptying result. Colloidal preparations are difficult to prepare, and their physicochemical properties offer disadvantages such as the formation of micelles and coagulation. The adsorption or absorption of the radiopharmaceutical by the stomach walls also provides an opportunity for error.

The recently introduced radiopharmaceutical ^{99m}Tc-labeled triethylenetetraamine–polystyrene resin (I) (14, 15) is free of these shortcomings and approximates the characteristics of an ideal radiopharmaceutical for assessing the rate and pattern of gastric emptying. These characteristics are: (*a*) not to influence the osmolality of the stomach content, (*b*) to be nonabsorbable and nonadsorbable, (*c*) to bind the radionuclide tenaciously and not to exchange it with food particles or the stomach wall, (*d*) to possess ideal food-mixing characteristics and have a particle size comparable to that of foods, and (*e*) to be nontoxic and inert and give reproducible and noninvasive estimates of the gastric emptying time without exposing the patient to a high radiation dose.

EXPERIMENTAL

Preparation of Popcorn Polystyrene—The polymer was prepared according to the procedure described by Letsinger *et al.* (16). It was washed three times with chloroform, dried, and broken down in a blender.

Preparation of Chloromethylated Polystyrene—Popcorn polystyrene (70 g, 40–100 mesh) was allowed to swell in 550 ml of chloroform for 1 hr. A mixture of 100 ml of chloromethyl methyl ether and 40 ml of stannic chloride was added to the polystyrene, and the contents were stirred at 25° for 2 hr. After filtration, the resin was washed with chloroform, dioxane, dioxane–water, water, and acetone. The product was dried at 60° under vacuum for 24 hr, yielding 86 g.

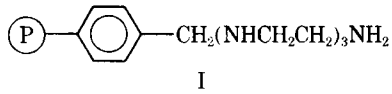
Anal.—Calc. for chloromethyl groups on 50% of the resin rings: Cl, 13.4%. Found: Cl, 13.3%.

Preparation of Triethylenetetraamine–Polystyrene Resin (I)—Chloromethylated polystyrene (10 g) was added to 30 ml of pyridine. After 30 min, a 10-fold excess of the amine was added; the mixture was maintained at 95° in an oil bath under a calcium chloride drying tube. The resin was recovered by filtration and washed with pyridine, water, and methanol. It was dried at 60° under vacuum.

Anal.—Calc. for: N, 14.87; Cl, 0.0. Found: N, 9; Cl, 0.08.

Uptake of Sodium [^{99m}Tc]Pertechnetate by Triethylenetetraamine–Polystyrene Resin—Triethylenetetraamine–polystyrene resin (0.20 g), with a bead size ranging between 0.16 and 0.4 mm, was placed in a 150-ml beaker, and 50 ml of double-distilled water was added. The pH was adjusted to a predetermined value with 0.1 *N* HCl. A 50-ml aliquot of sodium [^{99m}Tc]pertechnetate (52 μCi) in distilled water was added. The mixture was stirred magnetically, and the pH was kept constant and monitored by a pH meter[1]. The temperature was maintained at 23–25°. At the appropriate time intervals, 1-ml samples were withdrawn and counted by a γ-ray well counter[2].

Preparation of ^{99m}Tc-Labeled Triethylenetetraamine–Polystyrene Resin—Triethylenetetraamine–polystyrene resin (0.25 g, 40–100 mesh) was placed in a 50-ml beaker with 20 ml of distilled water and stirred for 1 min. A solution of sodium pertechnetate[3] (20 μCi–1 mCi) was added. The mixture was stirred for 10 min, and the labeled resin was



I

[1] Beckman Zeromatic SS-3.
[2] Picker Spector Scaler-4.
[3] Technetium 99*m*, obtained from a ^{99}Mo-generator (E. R. Squibb and Sons, Princeton, NJ 08540), as sodium [^{99m}Tc]pertechnetate in saline.

