Short Title:

KINETICS AND DYNAMICS OF FUROSEMIDE

IN PULMONARY EDEMA

THE KINETIC DISPOSITION AND DIURETIC EFFECT OF FUROSEMIDE IN PATIENTS WITH PULMONARY EDEMA

by

Jorge Perez Avila

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Department of Pharmacology and Therapeutics
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Short Title:

KINETICS AND DYNAMICS OF FUROSEMIDE IN PULMONARY EDEMA

DEDICATION:

To my Father:

For teaching me the principles

that have guided my life.

To my Mother:

For her patience and wonderful

dedication to her sons.

My country:

Much blood has been shed on this land,
on these very fields where we're standing,
for a sovereign and free country, shaping
its own future; for a country where
justice prevails!

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Jorge Perez Avila
Department of Pharmacology and Therapeutics M.Sc.

THE KINETIC DISPOSITION AND DIURETIC EFFECT OF FUROSEMIDE IN PATIENTS WITH PULMONARY EDEMA

Abstract

Furosemide (20 - 80mg) was administered intravenously over 5 minutes to 16 patients with the diagnosis of acute pulmonary edema due to left heart failure. Serum and urine samples collected at frequent intervals were assayed for unchanged and biotransformed furosemide, sodium, potassium, calcium, magnesium and chloride by specific techniques. There was a biexponential decay of serum furosemide concentration over time with wide variation in both alpha $t_{1/2}$ (range 15 - 79 min) and beta $t_{1/2}$ (range 127 - 1190 min). The beta $t_{1/2}$ values were inversely related to urinary creatinine clearance values. Recovery of furosemide and its metabolites from urine in 24 hr varied between 30 and 97% of the administered dose. Excretion of unchanged drug accounted for 23 - 73% of the dose. The glucuronide conjugate and 2-amino-4-chloro-5-sulfamoylanthranilic acid accounted for 3 - 40% and 0.13 - 3.92% of the dose respectively. Excretion of both metabolites were inversely related to creatinine clearance. The glucuronide conjugate of furosemide was the major metabolite found in these patients. Urine volume, renal clearance of furosemide and renal clearance of sodium were greater in the alpha than in the beta phase of serum furosemide disposition. For each

patient, a linear relationship was obtained between urinary excretion rate of unchanged furosemide and the excretion rate of sodium, chloride, calcium or urine volume. The serum concentrations of furosemide did not have a consistent relationship with the diuretic response. These results suggest that:

1. Furosemide disposition is altered in patients with pulmonary edema. This alteration is defined as a prolongation in the alpha and beta half-lives of furosemide, increase in metabolism and a decrease in the excretion of unchanged drug in urine.

2. Diuretic response to furosemide is associated with distribution of the drug outside of the serum to a compartment which is in rapid equilibrium with serum. The response to furosemide is determined by the concentration of drug in this peripheral kinetic compartment.

3. The excretion of furosemide into tubular urine is required to obtain a diuretic effect in patients with pulmonary edema.

Jorge Perez Avila
Département de Pharmacologie et Thérapeutique M.Sc.

LA CINETIQUE DE DISTRIBUTION ET D'ELIMINATION DU FUROSEMIDE ET SON EFFET DIURETIQUE CHEZ LES PATIENTS SOUFFRANT D'OEDEME PULMONAIRE

Condense

Le furosemide (20 - 80mg) a été administré par voie intraveineuse pendant 5 minutes à des patients dont le diagnostic indiquait un oedème pulmonaire aigu causé par une faiblesse du coeur gauche. Le furosemide intact et biotransformé a été mesuré par des techniques bien spécifiques dans des échantillons de sérum et d'urine prélevés à de fréquents intervalles; également ont été mesurés, le sodium, le potassium, le calcium, le magnésium et le chlorure. La diminution de la concentration du furosemide plasmatique est bi-exponentielle et est sujette à de fortes variations dans le sérum tant dans sa phase alpha ($t_{1/2}$, valeurs de 15 à 79 mn) que bêta ($t_{1/2}$, valeurs de 127 à 1190 mn). Les valeurs de la $t_{1/2}$ de la phase bêta sont inversement proportionnelles à la clearance rénale de la créatinine. Sur une période de 24 heures la récuperation du furosemide et de ses métabolites dans l'urine varie de 30 à 97% de la dose administrée. L'excrétion sous forme intacte est de 23 à 73%. Le conjugué glucuronidé et l'acide 2-amino-4-chloro-5-sulfamoylanthranilique sont respectivement de 3 à 40% et de 0.13 à 3.92% de la dose. L'excrétion des deux métabolites est inversement proportionelles à la clearance rénale de la créatinine. Le conjugué glucuronidé du furosemide est le

métabolite principal trouvé chez ces patients. Le volume urinaire, la clearance rénale du furosemide et du sodium, sont beaucoup plus élevés dans la phase alpha que dans la phase bêta. Pour chaque patient, une relation linéaire a été obtenue entre le taux d'excrétion urinaire du furosemide intact et le taux d'excrétion du sodium, du chlorure, du calcium et du volume urinaire. Les concentrations du furosemide dans le sérum ne sont pas en rapport constant avec la réponse diurétique.

Ces résultats suggèrent que:

1. La distribution du furosemide est modifiée chez les patients ayant un oedème pulmonaire. Cette modification est considérée courme étant une prolongation dans la demie-vie des phases alpha et bêta du furosemide, par un accroissement dans le métabolisme et une baisse dans l'excrétion urinaire de la forme intacte. La réponse diurétique au furosemide est associée à la distribution extraplasmatique du médicament dans un compartiment qui est en équilibre rapide avec le plasma. La réponse au furosemide est déterminée par la concentration du médicament dans ce compartiment cinétique périphérique.

3. L'excrétion du furosemide dans l'urine tubulaire est requise pour obtenir un effet diurétique chez les patients souffrant d'oedème pulmonaire.

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INTRODUCTION

The Pharmacokinetic Disposition of Furosemide

Furosemide (Lasix^{R}) is a potent diuretic commonly used in medical practice. It is effective when administered by both oral and parenteral routes (Kelly et al 1974, 1977). The use of furosemide has been recommended for several disease states such as renal failure (Muth, 1968, Allison et al 1971), congestive heart failure and pulmonary edema (Conn 1978 , Krupp and Chatton 1978) and in the treatment of ascites of hepatic cirrhosis (Fuller et al 1977). Although it is widely used in clinical practice, little is known about its pharmacokinetic disposition in different disease states.

The pharmacokinetics of furosemide have been studied by several investigators, but different methods for the determination of furosemide were used and there is still uncertainty about its fate in man. Calesnick et al (1966), using ^{35}S-furosemide, were the first to study its pharmacokinetic disposition in normal subjects. They reported the alpha and beta half-lives of furosemide to be 7 and 70 minutes, respectively. They were unable to find evidence for its metabolism in their study. The mean urinary excretion was 80% of the administered dose as the unchanged drug. Cutler et al (1974) studied the pharmacokinetic disposition of furosemide in normal subjects and in functionally anephric patients using unlabelled furosemide, and a fluorometric method to determine furosemide concentrations. They reported a mean beta half-life of 29.5 minutes in normal subjects and 80.7 minutes in anephric patients. A mean of 92% of the administered dose was reported to be excreted unchanged in urine of normal subjects. Metabolites were not reported in this study. Kelly et al (1974) studied

the pharmacokinetic disposition of orally and intravenously administered furosemide in normal subjects using a spectrofluorometric method to determine furosemide concentrations. They reported that their assay was sensitive to 0.5 ug/ml changes in serum furosemide concentrations. Due to fluorescent and quenching substances in urine, the assay was less precise for this fluid. Although they reported the sensitivity of their method as 0.5 ug/ml, in one of their figures (Fig. 5) one can see determination of plasma concentrations between 0.5 ug/ml and 0.1 ug/ml. They reported that the 24 hour excretion of ^{35}S-furosemide after oral administration was 30 to 50% of the administered dose. There was no mention of metabolites in their study. Huang et al (1974) studied the pharmacokinetic disposition of furosemide in patients with advanced renal failure using the fluorometric method to determine furosemide concentrations. They reported a mean beta half-life of 9.7 hours in these patients and a prolongation to 20 hours in one uremic patient with hepatic cirrhosis. They suggested that non-renal elimination of furosemide could account for 86 to 98% of the total dose in uremic patients without hepatic cirrhosis. Beermann et al (1975) studied the pharmacokinetic disposition of furosemide in normal subjects after oral and intravenous administration of ^{35}S-furosemide. The beta half-life of furosemide was found to vary from 47-53 minutes in these subjects. After intravenous administration, urinary excretion of unchanged furosemide varied from 82-84% of the dose and 6-9% was recovered from feces. They found no evidence of the 2-amino-4-chloro-5-sulfamoylanthranilic acid in this study. Treatment of these urine samples with β-glucuronidase resulted in the detection of more

furosemide. The suggestion was made that this was due to the conjugation of glucuronic acid with the administered drug. Benet et al (1976) studied the pharmacokinetic disposition of furosemide in patients with congestive heart failure after oral and intravenous administration. The plasma concentration of furosemide was measured by the fluorometric method. They reported the beta half-life of furosemide to be 76.7 ± 30.6 minutes (mean ± S.D.). Excretion of furosemide and its metabolites were not studied. These workers concluded that the absorption of furosemide was incomplete in patients with congestive heart failure, since the AUC in serum was less after an oral than an i.v. dose. Also, the diuretic response was greater when the drug was administered intravenously.

Kelly et al (1977) studied the diuretic response to oral and intravenous furosemide in "diuretic-resistant" patients (patients with varying degrees of hepatic or renal disease) using ^{35}S-furosemide. They reported that the beta half-life of furosemide varied from 30-91 minutes. There was no report of excretion or metabolism of furosemide. A similar diuretic action was observed after oral or intravenous administration of furosemide to these patients. Beermann et al (1977) studied the pharmacokinetic disposition in healthy subjects and in patients with renal failure using gas chromatography, high pressure liquid chromatography and ^{35}S for determination of furosemide. They reported that the beta half-life of furosemide was 47.4 minutes in normal subjects and varied between 69 and 1475 minutes in patients with renal failure. ^{35}S-furosemide was administered i.v. to one patient with renal failure. Only 24% of the radioactivity was found in urine and only a small proportion, 4.1%, corresponded to unchanged

furosemide. They found 60.4% of the administered dose in feces collected for 6 days. The concentration of furosemide metabolites in plasma exceeded that of furosemide 4 hours after the intravenous dose.

Branch et al (1977) studied determinants of the response to furosemide in normal subjects. Using a fluorometric method for furosemide determinations, they reported its beta half-life to be 50 minutes. They recovered 65% of the administered dose unchanged in urine. Thin-layer chromatographic analysis of urine from these subjects after both intravenous or oral administration of furosemide failed to reveal significant amounts of the 2-amino-4-chloro-5-sulfamoylanthranilic acid, reported previously as the main metabolite of this drug. The urine concentration of furosemide was not found to have a consistent relationship with the induced diuretic response. A linear correlation between furosemide plasma concentration and the rate of sodium excretion was found.

Homeida et al (1977) studied the influence of probenecid and spironolactone on furosemide kinetics and dynamics in normal subjects using a fluorometric method to determine furosemide concentration. Probenecid pretreatment was found to reduce the renal clearance of furosemide by 78% and extrarenal clearance by 56%. As a consequence, the furosemide beta half-life was increased by 54% (from 38.4 to 59.1 minutes). Probenecid significantly reduced the rate of sodium excretion at all plasma concentrations of furosemide, but the proportion of furosemide unchanged in urine compared to sodium was not changed markedly. They found no evidence for a pharmacokinetic interaction between spironolactone and furosemide.

Andreasen et al (1977) studied the distribution, elimination and effects of furosemide in normal subjects and in patients with heart failure using a fluorometric method to determine furosemide concentrations. They reported an alpha half-life for furosemide of 9.5 minutes in normal subjects and 18.9 minutes in patients with cardiac decompensation receiving chronic furosemide therapy. The beta half-life of furosemide was 71.8 minutes in normal subjects and 134.1 minutes in patients with heart failure receiving chronic furosemide therapy. The mean excretion of unchanged furosemide for patients and normal subjects was similar (63% of the administered dose recovered in the 24 hour collection). After treatment of urine samples with β-glucuronidase, a glucuronide metabolite of furosemide in amounts up to 4% of the administered dose was observed in normal subjects and up to 12% in patients with cardiac decompensation receiving chronic furosemide treatment. No visible spot was observed on any thin-layer chromatography plate characteristic of 2-amino-4-chloro-5-sulfamoylanthranilic acid. Andreasen et al (1978) studied the pharmacokinetic disposition of furosemide in anephric patients and in normal subjects using a modified fluorometric method and thin-layer chromatography. No alpha or beta half-life was reported for these patients. Very small amounts of the 2-amino-4-chloro-5-sulfamoylanthranilic acid were found in serum of both normal subjects and anephric patients. When they compared the new method with their old method of determining furosemide and its metabolites, they found a discrepancy in plasma clearance of up to 31%. Plasma clearance with the old method was 167 ml/min and with the new method 219 ml/min. This was associated with a reduction of almost 50% in the renal

clearance of furosemide which was reported as 118 ml/min by the old method and 66 ml/min by the new method in normal subjects. Lawrence et al (1978) compared the kinetics and dynamics of piretamide and furosemide in normal subjects using the fluorometric method. They reported the beta half-life of furosemide to be 36 minutes. No data for the urinary excretion of furosemide or its metablism were given. A linear correlation was found between the urinary excretion rate of furosemide and the concomitant sodium or potassium excretion rate.

Furosemide Assay Methodology

Analysis of the available literature on furosemide pharmacokinetics demonstrated that most of these studies performed in normal subjects or patients have utilized methods which lack specificity. The spectrofluorometric method described by Häussler and Hadju (1964) and used by most of the investigators mentioned above does not completely differentiate furosemide from its metabolites.

The radioactive labelling of furosemide is not itself specific if the label excreted is not subjected to chromatographic separation. A summary of pharmacokinetic parameters published to the present time is presented in Appendix 2 (Tables XVIII - XXI). Date concerning investigations of the metabolism of furosemide have been conflicting. Some authors have demonstrated metabolites of furosemide (Häussler and Wicha 1965, Rupp et al 1973, Andreasen et al 1977 and 1978), while others have been unable to find them Calesnick et al 1966; Branch et al 1977). In the present study, a very specific and sensitive gas chromatographic method that can distinguish furosemide and its metabolites was used.

Furosemide Kinetic Disposition and Effect in Pulmonary Edema

It has been demonstrated that disease states can affect drug pharmacokinetics (Benet 1976). A study of the pharmacokinetic disposition of furosemide in patients with pulmonary edema would be useful to determine if hemodynamic and physiological changes during pulmonary edema would affect the pharmacokinetics and pharmacodynamics of this potent diuretic.

Physiological Changes Occurring During Acute Pulmonary Edema Due to Left Ventricular Failure

Acute pulmonary edema of cardiogenic origin occurs in the presence of underlying heart disease and is precipitated by an event that causes either an acute decrease in left ventricular output or a rapid increase in venous return or right ventricular output. The principal factors causing acute pulmonary edema include coronary heart disease, myocardial infarction, systemic arterial hypertension and aortic or mitral valve disease.

The effects of left ventricular failure are manifested most predominantly in the lungs, although the function of other organs such as the kidney and brain may also be markedly impaired. As a consequence of left ventricular failure, a progressive pooling of blood within the pulmonary vasculature occurs and hydrostatic pressure in the pulmonary veins increases. This pressure is transmitted to the capillaries which normally have a pressure of 6-9 mm Hg. With an increase in hydrostatic pressure to 25-30 mm Hg, frank edema occurs. The anatomical structure of the lungs makes them particularly vulnerable to the development of edema since their honeycomb structure exerts

no significant tissue resistance to the escape of fluid. Formation of edema produces the clinical manifestation of dyspnea, probably because of inadequate oxygenation of blood in the functionally impaired lungs. However, it could also be due to hypoxemia of the respiratory center and carotid sinus. Cyanosis can be present because of the inadequate oxygenation of blood. Hemodynamic derangements occurring with failure of the left ventricle may markedly affect the kidneys. Reduction in cardiac output decreases blood flow to the renal arteries as well as increasing renal venous congestion. This results in renal hypoxia and reduction of arterial pulse pressure and glomerular filtration rate. Blood flow through the splanchnic circulation is markedly reduced to maintain flow and oxygenation of more important organs such as the heart and brain. How these changes affect the distribution, elimination and dynamic effect of furosemide is not presently known.

Mechanism for the Renal Action of Furosemide

Furosemide is an anthranilic acid diuretic which inhibits chloride reabsorption in the ascending limb of the loop of Henle (Burg et al 1973). Until a few years ago, it had been assumed that the ion being actively transported in the ascending limb of the loop of Henle was sodium and that chloride followed passively as is the case in other parts of the nephron. Direct assessment of this area was done by Burg and Green (1973) after developing a method to isolate and perfuse individual nephron segments. When the potential difference across the epithelium of the ascending limb of the loop of Henle was determined, it was found that the luminal membrane was positive in

relationship to the plasma membrane. This indicated an active anion transport system. Anion replacement studies showed that chloride was the anion actively transported. Burg et al (1973) tested furosemide in isolated rabbit segments of the ascending limb of the loop of Henle. They observed that furosemide caused a prompt decrease in the potential difference when it was perfused through the lumen, and when they measured chloride flux, a decrease in chloride transport was noted. When furosemide was removed from the perfusion solution, the potential difference and chloride transport returned to baseline values. When furosemide was perfused through a segment of the proximal or collecting tubules, no effect on potential difference was observed. When furosemide was placed into the bath and allowed to come in contact with the plasma membrane, no effect on potential difference was observed. Therefore two conclusions were derived from these experiments. First, furosemide inhibited chloride reabsorption in the ascending limb of the loop of Henle. Secondly, access to the luminal rather than the plasma membrane appeared to be necessary for the diuretic activity of furosemide.

The molecular basis for the action of furosemide has been actively sought, but up to the present time, is not well understood. However, three basic processes have been invoked:

1) Inhibition of Na^+-K^+ ATPase (Schmidt 1970)
2) Inhibition of synthesis of cyclic AMP (Ebel 1974)
3) Inhibition of glycolysis (Klahr et al 1973).

Definitive resolution of the molecular basis for the pharmacological effects of furosemide awaits identification of what role these factors

play in the control of salt and water homeostasis by the nephron.

Pharmacokinetic Disposition and Analysis

Pharmacokinetic analyses of furosemide disposition have been accomplished assuming a one or two compartment open model. The mathematical description of these models is presented in the following section.

Pharmacokinetics

The word "pharmacokinetics" derives its meaning from two stems; "kinetics", the study of movement and "pharmacon", the Greek word for drug and poison. The term was coined by Professor F. H. Dost in Germany. Pharmacokinetics includes the study of time-courses of drug and metabolite concentrations and amounts in biological fluids, tissues and excreta; pharmacological response; and the construction of suitable models to interpret such data. The goals of pharmacokinetics are as diverse as the disciplines that apply its principles to their efforts. These disciplines include the clinical sciences, particularly clinical pharmacology, as well as drug metabolism, pharmaceutical science, statistics, toxicology and pharmacology. Clinical pharmacology is concerned with the scientific study of drugs and their actions and effects on man. It emphasizes that effects of drugs are often characterized by significant interspecies variation and may be further modified by disease. Functions of clinical pharmacology are: (Wagner 1975)

1. To improve patient care by promoting safer and more effective use of drugs.
2. To increase knowledge through research.

It is usually stated that development of sophisticated mathematical models as well as specific chemical assays are virtually essential to the interpretation of the kinetic phenomena. Such mathematical models are described in the following pages.

One Compartment Open Model

The most simple approach to the pharmacokinetic characterization of a drug is to represent the body as a single compartment.

Assumptions

1. The body is considered as a single homogeneous unit with a volume Vd.
2. Any changes in plasma concentration (C_p) quantitatively reflect changes in tissue concentration of the drug.
3. Elimination occurs from this compartment by a first-order process represented by the rate constant k_e.
4. The dose of drug (D) is instantaneously distributed through the body.

Assuming that the rate of elimination of a drug is proportional to its concentration in plasma, it will be described by first order kinetics. This relationship may be expressed in the form of a linear differential equation:

$$\frac{C_p}{dt} = -kC_p \qquad (1)$$

where C_p is the plasma concentration at time t and

k is the rate constant for elimination, and for a one compartment open model approximates β.

