Furosemide kinetics and dynamics after kidney transplant

We examined differences between responder (R) (40 to 80 mg/day) and nonresponder (NR) (≥120 mg/day) patients after kidney transplant with respect to furosemide kinetics and dynamics. Nonresponders had reduced plasma clearance (NR 64 ± 21.4 and R 105 ± 23 ml/min, two-sample t test; $p < 0.05$), renal clearance (NR 18.4 ± 8.1 and R 47.1 ± 11.0 ml/min; $p < 0.005$), and renal clearance to creatinine clearance ratio (NR 0.43 ± 0.15 and R 0.80 ± 0.07; $p < 0.005$). Half-life rose in the nonresponders (NR 130 ± 13 and R 87.6 ± 16.3 min; $p < 0.005$). There was no difference between groups with respect to nonrenal clearance, extent of availability, volume of distribution steady state, and the fraction of the dose excreted unchanged in the urine after intravenous administration. These results suggest that nonresponders have less ability to secrete furosemide into tubular fluid as well as less ability to respond to drug.

David E. Smith, Ph.D.,* John G. Gambertoglio, Pharm.D., Flavio Vincenti, M.D., and Leslie Z. Benet, Ph.D. *San Francisco, Calif.*

Department of Pharmacy, School of Pharmacy, and Department of Medicine, School of Medicine, University of California

Furosemide is one of the most potent diuretics available today.[19, 20, 31] It exerts its effect at the luminal surface of the nephron where it inhibits the active reabsorption of chloride in the ascending limb of the loop of Henle.[8, 9, 16, 27] Because furosemide is highly protein bound,[2, 25] access to the kidney lumen occurs primarily through active secretion through the nonspecific organic acid secretory pathway.[8, 9, 13] Thus any drug or disease that prevents furosemide from reaching its site of action in the lumen could attenuate its natriuretic and diuretic action.

Furosemide is a valuable diuretic after kidney transplant in treatment of volume overload. Cumulation of extracellular fluid is common, usually occurring soon after transplantation, and may persist for months despite the absence of conditions usually associated with salt and water retention such as acute rejection of the transplant, congestive heart failure, hypoalbuminemia, and low glomerular filtration rate. Clinical observations* suggest that, although after kidney transplant some patients respond well to small doses of furosemide (responders), others (nonresponders) are refractory to the drug. In nonresponders larger doses of 120 mg or more may be needed to mobilize edema. After

This work was supported in part by National Institutes of Health (NIH) grant AM 20884 and by the Earl C. Anthony Fund.

During the course of this work Dr. Smith was supported as an NIH Predoctoral Scholar by NIH Training Grant GM 07175.

Received for publication Dec. 9, 1980.

Accepted for publication March 31, 1981.

Reprint requests to: Leslie Z. Benet, Ph.D., Department of Pharmacy, School of Pharmacy, 926-S, University of California, San Francisco, CA 94143.

*Present address: College of Pharmacy, The University of Michigan, Ann Arbor, MI.

*F. Vincenti: Unpublished observations.

0009-9236/81/070105+09$00.90/0

Table I. *Patient characteristics*

Patient	*Sex*	*Age (yr)*	*Weight (kg)*	*Cause of renal failure*	*CLcr* (ml/min)*	*Concomitant drugs*
C. T.	M	45	95.2	Nephrosclerosis	30.1	Prednisone, azathioprine, prazosin, calcium gluconate, bethanechol, minoxidil, aminophylline, isosorbide, metaproterenol
E. H.	M	53	89.5	Glomerulonephritis	61.5	Prednisone, cyclophosphamide, propranolol, clonidine
D. H.	F	25	65.5	Glomerulonephritis	37.3	Prednisone, azathioprine, cimetidine, hydralazine, propranolol
L. T.	M	31	68.5	Nephrosclerosis	41.7	Prednisone, azathioprine, cephradine, flurazepam, pseudoephedrine
V. W.	F	56	66.7	Glomerulonephritis	46.9	Prednisone, azathioprine, clonazepam, propranolol, isosorbide, diazepam, penicillin VK
S. J.	F	31	75.8	Glomerulonephritis	50.2	Prednisone, azathioprine, sulfisoxazole
P. D.	M	48	67.1	Glomerulonephritis	68.0	Prednisone, azathioprine, sulfisoxazole, bethanechol
W. J.	F	35	68.9	Glomerulonephritis	88.1	Prednisone, azathioprine, diazepam
F. R.	M	44	91.3	Unknown	47.7	Prednisone, azathioprine, flurazepam, sulfisoxazole, propranolol, nitroglycerin, acetaminophen
Mean (SD)		41 (11)	76.5 (12.1)		52.4 (17.7)	

