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The pharmacokinetics of furosemide

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THE PHARMACOKINETICS OF FUROSEMIDE

by

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DISSERTATION

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Abstract

THE PHARMACOKINETICS OF FUROSEMIDE

David Eric Smith

Furosemide is a potent diuretic agent which acts at the luminal surface of the nephron. There it inhibits the active reabsorption of chloride in the ascending limb of the loop of Henle; a process believed to be prostaglandin mediated. Since furosemide is over 95% plasma protein bound, access to the lumen occurs primarily through active secretion via the non-specific organic acid secretory pathway.

The relationship between the diuretic effect of furosemide and the drug's concentration/amount in a measurable sampling compartment has been poorly characterized. In addition, conflicting results have been reported concerning the pharmacokinetics and metabolism of furosemide due to problems inherent in the assay procedures. Therefore, a rapid, sensitive and specific HPLC assay, without prior extraction and/or derivatization was developed in an attempt to clarify the dose-response relationship of furosemide as well as the drug's disposition.

The relationship between urinary excretion rate, steady-state plasma levels and diuretic response of furosemide was studied in 28 rats. Results from this study demonstrate that the diuretic effect of furosemide is directly related to the drug's urinary excretion rate and not to its plasma concentration. In addition, furosemide exhibited capacity limited elimination at higher plasma concentrations (as evidenced by a reduced renal clearance), and this saturable process occurred in the rat at a level comparable to the therapeutic concentration range in humans.

The absorption and disposition of furosemide was studied in nine healthy volunteers after oral and iv dosing of the drug. No evidence of CSA, the putative metabolite of furosemide, was found and the results of this investigation conclusively demonstrate it to be an analytical artifact.

Glucuronidation accounted for approximately 14% of the available dose of furosemide, whether given orally or by iv administration. The bioavailability of furosemide was about 43%.

The role of probenecid and indomethacin in modifying furosemide's dose-response relationship was studied in four healthy volunteers. The results from the furosemide-probenecid interaction studies were consistent with previous animal studies and demonstrate that urinary excretion rate of furosemide is a better indicator of natriuresis and diuresis than is plasma concentration. The furosemide-indomethacin interaction studies demonstrate that the attenuation of furosemide's diuretic effect by indomethacin pretreatment is not due to a pharmacokinetic interaction. Inhibition of prostaglandin synthesis by indomethacin is the more probable mechanism.

The pharmacokinetics/dynamics of furosemide were evaluated in nine kidney transplant patients after oral and iv dosing of the drug. Similar values for mean bioavailability were observed between responder (50%) and non-responder (57%) patients. However, non-responders (in comparison to responders) had a reduced ability to secrete furosemide into tubular fluid as well as a decreased ability to respond to equivalent amounts of drug excreted in the urine. CSA was not found in any of the urine samples analyzed. Glucuronidation accounted for 8% of the available dose of furosemide and may be occurring in the kidney. Urinary recovery of furosemide and its glucuronide metabolite accounted for 45% of the intravenous dose in this patient population.

Plasma protein binding of furosemide after iv dosing was significantly reduced in kidney transplant patients as compared to healthy volunteers. Binding was further reduced in those patients concomitantly on sulfisoxazole.

Nevertheless, no correlation was observed between the fraction of the dose excreted in the urine unchanged and fraction free of furosemide. Therefore, the ability of kidney transplant patients to respond to furosemide treatment is independent of plasma protein binding. The response appears to be related to the ability of the kidney to secrete furosemide into the tubular fluid as well as the ability of the organ to respond.

To my parents George and Sylvia,

and to my brother Marc,

for their love, support and enduring confidence.

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Glossary

CHF	Congestive heart failure
CLcr	Creatinine clearance
CLnr	Non-renal clearance
CLp	Total plasma clearance
CLr	Renal clearance
CSA	2-amino-4-chloro-5-sulfamoylanthranilic acid
fe	Fraction of the intravenous dose excreted unchanged in the urine
Fluor	Fluorescence
fnr	Fraction of the intravenous dose excreted by non-renal routes
GLC	Gas liquid chromatography
HPLC	High performance liquid chromatography
^{35}S	Radiolabelled furosemide
Scr	Serum creatinine
Soln	Solution
Terminal $T_{\frac{1}{2}}$	Half-life determined from the log-linear terminal phase of elimination
tlag	Time prior to first measurable concentration of drug
TLC	Thin layer chromatography
tpeak	Time at which peak drug concentration is reached
Vdss	Volume of distribution steady-state

Chapter I

Introduction

Furosemide is one of the most potent diuretics available today (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 non-specific organic acid secretory pathway (5,6,10). Thus, any drug or disease state which prevents furosemide from reaching its site of action in the lumen could thereby attenuate its natriuretic and diuretic response. Although furosemide is widely used to treat edematous states of hepatic, cardiac, and renal origin (1-3), its disposition and dose-response relationship are still unclear and dosage regimens continue to be empiric. Therefore, the following overall research objectives are proposed:

1. To study the pharmacokinetics of furosemide after oral and intravenous administration in healthy volunteers and in kidney transplant patients.

2. To define a relationship between furosemide dose, its concentration and/or amount in a measurable sampling compartment and its natriuretic and diuretic effect.

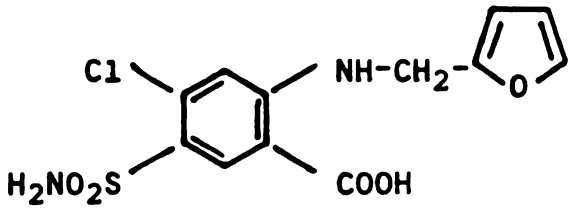
3. To determine the role of probenecid and indomethacin in modifying furosemide's dose-response relationship.

Specific objectives relating to each research project will be presented in the appropriate chapters.

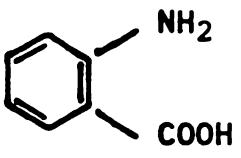
A. Chemistry

Furosemide is an anthranilic acid derivative (4-chloro-N-furfuryl-5-sulfamoylanthranilic acid) similar in chemical structure to the sulfonamide diuretics (Fig. I-1). The benzenesulfonamide diuretics, which include furosemide as well as the thiazides, share two common structural characteristics (11). Firstly, they both have a chlorine atom or trifluoromethylgroup in the position ortho to the sulfamyl ($-SO_2NH_2$) group. Secondly, an electronegative group, such as -CO- or $-SO_2-$ is located meta to the sulfamyl group or in this position as part of a condensed ring system. In addition, it has been shown that the ortho halogen may be replaced by a variety of lipophilic substituents. This modification results in retention of diuretic activity or enhancement as in the case of bumetanide.

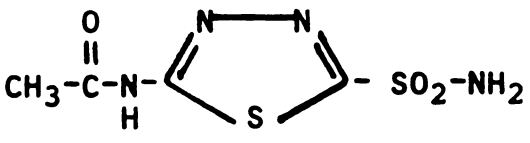
The empirical formula of furosemide is $C_{12}H_{11}ClN_2O_5S$ with a corresponding molecular weight of 330.7. Furosemide appears as a fine crystalline powder, is odorless and practically tasteless, and is white to slightly yellow in color. Furosemide melts between 203° and 205° and is unstable in light (12). Although soluble in acetone, methanol, dimethylformamide and aqueous solutions above pH 8.0, it is less soluble in ethanol and only slightly soluble in water and chloroform (13). Furosemide is a weak organic acid with a pK_a of 3.9 (14). It has three ultraviolet absorbance maxima in 0.01 N HCl which occur at 235 nm (ε = 45,000), 275 nm (ε = 21,000) and 340 nm (ε =5,800). The extinction coefficient (ε) at these maxima are also noted above. Absorption minima occur at 250 nm and at 300 nm.



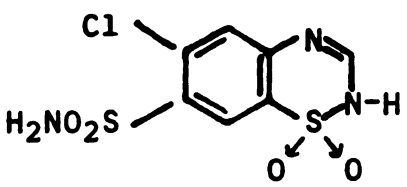
FUROSEMIDE



ANTHRANILIC ACID



ACETAZOLAMIDE



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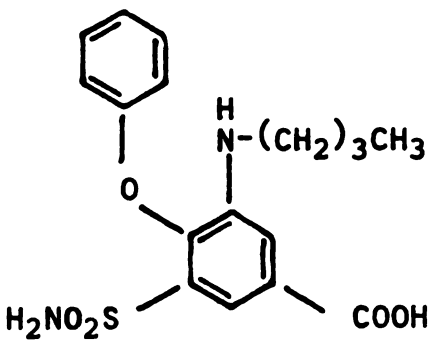


BUMETANIDE

Fig. I-1. Structural formula of furosemide, anthranilic acid and related sulfonamide diuretics.

B. Therapeutics

Furosemide has been classified as a "high-ceiling" diuretic due to its distinctive actions on renal tubular function (15). Features common to this class of diuretics include potency, a prompt onset of action, inhibition of sodium and chloride transport in the ascending limb of the loop of Henle and independence of action from changes in acid-base balance.

Furosemide has a rapid onset of action, 3 to 5 minutes when administered intravenously, about 30 minutes when administered orally; and a relatively brief duration of natriuresis, 1 to 2 hours when administered intravenously, 4 to 6 hours when administered orally (1). Similar to other diuretics, continuous administration of furosemide tends to diminish its effectiveness and intermittent therapy may prove more efficacious in mobilizing fluid.

Furosemide has been marketed in the United States since 1966 and has found clinical usefulness in edematous states associated with cardiac, hepatic and renal disease (1-3). However, one of its major advantages is its ability to effect a natriuretic response in patients with renal failure, even when the glomerular filtration rate is less than 5 ml/min (16,17). In contrast, organomercurial and thiazide diuretics become ineffective when the glomerular filtration rate falls below 15 to 20 ml/min (18). Additionally, chronic administration of organomercurials results in hypochloremic metabolic alkylosis which causes the patient to become refractory to the diuretic response (19).

Furosemide is supplied by Hoechst (Lasix) as 20 mg and 40 mg tablets for oral use, and as a sterile solution in 2 ml ampules, each containing

20 mg. The usual dosage range is from 20 mg to 80 mg daily, but dosage may differ considerably, especially in patients with renal failure.

C. Toxicology

Although furosemide is frequently administered in doses of 1 gm or greater to patients with acute (20,21) or chronic (16,22-24) renal failure, serious side effects are relatively few. The most common complications are fluid and electrolyte imbalance (1-3,25,26) which include hyponatremia, hypokalemia, hypochloremic metabolic alkylosis, hyperuricemia, volume depletion and hypotension. Gastrointestinal reactions including nausea, vomitting and anorexia are less common. Hyperglycemia, and hematologic and hypersensitivity reactions are rare (3). Furosemide-induced ototoxicity, although infrequent has been reported when the drug is administered in large doses to patients with renal impairment (27-29). However, it is unclear whether this is an effect of furosemide alone, or is related to the concomitant administration of other ototoxic agents such as ethacrynic acid and aminoglycoside antibiotics (22,27). Hearing loss appears to be reversible (27-29) and has been reported to occur only when furosemide is administered intravenously at a rate exceeding 4 mg/min as recommended by the manufacturer (27,30).

It has been shown that furosemide is converted by microsomal enzymes in the liver of mice and humans to a reactive arylating metabolite, and that this furan epoxide intermediate (Fig. I-2) leads to massive hepatic necrosis in mice (31-33). However, the implications of these findings with respect to clinical use of furosemide are uncertain since no incidences of furosemide-induced liver damage have been reported.

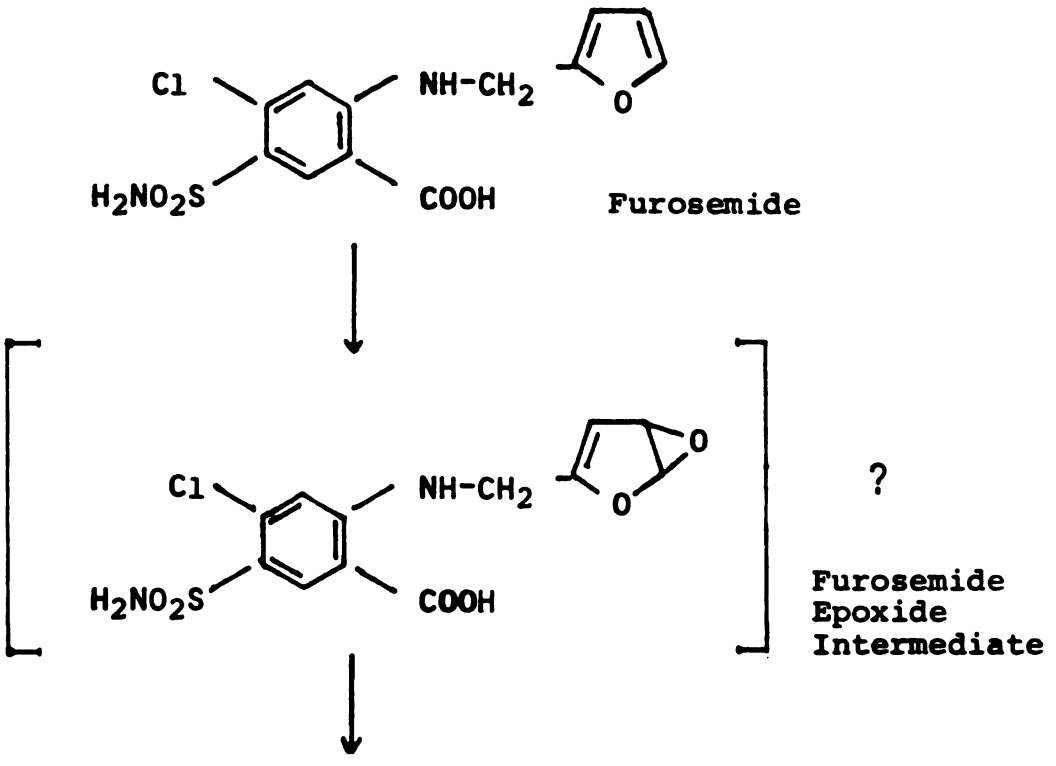


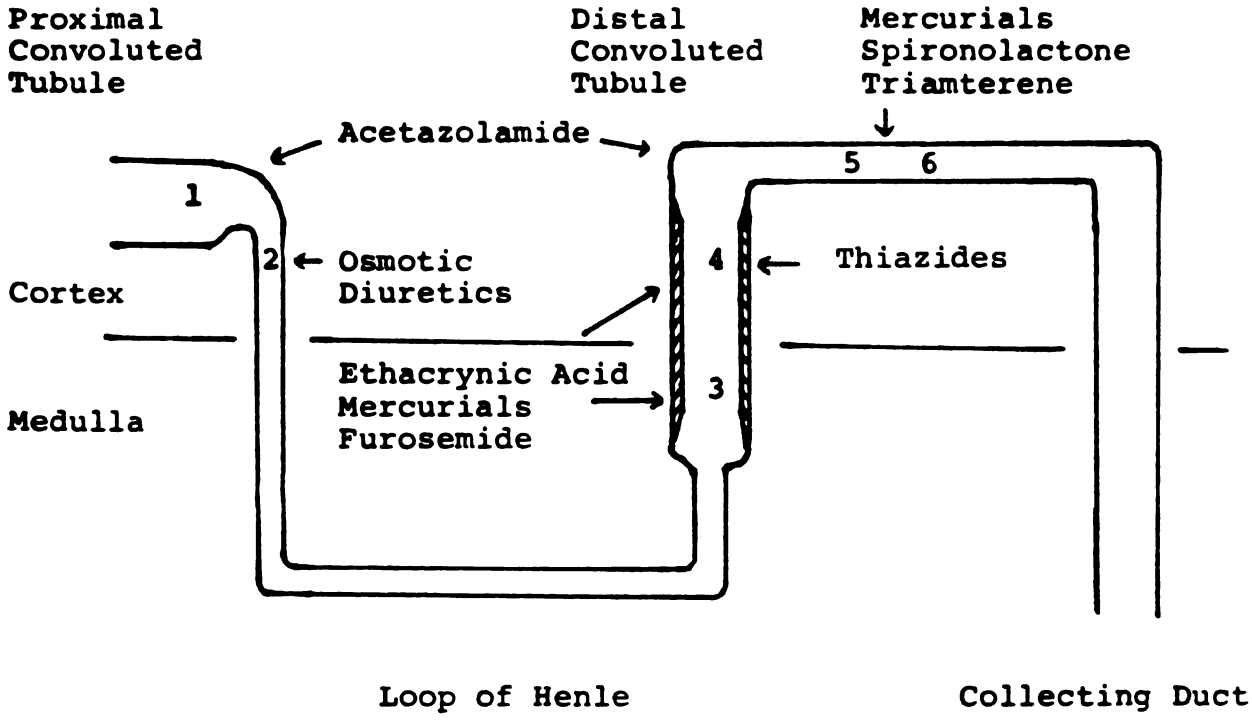
Fig. I-2. Proposed pathway for metabolism of furosemide to the hepatotoxic metabolite (32).

In addition, reproductive studies in animals have shown that furosemide may cause fetal abnormalities (34). It is therefore contraindicated in women of childbearing potential. An exception exists in the presence of life-threatening situations where the benefit of furosemide outweighs its potential risks. To my knowledge, published information concerning the teratogenicity of furosemide is not available except through the manufacturer.

D. Mechanism of Action

Furosemide exerts its natriuretic and diuretic effect at the luminal surface of the nephron (4-7,35). There it inhibits the active reabsorption of chloride in the thick segment of the ascending limb of the loop of Henle (Fig. I-3). Since furosemide is highly bound to plasma proteins (8,9), access to the kidney lumen is rather limited through glomerular filtration. However, as a weak organic acid furosemide can enter the tubular fluid in the proximal convoluted tubules via the non-specific organic acid secretory pathway (5,6,10,36,37). Furosemide also has a minor effect in the proximal tubule where it exhibits weak activity as a carbonic anhydrase inhibitor (4,35).

A number of indirect studies have implicated the prostaglandins, particularly PGE_2 as chemical mediator(s) of furosemide-induced natriuresis, diuresis, intrarenal hemodynamics and renin stimulation. It has been shown that parenteral administration of PGA and PGE to experimental animals and humans can cause a significant natriuresis and diuresis (38-40). Hemodynamically, furosemide causes an increase in renal blood flow (41-46) associated with a redistribution of flow from superficial to inner cortical



Site 1. Proximal tubule. Sensitive to carbonic anhydrase inhibitors.

Site 2. Proximal tubule. Sensitive to osmotic effects.

Site 3. Medullary diluting segment of ascending limb.

Site 4. Cortical diluting segment of ascending limb.

Site 5. Aldosterone-sensitive portion of distal tubule.

Site 6. Aldosterone-insensitive portion of distal tubule.

Fig. I-3. Schematic representation of sites of diuretic action in the nephron.

zones (41,44). Indomethacin, a potent inhibitor of prostaglandin synthetase can attenuate these hemodynamic as well as the natriuretic, diuretic and renin stimulating effects of furosemide (41,42, 47-54).

Circulating prostaglandins are not believed to be renally active since they are almost completely metabolized upon first pass through the lung (38,39,55). Instead, they function as local hormones and are primarily synthesized intracellularly in the renal medulla from the fatty acid precursor, arachidonic acid (38-40, 55) (Fig. I-4). However, the site of entry for prostaglandins into the tubular fluid still remains controversial. Some investigators propose that they diffuse into the luminal fluid at the ascending limb of the loop of Henle (39,56). Others suggest that prostaglandins are secreted into the urine by the classic anion transport system of renal proximal tubules (57-59). Prostaglandins synthesized in the medullary interstitial cells could theoretically diffuse into the interstitial fluid, vasa recta and urine. However, significant protein binding might limit the prostaglandin pool available for diffusion into the urine. Prostaglandins transported toward the renal cortex *via* the ascending vasa recta are in close proximity to the pars recta of the proximal convoluted tubule (Fig. I-5). The juxtaposition of the ascending vasa recta and pars recta would provide a constant supply of prostaglandins for secretion (59). Prostaglandins are metabolized in the renal cortex by the degradative enzymes PGE_2-15-hydroxydehydrogenase and PGE_2-9-ketoreductase (38-40). *In vitro* studies demonstrate that furosemide inhibits these degradative enzymes (60,61) as well as increasing free arachidonic acid levels (62). These two effects lead to increased amounts of prostaglandins *in vivo* (63-66), as determined by measuring urinary prostaglandins (55). In addition, a more recent study demonstrates that endogenous PGE_2

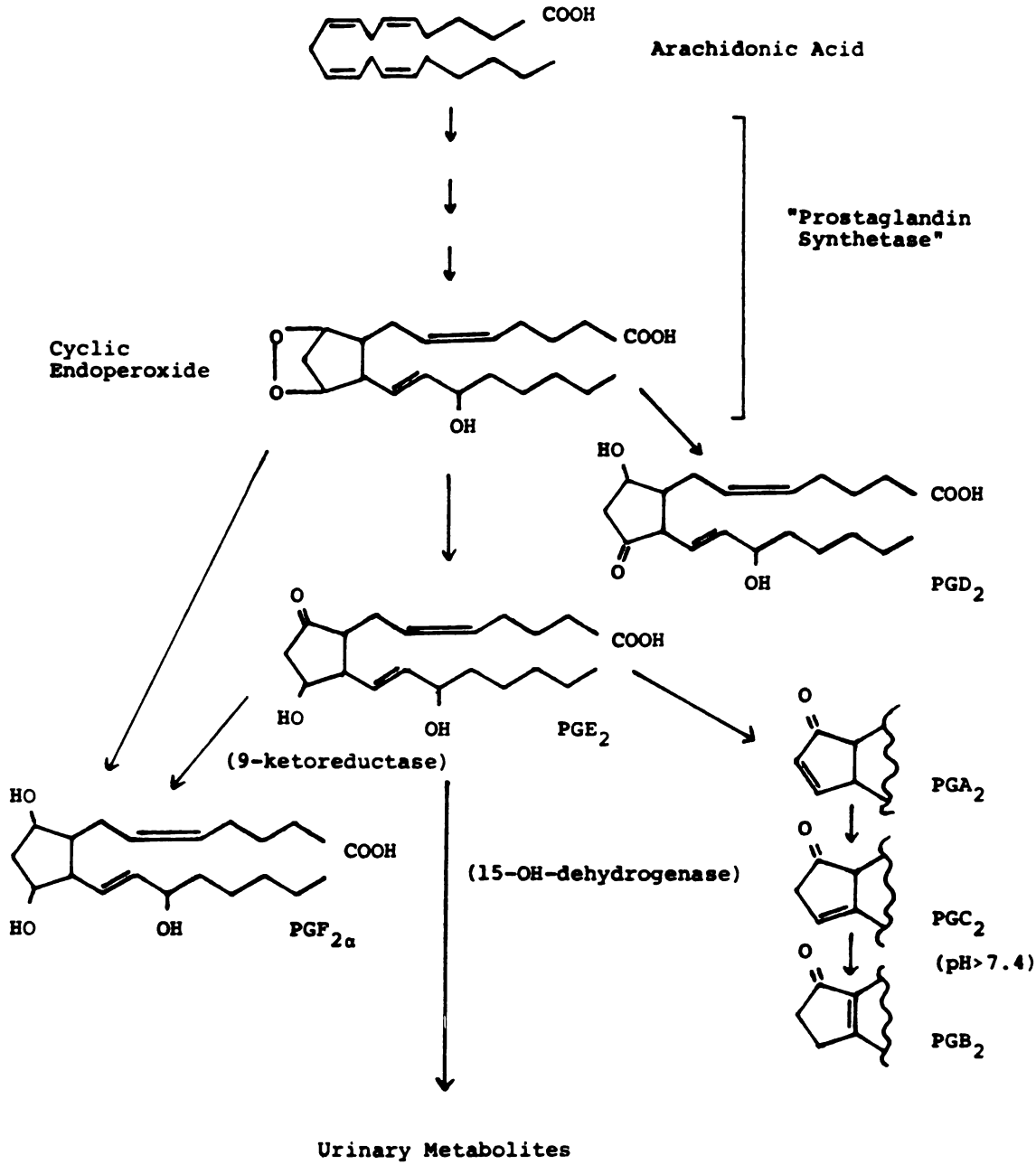


Fig. I-4. Synthesis of renal prostaglandins.

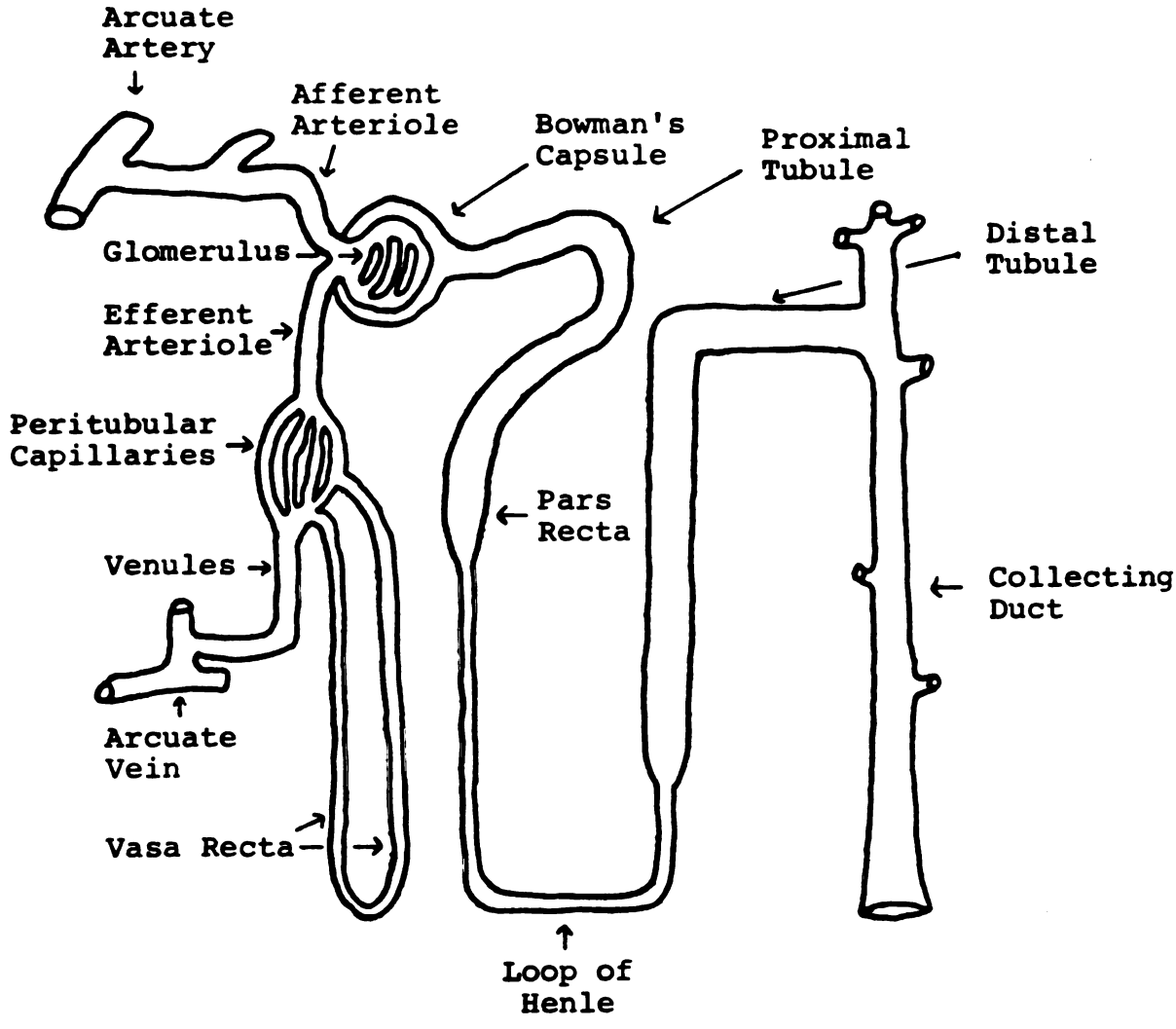


Fig. I-5. The functional nephron.

can inhibit net chloride transport across the medullary thick ascending limb of Henle's loop but has no effect on the cortical segment (67). This finding is consistent with the hypothesis that endogenous renal prostaglandins may be involved in the regulation of medullary tonicity and chloride secretion. It is also consistent with furosemide's mechanism of action, that is inhibition of active chloride reabsorption in the ascending limb of the loop of Henle.

A molecular basis of action has been sought for furosemide but is still unclear. Possible mechanisms include inhibition of Na-K ATPase, inhibition of cellular glycolysis, and inhibition or displacement of c-AMP (4,35).

E. Absorption

Bioavailability is defined as the relative amount of an administered drug that reaches the general circulation and the rate at which this occurs. The extent of absorption for furosemide in healthy volunteers, based on the literature reports summarized in Table I-1, is approximately 50-70% (9,68-72). However, the assay methodology and blood sampling schedules used in many of these reports are open to question as will be subsequently shown, and probably lead to uncertainties in the validity of the estimates. In three out of four studies in patients with renal impairment, decreased availability, 43-47%, was found (9,72,74). The exception was a study by Huang et al. (73) who found an average extent of availability for furosemide of 73% in patients with advanced renal failure. In fact, two patients in that study demonstrated complete oral absorption. This, however, may actually reflect biliary recycling of furosemide as well as

Table I-1. Bioavailability of Furosemide

Reference	Population	Number of Subjects	Dosage Form	Assay	Extent of Availability (%)
Rupp and Hajdú (68)	Healthy	7	Tablets	Fluor.	58
Kelly et al. (69)	Healthy	4	Tablets	Fluor.	65±24[a]
Beermann et al. (70)	Healthy	2	Aqueous Soln.	^{35}S	65
Branch et al.(71)	Healthy	6	Tablets	Fluor.	50
Tilstone and Fine (72)	Healthy	5	Aqueous Soln.	^{35}S	69±7
	Renal Failure, CLcr < 5 ml/min	11	Aqueous Soln.(n=5)		43±8
			Tablets (n=6)		43±7
Rane et al. (9)	Healthy	6	Tablets	HPLC	63±9
	Nephrotic Syndrome, CLcr = 74±11	7			46±9
	Uremia, CLcr = 11±1	6			46±9
Huang et al (73)	Renal Failure, Scr > 9 mg%	11	Tablets	Fluor.	73±20
Kelly et al.(74)	"Diuretic Resistant"	10	Tablets	^{35}S,HPLC	47±15
Greither et al.(75)	CHF	7	Tablets	Fluor.	61±16

[a]Values reported represent the mean ± standard deviation.

metabolite accumulation and therefore lead to an overestimation of the extent of availability. Patients with congestive heart failure (75) and renal failure (73) apparently show a higher relative standard deviation in extent of absorption than reported for normals. However, Kelly et al. (69) also reported a large relative standard deviation in the observed extent of absorption in healthy volunteers.

After oral administration of furosemide to healthy volunteers, peak plasma concentrations were reached in 60-120 minutes (68-71). However, in the study by Huang et al. (73) in patients with advanced renal failure, the mean peak time was 4.4 hours. This marked difference reflects not only a slower absorption rate but a reduction in furosemide elimination rate as well. It should also be noted that the extent of availability for orally administered furosemide is apparently independent of the dosage form employed (69,72) as well as the effect of meals (69). However, when administered postprandially, the peak concentration of furosemide develops later and is lower than when the drug is administered to a fasted individual (69), indicating slower absorption.

F. Pharmacokinetics

1. Healthy Volunteers

Calesnick et al. (76) were the first investigators to study the absorption, distribution and elimination of furosemide in humans. They reported the 24-hour urinary recovery of intravenously administered furosemide-^{35}S to range from 51-94% with considerably less being excreted following oral administration. They implied that the decreased excretion

following oral dosing was not due to incomplete absorption since only 2.1% of the label was recovered in the feces. However, these results are suspect since only one subject was studied and feces were collected for only 24 hours.

Rupp and Hajdu (68) described the plasma concentration - time profile of furosemide according to a three-exponential equation:

$$C_p = Ae^{-\alpha t} + Be^{-\beta t} + Ce^{-\gamma t}$$

where A,B,C are constant coefficients and α,β,γ are hybrid disposition rate constants. The average half-lives of furosemide for these three phases were 16.6 minutes, 57.2 minutes and 4.48 hours. Fractional areas under the curve, which determine the importance of a particular phase in defining drug kinetics during multiple dosing, were approximately 30%, 50%, and 20%, respectively. Since the terminal phase accounts for only 20% of the total area under the curve, this phase will probably not play a major role in drug kinetics or drug accumulation. More recent studies in fact have found a two-compartment open model to adequately fit the plasma concentration-time curve of furosemide (8,9,69,71,72,77-80).

The pharmacokinetic parameters found for furosemide in healthy volunteers after intravenous administration are presented in Table I-2. It can be readily seen that a great deal of interstudy variability exists in these kinetic parameters. The terminal half-life for example, varies four-fold between studies and ranges from 26-100 minutes. The half-life reported by Chennavasin et al. (80) most clearly defines the elimination phase since plasma samples were collected over an eight-hour period. Those studies (69,78,79,81) reporting shorter half-lives, determined this parameter over a four-hour collection period which does not adequately reflect

Table I-2. Furosemide Pharmacokinetics[a] in Healthy Volunteers Following Intravenous Dosing

Reference	Assay	Number of Subjects	Terminal $T_{\frac{1}{2}}$ (min)	CLp (ml/min)	CLr (ml/min)	fe	CLnr (ml/min)	Vdss (ml/kg)
Rupp & Hajdu (68)	Fluor.	7	57±6[b]	114				
Cutler et al. (81)	Fluor.	4	30±6	162±38	149±37	0.92±0.10	12±17	115±23
Kelly et al. (69)	Fluor.	4	26±10	142±38				
Branch et al. (71)	Fluor.	6	68	125	75	0.65	50	
Homeida et al. (79)	Fluor.	6	38±3	268±19	90±10	0.34	178±20	
Andreasen and Mikkelsen (77)	Fluor. + TLC	8	72±29	166±42	116±67	0.63±0.24	50	181±105
Honari et al. (78)	HPLC	4	36±5	155±24	134±23	0.87	21±3	176±21
Beermann et al. (82)	GLC, HPLC	5	52±15	194±35	95±24	0.45±0.20	99±48	210±56
Rane et al. (9)	HPLC	6	51±4	158	80	0.51	77	110±7
Andreasen et al. (8)	Fluor. + TLC	7	66±29	219±49				174 30
Chennavasin et al. (80,83)	HPLC	8	100±45	125±73	64±43	0.51	61±31	161±51

[a]Values reported represent the mean ± standard deviation.

[b]Value reported represents the half-life during the β-phase of elimination.

the slower elimination phase for furosemide.

Substantial differences between studies were observed in plasma, renal and non-renal clearances of furosemide, even when HPLC techniques were employed. Cutler and coworkers (69,78,81) reported consistent values for the pharmacokinetic parameters obtained in their three studies using both a fluorimetric and an HPLC assay. However, the fraction of drug excreted unchanged in the urine in these studies was significantly greater than the literature values reported in Table I-2, and consequently non-renal clearance values were quite low. This may reflect the difficulties of accurately evaluating furosemide from urinary metabolites even when HPLC methods are utilized. In contrast, the three studies by Branch and coworkers (9,71,79) reported significantly different values for plasma and non-renal clearances of furosemide using two different analytical techniques. In this series of studies, only renal clearance appeared to be consistent from study to study. In addition, unusual values for total, renal and non-renal clearances were reported by Homeida, Roberts and Branch (79) in which furosemide was assayed spectrofluorimetrically. This method is rather non-specific, especially in urine and may account for these unusual values.

Variations in the pharmacokinetic parameters reported in Table I-2 probably reflect the difference in methodological procedures and analytical techniques utilized. This aspect will be further discussed in the sections dealing with furosemide analysis (Chapter II) and metabolism (Chapter IV).

2. Renal Disease

Several studies have described the pharmacokinetics of furosemide in patients with impaired renal function (Table I-3). In those studies where

Table I-3. Furosemide Pharmacokinetics[a] in Patients with Renal Impairment

Reference	Assay	Population	Number of Subjects	Terminal $T_{\frac{1}{2}}$ (hr)	CLp (ml/min)	CLr (ml/min)	CLnr (ml/min)	Vdss (ml/kg)
Rupp et al. (84)	^{35}S	Chronic renal failure, CLcr<10 ml/min	8	13.5				
Cutler et al. (81)	Fluor.	Functionally anephric	5	1.35±0.20	104±29		104±29	179±38
Huang et al. (73)	Fluor.	Advanced renal failure, Scr > 14 mg%	7	7.8±2.1	38.3±5.0	2.6±1.6	35.8±6.1	327±98
Beermann et al. (82)	GLC,HPLC	CLcr range 0.6-53 ml/min	17	1.5-24.6	55.2± 1.08•CLcr	0.74•CLcr	79±32	229±50
Andreasen et al.(8)	Fluor. + TLC	Anephric	7	1.93±0.59	65.9±18.7		65.9±18.7	197±33
Rane et al.(9)	HPLC	Uremic, CLcr = 11±1	6	2.60±0.42	51±7	5±1	46±7	124±9
Tilstone and Fine (72)	^{35}S	Chronic renal failure, CLcr < 5 ml/min	13	14.2±2.3	15.5		15.5	367±63

[a]Values reported represent the mean ± standard deviation.

a comparison of healthy volunteers and patients were made (8,9,81,82), a significant increase in half-life was observed for the renally impaired patients. Rane et al. (9) for example, demonstrated a mean half-life of 2.60 hours in uremic patients compared to a half-life of 0.85 hours in healthy controls. In the four studies comparing patients and volunteers (8,9,81,82), a marked reduction in plasma and especially renal clearances were found for patients. However, the volume of distribution did not change significantly between these two groups. Studies by Huang et al. (73), Rupp et al. (84) and Tilstone and Fine (72) demonstrated extremely long half-lives in renal failure patients of 7.8 hours, 13.5 hours, and 14.2 hours, respectively. However, the assays used were rather non-specific and probably measured metabolites of furosemide as well. In patients with greatly reduced creatinine clearance, the metabolites could accumulate to a significant extent.

Rane et al. (9) reported a decrease in non-renal clearance when uremics were compared with healthy controls (46 vs. 77 ml/min, respectively). Beermann et al. (82) also noted this decrease in renally impaired patients (79 vs. 99 ml/min), although not to a statistically significant level. Rane et al. (9) indicate that the reason for this reduction is unclear. However, these workers speculate that endogenous substances which accumulate in uremia may compete with and inhibit furosemide from being actively transported via some loss process (possibly biliary excretion). Studies by Rupp and coworkers (84,85) and Beermann et al. (82) have demonstrated that in renal disease, biliary excretion becomes a more prominent mechanism of furosemide elimination. They reported that more than 60% of the dose can be recovered in the feces in renally impaired patients after intravenous administration of furosemide-^{35}S. In contrast, healthy subjects excreted

only 6-12% of an intravenous dose in the feces (70,85).

3. Congestive Heart Failure

Although furosemide is extensively used in the treatment of congestive heart failure, the magnitude of its diuretic effect is unpredictable in the patient group since few pharmacokinetic studies have been performed (Table I-4). Andreasen and Mikkelsen (77) studied the pharmacokinetics of furosemide after a 40 mg intravenous dose to eight healthy subjects (group A), six patients with acute congestive heart failure not previously treated with furosemide (group B), six patients with chronic congestive heart failure under long-term furosemide treatment (group C) and six patients with chronic congestive heart failure under long-term treatment with furosemide and an anti-coagulant (group D). Patients in group C had the longest half-lives and the lowest clearance values. Plasma clearance in this group was 1.02 ml/min·kg vs. 2.34 ml/min·kg in the healthy controls (group A). Andreasen and Mikkelsen (77) noted that the volume of distribution steady-state was not significantly different among the four groups studied. However, those patients also taking anti-coagulants (group D) had a lower plasma protein binding of furosemide than healthy volunteers and heart failure patients not on anti-coagulant therapy (groups B and C). This increase in furosemide free fraction apparently resulted in the greater plasma clearance reported for group D.

Andreasen and Mikkelsen (77) also followed the urinary excretion of furosemide in six healthy volunteers (group A) and in five patients with acute heart failure not previously on the drug (group B). They reported that the average renal clearance doubled, 39 to 77 ml/min, from the first

Table I-4. Furosemide Pharmacokinetics[a] in Patients with Congestive Heart Failure

Reference	Assay	Population	Number of Subjects	Terminal $T_{\frac{1}{2}}$ (min)	CLp (ml/min/kg)	CLr (ml/min)	Vdss (ml/kg)	tlag (min)	tpeak (min)
Greither et al. (75)	Fluor.	CHF	7	76.7 (30.5)	1.48 (0.35)		120 (17)	28 (15)	86 (32)
Andreasen and Mikkelsen(77)	Fluor. + TLC	Healthy, group A	8	71.8	2.34 (0.60)	116;117[b] (79);(54)	181 (105)		
		Acute CHF, group B	6	92.1	1.23 (0.23)	39;77[b] (17);(51)	140 (83)		
		Chronic CHF, >6 months furosemide, group C	6	134.1	1.02 (0.32)		176 (150)		
		Chronic CHF, >6 months furosemide + anti-coagulant, group D	6	115.9	1.86 (1.21)		188 (281)		
Perez et al. (86)	GLC	Acute pulmonary edema	16		1.53 (0.97)	52.1 (37.3)			

[a]Values reported represent the mean ± standard deviation in parenthesis.

[b]Average renal clearances estimated from 0-1 hours and from 1-2 hours, respectively with standard deviations in parenthesi

to the second one-hour collection periods after the intravenous injection. Andreasen and Mikkelsen (77) speculate that this may be due to a furosemide-induced improvement in renal circulation with a concomitant increase in renal tubular secretion. However, the possibility of a lag time for drug in plasma to reach the urine cannot be ruled out due to the limited number of incremental renal clearance estimates made by the authors.

Greither et al. (75) found furosemide to fit a two-compartment open model with average half-lives of 9.6 minutes and 76.7 minutes for the fast and slow disposition phases, respectively. In addition, they reported a reduced plasma clearance in patients with congestive heart failure (compare with Table I-2), reflecting the slightly increased half-lives found in these patients as opposed to healthy volunteers. The reduced plasma clearance reported by Greither et al. (75) in patients is consistent with studies by Andreasen and Mikkelsen (77) and Perez et al. (86).

An abstract by Tilstone and Lawson (87) reports a lower volume of distribution for the central compartment and a lower plasma clearance in heart failure patients. However, they do not provide any data, making comparisons between this and other studies difficult.

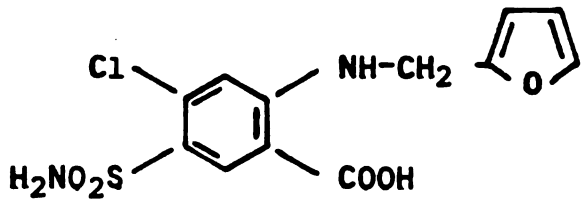
4. Hypertension

Andreasen et al. (88) are the only investigators who have reported the pharmacokinetics and natriuretic response of furosemide in hypertensive patients. Furosemide was administered as a 40 mg intravenous injection over 1 minute and its disappearance from the serum was described by a two-compartment open model. Those patients with clinical signs of congestive heart failure and those who had previously received furosemide were excluded

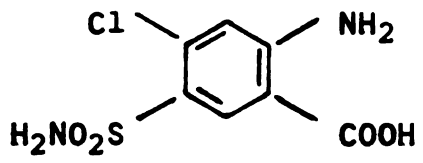
from the study. Andreasen et al. (88) noted a significant reduction in mean serum clearance in their hypertensive patients (1.83 ± 0.87 ml/min•kg) as compared to that of the healthy controls (2.96 ± 0.7 ml/min•kg). Volume of distribution steady-state however, was not significantly different between these two groups. Andreasen et al. (88) noted a highly significant correlation between renal clearance and serum clearance ($r = 0.9588$; $p < 0.001$) with an extrapolated y-intercept or non-renal clearance of about 50 ml/min. This value is in good agreement with the findings of several investigators in healthy volunteers (71,77,80). Andreasen et al. (8) reported a non-renal clearance of 65.9 ml/min in anephric patients, Rane et al. (9) reported a value of 46 ml/min in uremics and Huang et al. (73) reported a value of 35.8 ml/min in patients with advanced renal failure. A mean renal clearance of 59 ± 52 ml/min was also observed by Andreasen et al. (88) in hypertensive patients. This reduced value reflects the various degrees of impaired renal function present in the patient population as well.