The solution of this equation with the initial conditions C_p = when t=zero yields

$$C_p = \frac{X}{Vd} \qquad (2)$$

where x is the amount of drug in the body

and Vd is the apparent volume of distribution in this single compartment.

The exponential form of this equation is:

$$C_p = C_{p_o} e^{-kt} \qquad (3)$$

where e represents the base of the natural logarithm.

Therefore, $\ln C_p = \ln C_{p_o} - kt$ (4)

A plot of C_p versus time on semilogarithmic paper yields a straight line with the slope $-k/2.303$. This is described by the equation:

$$\text{Log } C_p = \text{Log } C_{p_o} - kt/2.303 \qquad (5)$$

The biological half-life, i.e., the time required to reduce a given plasma concentration by 50%, is described in the equation:

$$t_{1/2} = \frac{0.693}{k} \qquad (6)$$

Two Compartment Open Model

The two compartment open model describes the body as consisting of two spaces or volumes. These volumes do not necessarily correspond to specific anatomical structures. They are theoretical spaces postulated to account for the experimental observation that drugs are distributed through the body fluid and tissues at different rates. However, it has been postulated (Gibaldi and Perrier, 1975; Greenblatt and Koch-Weser, 1975) that physiologically one of these volumes can be described as a central compartment (V_1) probably consisting of blood together with the extracellular fluid of highly perfused tissues such as heart, lung, liver, kidney and endocrine glands. The other space or compartment is the peripheral compartment (V_2). It is said to be composed of less rapidly perfused tissues such as muscle, skin and body fat.

This model is represented by a bi-exponential decline in plasma concentration of drug as a function of time after intravenous injection. The initial, rapid fall in drug concentration called the alpha (α) or distribution phase represents distribution of drug from the central to peripheral compartment. When the distribution phase is completed, a dynamic equilibrium between drug concentration in the central and peripheral compartments is established and constantly maintained. The curve then enters into a relatively slow beta (β) or elimination phase. There are three types of two compartment open models (Wagner 1975). This arises because elimination can occur either from the central compartment, the peripheral compartment or from both. However, it is usually assumed that drug elimination in

this model occurs exclusively from the central compartment. The two compartment open model also specifies characteristics of drug passage into and out of the system. The central compartment with volume V_1 and drug concentration C_1 is open in the sense that elimination occurs from it by excretion and/or metabolism. This process is represented by the first order elimination rate constant k_{el}. The entire system is open since drug passes rapidly into and out of the peripheral compartment with the volume V_2 and drug concentration C_2 by the first order processes described by the rate constants k_{12} and k_{21}, respectively.

The drug concentration in the central compartment (C_1) depends upon several simultaneous and independent processes. Drugs can leave the central compartment by both distribution to the peripheral compartment and by elimination. Since they are first order processes, the rate at which they occur is directly proportional to C_1 with a proportionality constant equal to the sum of k_{12} and k_{el}. Drugs can return to the central compartment by redistribution from the peripheral space. This movement occurs at a rate proportional to the drug concentration in the peripheral compartment (C_2) with a rate constant k_{21}. The rate of change in C_1 can be represented by the equation:

$$\frac{dC_1}{dt} = -(k_{12} + k_{el})C_1 + k_{21} \cdot C_2 \qquad (7)$$

The rate of change in C_2 can be represented by the equation

$$\frac{dC_2}{dt} = -k_{21}C_2 + k_{12}C_1 \qquad (8)$$

These two simultaneous first-order, linear differential equations

are the mathematical basis for the two compartment model. They are appropriately modified for different methods of drug administration and solved with the use of Laplace transformations (Gibaldi and Perrier, 1975). If one administers a single dose (D) directly into the central compartment, and assumes instantaneous distribution throughout it, the concentration of drug (C_1) immediately after injection is equal to the dose divided by the volume of the central compartment. At the same time the amount of drug in the peripheral compartment is zero. At time (t) = 0 $C_1 = \frac{D}{V_1}$ and $C_2 = 0$.

Solving equations 7 and 8 yields the following relationship between C_p and time (t) after the end of the injection:

$$C_p = Ae^{-\alpha t} + Be^{-\beta t} \qquad (9)$$

where C_p is plasma concentration of the drug.

A = intercept with the ordinate obtained by extrapolating the α disposition curve back to time zero.

B = intercept with the ordinate obtained by extrapolating the β disposition curve back to time zero.

α = rate constant for the initial rapid phase of drug distribution

β = rate constant for the later slower phase of drug disposition (metabolism and excretion).

A plot of the logarithm of C_p versus time yields a curve with two distinct linear segments. The half-life of the initial fall in concentration called the alpha or distribution phase is represented by the equation

$$t_{1/2} = \frac{\ln 2}{\alpha} = \frac{0.693}{\alpha} \qquad (10)$$

The slow beta or elimination phase of drug disposition is determined by irreversible elimination from the central compartment and transfer

of drug from the peripheral to the central compartment. The slope of the curve during this phase is β. The elimination half-life is represented by the equation:

$$t_{1/2} = \frac{\ln 2}{\beta} = \frac{0.693}{\beta} \qquad (11)$$

If one knows the rate constants k_{12}, k_{21} and k_{el}, it is possible to derive α and β from the following equations: (Gibaldi and Perrier 1975, Wagner 1975)

$$\alpha = \frac{k_{12} + k_{21} + k_{el} + \sqrt{(k_{12} + k_{21} + k_{el})^2 - 4.k_{21}.k_{el}}}{2} \qquad (12)$$

$$\beta = \frac{k_{12} + k_{21} + k_{el} - \sqrt{(k_{12} + k_{21} + k_{el})^2 - 4.k_{21}.k_{el}}}{2} \qquad (13)$$

Volume Terms

The apparent volume of distribution (Vd) of a drug is defined as that volume in which the total amount of drug in the body would be uniformly distributed in order to give the observed plasma concentrations. It is usually referred to as the central compartment. The total apparent volume of distribution of a drug provides an estimate of the extent of its distribution through body fluid compartments and its uptake by tissues. Its main use is in relating the plasma concentration of drug to its total amount in the body at a given time. Therefore, the total amount of drug in the body = $Vd \cdot C_p$

If a single dose of drug (D) is administered directly into the central compartment (V_1) and is instantaneously distributed throughout it, then at time zero:

$$D = C_{p_o} \cdot V_1 \qquad (14)$$

Therefore $$V_1 = D/C_{p_o} = D/(A + B) \qquad (15)$$

The volume of distribution at steady state (Vd_{ss}) is determined when the rate of change of the amount of drug in the peripheral compartment is zero. From equation 7:

$$\frac{dC_1}{dt} = k_{12}C_1 - k_{21}.C_2 = 0$$

and substituting

$$C_1 \text{ by } V_1.C_p$$

then

$$k_{12}.V_1.C_p = k_{21}.C2 \tag{16}$$

and therefore $$\frac{k_{12}}{k_{21}} V_1 = \frac{C_2}{C_p} = V_2 \tag{17}$$

As C_2/C_p = volume of distribution of the drug in the peripheral compartment with respect to the concentration in the central compartment, then

$$Vd_{ss} = V_1 + V_2 \tag{18}$$

$$= V_1 + \frac{k_{12}}{k_{21}} \cdot V_1 \tag{19}$$

$$= V_1 (1 + \frac{k_{12}}{k_{21}}) \tag{20}$$

Vd_β and Vd_{area}

For a two compartment open model:

$$Vd_\beta = D/B \text{ where } B = \frac{D\ (k_{21} - \beta)}{V_1\ (\alpha + \beta)} \tag{21}$$

Therefore $$Vd_\beta = \frac{V_1\ (\alpha - \beta)}{(k_{21} - \beta)} \tag{22}$$

Vd_{area} is approximately equal to Vd_{β}.

$$Vd_{area} = \frac{D}{\beta\ (AUC)} = \frac{D}{(\frac{A}{\alpha} + \frac{B}{\beta})}$$

Since $AUC = \int_0^{\infty} C_p dt = \frac{A}{\alpha} + \frac{B}{\beta}$

AUC = area under the plasma concentration versus time curve from t=0 to t= infinity. It can be calculated by employing an approximate integration formula. The trapezoidal rule is one of such formulas.

As $\frac{A}{\alpha} + \frac{B}{\beta} = \frac{D}{k_{el} \cdot V_1}$

$$Vd_{area} = \frac{k_{el} \cdot V_1}{\beta}$$

during the beta phase of drug disposition $k_{el/\beta} = C_{p_o}/B$

Therefore $\frac{k_{el}}{\beta} \cdot V_1 = \frac{C_{p_o} \cdot V_1}{B} = \frac{D}{B} = Vd_{\beta}$

The Trapezoidal Rule

The trapezoidal rule involves the description of a given plasma concentration curve by a function that depicts the curve as a series of straight lines, thereby enabling the area under the curve to be divided into a number of trapezoids. The area of each trapezoid is calculated and the sum of the areas of all trapezoids yields an estimate of the true area under the curve of interest. It can be expressed by the equation: (Gibaldi and Perrier 1975)

$$\int_{t_o}^{t_n} \phi\ (t)\ dt = \sum_{i=0}^{n-1} \frac{t_{i+1} - t_i}{2} (C_1 + C_{i+1}) \qquad (23)$$

Utility of Calculation of Area Under the Concentration Versus Time Curve (AUC)

AUC is an important determination for the calculation of many pharmacokinetic parameters such as plasma clearance, renal clearance and the volume of distribution. In those calculations, AUC describes the plasma concentration of a drug. Determination of AUC is important for oral dose studies in which AUC measures bioavailability. It is also important in considering oral versus intravenous drug aministration in which AUC measures the relative bioavailability of oral doses.

Clearance

Serum clearance (Cl_s) Drugs can be cleared from serum by hepatic biotransformation, excretion by the kidney, exhalation by the lungs or fecal excretion. In a two compartment model, elimination of drug from the central compartment is governed by the rate constant k_{el}. This is related to serum clearance by the equation

$$Cl_s = V_1 . k_{el}$$

$$Cl_s = \beta . Vd_{area} = \frac{0,693}{t_{1/2}} . Vd_{area} \qquad (24)$$

$$Cl_s = \frac{D}{\int_0^\infty C \, dt} = \frac{D}{AUC} \qquad (25)$$

Clearance of a drug is inversely proportional to its elimination half-life ($t_{1/2}\beta$) and is directly proportional to the apparent volume of distribution (Vd).

Renal Clearance

When the drug is partially or entirely excreted unchanged by the

kidney, its renal clearance can be calculated by dividing the amount of drug found unchanged in the urine by the mean plasma concentration in the same time interval. However, the area under the plasma concentration versus time curve (AUC) reflects better its changing plasma concentration for the time interval described by the equation:

$$Cl_R = \frac{\text{urinary excretion of unchanged drug}}{\int_{t_1}^{t_2} C\,dt} \qquad (26)$$

$$= \frac{\text{urinary excretion of unchanged drug}}{\text{AUC from } t_1 \text{ to } t_2} \qquad (27)$$

AUC is calculated by the trapezoidal rule for the time interval of patient urine excretion.

Another way to describe elimination of a drug is to determine its excretion rate. It is calculated by:

$$Exc = \frac{U \cdot V}{t} \qquad (28)$$

where U is the concentration of the substance found in urine, V = urine volume excreted during the collection time and t = time or period of urine collection. The excretion rate does not require plasma concentration data for its determination. This is particularly important for drugs where plasma concentrations do not reflect drug action.

Utility of Clearance Determinations

Clearance of a substance describes the volume of plasma or serum per unit of time from which it is removed. For renal clearance, it is a measure of the efficiency of the kidney to clear a particular substance. Serum clearance includes both renal clearance and clearance

due to metabolic transformation. Determination of both plasma and renal clearance will indicate relative rates of metabolism and urinary excretion in the disposition of a substance. It is important to know how a drug is cleared from the body, at what rate it is cleared, and which organ is predominantly responsible for clearance of the drug, in order to derive optimal therapeutic regimens and to prevent toxicity.

GLOSSARY

GLOSSARY OF PHARMACOKINETIC ABBREVIATIONS

C_P	Plasma concentration at any time
C_{P_0}	Plasma concentration at t=0
k	Elimination rate constant, one compartment model
k_{el}	Elimination rate constant for two compartment model
k_{12}	Rate constant for transfer of drug from the central to the peripheral compartment
k_{21}	Rate constant for transfer of drug from the peripheral to the central compartment
D	Dose of drug administered
C_1	Drug concentration in the central compartment
C_2	Drug concentration in the peripheral compartment
α	Rate constant for the distribution (alpha) phase
β	Rate constant for the elimination (beta) phase
$\alpha t_{1/2}$	(Alpha) distribution half-life
$\beta t_{1/2}$	(Beta) elimination half-life
A	Intercept with the ordinate obtained by extrapolating the α disposition curve back to time=0.
B	Intercept with the ordinate obtained by extrapolating the β disposition curve back to time=0.
V_1	Volume of the central compartment
V_2	Volume of the peripheral compartment
Vd	Apparent volume of distribution
Vd_{ss}	Volume of distribution at steady state
Vd_{area}	Apparent volume of distribution calculated from the area under the plasma concentration curve

GLOSSARY

Vd_{β}	Apparent volume of distribution during the beta or elimination phase
AUC	Area under the plasma concentration versus time curve
Cl_S	Serum clearance
Cl_R	Renal clearance
Exc	Urinary excretion rate

MATERIALS AND METHODS

MATERIALS AND METHODS

Patient Selection and Diagnosis

Sixteen patients with the diagnosis of Acute Pulmonary Edema seen in the Montreal General Hospital volunteered to participate in this study. (Ethical approval protocol - Appendix 1). Criteria for the diagnosis of acute pulmonary edema and admission into the study were as follows:

1. Recent onset or acute exacerbation of severe dyspnea;
2. Acute respiratory distress with bilateral chest rales on auscultation;
3. Chest X-ray compatible with acute pulmonary edema of cardiac origin.

These criteria are similar to those accepted for the diagnosis of acute pulmonary edema (Lesch et al, 1968), which were:

1. Sudden recent onset of severe dyspnea.
2. Acute respiratory distress with bilateral rales or wheezes (patients with primary pulmonary disease were excluded), and respiratory rate greater than 30.
3. Clinical evidence of congestive heart failure.
4. X-ray film of the chest compatible with acute pulmonary edema.

Clinical diagnosis of acute pulmonary edema was agreed upon by at least two physicians. Severity of acute pulmonary edema was assessed on the basis of the X-ray film as follows:

Mild: Hilar and pulmonary vascular congestion and either perivascular (hazy and indistinguished vessels) or septal (curly B lines) pattern of interstitial edema.

Moderate: Alveolar edema with small patchy nonconfluent densities, estimated to involve less than half the area of the lung fields.

Severe: Alveolar edema with large fluffy confluent densities, estimated to involve more than half the area of the lung fields.

These criteria are identical to those accepted for the classification of severity of acute pulmonary edema (Aberman et al 1972).

Some of the patients with pulmonary edema had myocardial infarction. The diagnosis of myocardial infarction was made on the bases of:

1. Increased serum enzymes, creatinine phosphate kinase (CPK) glutamic oxaloacetic transaminase (SGOT), and lactic dehydrogenase (LDH), within 24-48 hours after onset of symptoms.

2. Electrocardiogram showing abnormal Q waves, changes in ST segment and later, symmetric inversion of T waves.

Clinical evaluation was completed within 20 minutes of the patient's arrival in the emergency ward. Routine treatment of acute pulmonary edema instituted in the emergency unit of the Montreal General Hospital may include placing the patient in semi-upright position, supplemental oxygen by mask, rotating tourniquets to the limbs as well as administration of morphine, theophylline and digoxin. In this study an intravenous (i.v.) infusion of furosemide (20 to 80 mg in 10 ml solution of 5% dextrose) was administered over five minutes by an infusion pump. The patient was asked to empty his bladder before the administration of the drug. Patients were then transferred to the Medical Intensive Care Unit (MICU) or coronary monitoring unit (CMU). Other therapy was the responsibility of the attending staff in the MICU or CMU. Administration of furosemide did not preclude other drug therapy.

Exclusions

Patients with pulmonary edema secondary to inhalation, aspiration or

ingestion of toxins and patients with acute cardiac dysrhythmias or cardiovascular shock were excluded from this study. As patients received a drug which was accepted treatment of this disease, their consent was obtained only for blood and urine sampling in conjunction with this investigation.

Collection of Biological Samples

Blood samples were taken from the arm opposite to the one used for drug infusion via an indwelling venous catheter in an antecubital vein. The catheter was filled with a solution of heparinized saline (10 U/ml) between sampling to prevent clotting. The catheter was withdrawn after the first 12 hours. Thereafter samples were taken by venipuncture.

Blood samples, placed in glass tubes without anticoagulant, were taken at the following intervals:

Pre-dose, 10, 20, 30, 40, 50, 60, 70, 80, 100, 120 minutes and then 3, 4, 6, 8, 12 and 24 hours after the dose. Serum was separated by centrifugation and frozen at -20° until analyzed for drug and ionic content.

Urine Samples

All urine excreted was collected during the following intervals for 24 hours after drug administration:

0-20, 20-40, 40-60, 60-80, 80-100, 100-120 minutes and then 2-3, 3-4, 4-6, 6-8, 8-12, 12-24 hours. The patient was asked to empty his bladder at the intervals described above. When that was not possible, urine was collected at the time the patient voided spontaneously.

After measurement of volume, aliquots of urine samples were frozen at -20° until subsequently analyzed.

Sample Analysis

Samples were analyzed during a period of 5 months and were checked

during that time at least twice to assure that there were no differences in the measurements during that time. Serum samples were analyzed for furosemide, 2-amino-4-chloro-5-sulfamoylanthranilic acid, sodium, potassium, chloride and creatinine content.

Urine: Samples were analyzed for furosemide, its metabolites (furosemide glucuronide and 2-amino-4-chloro-5-sulfamoylanthranilic acid), sodium, potassium, chloride, magnesium, calcium, creatinine, volume, pH and osmolality.

Assay for Furosemide and 2-amino-4-chloro-5-sulfamoylanthranilic acid

A gas-liquid chromatographic (GLC) method was used for the determination of serum and urine concentrations of furosemide and its acid metabolite (2-amino-4-chloro-5-sulfamoylanthranilic acid). This assay is a modification of the method reported by Lindstrom and Molander (1974). The major change involved inclusion of benzbromarone as the internal standard. Serum or urine (0.1 ml) was diluted with 0.2 ml of water and benzbromarone (200 mg in 10 µl 95% v/v ethanol) in a 15 ml round-bottom plastic stoppered tube. The mixture was acidified with 4 N hydrochloride acid (45 µl) and extracted with diethyl ether (2 x 3.0 ml) on a vortex mixer for one minute. The organic solvent (upper layer) was removed, combined and evaporated to dryness under a nitrogen atmosphere. A solution of 0.2 ml of 0.2 N sodium hydroxide, 5 µl of 0.1 M tetrahexylammonium iodide in dichloromethane and 0.5 ml of 0.5 M methyl iodide in dichloromethane were added to the residue. The tube was capped, mixed and then incubated at 50^{o} for 20 minutes. The contents of the tube were mixed twice during the incubation. The sample was centrifuged at 1000 x g for 10 minutes. The upper (aqueous) layer was removed and the organic solvent was evaporated in a nitrogen atmosphere. Hexane (0.10 ml) was added to the residue and the capped tube

was placed in an ultrasonic bath for 15 minutes. The tube was centrifuged at 1000 x g for 10 min and the hexane solution was transferred to a 0.2 ml vial which was sealed. An aliquot (5 µl) of this solution was injected for determination of furosemide and its acid metabolite concentration by GLC.

Principle

The basis for gas chromatographic separation is the distribution of a sample between two phases. One of these phases is a stationary fluid of large surface area (liquid phase) and the other phase is a gas which percolates through the stationary fluid. Heat is used to vaporize the components to be separated. They are then carried through the heated column by an inert gas (carrier gas). The sample mixture is partitioned between the carrier gas and a very low volatility liquid (stationary phase). The stationary phase selectively retards the sample components, according to their partition coefficients, until they form separate bands in the carrier gas. These component bands leave the column in a gas stream and are recorded as a function of time by the detector. The ^{63}Ni electron capture detector selectively measures compounds which have an affinity for electrons. It measures the loss of signal rather than a positively produced electrical current. If a sample containing electron absorbing molecules is then introduced, the current will be reduced. The loss of current is a measure of the amount and the electron affinity of the compound. These changes in current are recorded by an electronic integrator and are proportional to the peak area of the eluting component.