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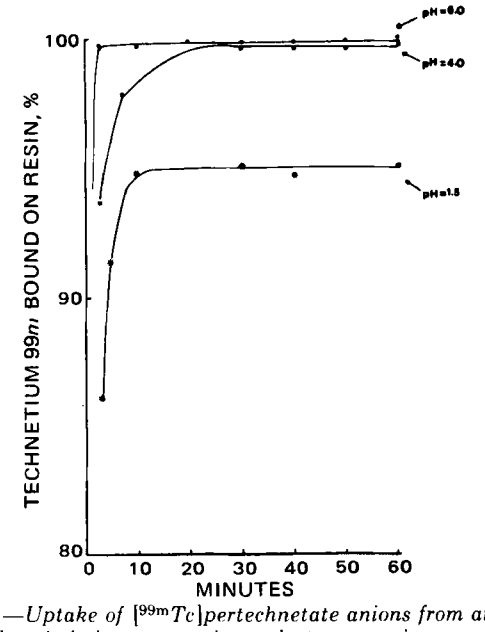

Figure 1—*Uptake of [^{99m}Tc]pertechnetate anions from an aqueous solution by triethylenetetraamine–polystyrene resin.*

recovered by filtration. The resin was washed with two 50-ml aliquots of distilled water and subsequently was counted to determine the specific activity.

Stability of ^{99m}Tc-Labeled Triethylenetetraamine–Polystyrene Resin in Simulated Gastric Juice—The ^{99m}Tc-labeled triethylenetetraamine–polystyrene resin (0.4 g, 2 μCi/g) was introduced in a beaker containing 50 ml of simulated gastric juice[4] (pH 1.4). The mixture was stirred magnetically, and the temperature was maintained at 25°. Samples of 0.5 ml were withdrawn at predetermined time intervals.

Human Testing—To evaluate the new radiodiagnostic agent, 20 adult male and female hospital patients were studied. Normal subjects who had never undergone GI tract surgery and subjects who had had surgery of the upper GI tract participated in the study. None of the subjects was treated with anticholinergic drugs prior to or during the study. No special preparation of the subject was needed. Two reference points were drawn on the abdominal skin of the subject and on the oscilloscope by the radioactive marker, cobalt 57, to ensure reproducibility of the position of the subject relative to the camera.

The ^{99m}Tc-labeled triethylenetetraamine–polystyrene resin was mixed with a test meal consisting of 33.3 g of cream of wheat and 2.4 g of sodium chloride in 235 ml of water. After the subject ingested the labeled test meal, the time was recorded and the subject was placed under the γ-camera[5,6] in the supine position. The positions of the γ-camera and the subject were adjusted so that the entire stomach appeared in the center of the monitoring oscilloscope. Several scintiphotographs of the stomach were obtained at certain time intervals.

In each time interval, four to nine points were collected. Each point represented the integrated relative radioactivity per minute of the desired flagged area of the stomach. The data were stored on a magnetic tape for future reference. The logarithm of the relative radioactivity of the stomach was plotted against time. From the slope of the graph, the half-time of the gastric emptying ($t_{1/2}$GE) of the test meal and the ^{99m}Tc-labeled triethylenetetraamine–polystyrene resin beads was determined. A whole-body survey at the end of each study failed to show any detectable activity outside of the GI tract.

RESULTS

Popcorn polystyrene, an opaque, low-density polymer, was prepared by copolymerization of styrene and divinylbenzene (99.8:0.2) according to a literature procedure (16). The particle size was reduced after a chloroform wash, and 40–100-mesh beads were used.

[4] USP.

[5] Baird atomic system 77 (Baird Atomic Nuclear Division, Bedford, MA 01730) equipped with a computer and magnetic tape and possessing storage and replay capability.

[6] Pho-Hp camera interfaced with a Nuclear data 50/50 computer, Nuclear of Chicago, Des Plaines, Ill.