*Creatinine clearance was determined over 24 hr.

kidney transplant, patients seem to respond better to intravenously administered doses of furosemide than to equivalent doses orally.

Although furosemide is widely used after kidney transplant, its disposition and dose-response relationship have not been studied, and dosage reigmens continue to be empiric. Our investigation was undertaken (1) to study furosemide kinetics after kidney transplant in patients after doses orally and intravenously, (2) to determine whether intravenous administration of furosemide is more efficacious in these patients than equal doses orally, and (3) to determine whether there are differences between responders (R) and nonresponders (NR) after kidney transplant with respect to furosemide kinetics and dynamics.

Methods

Materials. Furosemide tablets (40 mg) and intravenous solution (10 mg/ml), sodium phenobarbital, glass-distilled acetonitrile (Burdick and Jackson), and analytic reagent-grade phosphoric acid (Mallinckrodt) were used.

Patient studies. Characteristics of the nine patients studied are listed in Table I. Patients (five men, four women) ranged in age from 25 to 56 yr ($\bar{x}$ 41 yr) and in weight between 65.5 and 95.2 kg ($\bar{x}$ 76.5 kg). Creatinine clearance ranged from 30.1 to 88.1 ml/min ($\bar{x}$ 52.4 ml/min), and serum albumin and plasma electrolyte levels were normal. No subject had congestive heart failure (CHF), diabetes, nephrotic syndrome, or liver disease, except patient C. T., who had mild CHF. Patients were titrated to and studied at a dose

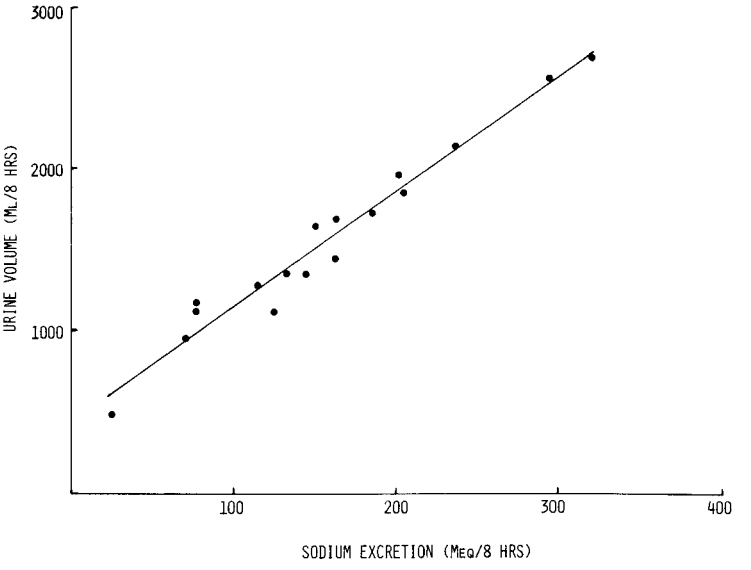

Fig. 1. Relationship between urine volume and sodium excretion in patients after kidney transplant.

capable of inducing an adequate response. Responders included those transplant patients who had an adequate natriuretic and diuretic response to a lower range of doses of furosemide (e.g., 40 to 80 mg). Nonresponders were more refractory and required 120 mg or more of furosemide for an adequate response. Although patient S. J. was studied at doses of 120 mg furosemide, she was assigned to group R because of her extensive natriuretic and diuretic output at this dose, with a weight loss of 3.2 kg after doses orally. She also had a substantial response with a 40 mg dose of furosemide orally.