All samples were analyzed in duplicate. A calibration curve was derived by adding known amounts of furosemide and its acid metabolite to

either serum or urine and analyzing the samples as described above.

The gas liquid chromatography analysis for each sample required 20 minutes.

Instrumental Conditions

The gas-liquid chromatograph was a Hewlett-Packard model 5713A instrument equipped with a pulsed electron capture ^{63}Ni detector, a model 7671A automatic injector and a model 3380A reporting integrator. The column was a glass tube (6 ft x 1/8 in i.d.) packed with 3% OV-17 on chromosorb W (AW-DMCS; 80-100 mesh). Operating conditions were: injector temperature 300°, column temperature 295° and detector temperature 350°. The carrier gas was 5% methane in argon at a flow rate of 30 ml/min. The method used to determine the concentration of furosemide was the peak area ratio between furosemide or its acid metabolite and benzbromarone, the internal standard.

Assay for Furosemide Glucuronide

One ml of urine was placed in a 5-ml round-bottom screw-capped tube. One ml of bacterial β-glucuronidase type I (450 units/ml dissolved in 9.0 ml of 0.1 M pH 7 phosphate buffer and 2-3-drops of chloroform) was added. Urine samples were then incubated at 37° for 17 hours. Simultaneously, samples without β-glucuronidase were incubated as controls. The differences in furosemide concentration between urine samples incubated with and without β-glucuronidase was used as a measure of the concentration of furosemide glucuronide which was then expressed in furosemide equivalents. An experiment was done to determine whether the urinary metabolite was due to the conjugation of glucuronic acid with furosemide. A sample was selected which was previously found to contain a significant amount of furosemide glucuronide. One ml of this urine sample was placed in each

of 18 tubes. Bacterial β-glucuronidase solution was added to six of these tubes. Bacterial β-glucuronidase solution in conjunction with 20 mg of glucuronolactone was added to six other tubes and phosphate buffer (0.1 M pH 7.0) was added to the remaining six tubes. One tube from each of these groups was frozen immediately. The remaining tubes were incubated at 37°. One tube of each group was removed after being incubated for 4, 8, 12 16 and 24 hours. Tubes were frozen immediately after removal from the bath. Samples were thawed and analyzed in duplicate for furosemide as described above.

Electrolyte Assay

Sodium and Potassium

Sodium and potassium were determined by flame emission spectrophotometry with an I.L. Model 151 atomic absorption/emission spectrophotometer.

Principle

Flame emission, like atomic absorption, uses a flame to disperse the sample and produce a cloud of neutral atoms. However, instead of a light source, emission analysis uses the flame itself as an energy source to excite the ground state neutral atoms. When the excited atoms return to the ground state, they emit light of a wavelength characteristic of the emitting species. The intensity of this emitted light is directly proportional to the concentration of that atom in the flame. For analysis, the wavelength of an emitted line is selected by a monochromator, and its intensity is measured by a detector and amplifier. The relative emission intensity of an element is displayed on a digital meter, which may be calibrated to read concentration. Lanthanium nitrate (1% w/v) was used as a diluent for sodium and potassium determinations to suppress

excessive ionization and enhance sensitivity. A calibration curve was determined by adding known amounts of either sodium chloride or potassium chloride to the diluent and determining emission intensity in the flame. All samples were analyzed in duplicate and the result for each sample was the mean of three readings of the digital display of emission intensity. The calibrated curve was checked with a standard control after every twenty samples. Plasma and urine samples were diluted 1:10,000 for sodium and 1:500 for potassium determinations. Instrumental conditions for sodium analysis were as follows: photo-multiplier 800 V, slit width 160 µm, wavelength 589 nm, fuel acetylene and oxidant air. The sample aspiration rate was 4.5 - 5.0 ml/min. Analysis was done with a lean flame. The optimal range for sodium determination was 7-700 ng/ml with a detection limit of 0.7 ng/ml. Instrumental conditions for potassium analysis were as follows: photo-multiplier 1000 V, slit width 320 µm, wavelength 766.6 nm, fuel acetylene and oxidant air. Sample aspiration rate was 4.5 - 5 ml/min. Analysis was done with a lean flame. The optimal range for potassium determinations was 50 - 500 ng/ml with a detection limit of 5 ng/ml.

Calcium and Magnesium

Calcium and magnesium were determined by atomic absorption spectrophotometry with an I.L. model 151 atomic absorption/emission spectrophotometer.

Principle

In atomic absorption analysis a solution of sample is sprayed into a flame which dries, volatilizes, and breaks the sample into clouds of neutral atoms. Light from a hollow cathode lamp passes through the flame, and the neutral atoms of the sample, by absorbing some of this light,

decrease the intensity of the beam. The amount of light absorbed by the neutral atoms is a function of three factors:

1. Absorptivity constant for the element at the wavelength measured.
2. Length of the light path through the flame.
3. Concentration of the element being measured, taken as a direct relative measure of the number of neutral atoms.

By making the first two factors constant, the amount of light absorbed is a measure of the concentration of the element. A given element absorbs at the same wavelength that it emits when excited. Since a large proportion of atoms in the flame exist in the unexcited neutral state, the wavelength absorbed is ground state radiation.

For analysis the wavelength of an emitted line is selected by a monochromator, and its absorbance is measured by a detector and amplifier. The relative absorbance of an element is displayed on a digital meter, which may be calibrated to read concentration. Deionized water was used as a diluent for magnesium and calcium. A calibration curve was determinated by adding known amounts of either calcium or magnesium standard (1.0 g/L) to the diluent and determining the absorbance. All samples were analyzed in duplicate and the result for each sample was the mean of three readings on the digital display. The calibration curve was checked with a standard control after every 20 samples.

Plasma and urine samples were diluted 1:5 for calcium and magnesium determination. Instrumental conditions for calcium samples were as follows: lamp current 7 mA, photo-multiplier 530 V, slit width 320 μm, wavelength 422.7 nm, fuel acetylene and oxidant air. The sample aspiration rate was 4.5 - 5.0 ml/min. Analysis was done with a lean flame. The optimal range for calcium determination was 20-10,000 ng/ml. The

detection limit was 2 ng/ml.

Instrumental conditions for magnesium analysis were as follows: lamp current 4 mA, photo-multiplier 530 V, slit width 320 μm, wavelength 285.2 nm, fuel acetylene and oxidant air. The sample aspiration rate was 4.5 - 5 ml/min. Analysis was done with a lean flame. The optimal range for magnesium determination was 3-1000 ng/ml with a detection limit of 0.1 ng/ml.

Chloride Determination

Chloride was determined by titration with a Buchler-Catlove chloridometer. The technique involves the coulometric generation of reagent. A constant direct current is passed between a pair of silver generator electrodes causing a release of silver ions into the titration solution at a constant rate. The end-point is indicated after all the chloride ion has been precipitated by the increasing concentration of free silver ion. This is detected by the amperometric circuit wherein the free silver ions cause a rising current to pass between two indicator electrodes and a meter relay in the indicator amperometric circuit. At the preset increment of indicator current the relay is activated, stopping a time which runs concurrently with the generation of silver ions. Since the rate of generation of silver ions is constant, the amount of chloride precipitated is proportional to the elapsed time.

Technical Aspects:

(a) Reagent (for high titration rate)

1. Nitric-acetic reagent

0.1 N nitric acid (HNO_3)

10% glacial acetic acid

2. Gelatin reagent

Dry mix:

Gelatin	60
Thymol blue, water soluble	1
Thymol reagent grade crystal	1

Titration times of 20 to 60 seconds per sample have a standard deviation of about 0.5 per cent of mean value and shorter times of about 5 seconds per sample have only slightly greater variability. The method accurately measures amounts as low as 0.25 mEq of chloride.

All the samples were analyzed in duplicate and the concentration of chloride in mEq was the mean of two readings. The calibration curve was checked with a standard control after every 20 samples.

Creatinine

Creatinine was determined by quantitating the red pigment, alkaline creatine picrate (Jaffé reaction). The formation of the red color characterized the reaction of creatinine with picrate in an alkaline solution and it is determined with a Spectronic 20 colorimetric/spectrophotometer (Bausch and Lomb) at 520 nm.

Principle

The Spectronic 20 is an instrument used for measuring the effective transmission of monochromatic light through a solution. This measurement determines the concentration of solute in solution. It contains a source of white light and an optical system which separates that light into its component wavelengths, collectively called its "spectrum". Any wavelength in this spectrum may be selected for use. The selected wavelength passes through the sample (contained in a test tube) and strikes a photosensitive vacuum tube. The resulting electronic signal is amplified

and displayed on the meter, indicating the percent transmittance or absorbance of that sample.

The standard curve was determined by adding known amounts of creatinine (stock creatinine standard; 2 mg/ml in 0.1 N HCl; stable indefinitely in the refrigerator) to the diluent and reading against reagent blank at 520 nm. All samples were analyzed in duplicate and the concentration of creatinine was the mean of two readings.

Osmolality

Osmolality was determined with a Fiske^{R} osmometer which is a simplified apparatus for accurate determination of osmotic pressure. The osmometer measures freezing point depression but it is calibrated to read osmotic pressure units directly.

Principle

If pure water is very carefully cooled without being disturbed, it may reach temperatures as low as -40^{o} without freezing. Water that is cooled below the normal freezing point is said to be supercooled. If a piece of ice or a speck of dust is added to such water, freezing takes place rapidly and the temperature rises to 0^{o}, the normal freezing point. This is due to the fact that each gram of water at 0^{o} that forms ice at 0^{o} gives off 80 calories of heat (latent heat). The formation of ice takes place readily at this temperature if there is some dust or other foreign matter on which the first crystals of ice can condense. Supercooling occurs when no such foreign matter is present. Solutes in a liquid solvent generally lower the freezing point of their solvent. The freezing point depression is usually proportional to the concentration of solutes.

The calibration curve is obtained by measuring the freezing point depression of solution of known solute concentration.

A calibration curve was derived from Fiske® standard solutions containing known amounts of sodium chloride. All samples were analyzed in duplicate and the osmolality for each sample was the mean of two readings. The calibration curve was checked with a standard control after every twenty samples.

Data Analysis

Pharmacokinetics calculations were based on the assumption that disappearance of furosemide from serum was consistent with a two compartment open-system model. Serum concentration versus time data were analyzed with an iterative least squares computer program, ASAAM-23 on an IBM 360/65 computer for derivation of pharmacokinetics constants (Berman and Weiss, 1966). This analysis yielded the pharmacokinetics constants k_{12} k_{21} k_{el} and V_1. Other pharmacokinetics parameters (α, β, V_2, Vd_{ss} and Cl_s were calculated from the formulae described in the introduction (Equations 12, 13, 17, 19, 24). This approach was possible for eleven of the sixteen patients. In the remaining five patients, due to multiple doses, it was not possible to estimate the alpha phase with precision, but it was possible to calculate β, $\beta t_{1/2}$, Vd_{area} and Cl_s as described in the Introduction (Equations 11, 23, 25).

Urinary excretion rate and renal clearance of furosemide were calculated for all the patients as described in the Introduction (Equations 26, 27, 28). A non-parametric method, Mann-Whitney U-test was used for the analysis of data. The possible relationship between the pharmacokinetics and pharmacodynamic disposition of furosemide was assessed by correlation analysis using least squares linear regression followed by analysis of variance for goodness of fit. The minimum level for significance was accepted to be $p \leq 0.05$.

CHEMICALS UTILIZED IN THIS INVESTIGATION

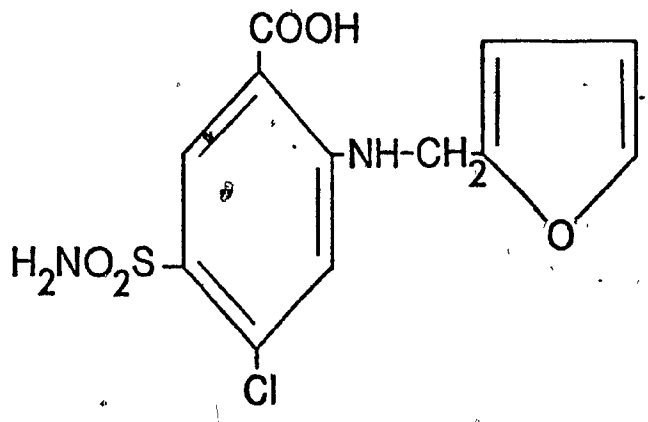
Chemical	Supplier
Furosemide 2-amino-4-chloro-5-sulfamoyl-anthranilic acid	Hoechst Pharmaceuticals, Montreal, Quebec
Bensbromarone	Mead Johnson & Co., Toronto, Ontario
Hydrochloric acid Sodium hydroxide Lanthanum Picric acid Lloyd reagent Oxalic acid Picric acid Methyl iodide Nitric acid	J. T. Baker 'Analyzed Reagent' supplied by Canadian Laboratory Supplies Ltd., Montreal, Quebec
Bacterial β-glucuronidase Glucuronolactone	Sigma Chemical Company, St. Louis, MO.
Sodium tungstenate Ether	Mallinckrodt Canada Ltd., Montreal, Quebec
Osmolality standards 100, 200, 400, 500, 1000 milliosmoles	Fiske Associates Inc., Uxbridge, Mass.

CHEMICALS - Continued

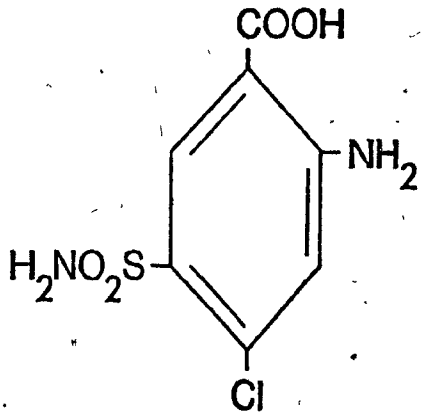
Chemical	Supplier
Nitrogen (high purity)	Medigas, Montreal, Quebec
Acetylene (purified)	
Hexane	Fisher Scientific Co. Ltd.,
Methylene chloride	Montreal, Quebec
Sodium chloride 'Analar'	B.D.H. The British Drug Houses, Ltd.,
Potassium chloride 'Analar'	Montreal, Quebec
Calcium standard	Harleco, A Division of American
Magnesium standard	Supply Co., supplied by Canadian Laboratory Supplies Ltd., Montreal, Quebec
Electrolytes standard	Dade Division of American Supply Co., supplied by Canadian Laboratory Supplies Ltd., Montreal, Quebec
Gelatine reagent	Searle, Division of Searle Analytic Inc., supplied by Canadian Laboratory Supplies Ltd., Montreal, Quebec
Tetra-N-hexyl ammonium iodide	ICN Pharmaceuticals Inc., Life Sciences Group, Montreal, Quebec

RESULTS

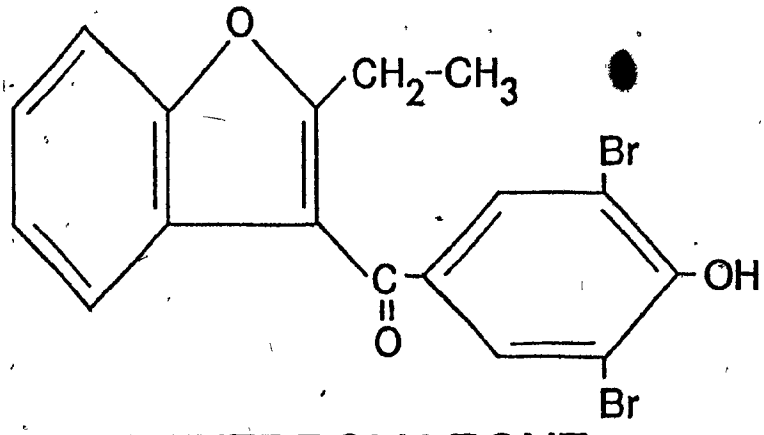
Figure 1. Chemical structures of furosemide, 2-amino-4-chloro-5-sulfamoylanthranilic acid and benzbromarone (internal standard).



FUROSEMIDE



2-AMINO-4-CHLORO-5-SULFAMOYL-
ANTHRANILIC ACID



BENZBROMARONE

RESULTS

Analysis for Furosemide and its Metabolites

Chemical structures of furosemide, 2-amino-4-chloro-5-sulfamoylanthranilic acid and benzbromarone are shown in Figure 1. The retention indices of furosemide/benzbromarone (F/B) and 2-amino-4-chloro-5-sulfamoylanthranilic acid/benzbromarone (acid/B) by gas-liquid chromatographic analysis were 1.68 and 0.73, respectively. Gas-liquid chromatograms of the extraction of furosemide and its metabolites from both urine and serum samples were similar. Representative examples are shown in Figure 2A and 2B. The standard curve of the area ratio F/B was linear within the range of 0.05 to 64 mg/L and for acid/B was linear within the range of 0.1 to 32 mg/L. Representative calibration curves are shown in Figures 3 and 4 respectively. The range of concentrations found either in serum or urine of the patient's never exceeded those limits. Correlation coefficient values for definition of the linearity of these standard curves was always greater than 0.99.

The relationship between the amount of furosemide glucuronide found in urine and the time of urine incubation with β-glucuronidase is presented in Figure 5. Neither samples which contained urine and buffer nor samples which contained β-glucuronidase mixed with glucuronolactone showed significant difference in the amount of furosemide due to the incubation. However, the amount of furosemide increased with time of incubation until sixteen hours had elapsed in urine samples containing β-glucuronidase. The differences between urinary furosemide content before and after hydrolysis for 16 hours with β-glucuronidase was attributed to the formation of the glucuronide conjugate of furosemide. The site of attachment of the glucuronic acid moiety to furosemide was not determined.

Figure 2. Representative gas-liquid chromatograms of extracts of serum or urine for furosemide and its acid metabolite 2-amino-4-chloro-5-sulfamoylanthranilic acid. A, sample blank; B, 2-amino-4-chloro-5-sulfamoylanthranilic acid (4.85 min), benzbromarone (6.66 min) and furosemide (11.19 min).

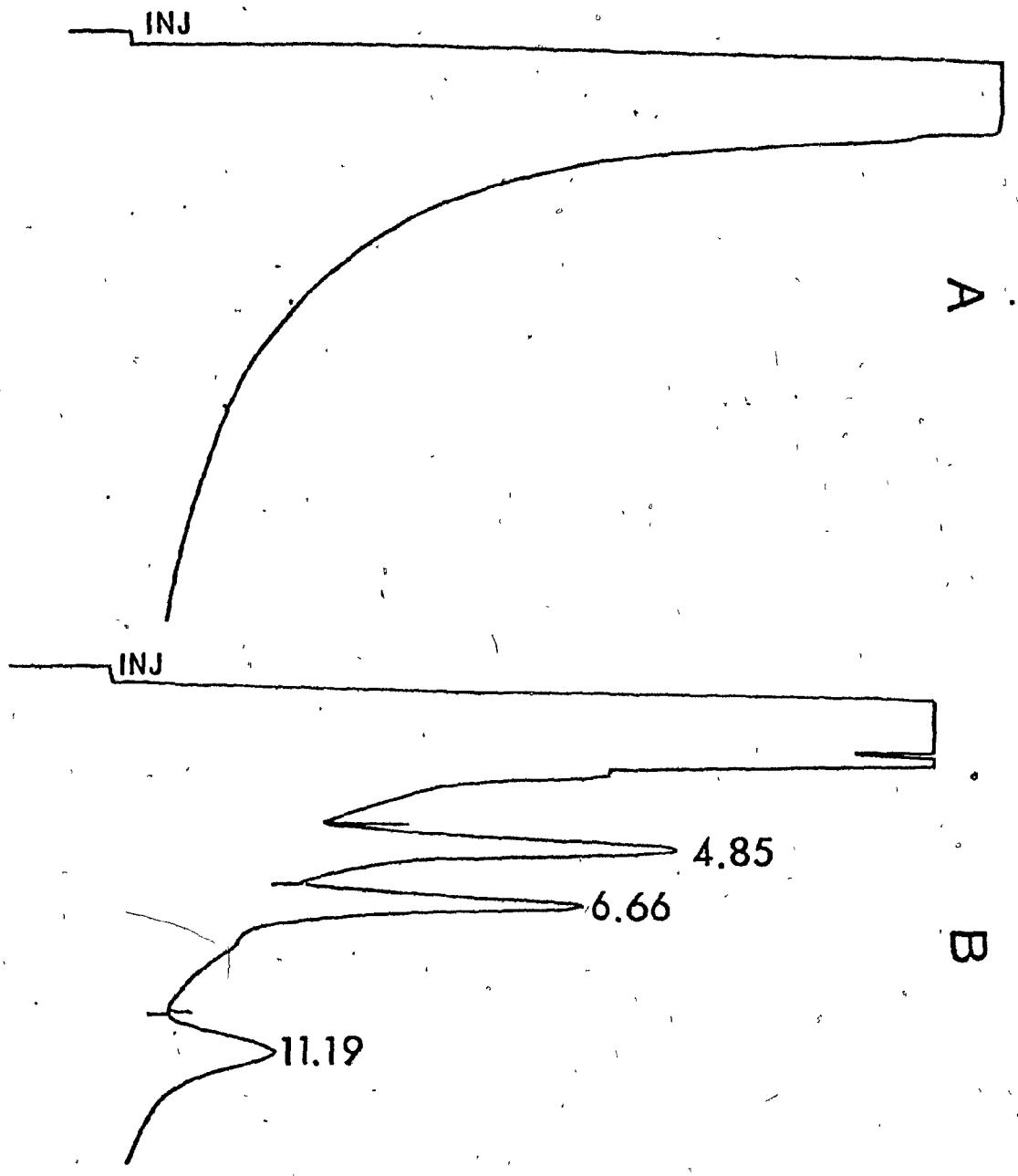

INJ
A
INJ
4.85
6.66
11.19
B

Figure 3. Representative standard curve for the extraction and gas-liquid chromatographic analysis of furosemide from serum or urine with benzbromarone as the internal standard.