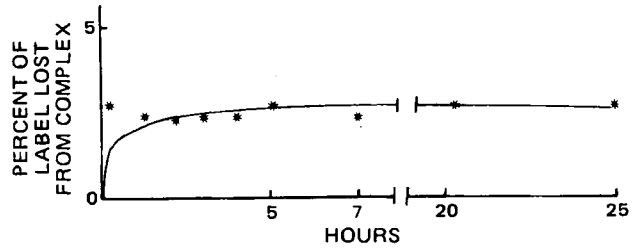

Figure 2—*Percent of radioactivity lost from ^{99m}Tc-labeled triethylenetetraamine–polystyrene in simulated gastric juice (pH 1.4).*

Introduction of chloromethyl functions into the insoluble polymer was carried out by reaction with chloromethyl methyl ether in the presence of anhydrous stannic chloride. The degree of chloromethylation of popcorn polystyrene was controlled by the amounts of stannic chloride and chloromethyl methyl ether and the reaction time. Chloromethyl groups were introduced in 50% of the polystyrene rings, producing a resin with 3.6 mEq of chloride/g.

Attachment of triethylenetetraamine was performed by swelling of the chloromethylated resin beads (40–100 mesh) in pyridine, followed by treatment with a 10-fold excess of triethylenetetraamine at 95°. Elemental analysis for nitrogen and chlorine indicated that triethylenetetraamine was bound to polystyrene by more than one attachment (17).

Triethylenetetraamine–polystyrene resin beads (40–100 mesh) efficiently and quantitatively bound the [^{99m}Tc]pertechnetate anions from aqueous solutions of ~10^{-9} *M* sodium [^{99m}Tc]pertechnetate. The anion uptake at three pH values (1.4, 4.0, and 6.0) was complete in 10 min (Fig. 1). When the popcorn polystyrene and chloromethylated polystyrene resins were used, the uptake of pertechnetate anions was 8.4 and 8.6%, respectively, after 60 min.

The ^{99m}Tc-labeled resin was stable in simulated gastric juice, releasing only 2.0% of the radioactivity in 25 hr at 37° (Fig. 2). The results of experiments in humans showed that <0.2% of the ingested radioactivity appeared in the blood and urine during the passage of the resin throughout the entire GI tract. A whole-body survey at the end of each study failed to show any detectable activity outside of the GI tract.

Twenty healthy adult volunteers were studied to establish a normal range of the gastric emptying rate (Table I). Invariably, the subjects exhibited a monoexponential pattern of gastric emptying. A typical curve is shown in Fig. 3. The ordinate represents the logarithm of relative radioactivity (expressed as counts per minute) and the abscissa denotes the time (minutes). The relative radioactivity was determined by selecting, with an electronic cursor, a region of interest over the whole gastric area excluding the pylorus and intestines. The slope of the curve was equal to $-\lambda\text{eff}/2.303$.

The observed constant, λeff, was equal to the sum of the technetium 99*m* decay constant, λ, and the rate constant of gastric emptying, λGE, where $\lambda\text{eff} = 0.693/t_{1/2}\text{eff}$, $\lambda = 0.693/t_{1/2}$, and $\lambda\text{GE} = 0.693/t_{1/2}\text{GE}$. The half-time of gastric emptying, $t_{1/2}$GE, was calculated from the following equation: $t_{1/2}\text{eff} = (t_{1/2}t_{1/2}\text{GE})/(t_{1/2} + t_{1/2}\text{GE})$. With this technique, the $t_{1/2}$GE for normal individuals ranged from 25 to 75 min (Table I). This

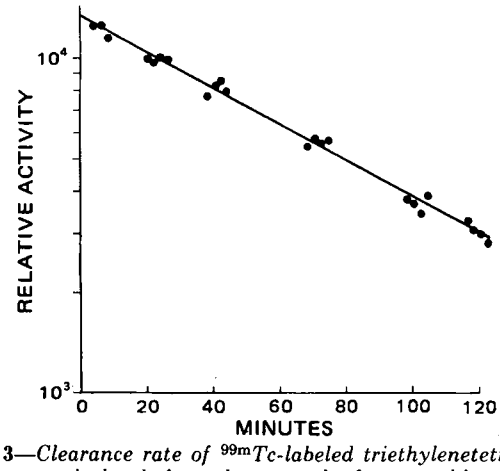

Figure 3—*Clearance rate of ^{99m}Tc-labeled triethylenetetraamine–polystyrene resin beads from the stomach of a normal human male, $t_{1/2}GE$ = 58 min.*