G. Metabolism

Despite the widespread clinical use of furosemide in a variety of edematous states (1-3), the data concerning its metabolism are sparse and controversial. Häussler and Hajdú (89) reported that 2-amino-4-chloro-5-sulfamoylanthranilic acid (CSA) was the only metabolite of furosemide in humans and dogs (Fig. I-6). Other investigators have also reported CSA as a metabolite of furosemide (8,85,86,90). However, Calesnick et al. (76), Kindt and Schmid (91) and Beerman et al. (70) found no evidence of CSA. Andreasen et al. (8), detected furosemide metabolites CSA as well as anthranilic acid in 1, 3, and 7 hour serum samples in both their healthy and anephric patients. Urine samples were not analyzed by these



FUROSEMIDE



CSA

Fig. I-6. Furosemide and its proposed metabolite, CSA.

coworkers for the proposed metabolites. Yakatan et al.(92) analyzed the urinary excretion of orally administered furosemide-^{35}S in dogs and monkeys using thin layer chromatographic separation. In both animal species, these investigators reported the label to be approximately 80% unchanged furosemide, 7% CSA and 7% attributable to two different unknown metabolites. CSA was reported by Rupp (85) to have approximately 25% of the diuretic activity of furosemide. In addition to the above postulated metabolites, a possible glucuronide metabolite of furosemide has been reported, although poorly quantitated in some studies (70,91), and found to vary with dosing history and renal function in others (77,88). Discrepancies in the metabolism of furosemide reflect problems inherent in the analytical methodologies employed. This aspect will be discussed in greater detail in the sections dealing with furosemide analysis (Chapter II) and metabolism (Chapter IV).

H. Dose-Response Relationships

Conflicting reports have appeared in the literature attempting to relate furosemide dose, as well as plasma concentration to the diuretic response. Most of the original work was carried out by Rupp and coworkers (68,84,85). They found the shape of the urine flow-time curves to parallel the plasma concentration-time curves following oral and intravenous administration of 40 mg of furosemide to healthy subjects. In addition, Rupp (85) observed the linear relationship between the logarithm of urine flow and the logarithm of furosemide plasma concentration to be virtually identical after both oral and intravenous dosing. However, only plasma levels following peak concentrations were considered in the evaluation of the oral data. Rupp and Hajdú (68) attempted to relate the oral availability

of furosemide to the observed diuretic response following oral and intravenous dosing. They reported the diuretic response after oral administration to be 64% of that observed following intravenous dosing. Area under the plasma concentration-time curves indicated an oral absorption of 58% for furosemide. The 64% estimate however, is quite tenuous. The value is on the increase in 8-hour urine volumes following oral and intravenous administration of furosemide compared to the control periods (no drug), respectively. Since the 8-hour difference in urine volumes between oral and intravenous treatments are no greater than the 8-hour control volumes, it is doubtful whether the diuresis observed is significantly different in the seven subjects studied. Subsequent studies in healthy subjects (69,71), heart failure patients (75) and "diuretic-resistant" patients (74) demonstrated that an equivalent diuretic response to furosemide was achieved whether the dose was administered orally by intravenous injection. However, in uremics, Huang et al. (73) found the diuresis produced by oral furosemide to always be less effective than after intravenous dosing.

In 1977, Branch et al. (71) attempted to define a measurable sampling site which better correlated with furosemide's diuretic effect. The authors hypothesized from work in healthy subjects that furosemide-induced diuresis was dependent on drug levels in a tissue compartment rather than in plasma or urine. This hypothesis was based upon the linear relationship between the logarithm of sodium excretion rate and the logarithm of plasma concentration of furosemide during the β-phase of elimination. Branch and coworkers (71) also noted that during the early distribution phase after an intravenous dose, the rate of sodium excretion was more closely related to the concentration of furosemide extrapolated from the β-phase of elimination rather than to the actual plasma concentration. However,

this relationship is suspect since pharmacokinetic models predict that drug concentration in a peripheral compartment cannot reach a log-linear phase prior to attainment of this linearity in the plasma compartment.

The relationship between furosemide dose and diuretic response is also ambiguous. Cutler et al. (81) studied four hydropenic healthy subjects after intravenous doses of approximately 0.5, 1.0 and 1.5 mg/kg of furosemide. Although the 6-hour excretion of water, sodium and potassium progressively increased as larger doses of furosemide were administered, the correlation of effect to dose were quite weak within each subject as well as with pooled data from all the subjects. Stallings et al. (93) in six healthy subjects, studied the natriuretic and diuretic effectiveness of oral furosemide as one - 40 mg dose or as two - 20 mg doses administered six hours apart. They reported no statistical differences between the two regimens in terms of the 24-hour excretion of sodium, potassium, chloride or water. In contrast, Wilson et al. (94) found a 20 mg, twice daily regimen of oral furosemide to be more natriuretic but not more diuretic than a single 40 mg dose in twelve healthy subjects. In addition, the single 20 mg oral dose of furosemide did not produce a significant weight loss, diuresis or natriuresis over the 24-hour collection period. Brater et al. (95) in a more recent study reported the 4-hour excretion of volume, sodium and potassium to be substantially greater than control values after 20 mg oral furosemide dosing to eight healthy subjects.

1. Role of Probenecid

Furosemide is highly protein bound (8,9) and gains access to the kidney lumen at the pars recta of the proximal tubule via the non-specific

organic acid secretory pathway (5,6,10). Thus, the amount of furosemide that reaches the tubular fluid could be significantly modified by changes in the capacity of this system to transport drug. In this respect, the coadministration of other weak organic acids such as probenecid could compete with furosemide for active transport. Consequently, a decreased delivery of drug to the site of action could result and change the relationship between furosemide dose and its natriuretic/diuretic effect.

Honari et al. (78) studied the effects of probenecid administration on furosemide kinetics and natriuresis in four healthy subjects. They reported that although a statistical reduction in natriuresis occurred after probenecid during the furosemide infusion studies, no effect of probenecid was noted when furosemide was given as a single intravenous injection. Probenecid administration during the infusion studies resulted in a reduced renal clearance of furosemide with a concomitant rise in plasma concentration. In addition, although probenecid pretreatment markedly altered the pharmacokinetics of furosemide during the single dose study, the fraction of the dose excreted unchanged was not different between the two treatments. In conclusion, the authors suggest that the amount of furosemide that reaches the tubular fluid rather than the plasma concentration is the main determinant of furosemide diuresis.

Homeida et al. (79) also studied the pharmacokinetics and pharmacodynamics of intravenous furosemide, with and without probenecid pretreatment, in six healthy subjects. These authors found the time course of furosemide response to be modified by probenecid due to changes in delivery rate of furosemide to the renal tubule. However, as both renal and non-renal clearance of furosemide were reduced, the total proportion of unchanged drug reaching the tubular fluid was not markedly altered with

concomitant probenecid administration. Thus, no change in total diuretic response was observed since the amounts of furosemide reaching the renal tubule were similar between studies.

2. Role of Indomethacin

An additional mechanism by which a change in the dose-response relationship of furosemide might occur is through a disruption of some mediator(s) in that response. As previously discussed, the prostaglandins have been implicated as chemical mediators of furosemide-induced natriuresis and diuresis. Consequently, studies in experimental animals and in humans have shown indomethacin, a potent inhibitor of prostaglandin synthetase, to attenuate the natriuretic, hemodynamic and renin-stimulating effects of furosemide. In addition, Rane et al. (96) have found the degree of inhibition of prostaglandin synthesis to correlate with indomethacin dose and plasma levels. However, an alternate explanation of this interaction is that indomethacin and furosemide (both are weak organic acids) may compete for active secretion into the renal tubule. This could prevent furosemide from reaching its site of action and thereby attenuate its diuretic response. Frolich et al. (49) recognized both possible mechanisms for the attenuation of furosemide's diuretic effect by indomethacin and on the basis of limited data, discounted the pharmacokinetic interaction. This aspect will be discussed in more detail in Chapter V.

3. Miscellaneous

A further modification of furosemide's dose-response relationship could occur in conditions not related to drug access to the lumen (97).

Interference with the amount of sodium and chloride delivered to the diluting segment of Henle's loop could alter the effectiveness of furosemide. Diseases such as congestive heart failure, nephrotic syndrome and cirrhosis result in a greater reabsorption of sodium in the proximal tubule. This decreased delivery of sodium to the loop of Henle could influence tubular response to furosemide, independent of the concentration of the drug present there. In addition, these same disease states are associated with shunting of blood from superficial to juxtamedullary nephrons. This shunting may similarly alter the response to furosemide, independent of drug delivery to the active site.

Chapter II

Analytical Methods

A. Previously Reported Techniques

A variety of assay methods have been developed for the analysis of furosemide in biological fluids.

1. Colorimetric Analysis

In 1964, Häussler and Hajdú (89) introduced the colorimetric determination of urinary furosemide concentrations by employing the Bratton-Marshall reaction for the detection of primary arylamines. This method is based upon the diazotization of furosemide followed by coupling with 2-dimethylaminoethyl-1-naphthalene for color development. Absorbance of the resultant dye complex is determined at 535 nm. Disadvantages of this method include time-consuming extraction and derivatization steps, in addition to problems of specificity with respect to other aromatic amines and potential furosemide metabolites.

2. Fluorescence Analysis

Häussler and Hajdú (89) also reported an assay procedure for the analysis of furosemide in serum samples from several animal species. The method involves an acid extraction step into ether using concentrated HCl followed by fluorimetric detection. Poor sensitivity (0.2 μg/ml),

large sample requirements (1 ml) and a time-consuming extraction step make this procedure less than desirable. Interference by extractable metabolites of furosemide as well as other fluorescent substances may also be a problem.

Several investigators (69,71,81,98,99) have subsequently modified the furosemide serum assay of Häussler and Hajdú (89). In addition to the original acid extraction step, furosemide was back extracted under basic conditions in an attempt to improve the specificity of the assay. Using this modification, analysis methods for furosemide in plasma (71,75) as well as plasma and urine samples (69,81,98,99) all required large sample volumes (1 ml). Other weaknesses of the assay include multiple extraction steps, poor sensitivity ($\geq$ 0.1 µg/ml) and specificity problems, particularly with urine samples. A quantitative thin layer chromatographic (TLC) method prior to fluorescence detection of furosemide in urine samples was reported by Kindt and Schmid (91). Mikkelsen and Andreasen (98) later modified the assay to allow for the simultaneous determination of furosemide, CSA and anthranilic acid. A sensitivity of 0.1 µg/ml for furosemide in plasma and 0.15 - 0.20 µg/ml for CSA and anthranilic acid was reported (98). In urine, only furosemide and anthranilic acid could be determined quantitatively by this method. Although designed to improve assay specificity, the assays are quite tedious, involving multiple extraction steps and TLC separation prior to fluorescence detection of furosemide. In addition, large sample volumes are needed (1 ml for urine, 2 ml for plasma) without obtaining improved sensitivity over less complex methods. Ironically, the specificity of the method must also be questioned. The ability to form anthranilic acid by removal of the 4-chloro and 5-sulfamoyl functional groups of furosemide by metabolic enzymic processes has no precedent and

is highly questionable.

3. Radioactive Analysis

Several investigators (70,72,74,76,92,99,100) have used furosemide-(^{14}C or ^{35}S) to describe the drug's absorption, disposition and protein binding. In general, the method is very sensitive but lacks the specificity to differentiate furosemide from its metabolite(s). In order to improve the assay specificity, prior extractions under acidic conditions (99), TLC separation (70,92) and paper chromatographic techniques (76) have been utilized. In addition to this time-consuming effort, the administration of a labelled drug makes the assay a poor choice in a clinical setting.

4. Gas Chromatographic Analysis

Lindström and Molander (101) reported a gas chromatographic method using electron capture detection for the analysis of furosemide in plasma. The method is based upon the conversion of furosemide to its trimethyl derivative by extractive alkylation. Perez et al. (86) later modified the assay and were able to determine furosemide in urine samples as well. Although the assay procedure is specific for furosemide, the prior acid extraction and derivatization steps make the method tedious and time-consuming. The sensitivity of the assay is about 0.1 μg/ml.

5. High Performance Liquid Chromatographic Analysis

High performance liquid chromatographic (HPLC) methods have more recently been developed in order to improve the assay specificity (102-107). Furosemide was separated from other substances in biologic fluids using ion-exchange (103,104) or reversed-phase (102,105-107) chromatography, and quantitated by ultraviolet (102) or fluorescence (103-107) detection. Several investigators (102,104-106) propose multiple extraction steps (acidic and basic) which are tedious and time-consuming. Other disadvantages of these assays include the use of large plasma volumes (1 to 2 ml) with a lower limit of sensitivity between 0.1 and 1.0 μg/ml (102-105,107). Swezey et al. (106) reported a HPLC method which can detect furosemide down to 20 ng/ml using 1 ml plasma or 0.1 ml urine samples. Blair et al. (103) reported an assay for furosemide where 5 μl aliquots of urine or serum can be injected directly onto a resin bed column. A precolumn in this chromatographic system was necessary; apparently to avoid plugging the main HPLC column by proteins contained in the serum. This method suffers from the lack of an internal standard as well as poor sensitivity (0.5 - 1.0 μg/ml). Nation et al. (107) also developed a simple and direct injection assay for furosemide in plasma. The only requirement for this method is prior precipitation of plasma proteins with acetonitrile. Using a 100 μl plasma sample, the sensitivity is 0.1 μg/ml. Disadvantages of the method are that no internal standard was used and that the analysis of furosemide does not apply to urine samples.

Discrepancies involving the pharmacokinetics as well as the metabolism of furosemide are prevalent in the literature. These discrepancies may reflect problems with different assay procedures as reviewed by Benet (97).

In particular, prior acidification and extraction steps may lead to the formation of the supposed CSA metabolite during the assay procedure. Therefore, a rapid, sensitive and specific HPLC assay was developed, without prior extraction and/or derivatization in an attempt to clarify the disposition of furosemide.

B. Experimental

1. Chemicals

Furosemide[1], sodium phenobarbital[2], chlorpromazine hydrochloride[3], indomethacin[3], probenecid[3], CSA[4], acetanilid[5], o-nitrobenzoic acid[6] and β-glucuronidase Type B-1[3] were used as received. Phosphoric acid[7] and glacial acetic acid[7] were both analytical reagent grade. The methanol[8] (HPLC grade), acetonitrile[9] (glass-distilled) and distilled water (glass redistilled and stored in glass) were filtered and degassed prior to HPLC use.

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[1]Hoechst-Roussel Pharmaceuticals, Inc., Somerville, NJ.

[2]Merck and Co., Inc., Rahway, NJ.

[3]Sigma Chemical Co., St. Louis, MO.

[4]U.S. Pharmacopeia, Rockville, MD.

[5]General Chemical Division, New York, NY.

[6]Eastman Organic Chemicals, Rochester, NY.

[7]Mallinckrodt, St. Louis, MO.

[8]Fisher Scientific Co., Fair Lawn, NJ.

[9]Burdick and Jackson Laboratories, Inc., Muskegon, MI.

2. Instrumentation

Method I

High performance liquid chromatographic[10] analyses were carried out on an instrument equipped with a U6-K universal injector and a dual-channel fixed wavelength, ultraviolet absorbance detector. The instrument was fitted with a 30 cm x 3.9 mm I.D. μBondapak C_{18} reversed-phase column[11], particle size 10 μm. The wavelengths of detection were fixed at 254 nm and 280 nm for the internal standard, sodium phenobarbital and furosemide, respectively. A dual-pen recorder[12] was used at a chart speed of 30 cm/hr.

Method II

Samples were analyzed using a liquid chromatograph[13] equipped with a syringe loading sample injector[14], a fluorescence spectrophotometer[15] and a dual-channel fixed wavelength, ultraviolet absorbance detector.[16]

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[10]Model ALC/GPC-244 w/Model 6000A, Waters Associates, Inc., Milford, Mass.

[11]Waters Associates, Inc., Milford, Mass.

[12]Omniscribe Model A5211-1, Houston Instruments, Austin, Texas

[13]Model 5000, Varian, Los Altos, CA.

[14]Model 7105, Perkin-Elmer, Mountainview, CA.

[15]Model 650-10S, Perkin-Elmer, Mountainview, CA.

[16]Model 440, Waters Associates, Inc., Milford, Mass.

A reversed-phase column[11] was fitted to the instrument as in Method I. The excitation and emission wavelengths of furosemide were set at 345 nm and 405 nm, respectively. The internal standard, sodium phenobarbital was measured by ultraviolet detection at 254 nm. A dual-pen recorder[17] was used at a chart speed of 20 cm/hr.

3. Preparation of Standard Solutions

Furosemide (4.1 mg) was dissolved in 50% acetonitrile/water to yield a stock solution of 41 μg/ml. This stock solution was then diluted 5-fold (8.2 μg/ml), 20-fold (2.0 μg/ml) and 100-fold (0.41 μg/ml) to give the working standard solutions. CSA (4.2 mg) was dissolved in acetonitrile to yield working standard solutions of 42 μg/ml for urine samples and diluted 20-fold (2.1 μg/ml) for plasma samples. Indomethacin (2.9 mg) and probenecid (140 mg) were dissolved in methanol to yield stock solutions of 29 μg/ml and 1.4 mg/ml, respectively. The hydrochloride salt of chlorpromazine (20 mg) was dissolved in acetonitrile to yield a 0.2 mg/ml stock solution. Sodium phenobarbital was dissolved in distilled water and varied in concentration (2.5 - 10 mg/ml) depending upon the furosemide concentration range to be measured. Acetanilid was dissolved in acetonitrile (0.5 mg/ml) and o-nitrobenzoic acid was dissolved in methanol (2.5 mg/ml).

4. Assay of Furosemide in Plasma Samples

A 50 μl aliquot containing the internal standard, sodium phenobarbital (2.5 mg/ml) was added to 0.20 ml furosemide plasma samples. The mixture

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[17]Model 585, Linear, Irvine, CA.

was shaken on a vortex mixer[18] and 0.40 ml acetonitrile was added. The mixture was shaken again on a vortex mixer and then centrifuged[19] for 10 minutes. The supernatant was transferred to a clean test tube and evaporated[20] under nitrogen until about 0.10 ml of the solution remained. An appropriate aliquot was then injected directly into the loop injector.

Method I

The mobile phase consisted of 25% acetonitrile-0.01 M glacial acetic acid, buffered to pH 5.0 with 4 N NaOH. The chromatograph was operated at a flow rate of 2 ml/min under isocratic and ambient temperature conditions. A dual-channel ultraviolet absorbance detector was used to simultaneously monitor furosemide at 280 nm and sodium phenobarbital at 254 nm.

Method II

The mobile phase consisted of 38% acetonitrile - 0.015 M phosphoric acid pumped isocratically at a flow rate of 2 ml/min, at ambient temperature. Fluorescent (EX λ = 345 nm, EM λ = 405 nm) and ultraviolet (254 nm) detection were utilized to simultaneously monitor furosemide and sodium phenobarbital, respectively.

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[18]Thermolyne Maxi-mix, Scientific Products, Menlo, CA.

[19]Model HN-SII, VWR Scientific, San Francisco, CA.

[20]High Speed Analytical Evaporator, Organomation Associates, Inc., Northborough, Mass.

5. Assay of Furosemide in Urine Samples

A 50 µl aliquot containing the internal standard, sodium phenobarbital (2.5 mg/ml) 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 appropriate volume was injected directly into the loop injector. The instrumentation and mobile phase requirements were identical to those previously discussed for Methods I and II. In one case, the solvent system for Method II consisted of 30% acetonitrile in 0.015 M phosphoric acid aqueous solution as detailed in Chapter V. All other conditions remained identical to those described above.

6. Assay of Glucuronide Metabolite in Urine Samples

Urine samples were measured in parallel for unchanged furosemide and its glucuronide metabolite. Duplicate urine samples (0.20 ml) were prepared and added to 0.80 ml buffer solutions (0.1 M sodium acetate, pH 4.5), one containing 2000 units β-glucuronidase and the other containing only buffer. Samples were sealed and incubated overnight (at least 17 hours) in a shaker bath[21] at 37°C. A 50 µl aliquot of the internal standard, sodium phenobarbital (10 mg/ml) was added to the mixture, which was then shaken on a vortex mixer. Acetonitrile (2.0 ml) was added to precipitate the enzyme proteins and the mixture was shaken again. After centrifugation for 10 minutes, the supernatant was transferred to a clean test tube. The supernatant was then evaporated under nitrogen until about 1.0 ml of the

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[21]Dubnoff Metabolic Shaking Incubator (Precision Scientific 66722), Scientific Products, Menlo, CA.

solution remained. An appropriate volume was injected into the loop injector. Detection and quantitation were effected using the same instrumentation and solvent system previously described for Method II.

7. Assay of CSA in Plasma Samples

Plasma samples containing CSA were prepared in a similar manner to that for furosemide in plasma. The instrumentation utilized was described in Method II. CSA was measured by fluorescence detection with excitation and emission wavelengths set at 325 nm and 390 nm, respectively. The internal standard, acetanilid (0.5 mg/ml) was measured by ultraviolet detection at 254 nm. Samples were eluted isocratically in 17% acetonitrile-0.015 M phosphoric acid at a flow rate of 2 ml/min. An appropriate volume was then introduced into the loop injector.

8. Assay of CSA in Urine Samples

Urine samples containing CSA were prepared in a similar manner to that for furosemide in urine. Method II describes the instrumentation utilized. The mobile phase used above was found unsuitable for measuring CSA in urine due to the presence of endogeneous interference peaks. This interference was resolved by changing the mobile phase (3% acetonitrile-0.015 M phosphoric acid), thereby causing greater retention of CSA on the column. In addition, o-nitrobenzoic acid (2.5 mg/ml) which was measured by ultraviolet detection at 254 nm was used as the internal standard for CSA. Volumes of 20 µl were introduced directly into the loop injector.

9. Assay of Probenecid in Plasma Samples

Probenecid plasma levels were determined by HPLC analysis for the furosemide-probenecid interaction studies in healthy volunteers (Chapter V). Sample preparation was identical to that described for furosemide in plasma. The instrumentation described for Methods I and II were simplified. Both probenecid and the internal standard, sodium phenobarbital were measured on one-channel of the ultraviolet detector at 254 nm. An appropriate volume was injected into the loop injector and samples were eluted at 2 ml/min. The mobile phase consisted of 30% acetonitrile-0.01 M glacial acetic acid, buffered to pH 5.0 with 4 N NaOH.

10. Assay of Indomethacin in Plasma Samples

Indomethacin plasma concentrations were determined by HPLC analysis for the furosemide-indomethacin interaction studies in healthy volunteers (Chapter V). Sample preparation was similar to that described for furosemide in plasma. However, chlorpromazine hydrochloride (0.2 mg/ml) was substituted as the internal standard for indomethacin. The instrumentation described for Methods I and II were simplified. Both indomethacin and chlorpromazine hydrochloride were measured on one-channel of the ultraviolet detector at 254 nm. An appropriate volume was injected into the loop injector and samples were eluted at 2 ml/min. The mobile phase consisted of 70% methanol-0.015 M phosphoric acid, buffered to pH 3.5 with 4 N NaOH.

C. Results and Discussion

Figure II-1 shows a chromatogram for the analysis of furosemide in plasma using Method I. The retention times for furosemide and sodium phenobarbital were 5.0 and 7.0 minutes, respectively. Standard curves were constructed by adding known amounts of furosemide and the internal standard, sodium phenobarbital, to plasma and urine. The peak height ratios of furosemide to sodium phenobarbital were then plotted against the concentration of furosemide. In plasma, furosemide concentration ranged from 0.08-2.45 μg/ml and in urine, from 0.20 - 10.2 μg/ml. Over a period of twelve days, six plasma standard curves were constructed. With 50 points the regression line for plasma was: $Y = (1.16 \pm 0.05)X + (0.02 \pm 0.05)$, with a coefficient of variation for the slope of 4% and a correlation coefficient of 0.99. For urine, five standard curves were constructed over a period of two months. With 34 points the regression line for urine was: $Y = (0.46 \pm 0.01)X + (0.03 \pm 0.04)$, with a coefficient of variation for the slope of 2% and a correlation coefficient of 0.99. With the above curves, a straight-line fit of the data was made by least squares linear regression analysis using the PROPHET system, a specialized computer resource developed by the Chemical/Biological Information Handling Program of the National Institutes of Health.

Figure II-2 shows a chromatogram for the analysis of furosemide in plasma using Method II. Under these conditions, furosemide and sodium phenobarbital had retention times of 6.0 and 4.0 minutes, respectively. A typical standard curve of furosemide/sodium phenobarbital peak height ratio over the furosemide concentration range 8.3 - 207 ng/ml resulted

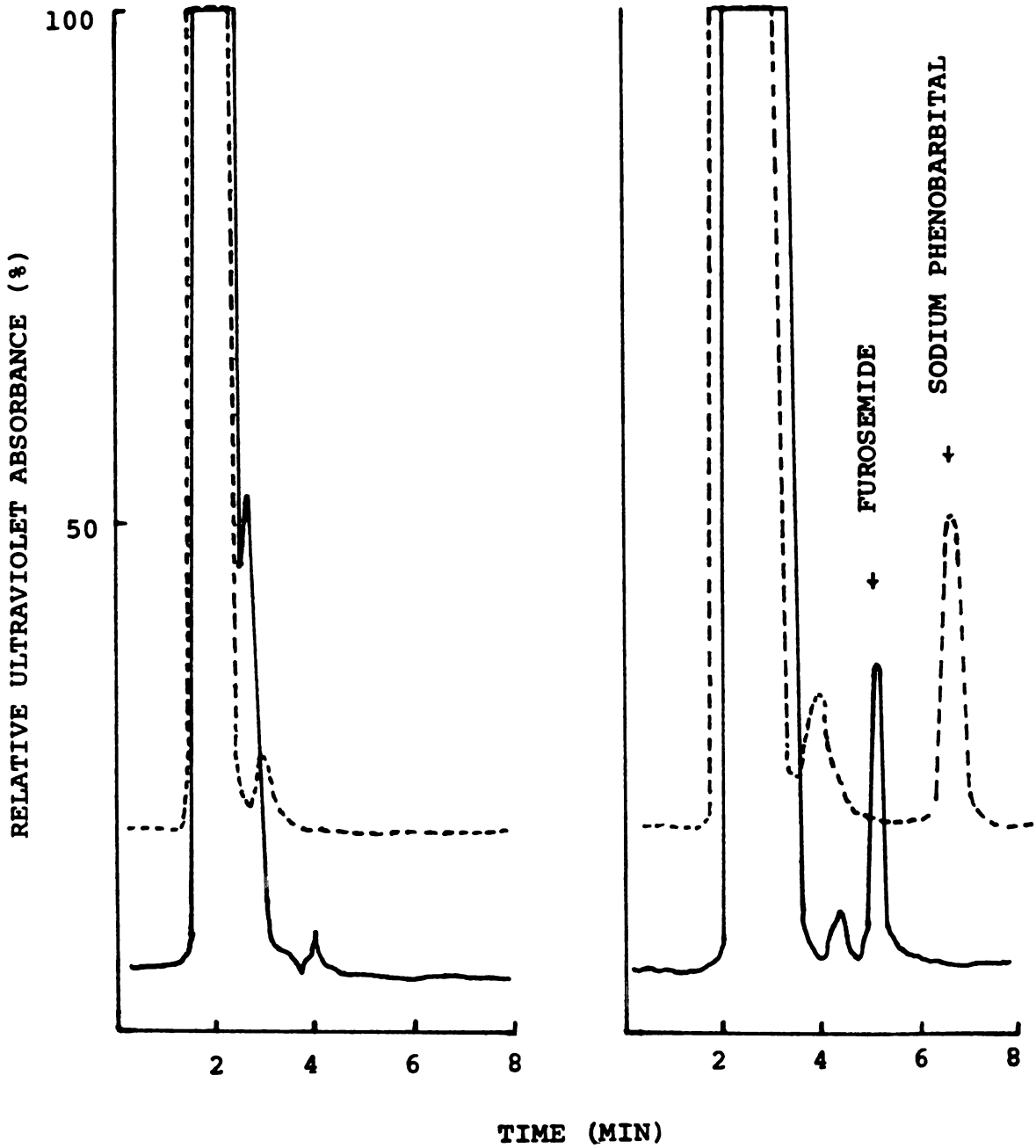

Fig. II-1. Chromatograms developed using Method I for blank plasma (left) and for plasma sample containing furosemide and the internal standard, sodium phenobarbital (right).

-------- 254 nm

———— 280 nm

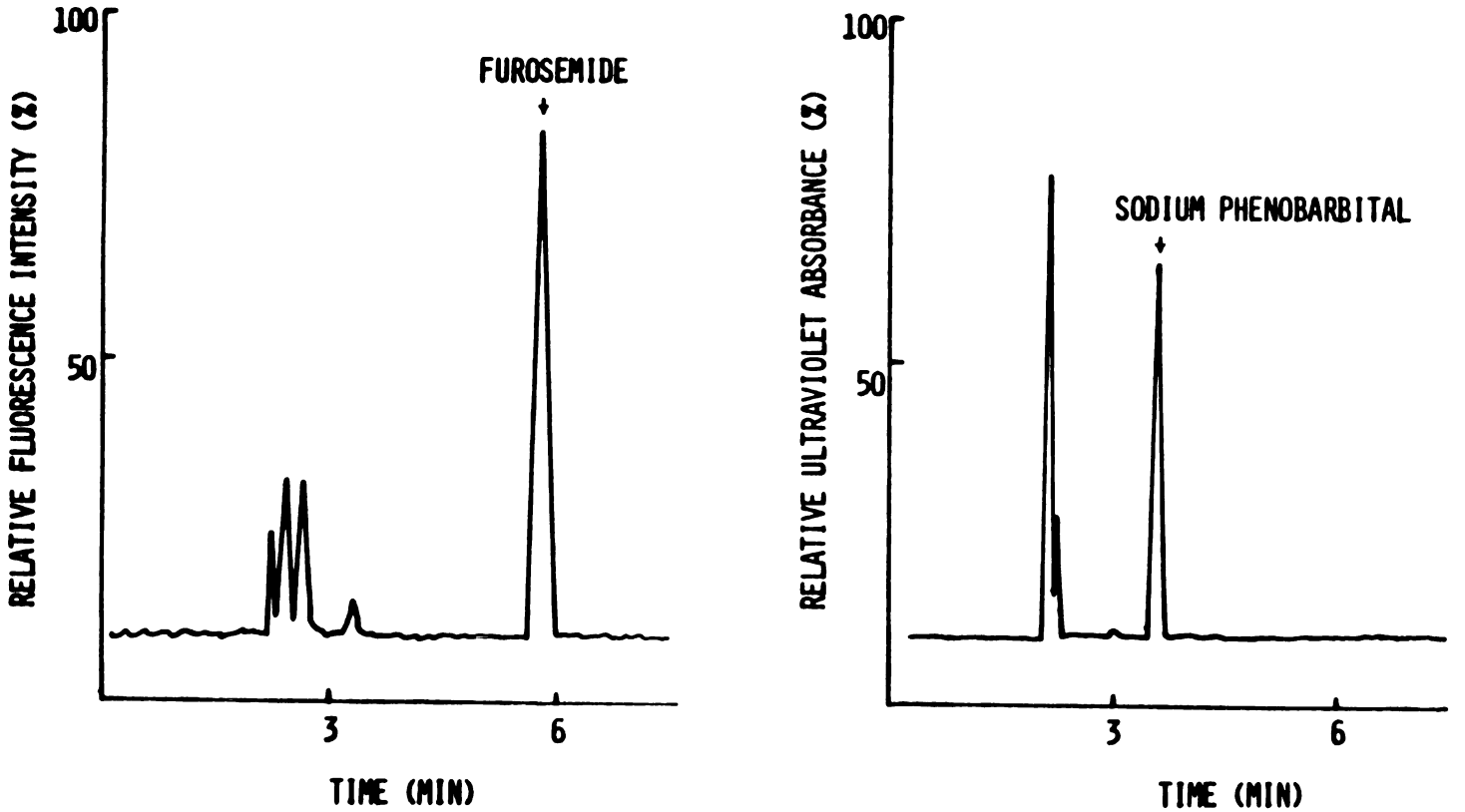

Fig. II-2. Chromatograms developed using Method II for plasma sample containing furosemide (left) and the internal standard, sodium phenobarbital (right).

in the following linear least squares regression equation: $Y = 0.019X - 0.021$; $r^2 = 0.999$ (Fig. II-3). With fluorescence detection, concentrations as low as 8.3 ng/ml have been measured for furosemide with 0.20 ml plasma samples. As previously noted, in one case the solvent system for Method II consisted of 30% acetonitrile-0.015 M phosphoric acid aqueous solution. Under these conditions, urine samples containing furosemide and sodium phenobarbital had retention times of 9.0 and 5.0 minutes, respectively (Fig. II-4).

Standard curves of furosemide in plasma (8.3 - 207 ng/ml) were constructed on seven different days to determine the variability of the slopes and the intercepts (Table II-1). The results show little day-to-day variability of slope and intercept as well as good linearity ($r^2 > 0.998$) over the concentration range studied. The coefficient of variation for the slope was 5.0%. Standard curves of furosemide in urine (1.7 - 41.4 µg/ml) were also constructed (Table II-2). The coefficient of variation for the slope was 3.5% with no significant intercept at zero furosemide concentration. In addition, all seven curves showed good linearity ($r^2 > 0.999$) over the concentration range studied.

Table II-3 shows the intra-and-interday precision and accuracy for the plasma assay of furosemide, assessed at three concentrations. A similar comparsion was made for the urine assay of furosemide in Table II-4. At plasma concentrations as low as 8.3 ng/ml, the intra-and-interday coefficient of variation was 9.0% and 5.0%, respectively. The precision of the assay, as determined by the coefficient of variation was less than 5.0% for all other concentrations. In addition, the plasma and urine assays were quite accurate with respect to the concentrations of furosemide tested (% error $\leq$ 6.0%).

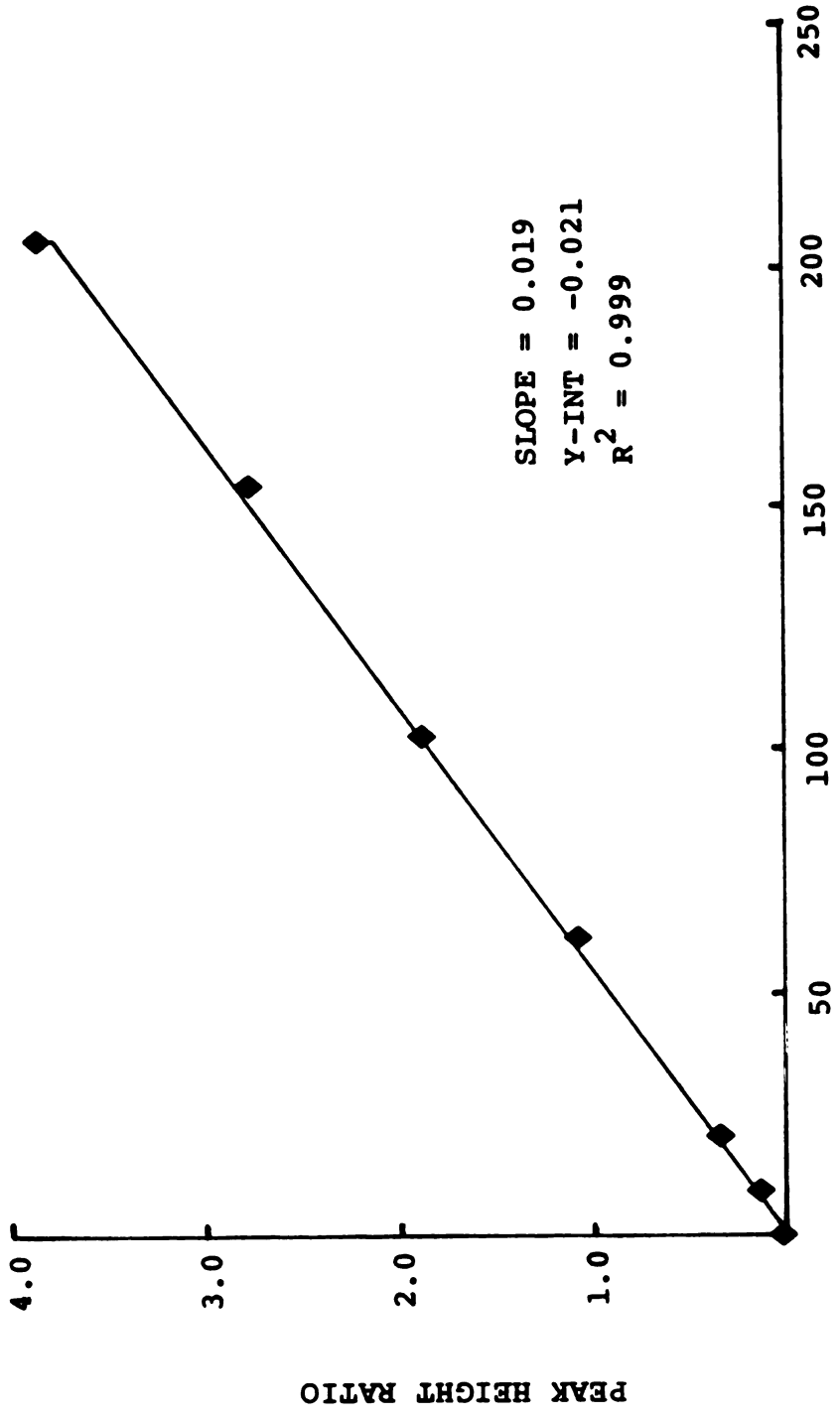

Fig. II-3. Standard curve relating the furosemide/sodium phenobarbital peak height ratio to the furosemide plasma concentration.

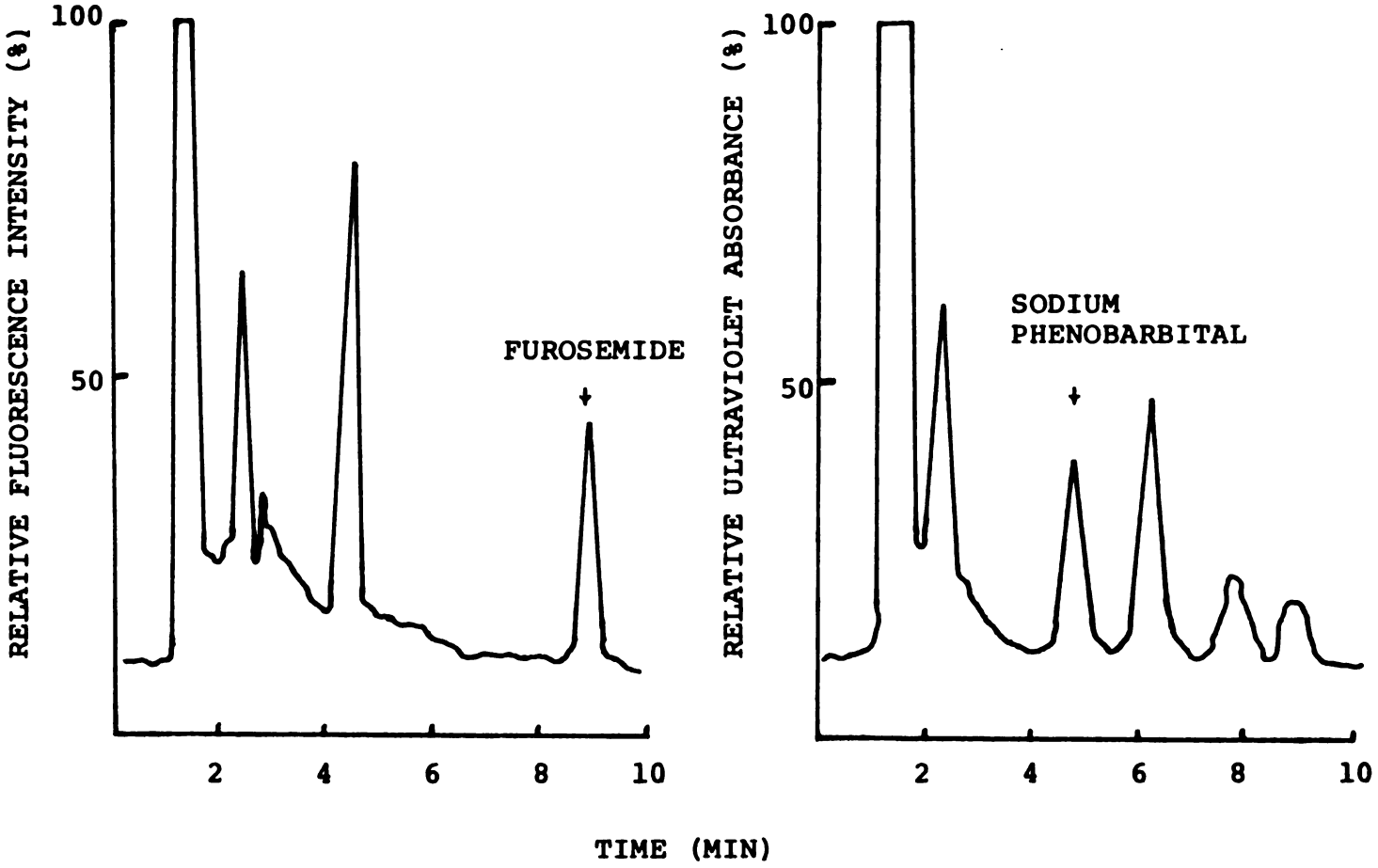

Fig. II-4. Chromatograms developed during the furosemide-probenecid interaction studies using Method II (modified) for urine sample containing furosemide (left) and the internal standard, sodium phenobarbital (right).

Table II-1. Interday[a] Variability of Slopes and Intercepts Derived from the Standard Curves of Furosemide[b] in Plasma

Curve #	Slope	Y-intercept	r^2
1	0.0162	0.0084	0.9997
2	0.0167	0.0140	0.9998
3	0.0154	0.0174	0.9990
4	0.0153	-0.0381	0.9989
5	0.0173	0.0001	0.9991
6	0.0155	0.0004	0.9986
7	0.0156	-0.0227	0.9989
MEAN	0.0160	-0.0029	0.9991
SD	0.0008	0.0203	0.0004

[a]Standard curves were constructed on seven different days over a 2-week period.

[b]Method II.

Table II-2. Interday[a] Variability of Slopes and Intercepts Derived from the Standard Curves of Furosemide[b] in Urine

Curve #	Slope	Y-intercept	r^2
1	0.0897	-0.0049	0.9999
2	0.0879	-0.0105	0.9998
3	0.0890	-0.0093	0.9999
4	0.0811	-0.0106	0.9998
5	0.0886	-0.0115	0.9995
6	0.0848	-0.0182	0.9996
7	0.0873	-0.0078	0.9999
MEAN	0.0869	-0.0104	0.9998
SD	0.0030	0.0041	0.0002

[a]Standard curves were constructed on seven different days over a 3-week period.

[b]Method II.

Table II-3. Intraday[a] and Interday[b] Variability of Furosemide Concentration[c] in Spiked Plasma Samples

Intraday			Interday		
Spiked Conc(ng/ml)	Measured Conc(ng/ml)	% error[d]	Spiked Conc(ng/ml)	Measured Conc (ng/ml)	% error[d]
8.3	Mean: 7.8	-6.0	8.3	Mean: 8.5	2.4
	SD : 0.7			SD : 0.4	
	CV(%): 9.0			CV(%): 5.0	
104	Mean: 104	-0.2	104	Mean: 102	-1.9
	SD : 2.1			SD : 4.5	
	CV(%): 1.2			CV(%): 4.4	
207	Mean: 210	1.4	207	Mean: 205	-1.0
	SD : 2.6			SD : 6.1	
	CV(%): 1.2			CV(%): 3.0	

[a]Mean values represent six different plasma samples.

[b]Mean values represent duplicate plasma samples analyzed on seven different days over a 2-week period.

[c]Method II.

[d]% error = 100 X (Measured Conc - Spiked Conc)/Spiked Conc.

Table II-4. Intraday[a] and Interday[b] Variability of Furosemide Concentration[c] in Spiked Urine Samples

Intraday			Interday		
Spiked Conc (μg/ml)	Measured Conc (μg/ml)	% error[d]	Spiked Conc(μg/ml)	Measured Conc(μg/ml)	% error[d]
1.7	Mean: 1.6	-4.1	1.7	Mean: 1.7	-0.3
	SD : 0.03			SD : 0.1	
	CV(%): 1.6			CV(%): 4.0	
20.7	Mean: 20.6	-0.5	20.7	Mean: 20.1	-2.9
	SD : 0.8			SD: 0.5	
	CV(%): 4.0			CV(%): 2.4	
41.4	Mean: 41.4	-0.04	41.4	Mean: 40.1	-3.1
	SD : 0.8			SD: 1.0	
	CV(%): 2.0			CV(%): 2.5	

[a]Mean values represent six different urine samples.

[b]Mean values represent duplicate urine samples analyzed on seven different days over a 3-week period.

[c]Method II.

[d]% error = 100 X (Measured Conc - Spiked Conc)/Spiked Conc.

Recovery of furosemide from plasma proteins was assessed by comparing the peak height of furosemide at three different concentrations when assayed in plasma samples versus samples prepared in water. As shown in Table II-5, recovery of furosemide in plasma was essentially complete at all concentrations.