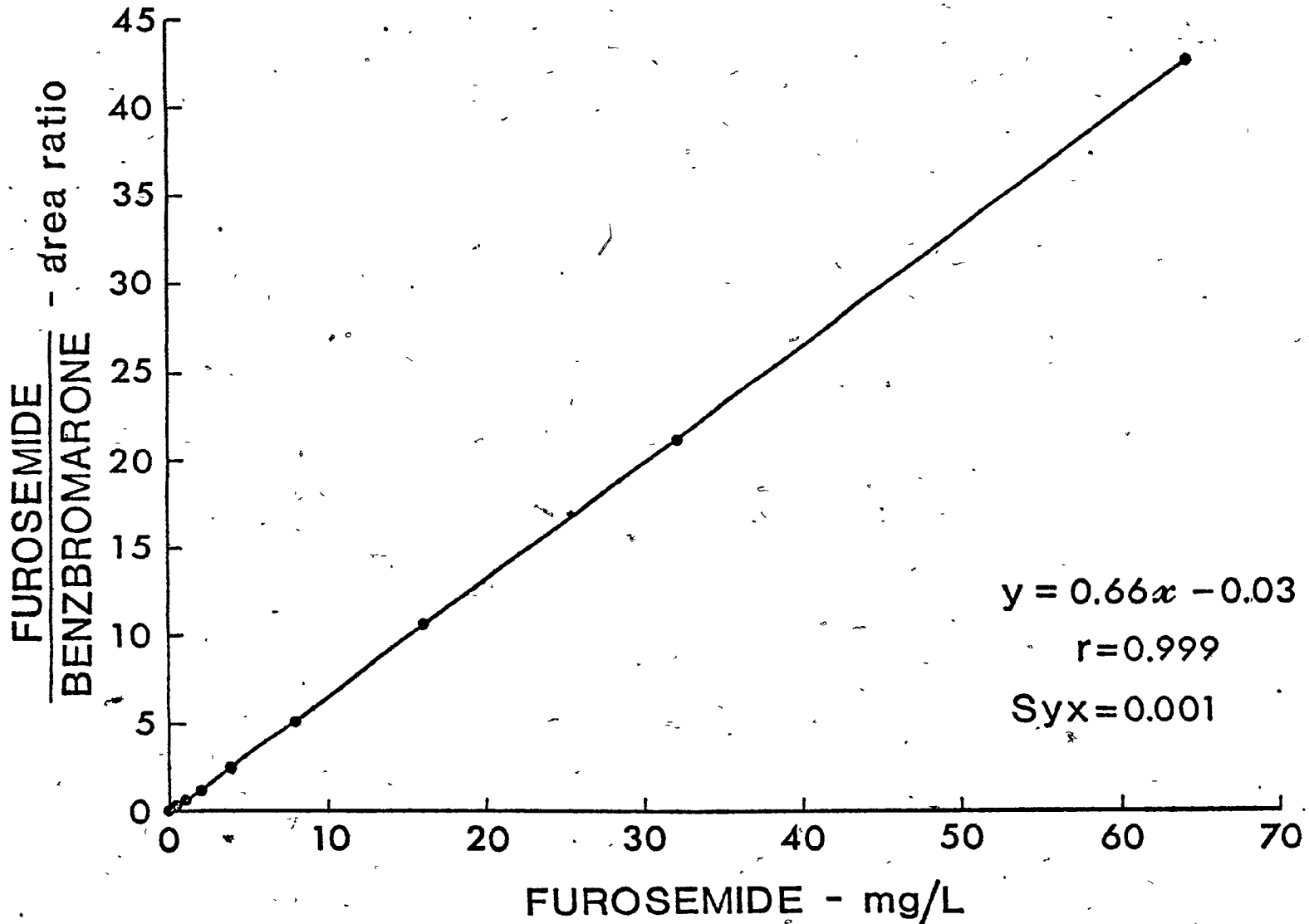

FUROSEMIDE / BENZBROMARONE - area ratio
45
40
35
30
25
20
15
10
5
0
0
10
20
30
40
50
60
70
FUROSEMIDE - mg/L
y = 0.66x −0.03
r=0.999
Syx=0.001

Figure 4. Representative standard curve for the extraction and gas-liquid chromatographic analysis of the acid metabolite of furosemide, 2-amino-4-chloro-5-sulfamoylanthranilic acid, from serum or urine with benzbromarone as the internal standard.

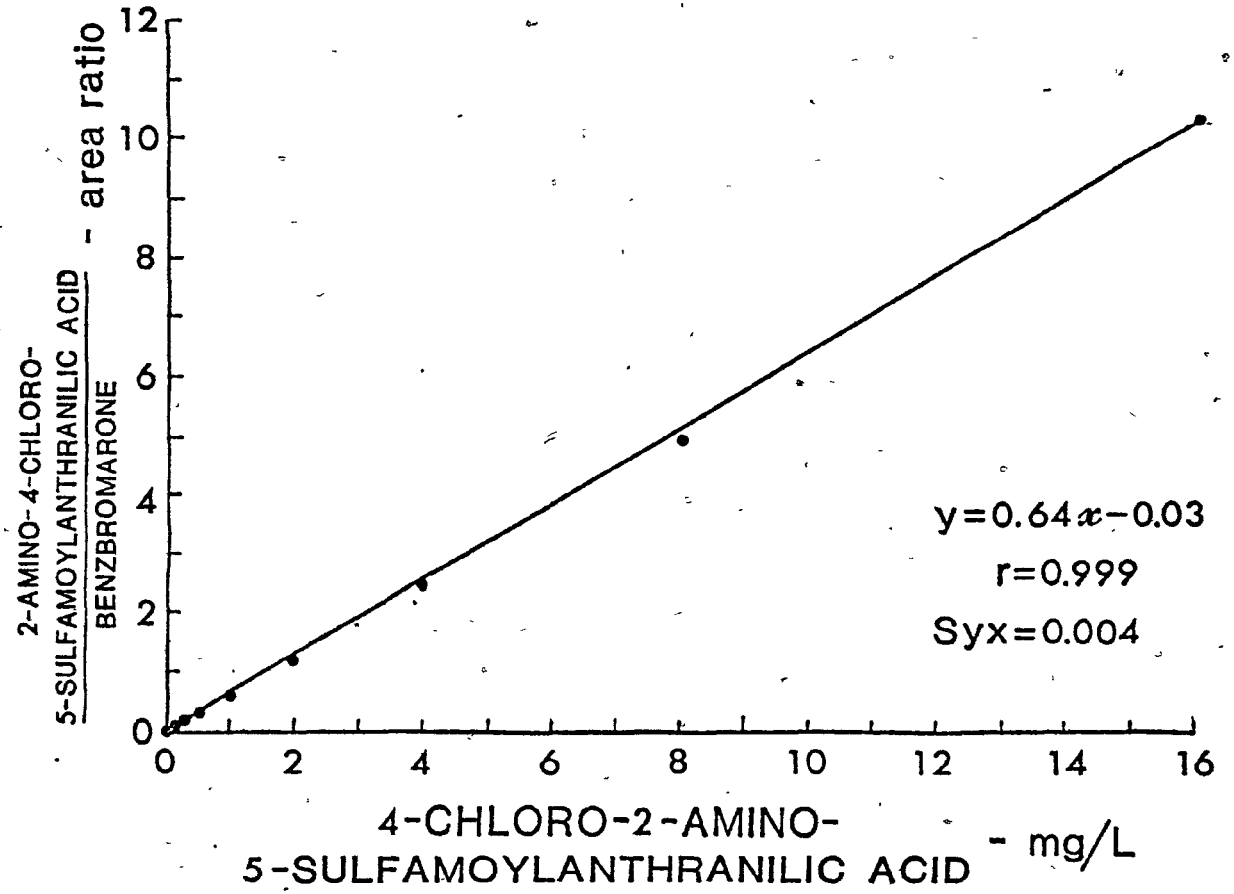
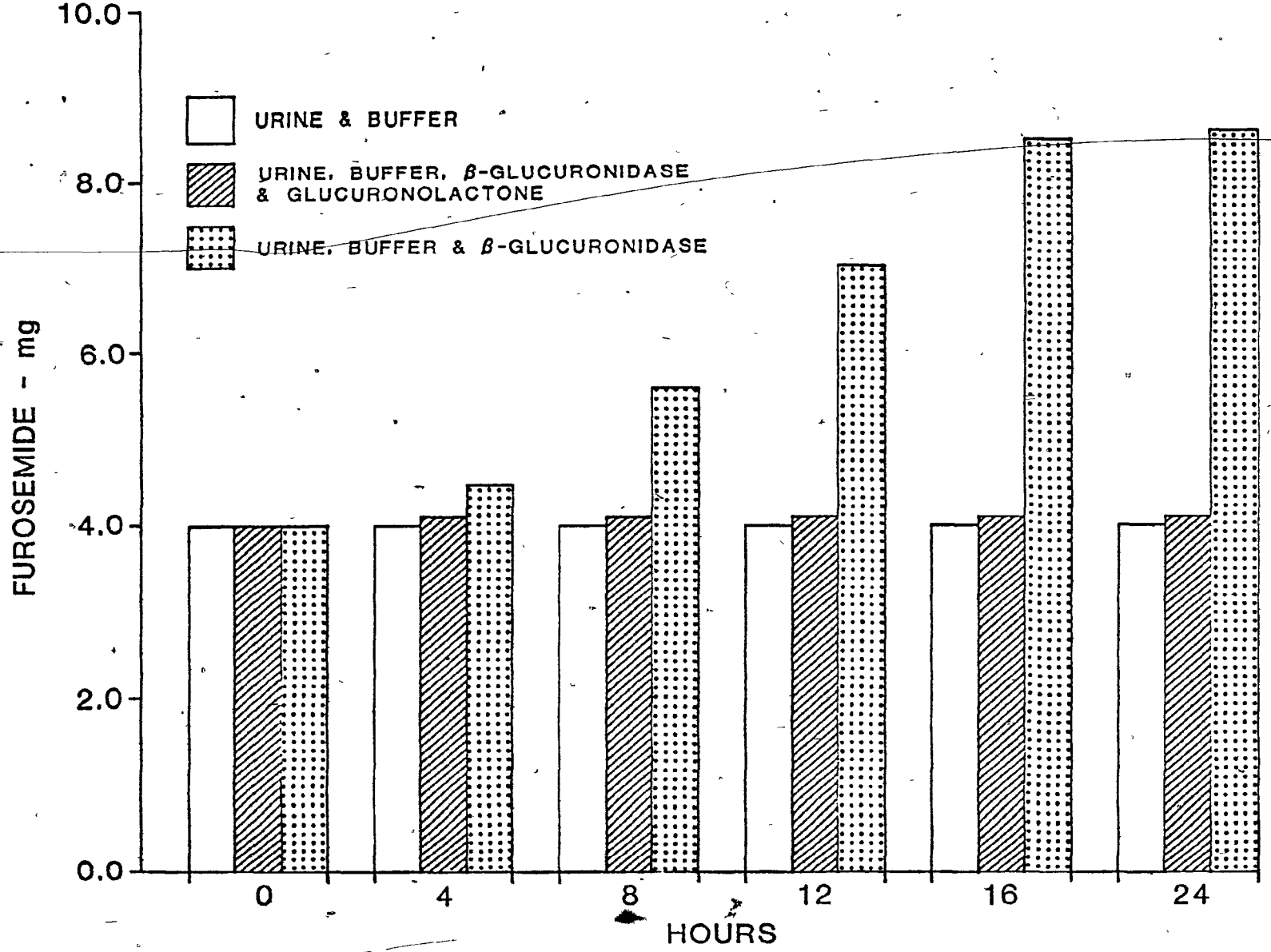

2-AMINO-4-CHLORO-5-SULFAMOYLANTHRANILIC ACID / BENZBROMARONE - area ratio
12
10
8
6
4
2
0
0
2
4
6
8
10
12
14
16
y=0.64x−0.03
r=0.999
Syx=0.004
4-CHLORO-2-AMINO-5-SULFAMOYLANTHRANILIC ACID - mg/L

Figure 5. Relationship between the hydrolysis of furosemide glucuronide and incubation time in the presence and absence of beta-glucuronidase with and without its specific inhibitor glucurnolactone.


10.0
8.0
6.0
4.0
2.0
0.0
FUROSEMIDE - mg
URINE & BUFFER
URINE, BUFFER, β-GLUCURONIDASE & GLUCURONOLACTONE
URINE, BUFFER & β-GLUCURONIDASE
0
4
8
12
16
24
HOURS

Patient Population

Demographic Data

Twenty patients with the diagnosis of acute pulmonary edema were considered for admission to this study. Four of these patients were excluded; three of them withdrew before the study was completed and one presented in cardiogenic shock. The 16 patients remaining in the study ranged from 28-88 years of age (median 69 years) and weighed from 46.8 to 100 kg (median 70.4 kg). There were 11 male and 5 female patients. Nine of them had suffered an acute myocardial infarction; four had coronary arteriosclerotic disease; two had hypertension and one had alcoholic cardiopathy. Four of the 16 patients had abnormal creatinine clearance (less than 60 ml/min). The characteristics of the patient population are presented in Table I. Other drugs received concomittantly with furosemide during 24 hours of the study are presented in Table II. Twelve of the patients received digoxin and six received lidocaine.

Kinetic Disposition of Furosemide

There was a biexponential decay of serum furosemide concentration over time. A representative semilogarithmic plot of disappearance of furosemide from serum after an 80 mg dose administered intravenously over 5 minutes to a 68-year old male patient (Patient No. 11) is shown in Figure 6. The alpha and beta disposition phases can be readily distinguished and a measurable quantity of furosemide was detected 24 hours after the single dose. The pharmacokinetic disposition of furosemide in the 16 patients is presented in Tables III and IV.

The serum furosemide concentration over time in 11 of the 16 patients

TABLE I. Description of patient population

Patient No.	Age yrs	Sex	Weight kg	Cause of the failure	Classification	Creatinine Clearance ml/min
1	67	M	72.7	Anterior MI	severe	59
2	81	M	90.9	Anterolateral MI	moderate	89
3	75	M	90.4	hypertension	mild	145
4	74	M	65.9	septal MI	severe	115
5	69	F	52.3	subendocardial MI	moderate	132
6	65	M	68.2	alcoholic cardiopathy atrial fibrillation	severe	161
7	51	M	72.7	C.A.D.	mild	107
8	75	M	77.3	hypertension	severe	25
9	28	F	68.1	anterior MI	moderate	142
10	65	M	77.3	subendocardial MI	moderate	107
11	68	M	63.6	anteroposterior MI	moderate	55
12	88	F	50	C.A.D. atrial fibrillation	moderate	134
13	70	M	81.8	anteroseptal MI	severe	114
14	75	M	100	C.A.D.	moderate	123
15	88	F	55.5	anterior MI	severe	70
16	64	F	46.8	C.A.D.	severe	50
mean	69		70.9			
median	69		70.4			
range	28-88		46.8-100			

TABLE II. Concomitant Drugs

Patient No.	
1	Lidocaine, digoxin
2	lidocaine, nitroglycerin, diazepam
3	---
4	lidocaine, digoxin, insulin, morphine
5	---
6	digoxin, aminophylline
7	digoxin
8	morphine, digoxin
9	lidocaine, digoxin
10	digoxin, nitroglycerin
11	digoxin, lidocaine
12	digoxin, heparin
13	xyloçaine, insulin
14	digoxin, aminophylline
15	morphine, digoxin, insulin
16	digoxin

Figure 6. Semilogarithmic plot of the disappearance of furosemide from serum of a male patient who received an intravenous dose of 80 mg of furosemide (number 11).

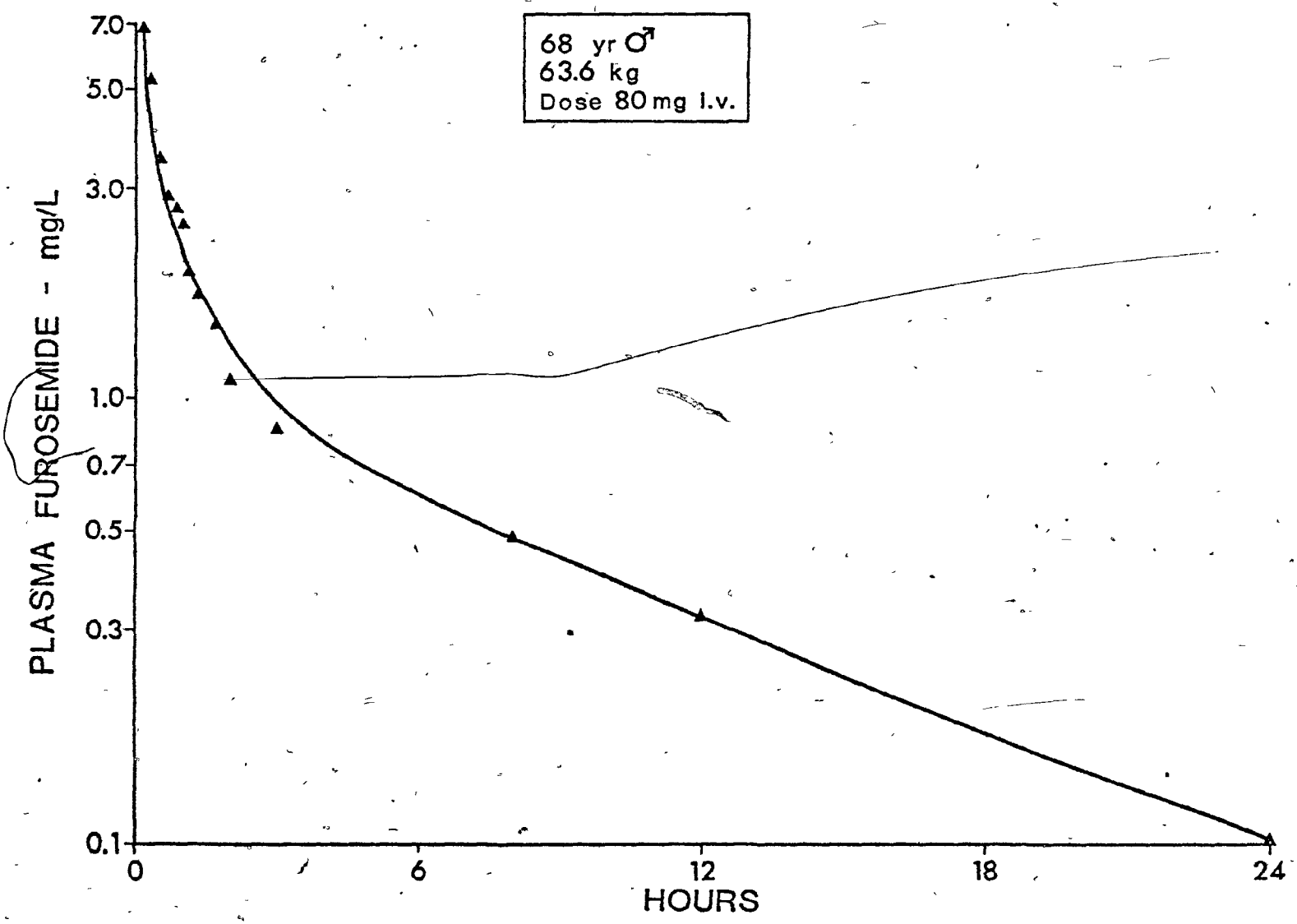

68 yr ♂
63.6 kg
Dose 80 mg I.v.
PLASMA FUROSEMIDE - mg/L
7.0
5.0
3.0
1.0
0.7
0.5
0.3
0.1
0
6
12
18
24
HOURS