After an overnight fast each subject received a dose of furosemide either orally or intravenously at approximately 8:00 A.M. Furosemide was taken with water or fruit juice, and patients fasted for at least 2 hr after the dose orally. The solution was infused intravenously over 10 min. Blood samples (3 ml) after doses intravenously were obtained by an indwelling heparinized scalp vein needle at 0, 10, 15, 20, 30, 45, 60, 80, 100, 120, 180, 240, 360, 480, and 1440 min, the end of the infusion period being 10 min. After doses orally blood samples were drawn at 0, 15, 30, 45, 60, 75, 90, 105, 120, 180, 240, 360, 480, and 1440 min. Voided urine was collected just before furosemide, hourly up to 8 hr, and then pooled for from 8 to 24 hr. Urine collection times differed depending on patient ability to void. Furosemide studies were carried out on consecutive days after doses orally and intravenously.

Preparation of standard solutions. Furosemide, 4.1 mg, was dissolved in acetonitrile to make a stock solution of 41 μg/ml. This stock solution was then diluted fivefold (8.2 μg/ml) and 100-fold (0.41 μg/ml) to make working standard solutions. Sodium phenobarbital was dissolved in distilled water at concentrations ranging from 0.05% to 1%, depending on the concentration range of furosemide to be analyzed in the samples.

Measurement of furosemide in plasma and urine. Plasma and urine samples containing furosemide were analyzed as described by us[30] with minor modifications. Samples were pumped through a μBondapak C18 reversed-phase column (30 cm × 3.9 mm i.d.) by a Varian Model 5000 Liquid Chromatograph, and furosemide was quantified using a Perkin-Elmer Fluorescence Spectrophotometer 650-10S. The excitation and emission wavelengths of furosemide were set at 345 and 405 nm. The internal standard, sodium phenobarbital, was measured by ultraviolet detection (254 nm) using a Waters Associates Model 440 absorbance detector. A 50-μl aliquot containing the internal standard, sodium phenobarbital (0.05%), was added to 0.20-ml furosemide plasma samples. The mix-

Table II. *Furosemide kinetics in patients after kidney transplant*

Patient	*Status*	*Treatment*	*CLp (ml/min)*	*Vdss (ml/kg)*	*T½ (min)*	*CLr (ml/min)*
C. T.	NR	160 mg po			138	10.8
		160 mg iv	60.4	77.5	138	10.7
E. H.	NR	120 mg po			120	22.1
		120 mg iv	84.6	110.0	116	23.0
D. H.	NR	120 mg po			174	10.3
		120 mg iv	35.6	92.9	143	12.4
L. T.	NR	120 mg po			137	23.8
		120 mg iv	75.4	167.0	122	27.4
Mean	NR	po			142*	16.8†
(SD)					(23)	(7.2)
Mean	NR	iv	64.0‡	112	130§	18.4§
(SD)			(21.4)	(39)	(13)	(8.1)
V. W.	R	80 mg po			70.5	45.9
		80 mg iv	80.5	102.0	99.8	35.1
S. J.	R	120 mg po			74.9‖	43.5
		120 mg iv	122	173.0	74.5	41.0
P. D.	R	40 mg po			85.0	65.6
		40 mg iv	135	127.0	66.4	54.2
W. J.	R	80 mg po			89.5	66.7
		80 mg iv	88.1	83.5	93.1	62.4
F. R.	R	80 mg po			119.0	50.4
		80 mg iv	98.9	95.1	104.0	42.8
Mean	R	po			87.8*	54.4†
(SD)					(19.0)	(11.0)
Mean	R	iv	105‡	116	87.6§	47.1§
(SD)			(23)	(36)	(16.3)	(11.0)

Level of significance between responders (R) and nonresponders (NR).

No difference was found between oral and intravenous administration of furosemide in R and NR.

*$p < 0.01$.

†$p < 0.001$.

‡$p < 0.05$.

§$p < 0.005$.