Stability studies with 2 μg/ml and 10 μg/ml of furosemide in plasma were performed over a 20 day period (Table II-6). Plasma samples were stored at -20°C up until the time of analysis. The results show that furosemide can be stored frozen in plasma for at least three weeks. In fact, urine samples containing furosemide that were reanalyzed more than one year since the original analysis,were found to be stable (Table II-7).

The specificity of β-glucuronidase enzyme for the glucuronide metabolite of furosemide was determined and the results are presented in Table II-8. Four aliquots of the same urine sample were treated with buffer only (control), denatured enzyme and β-glucuronidase, and then measured for unchanged furosemide as previously discussed. The denatured enzyme was obtained by heating β-glucuronidase on a steam bath at 70°C for 30 minutes. The results show almost identical values for unchanged concentrations of furosemide when urine was treated with buffer only or with the denatured enzyme. In contrast, treatment with β-glucuronidase showed a 15% increase in unchanged furosemide concentration as compared to the control urine. These results demonstrate a specificity of the enzyme protein for hydrolysis of furosemide glucuronide to the parent drug.

Table II-9 shows the effect of varying β-glucuronidase concentration on the enzyme's ability to hydrolyze furosemide glucuronide in urine. Concentrations of enzyme ranged 100-fold (100-10,000 units) per 0.20 ml urine sample and unchanged furosemide was measured as previously discussed.

Table II-5. Recovery of Furosemide[a] in Plasma Versus Water

Concentration (µg/ml)	Ratio Peak Height, Plasma vs. Water (%)	Mean ± SD (%)
0.2	99	
	103	
	99	
	90	
	99	
	99	
		98 ± 4.3
0.5	98	
	102	
	102	
	102	
	101	
	102	
		101 ± 1.6
2.0	98	
	99	
	95	
	96	
	98	
	96	
		97 ± 1.6

[a]Method II.

Table II-6. The Effect of Storage on Furosemide Concentration[a]

Furosemide (µg/ml)	Time (days)					
	0	1	2	6	10	20
2	2.10	2.00	1.95	2.00	1.95	1.95
10	9.95	--	10.0	10.1	9.75	9.60

[a]Method I.

Table II-7. Long-term Stability Study of Furosemide[a] Urine Samples from Healthy Volunteer DH

Furosemide conc.[b] (μg/ml)	Furosemide conc.[c] (μg/ml)	% Difference[d]
9.18	9.50	+3.5
7.06	6.72	-4.8

[a]Method I.

[b]Urine samples were originally analyzed on 12/20/77.

[c]Urine samples were reanalyzed on 3/18/79.

[d]% Difference = 100 X (Furosemide conc.[c] - Furosemide conc.[b])/Furosemide conc.[b]

Table II-8. Specificity of β-Glucuronidase for Furosemide Metabolite

Sample	Peak Height Ratio[a]	Mean ± SD
Control	1.65	
	1.65	
	1.67	
	1.66	
		1.66 ± 0.01
Denatured Enzyme (β-Glucuronidase)	1.64	
	1.64	
	1.61	
	1.78	
		1.67 ± 0.08
Enzyme (β-Glucuronidase)	1.88	
	1.93	
	1.93	
	1.90	
		1.91 ± 0.02

[a]Measures unchanged furosemide, Method II.

Table II-9. Effect of β-Glucuronidase Concentration on Furosemide Metabolite

β-Glucuronidase Enzyme (units)	Peak Height Ratio[a] (PHR)	% Difference[b]
0	1.23	0.0
100	1.53	24.4
200	1.51	22.8
500	1.55	26.0
1,000	1.55	26.0
2,000	1.55	26.0
5,000	1.52	23.6
10,000	1.52	23.6

[a]Value represents the mean of duplicate samples analyzed for unchanged furosemide, Method II.

[b]% Difference = 100 X [PHR (enzyme) - PHR (no enzyme)]/PHR (no enzyme).

Over the enzyme range tested the results show little difference in the ability of β-glucuronidase to effect complete conversion of furosemide glucuronide to parent compound. Therefore, 2000 units of β-glucuronidase per 0.20 ml urine sample was arbitrarily chosen in those analyses where the glucuronide metabolite of furosemide was to be measured. Under these conditions, complete conversion of furosemide glucuronide to parent compound was found as demonstrated by the chromatograph in Fig. II-5. With fluorescence detection and a flow rate of 2 ml/min, furosemide glucuronide and furosemide had retention times of 5.5 and 11.0 minutes, respectively (Fig. II-5-LEFT), in a 28% acetonitrile - 0.03 M phosphoric acid solvent system. After enzyme incubation, the peak corresponding to furosemide glucuronide completely disappeared with a concomitant increase in the furosemide peak (Fig. II-5-RIGHT). A stability study of furosemide incubated in buffer at 37°C showed the drug to be completely stable for as long as 48 hours (Table II-10), indicating that the parent drug did not degrade during the enzyme hydrolysis procedure.

Recovery of furosemide from urine samples containing β-glucuronidase was assessed. Duplicate urine samples spiked with furosemide and sodium phenobarbital were run in parallel; one containing only buffer (control) and the other containing the enzyme. A comparison of peak height ratios (furosemide/sodium phenobarbital) during both treatments (control versus enzyme) are presented in Table II-11. Both standard curves were virtually identical over the furosemide concentration range studied, indicating complete recovery of furosemide from urine samples containing β-glucuronidase protein. Therefore, a single standard curve, without enzyme present, was utilized in measuring furosemide concentrations in urine, before and after β-glucuronidase treatment.

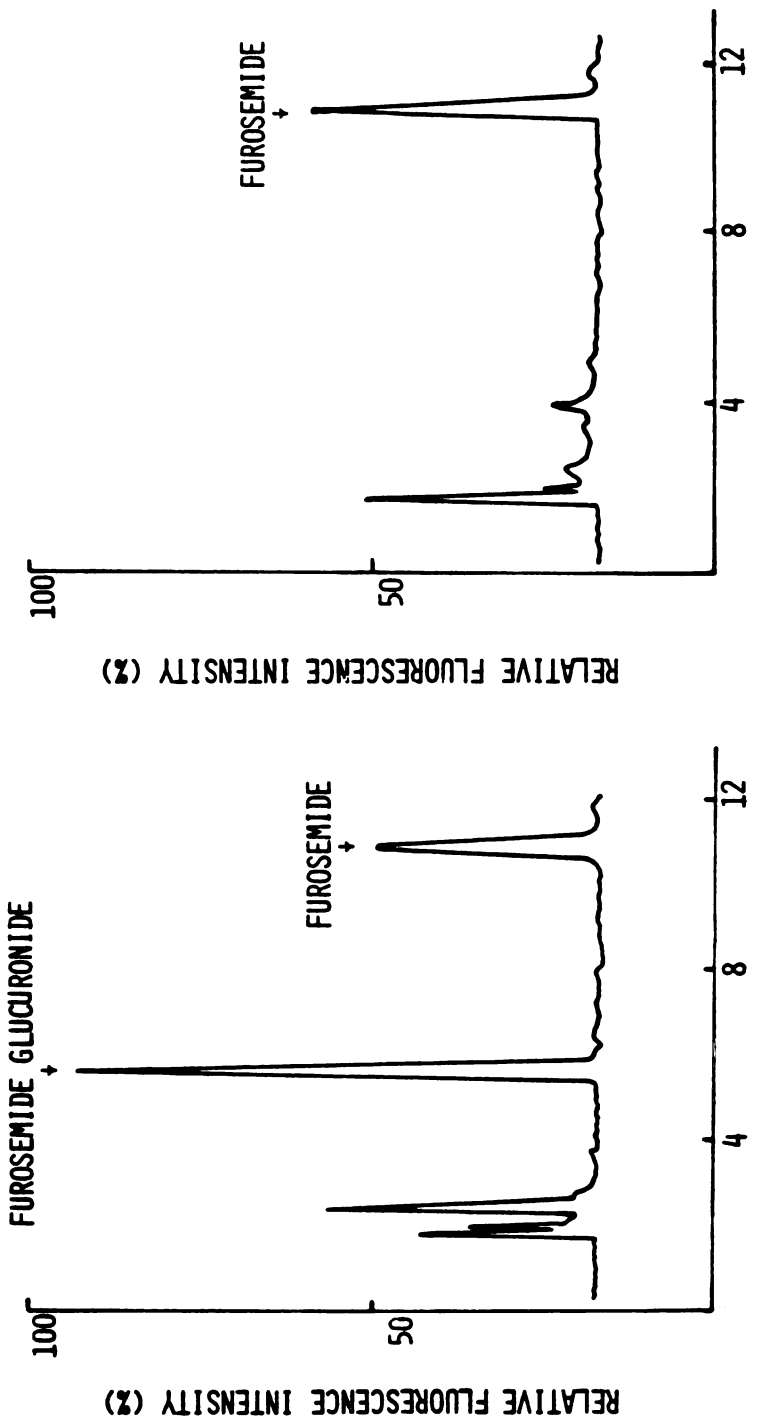

Fig. II-5. Chromatograms developed for urine sample containing furosemide and its glucuronide metabolite prior to enzyme treatment (left) and after enzyme treatment (right).

Table II-10. The Effect of Incubation on Furosemide Concentration[a]

Concentration (µg/ml)	Peak Height Ratio, Incubated /Frozen Sample (%) Time (hrs)					
	1	4	8	12	24	48
1	100	96.6	98.1	101	98.0	102
2	100	100	98.9	98.6	102	99.8

[a]Method I.

Table II-11. Recovery of Furosemide[a] in Urine Containing β-Glucuronidase

Concentration (μg/ml)	Peak Height Ratio (Buffer only)	Peak Height Ratio (Enzyme present)
0.00	0.000	0.000
0.50	0.090	0.093
2.50	0.396	0.409
5.00	0.900	0.882
10.0	1.76	1.79
15.0	2.61	2.63
25.0	4.39	4.45
Slope	0.176	0.178
Y-int	-0.005	-0.009
r^2	0.999	0.999

[a]Method I.

Figure II-6 shows a chromatogram for the analysis of CSA in urine. Under conditions previously described, CSA and the internal standard, o-nitrobenzoic acid had retention times of 16 and 14 minutes, respectively. A typical standard curve of CSA/o-nitrobenzoic acid peak height ratio over the concentration range 2.5 -84.0 μg/ml resulted in the following linear least squares regression equation: $Y = 0.052 - 0.041$; $r^2 = 0.999$ (Fig. II-7). It should be noted that in plasma, concentrations as low as 50 ng/ml can be detected for CSA.

Chromatograms for blank plasma and plasma spiked with probenecid and sodium phenobarbital are shown in Fig. II-8. Under conditions described previously, probenecid and the internal standard, sodium phenobarbital had retention times of 5.5 and 4.5 minutes, respectively. A typical standard curve of probenecid/sodium phenobarbital peak height ratio over the probenecid concentration range 35.0 - 280 μg/ml resulted in the following linear least squares regression equation: $Y= 0.0064X - 0.0166$; $r^2 = 0.999$ (Fig. II-9).

Chromatograms for blank plasma and plasma spiked with indomethacin and chlorpromazine hydrochloride are shown in Fig. II-10. Under conditions described previously, indomethacin and the internal standard, chlorpromazine hydrochloride had retention times of 5.5 and 4.0 minutes, respectively. A typical standard curve of indomethacin/chlorpromazine hydrochloride peak height ratio over the indomethacin concentration range 0.29 - 4.35 μg/ml resulted in the following linear least squares regression equation: $Y = 0.378X + 0.015$; $r^2 = 0.998$ (Fig. II-11).

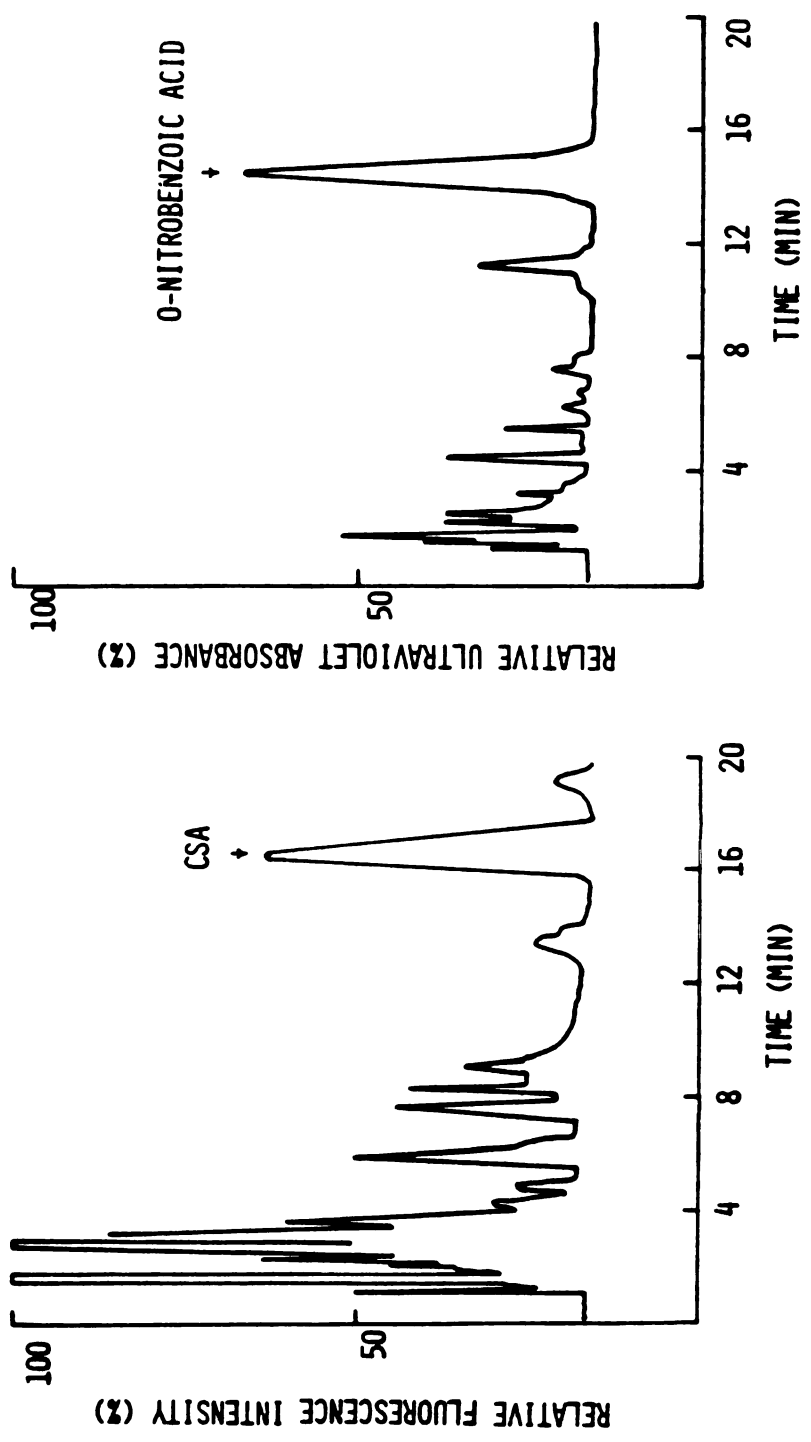

Fig. II-6. Chromatograms developed for urine sample spiked with CSA (left) and the internal standard, o-nitrobenzoic acid (right).

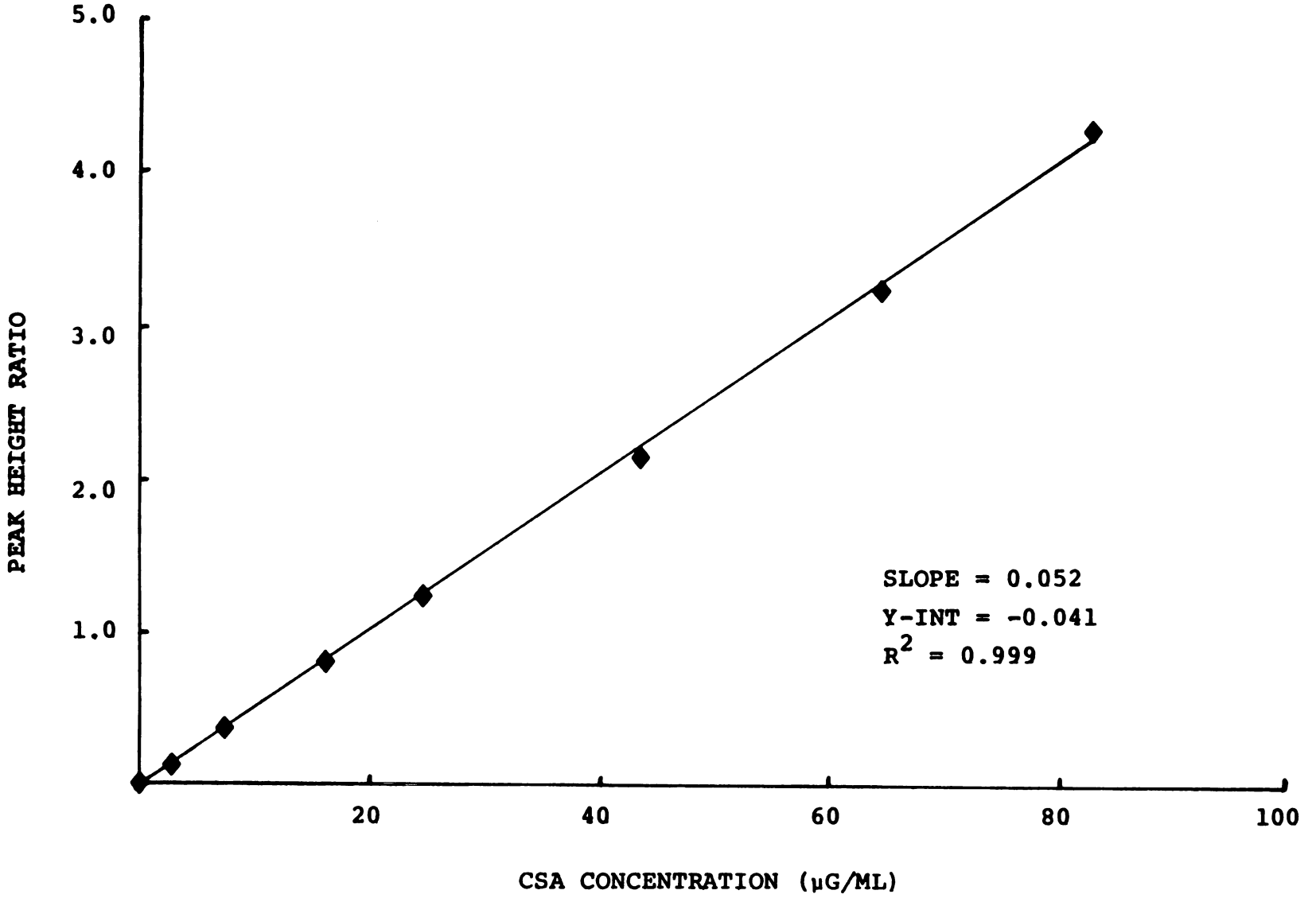

Fig. II-7. Standard curve relating the CSA/o-nitrobenzoic acid peak height ratio to the CSA urine concentration.

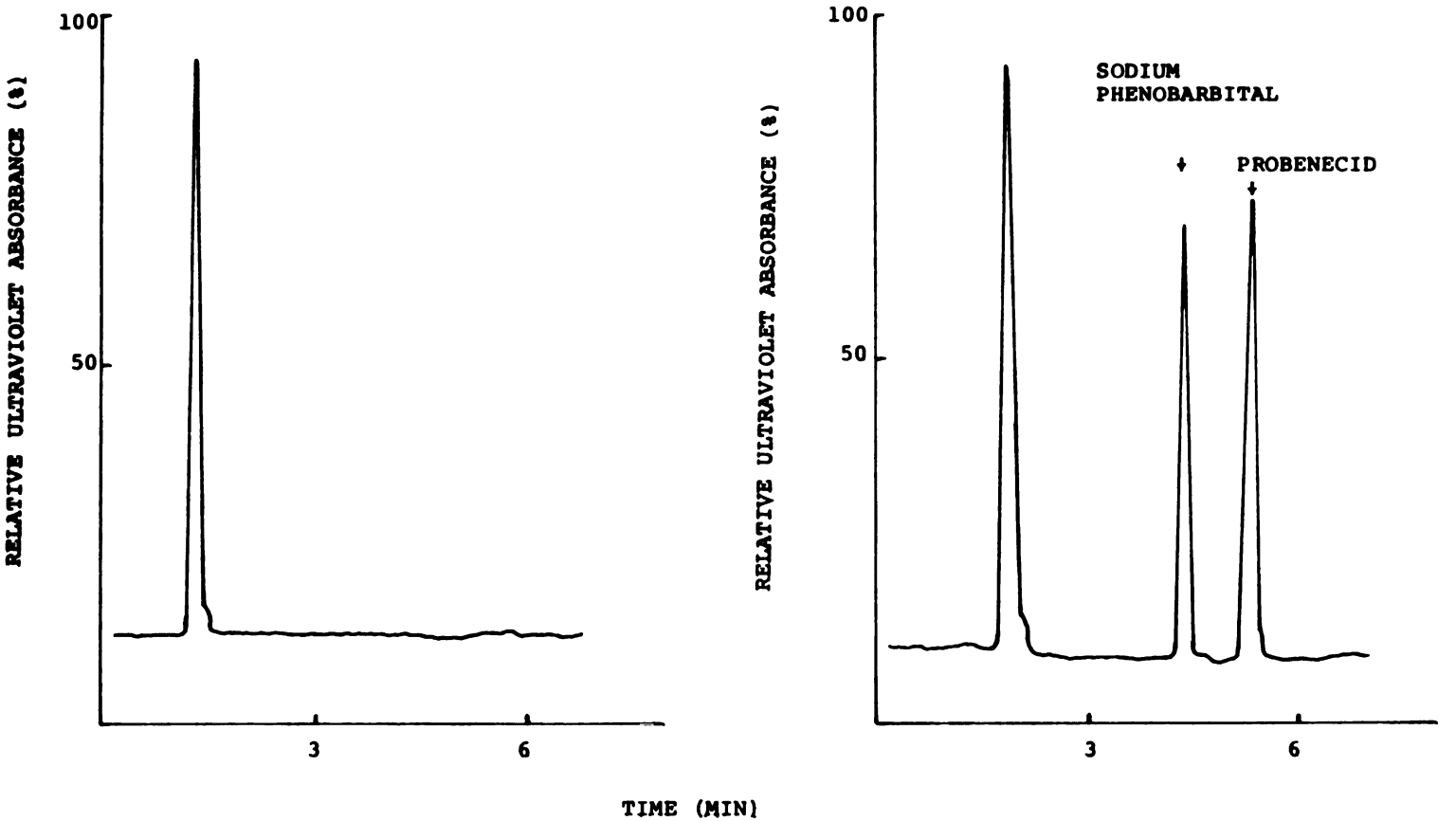

Fig. II-8. Chromatograms developed for blank plasma (left) and for plasma spiked with probenecid and the internal standard, sodium phenobarbital (right).

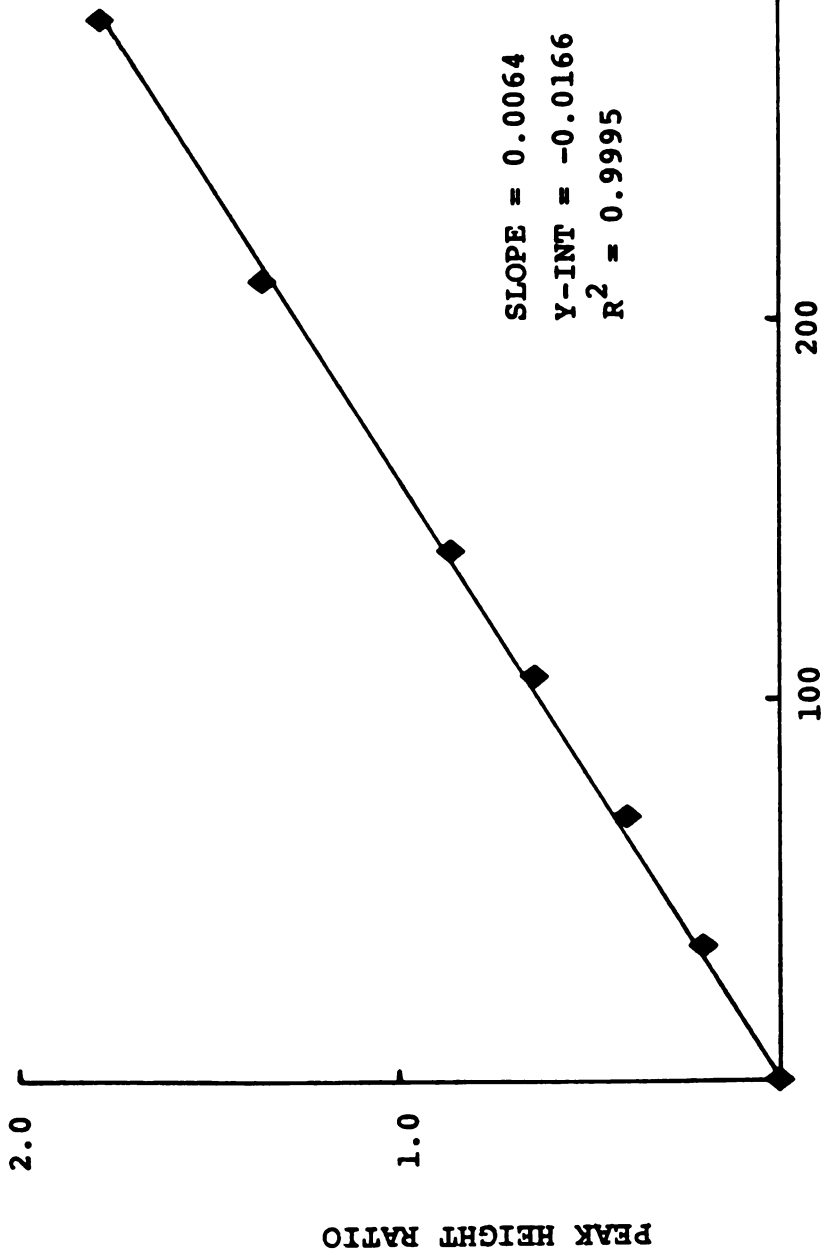

Fig. II-9. Standard curve relating the probenecid/sodium phenobarbital peak height ratio to the probenecid plasma concentration.

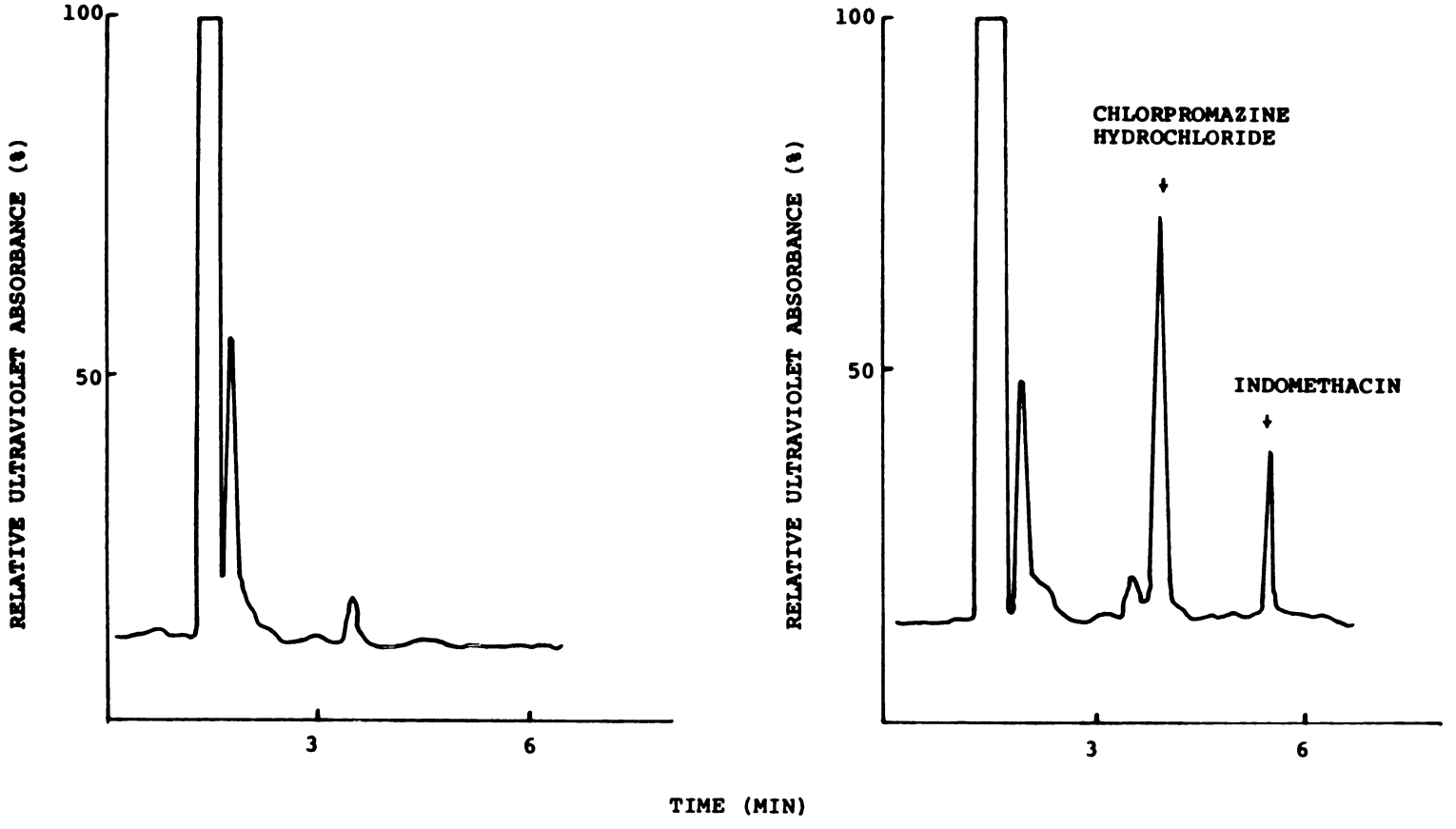

Fig. II-10. Chromatograms developed for blank plasma (left) and for plasma spiked with indomethacin and the internal standard, chlorpromazine hydrochloride (right).

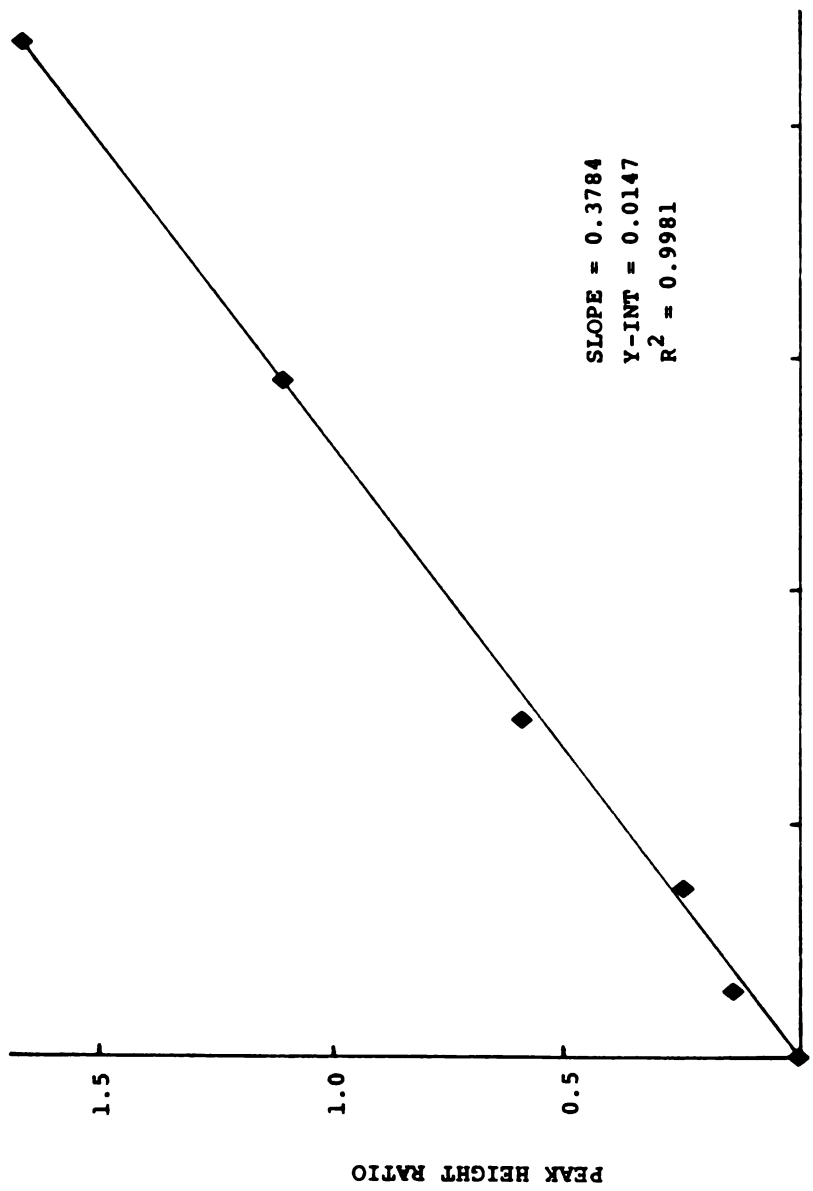

Fig. II-11. Standard curve relating the indomethacin/chlorpromazine hydrochloride peak height ratio to the indomethacin plasma concentration.

D. Summary

Two high performance liquid chromatographic methods have been developed for the analysis of furosemide in plasma and urine. Both methods are rapid, sensitive and specific. In addition, neither method requires prior extraction and/or derivatization. The only cleanup procedure involved is the precipitation of plasma proteins with acetonitrile. This results in reduced column pressures and band spreading as well as an overall increase in the life-time of the column. However, during the assay of urine furosemide samples following probenecid pretreatment (Chapter V), interfering peaks (possibly from probenecid metabolites) occurred in both the 280 nm and 254 nm detection channels. Therefore, it was necessary to develop a new assay system to separate the interfering peaks from those of furosemide and the internal standard, sodium phenobarbital. With fluorescence detection and a solvent system of 30% acetonitrile - 0.015 M phosphoric acid this was accomplished. The fluorescence assay was later refined to Method II and was used for the majority of the work presented in this thesis. The advantages of Method II as compared to Method I are two-fold. First, Method II is approximately 10-times more sensitive than Method I. And second, Method II is more specific with respect to clinical applications when other drugs are often coadministered.

Chapter III. Relationship Between Urinary Excretion Rate, Steady-State Plasma Levels and Diuretic Response of Furosemide in the Rat

A. Specific Objectives

Previous studies in animals have suggested that the luminal concentration/amount of furosemide in the renal tubule rather than the drug's plasma concentration may be the critical determinant with respect to natriuretic effect (10,108). Thus, any saturation process, drug interaction or disease state which prevents furosemide from reaching its site of action in the lumen could attenuate the drug's natriuretic and diuretic response. The present investigation was undertaken in order to satisfy two main objectives. The first was to determine if furosemide's active transport process could be saturated at therapeutic concentrations; the second was to define a relationship between furosemide in a measurable sampling compartment and its diuretic effect.

B. Experimental

1. Animal Model

The surgical preparation was described in detail by A.E. Till (109) in her Ph.D. dissertation on the renal excretion of pseudoephedrine in the rat. Briefly, male Sprague-Dawley rats[1], ranging in weight from

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[1]Charles River Breeding Laboratories, Inc., Wilmington, Mass.

248 to 313 gm, were anesthetized by intraperitoneal injection with sodium pentobarbital[2] (60 mg/kg). Supplemental injections were administered as needed. The femoral vein was cannulated with PE-50 polyethylene tubing[3] and continuously infused[4] with solution as subsequently detailed. The femoral artery was also cannulated with PE-50 tubing where 400 µl blood samples were collected. Urine samples were collected by cannulating the bladder with PE-90 polyethylene tubing[3] and a tracheotomy was performed when necessary using PE-205 polyethylene tubing.[3] Body temperature was maintained in the rat at 37 °C by connecting a rectal probe[5] to a temperature control unit[6] which was also connected to the heating element of the operating table. Mean blood pressure was monitored throughout the study with a mercury manometer. Upon completion of the experiments, the rats were sacrificed by injection of 0.5 ml euthanasia solution.[7] Figures III-1 and 2 provide a schematic representation of the animal preparation as well as a photograph of the entire experimental set-up, respectively.

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[2]Nembutal[R] sodium for veterinary use, 60 mg/ml, Abbott Laboratories, North Chicago, Ill.

[3]Intramedic[R] Non-Radiopaque Polyethylene Tubing, Clay Adams, Parsippany, NJ.

[4]Harvard Apparatus Compact Infusion Pump, Harvard Apparatus, Millis, Mass.

[5]YSI Model 402 Small Flexible Vinyl Rectal Probe, Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio

[6]YSI Model 73 ATD Indicating Controller, Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio

[7]Somlethol[R], sodium pentobarbital, 6 gr/ml, Med. Tech. Inc., Elwood, Kansas

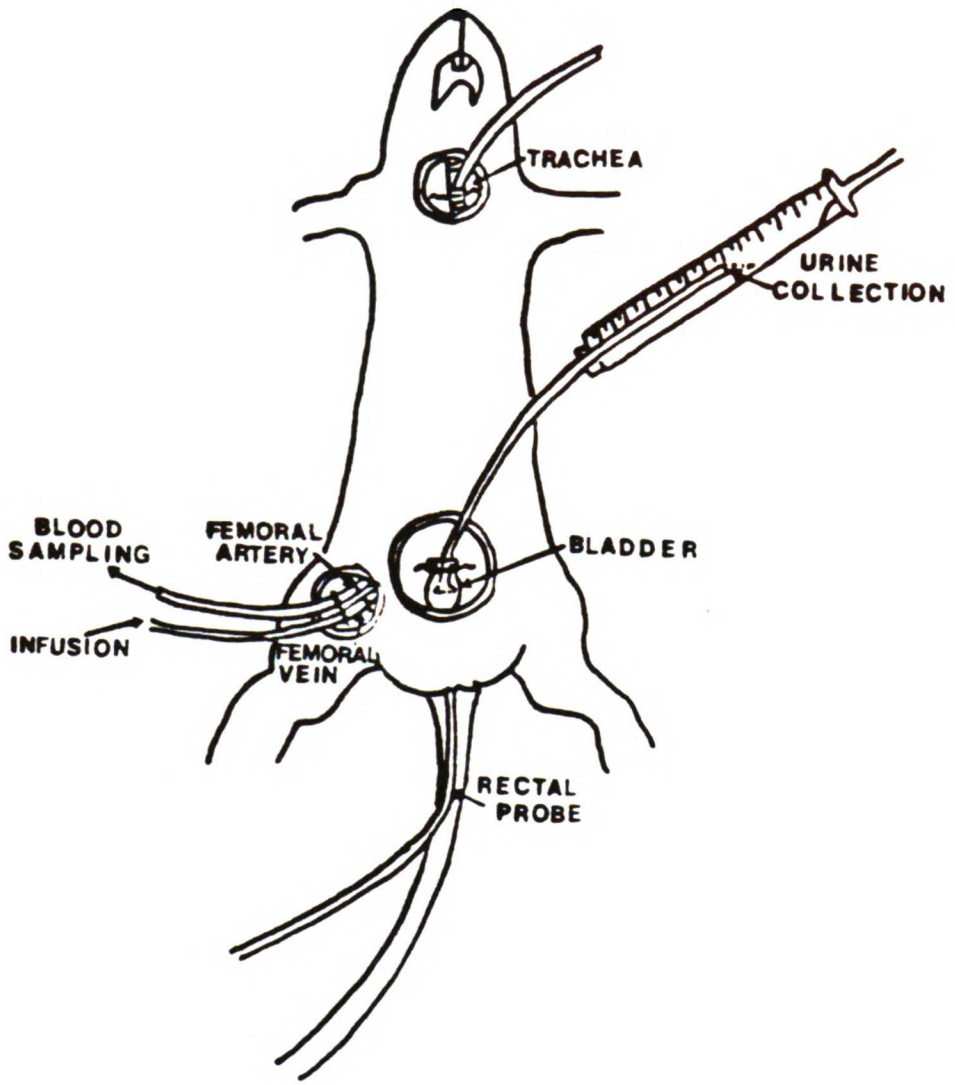

Fig. III-1. Schematic representation of rat preparation (109).

Fig. III-2. Photograph of the entire experimental set-up (109).

2. Methods

The infusion solution consisted of 4% inulin[8] in 0.9% NaCl with furosemide concentrations ranging from 25 to 350 μg/ml. To insure an adequate urine collection volume at low furosemide plasma concentrations (<3.0 μg/ml), 4% mannitol[9] was added to the infusion solution. Studies requiring mannitol were excluded in correlating furosemide plasma concentrations and urinary excretion rate with the diuretic response since mannitol itself is a diuretic. A loading dose of furosemide (0.5 - 1.5 mg/kg) was administered in order to reach steady-state levels more rapidly. Lower furosemide concentrations were infused at 60 μl/min and higher furosemide concentrations at 80 μl/min in a crude attempt to compensate for fluid loss.

After 90 minutes of infusion, samples were taken during four 20-minute clearance periods. Urine samples were collected at 0, 90-110, 110-130, 130-150, and 150-170 minutes. Blood samples (400 μl) were drawn at 0, 100, 120, 140, and 160 minutes; times which correspond to the midpoint of the urine collection intervals. Urinary pH[10,11] was determined immediately upon collection; 50 μl of urine were diluted 1,000-fold and refrigerated for subsequent inulin assay. Blood samples were centrifuged[12] immediately upon collection and the plasma frozen (as were undiluted urine samples) for subsequent analysis of furosemide concentrations.

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[8]Inulin from Dahlia Tubers, Sigma Chemical Company, St. Louis, MO.

[9]Mannitol N.F., Mallinckrodt Chemical Works, St. Louis, MO.

[10]Beckman Research pH Meter, Beckman Instruments, Inc., Fullerton, CA.

[11]Miramark Combination Electrode, Markson Science, Inc., Del Mar, CA.

[12]Beckman Spinco 152 Microfuge, Beckman Instruments, Inc., Fullerton, CA.

Plasma and urine samples of furosemide were analyzed as described under Method I (Chapter II).

The glomerular filtration rate (GFR) was determined by colorimetric assay (109) using inulin as a marker. Plasma samples (75 µl) were mixed with 1.5 ml distilled water and 0.75 ml of 9.3% trichloroacetic acid[13] to make a 31-fold dilution. The mixture was shaken on a vortex mixer and then centrifuged for 10 minutes. Urine samples were diluted 1,000-fold with distilled water at the time of collection as previously noted. A 0.20 ml aliquot of plasma supernatant, urine dilution, or standard solution (2-16 mg% inulin) was then mixed with 2.0 ml anthrone solution (0.2% anthrone[14] in 70% sulfuric acid[15]), and incubated for one hour at 37°C. The samples were cooled to room temperature and the absorbance[16] was read at 620 nm. Interference with the inulin assay by glucose and mannitol was found to be negligible or non-existent (109).

C. Calculations

The total renal clearance of furosemide was calculated by:

$$CLr = (\Delta Ae/\Delta t)/C_{pmid}$$

where $\Delta Ae/\Delta t$ is the urinary excretion rate of unchanged drug, and C_{pmid}

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[13]Trichloroacetic Acid Practical, Matheson Coleman and Bell, Norwood, Ohio

[14]Anthrone 'Baker Analyzed' Reagent, J.T. Baker Chemical Co., Phillipsburg, NJ.

[15]Sulfuric Acid, Analytical Reagent, Mallinckrodt Inc., Paris, KY.

[16]Beckman DB Spectrophotometer, Beckman Instruments, Inc., Fullerton, CA.

is the plasma concentration at the midpoint of the urine collection period. Using inulin as a marker, the GFR was determined in a manner analogous to that for total renal clearance. Each renal clearance, steady-state plasma concentration and urinary excretion rate of furosemide, as well as the GFR, urine flow rate and urinary pH reported in this work, represent the mean values of the four 20-minute clearance periods (Table III-1).