TABLE III. Pharmacokinetic disposition of furosemide in pulmonary edema patients using the two compartment open model

Patient	α-$t_{1/2}$ min	β-$t_{1/2}$ min	k_{12} hr^{-1}	k_{21} hr^{-1}	k_{el} hr^{-1}	Vd_{ss} L/kg	Cl_s ml/min	Vd_{area} L/kg	V_1 L/kg	V_2 L/kg
1	17.9	816	1.58	0.210	0.570	1.67	139	2.154	0.199	1.474
2	35.4	306	0.413	0.246	0.640	0.085	30.61	0.148	0.031	0.054
3	30.6	127	0.340	0.580	0.760	0.238	173	0.357	0.150	0.088
8	79.0	1190	0.257	0.090	0.196	0.317	20.92	0.463	0.082	0.235
9	29.4	554	0.811	0.253	0.415	0.268	27.50	0.323	0.058	0.210
10	28.4	290	0.477	0.241	0.866	0.853	295	1.53	0.278	0.575
11	25.2	414	0.903	0.300	0.540	0.617	89.6	0.201	0.156	0.461
12	25.8	203	0.600	0.760	0.420	0.368	73.27	0.204	0.205	0.163
13	15.1	145	1.14	0.610	1.28	0.210	129	0.338	0.073	0.137
14	46.4	265	0.260	0.270	0.517	0.292	128	0.488	0.144	0.148
16	35.4	344	0.351	0.188	0.749	0.355	72.6	0.774	0.124	0.231
mean	33.5	423	0.650	0.340	0.632	0.474	107.13	0.634	0.136	0.342
median	29.4	306	0.477	0.253	0.570	0.317	89.6	0.357	0.144	0.210
range	15-79	127-1190	0.257-1.58	0.09-0.760	0.196-1.28	0.085-1.67	20.92-173	0.148-2.154	0.03-0.278	0.054-1.47

TABLE IV. Pharmacokinetic disposition of furosemide in pulmonary edema patients using the one compartment open model

Patient	β-$t_{1/2}$ min	beta hr^{-1}	Cl_s ml/min	Vd_{area} L/kg
4	273.6	0.152	85.49	0.512
5	138.5	0.300	122.1	0.467
6	145	0.266	212	0.701
7	192	0.217	92.6	0.352
15	292.8	0.142	26.16	0.199
mean	210.6	0.215	107.6	0.446
median	192	0.217	92.6	0.467
range	156-293	0.142-0.300	26.6-122.1	0.199-0.701

was analyzed using a two compartment open kinetic model (Table III). In the remaining 5 patients, due to multiple doses of furosemide, it was difficult to analyze the data using a two compartment kinetic system. For this reason, a one compartment open model was used (Table IV). Model independent kinetic analyses were used to compare the two groups (Tables III and IV).

Alpha or Distribution Half-Life of Furosemide

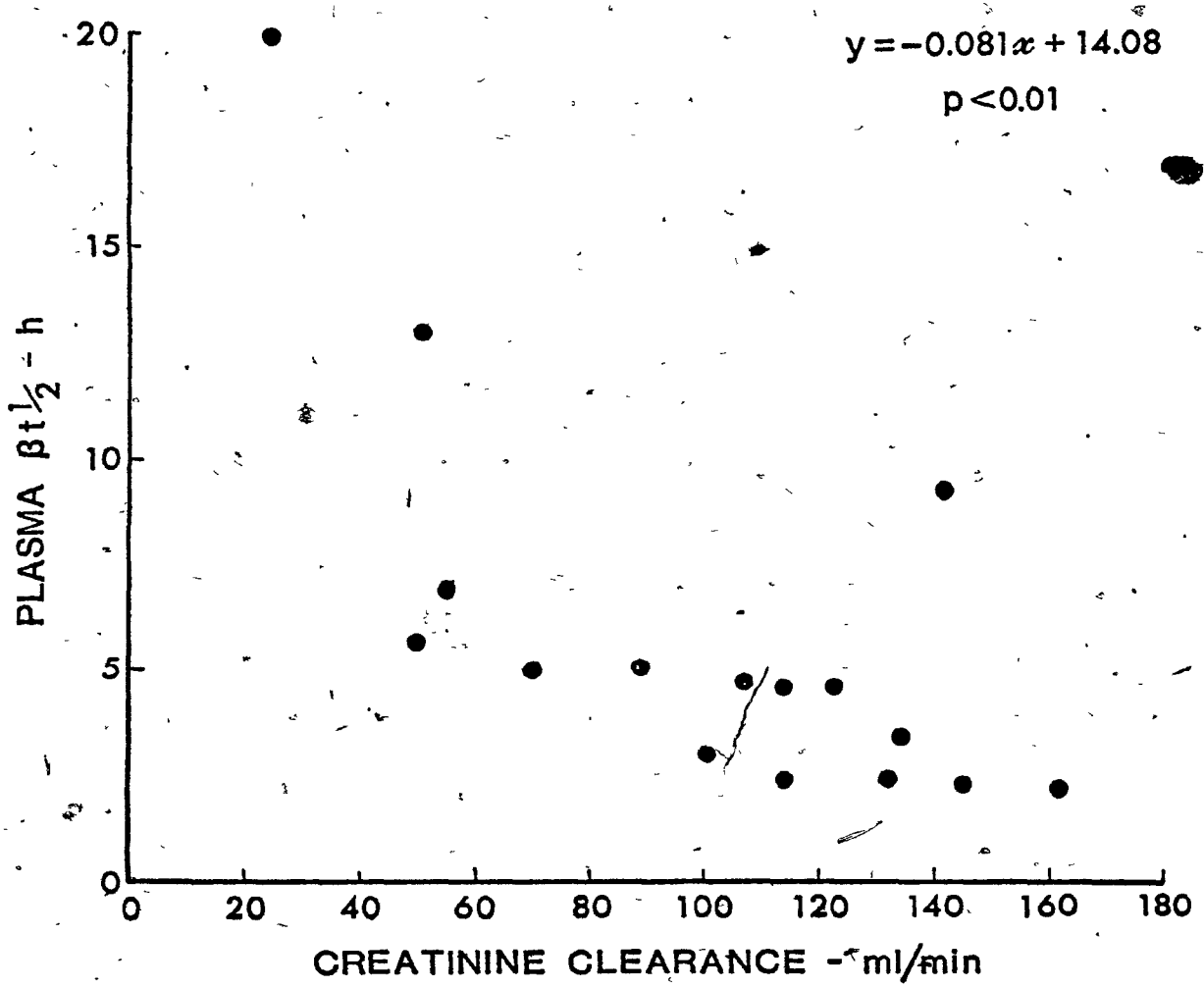
In the 11 patients analyzed by the two compartment open model, there was a wide variation in alpha half-life which ranged from 15 to 79 minutes, median 29.4 minutes. There was no apparent difference in the alpha $t_{1/2}$ of either patients with or without myocardial infarction, different degrees of renal function, or the severity of pulmonary edema.

Beta or Elimination Half-Life of Furosemide

There was wide variation in the β-$t_{1/2}$ which ranged from 127 to 1190 minutes, median 306 minutes. The β-$t_{1/2}$ in the 5 patients analyzed by the one compartment open model ranged from 138 to 293 minutes. There was no apparent difference in β-$t_{1/2}$ of the two groups of patients. The longest β-$t_{1/2}$ (1190 minutes) was observed in a patient with renal failure (patient #8, creatinine clearance 25 ml/min).

The relationship between β-$t_{1/2}$ and creatinine clearance is shown in Figure 7. Patients with poor renal function had a longer β-$t_{1/2}$ than those who had better renal function. Creatinine clearance was measured in the 24 hours of urine collection following the administration of furosemide. There was an inverse correlation between these parameters ($p < 0.01$, $F_{1,14} = 12.20$). There were no apparent differences in β-$t_{1/2}$ of either patients with or without myocardial infarction or patients with different severities of pulmonary edema.

Figure 7. Relationship between the beta half-life for the disappearance of furosemide from serum and creatinine clearance in patients with pulmonary edema.


y = −0.081x + 14.08
p<0.01
PLASMA βt½ - h
CREATININE CLEARANCE - ml/min
0
5
10
15
20
0
20
40
60
80
100
120
140
160
180

Volume of Distribution

There was a wide variation in the volume of distribution (Vd_{area}) which varied from 0.148 to 2.154 L/kg (median 0.407 L/kg) for the 16 patients. No apparent differences were noted between the volume of distribution in patients with either different degrees of renal function, with or without myocardial infarction or differences in severity of pulmonary edema.

Volumes of Central (V_1) and Peripheral (V_2) Compartments

Volume of the Central Compartment (V_1)

For the 11 patients analyzed with the two compartment open model the volume of the central compartment varied from 0.03 to 0.278 L/kg (median 0.144 L/kg). There was no apparent difference between the volume of the central compartment in the patients with different degrees of renal function. There was a great interindividual variation in the volume of the central compartment in patients with myocardial infarction and this volume tended to be smaller in these patients. In patients without myocardial infarction this volume tended to be larger and to show less variation. No significant difference was found in the volume of the central compartment in patients with or without myocardial infarction. No apparent relationship was found in the volume of the central compartment in these patients in relation to the severity of pulmonary edema.

Volume of the Peripheral Compartment (V_2)

The volume of the peripheral compartment for the 11 patients analyzed by the two compartment open model varied from 0.054 to 1.47 L/kg (median 0.21 L/kg). For all but two of the patients analyzed, the volume of the peripheral compartment was found to be larger than the central compartment. There was no apparent difference in the volume of the peripheral compartment in patients with different degrees of renal function.

In patients with myocardial infarction, the volume of the peripheral compartment was always found to be larger than the volume of the central compartment. In patients without myocardial infarction, the volume of the peripheral compartment was found to be smaller than the central compartment for two patients, the same in one patient, and larger in two patients. No apparent difference was found in the volume of the peripheral compartment in patients with different severities of pulmonary edema.

Distribution Rate Constants k_{21} and k_{12}

Rate Constant for Drug Transfer from the Central to the Peripheral Compartment (k_{12})

The rate constant for drug transfer from the central to the peripheral compartment (k_{12}) varied from 0.257 to 1.58 hr^{-1} (median 0.477 hr^{-1}). No apparent difference was found between k_{12} in patients with different degrees of renal function. The relationships between k_{12} and the excretion of unchanged furosemide in urine, in patients with and without myocardial infarction, is presented in Table V. The rate constant k_{12} was larger in patients with, than in those without, myocardial infarction ($p < 0.01$, Mann-Whitney U test). Consequently patients with myocardial infarction excreted less unchanged furosemide in urine than those without myocardial infarction during the first alpha (distribution) half-life ($p < 0.01$, Mann-Whitney U test). Patient #8 was omitted from this analysis because he had severely impaired renal function. No apparent differenoe was found in k_{12} in patients with different severities of pulmonary edema.

TABLE V. Relationship between k_{12} and furosemide excretion during one alpha $t_{1/2}$ in patients with and without M.I.

Patient No.	diagnosis	dose of furosemide mg	α-$t_{1/2}$ min	furosemide excretion in one $t_{1/2}$ interval mg	proportion of dose excreted	k_{12} hr^{-1}
1	M.I.	60	17.8	6.10	0.102	1.58
2	M.I.	20	35.4	1.78	0.089	0.413
9	M.I.	20	29.4	1.95	0.097	0.811
10	M.I.	80	28.4	6.57	0.082	0.477
11	M.I.	80	25.2	9.05	0.113	0.903
13	M.I.	40	15.6	1.39	0.034	1.14
3	hypertension	40	30.6	7.64	0.121	0.340
12	C.A.D.	40	25.8	7.78	0.117	0.600
14	C.A.D.	40	46.3	4.86	0.191	0.260
16	C.A.D.	40	35.4	4.68	0.194	0.351

Rate Constant for Drug Transfer from the Peripheral to the Central Compartment (k_{21}).

For the 11 patients analyzed by the two compartment open model k_{21} varied from 0.090 to 0.760 hr^{-1} (median 0.253 hr^{-1}). No apparent difference was found in k_{21} in patients with different degrees of renal function, with or without myocardial infarction or due to severity of pulmonary edema.

Serum Clearance of Furosemide

In the 16 patients analyzed, the serum clearance of furosemide ranged from 20.9 to 295 ml/min (median 91.1 ml/min). The mean serum furosemide clearance was twice as great as the mean renal furosemide clearance. Although there was a large interindividual variation in serum clearance among the patients with pulmonary edema, a significant relationship between serum furosemide clearance and creatinine clearance was found in all these patients ($p < 0.05$ $F_{1,15} = 5.24$). No apparent difference was found in serum clearance of the patients with or without myocardial infarction or patients with different severities of pulmonary edema.

Renal Clearance of Furosemide

The renal furosemide clearance varied from 6.38 to 112.8 ml/min, median 46.4 ml/min. There was a large interindividual variation in renal furosemide clearance among the patients with pulmonary edema. Therefore, if all patients are plotted, no significant relationship is found between renal furosemide clearance and creatinine clearance. If patients numbers 1 and 9 are withdrawn, this relationship is then significant ($p < 0.05$, $F_{1,13} = 5.4$). No apparent difference was found in the renal furosemide clearance in patients with or without myocardial infarction.

TABLE VI. Clearance of furosemide in patients with pulmonary edema

Patient No.	Cl_s ml/min	Cl_R ml/min	Non-renal clearance ml/min	Cl_s/Cl_R
1	139.0	100.6	38.4	1.38
2	30.6	16.43	14.18	1.86
3	173.0	112.8	60.2	1.53
4	89.5	47.9	41.6	1.86
5	122.1	54.8	67.3	2.22
6	212.0	33.4	178.6	6.34
7	92.6	66.6	26.0	1.39
8	20.9	6.38	14.5	3.28
9	27.5	15.4	12.1	1.79
10	295.0	132.7	163.0	2.22
11	89.6	30.3	59.3	2.95
12	73.3	46.7	26.6	1.57
13	129.0	27.1	101.9	4.75
14	128.0	76.6	51.4	1.67
15	26.2	17.6	8.60	1.50
16	72.5	46.8	25.8	1.55
mean	107.55	52.00	55.59	2.36
median	91.6	46.7	40.0	1.82
range	20.9-295	6.38-112.8	8.6-178	1.38-6.34

TABLE VII. Renal clearance of furosemide during the alpha and beta phase of furosemide disposition in patients with pulmonary edema

Patient No.	Renal clearance of furosemide	
	alpha	beta
1	15.57	7.51
2	5.38	2.29
3	7.71	10.76
4	4.30	3.18
5	8.03	3.63
6	4.67	2.88
7	6.64	5.53
8	-	3.01
9	2.59	1.54
10	20.94	18.79
11	7.25	2.98
12.	8.0	3.91
13	2.60	2.08
14	6.93	3.42
15	1.22	1.28
16	4.44	3.98

TABLE VIII. Excretion of furosemide and metabolites in urine for 24 hours after an intravenous dose of furosemide to pulmonary edema patients

Patient	Dose total	Unchanged furosemide	% of dose	Conjugated furosemide	% of dose	Acid	% of dose
	mg	mg		mg		mg	
1	60	33.50	55.83	16.0	26.66	0.06	0.13
2	20	11.20	56.00	4.0	20	0.31	2.05
3	40	22.40	56.00	1.82	4.55	0.37	1.22
4	160	89.00	55.62	64.26	40.45	1.28	1.05
5	80	35.86	44.82	6.07	7.58	1.10	1.80
6	80	46.12	57.65	5.24	6.55	0.66	1.07
7	120	86.27	71.82	20.94	17.45	1.39	1.52
8	40	9.04	22.60	1.32	3.3	1.20	3.92
9	20	9.75	48.75	3.08	15.4	0.12	0.80
10	80	53.76	67.20	6.81	8.51	1.07	1.75
11	80	25.01	31.26	7.10	8.78	1.72	2.81
12	60	44.03	73.38	2.88	4.80	0.92	2.00
13	60	27.83	46.38	5.21	8.68	1.11	2.42
14	40	24.49	61.22	6.24	15.6	0.70	2.30
15	80	54.02	67.52	10.23	12.78	1.80	2.95
16	40	25.01	62.52	7.59	18.97	1.05	3.45
median			56.0		10.78		1.88
range			22.6-73.4		3.3-40.4		0.13-3.92

% of dose excreted as the acid metabolite was corrected for the changes in molecular weight from that of furosemide (1.31)

The ratio between serum and renal clearance of furosemide is presented in Table VI. The highest rates were found in 3 patients with severe pulmonary edema, patients numbers 6, 8, and 13. In all but two patients this ratio was found to be greater than 1.5.

Renal Clearance of Furosemide During the α and β Phases of Furosemide Disposition

Sequential urine samples were collected and analyzed for furosemide and its metabolites after an intravenous administration of the drug. The end of the alpha phase was determined from a plot, on semilogarithmic paper, of the serum concentrations of furosemide versus time. The end of the alpha phase for each patient was estimated as the time when the alpha phase crossed the extrapolated beta phase of the curve. The urine collected and analyzed for furosemide and its metabolite content during this time was considered the urine excreted during the alpha phase for all kinetic calculations. Urine excreted after the end of the alpha phase until 24 hours after the furosemide dose was considered urine excreted during the beta phase for all the kinetic analyses. The renal clearance of unchanged furosemide was found to be greater during the alpha phase than the beta phase of serum drug disposition for 13 of the 16 patients. This relationship is presented in Table VII.

Urinary Excretion of Furosemide and Its Metabolites

Excretion of furosemide and its metabolites is presented in Table VIII.

Urinary Excretion of Unchanged Furosemide

The range of excretion of unchanged furosemide varied between 22.6 and 73.4 percent of the administered dose, median 56 percent. The percentage of the dose of furosemide excreted unchanged in urine, related to creatinine clearance, is illustrated in Figure 8. Since all patients

are included in the plot of this relationship, and since individual variation in the excretion of furosemide in patients with approximately the same creatinine clearance is large, no significant correlation was found. The excretion of unchanged furosemide was found to be less in patients with, than in those without, myocardial infarction ($p < 0.05$, Mann-Whitney U test). Patient number 8 was excluded from this analysis because of his impaired renal function. No apparent difference was found in the excretion of unchanged furosemide and the severity of pulmonary edema.

Urinary Excretion of Furosemide Glucuronide

The excretion of furosemide glucuronide represented 3.3 to 40.4 percent of the administered dose, median 10.78 percent. The relationship between the excretion of furosemide glucuronide and creatinine clearance is presented in Fig. 9. Patients with poor renal function excreted more conjugated furosemide than patients with normal renal function as indicated by creatinine clearance. There was an inverse relationship between the excretion of furosemide glucuronide and creatinine clearance ($p < 0.05$, $F_{1,12} = 7.97$). Two patients were excluded from this analysis, one of them due to multiple doses of furosemide (patient 4), the other one because of impaired renal function (patient 8). There was no apparent relationship between the excretion of furosemide glucuronide in patients with or without myocardial infarction or with different severities of pulmonary edema.

Analysis of the percent of the dose of furosemide conjugated with glucuronic acid, versus dose, is presented in Figure 10. No dose dependent relationship for the formation and excretion of furosemide glucuronide was observed in these patients. The mean proportion of furosemide conjugated with glucuronic acid was similar for all doses but

Figure 8. Relationship between excretion of unchanged furosemide in urine and creatinine clearance in patients with pulmonary edema.