Table I—Gastric Emptying Half-Time in Normal Volunteers

Patient (Sex)	$t_{1/2}$GE, min
KM (M)	63
TF (M)	57
LW (M)	75
AM (M)	25
RB (M)	43
FD (M)	63
MS (F)	34
MT (F)	41
HF (M)	60
AM (M)	37
RL (F)	39
BM (F)	58
JW (M)	35
NF (F)	41
CE (F)	39
MV (F)	55
VS (F)	45
WG (M)	51
GD (M)	57
MM (M)	62

range was in agreement with results obtained using other radiopharmaceuticals (11). The $t_{1/2}$eff was not affected by the duodenal or jejunal overlap with the stomach (9). The gradual movement of radioactivity from the stomach through the pylorus into the intestines was indicated by a series of sequential scintigraphic images (Fig. 4). The entire stomach and the intestines were visualized. The absorbed radiation dose by the stomach was 102 mrads/250 μCi.

A second group of three individuals suffering from various disorders of the GI tract was used to demonstrate the difference in gastric emptying rates between normals and patients. Two patients suffering from pyloric stenosis exhibited a monoexponential pattern of gastric emptying with half-times of 115 and 136 min, respectively. A third patient with a 70% gastric resection and gastrojejunostomy exhibited a gastric emptying pattern consisting of two components: a component with a high rate of decrease, $t_{1/2}$GE = 5 min, representing dumping, and a component with a low rate of decrease, $t_{1/2}$GE = 91 min (Fig. 5).

DISCUSSION

The purpose of this study was to demonstrate the suitability of ^{99m}Tc-labeled triethylenetetraamine–polystyrene resin as an agent for investigating the pattern and rate of gastric emptying in humans. This study ascertained that the ^{99m}Tc-labeled resin was an ideal agent for measuring the gastric emptying rate of a solid meal in normal subjects and patients. The agent fulfilled the criteria of an ideal agent. It tenaciously bound [^{99m}Tc]pertechnetate anions; it was not absorbed or adsorbed on the gastric mucosa; the polystyrene beads had a particle size comparable to that of solid food and mixed homogeneously with the solid phase of the gastric content; it was nontoxic; it was an easily prepared radiopharmaceutical with the ideal radiation characteristics of technetium 99*m*; and, finally, the technique was noninvasive and comfortable to the patient. The use of the γ-camera coupled with a fast data-acquisition device allowed rapid and convenient observation of second-by-second alterations in the pattern and rate of gastric emptying.

Two fundamental assumptions were made in using this technique: the stomach was presumed to remain in the same position and retain the same shape throughout the study, and no correction was made for changes of depth of the radioisotope below the abdominal wall. The error that was

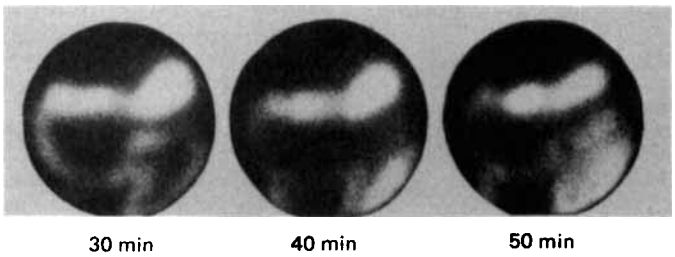

Figure 4—*Anterior scintigraphic series at 10-min intervals illustrating the gastric elimination and bowel distribution of the ^{99m}Tc-labeled triethylenetetraamine–polystyrene resin, $t_{1/2}GE$ = 36 min.*