D. Results

Twenty-eight rats were infused to steady-state plasma furosemide levels over the therapeutic concentration range 0.8 - 25.1 μg/ml. The total renal clearance (corrected for kidney function as measured by inulin clearance) showed a negative correlation with plasma concentration ($r = -0.655$, $p < 0.001$) and differed by a factor of 2 over the given plasma concentration range (Fig. III-3). This result would be tenuous if the change in the clearance ratio were due to an increase in GFR only. The results in Fig. III-4 indicate the opposite conclusion with GFR showing a weak negative correlation with plasma concentration ($r = -0.374$, $p = 0.05$). Brennan *et al.* (110) reported that furosemide produced a transient drop in the GFR of rats, but the authors note that this drop was not statistically significant. In Figs. III-5 and 6, an attempt was made to correlate the diuretic response in the rat, as measured by urine flow rate, with steady-state plasma levels and the urinary excretion rate of furosemide, respectively. Steady-state plasma levels (Fig. III-5) showed a poor correlation with the urine flow rate ($r = 0.377$, $p > 0.10$). On the other hand, a good correlation was found between urine flow rate and the urinary excretion rate of furosemide; $r = 0.777$, $p < 0.001$ (Fig. III-6). It should be noted that the renal clearance of furosemide was found to be independent of urine flow rate

Table III-1. Renal Clearance Parameters Obtained Following I.V. Infusion of Furosemide in 28 Rats

Rat	Weight (kg)	Urine Flow (ml/min)	$\Delta Ae/\Delta t$ (μg/min)	C_{pmid} (μg/ml)	CLr (ml/min·kg)	Urinary pH	GFR (ml/min·kg)	$\frac{CLr}{GFR}$
1	0.270	0.0612	6.76	15.2	1.67	5.80	6.28	0.263
2	0.253	0.0556	5.69	16.0	1.41	5.21	5.85	0.249
3	0.248	0.0287	2.72	18.8	0.637	4.98	3.72	0.157
4	0.290	0.0585	4.44	15.7	0.985	4.94	4.38	0.236
5	0.255	0.0530	5.54	9.90	2.22	5.30	6.36	0.355
6	0.250	0.0640	5.92	11.8	2.02	5.61	7.59	0.268
7	0.277	0.0586	6.76	9.13	2.67	5.22	7.83	0.343
8	0.276	0.102	6.76	10.9	2.26	5.35	6.98	0.339
9	0.266	0.0537	4.67	11.5	1.56	4.58	5.68	0.267
10	0.264	0.0618	5.28	12.2	1.63	5.14	5.98	0.275
11[a]	0.313	0.0809	0.550	0.783	2.26	5.03	7.82	0.291
12[a]	0.304	0.0530	0.435	0.798	1.88	5.66	4.86	0.377
13[a]	0.304	0.0612	0.995	1.04	3.14	5.15	7.08	0.442
14[a]	0.296	0.0588	0.878	1.18	2.51	5.56	6.18	0.411
15[a]	0.299	0.0915	1.69	2.92	1.96	4.94	6.73	0.291

Table III-1 (Continued)

Rat	Weight (kg)	Urine Flow (ml/min)	$\Delta Ae/\Delta t$ (μg/min)	C_{pmid} (μg/ml)	CLr (ml/min·kg)	Urinary pH	GFR (ml/min·kg)	$\frac{CLr}{GFR}$
16[a]	0.298	0.103	2.12	2.21	3.08	5.38	8.46	0.383
17	0.294	0.0956	7.77	11.5	2.30	5.18	7.16	0.325
18	0.256	0.105	8.05	12.3	2.58	5.19	7.44	0.358
19	0.291	0.0800	6.54	19.4	1.16	4.89	4.97	0.235
20	0.278	0.0592	8.02	16.4	1.76	5.50	5.48	0.328
21[b]	0.296	0.0452	1.99	3.01	2.30	5.51	6.00	0.458
22[b]	0.252	0.0320	1.94	6.79	1.14	5.38	6.28	0.183
23[b]	0.269	0.0612	2.76	4.78	2.17	5.32	8.61	0.254
24[b]	0.267	0.0502	2.26	5.77	1.58	5.35	5.50	0.289
25[b]	0.264	0.0340	3.18	5.00	2.36	5.44	5.46	0.443
26[b]	0.256	0.0496	5.02	7.96	2.53	5.12	7.62	0.309
27	0.273	0.0884	8.70	15.3	2.08	5.57	6.77	0.308
28	0.280	0.0826	7.06	25.1	1.00	4.90	6.32	0.163

[a]The infusion solution contained 4% mannitol in addition to the 4% inulin.

[b]Solution was infused at 60 μl/min. All other rats were infused at 80 μl/min.

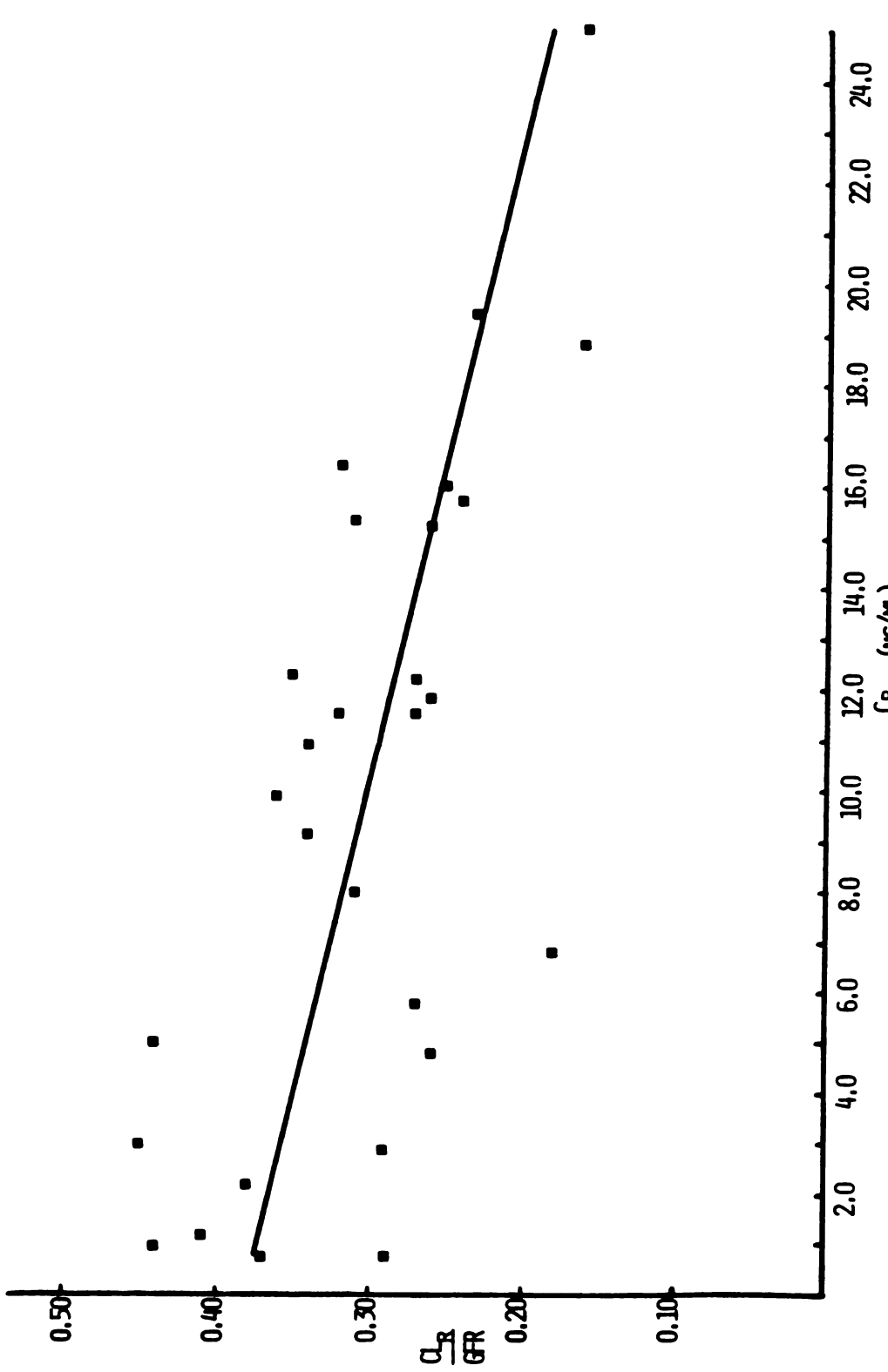

Fig. III-3. Correlation between the total renal clearance (corrected for kidney function) and the steady-state plasma concentrations of furosemide ($r = -0.655$, $p < 0.001$).

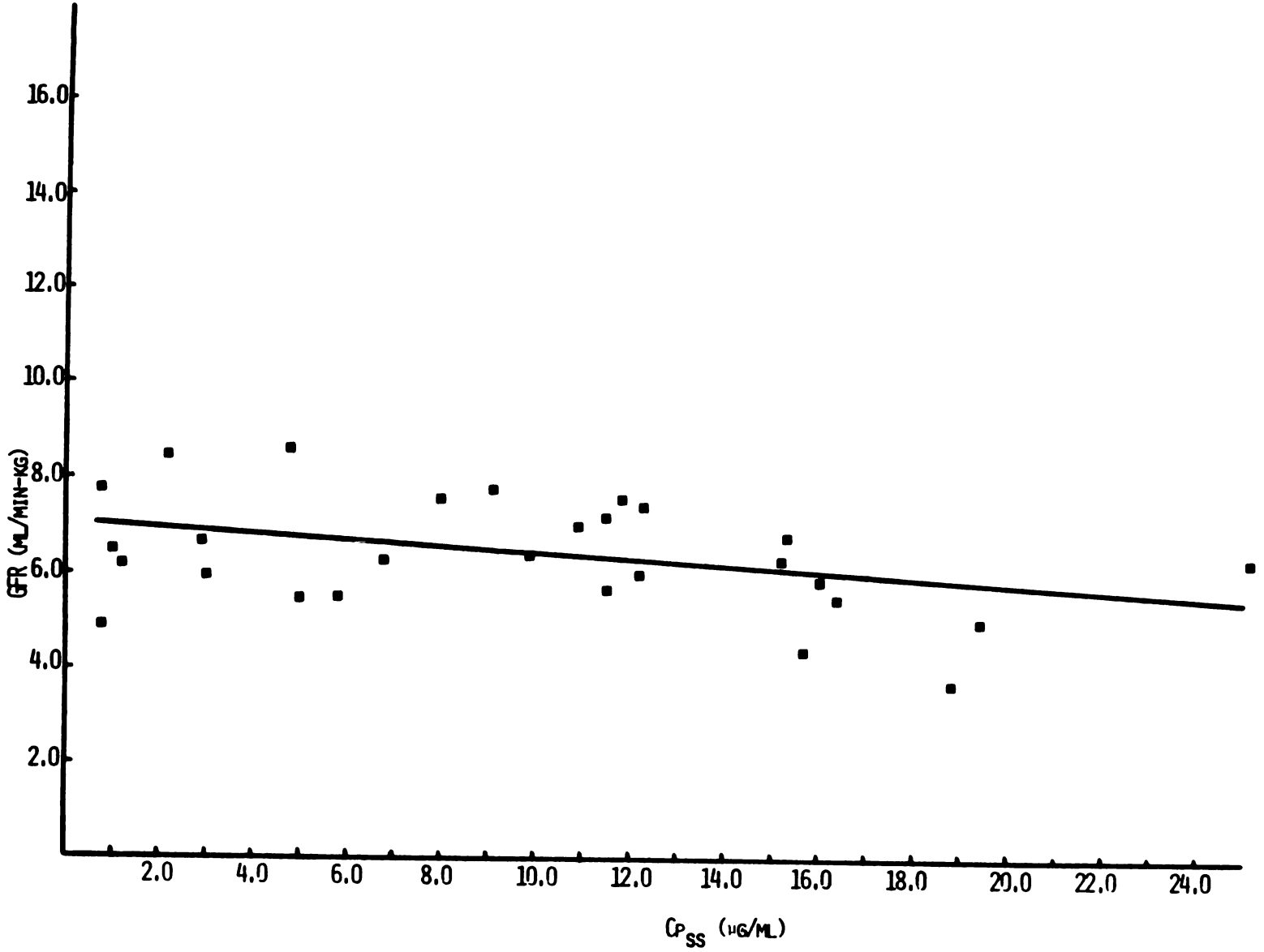

Fig. III-4. Correlation between the glomerular filtration rate and the steady-state plasma concentrations of furosemide ($r = -0.374$, $p = 0.05$).

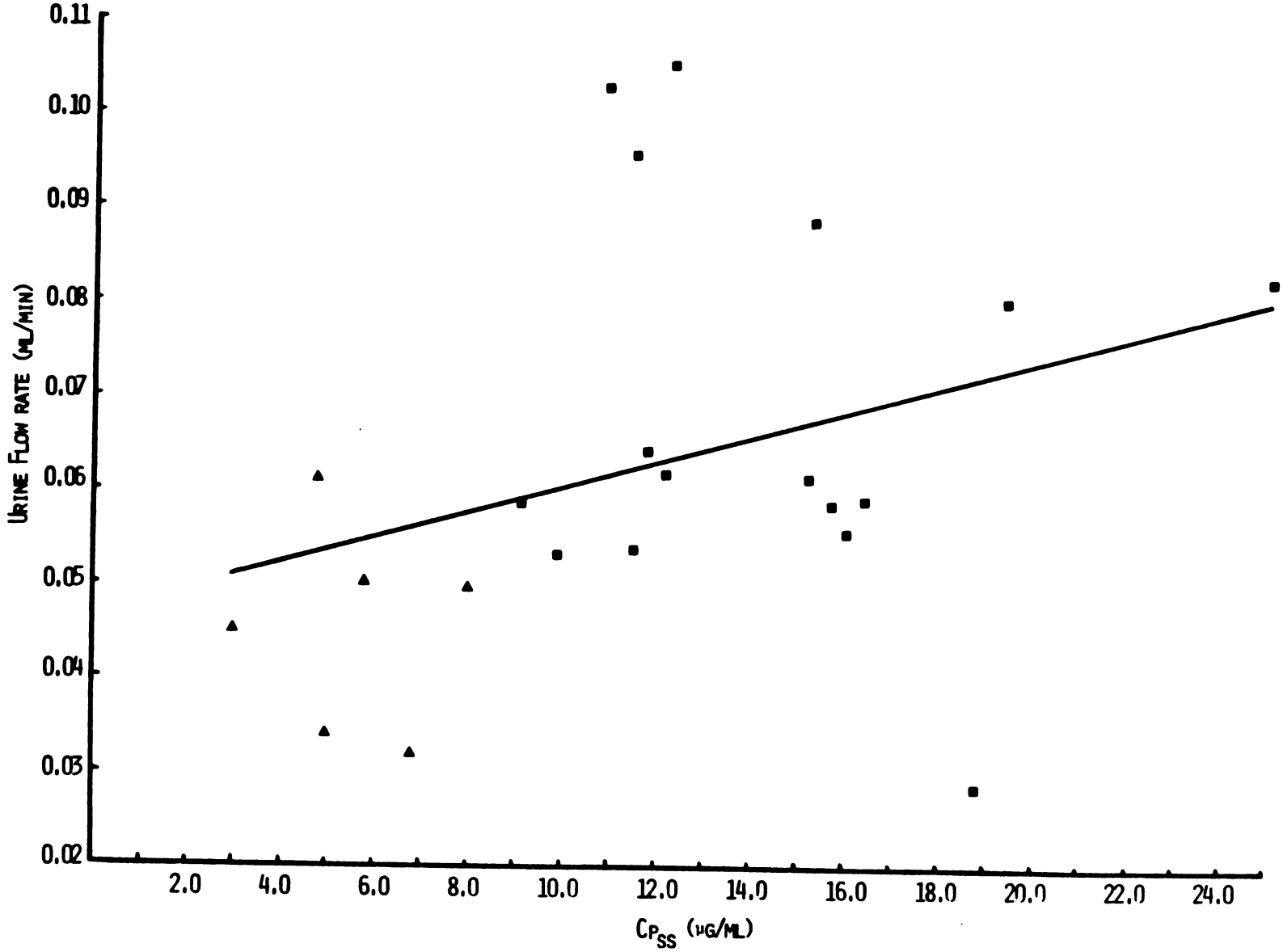

Fig. III-5. Correlation between the urine flow rate and the steady-state plasma concentrations of furosemide ($r = 0.377$, $p > 0.10$). ▲ infusion rate of 60 µl/min ■ infusion rate of 80 µl/min

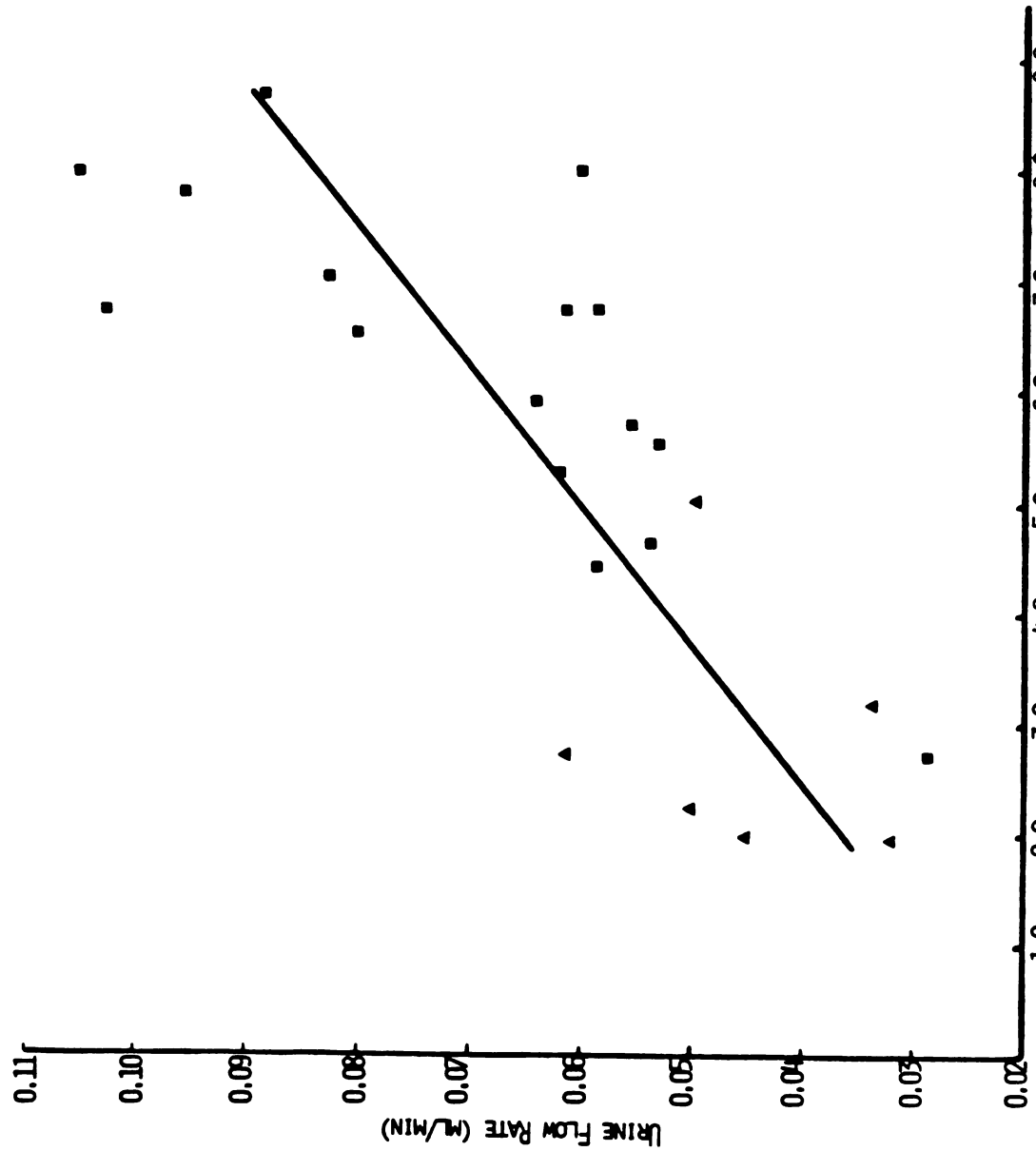

Fig. III-6. Correlation between the urine flow rate and the urinary excretion rate of furosemide ($r = 0.777$, $p < 0.001$). ▲ infusion rate of 60 µl/min ■ infusion rate of 80 µl/min

($r = 0.343$, $p > 0.05$) and urinary pH ($r = 0.314$, $p > 0.10$) in the rat. This indicates that passive reabsorption of furosemide in the renal tubule constitutes a minor or negligible component of its renal clearance.

E. Discussion

Previous studies have been conflicting and insufficient in establishing a relationship between the diuretic effect of furosemide and its concentration/amount in a measurable sampling compartment (97). This may be primarily due to nonspecific assay techniques and because previous attention has focused on relating serum concentrations to the diuretic response rather than considering other drug compartments, such as urine, which may better reflect furosemide at its site of action.

Furosemide has an oral availability in healthy volunteers of 50-65% (Table I-1). If a relationship exists between plasma concentrations of furosemide and diuretic response, then one would expect an intravenous dose to exert a greater diuretic response than that of an equivalent oral dose. Kelly et al. (69) observed no such difference in the diuretic effect of healthy subjects following 80-mg single doses of furosemide given both orally and intravenously. Branch et al. (71) as well as Kelly et al. (74) also observed an equivalent diuretic response in healthy subjects and 'diuretic-resistant' patients, respectively, when the same dose of furosemide was taken by oral and intravenous administration. However, in uremic patients, Huang et al. (73) found that the oral dose of furosemide was always less effective than the same intravenous dose. Although there was considerable variation in the diuretic response of their uremic patients, the authors note that the magnitude of the response after the intravenous dose of furosemide did not correlate with either the peak or mean plasma

furosemide concentration. In addition, no good correlation was found between the relative effectiveness of oral therapy with either the rate or completeness of furosemide absorption.

The above discussion points out some of the discrepancies found when comparisons are made between blood level and diuretic response following single doses given both orally and intravenously. Results in this study have confirmed that the diuretic effect of furosemide is directly related to its urinary excretion rate and not to its plasma concentration (Figs. III-5 and 6). This observation is consistent with *in vitro* studies indicating that furosemide exerts its effect on the luminal, rather than the basal side of the kidney tubule (6).

This study also shows that furosemide exhibits capacity limited elimination at higher plasma concentrations (as evidenced by a reduced renal clearance), and that this saturable process occurs in the rat at a level comparable to the therapeutic concentration range in humans. Plasma concentrations may correlate with diuretic response of furosemide only when they parallel what is happening in the urine. Thus any change in plasma levels of furosemide should reflect a similar change in both urinary excretion rate and diuretic response. This assumes that the renal clearance has remained constant and that there exists a direct and linear relationship between the excretion rate of furosemide and diuretic response. This delicate balance between plasma and urine levels of furosemide no longer holds when the active transport mechanism for the drug reaches the concentrations where capacity limited kinetics are involved, thereby changing the renal clearance of the drug. Thus, changes in the dose or plasma concentrations of furosemide may not show proportionate changes in excretion rate of the drug and, therefore, diuretic response. Although speculative, it is possible that this dose-dependent phenomenon may in part

be responsible for previous discrepancies relating plasma levels as well as dose to the diuretic response. This presumes that capacity limited renal excretion may occur in humans at therapeutic concentrations.

Chapter IV. Absorption and Disposition of Furosemide in Healthy Volunteers

A. Specific Objectives

The data concerning the metabolism of furosemide are sparse and controversial. As previously noted, these discrepancies probably reflect problems with the different assay procedures. By using a rapid, sensitive and specific HPLC assay, without prior extraction and/or derivatization, an attempt was made to clarify the metabolic fate of furosemide. Specifically, the objectives of this study are two-fold: 1) to compare the metabolism of furosemide after intravenous and oral administration; and 2) to compare the pharmacokinetics and bioavailability of furosemide as a function of route of administration.

B. Methods

1. Subject Selection

Nine male volunteers, aged 21-40, and weighing 70-130 kg participated as outpatients in our study. Each subject had a normal medical history, physical examination and standard laboratory tests, including a creatinine clearance (CLcr) determination. Informed consent was obtained from each subject prior to participation in the study.

2. Study Design

After fasting overnight (at least 10 hours), volunteers drank 250 ml

of water and voided prior to drug administration. The sequence of studies was randomized and at 8 a.m., each volunteer received either 80 mg of furosemide[1] (2 tabs x 40 mg/tab, lot #602498) by mouth with 250 ml of water, or 40 mg of furosemide[1] (10 mg/ml, lot #618222) intravenously infused[2] over a three minute period (time zero being considered the midpoint of the infusion) together with 250 ml of water given orally. Blood samples (5 ml) were obtained by an indwelling heparinized scalp vein needle[3] at 0, 10, 20, 30, 45, 60, 80, 100, 120, 150, 180 minutes and 4, 6, 8, 12 and 24 hours. Plasma for drug analysis was separated from red blood cells within one hour of collection and immediately frozen. Voided urine was collected at -1 to 0, 0 to 0.5, 0.5 to 1.0, 1.0 to 1.5, 1.5 to 2.0, 2.0 to 3.0, 3.0 to 4.0, 4.0 to 6.0, 6.0 to 8.0, 8.0 to 12.0, 12.0 to 24.0, and 24.0 to 48.0 hours. Additional urine collections from 48.0 to 72.0 hours were obtained from volunteers 1, 4, and 5. To avoid dehydration and electrolyte depletion, subjects drank juices or flavored Lactated Ringer's Injection or were given Lactated Ringer's Injection by vein in an amount approximately equal to the urine volume produced. Four hours after dosing, volunteers were given a clear liquid standard hospital diet and were allowed to eat solid food eight hours after the dose. No fluids or food were permitted for the first four hours of the study with the exception of water and balanced salt solution replacement as already described.

During the entire study, volunteers were not permitted any medications other than furosemide, and were to refrain from drinking alcohol. For

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[1]Hoechst-Roussel Pharmaceuticals, Inc., Somerville, NJ.

[2]Harvard Apparatus Compact Infusion Pump, Harvard Apparatus, Millis, Mass.

[3]E-Z Set^{R} - PRN Intermittent Infusion Set, Deseret Pharmaceutical Co., Inc., Sandy, Utah

the three days prior to the administered dose of furosemide, volunteers were maintained on a controlled daily diet of 150 meq of sodium, 80 meq of potassium, and at least 2000 ml of fluid. An interval of at least one week elapsed between studies and identical lot numbers were used throughout. Smoking was not permitted on collection days.

3. Assay Procedures

Furosemide concentrations in plasma and urine samples (with and without β-glucuronidase treatment) were measured as described for Method II (Chapter II). CSA concentrations in plasma and urine samples were also measured as outlined in Chapter II.

4. Calculations

The half-life of furosemide, Terminal $T_{\frac{1}{2}}$, was determined by linear regression using at least four data points from the terminal portion of the intravenous plasma versus time plots. The Terminal $T_{\frac{1}{2}}$ from the oral data was not calculated since long lag times for absorption and possible biliary recycling in some volunteers made estimates difficult. The area under the plasma concentration time curve, AUC, was calculated using the trapezoidal rule, extrapolated to infinity from the last measured concentration. The extrapolated AUC for the oral data was estimated using the Terminal $T_{\frac{1}{2}}$ obtained from intravenous administration. The potential error in this estimation is quite small since the extrapolated AUCs represent only 1.4 - 8.0% of the total AUCs (mean ± SD = 3.8 ± 2.4%).

The absolute bioavailability, F, was calculated using both plasma

(F_p) and urine (F_u) data:

$$F_p = AUC_{oral}\ DOSE_{iv} / AUC_{iv}\ DOSE_{oral}$$

$$F_u = Ae^{\infty}_{oral}\ DOSE_{iv} / Ae^{\infty}_{iv}\ DOSE_{oral}$$

where the amount of unchanged drug recovered in the urine at time infinity is represented by Ae^{∞}. In this study, the reported F represents the averaged availability of F_p and F_u.

The volume of distribution steady-state, Vdss, was determined from the intravenous plasma data by the compartment independent method of Benet and Galeazzi (111):

$$Vdss = DOSE\ (AUMC) / (AUC)^2$$

where AUMC is the area under the curve of the first moment of the concentration time curve, i.e $\int_0^{\infty} tC_p dt$. Total plasma clearance of intravenously administered furosemide, CLp was calculated as:

$$CLp = DOSE/AUC$$

Total renal clearance, CLr, was estimated following intravenous and oral dosing by:

$$CLr = Ae^{\infty}/AUC$$

The fraction of the intravenous dose of furosemide excreted unchanged in the urine, fe, was calculated as:

$$fe = Ae^{\infty}/DOSE$$

Non-renal plasma clearance, CLnr, was calculated as the difference between the plasma and renal clearances. The percent of the available dose of furosemide excreted as the glucuronide metabolite; fGL, was estimated by:

$$fGL = 100X[(Ae^{\infty})enzyme - (Ae^{\infty}) \text{ no enzyme}]/F \cdot Dose$$

where the amount of unchanged drug recovered in the urine at time infinity after treatment with β-glucuronidase is represented by (Ae^{∞}) enzyme. The amount of unchanged drug recovered in the urine after treatment with buffer is represented by (Ae^{∞}) no enzyme. F was assumed to be equal to one for all intravenous doses.

Data throughout the study are expressed as the mean ± standard deviation. Statistical differences were determined using a paired t-test.

C. Results

The pharmacokinetics of oral and IV administered furosemide are presented in Table IV-1. The volume of distribution steady-state was 109 ± 19 ml/kg and the half-life was 92 ± 7 min. The total plasma clearance was 164 ± 26 ml/min, the non-renal plasma clearance was 54.5 ± 9.6 ml/min, and the fraction of furosemide excreted unchanged in the urine was 0.662 ± 0.068. In addition, the plasma renal clearance (111 ± 17 ml/min for oral, 110 ± 24 ml/min for IV; $p > 0.50$) was consistent between the two routes of administration. It should be noted that the incremental renal clearances of furosemide were constant throughout each study for all the volunteers. There was also no significant difference between (AUC)oral and (AUC)iv ($p > 0.05$) indicating that the extent of absorption was approximately 50%

Table IV-1. Pharmacokinetics of Oral and IV Furosemide

SUBJECT	Wt.	CLcr (ml/min)	TREATMENT	AUC ($\frac{\mu g \cdot min}{ml}$)	CLp ($\frac{ml}{min}$)	Vdss (ml/min)	Terminal $T_{\frac{1}{2}}$ (min)	CLr ($\frac{ml}{min}$)	CLnr ($\frac{ml}{min}$)	fe
1	93	85.2	80 mg po	209	--	--	--	125	--	--
			40 mg iv	211	190	99.1	92	123	67.0	0.650
2	130	131	80 mg po	164	--	--	--	110	--	--
			40 mg iv	215	186	88.5	103	115	71.0	0.618
3	80	164	80 mg po	191	--	--	--	83.8	--	--
			40 mg iv	351	114	73.5	98	60.7	53.3	0.532
4	80	92.1	80 mg po	207	--	--	--	143	--	--
			40 mg iv	254	157	117	86	113	44.0	0.715
5	71	71.0	80 mg po	273	--	--	--	98.2	--	--
			40 mg iv	289	138	117	83	88.9	49.1	0.642
6	72	85.9	80 mg po	149	--	--	--	108	--	--
			40 mg iv	225	178	127	85	130	48.0	0.732
7	70	106	80 mg po	296	--	--	--	105	--	--
			40 mg iv	253	158	120	91	97.6	60.4	0.618
8	77	83.8	80 mg po	220	--	--	--	117	--	--
			40 mg iv	210	190	130	100	139	51.0	0.728
9	79	112	80 mg po	182	--	--	--	105	--	--
			40 mg iv	239	167	106	87	121	46.0	0.722
MEAN	84	103	80 mg po	210	--	--	--	111	--	--
			40 mg iv	250	164	109	92	110	54.4	0.662
SD	19	29	80 mg po	48	--	--	--	17	--	--
			40 mg iv	46	26	19	7	24	9.6	0.068

since the oral dose was twice that of the intravenous dose.

Furosemide tablets and intravenous solution were assayed as well for purity. Mean values ± SD for the tablets (40 mg) and the solution (20 mg) were 40.2 ± 0.7 and 19.9 ± 0.7, respectively.

Table IV-2 describes the urinary excretion of furosemide and its glucuronide metabolite after oral and intravenous administration, as well as its bioavailability. During the first 24 hours, approximately 95% of the amount of furosemide excreted unchanged in the urine was recovered after oral dosing and over 99% after intravenous administration. The total amount recovered after 72 hours (considered time infinity) was not significantly different between the two treatments ($p > 0.10$). There was also no difference between availabilities determined with either plasma or urine data (0.428 ± 0.099 for F_p, 0.440 ± 0.113 for F_u; $p > 0.50$). The glucuronide metabolite of furosemide accounted for approximately 14% of the absorbed dose following both oral and intravenous administration.

The proposed metabolite of furosemide, CSA was sought in plasma and urine samples for all nine volunteers after both oral and i.v. treatments. No evidence of this metabolite was found in any of the samples.

D. Discussion

The data available concerning the metabolism of furosemide are sparse and controversial. Häussler and Hajdú (89), using paper chromatography with spectrofluorimetric detection of urine samples, reported that CSA was the only metabolite of furosemide in humans and dogs. Häussler and Wicha (90) and Rupp (85) corroborate the existence of this metabolite in humans but do not give any information about concentrations or amounts. Andreasen *et al.* (8), using thin-layer chromatography followed by fluorimetric

Table IV-2. Urinary Excretion, Bioavailability and Metabolism of Oral and IV Furosemide

SUBJECT	TREATMENT	Ae 0-24 (mg)	Ae 24-48 (mg)	Ae 48-72 (mg)	Ae^{∞} (mg)	F_p	F_u	F	fGL
1	80 mg po	25.7	0.5	0.0	26.2				0.175
	40 mg iv	26.0	0.0	0.0	26.0	0.495	0.504	0.500	0.132
2	80 mg po	17.6	0.4	--	18.0				0.154
	40 mg iv	24.7	0.0	--	24.7	0.381	0.364	0.372	0.190
3	80 mg po	14.4	1.6	--	16.0				0.197
	40 mg iv	21.3	0.0	--	21.3	0.272	0.376	0.324	0.123
4	80 mg po	27.1	1.3	1.3	29.7				0.184
	40 mg iv	28.6	0.0	0.0	28.6	0.407	0.519	0.463	0.155
5	80 mg po	24.5	1.2	1.1	26.8				0.116
	40 mg iv	25.2	0.5	0.0	25.7	0.472	0.521	0.496	0.105
6	80 mg po	13.9	2.2	--	16.1				0.120
	40 mg iv	29.3	0.0	--	29.3	0.331	0.275	0.303	0.158
7	80 mg po	31.1	0.0	--	31.1				0.136
	40 mg iv	24.7	0.0	--	24.7	0.585	0.630	0.608	0.122
8	80 mg po	25.0	0.8	--	25.8				0.127
	40 mg iv	29.1	0.0	--	29.1	0.524	0.443	0.484	0.134
9	80 mg po	18.5	0.6	--	19.1				0.126
	40 mg iv	28.9	0.0	--	28.9	0.381	0.330	0.356	0.118
Mean	80 mg po	22.0	1.0	0.8	23.2				0.148
	40 mg iv	26.4	0.06	0.0	26.5	0.428	0.440	0.434	0.137

Table IV-2 (Continued)

SUBJECT	TREATMENT	Ae 0-24 (mg)	Ae 24-48 (mg)	Ae 48-72 (mg)	Ae^{∞} (mg)	F_p	F_u	F	fGL
SD	80 mg po	6.0	0.7	0.7	5.9				0.030
	40 mg iv	2.7	0.2	0.0	2.7	0.099	0.113	0.101	0.026
*Level of significance		NS (p>0.50)	S (p<0.005)	NS (p>0.10)	NS (p>0.10)	NS (p>0.50)		NA	NS (p>0.20)

*S = significant

NS = not significant

NA = not applicable

detection, were able to simultaneously determine the serum concentrations of furosemide, CSA, and anthranilic acid. In both their normal subjects and anephric patients, CSA as well as anthranilic acid were detected in 1, 3, and 7 hour serum samples. Recently, Perez et al. (86), using gas-liquid chromatography with prior acid extraction reported that CSA accounted for 0.13 - 3.92% of the dose in patients with acute pulmonary edema. Control samples of serum or urine spiked with furosemide were not analyzed by Andreasen et al. (8) or Perez et al. (86), to determine whether the supposed metabolite CSA had developed as a consequence of the analytical procedure. A recent in vitro study by Cruz et al. (112) demonstrated the acid labile hydrolysis of furosemide to CSA.

It was therefore decided to run a control study with a urine sample known to contain about 20 μg/ml of furosemide with no CSA present as determined by our assay (Fig. IV-1-LEFT). Using the acid extraction procedure on this urine sample as outlined by Perez et al. (86), it was now possible to detect a CSA peak indicating the putative metabolite to be an analytical artifact (Fig. IV-1-RIGHT). Identical results were obtained by treating furosemide stock solution with acid extraction as well. The results of this study are in agreement with those of Calesnick et al. (76), Kindt and Schmid (91), and Beermann et al. (70). Using a direct injection assay method, CSA was not detected in any of the samples.

Kindt and Schmid (91) as well as Beermann et al. (70) have reported the possibility of a glucuronide conjugate of furosemide. However, their results are somewhat vague and poorly quantitated. Andreasen and Mikkelsen (77) analyzed urine samples for furosemide and furosemide metabolites in their study of normal volunteers and heart failure patients. Following 40 mg intravenous doses to volunteers and patients not previously receiving

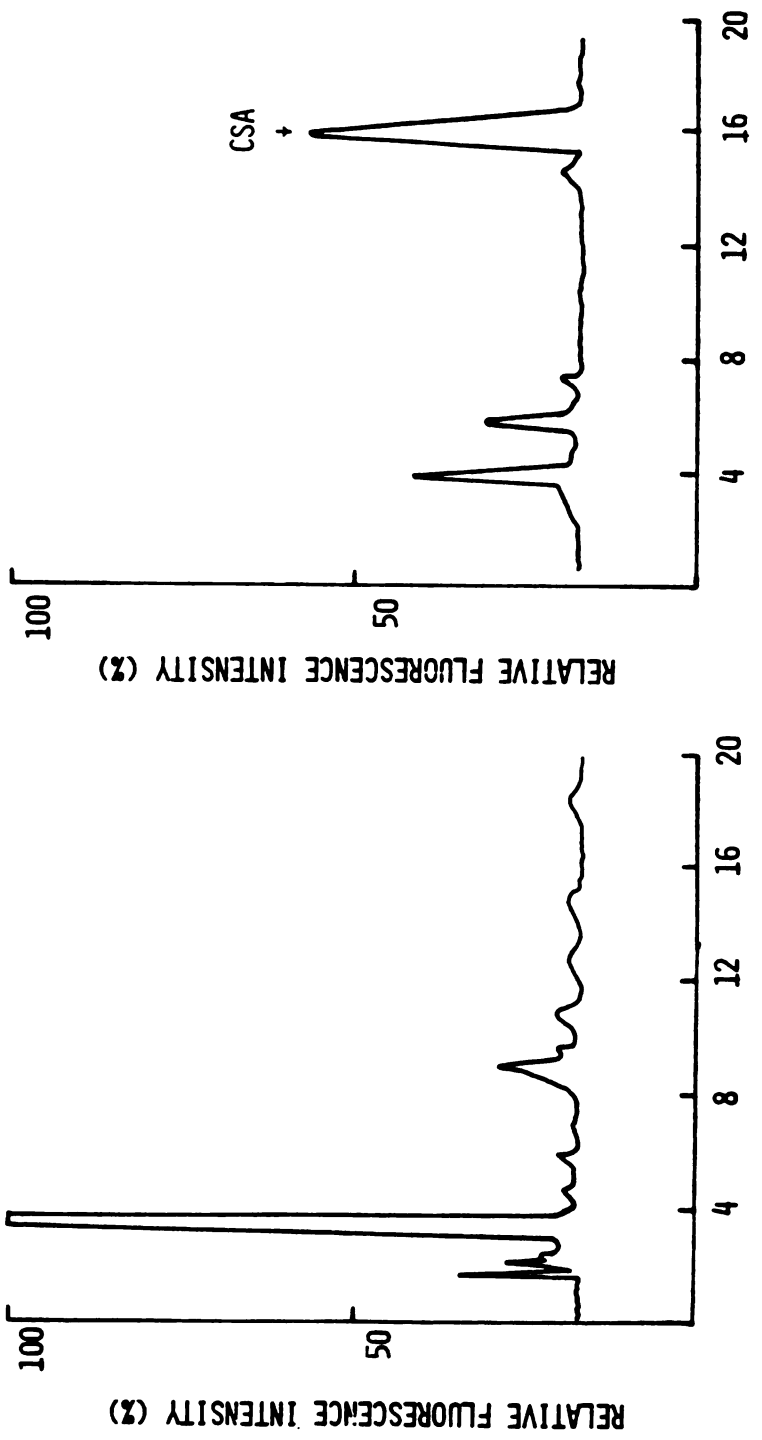

Fig. IV-1. Chromatograms developed for urine sample of volunteer which did not contain any CSA as determined by the direct injection method (left), and after the acid extraction procedure as outlined by Perez et al. (86) (right).

the drugs, only 0.7 - 0.8 mg of the furosemide dose could be accounted for as the glucuronide. However, in patients receiving furosemide chronically for at least 6 months, an average 6.4 mg of furosemide was excreted as the glucuronide in the same 24 hour period after a 40 mg intravenous dose. The authors (77) speculate that chronic administration of furosemide may be able to induce the glucuronidation process. In seven patients with severe arterial hypertension, Andreasen et al. (88) demonstrated a highly significant negative correlation between serum clearance and the fraction of furosemide excreted as glucuronide. They note that approximately 15-20% of an intravenously administered dose was excreted as a glucuronide metabolite when serum clearances fell below 2 ml/min·kg. Recently, Perez et al. (86) reported the excretion of furosemide glucuronide to account for 3.3 - 40.4% of the dose in patients with acute pulmonary edema. However, alkaline conditions used in their analytical procedure may lead to possible errors. Recent unpublished research (113) in our laboratory has demonstrated that basic conditions will lead to degradation of furosemide glucuronide and to a misrepresentation of the data. In the work detailed here approximately 5.5 mg of furosemide was excreted as the glucuronide conjugate after intravenous administration and about 5.1 mg after oral administration. A plot of the urinary excretion rate of unchanged furosemide and furosemide glucuronide after intravenous administration of furosemide is shown in Fig. IV-2. The terminal slopes decline in parallel indicating that metabolite formation is the rate limiting step in its elimination.

Plasma analysis after the 80 mg oral dose indicated a secondary peak in four out of the nine volunteers studied. These peaks occurred at approximately 240 min or 480 min (or both times in one subject), and may be reflective of biliary recycling. Figure IV-3 demonstrates this unusual

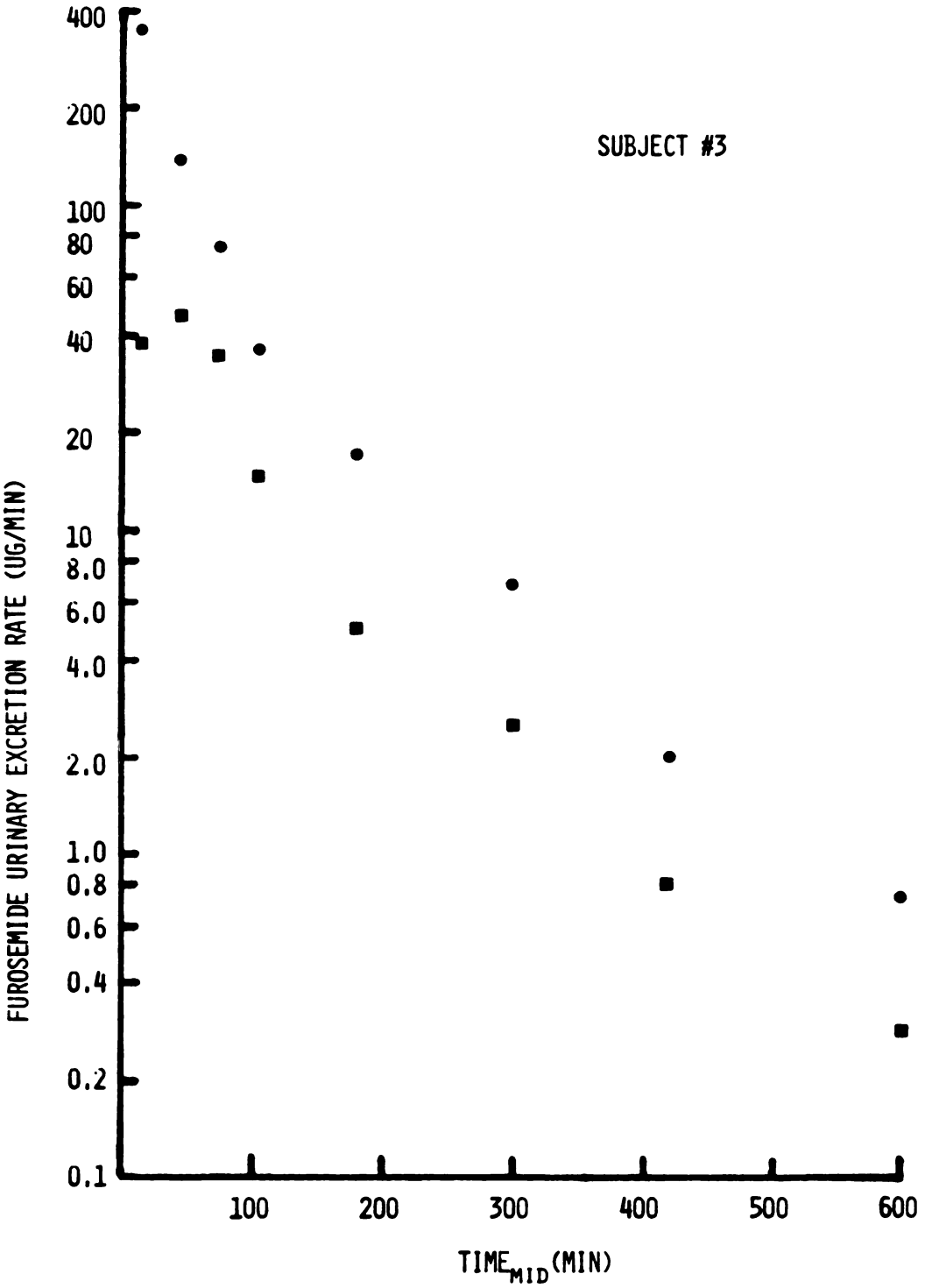

Fig. IV-2. Urinary excretion rate vs. midpoint time plots of unchanged furosemide (●) and furosemide glucuronide(■) after intravenous administration of 40 mg furosemide to subject #3 (expressed in furosemide weight units).