‖Determined from the residual slope of the feathered oral plasma curve (*see* Discussion).

ture was shaken on a vortex mixer, and 0.40 ml acetonitrile was added. The mixture was shaken again on a vortex mixer and then centrifuged for 10 min. The supernatant was transferred to a clean test tube and evaporated under nitrogen until about 0.10 ml solution remained. Urine samples were prepared similarly, but no acetonitrile was added and the evaporation step was omitted. A 50-μl aliquot containing the internal standard, sodium phenobarbital (1.0%), was added to 0.05-ml furosemide urine samples and 0.20 ml distilled water. The mixture was shaken on a vortex mixer, and an aliquot was introduced directly into the loop injector. At a flow rate of 2 ml/min furosemide and sodium phenobarbital had retention times of 6 and 4 min in a 38% acetonitrile–0.015 M phosphoric acid solvent system. The detection limit of furosemide in plasma under the described assay conditions is 8 ng/ml with a peak to noise ratio of 5. Chlorpromazine (0.02%) was substituted as the internal standard in patients taking sulfisoxazole, because sodium phenobarbital and sulfisoxazole have similar retention times and interfere with each other. Under conditions identical to those described above, chlorpromazine was measured by UV detection (254 nm) and had a retention time of 8.5 min.

Measurement of sodium. Urine samples containing sodium were analyzed by Corning Model 450 flame photometer.[29] Sodium concentrations were not measured for patient V. W. because

CLnr (ml/min)	*fe*	*F (%)*	*CLr / CLcr*
			0.36
49.7	0.176	74.6	0.36
			0.36
61.6	0.272	30.4	0.37
			0.28
23.3	0.348	81.6	0.33
			0.57
48.0	0.363	42.4	0.66
			0.39†
			(0.12)
45.6	0.290	57.2	0.43§
(16.1)	(0.086)	(24.7)	(0.15)
			0.98
45.4	0.436	53.0	0.75
			0.87
81.0	0.336	38.6	0.82
			0.96
80.8	0.402	48.2	0.80
			0.76
25.7	0.709	54.4	0.71
			1.06
56.1	0.432	55.3	0.90
			0.93†
			(0.11)
57.8	0.463	49.9	0.80§
(23.7)	(0.143)	(6.9)	(0.07)

the samples were lost. An estimate of urinary sodium was therefore made for her based on the strong correlation between sodium excretion and urine output in the other eight patients (Fig. 1; $r = 0.981$, $p < 0.001$).

Calculations. The furosemide half-life ($t_{1/2}$) was determined by linear regression using at least four data points from the terminal portion of the plasma versus time plots. The area under the plasma concentration time curve (AUC) was calculated by the trapezoidal rule, extrapolated to infinity from the last measured concentration. The absolute bioavailability (F) was calculated using both plasma (Fp) and urine (Fu) data: $Fp = AUC_{oral}/AUC_{iv}$ and $Fu = Ae^{\infty}_{oral}/Ae^{\infty}$ where the amount of unchanged drug recovered in the urine at time infinity is represented by Ae^{∞}. In our study the reported F represents the averaged availability of Fp and Fu. Total plasma clearance of intravenously administered furosemide (CLp) was calculated as CLp = dose/AUC. Total renal clearance (CLr) was estimated after doses intravenously and orally by $CLr = Ae^{\infty}/AUC$. The fraction of the furosemide dose intravenously that was excreted unchanged in the urine (fe) was calculated as $fe = Ae^{\infty}/Dose$. Nonrenal plasma clearance (CLnr) was calculated as the difference between the plasma and renal clearances. The volume of distribution steady state (Vd_{ss}) was determined from the intravenous plasma data by the compartment independent method of Benet and Galeazzi[6] corrected for infusion administration:

$$Vdss = \frac{Dose\ (AUMC)}{(AUC)^2} - \frac{\tau Dose}{2\ (AUC)}$$

where AUMC is the area under the curve of the first moment of the concentration time curve (i.e., $\int_0^{\infty} tCpdt$) and τ is the infusion time.

Data throughout the study are expressed as $\bar{x} \pm SD$. Statistical differences between groups R and NR were determined using a two-sample t test. Statistical differences between treatments given orally and intravenously within groups R and NR were determined by a paired t test.