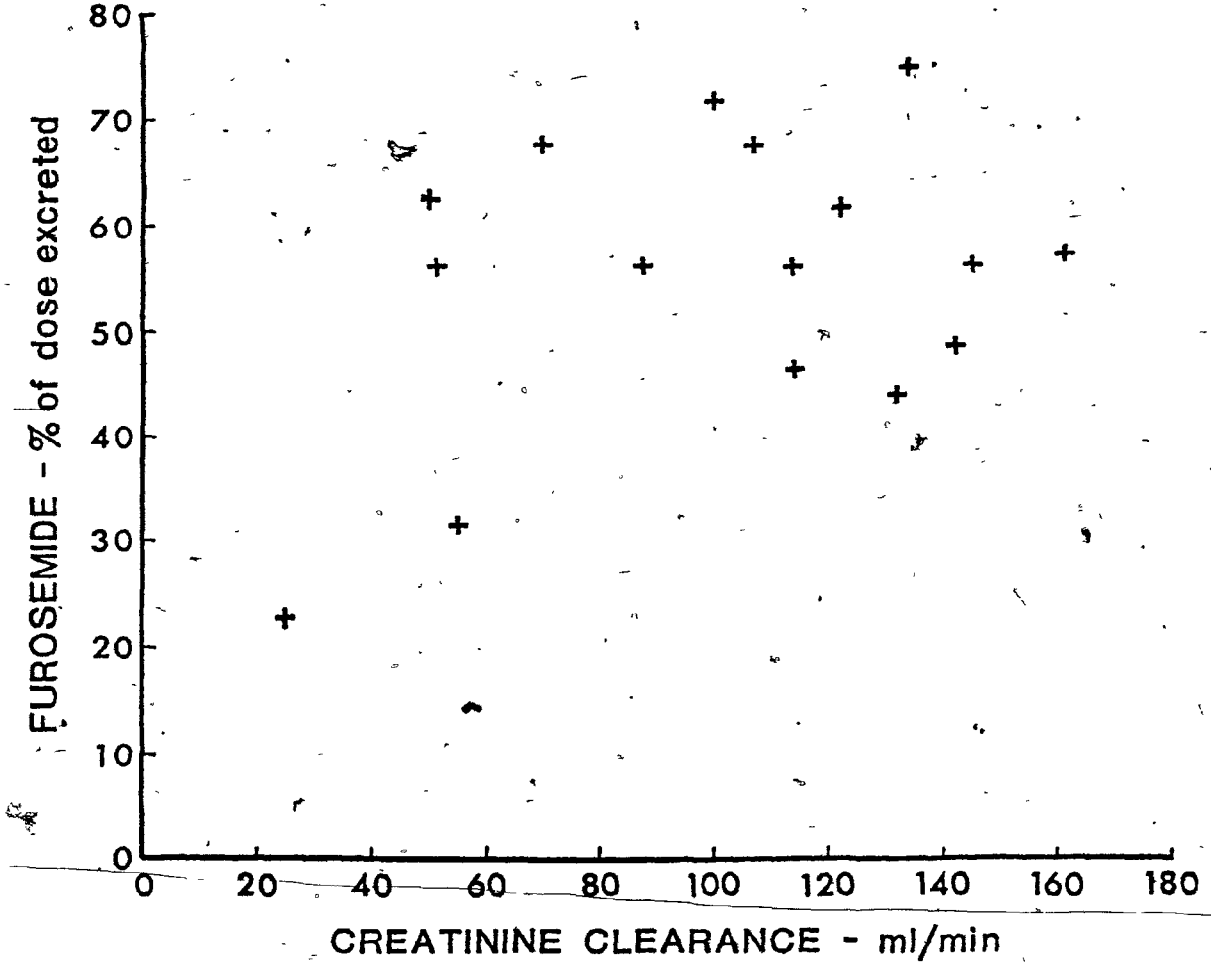

FUROSEMIDE - % of dose excreted
80
70
60
50
40
30
20
10
0
0
20
40
60
80
100
120
140
160
180
CREATININE CLEARANCE - ml/min

Figure 9. Relationship between the urinary excretion of furosemide glucuronide and creatinine clearance in patients with pulmonary edema.

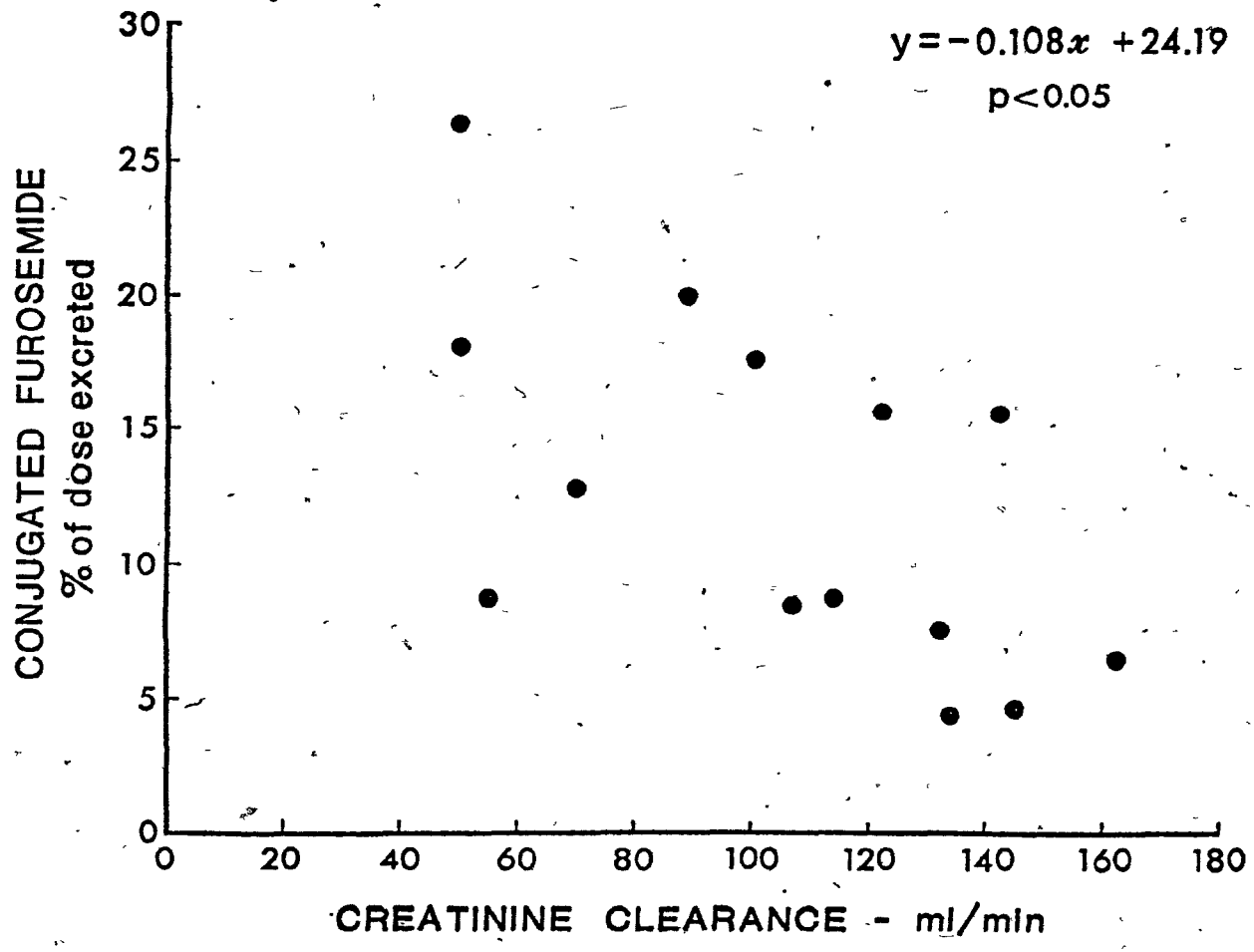
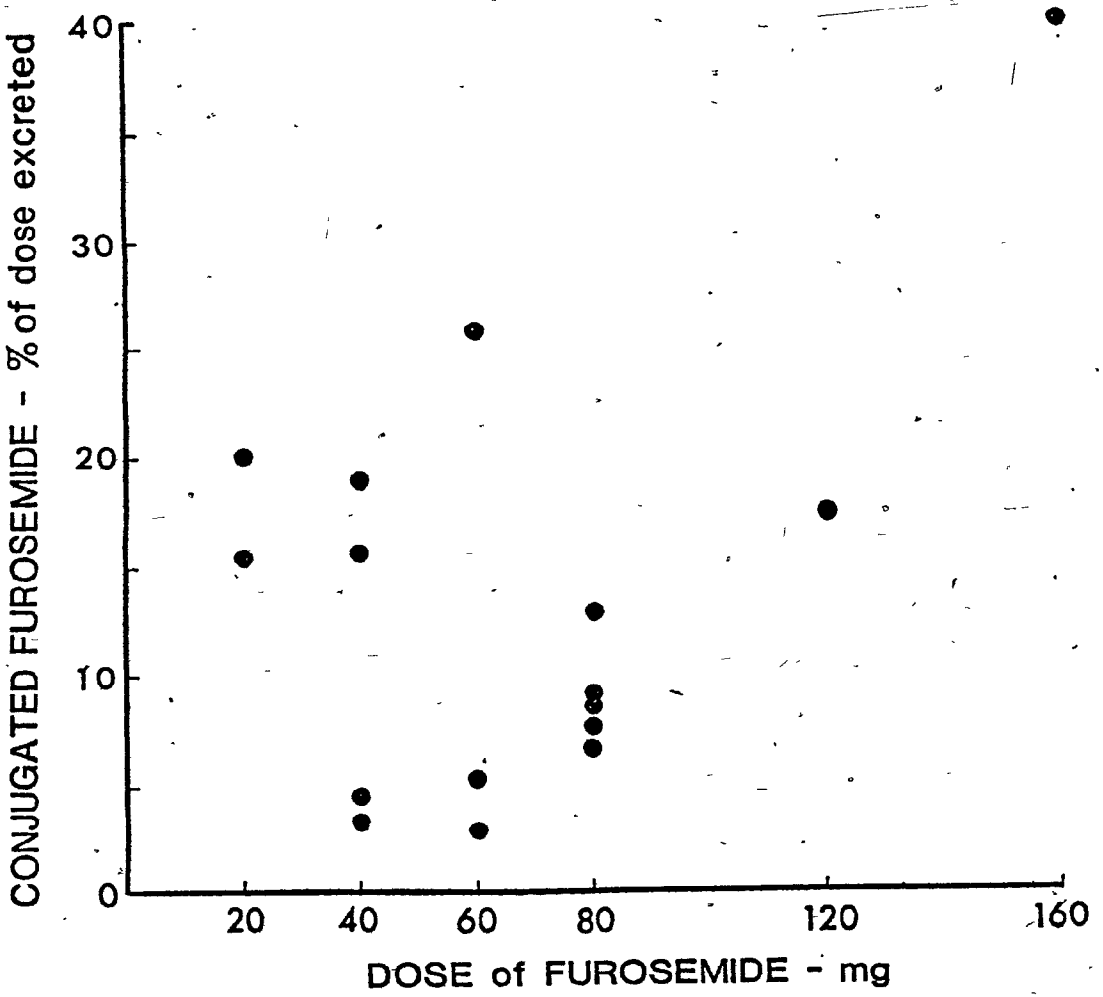

y = −0.108x + 24.19
p<0.05
CONJUGATED FUROSEMIDE
% of dose excreted
30
25
20
15
10
5
0
0
20
40
60
80
100
120
140
160
180
CREATININE CLEARANCE - ml/min

Figure 10. Relationship between the dose of furosemide and the proportion excreted in urine as the glucuronide conjugate.


CONJUGATED FUROSEMIDE - % of dose excreted
40
30
20
10
0
20
40
60
80
120
160
DOSE of FUROSEMIDE - mg

was highly variable between patients receiving the same dose.

Urinary Excretion of the Acid Metabolite of Furosemide

Excretion of the acid metabolite of furosemide ranged from 0.13 to 3.92 percent of the administered dose (median 1.88 percent). The relationship between excretion of the acid metabolite and creatinine clearance is presented in Figure 11. Patients with poor renal function excreted more acid metabolite than patients with normal renal function as indicated by creatinine clearance. There was an inverse relationship between excretion of the acid metabolite of furosemide and creatinine clearance ($p < 0.05$, $F_{1,14} = 6.43$). No apparent relationship was found between excretion of the acid metabolite in patients with or without myocardial infarction or in patients with different severities of pulmonary edema.

Relationship between the Pharmacodynamics and Pharmacokinetics of Furosemide Disposition

The 24 hour urine volume and excretion of electrolytes after an intravenous dose of furosemide are presented in Table IX. Excretion of electrolytes in some patients was similar to the values reported as normal for 24 hour urine, but lower in other patients who were chronically ill with restricted intake of water and salt.

Urine Osmolality

The urinary osmolality during the alpha and beta phases of serum furosemide disposition are presented in Table X. The lowest osmolality was found during the alpha phase for all patients. No apparent difference was found in urinary osmolality in patients with either different degrees of renal function, with or without myocardial infarction, or with different severities of pulmonary edema.

Figure 11. Relationship between the urinary excretion of the acid metabolite of furosemide and creatinine clearance.

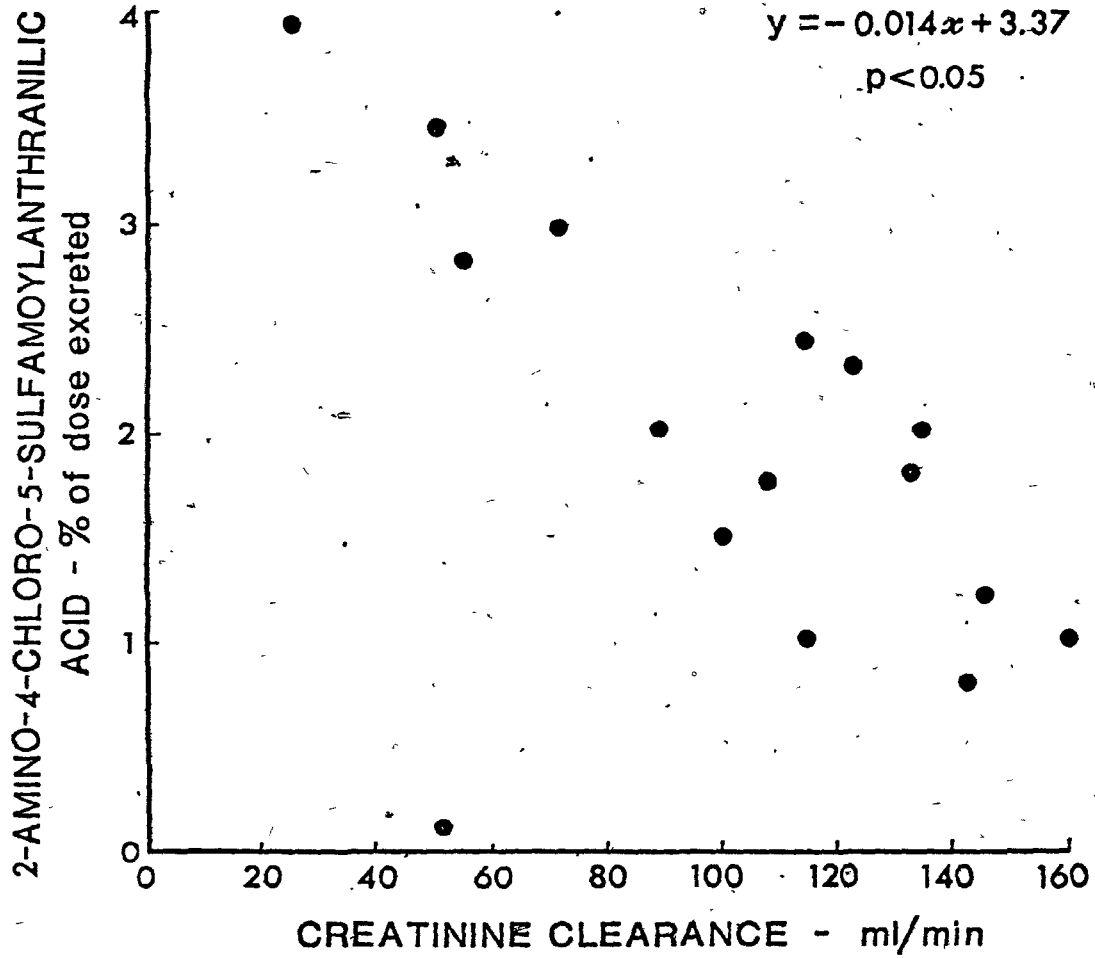

y = − 0.014x + 3.37
p<0.05
2-AMINO-4-CHLORO-5-SULFAMOYLANTHRANILIC ACID - % of dose excreted
0
1
2
3
4
0
20
40
60
80
100
120
140
160
CREATININE CLEARANCE - ml/min

TABLE IX. Total urine volume and electrolyte excretion for 24 hours in pulmonary edema patients given intravenous furosemide

Patient	Urine volume ml	Na^{+} mEq	K^{+} mEq	Cl^{-} mEq	Mg^{++} mg	Ca^{++} mg
1	1174	163	37	112	242	59
2	2411	236	105	208	436	152
3	1878	167	50	201	65	100
4	3270	246	98	309	292	265
5	2680	325	114	344	268	78
6	6390	568	69	643	183	231
7	2340	217	31	248	139	90
8	2060	151	60	138	113	130
9	1030	66	30	106	103	72
10	2490	205	70	250	168	113
11	1398	52	62	74	22	21
12	2330	182	40	234	58	59
13	4290	166	108	341	155	308
14	5070	248	118	466	144	232
15	2385	170	83	258	144	157
16	1260	50	48	88	52	57

TABLE X. Urine osmolality during furosemide disposition in pulmonary edema patients

Patient No.	Time of minimum osmolality min	Alpha Phase median	Alpha Phase range		Beta Phase median	Beta Phase range	
1	20-40	305	250	460	478	400	525
2	20-40	250	166	320	290	275	305
3	60-80	300	280	345	315	310	320
4	40-60	325	260	445	390	380	424
5	0-80	324	279	375	376	345	538
6	20-40	212	166	474	300	290	320
7	60-80	285	269	300	325	320	395
8					440	385	506
9	40-60	255	200	309	600	435	760
10	60-80	300	225	460	421	400	497
11	30-60	327	285	420	475	360	525
12	20-40	290	279	375	325	310	385
13	30-50	325	231	365	380	370	410
14	50-70	300	280	410	379	345	425
15	30-60	280	200	360	345	310	420
16	30-60	317	280	345	425	425	440

Urinary Furosemide Excretion and Effect on Renal Function

Ratios of renal sodium to furosemide clearance during the alpha and the beta phases of furosemide disposition are presented in Table XI. If the action of furosemide was constant through the alpha and beta phases, one would expect that the ratio between clearance of sodium to clearance of furosemide would be similar in the two phases. However, this ratio is greater in the alpha phase than in the beta phase, consistent with the clinical observation that the diuretic effect of furosemide is largely over by the end of the alpha phase. However, clearance of furosemide by the kidney is still continuing during the beta phase of the serum furosemide over time curve. Since drug effect is said to be related to its concentration at the site of action, a comparison between the excretion of sodium, chloride and urine volume, and the excretion of furosemide was made. A plot of 3 representative patients is shown in Figures 12, 13 and 14, where the excretion of sodium chloride and urine volume is compared to furosemide excretion from sequential urine samples. For each patient a positive and linear relationship was found. Tables XII, XIII and XIV present correlation analyses for these relationships. They were significant for all patients. Therefore the excretion of urine volume, sodium and chloride increase linearly with the excretion of furosemide.