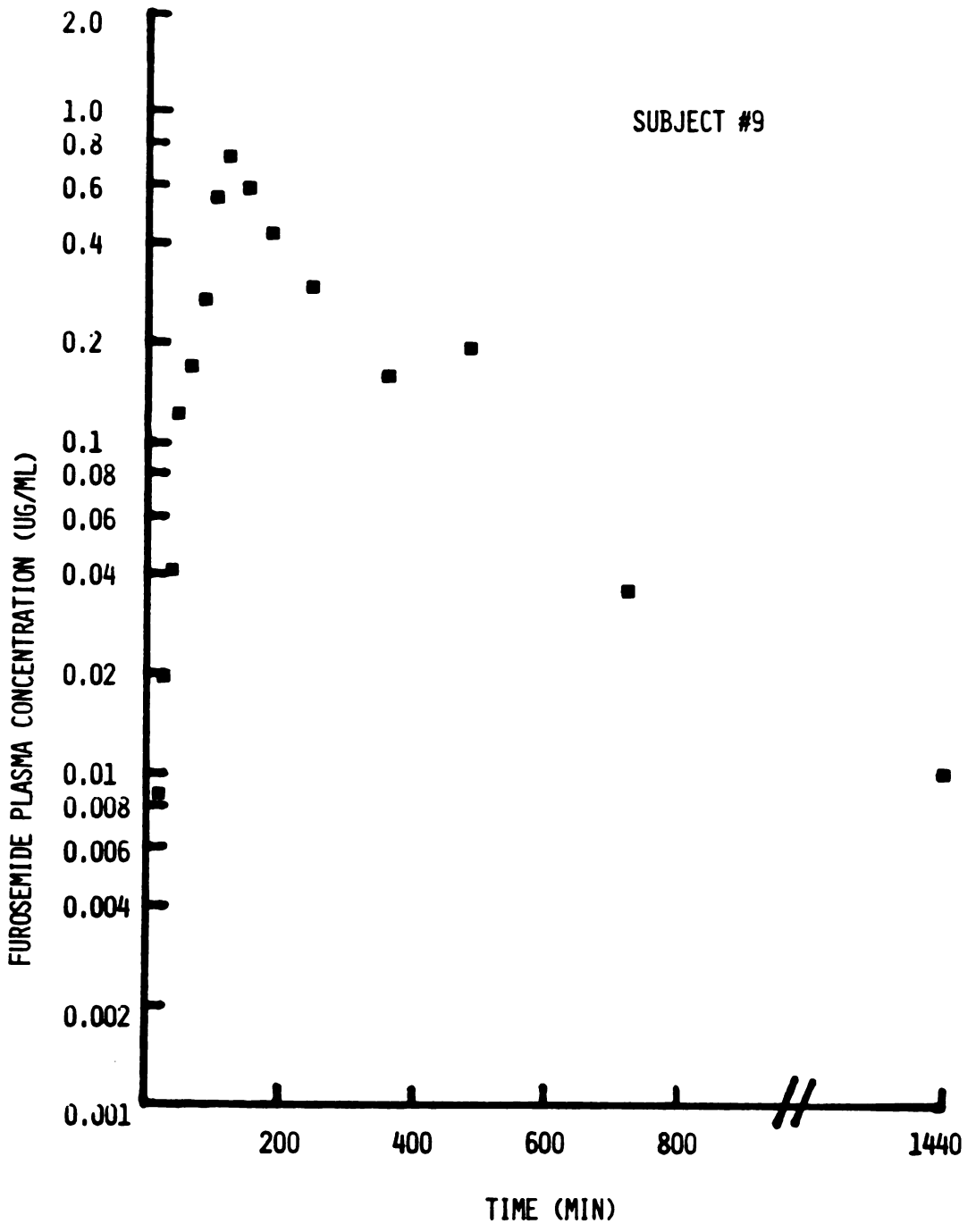

Fig. IV-3. Plasma concentration vs. time plot after oral administration of 80 mg furosemide to subject #9.

plasma profile for subject #9. Although these plasma samples were supposedly taken prior to lunch and dinner, anticipation of a meal (especially in a fasted subject), can cause the gall bladder to empty. Biliary excretion of furosemide, as evidenced by its recovery in the feces after intravenous administration, has been shown to account for 6-12% of the dose (70,85).

It should be noted that the absolute bioavailability of furosemide found in this study (0.434 ± 0.101) is somewhat lower than other literature values (Table I-1). This however, may reflect the specificity of the assay method utilized, the lot of furosemide studied, or the fact that volunteers in this study were in a supine position during the intravenous replacement of fluid and electrolytes lost in the voided urine.

E. Summary

Discrepancies involving furosemide metabolism and pharmacokinetics may reflect errors inherent in the assay procedure. No evidence of CSA, the putative metabolite of furosemide, was found and the results of this present investigation conclusively demonstrate it to be an analytical artifact. In addition, glucuronidation accounted for approximately 14% of the available dose of furosemide, whether given orally or by intravenous administration.

Chapter V. Drug-Interaction Studies in Healthy Volunteers

A. Furosemide - Probenecid Interaction

1. Specific Objectives

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 support this hypothesis and show that probenecid can decrease the natriuretic action of furosemide (10,114). Studies in humans evaluating the effect of probenecid on the pharmacokinetics and pharmacodynamics of furosemide are limited and less clear (78, 79, 115).

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.

2. Methods

a. Subject Selection

Four males, 21-33 years and 65-77 kg, volunteered as outpatients in the study. Each subject had a normal medical history, physical examination,

and standard laboratory tests. Informed consent was obtained from each subject prior to participation in the study.

b. Study Design

Each subject received 40 mg of furosemide[1] alone and after pretreatment with probenecid[2]. Subjects fasted the night before and until at least 2 hours after administration of the diuretic. Furosemide was administered intravenously over 3 minutes, with the midpoint of the infusion[3] considered as time zero. One gram of probenecid (2 tabs x 0.5 gm/tab) was ingested at bedtime the night before and on arising the morning of the study (30-60 minutes prior to furosemide administration). An interval of at least one week elapsed between studies and identical lot numbers for each drug were used throughout.

Blood samples (3 ml) to determine the drug concentration were obtained with an indwelling heparinized scalp vein needle[4] at 0, 5, 10, 20, 30, 45, 60, 80, 100, 120, 180, 240, 300, 360, and 480 minutes and at 24 hours. 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 hours and at two times of spontaneous voiding at home between the 8 and 24 hour 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.

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[1]Hoechst-Roussel Pharmaceuticals, Inc., Somerville, NJ.

[2]Merck Sharp and Dohme, West Point, Pa.

[3]Harvard Apparatus Compact Infusion Pump, Harvard Apparatus, Millis, Mass.

[4]E-Z Set^{R} - PRN Intermittent Infusion Set, Deseret Pharmaceutical Co., Inc., Sandy, Utah

All 24 hour blood samples showed normal electrolytes, urea nitrogen and creatinine. Sodium concentrations were measured with a flame photometer[5]. Statistical differences were determined using a paired t-test.

c. Assay Procedures

Plasma samples of furosemide, with and without probenecid pretreatment, as well as urine samples of furosemide administered alone were analyzed by Method I (Chapter II). Urine furosemide samples following probenecid pretreatment were measured by a minor modification of Method II as previously noted. The analysis of plasma samples containing probenecid were also discussed previously (Chapter II).

d. Calculations

The half-life of furosemide, Terminal $T_{\frac{1}{2}}$, was determined by linear regression from the terminal portion of the urinary excretion rate versus midpoint time plots. All other pharmacokinetic parameters were calculated as discussed in Chapter IV.

3. Results

The effects of probenecid on the pharmacokinetics of furosemide were studied in both plasma and urine (Table V-1). Mean plasma concentrations of furosemide with probenecid pretreatment were significantly increased at all time points except at 5 min (Fig. V-1). This resulted in a significant increase in AUC [252 ± 24 (μg·min)/ml for furosemide alone

[5]Model 450, Corning Scientific Instruments, Medfield, Mass.

Table V-1 Pharmacokinetic Effects of Probenecid on Furosemide

Subject	Treatment	AUC (μg·min/ml)	CLp (ml/min)	Vdss (liters)	Terminal $T_{\frac{1}{2}}$ (min)	CLr (ml/min)	CLnr (ml/min)	fe	fnr
TP	Furosemide	225	178	8.85	85	122	56	0.68	0.31
	Furosemide with probenecid	723	55.3	6.00	185	22.8	32.5	0.41	0.59
RP	Furosemide	280	143	9.55	85	101	42	0.71	0.29
	Furosemide with probenecid	907	44.1	5.50	193	22.7	21.4	0.52	0.49
TT	Furosemide	242	165	7.62	75	139	26	0.84	0.16
	Furosemide with probenecid	787	50.9	5.81	160	22.3	28.6	0.44	0.56
DH	Furosemide	261	153	7.73	82	109	44	0.71	0.29
	Furosemide with probenecid	724	55.3	9.31	161	24.6	30.7	0.45	0.56
Mean±SD	Furosemide	252±24	160±15	8.44±0.93	82±5	118±17	42±12	0.74±0.07	0.26±0.07
	Furosemide with probenecid	785±87	51.4±5.3	6.66±1.78	175±17	23.1±1.0	28.3±4.9	0.46±0.05	0.55±0.04
Level of significance		S ($p<0.001$)	S ($p<0.001$)	NS ($p>0.20$)	S ($p<0.001$)	S ($p<0.002$)	NS ($p>0.10$)	S ($p<0.01$)	S ($p<0.01$)

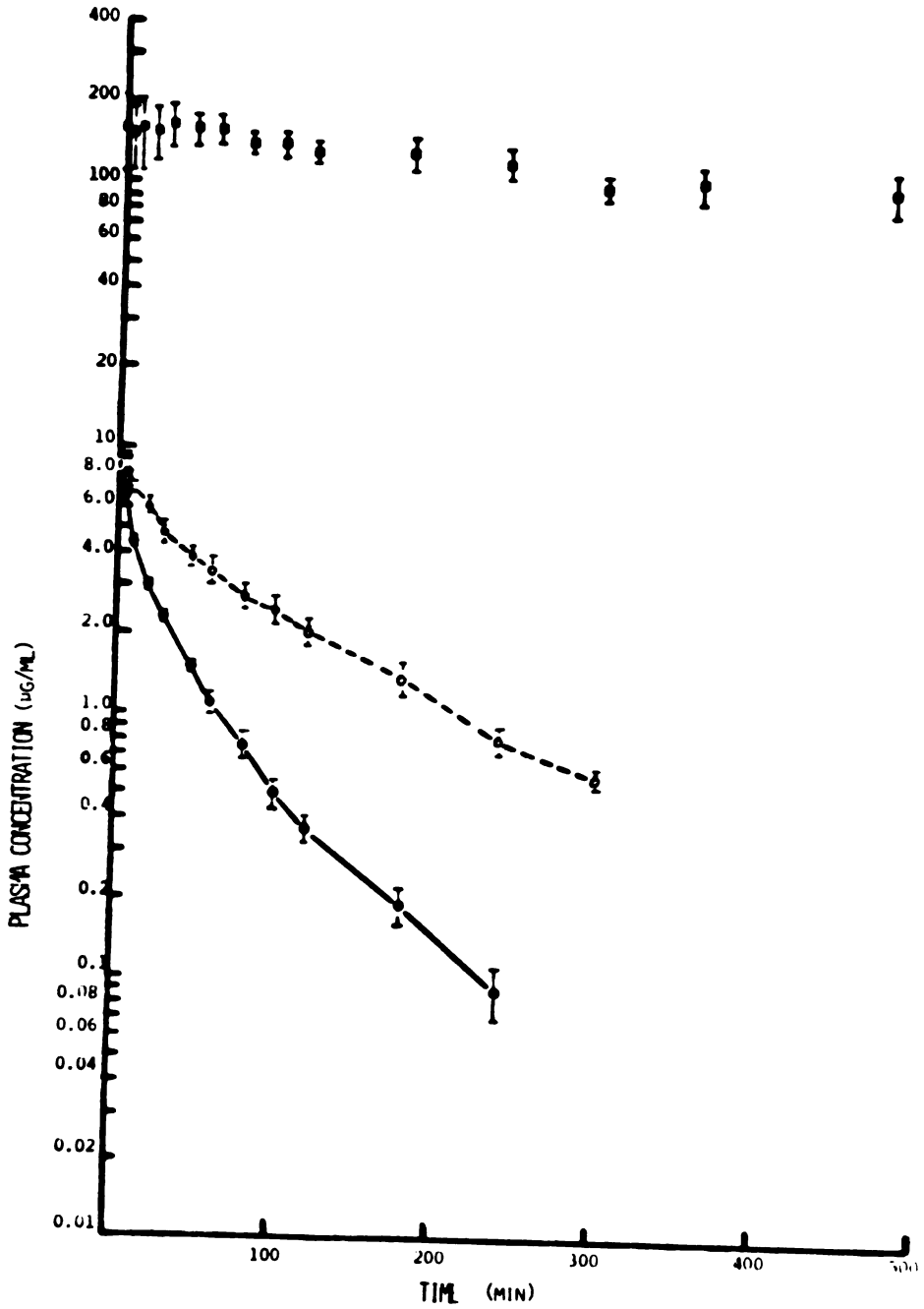

Fig. V-1. Plasma concentration vs. time plots of furosemide alone (●), furosemide with probenecid pretreatment (○), and probenecid (■); data are expressed as the mean ± SEM.

(Treatment I) and 785 ± 87 μg·min)/ml for furosemide with probenecid pretreatment (Treatment II); $p < 0.001$] as a consequence of the significantly reduced total plasma clearance of furosemide in the presence of probenecid (160 ± 15 ml/min for Treatment I and 51.4 ± 5.3 ml/min for Treatment II; $p < 0.001$). This difference in total plasma clearance was reflected by the significant increase in the half-life of furosemide (82 ± 5 min for Treatment I and 175 ± 17 min for Treatment II; $p < 0.001$) since the Vdss value was not altered significantly (8.44 ± 0.93 liters for Treatment I and 6.66 ± 1.78 liters for Treatment II; $p > 0.20$).

The total renal clearance of furosemide was reduced markedly with probenecid pretreatment (118 ± 17 ml/min for Treatment I and 23.1 ± 1.0 ml/min for Treatment II; $p < 0.002$), while the nonrenal plasma clearance did not change significantly (42 ± 12 ml/min for Treatment I and 28.3 ± 4.9 ml/min for Treatment II; $p > 0.10$). No measurements of furosemide glucuronide were made since this study was carried out prior to the development of that assay. In addition, the fraction of furosemide excreted unchanged in the urine in the presence of probenecid was reduced significantly (0.74 ± 0.07 for Treatment I and 0.46 ± 0.05 for Treatment II; $p < 0.01$) with a corresponding increase in the fraction excreted by nonrenal routes (0.26 ± 0.07 for Treatment I and 0.55 ± 0.04 for Treatment II; $p < 0.01$).

Analysis of the urinary excretion rate of furosemide, with and without probenecid pretreatment, is shown in Fig. V-2. Initially, the urinary excretion rate of furosemide with probenecid was significantly lower than that of furosemide when administered alone. However, after ∿ 125 min, the two curves (Treatments I and II) intersect; at subsequent times, the urinary excretion rate of furosemide with probenecid was significantly greater than that of furosemide alone. This result was primarily due to

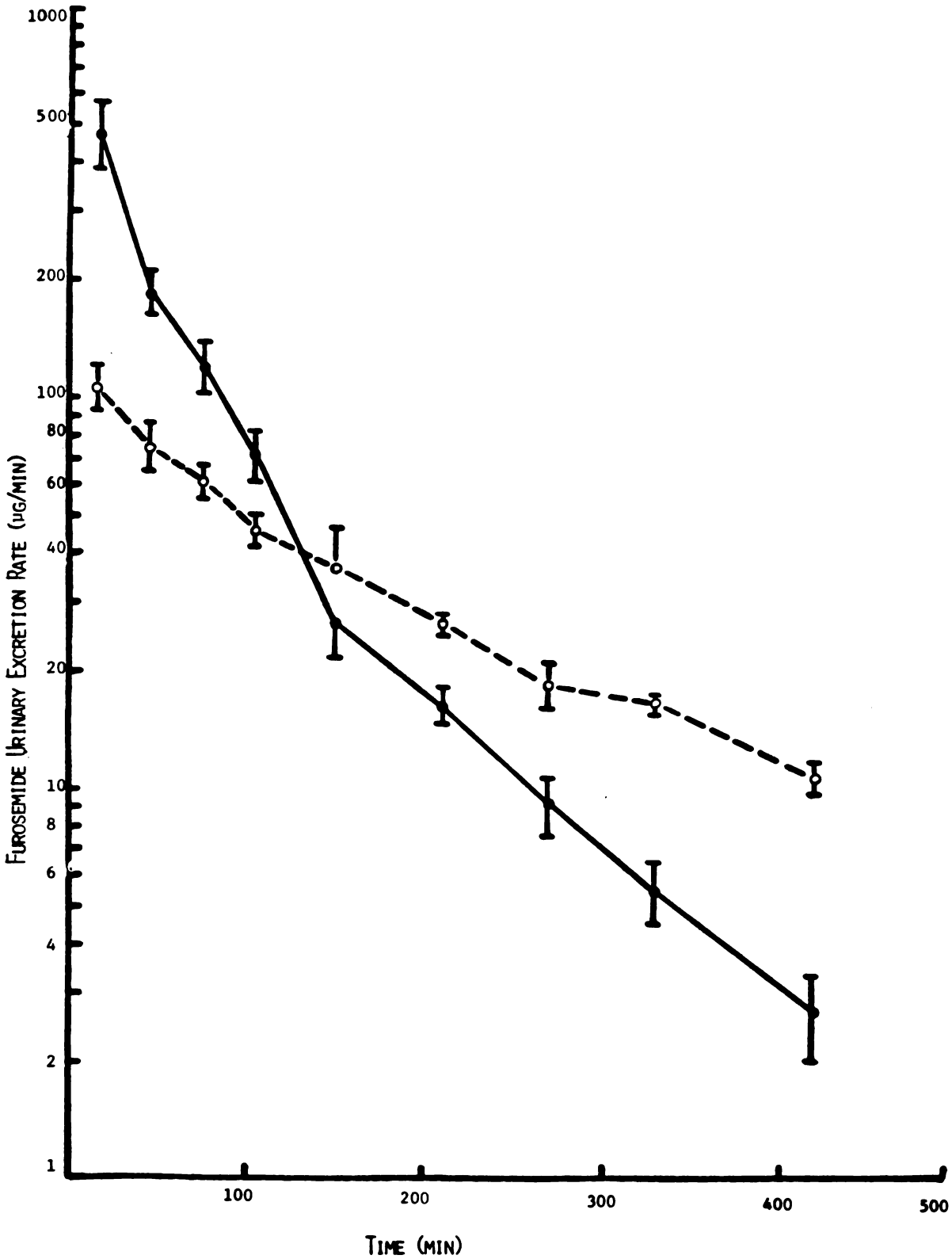

Fig. V-2. Urinary excretion rate vs. midpoint time plots of furosemide alone (●) and furosemide with probenecid pretreatment (O); data are expressed as the mean ± SEM.

the large difference in plasma furosemide concentrations at subsequent times between Treatments I and II (Fig. V-I) since the renal clearance was reduced but was constant throughout each study.

The effect of probenecid on furosemide-induced natriuresis is shown in Fig. V-3. The initial natriuretic response to furosemide when it was given concomitantly with probenecid was reduced compared to that of furosemide administered alone. The two curves (Treatments I and II) intersect at 100 min; at subsequent times, the natriuretic response to furosemide with probenecid was greater than to furosemide alone, similar to that seen for the urinary excretion rate of furosemide. Although differences in the sodium excretion rate were seen with and without probenecid pretreatment, they did not appear to be statistically different. Table V-2 shows that the 8-hr sodium excretion (milliequivalents) was 291 ± 53 for Treatment I and 323 ± 106 for Treatment II ($p > 0.50$). The diuretic response (milliliters per 8 hr) was 2257 ± 422 for Treatment I and 2637 ± 632 for Treatment II ($p > 0.20$).

4. Discussion

Experiments in animals suggested that the luminal concentration or amount of furosemide rather than its plasma concentration may be the critical determinant with respect to its natriuretic and diuretic effect (10, 108, 114, 116). Hook and Williamson (10) and Friedman and Roch-Ramel (114) demonstrated in the dog and cat, respectively, that probenecid (50 mg/kg iv) significantly inhibited furosemide-induced natriuresis. Since probenecid is highly secreted (117), it can compete for active transport and prevent furosemide from reaching the tubular fluid, thereby attenuating furosemide's natriuretic effect. However, human studies do not corroborate

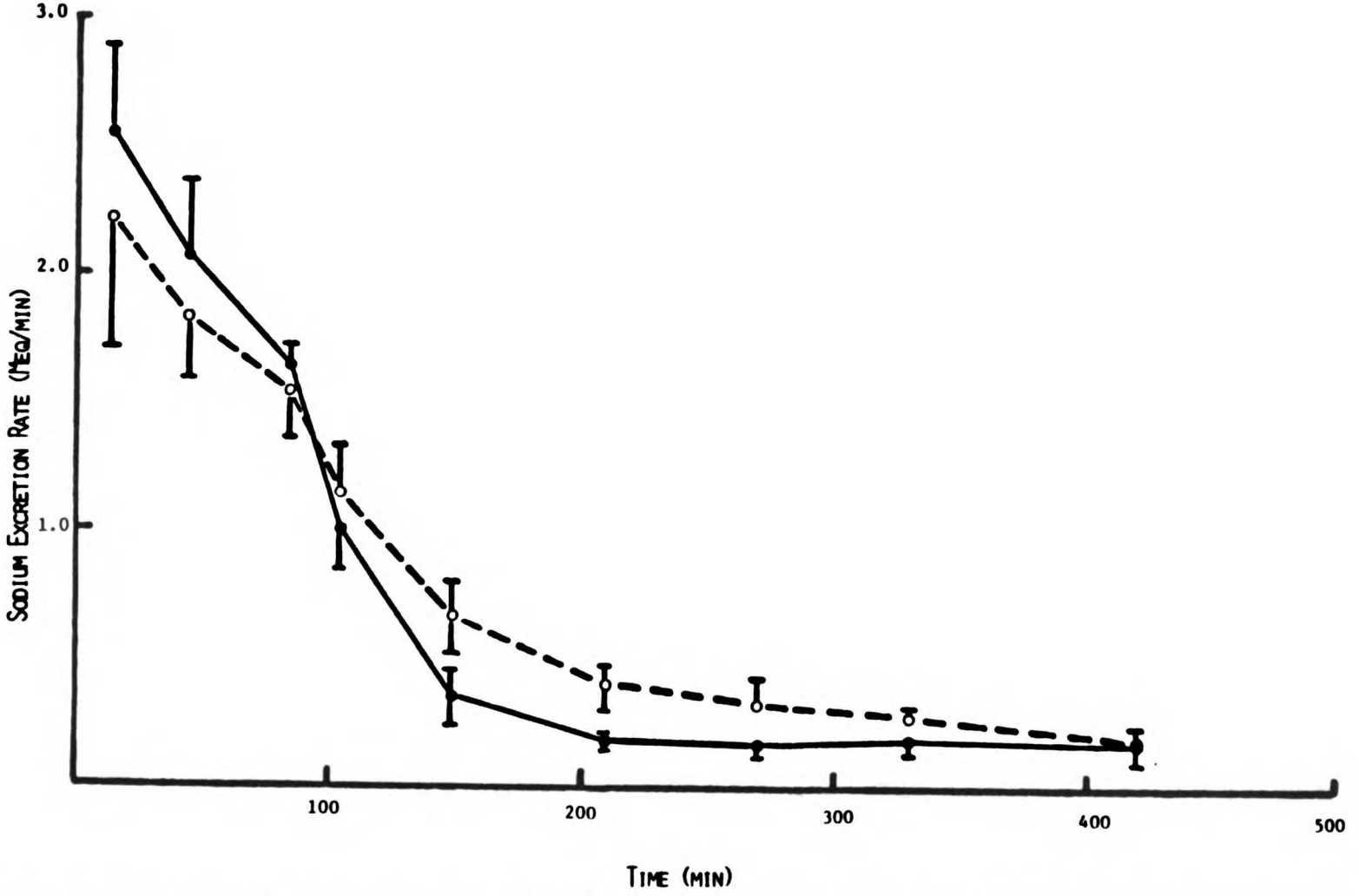

Fig. V-3. Sodium excretion rate vs. midpoint time plots of furosemide alone (●) and furosemide with probenecid pretreatment (O); data are expressed as the mean ± SEM.

Table V-2. Effects of Probenecid on Furosemide Diuresis and Natriuresis

Subject	Treatment	Urine Volume (ml/8 hr)	Sodium Excretion (mEq/8 hr)
TP	Furosemide	2451	328
	Furosemide with probenecid	2117	232
RP	Furosemide	2251	262
	Furosemide with probenecid	2555	288
TT	Furosemide	1674	232
	Furosemide with probenecid	2329	296
DH	Furosemide	2653	343
	Furosemide with probenecid	3546	477
Mean ± SD	Furosemide	2257 ± 422	291 ± 53
	Furosemide with probenecid	2637 ± 632	323 ± 106
Level of significance		NS ($p > 0.20$)	NS ($p > 0.50$)

these findings in animals. In contrast, probenecid caused either no change or a significant increase in the natriuretic response to furosemide.

Honari et al. (78) showed that probenecid significantly decreased the total plasma clearance (155 ml/min for Treatment I* and 85 ml/min for Treatment II*) and the total renal clearance (134 ml/min for Treatment I and 63 ml/min for Treatment II) of furosemide, and significantly increased the furosemide half-life (35.8 min for Treatment I and 60.8 min for Treatment II) in humans. However, the 6-hr urine volume (5098 ml for Treatment I and 6164 ml for Treatment II) and the sodium excretion (578 mEq for Treatment I and 694 mEq for Treatment II) were not significantly different between treatments. In addition, the fraction of the dose excreted unchanged in the urine was not statistically altered with probenecid pretreatment, although three of the four subjects studied did excrete a smaller percentage of the drug. Therefore, the investigators (78) concluded that their results were consistent with the findings of a previous study by Hook and Williamson (10), who suggested that the amount of furosemide in the tubular fluid is the main determinant of furosemide diuresis. However, both groups of investigators did not fully characterize the mechanism of this interaction between furosemide and probenecid. Since the time course of the natriuretic and diuretic response was not described, the previous investigators were considering only gross effects.

Homeida et al. (79) also demonstrated marked changes in furosemide pharmacokinetics with probenecid pretreatment. Similarly, these investigators noted that since the total proportion of unchanged drug reaching the renal tubule was not changed markedly, the total diuretic effect remained

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*Treatment I - Furosemide alone.

Treatment II - Furosemide with probenecid pretreatment.

unaltered. However, this conclusion is suspect since one can calculate from their data that the fraction of the furosemide dose excreted unchanged in the urine decreased about 41% (from 0.34 to 0.20) when the subjects were pretreated with probenecid. In addition, furosemide was assayed spectrofluorometrically in their study, which is rather nonspecific, especially in urine. This method may account for the unusual values for the total, renal, and nonrenal plasma clearances reported in their control subjects, as suggested by Benet (97).

In a more recent study, the pharmacodynamic effect of probenecid on the response to furosemide in humans was quantified (115). Analysis of the time course of natriuresis and diuresis showed that probenecid actually decreased the response of furosemide for the first 60-90 min but increased the subsequent response sufficiently to result in a statistically greater overall effect. However, it was noted (115) that since the concentrations or amounts of furosemide in the urine were not compared with the response, a unifying hypothesis to explain the mechanism of a furosemide-probenecid interaction was not possible.

In the present investigation, the time course of furosemide in plasma and urine was compared with that of the natriuretic effect in an attempt to explain the mechanism for a furosemide-probenecid interaction. Although probenecid caused marked changes in the pharmacokinetic parameters of furosemide (Table V-1), there was no significant difference in its gross natriuretic and diuretic effect (Table V-2). Analysis of the time course for natriuresis (Fig. V-3) shows that probenecid actually decreased the response for the first 100 min after furosemide administration. However, the subsequent response was increased sufficiently to result in no statistical difference in the mean 8-hr value for sodium excretion. Although a similar pattern was seen with respect to the urinary excretion of furo-

semide, the magnitude of this difference between treatments was statistically significant (Fig. V-2).

Figure V-4 shows that probenecid caused a significant shift to the right in the relationship between sodium excretion rate and the logarithm of furosemide plasma concentration. This observation suggests that higher plasma furosemide concentrations are needed in the presence of probenecid to produce a natriuretic response equivalent to that produced by lower concentrations when probenecid is absent. Figure V-5 shows the relationship between sodium excretion rate and the logarithm of furosemide urinary excretion rate. Although Treatments I* and II* were not parallel over the entire dose-response curve, the amount of furosemide excreted into the urine per unit time was more closely correlated with response than was the plasma furosemide concentration.

The shift to the left between the urinary excretion rate of furosemide and the effect (upper portion of Fig. V-5) may be real or may be an artifact due to the limited number of subjects. However, a possible explanation for this finding may involve an interaction between probenecid and prostaglandins. Previous investigators hypothesized that prostaglandins mediate the natriuretic-diuretic effect of furosemide (40, 49, 50, 66, 118). Renal prostaglandins are synthesized primarily in the medulla (38-40, 55) and are released into the extracellular fluids (119). In vitro studies showed that prostaglandins accumulate in several tissues, including the renal cortex, as a result of an active transport mechanism (120,121). In addition, it was shown that probenecid can inhibit the renal tubular trans-

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*Treatment I - Furosemide alone.

Treatment II - Furosemide with probenecid pretreatment.

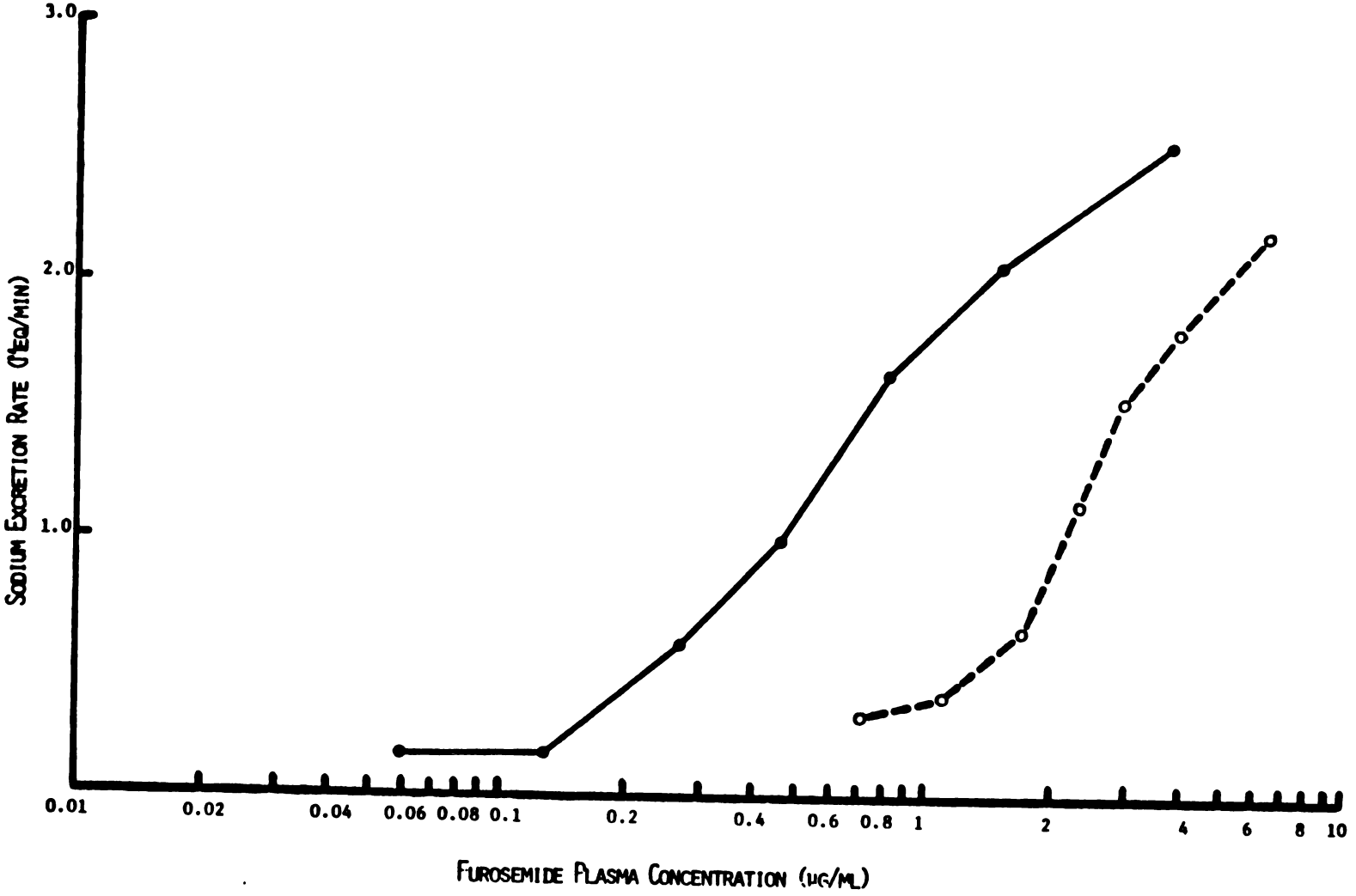

Fig. V-4. Sodium excretion rate vs. plasma concentration plots of furosemide alone (●) and furosemide with probenecid pretreatment (O).

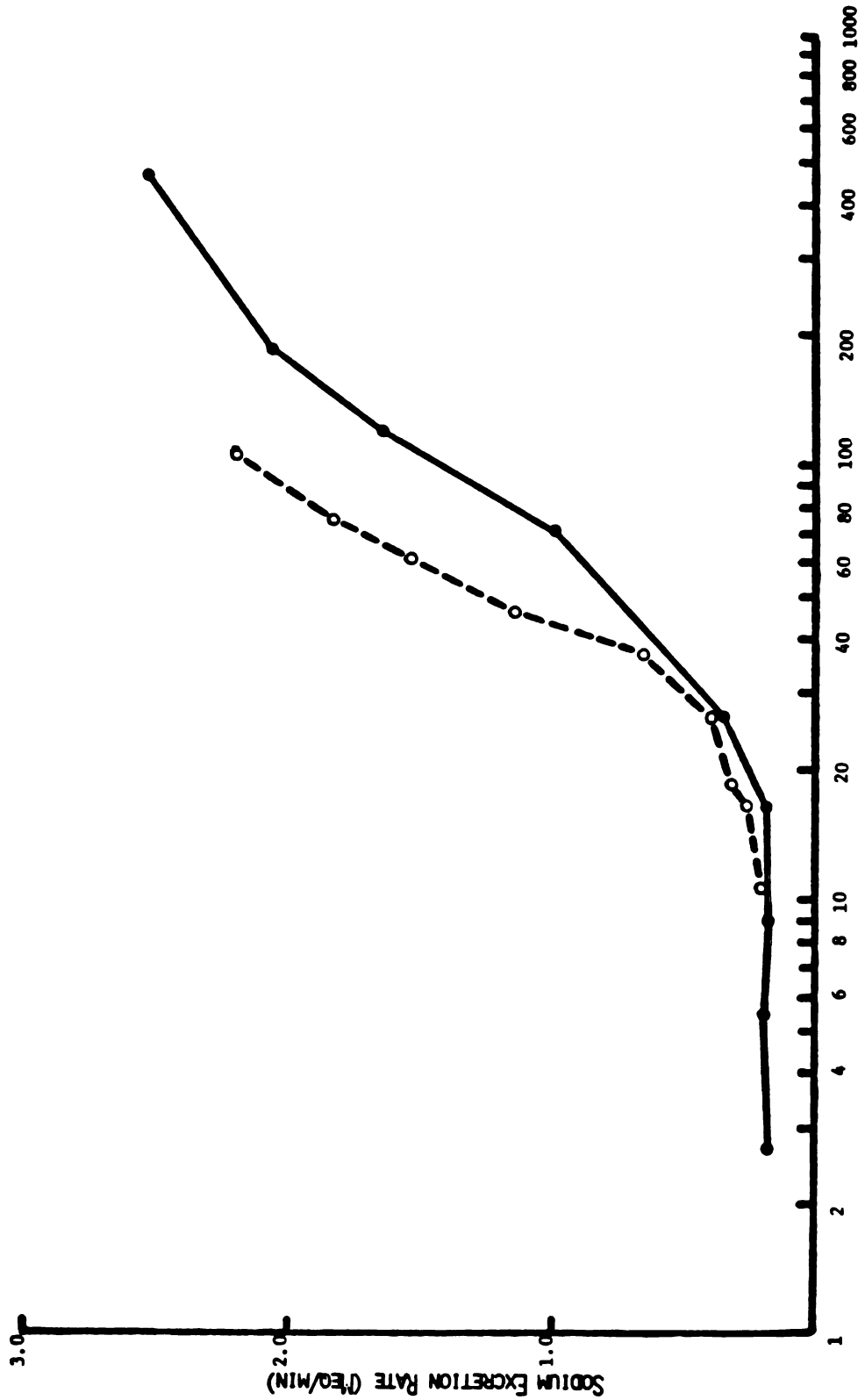

Fig. V-5. Sodium excretion rate vs. urinary excretion rate plots of furosemide alone (●) and furosemide with probenecid pretreatment (O).

port of prostaglandins, presumably by competing for active transport into the urine (57,58). Although this conclusion is speculation, this inhibition by probenecid of prostaglandin transport may result in a tubule that is more responsive to smaller amounts of furosemide in the urine and thus account for the shift to the left as described.

5. Summary

The mechanism by which probenecid alters furosemide-induced natriuresis is consistent with *in vitro* studies (6) indicating that furosemide acts at the luminal surface of the nephron. This study in humans, as well as previous animal studies (116), demonstrate that the urinary excretion rate of furosemide is a better indicator of natriuresis and diuresis than is the plasma concentration.

B. Furosemide-Indomethacin Interaction

1. Specific Objectives

Indomethacin, a potent inhibitor of prostaglandin synthetase, has been shown to attenuate the natriuretic, hemodynamic and renin-stimulating effects of furosemide (41,42, 47-54), whose diuretic response is believed to be prostaglandin mediated. However, indomethacin is also a weak organic acid which can compete with furosemide for active secretion into the kidney lumen. This could prevent furosemide from reaching its site of action and thereby attenuate its diuretic response. The present study was undertaken in order to evaluate the role of a pharmacokinetic interaction as a possible explanation for the attenuation of furosemide's diuretic effect by indomethacin.

2. Methods

a. Subject Selection

Participants were the same volunteers as described for the furosemide-probenecid interaction study.

b. Study Design

Each subject received 40 mg of furosemide[1] alone and after pretreatment with indomethacin[2]. A 50 mg tablet of indomethacin was ingested at bedtime the night before and on arising the morning of the study (30-60 minutes prior to furosemide administration). All other conditions were identical to those described in the furosemide-probenecid interaction study.

c. Assay Procedures

Plasma and urine samples of furosemide (Method I), as well as plasma samples containing indomethacin were analyzed as previously discussed (Chapter II).

d. Calculations

The half-life of furosemide, Terminal $T_{\frac{1}{2}}$, was determined by linear

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[1] Hoechst-Roussel Pharmaceuticals, Inc., Somerville, NJ.

[2] Merck Sharp and Dohme, West Point, Pa.

regression from the terminal portion of the urinary excretion rate versus midpoint time plots. All other pharmacokinetic parameters were calculated as discussed in Chapter IV.

3. Results

The analysis of the plasma and urine data in terms of various pharmacokinetic parameters is presented in Table V-3. Pretreatment with indomethacin caused increased plasma concentrations of furosemide in all subjects (see Fig. V-6 for data of subject R.P.), as well as a significant increase in the AUC [252 ± 24 for furosemide alone (F) and 344 ± 47 μg·min/ml for furosemide with indomethacin (F + I); $p < 0.01$]. In addition, the total furosemide plasma clearance after pretreatment with indomethacin significantly decreased [160 ± 15 for F, 118 ± 16 ml/min for F + I; $p < 0.005$]. Similarly, the total renal clearance of furosemide dramatically decreased with indomethacin pretreatment (118 ± 17 for F and 77.0 ± 8.7 ml/min for F + I; $p < 0.01$), but was constant throughout each study. Although the half-life for furosemide increased in each of the four subjects with concomitant indomethacin administration, the level of significance of this change was less than 95%. This lack of significance was probably due to the limited number of subjects. The parameters Vdss, fe and fnr did not differ significantly between treatments.