Results

The kinetic data on furosemide administered orally and intravenously in patients after kidney transplant are presented in Table II. The volume of distribution steady state did not differ between responders and nonresponders (R 116 ± 36 and NR 112 ± 39 ml/kg; $p > 0.50$) and was in good agreement with data published by our group in healthy subjects.[29, 30] Nonresponders had a reduced plasma clearance (NR 64.0 ± 21.4 and R 105 ± 23 ml/min; $p < 0.05$) and renal clearance (NR 18.4 ± 8.1 and R 47.1 ± 11.0 ml/min; $p < 0.005$), but nonrenal clearance did not differ from responders (NR 45.6 ± 16.1 and R 57.8 ± 23.7 ml/min; $p > 0.20$). $T_{1/2}$ in responders was of the same order as in healthy subjects[29, 30] but were lower than in the nonresponders (R 87.6 ± 16.3 and NR 130 ± 13 min; $p < 0.005$). Although the fraction excreted unchanged in the urine after intravenous administration was approximately 37% lower in nonresponders, the magnitude of this change was no different (NR 0.290 ± 0.086 and R 0.463 ± 0.143; $p > 0.05$). There was no difference in the extent of absorption orally between responders and nonrespond-

Table III. *Furosemide dynamics in patients after kidney transplant*

Patient	*Status*	*Treatment*	*Sodium excretion (mEq/8 hr)*	*Urine volume (Ml/8 hr)*	*Ae^{∞} (mg)*
C. T.	NR	160 mg po	77.8	1185	21.2
		160 mg iv	77.4	1129	28.2
E. H.	NR	120 mg po	25.5	489	9.7
		120 mg iv	116.0	1277	32.6
D. H.	NR	120 mg po	69.2	949	31.0
		120 mg iv	151.0	1644	41.8
L. T.	NR	120 mg po	133.0	1360	17.2
		120 mg iv	237.0	2136	43.6
Mean	NR	po	76.4*	996	19.8
(SD)			(44.2)	(377)	(8.9)
Mean	NR	iv	145	1546	36.6
(SD)			(68)	(449)	(7.4)
V. W.	R	80 mg po	118†	1278	21.8
		80 mg iv	167†	1627	34.9
S. J.	R	40 mg po	145‡	1353‡	5.3‡
		120 mg po	322	2686	16.1
		120 mg iv	185	1717	40.3
P. D.	R	40 mg po	125	1108	8.5
		40 mg iv	163	1456	16.1
W. J.	R	80 mg po	296	2579	31.9
		80 mg iv	204	1854	56.7
F. R.	R	80 mg po	164	1695	20.7
		80 mg iv	203	1979	34.6
Mean	R	po	205*	1869	19.8
(SD)			(97)	(730)	(8.6)
Mean	R	iv	184	1727	36.5
(SD)			(19)	(202)	(14.5)

*Level of significance $p < 0.05$.

†Derived from linear regression analysis in Fig. 1.

‡Values not included in the mean (SD) data.

ers (R 49.9 ± 6.9 and NR 57.2 ± 24.7%; $p > 0.50$) as well as in our data in healthy subjects.[30] When renal clearance was corrected for kidney function (as determined by creatinine clearance) there were clear differences between responders and nonresponders (R 0.80 ± 0.07 and NR 0.43 ± 0.15; $p < 0.005$).

Furosemide dynamics in patients after kidney transplant following oral and intravenous administration as well as the amount of furosemide excreted unchanged in the urine after both treatments are presented in Table III. Nonresponders had reduced sodium excretion after furosemide orally (NR 76.4 ± 44.2 and R 205 ± 97 mEq/8 hr; $p < 0.05$), although equivalent amounts of unchanged drug were excreted in the urine (NR 19.8 ± 8.9 and R 19.8 ± 8.6 mg; $p > 0.50$). Urine volume after furosemide orally was also less in nonresponders but not statistically significantly so (NR 996 ± 377 and R 1869 ± 730 ml/8 hr; $0.10 > p > 0.05$); but after intravenous administration there was no difference between responders and nonresponders with respect to furosemide-induced natriuresis (R 184 ± 19 and NR 145 ± 68 mEq/8 hr; $p > 0.20$), diuresis (R 1727 ± 202 and NR 1546 ± 449 ml/8 hr; $p > 0.20$), and amount excreted unchanged in the urine (R 36.5 ± 14.5 and NR 36.6 ± 7.4 mg; $p > 0.50$).