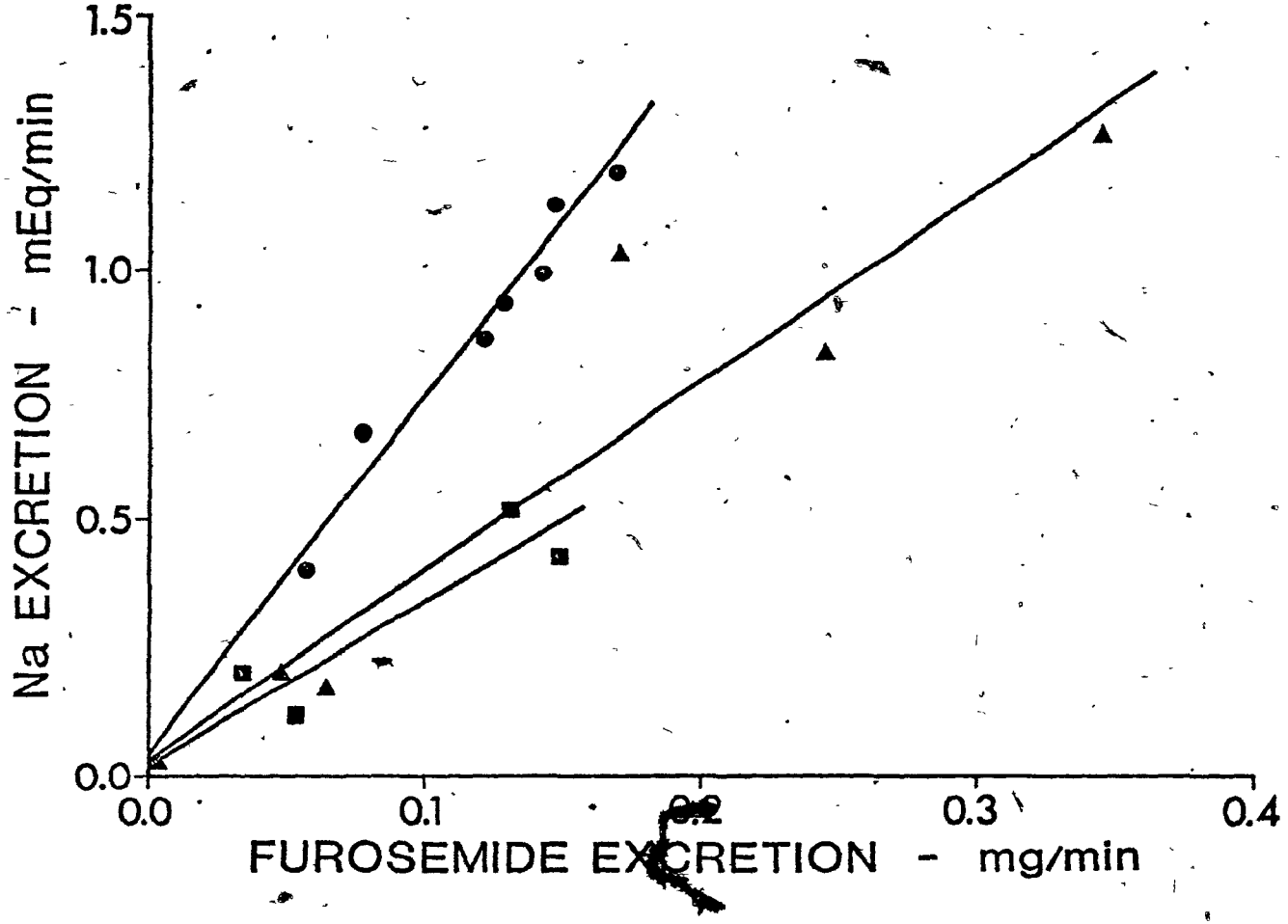
Effect of Furosemide on the Excretion of Other Electrolytes

A comparison between the excretion of calcium and the excretion of furosemide is presented in Table XV. This correlation was found to be linear for 14 of the 16 patients. No correlation was found between the excretion of potassium or magnesium and the excretion of furosemide for any of the patients.

TABLE XI. Renal clearance of sodium and furosemide during the alpha and beta phase of furosemide disposition in pulmonary edema patients

Patient No.	Renal clearance of sodium		Renal clearance of furosemide		Clearance ratios sodium/furosemide	
	alpha	beta	alpha	beta	alpha	beta
1	7.43	0.92	15.57	7.51	0.47	0.12
2	8.13	0.63	5.38	2.29	1.51	0.27
3	7.35	3.87	7.71	10.76	0.95	0.36
4	3.09	1.13	4.30	3.18	0.72	0.35
5	5.92	1.50	8.03	3.63	0.73	0.41
6	4.36	2.56	4.67	2.88	0.93	0.88
7	5.35	3.86	6.64	5.53	0.81	0.70
8	-	1.03	-	3.01	-	0.34
9	1.76	0.29	2.59	1.54	0.68	0.19
10	10.31	0.39	20.94	18.79	0.49	0.02
11	4.89	0.14	7.25	2.98	0.67	0.05
12	5.30	1.42	8.0	3.91	0.66	0.36
13	5.15	0.89	2.60	2.08	1.98	0.40
14	9.32	1.38	6.93	3.42	1.34	0.35
15	3.41	1.68	1.22	1.28	2.80	1.31
16	2.00	0.89	4.44	3.98	0.45	0.22

Figure 12. Relationship between the urinary excretion of sodium and furosemide in three representative patients with acute pulmonary edema (numbers 3 ●, 4 ■ and 7 ▶).


1.5
1.0
0.5
0.0
Na EXCRETION - mEq/min
0.0
0.1
0.2
0.3
0.4
FUROSEMIDE EXCRETION - mg/min

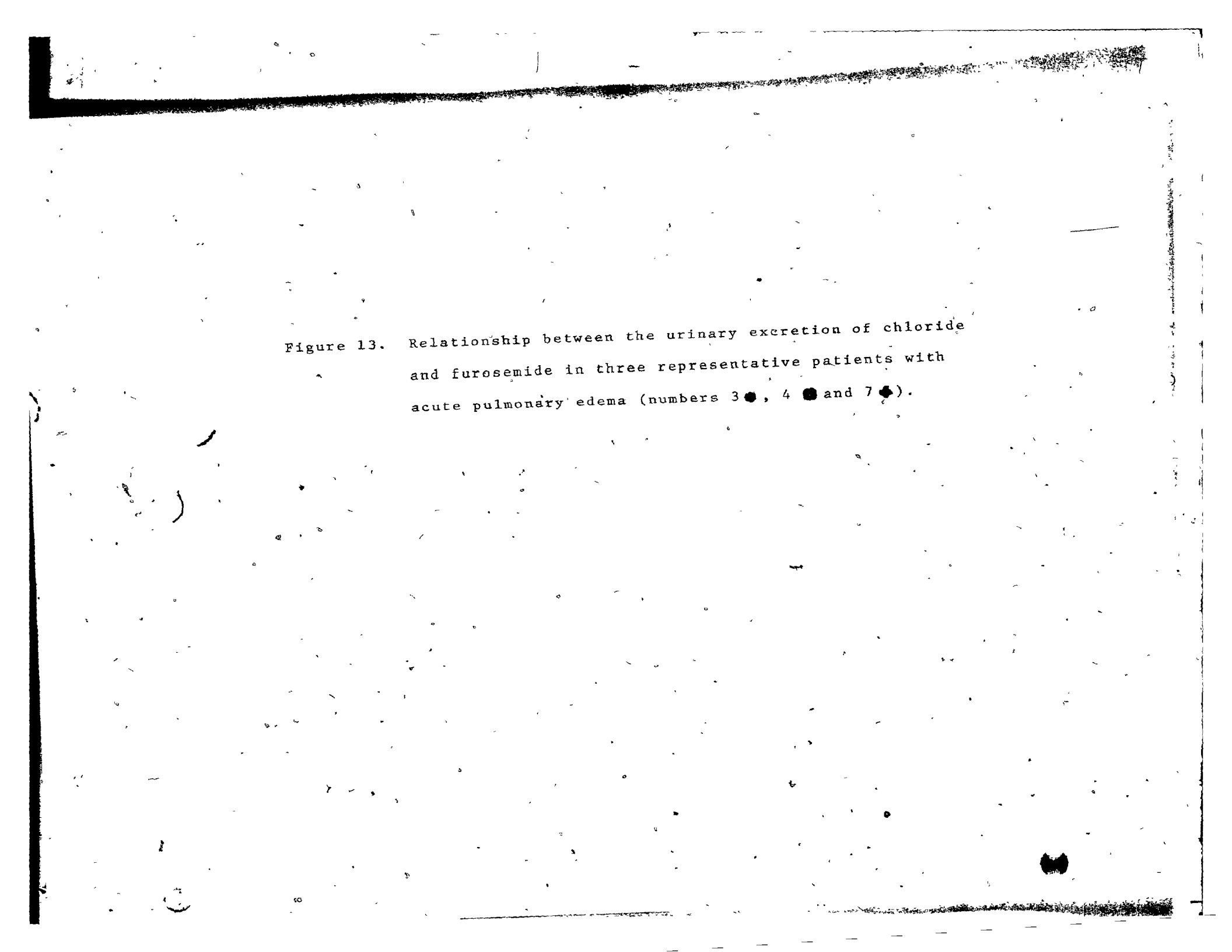


Figure 13. Relationship between the urinary excretion of chloride and furosemide in three representative patients with acute pulmonary edema (numbers 3 ●, 4 ■ and 7 ◆).

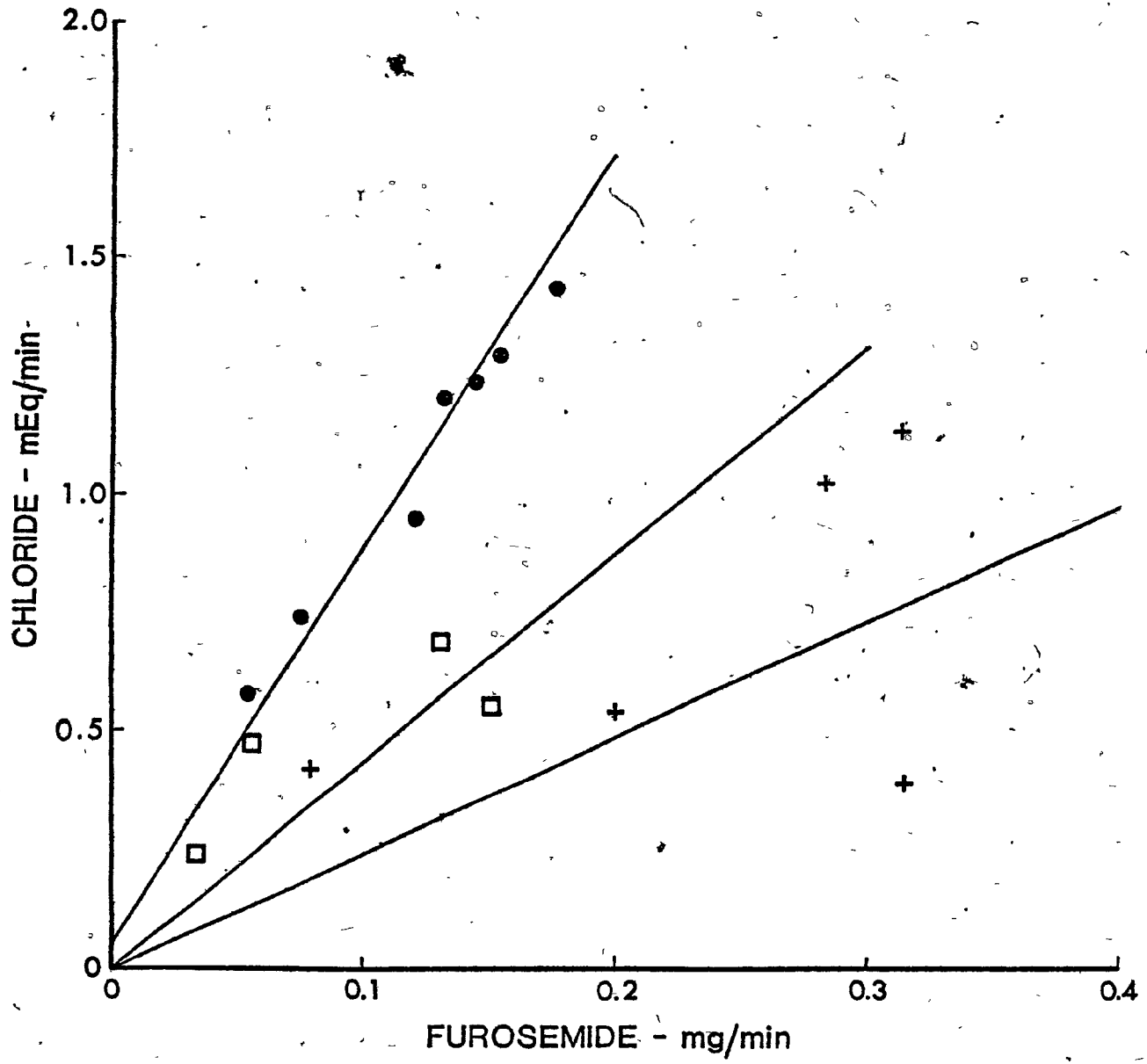

2.0
1.5
1.0
0.5
0
CHLORIDE - mEq/min-
0
0.1
0.2
0.3
0.4
FUROSEMIDE - mg/min

Figure 14. Relationship between urinary volume and furosemide excretion rate in three representative patients with acute pulmonary edema (numbers 3 ●, 4 ●· and 7 ●).

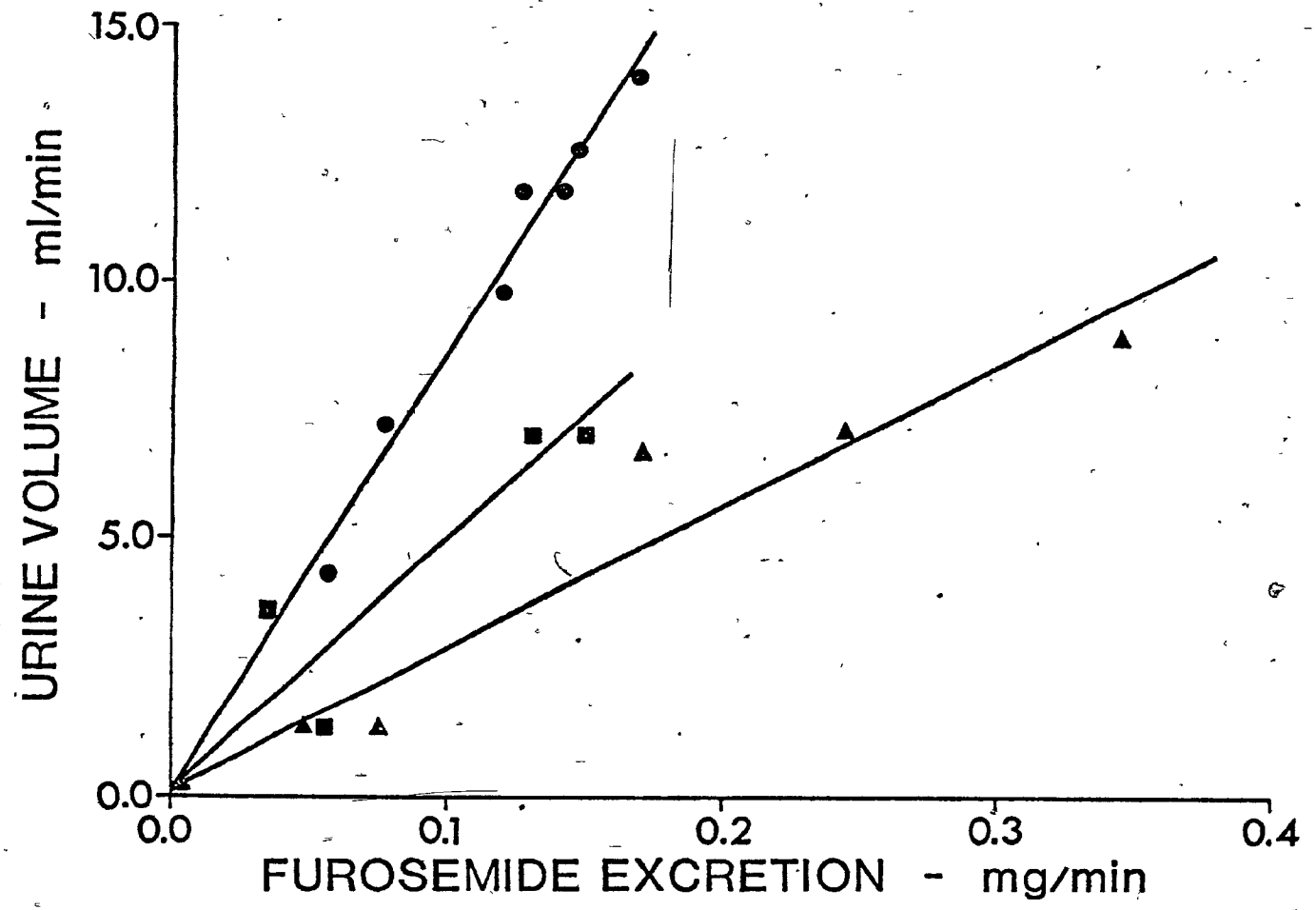

15.0
10.0
5.0
0.0
URINE VOLUME - ml/min
0.0
0.1
0.2
0.3
0.4
FUROSEMIDE EXCRETION - mg/min

TABLE XII. Regression analysis for sodium excretion rate to furosemide excretion rate

Patient	Slope	Intercept	r	P*
1	3.77	0.02	0.94	<0.01
2	21.85	0.09	0.91	<0.01
3	7.08	0.03	0.99	<0.01
4	3.18	0.02	0.94	<0.01
5	7.49	-0.08	0.98	<0.01
6	0.048	0.44	0.90	<0.01
7	2.55	0.01	0.99	<0.01
8	14.67	0.02	0.96	<0.01
9	5.03	-0.04	0.92	<0.05
10	6.99	-0.14	0.88	<0.05
11	3.24	-0.02	0.99	<0.01
12	4.09	0.01	0.97	<0.01
13	9.73	-0.04	0.93	<0.01
14	8.37	0.03	0.98	<0.01
15	4.17	0.03	0.90	<0.01
16	2.09	-0.01	0.99	<0.01

*Analysis of variance

TABLE XIII. Regression analysis for chloride excretion rate to furosemide excretion rate

Patient	Slope	Intercept	r	P*
1	2.85	0.03	0.91	<0.01
2	23.25	-0.11	0.96	<0.01
3	16.33	0.00	0.99	<0.01
4	4.20	0.01	0.97	<0.01
5	6.35	0.01	0.99	<0.01
6	2.57	0.01	0.90	<0.01
7	2.44	0.03	0.97	<0.01
8	16.33	-0.01	0.99	<0.01
9	9.24	-0.07	0.91	<0.05
10	6.75	-0.07	0.88	<0.05
11	3.00	0.00	0.99	<0.01
12	3.77	0.08	0.94	<0.01
13	12.89	0.00	0.98	<0.01
14	15.21	0.09	0.94	<0.01
15	4.39	-0.02	0.88	<0.05
16	4.14	-0.03	0.99	<0.01

*Analysis of variance

TABLE XIV. Regression analysis for excretion rate of urine volume and furosemide excretion rate

Patient	Slope	Intercept	r	P*
1	27.36	0.126	0.97	<0.01
2	168.02	-0.53	0.92	<0.01
3	84.5	0.04	0.99	<0.01
4	47.31	0.19	0.91	<0.01
5	48.34	0.21	0.99	<0.01
6	77.7	1.23	0.90	<0.01
7	24.66	0.66	0.90	<0.01
8	129.56	0.01	0.95	<0.01
9	75.50	0.07	0.92	<0.05
10	67.40	-1.11	0.90	<0.05
11	27.12	1.02	0.95	<0.01
12	39.59	1.04	0.96	<0.01
13	155.67	-0.47	0.98	<0.01
14	140.86	1.36	0.96	<0.01
15	39.81	1.22	0.90	<0.01
16	30.91	0.33	0.93	<0.01

*Analysis of variance

TABLE XV. Regression analysis for excretion rate of calcium and the excretion rate of furosemide

Patient	Slope	Intercept	r	P*
1	0.93	0.04	0.90	<0.01
2	4.18	0.00	0.99	<0.01
3	4.94	0.00	0.98	<0.01
4	2.93	-0.04	0.95	<0.01
5	3.31	0.00	0.99	<0.01
6	0.93	0.16	0.80	>0.05
7	0.88	0.01	0.80	>0.05
8	12.43	-0.04	0.99	<0.01
9	10.43	-0.04	0.94	<0.05
10	6.79	0.01	0.92	<0.05
11	2.44	0.01	0.98	<0.01
12	0.42	0.02	0.88	<0.05
13	0.82	0.02	0.98	<0.01
14	9.68	-0.02	0.98	<0.01
15	9.69	0.15	0.88	<0.05
16	1.11	0.02	0.97	<0.01

*Analysis of variance

TABLE XVI. Correlation analyses to determine the relationship between furosemide and sodium disposition in pulmonary edema patients