In three subjects both the natriuretic and diuretic responses of furosemide were significantly attenuated when the subjects were pretreated with indomethacin. The 8-hour sodium excretion (mEq) was 274 ± 50 for F and 180 ± 20 for F + I ($p < 0.02$), and the diuretic response (ml/8 hr) was 2112 ± 407 for F and 1583 ± 308 for F + I ($p < 0.05$). Analyses of the sodium excretion rate over time (Fig. V-7) and the urine flow rate over time

Table V-3. Pharmacokinetic Effects of Indomethacin (I) on Furosemide (F)

Subject	AUC (μg·min/ml)	CLp (ml/min)	Vdss (L)	Terminal $T_{\frac{1}{2}}$ (min)	CLr (ml/min)	CLnr (ml/min)	fe	fnr
T.P.								
F	225	178	8.85	85	122	56	0.68	0.31
F+I	302	132	12.2	170	88.7	43.3	0.67	0.33
R.P.								
F	280	143	9.55	85	101	42	0.71	0.29
F+I	374	107	7.32	105	68.4	38.6	0.64	0.36
T.T.								
F	242	165	7.62	75	139	26	0.84	0.16
F+I	305	131	8.95	105	78.0	53.0	0.60	0.40
D.H.								
F	261	153	7.73	82	109	45	0.71	0.29
F+I	394	101	8.64	120	73.1	27.9	0.72	0.28
Mean ± SD								
F	252±24	160±15	8.44±0.93	82±5	116±16	42±12	0.74±0.07	0.26±0.07
F+I	344±47	118±16	9.28±2.07	125±31	77±8.7	40.7±10.4	0.66±0.05	0.34±0.05
Level of significance	S ($p<0.01$)	S ($p<0.005$)	NS ($p>0.50$)	NS ($p>0.05$)	S ($p<0.01$)	NS ($p>0.50$)	NS ($p>0.20$)	NS ($p>0.20$)

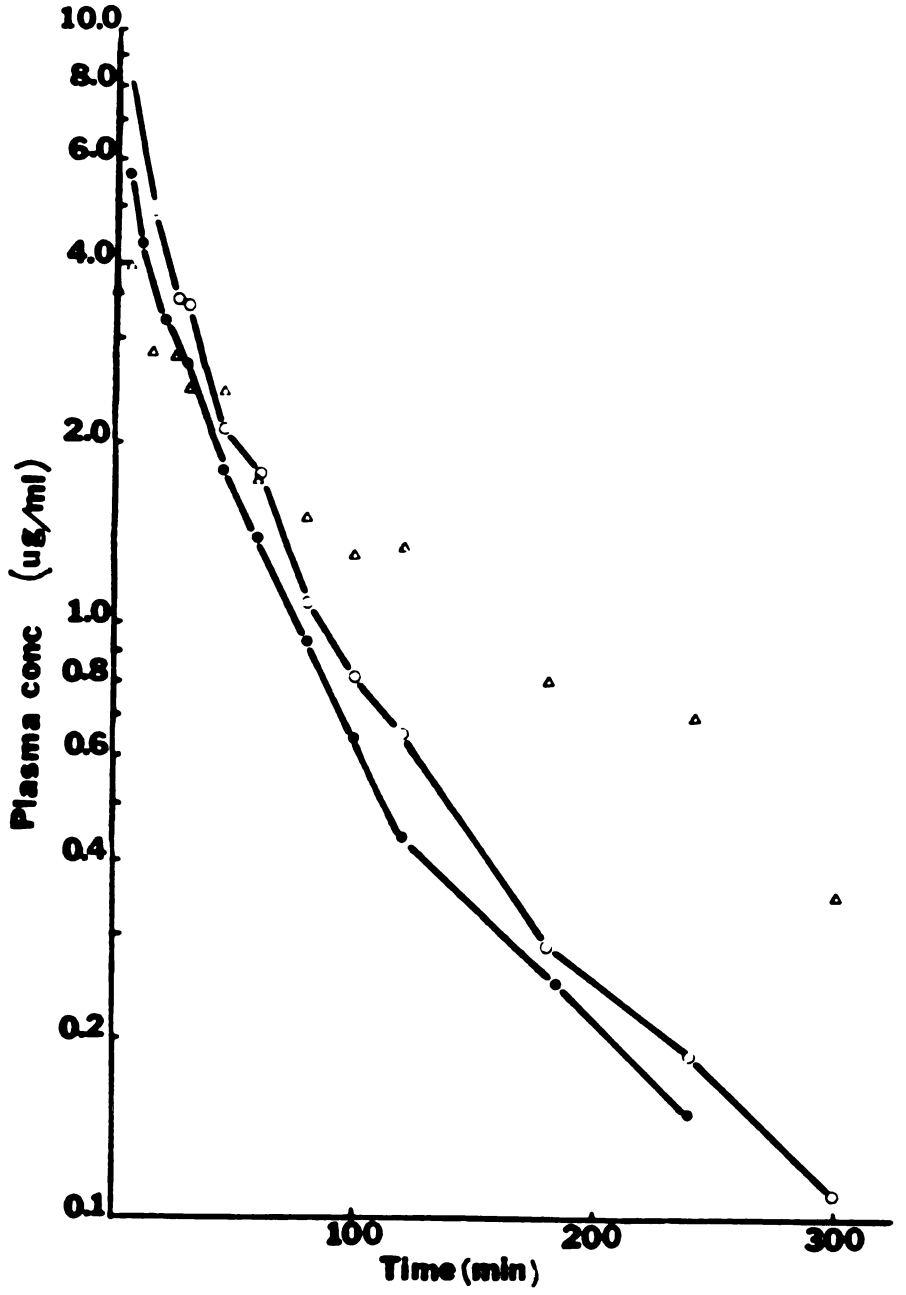

Fig. V-6. Plasma concentration vs. time plots of furosemide alone (●), furosemide with indomethacin pretreatment (O), and indomethacin (△) for subject RP.

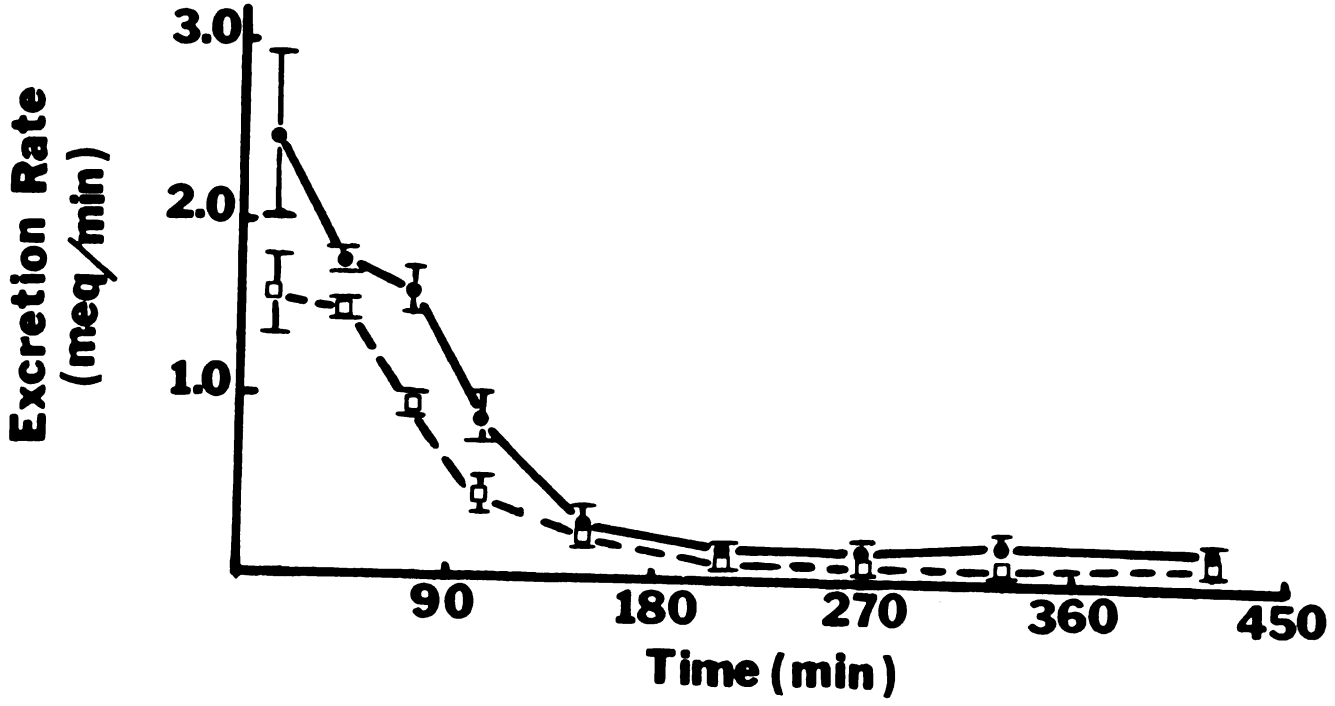

Fig. V-7. Sodium excretion rate vs. midpoint time plots of furosemide alone (●) and furosemide with indomethacin pretreatment (□); data are expressed as the mean ± SEM.

(Fig. V-8) show that the inhibiting effect of indomethacin was most pronounced during the first 2 hours. Slopes of the sodium excretion and urine flow rate vs. time plots were parallel, corresponding to half-lives of 80 minutes, which is similar to that determined for furosemide. The pharmacodynamic data for subject D. H. have been omitted since large quantities of liquid were consumed by this subject on the day of the F + I study. It was learned at the conclusion of the study that the subject had engaged in strenuous exercise the previous day and was feeling sick and dehydrated on the study day. The drinking of excess fluids was contrary to our protocol, thereby producing unreliable data. Brater (95) subsequently studied the pharmacodynamic interaction of furosemide and indomethacin in a similar manner in six additional normal volunteers. In this group the 8-hour sodium excretion was measured, and the results support data from the current study (245 ± 16 for F and 175 ± 18 mEq/8 hr for F + I; $p < 0.02$).

4. Discussion

Two possible mechanisms for the attenuation of the diuretic effect of furosemide by indomethacin have been suggested. Patak et al. (50) have proposed that indomethacin inhibits prostaglandin synthetase and therefore decreases the protaglandin mediated diuretic effect of furosemide. Frolich et al. (49) recognized this possibility but also suggested that indomethacin could compete for the active secretion of furosemide into the lumen of the kidney tubule thus decreasing the amount of furosemide available to the intraluminal site of action. Frolich et al . (49) attempted to quantify this interaction and found furosemide plasma levels to be higher after

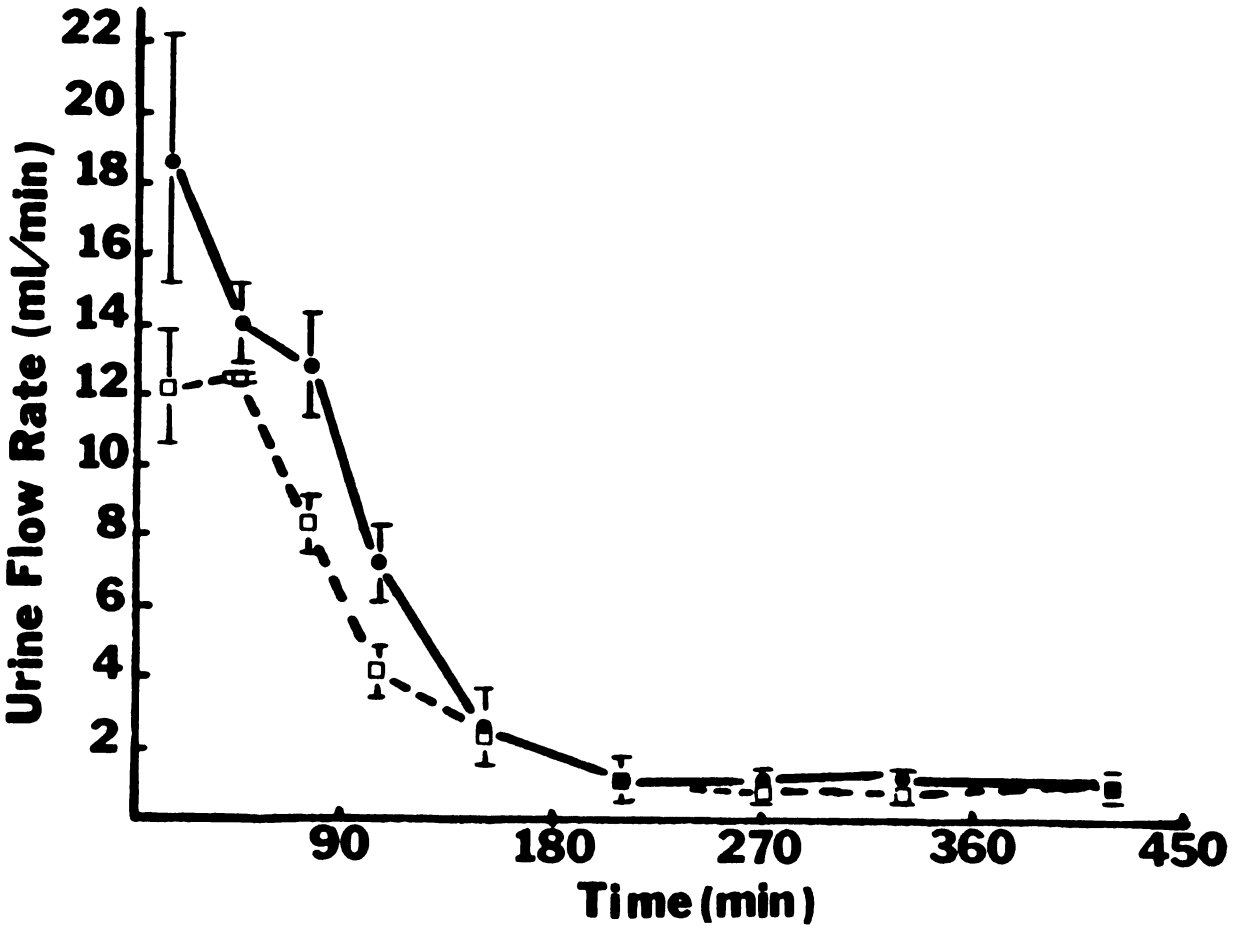

Fig. V-8. Urine flow rate vs. midpoint time plots of furosemide alone (●) and furosemide with indomethacin pretreatment (□); data are expressed as the mean ± SEM.

administration of indomethacin, although the differences were not significant. However, these authors compared plasma levels only at 10 and 30 minutes after drug administration. Furosemide obeys multicompartment kinetics with a half-life of the fast distribution phase ranging from about 5 to 15 minutes (83). Thus changes in plasma levels at 10 and 30 minutes may be more reflective of drug distribution than of drug elimination.

Frolich et al. (49) also compared the amount of furosemide excreted in the urine during the first 2 hours following administration of the drug. They found that furosemide excretion was reduced 18% when administered in conjunction with indomethacin and that this reduction was significant at the 0.01 level. In addition, urine volume was decreased 23% and sodium excretion was reduced 28% during this same period. On the evidence derived from limited plasma and urine measurements, Frolich et al. (49) concluded that indomethacin's effect on the diuretic response of furosemide was not due to a pharmacokinetic drug interaction. Although this statement may be true, I do not believe that their data proves this point.

Our results indicate that indomethacin significantly decreases the diuretic and natriuretic response to furosemide. Higher plasma concentrations of furosemide are noted throughout the time course of the F + I studies in comparison to those after F alone (Fig. V-6). Indomethacin also significantly decreases the renal clearance of furosemide (Table V-3) but has little effect on the nonrenal clearance. Under these conditions one would expect a decrease in the fraction of the dose excreted unchanged in the urine when indomethacin is administered concomitantly. The average results in the four subjects do indicate a decrease in fe of approximately 10% (Table V-3). However, this difference is not significant. Thus, al-

though renal clearance decreases significantly, the increase in plasma levels causes the amount of diuretic excreted in the urine (the product of renal clearance and plasma concentration) to decrease only slightly. This is illustrated in Fig. V-9, where plots of renal excretion rate vs. time for the two studies indicate no significant differences in furosemide elimination except when 90-120 minute urine collections are compared, although average excretion rates of furosemide for the F + I studies are slightly lower than those for the F alone at all times. Figure V-10 shows that indomethacin caused a shift to the right in the relationship between sodium excretion rate and the logarithm of furosemide urinary excretion rate. This observation supports a prostaglandin interaction and suggests that in the presence of indomethacin, greater amounts of unchanged furosemide (per unit time) are needed in the urine to produce a natriuretic response equivalent to that produced when furosemide is administered alone.

5. Summary

In conclusion, indomethacin does affect the disposition kinetics of furosemide. However, the extent of this pharmacokinetic change is minimal in comparison to the marked effect of indomethacin on natriuresis and diuresis. Therefore, it appears unlikely that a pharmacokinetic interaction can explain the magnitude of the pharmacodynamic results observed; it appears that a prostaglandin interaction is the more probable mechanism.

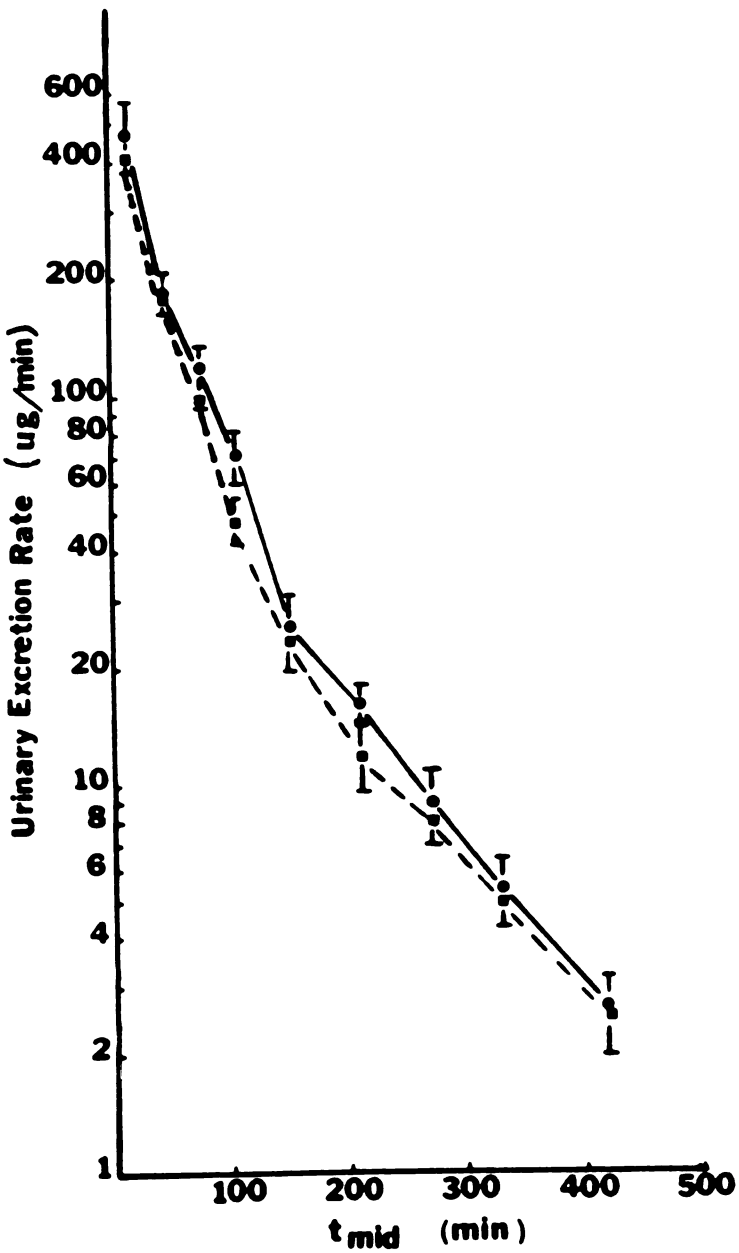

Fig. V-9. Urinary excretion rate vs. midpoint time plots of furosemide alone (●) and furosemide with indomethacin pretreatment (■); data are expressed as the mean ± SEM.

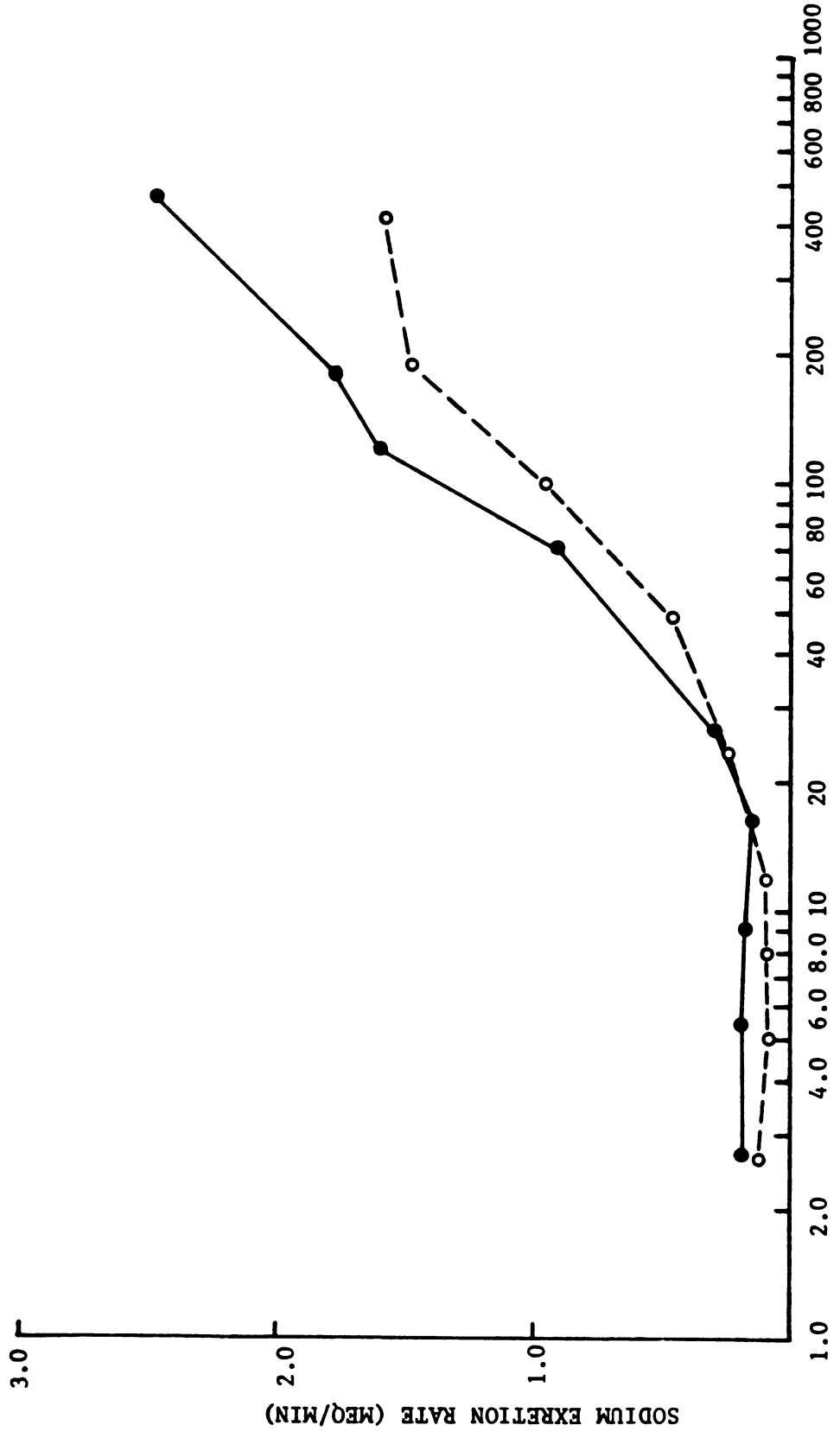

Fig. V-10. Sodium excretion rate vs. urinary excretion rate plots of furosemide alone (●) and furosemide with indomethacin pretreatment (○).

Chapter VI. Human Studies in Kidney Transplant Patients

A. Pharmacokinetic/Pharmacodynamic Evaluation of Furosemide

1. Specific Objectives

Furosemide is a valuable diuretic in kidney transplant patients for the treatment of volume overload. The accumulation of extracellular fluid is a common occurrence in these patients and usually occurs early post transplant. However, the fluid accumulation may persist for months despite the absence of conditions usually associated with salt and water retention such as acute rejection, congestive heart failure, hypoalbuminemia and low glomerular filtration rate. Clinical observations suggest that while some kidney transplant patients respond well to small doses of furosemide (responders), others are more refractory even when renal function is optimal. In these patients (non-responders), larger doses of 120 mg or greater may be needed to mobilize edematous fluid. In addition, kidney transplant patients seem to respond better to intravenous doses of furosemide compared to equivalent oral doses.

Although furosemide is widely used in kidney transplant patients, its disposition and dose-response relationship have not been studied and dosage regimens continue to be empiric. The present investigation was undertaken in order to satisfy the following objectives: 1) to study the pharmacokinetics of furosemide in kidney transplant patients after oral and intravenous administration; 2) to determine if intravenous adminis-

tration of furosemide is more efficacious in these patients than an equivalent oral dose; and 3) to investigate whether differences exist between responder and non-responder kidney transplant populations with respect to furosemide pharmacokinetics and pharmacodynamics.

2. Methods

a. Patient Selection

Characteristics of the nine kidney transplant patients (5 males, 4 females) studied are listed in Table VI-1. Patients ranged in age from 25-56 years (mean 41) and weighed between 65.5 and 95.2 kg (mean 76.5). Creatinine clearances ranged from 30.1 - 88.1 ml/min (mean 52.4) and the patients were normal with respect to serum albumin and plasma electrolyte levels. In addition, all patients were devoid of congestive heart failure (CHF), diabetes, nephrotic syndrome and liver disease, except patient CT who had mild CHF when studied. Patients were titrated to, and studied at a dose capable of inducing an adequate pharmacodynamic response. Responders (group R) included those transplant patients who elicited an adequate natriuretic and diuretic response to smaller doses of furosemide such as 40-80 mg. Non-responders (group NR) were more refractory and required 120 mg or greater of furosemide in order to elicit an adequate response. Although patient SJ was studied at 120 mg oral and intravenous furosemide, she was assigned to group R. This clinical designation was based upon her extensive natriuretic and diuretic output at this dose, with a concomitant weight loss of 3.2 kg after oral administration. In addition, she had a substantial pharmacodynamic response with a 40 mg oral dose of furosemide, as will be shown subsequently.

Table VI-1. Patient Characteristics

Patient	Sex	Age (yr)	Weight (kg)	Cause of Renal Failure	CLcr[a] (ml/min)	Concomitant Drugs
CT	M	45	95.2	Nephrosclerosis	30.1	Prednisone, azathioprine, prazosin, calcium gluconate, bethanechol, minoxidil, aminophylline, isosorbide, metaproterenol.
EH	M	53	89.5	Glomerulonephritis	61.5	Prednisone, cyclophosphamide, propranolol, clonidine.
DH	F	25	65.5	Glomerulonephritis	37.3	Prednisone, azathioprine, cimetidine, hydralazine, propranolol.
LT	M	31	68.5	Nephrosclerosis	41.7	Prednisone, azathioprine, cephradine, flurazepam, pseudoephedrine.
VW	F	56	66.7	Glomerulonephritis	46.9	Prednisone, azathioprine, clonazepam, propranolol, isosorbide, diazepam, penicillin VK.
SJ	F	31	75.8	Glomerulonephritis	50.2	Prednisone, azathioprine, sulfisoxazole.
PD	M	48	67.1	Glomerulonephritis	68.0	Prednisone, azathioprine, sulfisoxazole, bethanechol.
WJ	F	35	68.9	Glomerulonephritis	88.1	Prednisone, azathioprine, diazepam.
FR	M	44	91.3	Unknown etiology	47.7	Prednisone, azathioprine, flurazepam, sulfisoxazole, propranolol, nitroglycerin, acetaminophen.
MEAN		41	76.5		52.4	
SD		11	12.1		17.7	

[a]Creatinine clearance was determined over a 24-hour period.

b. Study Design

After an overnight fast, each patient received either an oral or intravenous dose of furosemide at approximately 8 a.m. Furosemide tablets[1] (40 mg, lot # 601549) were taken with water or fruit juice; the intravenous solution[1] (10 mg/ml, lot #'s X619222 and 613379) was infused[2] over a 10 minute period. Blood samples (3 ml) after intravenous administration were obtained by an indwelling heparinized scalp vein needle[3] at 0, 10, 15, 20, 30, 45, 60, 80, 100, 120, 180, 240, 360, 480, and 1440 minutes; the end of the infusion period being 10 minutes. After oral administration, blood samples were taken at 0, 15, 30, 45, 60, 75, 90, 105, 120, 180, 240, 360, 480 and 1440 minutes. Voided urine was collected from -1 to 0, 0-1, 1-2, 2-3, 3-4, 4-5, 5-6, 6-7, 7-8, and 8-24 hours. Urine collection times differed in some patients depending upon the urge to void.

Patients fasted for at least two hours after oral administration of furosemide. In addition, furosemide was studied on consecutive days after oral and intravenous dosing, respectively. All patients signed the Consent Form approved by the Human Research Committee of the University of California, San Francisco.

c. Assay Procedures

Furosemide concentrations in plasma and urine samples were measured as described for Method II (Chapter II). However, chlorpromazine hydro-

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[1]Hoechst-Roussel Pharmaceuticals, Inc., Somerville, NJ.

[2]Harvard Apparatus Compact Infusion Pump, Harvard Apparatus, Millis, Mass.

[3]E-Z Set^{R} - PRN Intermittent Infusion Set, Deseret Pharmaceutical Co., Inc., Sandy, Utah

chloride (0.02%) was substituted as the internal standard for the analysis of furosemide in those patients concomitantly taking sulfisoxazole. This was necessary since sodium phenobarbital, the usual internal standard for furosemide, and sulfisoxazole have similar retention times and will interfere with each other. Under conditions identical to those described previously for Method II (Chapter II), chlorpromazine hydrochloride was measured by ultraviolet detection (254 nm) and had a retention time of 8.5 minutes (Fig. VI-1).

Sodium concentrations in urine samples were analyzed by flame photometry.[4] However, sodium concentrations were not measured for patient VW due to loss of the samples prior to the availability of the flame photometer. An estimate of urinary sodium was therefore made for patient VW based on the strong correlation between urine output and sodium excretion in the eight kidney transplant patients (Fig. VI-2; $r = 0.981$, $p < 0.001$).

d. Calculations

The half-life of furosemide, Terminal $T_{\frac{1}{2}}$, was determined by using at least four data points from the terminal portion of the plasma versus time plots following oral and intravenous administration.

The volume of distribution steady-state, Vdss, was determined from the intravenous plasma data by the compartment independent method of Benet and Galeazzi (111), corrected for infusion administration:

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

[4]Model 450, Corning Scientific Instruments, Medfield, Mass.

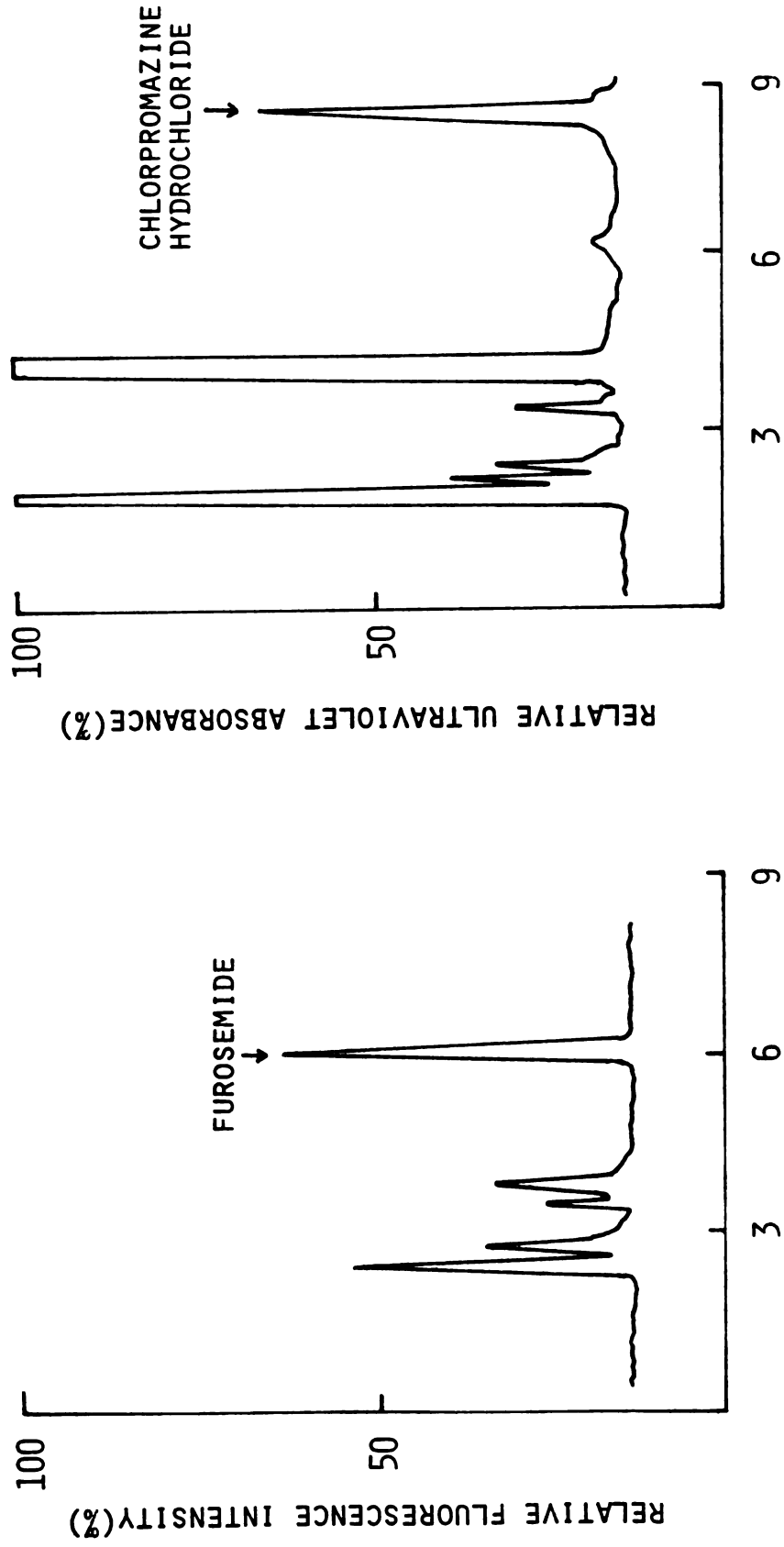

Fig. VI-1. Chromatograms developed using Method II for plasma sample containing furosemide (left) and the internal standard, chlorpromazine hydrochloride (right).

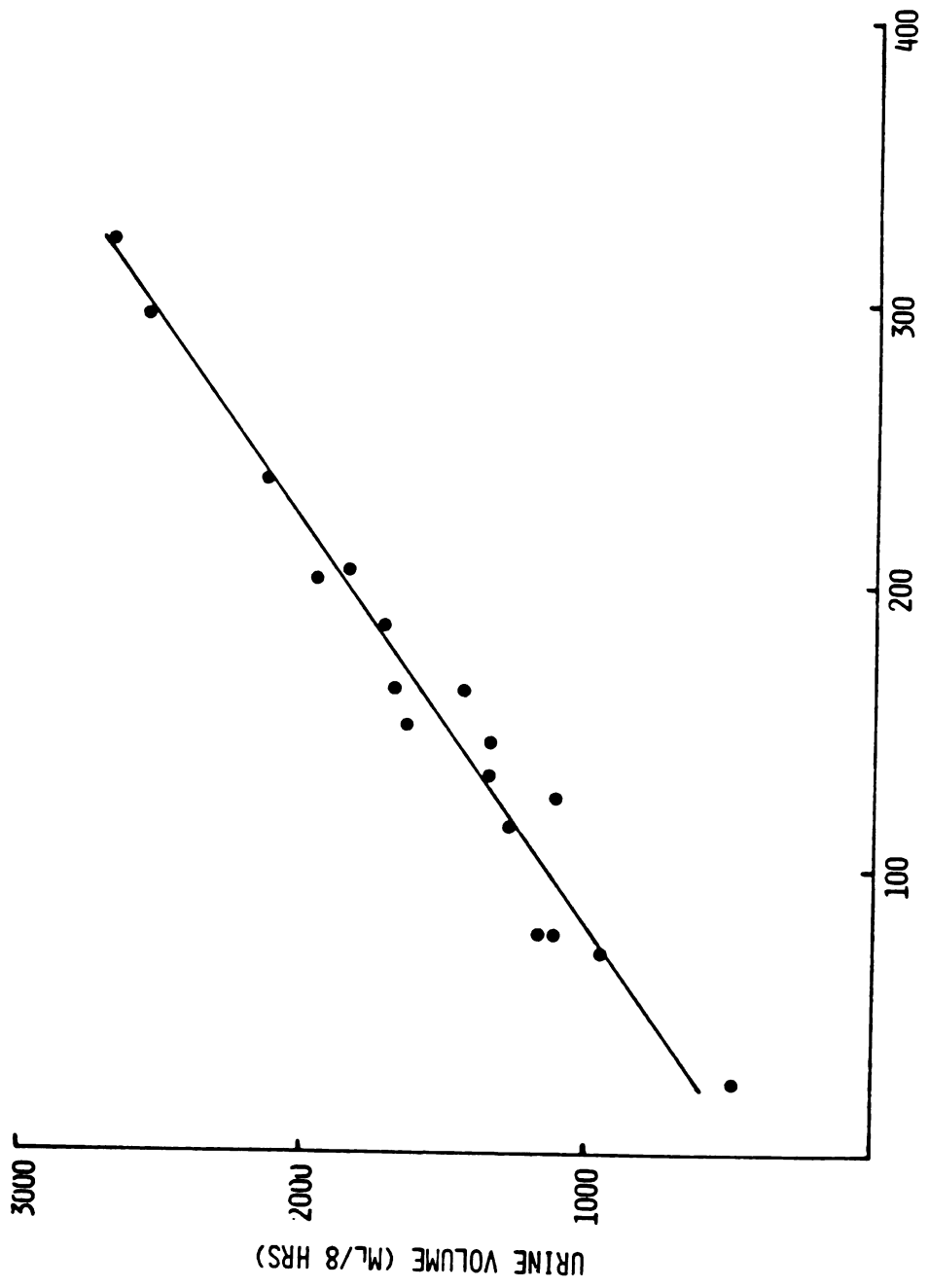

Fig. VI-2. Correlation between urine output and sodium excretion in kidney transplant patients ($r = 0.981$, $p < 0.001$).

where τ is the length of time during which the intravenous infusion was administered. All other pharmacokinetic parameters, including AUMC and AUC, were calculated as discussed in Chapter IV.

3. Results

The pharmacokinetics of oral and intravenous furosemide administration in kidney transplant patients are presented in Table VI-2. The volume of distribution steady-state was not significantly different between responders and non-responders (116 ± 36 for R vs. 112 ± 39 ml/kg for NR; $p > 0.50$) and was in good agreement with data previously published by Smith et al. (122, 123) in healthy volunteers. Non-responders had a significantly reduced plasma clearance (64.0 ±21.4 for NR vs. 105 ± 23 ml/min for R; $p < 0.05$) and renal clearance (18.4 ± 8.1 for NR vs. 47.1 ± 11.0 ml/min for R; $p < 0.005$) while non-renal clearance was lower (45.6 ± 16.1 for NR vs. 57.8 ± 23.7 ml/min for R; $p > 0.20$), but not to a statistically significant level. Values for half-life in responders were consistent with values previously reported by Smith et al. (122, 123) in healthy volunteers, but were significantly less than the half-lives in the non-responder population (87.6 ± 16.3 for R vs. 130 ± 13 min for NR; $p < 0.005$). Although the fraction excreted unchanged in the urine after intravenous administration was approximately 37% lower in non-responders, the magnitude of this change was not statistically significant (0.290 ± 0.086 for NR vs. 0.463 ± 0.143 for R; $p > 0.05$). In addition, no difference was observed in the extent of oral absorption between responder and non-responder populations (49.9 ± 6.9 for R vs 57.2 ± 24.7% for NR; $p > 0.50$) as well as compared to values in healthy volunteers (Table I-1). When renal clearance was corrected for kidney function (as determined by creatinine clearance), marked differences were observed in this value

Table VI-2. Furosemide Pharmacokinetics in Kidney Transplant Patients

Patient	Status	Treatment	CLp (ml/min)	Vdss (ml/kg)	Terminal $T_{\frac{1}{2}}$ (min)	CLr (ml/min)	CLnr (ml/min)	fe	F (%)	$\frac{CLr}{CLcr}$
CT	NR	160 mg po			138	10.8				0.36
		160 mg iv	60.4	77.5	138	10.7	49.7	0.176	74.6	0.36
EH	NR	120 mg po			120	22.1				0.36
		120 mg iv	84.6	110	116	23.0	61.6	0.272	30.4	0.37
DH	NR	120 mg po			174	10.3				0.28
		120 mg iv	35.6	92.9	143	12.4	23.3	0.348	81.6	0.33
LT	NR	120 mg po			137	23.8				0.57
		120 mg iv	75.4	167	122	27.4	48.0	0.363	42.4	0.66
MEAN	NR	po			142[b]	16.8[d]				0.39[d]
(SD)					(23)	(7.2)				(0.12)
MEAN	NR	iv	64.0[a]	112	130[c]	18.4[c]	45.6	0.290	57.2	0.43[c]
(SD)			(21.4)	(39)	(13)	(8.1)	(16.1)	(0.086)	(24.7)	(0.15)
VW	R	80 mg po			70.5	45.9				0.98
		80 mg iv	80.5	102	99.8	35.1	45.4	0.436	53.0	0.75
SJ	R	120 mg po			74.9*	43.5				0.87
		120 mg iv	122	173	74.5	41.0	81.0	0.336	38.6	0.82
PD	R	40 mg po			85.0	65.6				0.96
		40 mg iv	135	127	66.4	54.2	80.8	0.402	48.2	0.80
WJ	R	80 mg po			89.5	66.7				0.76
		80 mg iv	88.1	83.5	93.1	62.4	25.7	0.709	54.4	0.71
FR	R	80 mg po			119	50.4				1.06
		80 mg iv	98.9	95.1	104	42.8	56.1	0.432	55.3	0.90
MEAN	R	po			87.8[b]	54.4[d]				0.93[d]
(SD)					(19.0)	(11.0)				(0.11)
MEAN	R	iv	105[a]	116	87.6[c]	47.1[c]	57.8	0.463	49.9	0.80[c]
(SD)			(23)	(36)	(16.3)	(11.0)	(23.7)	(0.143)	(6.9)	(0.07)

[a]Level of significance between responder (R) and non-responder (NR) patients:

[a]$p<0.05$ [b]$p<0.01$ [c]$p<0.005$ [d]$p<0.001$

No significant difference was found between oral (po) and intravenous (iv) administration of furosemide to both R and NR patients.

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

(CLr/CLcr) between responder and non-responder patients (0.80 ± 0.07 for R vs. 0.43 ± 0.15 for NR; $p < 0.005$). There were no significant differences in the pharmacokinetic parameters between oral and intravenous treatments. It should be noted that the incremental renal clearances of furosemide were constant throughout each study for all the kidney transplant patients.

The pharmacodynamics of furosemide in kidney transplant patients after oral and intravenous administration as well as the amount of furosemide excreted unchanged in the urine after both treatments are presented in Table VI-3. As previously stated, transplant patients were titrated to, and studied at a dose capable of eliciting a sufficient natriuretic and diuretic response. Non-responders had a significant reduction in sodium excretion after oral dosing of furosemide (76.4 ± 44.2 for NR vs. 205 ± 97 Meq/8 hrs for R; $p < 0.05$), although equivalent amounts of unchanged drug were excreted in the urine as compared to responders (19.8 ± 8.9 for NR vs. 19.8 ± 8.6 mg for R; $p > 0.50$). Urine volume after oral administration of furosemide was also reduced in non-responders but not to a statistically significant level (996 ± 377 for NR vs. 1869 ± 730 ml/8 hrs for R; $0.10 > p > 0.05$). However, following intravenous administration, no difference was observed between responder and non-responder patients with respect to furosemide-induced natriuresis (184 ± 19 for R vs. 145 ± 68 Meq/8 hrs for NR; $p > 0.20$), diuresis (1727 ± 202 for R vs. 1546 ± 449 ml/8 hrs for NR; $p > 0.20$) and amount excreted unchanged in the urine (36.5 ± 14.5 for R vs. 36.6 ± 7.4 mg for NR; $p > 0.50$). In addition, an equivalent natriuretic (148 ± 100 for po vs. 167 ± 48 Meq/8 hrs for iv; $p > 0.50$) and diuretic (1481 ± 729 for po vs. 1647 ± 324 ml/8 hrs for iv; $p > 0.20$) response was observed for oral and intravenous dosing when data is averaged over all nine kidney transplant patients.

Table VI-3. Furosemide Pharmacodynamics in Kidney Transplant Patients

Patient	Status	Treatment	Sodium Excretion (Meq/8 hrs)	Urine Volume (Ml/8 hrs)	Ae^{∞} (mg)
CT	NR	160 mg po	77.8	1185	21.2
		160 mg iv	77.4	1129	28.2
EH	NR	120 mg po	25.5	489	9.7
		120 mg iv	116	1277	32.6
DH	NR	120 mg po	69.2	949	31.0
		120 mg iv	151	1644	41.8
LT	NR	120 mg po	133	1360	17.2
		120 mg iv	237	2136	43.6
MEAN	NR	po	76.4[c]	996	19.8
(SD)			(44.2)	(377)	(8.9)
MEAN	NR	iv	145	1546	36.6
(SD)			(68)	(449)	(7.4)
VW	R	80 mg po	118[a]	1278	21.8
		80 mg iv	167[a]	1627	34.9
SJ	R	40 mg po	145[b]	1353[b]	5.3[b]
		120 mg po	322	2686	16.1
		120 mg iv	185	1717	40.3
PD	R	40 mg po	125	1108	8.5
		40 mg iv	163	1456	16.1
WJ	R	80 mg po	296	2579	31.9
		80 mg iv	204	1854	56.7
FR	R	80 mg po	164	1695	20.7
		80 mg iv	203	1979	34.6
MEAN	R	po	205[c]	1869	19.8
(SD)			(97)	(730)	(8.6)
MEAN	R	iv	184	1727	36.5
(SD)			(19)	(202)	(14.5)

[a]Derived from linear regression analysis in Fig. VI-2.

[b]Values not included in the mean (SD) data.

[c]Level of significance, $p < 0.05$.

Ae^{∞} represents the amount of furosemide excreted in the urine unchanged.

4. Discussion

The therapeutic efficacy of furosemide varies widely among patients with different degrees of renal impairment (16, 22, 124). The ability of kidney transplant patients to respond to furosmide is quite unpredictable and higher doses of the drug are often needed in order to elicit an adequate diuresis and natriuresis. Possible mechanisms which may explain this resistance to furosemide effect include a reduced bioavailability, changes in drug metabolism, a decreased glomerular filtration rate and a reduction in renal tubular transport.