Discussion

The therapeutic efficacy of furosemide varies widely among patients with different degrees of renal impairment.[1, 21, 22] The ability of patients after kidney transplant to respond to furosemide is unpredictable, and larger doses are often needed to induce adequate diuresis and natri-

uresis. Mechanisms that may explain the resistance to furosemide include reduced bioavailability, changes in drug metabolism, decreased glomerular filtration rate, and reduction in renal tubular transport.

In healthy subjects renal clearance of furosemide is about 120 ml/min[4, 29, 30] and the fraction of the dose excreted unchanged in the urine about 60% to 75%.[4, 7, 29, 30] We found that the renal clearance for the nine patients ranged from 10.3 to 66.7 ml/min, which was 0.086 to 0.56 that of healthy subjects, but there were marked differences between responders and nonresponders with respect to renal clearances alone as well as when corrected for kidney function. Mean corrected renal clearances (CLr/CLcr) for the nonresponders (0.43 ± 0.15) were approximately half the values for responders (0.80 ± 0.07). Because furosemide is over 95% protein bound in plasma,[2–4, 24, 25, 30] glomerular filtration contributes minimally to its total renal clearance. Thus attenuated renal clearance of furosemide suggests impairment in the secretory component of the organic acid transport system. Such depression in renal transport can affect urinary excretion rate of furosemide, which has been shown to be the critical determinant with respect to diuretic and natriuretic effect.[10, 11, 26, 28, 29]

In our study the attenuated renal clearance in nonresponders necessitates larger doses of furosemide to achieve equivalent unchanged drug in urine and therefore an equivalent dynamic effect to that of responders. This is shown in Table III, where responders and nonresponders have virtually identical amounts of unchanged furosemide in the urine after doses intravenously, and also by a similar response between the two groups. After furosemide orally, however, nonresponders have less natriuresis than responders, although both groups ultimately excrete identical amounts of unchanged drug in the urine. Although speculative, it is possible that the "critical" luminal concentration–amount of furosemide needed for an adequate dynamic effect is higher in nonresponders such that this "critical" level is reached after doses intravenously but not orally. This may explain the apparent discrepancy in the differences in natriuresis and diuresis between responders and

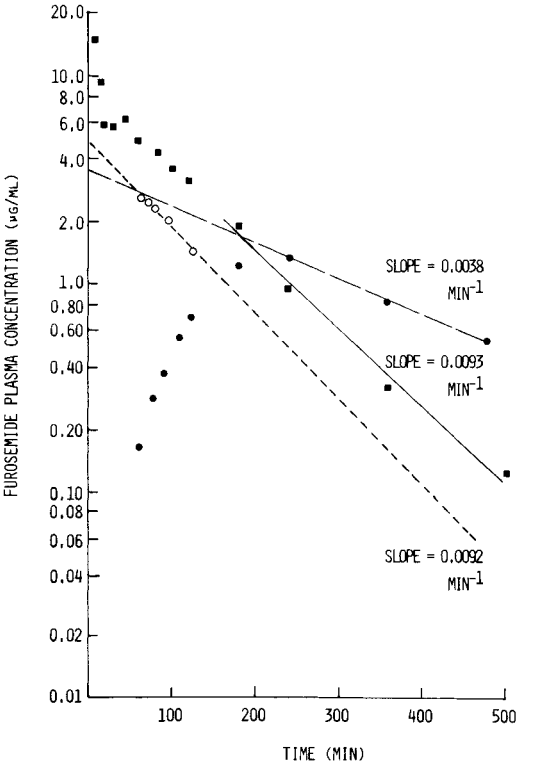

Fig. 2. Furosemide plasma concentration:time plot in patient S. J. after oral (●) and intravenous (■) administration of furosemide. (○), residual slope of the feathered oral curve.

nonresponders after doses orally but not intravenously.