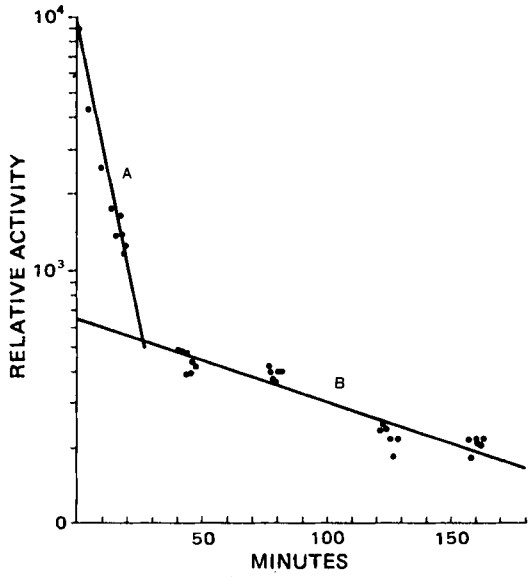

Figure 5—*Clearance rate of ^{99m}Tc-labeled triethylenetetraamine–polystyrene from the stomach of a male patient with Billroth II. Key: A, $t_{1/2}GE$ = 5 min; and B, $t_{1/2}GE$ = 99 min.*

introduced by these assumptions was minimal (13). Another source of error was the variations in tissue absorption of the γ-rays and the overlap of the duodenal–jejunal flexure with the gastric antrum, which tended to increase the count when, at the time of reading, the labeled gastric contents went through the duodenum or jejunum. However, this error did not change the slope of the linear logarithmic equation that described the gastric emptying process (3).

This study demonstrated that the gastric emptying of the solid phase of the ^{99m}Tc-labeled triethylenetetraamine–polystyrene gastric content can be described by a monoexponential function, at least for the first 120 min for normal subjects and patients suffering from pyloric stenosis. Moreover, it was shown that GI surgery, such as gastrojejunostomy, may drastically change the pattern and rate of gastric emptying (Fig. 5). Since the gastric emptying rate was affected by various factors, the position of the patient, the test meal, the position of camera, and the administered radioactivity dose were kept as constant as possible.

The use of ^{99m}Tc-labeled triethylenetetraamine–polystyrene in combination with a scintillation camera is a better technique than the isotopic and nonisotopic techniques that have been used for the investigation of the pattern and rates of gastric emptying of the solid phase of the gastric content. The disadvantages of the previous techniques and agents were discussed extensively elsewhere (9). Clinicians may use this technique to study GI diseases such as gastric and duodenal ulcers, gastric neoplasms, pyloric stenosis, dumping syndrome, and postsurgical status of gastrectomy, gastrojejunostomy, vagotomy, and pyloroplasty.

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Preliminary Evaluation of Furosemide–Probenecid Interaction in Humans

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Abstract □ The pharmacokinetics and pharmacodynamics of intravenous furosemide, 40 mg, were studied in four healthy male subjects in a crossover fashion with and without probenecid pretreatment. In each study, 16 plasma and 10 urine samples were collected over 24 hr. Fluid and electrolyte urinary losses were replaced orally throughout the study. Unchanged furosemide and probenecid were measured using high-pressure liquid chromatography; urinary sodium was measured by flame photometry. Although probenecid caused marked changes in the pharmacokinetic parameters of furosemide (increased area under the curve, decreased plasma and renal clearance, increased half-life, and decreased fraction excreted unchanged in the urine), there was no significant difference in its gross 8-hr natriuretic and diuretic effect. However, analysis of the time course of natriuresis showed a pattern similar to that of the urinary furosemide excretion rate, whereas the plasma concentration was poorly correlated over the entire dose–response curve.