In healthy volunteers, the renal clearance of furosemide is about 120 ml/min (77, 122, 123) and the fraction of the dose excreted unchanged in the urine about 60-75% (71, 77, 122, 123). In the present study, the renal clearance for all nine kidney transplant patients ranged from 10.3 to 66.7 ml/min which is 8.6 to 56% the value found in healthy volunteers. However, marked differences were observed between responder and non-responder kidney transplant patients with respect to their renal clearances alone and when corrected for kidney function. In fact, the mean corrected renal clearance (CLr/CLcr) for the non-responder patients (0.43 ± 0.15) was approximately one-half the value found for responders (0.80 ± 0.07). Since furosemide is over 95% protein bound in plasma (8, 9, 77, 88, 123, 125, 126), glomerular filtration contributes minimally to the total renal clearance of the drug. Thus, the attenuated renal clearance of furosemide reflects an impairment in the secretory component of the organic acid transport system. This depression in renal transport can affect the urinary excretion rate of furosemide which has previously been shown to be the critical determinant with respect to diuretic and natriuretic effect (80, 108, 116, 122, 127).

In the present study, the attenuated renal clearance in non-responders necessitates the administration of larger doses of furosemide in order to achieve equivalent amounts of unchanged drug in the urine and therefore an equivalent pharmacodynamic effect to that of responders. This is demonstrated in Table VI-3 where responder and non-responder patients have virtually identical amounts of unchanged furosemide excreted in the urine after intravenous administration and is reflected by a similar response between the two groups. However, after oral administration of furosemide, non-responders have a significantly reduced natriuresis compared to responders although both groups excrete identical amounts of unchanged drug in the urine. This implies that non-responders (in comparison to responders) have a decreased ability to respond to equivalent amounts of furosemide excreted in the urine after oral dosing. Although speculative, it is possible that the "critical" luminal concentration/amount of furosemide needed for an adequate pharmacodynamic effect is higher in non-responders such that this "critical" level is reached after intravenous but not oral dosing. This may explain the apparent discrepancy as to why differences in natriuresis and diuresis exist between responders and non-responders after oral dosing but not after intravenous administration.

Although furosemide shows a trend toward reduced bioavailability in patients with renal impairment (9, 72, 74), this has not been a factor with respect to diuretic resistance (74). However, a recent case report (128) shows that an apparent resistance to oral furosemide treatment can be explained by reduced bioavailability of the drug in the edematous, as opposed to non-edematous state. In the present study, similar values for bioavailability were observed between responder and non-responder kidney transplant patients as well as compared with healthy volunteers (Table I-1).

Therefore, changes in the extent of oral absorption for furosemide as a viable explanation for its reduced effectiveness in kidney transplant patients must be discounted.

Previous studies in healthy volunteers (69, 71), heart failure patients (75) and "diuretic-resistant" patients (74) demonstrated that an equivalent diuretic response to furosemide was achieved whether the dose was administered orally or by intravenous injection. However, in uremics, Huang et al. (73) found the diuresis produced by oral furosemide to always be less effective than after intravenous dosing. In the present study, five out of the nine kidney transplant patients demonstrated a substantial increase (>25%) in natriuresis and diuresis after intravenous administration of furosemide as compared to oral dosing. In patients EH, DH and LT the difference in response between oral and intravenous treatments was quite substantial (about two-fold or greater). However, patients CT and FR showed an equivalent natriuretic and diuretic effect between treatments while patients SJ and WJ had a more pronounced response after oral furosemide administration. These results demonstrate a considerable variability in the natriuretic and diuretic response of kidney transplant patients to oral and intravenous dosing of furosemide. Nevertheless, no significant difference in pharmacodynamic response was observed whether furosemide was given orally or by intravenous infusion when all nine patients are considered as a group. Factors such as uncontrolled fluid intake and lack of electrolyte/water replacement may have contributed to this variability and thereby complicate interpretation of the pharmacodynamic data. However, a more controlled study was not ethically possible due to the clinical condition of the patient population involved. These studies were carried out as the drug is used clinically.

An unusual plasma concentration vs. time profile of furosemide was observed in patient SJ in which the terminal slopes after oral and intravenous

dosing were found to be dissimilar (Fig. VI-3). Upon 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 greater than zero suggests a lag time before absorption. In this case, there was a lag time of about 50 minutes with a peak concentration of furosemide in plasma not being reached until 4 hours after dosing. The delayed absorption of furosemide in patient SJ may also be present in other kidney transplant patients, perhaps to a lesser degree, and contribute to the unpredictability of assessing the diuretic and natriuretic response to furosemide.

A recent case (129) reported furosemide to have a half-life around 4 days in a 39 year old kidney transplant patient studied post-operatively for 26 days. During the first 10 days after transplantation the patient had lost 172 liters of urine. The authors speculate that this massive diuresis may be due to a depot effect of furosemide in which the drug accumulated in body tissues during high dose furosemide treatment prior to transplantation. In the present study, kidney transplant patients were studied at least 18 days after surgery. The mean half-lives for furosemide in responder and non-responder patients were 87.6 and 130 minutes, respectively. These values are in sharp contrast to the 4 day half-life reported above (129) and would argue against a similar depot effect being present in the nine kidney transplant patients of this study.

The fractional areas under the plasma concentration-time profile determine the importance of a particular phase in defining drug kinetics during multiple dosing since area under the curve is inversely proportional to

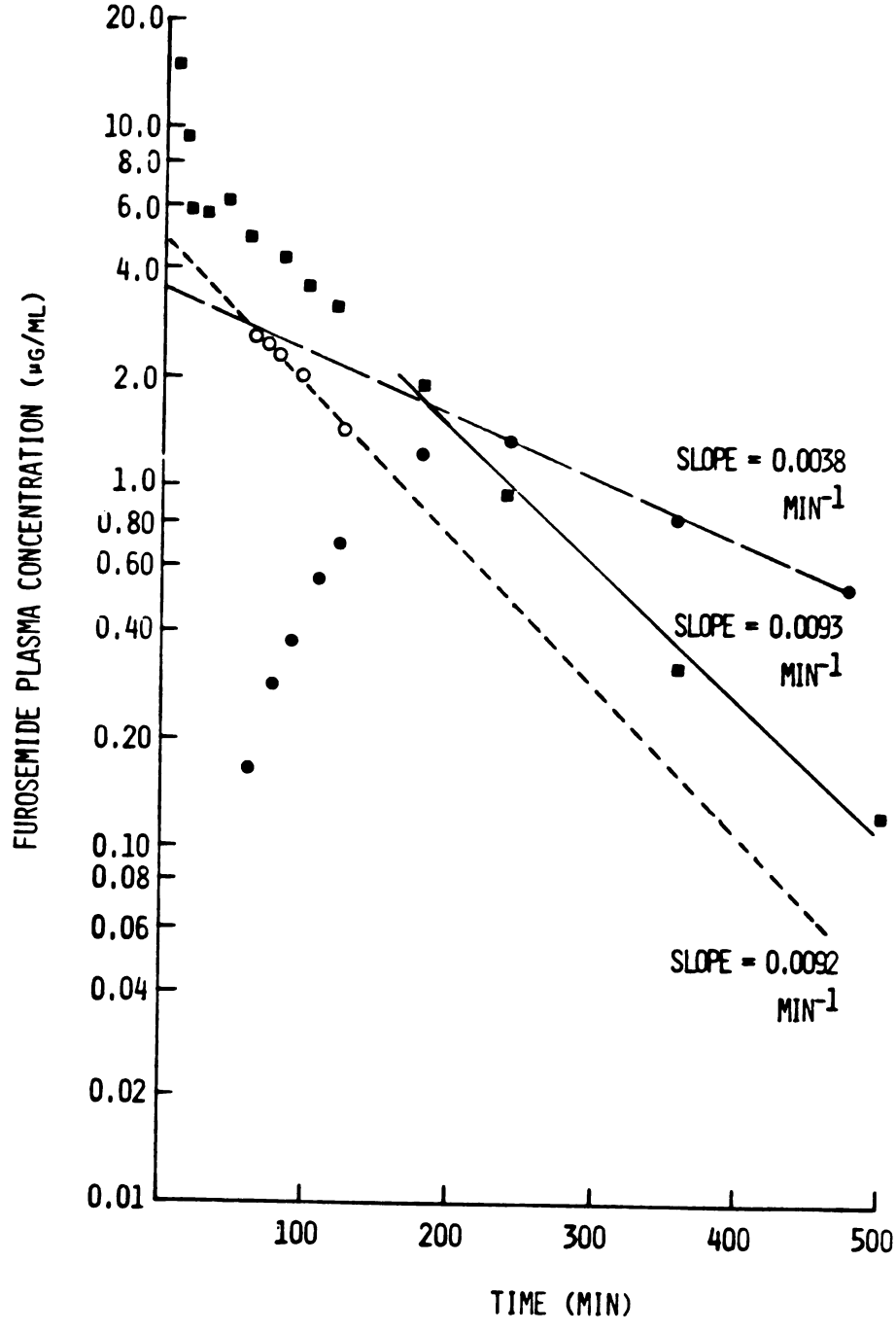

Fig. VI-3. Plasma concentration vs. time profile of furosemide in patient SJ after oral (●) and iv (■) dosing; (○) represents the residual slope from the feathered oral curve.

the total plasma clearance of the drug. Table VI-4 demonstrates that the area under the curve of furosemide during the terminal phase of elimination (A_T/λ_T) comprises a greater percentage of the total area under the curve (AUC) in transplant patients than in healthy volunteers (64.1 ± 15.5 for patients vs. 27.8 ± 4.6 % for volunteers; $p < 0.001$). This indicates that in contrast to healthy volunteers, the terminal phase of furosemide elimination in transplant patients plays a major role in defining its kinetics. However, accumulation of furosemide in kidney transplant patients during multiple dosing is highly unlikely since the half-life of the drug is much smaller than its usual dosing interval of one day.

5. Summary

The results of this study imply that non-responder kidney transplant patients (in comparison to responders) have a reduced ability to secrete furosemide into tubular fluid as well as a decreased ability to respond to equivalent amounts of drug excreted in the urine. In addition, the intravenous administration of furosemide offers no real advantages over oral dosing for continued therapy except when the oral route is not possible or a rapid onset of diuresis is required.

B. Biotransformation of Furosemide

1. Specific Objectives

In patients with renal disease, the urinary excretion of unchanged furosemide is impaired and other non-renal elimination pathways become

Table VI-4. Fractional Area Under the Curve of Furosemide during the Terminal Phase of the Plasma Concentration-Time Profile in Healthy Volunteers and Kidney Transplant Patients

Volunteers[a]	AUC ($\frac{\mu g \cdot min}{ml}$)	(A_T/λ_T) ($\frac{\mu g \cdot min}{ml}$)	$(A_T/\lambda_T)/AUC$ (%)	Patients	AUC ($\frac{\mu g \cdot min}{ml}$)	A_T/λ_T ($\frac{\mu g \cdot min}{ml}$)	$(A_T/\lambda_T)/AUC$ (%)
1	211	46.5	22.0	CT	2649	1589	60.0
2	215	66.9	31.1	EH	1418	921	65.0
3	351	84.8	24.2	DH	3371	2703	80.2
4	254	84.4	33.2	LT	1592	1327	83.4
5	289	103	35.6	VW	994	580	58.4
6	225	61.3	27.2	SJ	984	839	85.3
7	253	68.3	27.0	PD	296	144	48.6
8	210	49.8	23.7	WJ	908	403	44.4
9	239	62.8	26.3	FR	809	419	51.8
		MEAN	27.8[b]			MEAN	64.1[b]
		(SD)	(4.6)			SD	(15.5)

[a]Furosemide was administered as a 40 mg intravenous dose (Chapter IV).

[b]Level of significance, $p < 0.001$.

more prominent (82, 85). The metabolism of furosemide has been studied in healthy volunteers and in various patient populations (83, 97, 123), but not in kidney transplant patients. In addition, the data concerning the metabolic fate of furosemide is controversial due to analytical problems as previously discussed in Chapters II and IV. The present investigation was undertaken in order to define, in kidney transplant patients, the metabolism of furosemide using a specific HPLC assay.

2. Methods

a. Patient Selection

The same kidney transplant patients were participants as described for the pharmacokinetic/pharmacodynamic evaluation of furosemide in Section A of this chapter.

b. Study Design

All conditions were identical to those described in Section A of this chapter.

c. Assay Procedures

Furosemide concentrations in urine samples (with and without β-glucuronidase treatment) were measured as described for Method II (Chapter II). Chlorpromazine hydrochloride was substituted for sodium phenobarbital as the internal standard for the analyses of furosemide in those patients con-

comitantly taking sulfisoxazole as discussed in Section A of this chapter.

Urine samples were analyzed for CSA using HPLC with fluorescence detection as previously discussed (Chapter II). The sensitivity for this direct injection method is 2.5 μg/ml using 0.05 ml urine samples.

d. Calculations

The percent of the available dose of furosemide excreted as the glucuronide metabolite; fGL was calculated as in Chapter IV. The equation assumes a negligible first-pass effect for the metabolism of furosemide to its glucuronide metabolite, as will be discussed subsequently.

3. Results

The putative metabolite of furosemide, CSA was sought in the urine samples of kidney transplant patients after both oral and intravenous administration. No evidence of this metabolite was found in any of the samples analyzed.

Table VI-5 details the urinary excretion of furosemide and its glucuronide metabolite after both oral and intravenous treatments. Urinary recovery of the parent compound and metabolite accounted for only 45.2 ± 17.4 % of the intravenous dose. The amount of furosemide excreted as the glucuronide metabolite was approximately 8% of the available dose and varied considerably between patients, as reflected by a coefficient of variation of almost 50%. Nevertheless, as shown in Fig. VI-4 a significant positive correlation was observed between the percent of the available dose

Table VI-5. Urinary Excretion[a] of Furosemide and its Conjugated Metabolite in Kidney Transplant Patients

Patient	Treatment	Unchanged Furosemide (mg)[b]	Conjugated Furosemide (mg)[c]	fGL (%)
CT	160 mg po	21.2	3.2	2.7
	160 mg iv	28.2	7.6	4.8
EH	120 mg po	9.7	3.6	9.9
	120 mg iv	32.6	9.7	8.1
DH	120 mg po	31.0	8.9	9.1
	120 mg iv	41.8	8.4	7.0
LT	120 mg po	17.2	1.8	3.5
	120 mg iv	43.6	5.9	4.9
VW	80 mg po	21.8	--[d]	--[d]
	80 mg iv	34.9	--	--
SJ	120 mg po	16.1	2.4	5.2
	120 mg iv	40.3	2.4	2.0
PD	40 mg po	8.5	2.9	15.0
	40 mg iv	16.1	3.9	9.8
WJ	80 mg po	31.9	4.5	10.3
	80 mg iv	56.7	8.2	10.2
FR	80 mg po	20.7	6.0	13.6
	80 mg iv	34.6	8.5	10.6
		MEAN (SD)	po	8.7 (4.5)
		MEAN (SD)	iv	7.2 (3.1)

[a]Collection period of 24 hours.

[b]Values were previously reported (Table VI-3).

[c]Expressed in furosemide weight units.

[d]Insufficient sample.

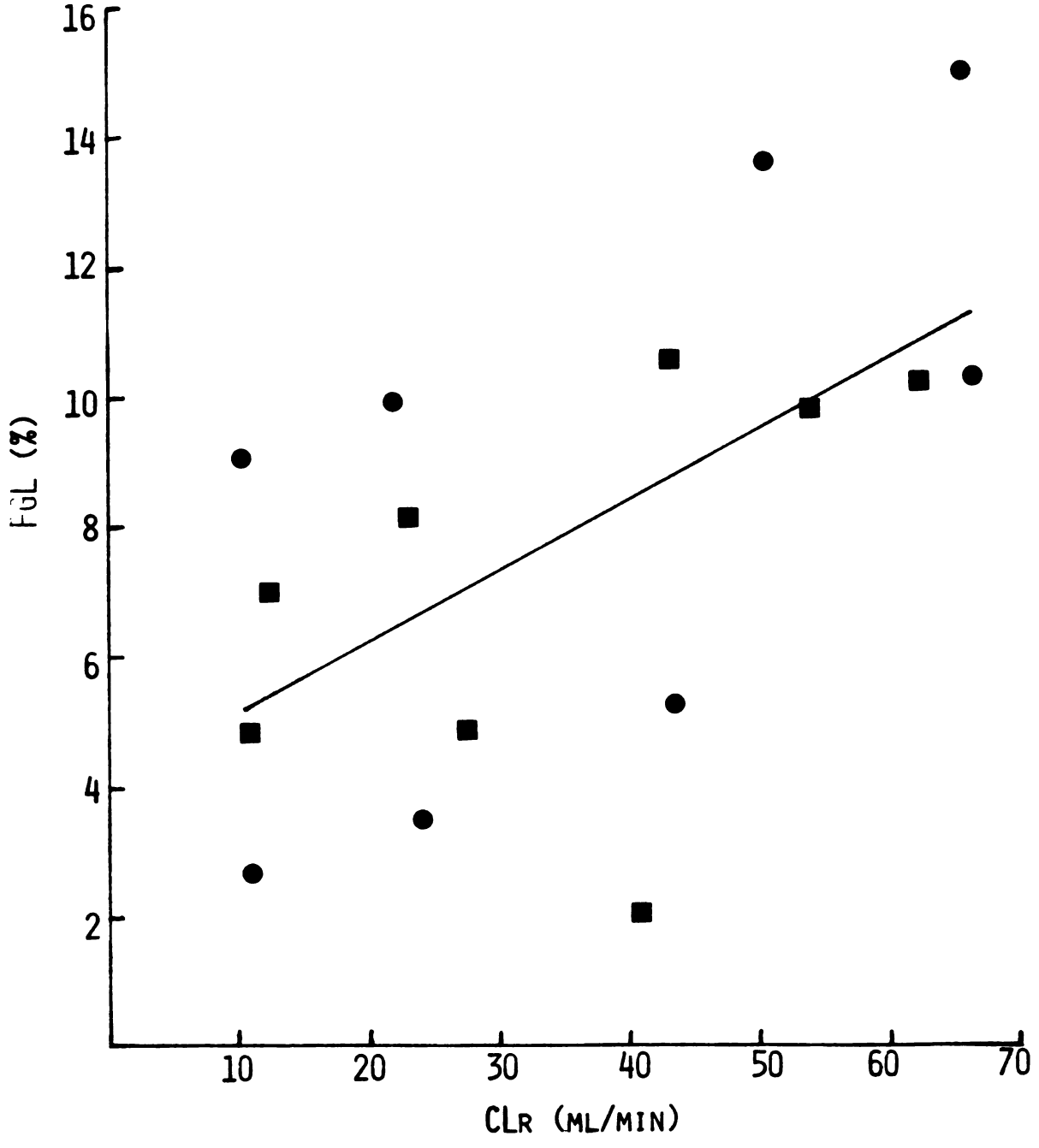

Fig. VI-4. Correlation between the percent of the available dose of furosemide excreted as glucuronide metabolite (fGL) and furosemide renal clearance (CLr) after oral (●) and intravenous (■) dosing of furosemide to kidney transplant patients ($r = 0.581$, $p < 0.02$).

excreted as furosemide glucuronide and the renal clearance of furosemide in the kidney transplant patients studied ($r = 0.581$, $p < 0.02$). In addition, no significant difference was found in the percent of the available dose excreted as furosemide glucuronide with respect to route of administration (8.7 ± 4.5 for po vs. 7.2 ± 3.1% for iv; $p > 0.10$; paired t-test).

4. Discussion

It has been clearly demonstrated in healthy volunteers that CSA is not formed *in vivo*, but is an analytical artifact formed during an acid extraction procedure (Chapter IV). In the present study, the putative metabolite of furosemide, CSA was not detected in the urine samples of kidney transplant patients. This finding supports the contention that CSA is not a metabolite of furosemide.

In healthy volunteers, the urinary excretion of furosemide glucuronide accounted for approximately 14% of the available dose, whether given orally or by intravenous administration (Chapter IV). In contrast, Andreasen and Mikkelsen (77) found the urinary excretion of the glucuronide metabolite to account for only 2% of the intravenous dose in volunteers and heart failure patients not previously receiving furosemide. However, in patients on chronic furosemide treatment ($\geq$ 6 months), approximately 16% of the intravenous dose of furosemide was excreted in the urine as a glucuronide. The authors (77) speculate that chronic administration of furosemide may be able to induce the glucuronidation process. Recently, Perez *et al.* (86) reported the glucuronide metabolite of furosemide to be the major biotransformation product in patients with acute pulmonary edema. The excretion of furosemide glucuronide accounted for 3.3-40.4% of the intravenous dose and was not

related to creatinine clearance, the severity of pulmonary edema or the presence of myocardial infarction. However, their results are suspect since alkaline conditions used in their analytical procedure may lead to degradation of furosemide glucuronide to the parent drug (Chapter IV).

The significant positive correlation between the percent of the available dose excreted as furosemide glucuronide and the renal clearance of furosemide (Fig. VI-4) suggests that the biotransformation of furosemide to its glucuronide metabolite may be occurring in the kidney. This hypothesis is supported by the fact that the ratio of the amount of furosemide glucuronide to unchanged furosemide in the urine is similar between the kidney transplant patients (0.23 ± 0.10 for po; 0.21 ± 0.08 for iv) in this study and the healthy volunteers (0.22 ± 0.05 for po; 0.21 ± 0.04 for iv) reported in Chapter IV. In addition, no significant difference was observed in the percent of the available dose of furosemide excreted as glucuronide metabolite, whether the drug was administered orally or intravenously to kidney transplant patients and healthy volunteers (Tables VI-5 and IV-2, respectively). This implies that the first-pass effect for hepatic and gut wall metabolism of furosemide to its glucuronide metabolite is probably negligible. In contrast, Andreasen et al. (88) demonstrated a highly significant negative correlation between fraction of furosemide excreted as glucuronide and the serum clearance in patients with severe arterial hypertension, which would seem to indicate that greater metabolism results from prolonged drug residence in the body. One can calculate from their data that a highly significant negative correlation also exists between fraction excreted as furosemide glucuronide and the renal clearance.

Total urinary recovery of furosemide and its glucuronide metabolite accounted for only 45% of the intravenous dose. The remainder of the dose

was probably excreted into the feces via the biliary route, either as unchanged furosemide and/or furosemide glucuronide. This is consistent with previous studies (82,85) which demonstrate that over 60% of furosemide-S^{35} can be recovered in the feces after intravenous administration of drug to patients with impaired renal function.

5. Summary

No evidence of CSA, the putative metabolite of furosemide was found in the urine samples of kidney transplant patients. This supports previous studies in healthy volunteers (Chapter IV) which demonstrate it to be an analytical artifact. Glucuronidation accounted for about 8% of the available dose of furosemide and may be occurring in the kidney. In addition, only 45% of the intravenous dose could be recovered in the urine (furosemide and glucuronide metabolite). The remainder is probably excreted in the feces via the biliary route.

Chapter VII. Plasma Protein Binding and Red Blood Cell Partitioning of Furosemide

A. Healthy Volunteers

1. Plasma Protein Binding

a. Objective

The degree of binding of furosemide to plasma proteins has been reported for healthy volunteers and various patient populations (83). However, the accuracy of these reported values must be questioned since those assays employing acid and/or base extractions (8, 77, 88, 125) may be inaccurate due to analytical problems as previously discussed in Chapter IV. In addition, studies using radiolabelled drug (9, 126, 130) are not suitable for *in vivo* protein binding determinations since they will not differentiate between furosemide and its metabolite(s). The present study was undertaken in order to determine the *in vivo* binding of furosemide to plasma proteins using a sensitivie and specific HPLC assay.

b. Methods

(i) Subject Selection

Participants were the same volunteers as previously described in Chapter IV.

(ii) Study Design

All conditions were identical to those described in Chapter IV. However, only furosemide plasma samples following intravenous administration were assayed for the protein binding determinations.

(iii) Assay Procedures

Equilibrium dialysis[1] was performed on furosemide plasma samples taken after intravenous administration which yielded a concentration range of 0.34 - 7.40 µg/ml. One-half ml of plasma was dialyzed against 0.5 ml of isotonic Krebs Ringer Bicarbonate buffer (pH 7.4) in a shaking incubator bath[2] at 37°C for 5 hours. Preliminary studies indicated that equilibrium was achieved within 2 hours and remained constant for 24 hours using Spectrapor 2 membrane tubing[3] (45 mm x 50 ft; 12,000-14,000 MWCO).

Free furosemide concentrations were determined in the following manner. Three-tenths ml of dialyzed buffer was mixed with 50 µl of the internal standard, sodium phenobarbital (2.5 mg/ml) and evaporated under nitrogen gas until about 0.10 ml remained. The mixture was then injected directly onto the HPLC system. Instrumentation settings and solvent strength were the same as detailed in Chapter II on measurement of furosemide in plasma (Method II). A typical standard curve of furosemide/sodium phenobarbital

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[1]Model 260 Equilibrium Type Dialysis Cells, Technilab Instruments, Inc. Pequannock, NJ.

[2]Dubnoff Metabolic Shaking Incubator (Precision Scientific 66722), Scientific Products, Menlo, CA.

[3]Spectrum Medical Industries, Inc., Los Angeles, CA.

peak height ratio over the buffer concentration range 5.5-137 ng/ml resulted in the following linear least squares regression equation: $Y = 0.075\ X + 0.011$ ($r^2 = 0.999$). Plasma samples were assayed for total furosemide (bound and free) for the volunteers as previously described in Chapter IV.

(iv) Calculations

The percent free or percent of furosemide unbound to plasma proteins (α) was calculated as:

$$\alpha = 100\ X\ 1/(Cp/Cu' - 1) \qquad \text{(Eq. 1)}$$

where Cp represents the total plasma concentration of furosemide prior to dialysis and Cu' represents the unbound or free concentration of furosemide in buffer after dialysis. Equation 1 assumes that the initial plasma and buffer volumes are equal prior to dialysis, that there is negligible binding of drug to the dialysis membrane (less than 2% for furosemide) and that protein binding is linear.

c. Results

The binding of furosemide to plasma proteins in nine healthy volunteers is presented in Table VII-1. Plasma samples taken from 10-120 minutes after intravenous administration ranged from 0.34 - 7.40 μg/ml total furosemide. The range in percent free of furosemide between volunteers was 0.9 - 1.5%. The intersubject variability in α (Mean ± SD) was 1.2 ± 0.2.

Table VII-1. Plasma Protein Binding of IV Furosemide to Healthy Volunteers

Subject	Percent[a] Free	SD
1	1.5	0.2
2	1.5	0.2
3	1.3	0.2
4	1.2	0.1
5	1.3	0.1
6	1.0	0.03
7	1.1	0.2
8	1.2	0.2
9	0.9	0.05
Intersubject Variability	1.2±0.2	

[a]The percent free for each subject represents the mean value for plasma samples taken from 10-120 minutes after intravenous administration (intrasubject variability).

d. Discussion

The binding of furosemide to plasma proteins was found to be about 98-99%. This value, although somewhat higher is consistent with values from previous in vitro (9, 88, 125, 126, 130) and in vivo (8, 77) studies. Differences most probably reflect the methodologies utilized in studying protein binding as well as the fact that some investigators (8, 9, 77, 88, 125, 130) incubated the samples at room temperature which tends to decrease the protein binding of furosemide (126).

2. Red Blood Cell Partitioning

a. Methods

Duplicate samples of heparinized whole blood (2 ml) from a healthy volunteer were spiked with furosemide (concentrations 1, 2 and 3 μg/ml) and incubated in a shaker bath[2] for 1 hour at 37 °C. After incubation, the plasma was separated from the red blood cells (RBC) by centrifuging[4] for 10 minutes, and assayed for furosemide (Method I, Chapter II).

b. Calculations

The relationship between the blood (Cblood) and plasma (Cplasma) concentrations may be expressed as:

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[4]Model HN-SII, VWR Scientific, San Francisco, CA.

$$\frac{Cblood}{Cplasma} = (1-H) + H \cdot \lambda \qquad \text{(Eq. 2)}$$

where H is the hematocrit and λ represents the ratio between drug concentration in the red blood cells (Crbc) and Cplasma. Equation 2 is simply a rearrangement of the mass balance statement that the total amount of drug in the blood equals the amount in the plasma plus the amount in the red blood cells. Rearrangement of Equation 2 allows one to solve for λ:

$$\lambda = (\frac{Cblood}{Cplasma} + H - 1)/H \qquad \text{(Eq. 3)}$$

c. Results

The partitioning of furosemide between red blood cells and plasma at three different concentrations is shown in Table VII-2. The average partitioning of furosemide into red blood cells, λ, for 6 measurements was 0.50 ± 0.06.

B. Kidney Transplant Patients

1. Plasma Protein Binding

a. Objective

It is generally recognized that the binding of a drug to plasma proteins can affect its distribution, elimination and ultimately its therapeutic or toxic response since only the unbound drug is pharmacologically active.

Table VII-2. Red Blood Cell Partitioning of Furosemide[a]

Cblood (μg/ml)	Cplasma (μg/ml)	λ[b]
1.0	1.42	0.39
1.0	1.30	0.53
2.0	2.59	0.53
2.0	2.61	0.53
3.0	3.93	0.51
3.0	3.92	0.53

[a]Volunteer had a hematocrit of 0.49.

[b]λ = Crbc/Cplasma (estimated using Equation 3).

It has also been established that renal impairment may alter drug binding to plasma proteins (131-135), particularly with respect to acidic drugs (133). Possible explanations for reduced drug binding in patients with renal dysfunction include hypoalbuminemia (132), the presence of irreversible and competitive inhibitors in the plasma (133, 135) and altered albumin composition (131).

Furosemide is highly bound to plasma proteins (8,9) and gains access to its site of action in the kidney lumen primarily through active secretion via the non-specific organic acid secretory pathway (5,6,10). Previous studies have shown that renal disease can effect dramatic changes in the pharmacokinetics of furosemide (Table I-3), including impaired plasma protein binding in uremics (9,125), nephrotics (9,126) and anephric patients (8). The degree of binding of furosemide to plasma proteins in kidney transplant patients has not been reported. Since only the free drug is presumed to be transported by the kidney to its site of action in the tubular fluid, it may be important to understand the role of plasma protein binding with respect to the natriuretic and diuretic response to furosemide.

b. Methods

(i) Patient Selection

Participants were the same kidney transplant patients as previously described in Chapter VI.

(ii) Study Design

All conditions were identical to those described in Chapter VI. However, only furosmide plasma samples following intravenous administration were assayed for the protein binding determinations.

(iii) Assay Procedures

The in vivo binding of furosemide to plasma proteins was determined using the equilibrium dialysis method previously discussed in Section A of this chapter.

Free furosemide concentrations were determined as described in Section A of this chapter and total furosemide concentrations as described in Section A of Chapter VI. Chlorpromazine hydrochloride was substituted for sodium phenobarbital as the internal standard for the analyses of furosemide (free and total) in those patients concomitantly taking sulfisoxazole as discussed in Section A of Chapter VI.

(iv) Calculations

In cases of nonlinear plasma protein binding, Equation 1 (Section A of this chapter) is inappropriate and will underestimate the true value for the percent free of drug in the original plasma sample. Patient EH displayed nonlinear binding of furosemide to plasma proteins and values for percent free were determined in this patient according to the procedure of Behm and Wagner (136), as described below.

The total plasma concentration of furosemide after dialysis (C_p') was calculated using mass balance and is given by Equation 4:

$$Cp' = (Vp \cdot Cp - Vu' \cdot Cu')/Vp' \qquad \text{(Eq. 4)}$$

where Cp and Cu′ were experimentally determined. The plasma volumes prior to (Vp) and after dialysis (Vp′) as well as the buffer volume after dialysis (Vu′) were assumed to remain constant during the dialysis experiment.

The bound plasma concentration of furosemide after dialysis (Cb′) was also calculated using mass balance and is given by Equation 5:

$$Cb' = Cp' - Cu' \qquad \text{(Eq. 5)}$$

The free and bound equilibrium concentrations of furosemide were best fitted to a conventional protein binding model for a single Langmuir term plus a linear term:

$$Cb' = P1 \cdot Cu'/(P2 + Cu') + P3 \cdot Cu' \qquad \text{(Eq. 6)}$$

Other protein binding models were tested (single Langmuir and double Langmuir), but the data did not fit them as well, as determined by the values for the coefficient of determination and the residual sum of squares. The above Langmuir-type protein binding model can be modified to give the quadratic equation of Equation 7:

$$(1 + P3) \cdot Cu^2 + (P1 + P2 + P2 \cdot P3 - Cp) \cdot Cu - P2 \cdot Cp = 0 \qquad \text{(Eq. 7)}$$

where the binding parameters P1, P2 and P3 were obtained from a computer fit to the Langmuir-type model in Equation 6. Values for the free plasma concentrations of furosemide prior to dialysis or in the original plasma sample (Cu) were obtained by finding the positive root of the quadratic

Equation 7 for a given value of Cp.

The percent free of drug in the original plasma sample (α) can now be calculated using Equation 8:

$$\alpha = 100 \text{ X } Cu/Cp \qquad \text{(Eq. 8)}$$

The above equations were used for patient EH who demonstrated nonlinear binding of furosemide to plasma proteins. Values for percent free of furosemide in plasma for all other kidney transplant patients were determined using Equation 1.

c. Results

The plasma protein binding of furosemide in kidney transplant patients is presented in Table VII-3. Serial plasma samples taken after intravenous administration ranged from 0.32 - 124 μg/ml total furosemide. The variability between patients in percent free of furosemide was substantial as evidenced by an approximate 50% coefficient of variation.

Patient EH demonstrated nonlinear protein binding as displayed in Fig. VII-1. The bound and free equilibrium plasma concentrations were fitted to Equation 6 by nonlinear least squares using the MULTIFUN procedure of Prophet (a specialized computer resource developed by the Chemical/Biological Information Handling Program of the National Institutes of Health). The parameters obtained by computer fitting were P1 = 4.02, P2 = 0.0525 and P3 = 4.54 (r^2 = 0.980). Using the above parameters, and the original total plasma concentrations, the corresponding free concentrations of furosemide were estimated and appropriate values for percent free were obtained. Patient EH had an approximate 10-fold range in percent free

Table VII-3. Plasma Protein Binding of IV Furosemide in Kidney Transplant Patients.

Patient	Status	Treatment	Serum Albumin Conc. (gm %)	Percent[a] Free	SD
CT	NR	160 mg iv	4.4	1.6	0.3
EH	NR	120 mg iv	3.4	1.3-12.9[b]	---[b]
DH	NR	120 mg iv	4.2	1.6	0.2
LT	NR	120 mg iv	3.9	2.2	0.1
VW	R	80 mg iv	4.4	--[c]	--[c]
SJ	R	120 mg iv	4.1	5.2	2.2
PD	R	40 mg iv	3.7	3.7	1.2
WJ	R	80 mg iv	4.5	1.5	0.1
FR	R	80 mg iv	3.5	4.2	1.4
Intersubject Variability					
	Total (n=7)			2.9±1.5	

[a]The percent free for each patient represents the mean value for at least 8 serial plasma samples taken after iv administration (intrasubject variability).

[b]The range of values for percent free in patient EH are reported due to nonlinear plasma protein binding. These values were calculated as described by Behm and Wagner (136) and were excluded from the intersubject variability results.

[c]Insufficient plasma sample.

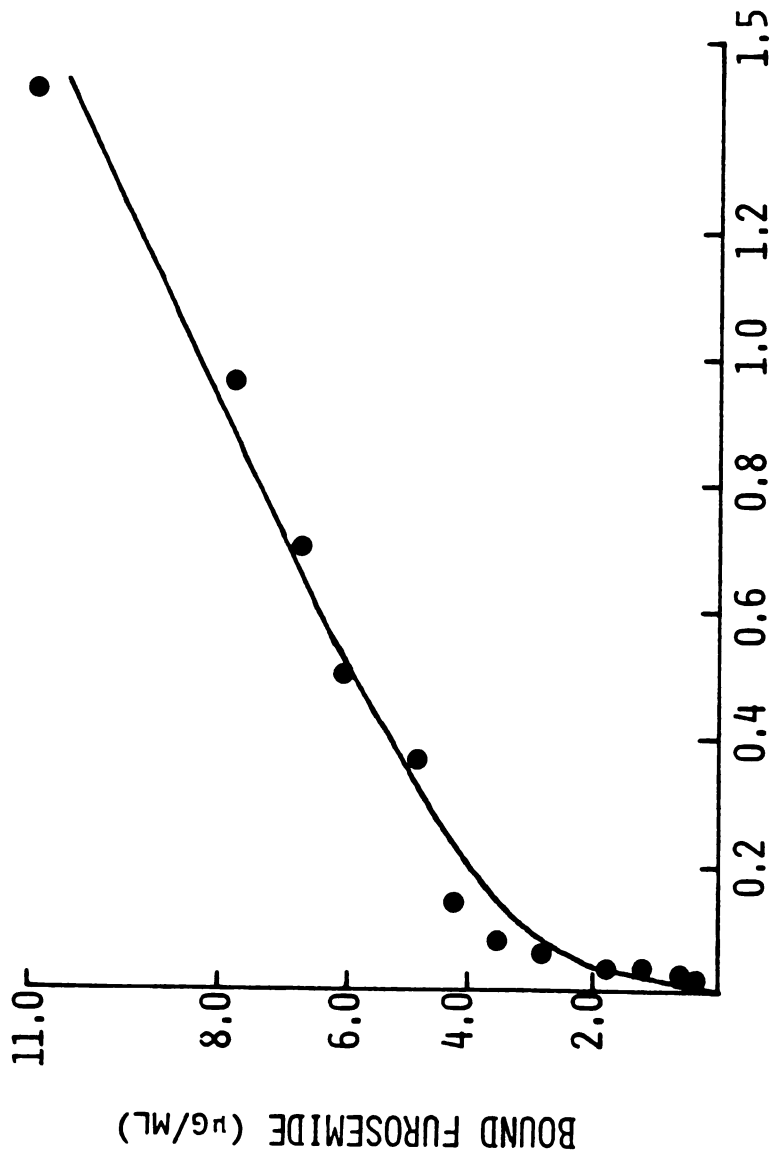

Fig. VII-1. Relationship between bound and free equilibrium plasma concentrations of furosemide in kidney transplant patient EH.

over the total furosemide plasma concentrations studied (Fig. VII-2).

Table VII-4 compares the values for percent free of furosemide between healthy volunteers (controls), kidney transplant patients not taking sulfisoxazole concomitantly (KT-) and transplant patients taking sulfisoxazole concomitantly (KT+) with furosemide. The results demonstrate that those kidney transplant patients who are on concomitant sulfisoxazole treatment have a significantly greater percent free of furosemide as compared to transplant patients not on sulfisoxazole (4.4 ± 0.8 for KT+ vs. 1.7 ± 0.3% for KT-; $p < 0.01$) as well as to healthy volunteers (4.4 ± 0.8 for KT+ vs. 1.2 ± 0.2% for controls; $p < 0.01$). In addition, kidney transplant patients not on concomitant sulfisoxazole treatment had a significantly higher value for percent free of furosemide with respect to healthy volunteers (1.7 ± 0.3 for KT- vs. 1.2 ± 0.2% for controls; $p < 0.05$).

d. Discussion

Impaired binding of furosemide to plasma proteins has been reported in uremics (9, 125), nephrotics (9, 126) and anephric patients (8). In the present study, the percent free of furosemide in plasma was significantly greater in kidney transplant patients (KT- and KT+) than in healthy volunteers. This was probably due to the presence of endogeneous and exogenous (drugs) substances which compete with furosemide for binding sites on the plasma proteins. Since furosemide is exclusively bound to albumin (125, 126), the presence of hypoalbuminemia has been postulated (9) as a possible cause for reduced drug binding of furosemide in patients with renal dysfunction. However, the kidney transplant patients in this study were normal with respect to serum albumin levels and no significant correlation was observed between percent free of furosemide and albumin

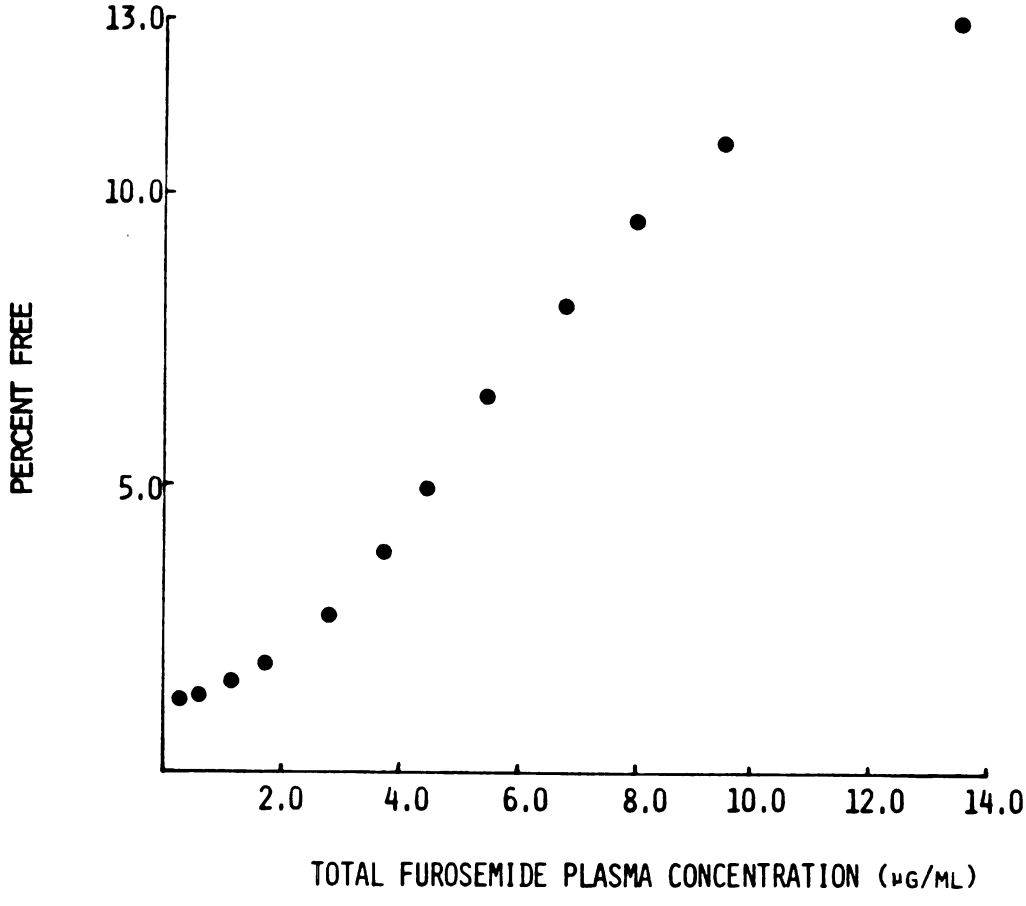

Fig. VII-2. Relationship between percent free and total furosemide plasma concentrations in kidney transplant patient EH.

Table VII-4. Comparison of Plasma Protein Binding of IV Furosemide in Healthy Volunteers and Kidney Transplant Patients

	Controls[a] (n = 9)	KT-[b] (n=4)	KT+[c] (n=3)
Percent free	1.2	1.7	4.4
SD	0.2	0.3	0.8
Level of Significance[d]		($p < 0.01$)	

Comparison	Inference[e]
KT+ vs. Controls	$p < 0.01$
KT+ vs. KT-	$p < 0.01$
KT- vs. Controls	$p < 0.05$

[a]Values were previously reported (Table VII-1).

[b]Kidney transplant patients not concomitantly taking sulfisoxazole.

[c]Kidney transplant patients concomitantly taking sulfisoxazole.

[d]Determined by single factor analysis of variance.

[e]Level of significance determined by Newman-Keuls multiple range test.

concentration (Fig. VII-3). This lack of correlation was in agreement with results from a previous study in anephric patients (8).

Although the mean values for percent free were 2-fold greater in responder than in non-responder kidney transplant patients, the difference was not statistically significant (3.6 ± 1.6 for R vs. 1.8 ± 0.3% for NR; $p > 0.10$). This was due to the large variability in this parameter between responders (CV = 44.4%) and probably reflects the effect of concomitant sulfisoxazole administration in 3 out of the 4 patients studied in this population. The effect of sulfisoxazole on furosemide protein binding was assessed by comparing the percent free of those patients concomitantly taking sulfisoxazole with those patients on furosemide without sulfisoxazole (Table VII-4). Not only were these two groups (KT- and KT+) different from one another with respect to percent free, but their within group variability (CV = 17.6% for KT-; CV = 18.2% for KT+) was substantially reduced, compared to the total transplant population, and in good agreement with the variability observed for percent free in healthy volunteers (CV = 16.7%). This displacing effect by sulfisoxazole has previously been demonstrated *in vitro* and results in significantly reduced binding of furosemide to human albumin (126).