Although there is a trend toward reduced bioavailability of furosemide in patients with renal impairment,[5] this has not been a factor in diuretic resistance.[17] However, a recent case report[23] shows that apparent resistance to furosemide orally can be explained by reduced bioavailability in the edematous as opposed to the nonedematous state. We found similar values for bioavailability in responder and nonresponder patients after kidney transplant as well as in healthy subjects.[5] Therefore change in the extent of oral absorption is not a viable explanation for its reduced effectiveness after kidney transplant.

Reports on healthy subjects,[7, 18] patients with heart failure,[12] and "diuretic-resistant" patients[17] demonstrated that an equivalent diuretic response to furosemide was achieved whether the dose was given by mouth or by intravenous

injection. In uremic patients Huang et al.[14] found the diuresis induced by furosemide orally was always less effective than after doses intravenously. This also appeared to be the case in our study when average sodium and water excretion values after doses orally and intravenously are compared for nonresponders but not for responders. The individual results in Table III demonstrate considerable variability in the natriuretic and diuretic response of patients after kidney transplant to furosemide orally and intravenously. Factors such as uncontrolled fluid intake and lack of electrolyte-water replacement may have contributed to this variability, but a more controlled study was not ethically possible because of the clinical condition of the patients. Our studies were carried out as the drug is used clinically.

An unusual plasma concentration: time profile of furosemide was observed in patient S. J., in whom the terminal slopes after doses orally and intravenously were not similar (Fig. 2). On feathering the oral curve the residual slope was virtually identical to that of the terminal slope after intravenous administration of furosemide. This is indicative of a "flip-flop" model in which the elimination of the drug is rate limited by its absorption. In addition, intersection of the terminal and residual slopes of the oral curve at some point in time above zero suggests lag time before absorption. In this case there was a lag time of about 50 min, with a peak furosemide concentration in plasma not reached until 4 hr after dosing orally. Delayed absorption of furosemide such as in patient S. J. may also be present in other patients after kidney transplant, perhaps to a lesser degree, and contributes to the unpredictability of assessing diuretic and natriuretic response to furosemide.

A recent study[15] reported furosemide to have a t½ of about 4 days in a 39-year-old patient studied postoperatively for 26 days. During the first 10 days after kidney transplantation the patient lost 172 *l* urine. The authors speculated that this massive diuresis may have been caused by a depot effect of furosemide in which the drug cumulated in body tissues during high-dose furosemide treatment before transplantation. In our study patients were studied at least 18 days after surgery. The mean furosemide t½ in responders and nonresponders was 87.6 and 130 min, in sharp contrast to the 4-day t½ reported[15] and would argue against a similar depot effect being present in our nine patients.

Our results imply that after kidney transplant nonresponders have less ability to secrete furosemide into tubular fluid as well as less ability to respond to equivalent amounts of drug excreted in the urine. Furosemide intravenously offers no real advantages over oral drug for continued therapy.

References

1. Allison MEM, Kennedy AC: Diuretics in chronic renal disease: A study of high dosage furosemide. Clin Sci **41:**171-187, 1971.
2. Andreasen F, Hansen HE, Mikkelsen E: Pharmacokinetics of furosemide in anephric patients and in normal subjects. Eur J Clin Pharmacol **13:**41-48, 1978.
3. Andreasen F, Jakobsen P: Determination of furosemide in blood plasma and its binding to proteins in normal plasma and in plasma from patients with acute renal failure. Acta Pharmacol Toxicol **35:**49-57, 1974.
4. Andreasen F, Mikkelsen E: Distribution, elimination and effects of furosemide in normal subjects and in patients with heart failure. Eur J Clin Pharmacol **12:**15-22, 1977.
5. Benet LZ: Pharmacokinetics/pharmacodynamics of furosemide in man: A review. J Pharmacokinet Biopharm **7:**1-27, 1979.
6. Benet LZ, Galeazzi RL: Noncompartmental determination of the volume of distribution steady-state. J Pharm Sci **68:**1071-1074, 1979.
7. Branch RA, Roberts CJC, Homeida M, Levine D: Determinants of response to furosemide in normal subjects. Br J Clin Pharmacol **4:**121-127, 1977.
8. Burg MB: Tubular chloride transport and the mode of action of some diuretics. Kidney Int **9:**189-197, 1976.
9. Burg M, Stoner L, Cardinal J, Green N: Furosemide effect on isolated perfused tubules. Am J Physiol **225:**119-124, 1973.
10. Chennavasin P, Seiwell R, Brater DC, Liang WMM: Pharmacodynamic analysis of the furosemide-probenecid interaction in man. Kidney Int **16:**187-195, 1979.
11. Green TP, Mirkin BL: Resistance of proteinuric rats to furosemide: Urinary drug protein binding as determinant of drug effect. Life Sci **26:**623-630, 1980.
12. Greither A, Goldman S, Edelen JS, Benet LZ, Cohn K: Absorption of furosemide in patients