Keyphrases □ Furosemide—pharmacokinetics and pharmacodynamics with and without probenecid pretreatment, humans □ Probenecid—pharmacokinetics and pharmacodynamics of furosemide with and without probenecid pretreatment, humans □ Drug–drug interactions—pharmacokinetics and pharmacodynamics of furosemide with and without probenecid pretreatment, humans □ Pharmacokinetics—furosemide with and without probenecid pretreatment, humans

Furosemide is an anthranilic acid derivative used to treat edematous states of hepatic, cardiac, and renal origin (1–3). It is believed to act at the luminal surface of the nephron where it inhibits the active reabsorption of chloride in the ascending limb of the loop of Henle (4–7). Since furosemide is highly protein bound (8, 9), access to the lumen occurs primarily through active secretion *via* the nonspecific organic acid secretory pathway (5, 6, 10). Thus, any drug or chemical substance that competes for this pathway could prevent furosemide from reaching its site of action and thereby attenuate its diuretic response.

Probenecid is a weak organic acid that competes with furosemide for active secretion into the kidney lumen. This competition can prevent furosemide from achieving an adequate cellular or luminal concentration and thereby diminish its natriuretic and diuretic response. Previous studies in experimental animals supported this hypothesis and showed that probenecid can decrease the natriuretic action of furosemide (10, 11). Studies in humans evaluating the effect of probenecid on the pharmacokinetics and pharmacodynamics of furosemide are limited and less clear (12–14).

The present investigation was undertaken to clarify the mechanism by which probenecid alters the diuretic response of furosemide. An additional objective was to define, in humans, a relationship between the dose of furosemide, its concentration or amount in a measurable sampling compartment, and its diuretic effect.

EXPERIMENTAL

Materials and Methods—Four males, 21–33 years and 65–77 kg, participated as outpatients in this study. Each subject had a normal medical history, physical examination, and standard laboratory tests. Each subject received 40 mg of furosemide alone and after pretreatment with probenecid. An interval of at least 1 week elapsed between studies. Subjects fasted the night before and until at least 2 hr after administration of the diuretic. Identical lot numbers for each drug were used throughout the study.

Furosemide was administered intravenously over ~3 min, with the midpoint of the infusion considered as time zero. Probenecid (1 g) was ingested at bedtime the night before and on arising the morning of the study (30–60 min prior to furosemide administration). Blood samples (3 ml) to determine the drug concentration were obtained with an indwelling heparinized scalp vein needle at 0, 5, 10, 20, 30, 45, 60, 80, 100, 120, 180, 240, 300, 360, and 480 min and ~24 hr.

Voided urine was collected at 0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, and 24 hr and at two times of spontaneous voiding at home between the 8- and 24-hr collections. After each voiding, subjects drank a volume of balanced electrolyte solution flavored with fruit syrup equal to their urinary volume to avoid dehydration and electrolyte depletion.

All 24-hr blood samples showed normal electrolytes, urea nitrogen, and creatinine. Sodium concentrations were measured with a flame photometer[1]. Statistical differences were determined using a paired t test.

Plasma samples of furosemide, with and without probenecid pretreatment, were analyzed using a rapid, sensitive, and specific high-pressure liquid chromatographic[2] (HPLC) assay developed in this laboratory (15). Samples were run on a μBondapak C_{18} reversed-phase column[3] (30 cm × 3.9 mm i.d.) using dual-channel UV detection[4] (0.01 aufs). A 20-μl aliquot containing the internal standard, phenobarbital sodium (0.25 mg/ml), was added to 0.20-ml plasma furosemide samples. The mixture was vortexed, and 0.40 ml of acetonitrile was added. The mixture was vortexed again and then centrifuged for 10 min. The supernate was transferred to a clean test tube and evaporated under nitrogen gas until ~0.15 ml of the solution remained. A solvent system of 25% acetonitrile in 0.01 *M* acetic acid, adjusted to pH 5.0 with sodium hydroxide, was employed to measure furosemide at 280 nm and pheno-

[1] Model 450, Corning Scientific Instruments, Medfield, Mass.
[2] Model 6000A, Waters Associates, Milford, Mass.
[3] Waters Associates, Milford, Mass.
[4] Model 440 absorbance detector, Waters Associates, Milford, Mass.

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