A positive correlation was observed in kidney transplant patients between the corrected renal clearance of furosemide (CLr/CLcr) and the percent free in plasma (Fig. VII-4; $r = 0.762$, $p < 0.05$). This finding was consistent with a study by Yacobi and Levy (137) who found the renal clearance of sulfisoxazole in rats to be positively correlated with the serum free fraction of the drug. This suggests that furosemide renal clearance may be rate limited by its plasma protein binding. However, in patient EH, the incremental renal clearances of furosemide were

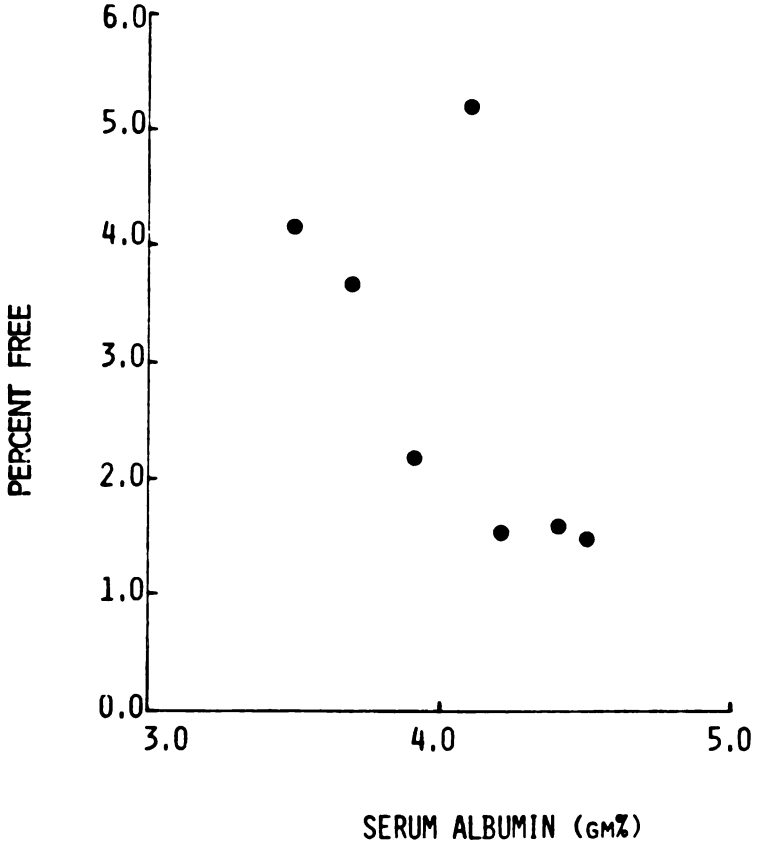

Fig. VII-3. Correlation between percent of furosemide free in plasma and albumin concentration in kidney transplant patients ($r = -0.629$, $p > 0.10$).

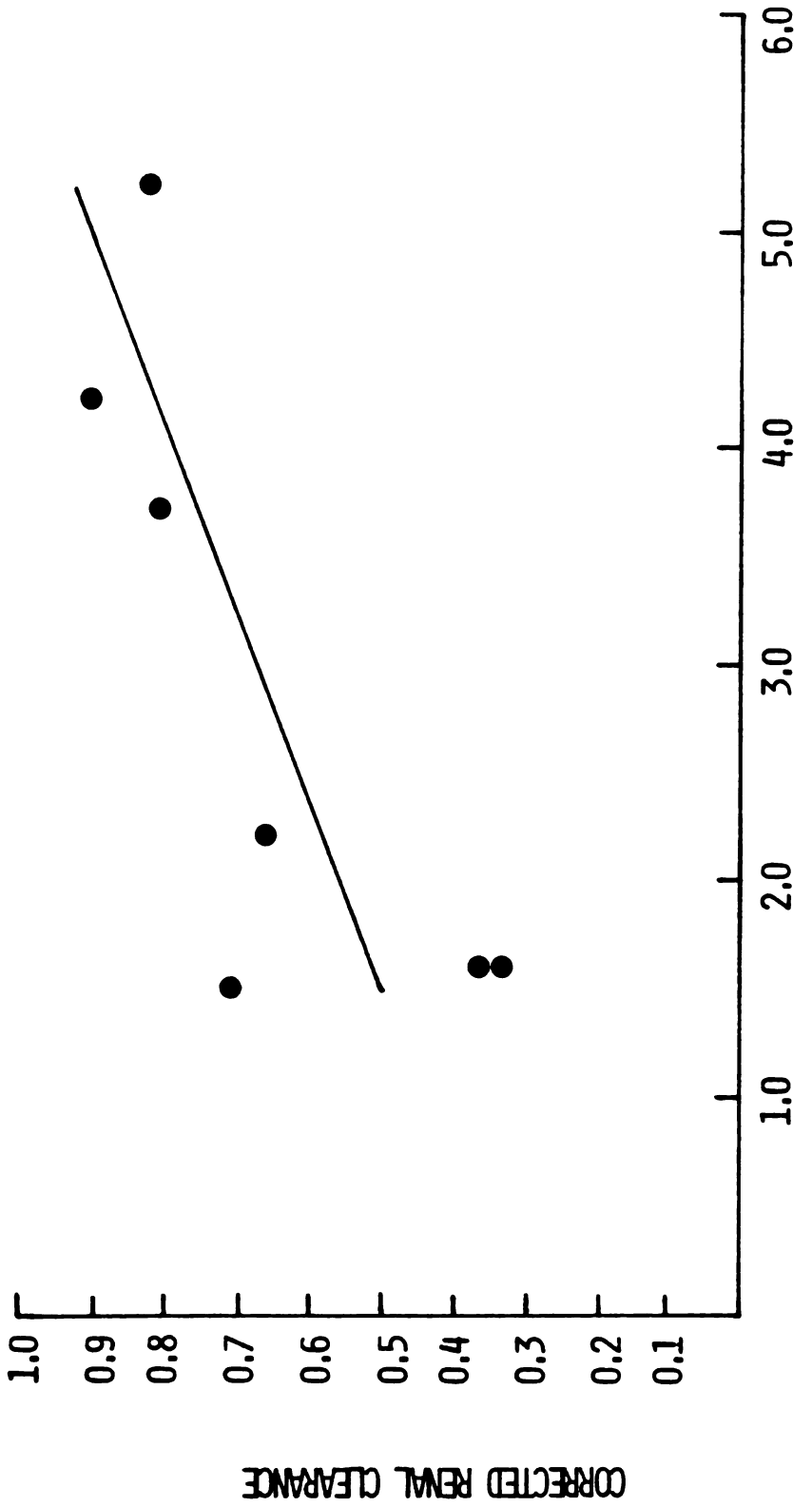

Fig. VII-4. Correlation between the corrected renal clearance of furosemide (CLr/CLcr) and percent free in plasma in kidney transplant patients ($r = 0.762$, $p < 0.05$).

found to remain relatively constant even though the percent free values varied from 1.3 - 12.9% (Table VII-5). This apparent discrepancy is difficult to explain, especially since other drugs were coadministered and may complicate the interpretation of the data. Nevertheless, no significant correlation was observed between the fraction of the dose excreted in the urine unchanged and percent free of furosemide in the kidney transplant patients (Fig. VII-5; $r = -0.106$, $p > 0.50$).

It is interesting to note that 3 out of the 5 kidney transplant patients designated as responders were concomitantly taking sulfisoxazole with furosemide. However, it is doubtful that this drug interaction was a factor in these patients being more responsive to furosemide treatment. Although responder patients on concomitant sulfisoxazole had reduced binding of furosemide to plasma proteins, this effect was not translated into greater values for fraction of the dose excreted unchanged in the urine (compare patients SJ, PD, FR to patients VW, WJ; Table VI-2). In fact, responder patient WJ (KT-) excreted over 70% of the unchanged drug in the urine with a percent free for furosemide of only 1.5%.

Table VII-6 reports the intrinsic metabolic clearances of furosemide in healthy volunteers and kidney transplant patients. Since furosemide is a low extraction ratio drug with respect to hepatic elimination, intrinsic metabolic clearances were estimated by correcting the non-renal clearance values for protein binding (CLnr/fraction free). The relationship assumes that the non-renal clearances were due solely to hepatic metabolism. The results demonstrate that the intrinsic metabolic clearance of furosemide was significantly lower in kidney transplant patients than in healthy volunteers (1934 ± 616 for patients vs. 4488 ± 606 ml/min for volunteers; $p <$

Table VII-5. Incremental Renal Clearances[a] for Patient EH

Time (min)	ΔCp(μg/ml)	Percent Free	CLr(ml/min)
0 - 40	7.5 - 13.6	9.1 - 12.9	18.5
40 - 94	3.8 - 7.5	3.9 - 9.1	33.9
94 - 130	2.6 - 3.8	2.5 - 3.9	29.6
130 - 208	1.6 - 2.6	1.8 - 2.5	18.9
208 - 328	0.8 - 1.6	1.5 - 1.8	14.5
328 - 480	0.3 - 0.8	1.3 - 1.5	16.9

[a]Determined following 120 mg intravenous dose of furosemide. ΔCp represents the total plasma concentrations of furosemide during the specified time interval (ΔTime).

CLr represents the incremental renal clearance.

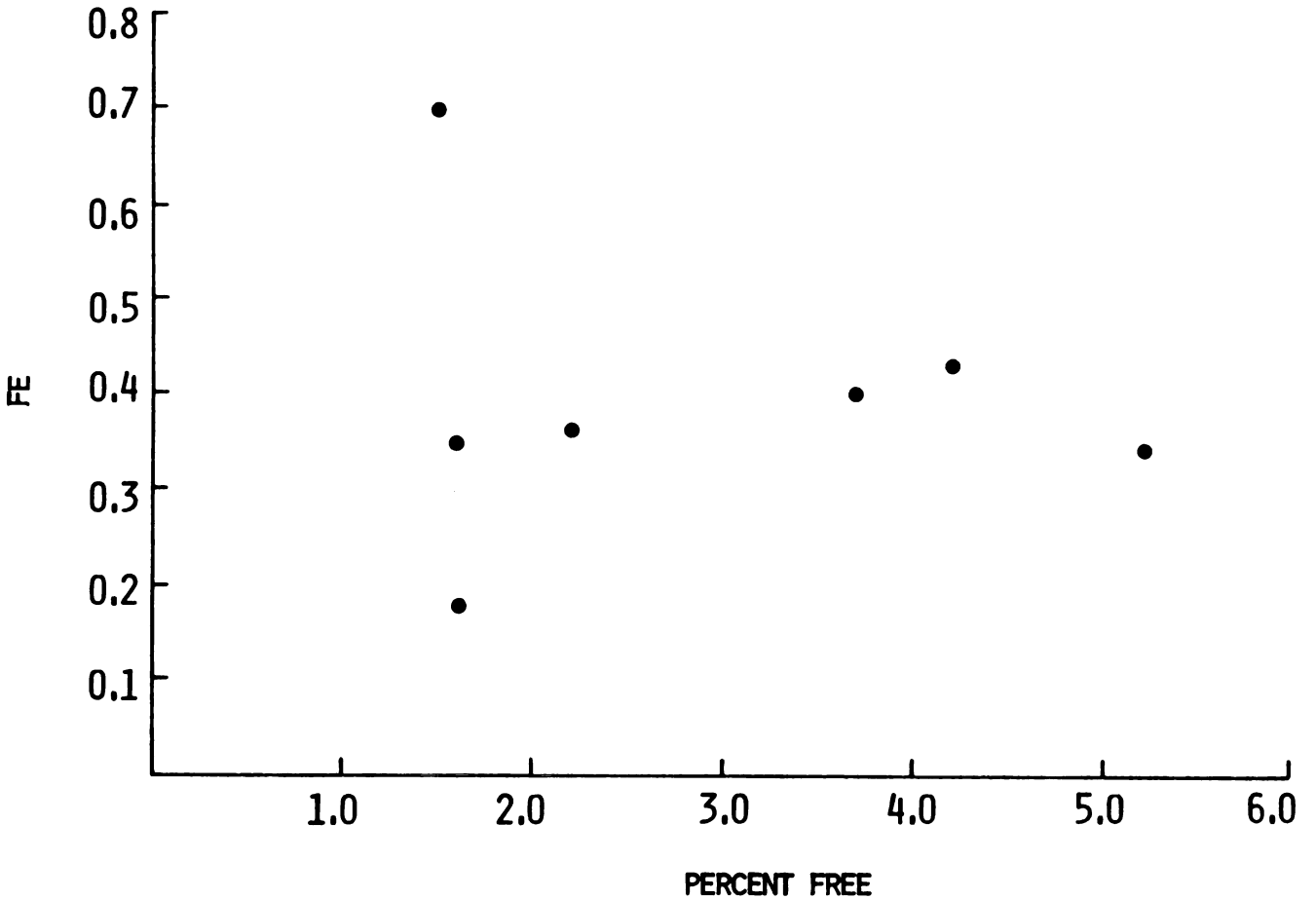

Fig. VII-5. Correlation between the fraction of the dose excreted in the urine unchanged (FE) and percent free of furosemide in plasma in kidney transplant patients ($r = -0.106$, $p > 0.50$).

Table VII-6. Intrinsic Metabolic Clearances of Furosemide

Volunteer[a]	CLint, m[c] (ml/min)	Patient[b]	CLint, m[c] (ml/min)
1	4467	CT	3106
2	4733	DH	1456
3	4100	LT	2182
4	3667	SJ	1558
5	3777	PD	2184
6	4800	WJ	1713
7	5491	FR	1336
8	4250		
9	5111		
MEAN	4488[d]	MEAN	1934[d]
(SD)	(606)	(SD)	(616)

[a]Previously described in Chapter IV.

[b]Previously described in Chapter VI.

[c]Intrinsic metabolic clearance (CLint, m = CLnr/fraction free).

[d]Level of significance, $p < 0.001$.

0.001). This may be due to the presence of endogenous substances which accumulate in renal impairment and compete with furosemide for the metabolic enzymes.

C. Summary

The present studies have shown that the binding of furosemide to plasma proteins is significantly reduced in kidney transplant patients as compared to healthy volunteers. This binding is further reduced in those patients concomitantly on sulfisoxazole. In addition, furosemide may exhibit nonlinear protein binding as evidenced by patient EH. Although differences may exist in the percent free of furosemide in kidney transplant patients, the fraction of the dose excreted in the urine unchanged does not appear to be influenced by its protein binding. Therefore, the ability of kidney transplant patients to respond to furosemide treatment is independent of plasma protein binding. Instead, the response appears to be related to the ability of the kidney to secrete furosemide into the tubular fluid as well as the ability of the organ to respond.

Chapter VIII. Summary and Conclusions

Furosemide is a potent diuretic agent which acts at the luminal surface of the nephron. There it inhibits the active reabsorption of chloride in the ascending limb of the loop of Henle; a process believed to be prostaglandin mediated. Since furosemide is over 95% plasma protein bound, access to the lumen occurs primarily through active secretion via the non-specific organic acid secretory pathway.

The relationship between the diuretic effect of furosemide and the drug's concentration/amount in a measurable sampling compartment has been poorly characterized. In addition, conflicting results have been reported concerning the pharmacokinetics and metabolism of furosemide due to problems inherent in the assay procedures. Therefore, a rapid, sensitive and specific HPLC assay, without prior extraction and/or derivatization was developed in an attempt to clarify the dose-response relationship of furosemide as well as the drug's disposition.

The relationship between urinary excretion rate, steady-state plasma levels and diuretic response of furosemide was studied in 28 rats. Results from this study demonstrate that the diuretic effect of furosemide is directly related to the drug's urinary excretion rate and not to its plasma concentration. In addition, furosemide exhibited capacity limited elimination at higher plasma concentrations (as evidenced by a reduced renal clearance), and this saturable process occurred in the rat at a level comparable to the therapeutic concentration range in humans.

The absorption and disposition of furosemide was studied in nine healthy volunteers after oral and iv dosing of the drug. No evidence of CSA, the putative metabolite of furosemide, was found and the results of this investigation conclusively demonstrate it to be an analytical artifact.

Glucuronidation accounted for approximately 14% of the available dose of furosemide, whether given orally or by iv administration. The bioavailability of furosemide was about 43%.

The role of probenecid and indomethacin in modifying furosemide's dose-response relationship was studied in four healthy volunteers. The results from the furosemide-probenecid interaction studies were consistent with previous animal studies and demonstrate that urinary excretion rate of furosemide is a better indicator of natriuresis and diuresis than is plasma concentration. The furosemide-indomethacin interaction studies demonstrate that the attenuation of furosemide's diuretic effect by indomethacin pretreatment is not due to a pharmacokinetic interaction. Inhibition of prostaglandin synthesis by indomethacin is the more probable mechanism.

The pharmacokinetics/dynamics of furosemide were evaluated in nine kidney transplant patients after oral and iv dosing of the drug. Similar values for mean bioavailability were observed between responder (50%) and non-responder (57%) patients. However, non-responders (in comparison to responders) had a reduced ability to secrete furosemide into tubular fluid as well as a decreased ability to respond to equivalent amounts of drug excreted in the urine. CSA was not found in any of the urine samples analyzed. Glucuronidation accounted for 8% of the available dose of furosemide and may be occurring in the kidney. Urinary recovery of furosemide and its glucuronide metabolite accounted for 45% of the intravenous dose in this patient population.

Plasma protein binding of furosemide after iv dosing was significantly reduced in kidney transplant patients as compared to healthy volunteers. Binding was further reduced in those patients concomitantly on sulfisoxazole.

Nevertheless, no correlation was observed between the fraction of the dose excreted in the urine unchanged and fraction free of furosemide. Therefore, the ability of kidney transplant patients to respond to furosemide treatment is independent of plasma protein binding. The response appears to be related to the ability of the kidney to secrete furosemide into the tubular fluid as well as the ability of the organ to respond.

REFERENCES

1. W.B. Stason, P.J. Cannon, H.O. Heinemann and J.H. Laragh. Furosemide: A clinical evaluation of its diuretic action. Circulation 34: 910-920 (1966).
2. W.M. Kirkendall and J.H. Stein. Clinical pharmacology of furosemide and ethacrynic acid. Am. J. Cardiol. 22: 162-167 (1968).
3. K.E. Kim, G. Onesti, J.H. Moyer and C. Swartz. Ethacrynic acid and furosemide: Diuretic and hemodynamic effects and clinical uses. Am. J. Cardiol. 27: 407-415 (1971).
4. H.R. Jacobson and J.P. Kokko. Diuretics: Sites and mechanisms of action. Ann. Rev. Pharmacol. Toxicol. 16: 201-204 (1976).
5. M.B. Burg. Tubular chloride transport and the mode of action of some diuretics. Kidney Int. 9: 189-197 (1976).
6. M. Burg, L. Stoner, J. Cardinal and N. Green. Furosemide effect on isolated perfused tubules. Am. J. Physiol. 225: 119-124 (1973).
7. J.F. Seely and J.H. Dirks. Site of action of diuretic drugs. Kidney Int. 11: 1-8 (1977).
8. F. Andreasen, H.E. Hansen and E. Mikkelsen. Pharmacokinetics of furosemide in anephric patients and in normal subjects. Eur. J. Clin. Pharmacol. 13: 41-48 (1978).
9. A. Rane, J.P. Villeneuve, W.J. Stone, A.S. Nies, G.R. Wilkinson and R.A. Branch. Plasma binding and disposition of furosemide in the nephrotic syndrome and in uremia. Clin. Pharmacol. Ther. 24: 199-207 (1978).

10. J.B. Hook and H.E. Williamson. Influence of probenecid and alterations in acid-base balance of the saluretic activity of furosemide. J. Pharmacol. Exp. Ther. 149: 404-408 (1965).

11. T.C. Daniels and E.C. Jorgensen. Diuretics. In Textbook of Organic Medicinal and Pharmaceutical Chemistry, 7th edition, C.O. Wilson, O. Gisvold and R.F. Doerge (eds), J.B. Lippincott Co., Philadelphia, 1977, p. 589.

12. E.A. Swinyard. Diuretic Drugs. In Remington's Pharmaceutical Sciences, 15th edition, A. Osol (ed), Mack Publishing Co., Easton, 1975, p. 872.

13. The Merck Index, 9th edition, M. Windholz (ed), Merck and Co., Inc., Rahway, 1976, p. 4163.

14. V.P. Hajdú and A. Häussler. Untersuchungen mit dem Salidiureticum 4-Chloro-N-(2-furylmethyl)-5-sulfamylanthranilsäure, I. Arzneim. Forsch. 14: 709-710 (1964).

15. G.H. Mudge. Diuretics and other agents employed in the mobilization of edema fluid. In The Pharmacological Basis of Therapeutics, 5th edition, L.S. Goodman and A. Gilman (eds), Macmillan Publishing Co., Inc., New York, 1975, p. 833.

16. M.E. Allison and A.C. Kennedy. Diuretics in chronic renal disease: A study of high dosage furosemide. Clin. Sci. 41: 171-187 (1971).

17. N.W. Levin. Furosemide and ethacrynic acid in renal insufficiency. Med. Clin. N. Am. 55: 107-120 (1971).

18. F.C. Reubi and P.T. Cottier. Effects of reduced glomerular filtration rate on responsiveness to chlorothiazide and mercurial diuretics. Circulation 23: 200-210 (1961).

19. R.I. Levy, I.M. Weiner and G.H. Mudge. The effects of acid-base

balance on the diuresis produced by organic and inorganic mercurials. J. Clin. Invest. 37:1016-1023 (1958).

20. F. Cantarovich, A. Locatelli, J.C. Fernandez, J.P. Loredo and J. Cristhot. Furosemide in high doses in the treatment of acute renal failure. Postgrad. Med. J. Suppl. 47: 13-17 (1971).

21. D. Fries, N. Pozet, N. Dubois and J. Traeger. The use of large doses of furosemide in acute renal failure. Postgrad. Med. J. Suppl. 47: 18-20 (1971).

22. R.G. Muth. Furosemide in severe renal insufficiency. Postgrad. Med. J. Suppl. 47: 21-25 (1971).

23. O.H. Morelli, L.I. Moledo, E. Alanis, O.L. Gaston and O. Terzaghi. Acute effects of high doses of furosemide in patients with chronic renal failure. Postgrad. Med. J. Suppl. 47: 29-35 (1971).

24. S.P. Rastogi, G. Volans, R.W. Elliott, D.W. Eccleston, R. Ashcroft, D. Webster and D.N.S. Kerr. High dose furosemide in the treatment of hypertension in chronic renal insufficiency and of terminal renal failure. Postgrad. Med. J. Suppl. 47: 45-53 (1971).

25. D.J. Greenblatt, D.W. Duhme, M.D. Allen and J. Koch-Weser. Clinical toxicity of furosemide in hospitalized patients. Am. Heart J. 94: 6-13 (1977).

26. J. Lowe, J. Gray, D.A. Henry and D.H. Lawson. Adverse reactions to furosemide in hospital inpatients. Br. Med. J. 2: 360-362 (1979).

27. K.L. Gallagher and J.K. Jones. Furosemide-induced ototoxicity. Ann. Int. Med. 91: 744-745 (1979).

28. M.E. Wigand and A. Heidland. Ototoxic side-effects of high doses of furosemide in patients with uremia. Postgrad. Med. J. Suppl. 47: 54-56 (1971).

29. G.H. Schwartz, D.S. David, R.R. Riggio, K.H. Stenzel and A.L. Rubin. Ototoxicity induced by furosemide. N. Engl. J. Med. 282: 1413-1414 (1970).

30. L.B. Cooperman and I.L. Rubin. Toxicity of ethacrynic acid and furosemide. Am. Heart J. 85: 831-834 (1973).

31. J.R. Mitchell, W.Z. Potter, Z.A. Hinson and D.J. Jollow. Hepatic necrosis caused by furosemide. Nature, Lond. 251: 508-510 (1974).

32. J.R. Mitchell, W.L. Nelson, W.Z. Potter, H.A. Sasame and D.J. Jollow. Metabolic activation of furosemide to a chemically reactive, hepatotoxic metabolite. J. Pharmacol. Exp. Ther. 199: 41-52 (1976).

33. P.J. Wirth, C.J. Bettis and W.L. Nelson. Microsomal metabolism of furosemide: Evidence for the nature of the reactive intermediate involved in covalent binding. Mol. Pharmacol. 12: 759-768 (1976).

34. Physician's Desk Reference, 33rd edition, Medical Economics Co., Oradell, 1979, p. 915.

35. M. Goldberg. The renal physiology of diuretics. In Handbook of Physiology, Section 8: Renal Physiology, J. Orloff and R.W. Berliner (eds), American Physiological Society, Wash. D.C., 1973, pp. 1003-1031.

36. G.H. Hirsch, A.P. Pakuts and A.J. Bayne. Furosemide accumulation by renal tissue. Biochem. Pharmacol. 24: 1943-1946 (1975).

37. P. Deetjen. Micropuncture studies on site and mode of diuretic action of furosemide. Ann. N.Y. Acad. Sci. 139: 408-415 (1966).

38. J.C. McGiff, K. Crowshaw and H.D. Itskovitz. Prostaglandins and renal function. Federation Proc. 33: 39-47 (1974).

39. G.R. Zins. Renal prostaglandin. Am. J. Med. 58: 14-24 (1975).

40. J.B. Lee, R.V. Patak and B.K. Mookerjee. Renal prostaglandins and the regulation of blood pressure and sodium and water homeostasis. Am. J. Med. 60: 798-816 (1976).

41. M.D. Bailie, J.A. Barbour and J.B. Hook. Effects of indomethacin on furosemide-induced changes in renal blood flow. Proc. Soc. Exp. Biol. Med. 148: 1173-1176 (1975).

42. H.E. Williamson, W.A. Bourland and G.R. Marchand. Inhibition of furosemide induced increase in renal blood flow by indomethacin. Proc. Soc. Exp. Biol. Med. 148: 164-165 (1975).

43. J.B. Hook, A.H. Blatt, M.J. Brody and H.E. Williamson. Effects of several saluretic-diuretic agents on renal hemodynamics. J. Pharmacol. Exp. Ther. 154: 667-673 (1966).

44. J. H. Stein, R.C. Mauk, S. Boonjarern and T.F. Ferris. Differences in the effect of furosemide and chlorothiazide on the distribution of renal cortical blood flow in the dog. J. Lab. Clin. Med. 79: 995-1003 (1972).

45. N.T. Stowe, L.F. Wolterink, A.E. Lewis and J.B. Hook. Diuretic and hemodynamic effects of furosemide in the isolated dog kidney. Naunyn-Schmiedeberg's Arch. Pharmacol. 277: 13-26 (1973).

46. J.H. Ludens, J.B. Hook, M.J. Brody and H.E. Williamson. Enhancement of renal blood flow by furosemide. J. Pharmacol. Exp. Ther. 163: 456-460 (1968).

47. M.D. Bailie, K. Crosslan and J.B. Hook. Natriuretic effect of furosemide after inhibition of prostaglandin synthetase. J. Pharmacol. Exp. Ther. 199: 469-476 (1976).

48. E. Oliw, G. Kövér, C. Larsson and E. Änggård. Reduction by indomethacin of furosemide effects in the rabbit. Eur. J. Pharmacol. 38: 95-100 (1976).

49. J.C. Frölich, J.W. Hollifield, J.C. Dormois, B.L. Frölich, H. Seyberth, A.M. Michelakis and J.A. Oates. Suppression of plasma renin activity by indomethacin in man. Circ. Res. 39: 447-452 (1976).

50. R.V. Patak, B.K. Mookerjee, C.J. Bentzel, P.E. Hysert, M. Babet and J.B. Lee. Antagonism of the effects of furosemide by indomethacin in normal and hypertensive man. Prostaglandins 10: 649-659 (1975).

51. G. Kövér and H. Tost. The effect of indomethacin on kidney function: Indomethacin and furosemide antagonism. Pflügers Arch. 372: 215-220 (1977).

52. J.B. Hook and M.D. Bailie. Release of vasoactive materials from the kidney by diuretics. J. Clin. Pharmacol. 17: 673-680 (1977).

53. D.C. Brater. Analysis of the effect of indomethacin on the response to furosemide in man: Effect of dose of furosemide. J. Pharmacol. Exp. Ther. 210: 386-390 (1979).

54. J.L. Data, A. Rane, J. Gerkens, G.R. Wilkinson, A.S. Nies and R.A. Branch. The influence of indomethacin on the pharmacokinetics, diuretic response and hemodynamics of furosemide in the dog. J. Pharmacol. Exp. Ther. 206: 431-438 (1978).

55. J.C. Frölich, T.W. Wilson, B.J. Sweetman, M. Smigel, A.S. Nies, K. Carr, J.T. Watson and J. A. Oates. Urinary prostaglandins: Identification and origin. J. Clin. Invest. 55: 763-770 (1975).

56. W.M. Williams, J.C. Frölich, A.S. Nies and J.A. Oates. Urinary prostaglandins: Site of entry into renal tubular fluid. Kidney Int. 11: 256-260 (1977).

57. L.Z. Bito. Inhibition of renal prostaglandin metabolism and excretion by probenecid, bromcresol green and indomethacin. Prostaglandins 12: 639-646 (1976).

58. B.R. Rennick. Renal tubular transport of prostaglandins: Inhibition by probenecid and indomethacin. Am. J. Physiol. 233: F133-F137 (1977).

59. J.M. Irish III. Secretion of prostaglandin E_2 by rabbit proximal tubules. Am. J. Physiol. 237: F268-F273 (1979).

60. K.J. Stone and M. Hart. Inhibition of renal PGE_2-9-ketoreductase by diuretics. Prostaglandins 12: 197-207 (1976).

61. J.R. Paulsrud and O.N. Miller. Inhibition of 15-OH prostaglandin dehydrogenase by several diuretic drugs. Federation Proc. 33: 590 (1974).

62. P.C. Weber, B. Scherer and C. Larsson. Increase of free arachidonic acid by furosemide in man as the cause of prostaglandin and renin release. Eur. J. Pharmacol. 41: 329-332 (1977).

63. E. Oliw and E. Änggård. Different effects of furosemide on urinary excretion of prostaglandin E_2 and $F_{2\alpha}$ in rabbits. Acta Physiol. Scand. 105: 367-373 (1979).

64. B. Scherer, J. Schnermann, M. Sofroniev and P.C. Weber. Prostaglandin (PG) analysis in urine of humans and rats by different radioimmunoassays: Effect of PG - excretion by PG - synthetase inhibitors, laparotomy and furosemide. Prostaglandins 15: 255-266 (1978).

65. K. Abe, M. Yasujima, S. Chiba, N. Irokawa, T. Ito and K. Yoshinaga. Effect of furosemide on urinary excretion of prostaglandin E in normal volunteers and patients with essential hypertension. Prostaglandins 14: 513-521 (1977).

66. B. Scherer and P.C. Weber. Time-dependent changes in prostaglandin excretion in response to furosemide in man. Clin. Sci. 56: 77-81 (1979).

67. J.B. Stokes. Effect of prostaglandin E_2 on chloride transport across the rabbit thick ascending limb of Henle. J. Clin. Invest. 64: 495-502 (1979).

68. W. Rupp and P. Hajdú. Pharmacokinetics and pharmacodynamics of a diuretic agent. In Symposium on Pharmacological and Clinical Significance of Pharmacokinetics, H.J. Dengler (ed), F.K. Schattauer Verlag, Stuttgart, 1970, pp. 105-111.

69. M.R. Kelly, R.E. Cutler, A.W. Forrey and B.M. Kimpel. Pharmacokinetics of orally administered furosemide. Clin. Pharmacol. Ther. 15: 178-186 (1974).

70. B. Beermann, E. Dalén, B. Lindström and A. Rosén. On the fate of furosemide in man. Eur. J. Clin. Pharmacol. 9: 57-61 (1975).

71. R.A. Branch, C.J.C. Roberts, M. Homeida and D. Levine. Determinants of response to furosemide in normal subjects. Br. J. Clin. Pharmacol. 4: 121-127 (1977).

72. W.J. Tilstone and A. Fine. Furosemide kinetics in renal failure. Clin. Pharmacol. Ther. 23: 644-650 (1978).

73. C.M. Huang, A.J. Atkinson, M. Levin, N. Levin and A. Quintanilla. Pharmacokinetics of furosemide in advanced renal failure. Clin. Pharmacol. Ther. 16: 659-666 (1974).

74. M.R. Kelly, A.D. Blair, A.W. Forrey, N.A. Smidt and R.E. Cutler. A comparison of the diuretic response to oral and intravenous furosemide in "diuretic-resistant" patients. Current Ther. Res. 21: 1-9 (1977).

75. A. Greither, S. Goldman, J.S. Edelen, L.Z. Benet and K. Cohn. Absorption of furosemide in patients with congestive heart failure. Pharmacology 19: 121-131 (1979).

76. B. Calesnick, J.A. Christensen and M. Richter. Absorption and excretion of furosemide - S^{35} in human subjects. Proc. Soc. Exp. Biol. Med. 123: 17-22 (1966).

77. F. Andreasen and E. Mikkelsen. Distribution, elimination and effects of furosemide in normal subjects and in patients with heart failure. Eur. J. Clin. Pharmacol. 12: 15-22 (1977).

78. J. Honari, A.D. Blair and R.E. Cutler. Effects of probenecid on furosemide kinetics and natriuresis in man. Clin. Pharmacol. Ther. 22: 395-401 (1977).

79. M. Homeida, C. Roberts and R.A. Branch. Influence of probenecid and spironolactone on furosemide kinetics and dynamics in man. Clin. Pharmacol. Ther. 22: 402-409 (1977).

80. P. Chennavasin,R. Seiwell, D.C. Brater and W.M.M. Liang. Pharmacodynamic analysis of the furosemide-probenecid interaction in man. Kidney Int. 16: 187-195 (1979).

81. R.E. Cutler, A.W. Forrey, T.G. Christopher and B.M. Kimpel. Pharmacokinetics of furosemide in normal subjects and functionally anephric patients. Clin. Pharmacol. Ther. 15: 588-596 (1974).

82. B. Beermann, E. Dalén and B. Lindström. Elimination of furosemide in healthy subjects and in those with renal failure. Clin. Pharmacol. Ther. 22: 70-78 (1977).

83. R.E. Cutler, and A.D. Blair. Clinical pharmacokinetics of furosemide. Clin. Pharmacokinet. 4: 279-296 (1979).

84. W.A. Rupp, A. Heidland, P. Hajdú and G. Neuhaus. Pharmacokinetic von Furosemid bei normaler und eingeschrankter Nierenfunktion. In 4. Freiburger Tagung über Nephrologie, 1970, Thieme Verlag, Stuttgart, 1971, pp. 55-64.

85. W. Rupp. Pharmacokinetics and pharmacodynamics of lasix. Scot. Med. J. 19: 5-13 (1974).

86. J. Perez, D.S. Sitar and R.I. Ogilvie. Biotransformation of furosemide in patients with acute pulmonary edema. Drug Metab. Dispos. 7: 383-387 (1979).

87. W.J. Tilstone and D.H. Lawson. Pharmacokinetics of furosemide in patients with heart failure. Clin. Res. 24: 513A (1976).

88. F. Andreasen, O.L. Pedersen and E. Mikkelsen. Distribution, elimination and natriuretic effect of furosemide in patients with severe arterial hypertension. Eur. J. Clin. Pharmacol. 14: 237-244 (1978).

89. A. Häussler and P. Hajdú. Untersuchungen mit dem Salidiureticum 4-Chloro-N-(furylmethyl)-5-sulfamylanthranilsäure, II. Arzneim. Forsch. 14: 710-713 (1964).

90. A. Häussler and H. Wicha. Untersuchungen mit dem Salidiureticum 4-Chloro-N-(furylmethyl)-5-sulfamylanthranilsäure. Arzneim. Forsch. 15: 81-83 (1965).

91. H. Kindt and E. Schmid. Über die Harnausscheidung von Furosemid bei gesunden und kranken mit Lebercirrhose. Pharmacol. Clin. 2: 221-226 (1970).

92. G.J. Yakatan, D.D. Maness, J. Scholler, W.J. Novick, Jr., and J.T. Doluisio. Absorption, distribution, metabolism and excretion of furosemide in dogs and monkeys. I: Analytical methodology, metabolism, and urinary excretion. J. Pharm. Sci. 65: 1456-1460 (1976).

93. S.B. Stallings, K.J. Childress, S.M. Maynard and W.T. Sawyer. Comparison of natriuretic and diuretic effects of single and divided doses of furosemide. Am. J. Hosp. Pharm. 36: 68-71 (1979).

94. T.W. Wilson, K.J. Falk, J.L. Labelle and K.B. Nguyen. Effect of dosage regimen on natriuretic response to furosemide. Clin. Pharmacol. Ther. 18: 165-169 (1975).

95. D.C. Brater. Analysis of the effect of indomethacin on the response to furosemide in man: Effect of dose of furosemide. J. Pharmacol. Exp. Ther. 210: 386-390 (1979).

96. A. Rane, O. Oelz, J.C. Frolich, H.W. Seyberth, B.J. Sweetman, J.T. Watson, G.R. Wilkinson, and J.A. Oates. Relation between plasma concentration of indomethacin and its effect on prostaglandin synthesis and platelet aggregation in man. Clin. Pharmacol. Ther. 23: 658-668 (1978).

97. L.Z. Benet. Pharmacokinetics/pharmacodynamics of furosemide in man: A review. J. Pharmacokinet. Biopharm. 7: 1-27 (1979).

98. E. Mikkelsen and F. Andreasen. Simultaneous determination of furosemide and two of its possible metabolites in biological fluids. Acta Pharmacol. Toxicol. 41: 254-262 (1977).

99. H.J. Rose, A.W. Pruitt, and J.L. McNay. Effect of experimental azotemia on renal clearance of furosemide in the dog. J. Pharmacol. Exp. Ther. 196: 238-247 (1976).

100. V.S. Chungi, L.W. Dittert and R.B. Smith. Gastrointestinal sites of furosemide absorption in rats. Int. J. Pharm. 4: 27-38 (1979).

101. B. Lindström and M. Molander. Gas chromatographic determination of furosemide in plasma using an extractive alkylation technique and an electron capture detector. J. Chromatogr. 101: 219-221 (1974).

102. B. Lindström. Determination of furosemide concentrations in plasma and urine using high-speed liquid chromatography. J. Chromatogr. 100: 189-191 (1974).

103. A.D. Blair, A.W. Forrey, B.T. Meijsen and R.E. Cutler. Assay of flucytosine and furosemide by high-pressure liquid chromatography. J. Pharm. Sci. 64: 1334-1339 (1975).

104. M.L. MacDougall, D.W. Shoeman and D.L. Azarnoff. Separation and quantitative analysis of furosemide and 4-Chloro-5-sulfamoylanthranilic acid (CSA) by high-pressure liquid chromatography. Res. Comm. Chem. Pathol. Pharmacol. 10: 285-292 (1975).

105. K. Carr, A. Rane and J.C. Frölich. A simplified assay of furosemide in plasma and urine by high-pressure liquid chromatography. J. Chromatogr. 145: 421-427 (1978).

106. S.E. Swezey, P.J. Meffin and T.F. Blaschke. Measurement of furosemide by high-performance liquid chromatography. J. Chromatogr. 174: 469-473 (1979).

107. R.L. Nation, G.W. Peng, and W.L. Chiou. Quantitative analysis of furosemide in micro plasma volumes by high-performance liquid chromatography. J. Chromatogr. 162: 88-93 (1979).

108. H.J. Rose, A.W. Pruitt, P.G. Dayton and J.L. McNay. Relationship of urinary furosemide excretion rate to natriuretic effect in experimental azotemia. J. Pharmacol. Exp. Ther. 199: 490-494 (1976).

109. A.E. Till. Renal excretion of pseudoephedrine in the rat. Dissertation for Doctor of Philosophy in Pharmaceutical Chemistry, University of California, San Francisco, 1979, p. 26.

110. F.T. Brennan, G.F. Sosnowski, W.A. Mann, L. Sulat and V.D. Wiebelhaus. Renal clearance procedure for the rat. Effect of dopamine and standard saluretics. J. Pharm. Pharmac. 29: 744-747 (1977).

111. L.Z. Benet and R.L. Galeazzi. Noncompartmental determination of the steady-state volume of distribution. J. Pharm. Sci. 68: 1071-1074 (1979).

112. J.E. Cruz, D.D. Maness and G.J. Yakatan. Kinetics and mechanism of hydrolysis of furosemide. Int. J. Pharm. 2: 275-281 (1979).

113. F. Sorgel, D.E. Smith, E.T. Lin and L.Z. Benet. (in preparation).

114. P.A. Friedman and F. Roch-Ramel. Hemodynamic and natriuretic effects of bumetanide and furosemide in the cat. J. Pharmacol. Exp. Ther. 203: 82-91 (1977).

115. D.C. Brater. Effects of probenecid on furosemide response. Clin. Pharmacol. Ther. 24: 548-554 (1978).

116. D.E. Smith and L.Z. Benet. Relationship between urinary excretion rate, steady-state plasma levels and diuretic response of furosemide in the rat. Pharmacology 19: 301-306 (1979).

117. I.M. Weiner. Transport of weak acids and bases. In Handbook of Physiology, Section 8: Renal Physiology, J. Orloff and R.W. Berliner (eds), American Physiological Society, Wash. D.C., 1973, pp. 521-554.

118. D.E. Smith, D.C. Brater, E.T. Lin and L.Z. Benet. Attenuation of furosemide's diuretic effect by indomethacin: Pharmacokinetic evaluation. J. Pharmacokinet. Biopharm. 7: 265-274 (1979).

119. L.Z. Bito. Are prostaglandins intracellular, transcellular or extracellular autocoids? Prostaglandins 9: 851-855 (1975).

120. L.Z. Bito, M. Wallerstein and R. Baroody. The role of transport processes in the distribution and disposition of prostaglandins. In Advances in Prostaglandin and Thromboxane Research, Volume 1, B. Samuelsson and R. Paoletti (eds), Raven Press, New York, 1976, pp. 297-303.

121. L.Z. Bito, Accumulation and apparent active transport of prostaglandins by some rabbit tissues in vitro. J. Physiol. 221: 371-387 (1972).

122. D.E. Smith, W.L. Gee, E.T. Lin and L.Z. Benet. Preliminary evaluation of furosemide-probenecid interaction in humans. J. Pharm. Sci. 69: 571-575 (1980).

123. D.E. Smith, E.T. Lin and L.Z. Benet. Absorption and disposition of furosemide in healthy volunteers, measured with a metabolite-specific assay. Drug Metab. Dispos. 8: 337-342 (1980).

124. R.G. Muth. Diuretic properties of furosemide in renal disease. Ann. Intern. Med. 69: 249-261 (1968).

125. F. Andreasen and J. Jakobsen. 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).

126. J. Prandota and A.W. Pruitt. Furosemide binding to human albumin and plasma of nephrotic children. Clin. Pharmacol. Ther. 17: 159-166 (1975).

127. T.P. Green and B.L. Mirkin. Resistance of proteinuric rats to furosemide: Urinary drug protein binding as a determinant of drug effect. Life Sci. 26: 623-630 (1980).

128. B.G. Odlind and B. Beermann. Diuretic resistance: Reduced bioavailability and effect of oral furosemide. Br. Med. J. 208: 1577 (1980).

129. B. Husberg, S. Hellsten, S. Bergentz, T. Hansen and K. Möller-Jensen. Massive diuresis after renal transplantation due to retention of furosemide. Transplantation 23: 101-103 (1977).

130. A.W. Forrey, B. Kimpel, A.D. Blair and R.E. Cutler. Furosemide concentrations in serum and urine, and its binding by serum proteins as measured fluorometrically. Clin. Chem. 20: 152-158 (1974).

131. S.W. Boobis. Alteration of plasma albumin in relation to decreased drug binding in uremia. Clin. Pharmacol. Ther. 22: 147-153 (1977).

132. R. Gugler and D.L. Azarnoff. Drug protein binding and the nephrotic syndrome. Clin. Pharmacokinet. 1: 25-35 (1976).

133. M.M. Reidenberg. The binding of drugs to plasma proteins from patients with poor renal function. Clin. Pharmacokinet. 1: 121-125 (1976).

134. M.M. Reidenberg, I. Odar-Cederlöf, C. Von Bahr, O. Borgå and F. Sjoqvist. Protein binding of diphenylhydantoin and desmethylimipramine in plasma from patients with poor renal function. N. Engl. J. Med. 285: 264-267 (1971).

135. I. Sjöholm, A. Kober, I. Odar-Cederlöf and O. Borgå. Protein binding of drugs in uremic and normal serum: The role of endogenous binding inhibitors. Biochem. Pharmacol. 25: 1205-1213 (1976).

136. H.L. Behm and J.G. Wagner. Errors in interpretation of data from equilibrium dialysis protein binding experiments. Res. Comm. Chem. Pathol. Pharmacol. 26: 145-160 (1979).

137. A. Yacobi and G. Levy. Effect of serum protein binding on sulfisoxazole distribution, metabolism, and excretion in rats. J. Pharm. Sci. 68: 742-746 (1979).


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