with congestive heart failure. Pharmacology **19:**121-131, 1979.
13. Hook JB, Williamson HE: Influence of probenecid and alterations in acid-base balance of the saluretic activity of furosemide. J Pharmacol Exp Ther **149:**404-408, 1965.
14. Huang CM, Atkinson AJ, Levin M, Levin N, Quintanilla A: Pharmacokinetics of furosemide in advanced renal failure. CLIN PHARMACOL THER **16:**659-666, 1974.
15. Husberg B, Hellsten S, Bergentz S, Hansen T, Möller-Jensen K: Massive diuresis after renal transplantation due to retention of furosemide. Transplantation **23:**101-103, 1977.
16. Jacobson HR, Kokko JP: Diuretics: Sites and mechanisms of action. Ann Rev Pharmacol Toxicol **16:**201-204, 1976.
17. Kelly MR, Blair AD, Forrey AW, Smidt NA, Cutler RE: A comparison of the diuretic response to oral and intravenous furosemide in "diuretic-resistant" patients. Curr Ther Res **21:**1-9, 1977.
18. Kelly MR, Cutler RE, Forrey AW, Kimpel BM: Pharmacokinetics of orally administered furosemide. CLIN PHARMACOL THER **15:**178-186, 1974.
19. Kim KE, Onesti G, Moyer JH, Swartz C: Ethacrynic acid and furosemide: Diuretic and hemodynamic effects and clinical uses. Am J Cardiol **27:**407-415, 1971.
20. Kirkendall WM, Stein JH: Clinical pharmacology of furosemide and ethacrynic acid. Am J Cardiol **22:**162-167, 1968.
21. Muth RG: Diuretic properties of furosemide in renal disease. Ann Intern Med **69:**249-261, 1968.
22. Muth RG: Furosemide in severe renal insufficiency. Postgrad Med J **47:**21-25, 1971. (Suppl.)
23. Odlind BG, Beermann B: Diuretic resistance: Reduced bioavailability and effect of oral furosemide. Br Med J **208:**1577, 1980.
24. Prandota J, Pruitt AW: Furosemide binding to human albumin and plasma of nephrotic children. CLIN PHARMACOL THER **17:**159-166, 1975.
25. Rane A, Villeneuve JP, Stone WJ, Nies AS, Wilkinson GR, Branch RA: Plasma binding and disposition of furosemide in the nephrotic syndrome and in uremia. CLIN PHARMACOL THER **24:**199-207, 1978.
26. Rose HJ, Pruitt AW, Dayton PG, McNay JL: Relationship of urinary furosemide excretion rate to natriuretic effect in experimental azotemia. J Pharmacol Exp Ther **199:**490-494, 1976.
27. Seely JF, Dirks JH: Site of action of diuretic drugs. Kidney Int **11:**1-8, 1977.
28. Smith DE, Benet LZ: Relationship between urinary excretion rate, steady-state plasma levels and diuretic response of furosemide in the rat. Pharmacology **19:**301-306, 1979.
29. Smith DE, Gee WL, Brater DC, Lin ET, Benet LZ: Preliminary evaluation of furosemide-probenecid interaction in humans. J Pharm Sci **69:**571-575, 1980.
30. Smith DE, Lin ET, Benet LZ: Absorption and disposition of furosemide in healthy volunteers using a metabolite specific assay. Drug Metab Dispos **8:**337-342, 1980.
31. Stason WB, Cannon PJ, Heinemann HO, Laragh JH: Furosemide: A clinical evaluation of its diuretic action. Circulation **34:**910-920, 1966.