Patient No.	Cl_{Na}/Cl_F r	P*	Cl_{Na}/Cs_F r	P*	Exc_{Na}/Exc_F r	P*
1	0.77	>0.05	0.84	>0.05	0.94	<0.01
2	0.53	>0.05	0.98	<0.01	0.91	<0.01
3	0.47	>0.05	0.78	>0.05	0.99	<0.01
4	0.54	>0.05	0.72	>0.05	0.94	<0.01
5	0.80	>0.05	0.97	<0.01	0.98	<0.01
6	0.80	>0.05	0.42	>0.05	0.90	<0.01
7	0.82	>0.05	0.48	>0.05	0.99	<0.01
8	0.75	>0.05	0.90	<0.01	0.96	<0.01
9	0.87	>0.05	0.99	<0.01	0.92	<0.05
10	0.66	>0.05	0.57	>0.05	0.88	<0.05
11	0.81	>0.05	0.96	<0.01	0.99	<0.01
12	0.49	>0.05	0.95	<0.01	0.97	<0.01
13	0.29	>0.05	0.98	<0.01	0.93	<0.01
14	0.50	>0.05	0.84	>0.05	0.98	<0.01
15	0.70	>0.05	0.11	>0.05	0.90	<0.01
16	0.72	>0.05	0.99	<0.01	0.99	<0.01

* Analysis of variance

TABLE XVII. Correlation analyses to determine the relationship between furosemide and chloride disposition in pulmonary edema patients

Patient No.	Cl_{Cl}/Cl_F r	P*	Cl_{Cl}/C_{S_F} r	P*	Exc_{Cl}/Exc_F r	P*
1	0.68	>0.05	0.72	>0.05	0.91	<0.01
2	0.87	>0.05	0.99	<0.01	0.96	<0.01
3	0.51	>0.05	0.76	>0.05	0.99	<0.01
4	0.47	>0.05	0.66	>0.05	0.97	<0.01
5	0.99	<0.01	0.98	<0.01	0.99	<0.01
6	0.84	>0.05	0.69	>0.05	0.90	<0.01
7	0.87	<0.05	0.43	>0.05	0.97	<0.01
8	0.59	>0.05	0.99	<0.01	0.99	<0.01
9	0.81	>0.05	0.98	<0.01	0.91	<0.05
10	0.58	>0.05	0.52	>0.05	0.88	<0.05
11	0.40	>0.05	0.97	<0.01	0.99	<0.01
12	0.26	>0.05	0.90	<0.05	0.94	<0.01
13	0.45	>0.05	0.75	>0.05	0.98	<0.01
14	0.33	>0.05	0.72	>0.05	0.94	<0.01
15	0.78	>0.05	0.40	>0.05	0.88	<0.05
16	0.35	>0.05	0.99	<0.01	0.99	<0.01

*Analysis of variance

Disposition of Furosemide and Its Pharmacological Effect

An attempt to determine the most satisfactory relationship between the disposition of furosemide and its pharmacological effect on the renal excretion of salt and water in these patients is presented in Tables XVI and XVII. Table XVI presents a comparison of the correlation of the renal clearance of sodium to the renal clearance of furosemide, the renal clearance of sodium to serum concentration of furosemide, and the excretion of sodium to the excretion of furosemide. Comparison of renal clearance of sodium to the renal clearance of furosemide resulted in an unsatisfactory explanation for the pharmacological effect of the drug, since a poor correlation was found for all patients analyzed. The renal clearance of sodium was compared to the serum concentration of furosemide on the basis that the pharmacological effect might be related to the serum furosemide concentration. This relationship produced a better correlation than that observed between the renal clearance of sodium and the renal clearance of furosemide. However, it was still less than satisfactory for 8 of the 16 patients. The plasma concentration of furosemide did not have a consistent relationship with the diuretic response. Finally, a comparison between the urinary excretion of sodium and the urinary excretion of furosemide correlated most satisfactorily with furosemide disposition and its diuretic effect. This comparison was found to be satisfactory for all 16 patients. Table XVII presents the correlation analyses between clearance of chloride and clearance of furosemide, clearance of chloride and plasma concentration of furosemide and excretion of chloride and excretion of furosemide. This relationship was examined on the basis that the pharmacological effect of furosemide is exerted on the loop of Henle by inhibiting chloride reabsorption. The

best relationship was found between chloride and furosemide excretion in urine. This comparison resulted in a similar relationship to that found for sodium and furosemide.

DISCUSSION

DISCUSSION

Analysis for Furosemide and its Metabolites

The gas chromatographic method used for determination of furosemide and its acid metabolite is a modification of the method used by Lindström and Molander (1974). The major change involves the use of benzbromarone as the internal standard. The method used by Lindström and Molander incorporated the triethyl analogue of furosemide as an external standard. The use of an external standard does not allow for compensation in errors due to extraction since this external standard is added after the extraction. The use of benzbromarone as an internal standard compensates for extraction errors since it is added to the plasma or urine sample and is extracted with furosemide and its metabolites at the same time. The acid metabolite of furosemide is also extracted by this method. This modified method has described for the first time, the extraction and chromatographic characteristics of the acid metabolite of furosemide.

This modified method for determination of furosemide is more specific and sensitive than the fluorometric method. The spectrophotofluorometric method reported by Haussler and Hadju (1964), and used by Kelly et al (1973) and Anderson and Mikkelsen (1977) for the determination of furosemide has a detection limit of from 0.2 to 0.5 mg/L. Due to fluorescent and quenching substances in urine this assay is less precise for urinary determinations. The gas chromatographic method is specific, sensitive and linear from 0.05 to 64 mg/L for furosemide and from 0.1 to 32 mg/L for the acid metabolite. The amount of furosemide found in samples from these patients did not

exceed these limits.

Relationship Between the Amount of Furosemide Glucuronide Found in Urine and Time of Urine Incubation

The presence of a metabolite of furosemide, possibly a glucuronide conjugate was reported by Kindt and Schmid (1970), Beermann et al (1975) and Andreasen and Mikkelsen (1977) after treatment of urine samples with β-glucuronidase for different periods of time.

The present experiment demonstrates that urine must be incubated for at least 16 hours in order to hydrolyze all the glucuronide conjugated with furosemide and at the same time this hydrolysis can be prevented with the use of glucuronolactone, a specific inhibitor of β-glucuronidase. This is confirmed from incubation studies on the samples treated with glucuronolactone and β-glucuronidase, that showed no differences from control samples for furosemide content. Samples treated with β-glucuronidase alone showed a marked increase in the amount of furosemide with time. Therefore, the control experiment offers more direct evidence, for the presence in urine of a glucuronide metabolite of furosemide than has been reported previously.

Patient Population

Demographic Data

The patient population studied is heterogeneous in that there are patients with different degrees of renal function and with or without myocardial infarction. Interpatient comparisons were done in order to determine whether renal impairment and myocardial infarction could alter the pharmacokinetic disposition and pharmacodynamic effect of furosemide in patients with pulmonary edema.

Kinetic Disposition of Furosemide

In the present study, following an intravenous dose of furosemide in pulmonary edema patients, there was biexponential decay in serum furosemide concentration over time. A measurable quantity of furosemide was detected 24 hours after a single dose in these patients. Therefore the use of a two compartment open system model to determine the pharmacokinetic disposition of furosemide was used where possible.

The use of a one compartment open kinetic model for the analyses of five of these patients was justified on the basis that these patients received more than one dose of furosemide over a short period of time. It was difficult to do a precise estimation of the alpha half-life, but it was possible to determine when the alpha phase was finished and then to analyze the β half-life from the terminal phase of the concentration versus time phase. The use of a one compartment kinetic model to determine pharmacokinetic parameters for furosemide has been used by others (Cutler et al 1974; Beermann et al 1977, Aranda et al 1978). The use of model independent kinetic analyses to calculate Vd_{area}, Cl_s and Cl_R allowed for a comparison between all of the patients analyzed.

Alpha or Distribution Half-Life

Calesnick et al (1966) used furosemide-^{35}S and a two compartment open model in normal subjects. They reported the alpha or distribution half-life of furosemide to be 7 minutes. Andreasen et al (1977), using a two compartment open model for pharmacokinetic analysis of furosemide in normal subjects and in patients with heart failure, reported the alpha or distribution half-life to be 9.5 and 18.9 minutes

respectively. The alpha or distribution half-life of furosemide in patients with pulmonary edema varied from 15 to 79 minutes. The shortest alpha half-life in these patients was found to be twice as long as the mean value described for normal subjects (Calesnick et al 1966, Andreasen et al 1977). The median alpha half-life in patients with pulmonary edema was found to be 1.5 times greater than the mean alpha half-life reported in patients with cardiac decompensation who were receiving chronic furosemide therapy (Andreasen et al 1977). The difference could be explained by the fact that none of the patients investigated by Andreasen et al had myocardial infarction or pulmonary edema. The alpha phase of drug disposition represents its distribution throughout the organism. It is well documented (Guyton 1976, Harrison 1977, Robbins 1977) that when pulmonary edema is present secondary to heart failure, it is due to failure of the left ventricle which loses its efficiency as a pump. Therefore cardiac output is considerably decreased. In that case one would expect that distribution of drug through the body would take longer. Therefore the alpha half-life would be longer.

Beta or Elimination Half-Life of Furosemide

Calesnick et al (1966) using furosemide - ^{35}S and a two compartment open kinetic model in normal subjects reported the beta or elimination half-life of furosemide to be 70 minutes. Rupp and Zapf (1973) used a three compartment open model to describe the disposition of furosemide-^{35}S. They reported apparent half-lives of 11, 33, and 148 minutes respectively for these compartments after an intravenous dose. Cutler et al (1974) used a one compartment model to determine

furosemide disposition after an intravenous dose. The mean half-life of furosemide was reported to be 29.5 minutes in healthy subjects. They followed the plasma concentration of furosemide for only 6 hours. These discrepancies might be explained by technical differences in drug analysis. Rupp and Zapf based their data on plasma concentrations of radioactivity, and their lower limit of detection was 5 ng attributed to furosemide. Cutler et al (1974) used a fluorometric assay sensitive to 0.5 µg/ml changes in serum furosemide concentration. They calculated a half-life from several serum concentrations less than 1 µg/ml. Huang et al (1974), using a two compartment open model for pharmacokinetic analyses in patients with advanced renal failure, reported that the beta half-life of furosemide varied between 3.6 and 20 hours. Cutler et al (1974) reported beta half-lives of furosemide less than 2 hours for anephric patients in their study. The reason for these contradictory results might be that Cutler et al followed the plasma concentration of furosemide for only 6 hours. Beermann et al (1975), using furosemide-^{35}S and a two compartment open system model for pharmacokinetic analysis in normal subjects, reported that the beta half-life of furosemide was 47 to 53 minutes. Beermann et al (1977) used a gas chromatographic method for analysis of furosemide and a two compartment open model for pharmacokinetic analysis in normal subjects and in patients with renal failure. They reported the beta half-life of furosemide to be from 29.4 to 72 minutes in normal subjects and from 1.15 to 24.58 hours in patients with renal failure. Andreasen et al (1977), using a two compartment model for pharmacokinetic analysis of furosemide in normal subjects and in patients with heart failure, reported the beta or elimination half-life of furosemide

to be 71.8 and 134.1 minutes respectively.

In the present study there was a wide variation in the beta half-life of furosemide that ranged from 127 to 1190 minutes, median 283 minutes. The longest beta half-life (1190 minutes) found in patient 8, can be explained on the basis of his renal impairment (creatinine clearance 25 ml/min). Similar beta half-lives of furosemide have been reported in patients with renal failure (Huang et al 1974, Beermann et al 1977). The beta or elimination half-life of furosemide in patients with pulmonary edema was found to be longer than that for normal subjects reported previously by Calesnick et al (1966), Beermann et al (1975) and Andreasen et al (1977). The median beta half-life of furosemide in patients with pulmonary edema was found to be 2.1 times longer than the mean beta half-life of furosemide reported by Andreasen et al in patients with cardiac decompensation receiving chronic furosemide therapy. The difference can be explained by the fact that, in patients with pulmonary edema, left ventricular failure is greater. Also Andreasen et al (1977) followed plasma concentrations of furosemide for 8 hours using a fluorometric method. Benet et al (1976), using a fluorometric assay, reported the elimination half-life of furosemide was 76 minutes in patients with congestive heart failure. These data are completely different to previous reports. Data from the present study suggest that the alpha and the beta half-lives of furosemide are prolonged in patients with pulmonary edema.

Volume of Distribution
Vd_{area}

The volume of distribution (Vd_{area}) in pulmonary edema patients

(median 0.407 L/kg), was greater than that reported for patients with cardiac decompensation who were receiving chronic furosemide therapy (mean 0.28 L/kg, Andreasen et al 1977). This discrepancy might be explained by the use of a fluorometric method to determine furosemide concentrations in plasma or it may in fact be due to the difference in disease state. The highest apparent volume of distribution was found in two patients with myocardial infarction, patient numbers 1 and 10. This could not be explained by any physiological reason. It might be expected that with myocardial infarction, cardiac output would have been reduced and thereby reduced the apparent volume of distribution. The fact that Vd_{ss} also had a tendency to be greater in patients with myocardial infarction could not be explained for the same reasons described above.

Volume of the Central (V_1) and Peripheral (V_2) Compartments

The volume of the central compartment, median 0.144 L/kg, was found to be similar to that described in patients with renal or heart failure (0.117 L/kg, Huang et al 1974 and 0.100 L/kg Andreasen et al 1977). From data of Huang et al (1974) the volume of the central compartment was almost always smaller than the peripheral compartment. From data of Andreasen et al, the volume of the central compartment was similar to the volume of the peripheral compartment. However, Benet et al (1976) reported a larger volume of the central compartment in his patients with heart failure. They proposed that there was almost no distribution to the peripheral compartment. The increase in apparent volume of distribution, Vd_{area} or Vd_{ss} for furosemide in patients with pulmonary edema and myocardial infarction

was not due to expansion of the central compartment.

Volume of the Peripheral Compartment (V_2)

The volume of the peripheral compartment in pulmonary edema patients, median 0.21 L/kg, was larger than the mean, 0.09 L/kg, reported previously (Andreasen et al 1977) and was found to be similar to that (mean 0.198 L/kg) reported by Huang et al (1974). In 8 of the 11 pulmonary edema patients analyzed by the two compartment open model system, the volume of the peripheral compartment was found to be larger than the central compartment. This would support the interpretation that furosemide is distributed into the peripheral compartment and contradicts the findings of Benet et al (1976). This difference might be due to the difference in disease state in these two studies.

Distribution of Furosemide Between the Central and Peripheral Compartments

The rate constant k_{12} (median $0.477hr^{-1}$) in pulmonary edema patients was smaller than that (mean $0.924hr^{-1}$) reported by Andreasen et al (1977) and (mean of $2.91\ hr^{-1}$) Lawrence et al (1978) in normal subjects. It was found to be similar to that (mean $0.482hr^{-1}$) reported in patients with heart failure (Andreasen et al 1977) and lower than that (mean $0.603hr^{-1}$) reported in patients with renal failure (Huang et al 1973). Comparison of data in pulmonary edema patients with that from other investigators in normal subjects indicates that the rate of transfer from the central to the peripheral compartment is decreased in patients with pulmonary edema.

The rate constant k_{12} was larger in patients with myocardial infarction than in those without infarction. This is supported by the

fact that in patients with myocardial infarction the volume of the peripheral compartment was found to be larger and these patients excreted less unchanged furosemide in urine during the first alpha phase and for 24 hours after the dose. This is consistent with the possibility that furosemide is not excreted from the peripheral compartment and that increased metabolism may be associated with distribution into it. This interpretation is supported by the finding that there is little distribution of furosemide to a peripheral compartment in normal subjects, and they excreted most of the dose unchanged in urine (Calesnick et al 1966, Beermann et al 1975).

Rate Constant for Drug Transfer from the Peripheral to the Central Compartment (k_{21})

The rate constant k_{21} (median $0.253hr^{-1}$) was found to be smaller than that described in normal subjects (mean $3.2hr^{-1}$) by Andreasen et al 1977 and (mean $4.67hr^{-1}$) Lawrence et al 1978. In patients with pulmonary edema k_{21} was found to be smaller than that (mean $3.95hr^{-1}$) reported in patients with heart failure (Andreasen et al 1977). It was also smaller than that (mean $0.333hr^{-1}$) reported in patients with renal failure by Huang et al (1974).

Comparison of the rate constant of drug transfer from the peripheral to the central compartment (k_{21}) with data from other investigators in normal subjects suggests that rate of drug transfer from the peripheral to the central compartment is decreased in patients with pulmonary edema.

Relationship Between k_{12} and k_{21} in Patients with Pulmonary Edema

Data from the present study suggest that in patients with pulmonary edema the transfer of drug from the central to the peripheral and from

the peripheral to the central compartment is lower than has been reported previously for normal subjects. This is expected in patients with pulmonary edema who would have a lower cardiac output and reduced tissue perfusion. It would be expected to take longer to distribute the dose throughout the body. The fact that k_{12} was found to be larger in patients with myocardial infarction and pulmonary edema than in patients without myocardial infarction would not be explained by any physiological reason. Since it may be expected that in patients with myocardial infarction and pulmonary edema cardiac output would have been reduced, this might have reduced the transfer of drug from the central to the peripheral compartment. It appears that even in this state, furosemide is rapidly distributed into the peripheral compartment.

Serum Furosemide Clearance

The median serum clearance of furosemide (91.6 ml/min) in pulmonary edema patients was smaller than the mean values reported for normal subjects by Kelly et al (138 ml/min; 1974), Beermann et al (194 ml/min; 1977) and Andreasen et al (166 ml/min; 1977, and 219 ml/min; 1978). The median serum clearance of furosemide in patients with pulmonary edema was smaller than or approximately the same (means 83.5 and 126.5 ml/min) as that reported in a series of patients with heart failure by Andreasen et al (1977). The finding of lower serum clearance in patients with pulmonary edema than in normal subjects can be explained by hemodynamic changes expected in patients with severe heart failure, in which tissue perfusion is reduced and therefore the time to clear the body of drug would be increased.

A good correlation was found between serum clearance of furosemide and creatinine clearance among the patients with pulmonary edema. The substantial interindividual variation suggests that it is not possible to predict serum clearance of furosemide for a given value of creatinine clearance. This variation could be explained in part by the variation in nonrenal clearance. This variation also has been reported in normal subjects and in patients with renal failure (Beermann et al 1977).

Renal Clearance of Furosemide

The median renal clearance of unchanged furosemide (46.7 ml/min) in patients with pulmonary edema was less than the mean values (95 ml/min) reported by Beermann et al (1977) and (116 ml/min) Andreasen et al (1977) in normal subjects. The median renal clearance of furosemide in patients with pulmonary edema was found to be less than the mean renal clearance (80 ml/min) reported by Andreasen et al (1977) for patients with heart failure. This could be explained by differences in disease state and by the fact that the fluorometric method used to determine furosemide concentration is even less sensitive for urine than for serum determinations because of the presence of fluorescent and quenching substances, including furosemide metabolites in urine (Kelly et al 1974). The renal clearance of furosemide did not have a significant relationship with creatinine clearance when data from all patients with pulmonary edema were plotted. This might be explained by the interindividual variation in renal and nonrenal excretion of furosemide among patients with pulmonary edema and similar creatinine clearance.

It has been reported (Greenblatt et al 1976) that creatinine

excretion is not constant over time in normal subjects and can vary as much as threefold although the standard deviation of mean values for their population was only 10.5 to 14.4 percent. Since pulmonary edema is an acute state that must be resolved within hours, the patient may excrete the drug differently than creatinine. If patient numbers 1 and 9 are taken out of this analysis, then the relationship between renal clearance of furosemide and creatinine clearance is significant. Although it is possible that creatinine clearance was incorrectly estimated for these patients, this is unlikely since creatinine clearance determinations were done by two different laboratories with similar results to each other. It was not necessary to eliminate these patients from other comparisons of renal clearance of furosemide metabolites and creatinine clearance. Patient number 9 was a 28 year old pregnant woman with myocardial infarction. Whether this factor altered the excretion of furosemide is not known. Patient number 1 also had myocardial infarction and a low creatinine clearance, but he had a high clearance of furosemide. The fact that patients with normal or approximately normal creatinine clearance can clear different amounts of furosemide has been reported by Beermann et al (1977). The data of the present study are limited with respect to the numbers of patients with severe renal impairment. More patients with severely impaired renal function and pulmonary edema should be studied in order to resolve the importance of impaired renal function and furosemide renal clearance in this disease state.

Relationship Between Renal and Serum Clearance of Furosemide

The serum clearance of furosemide in pulmonary edema patients was

always higher than the renal clearance. The average of the ratio between serum clearance and renal clearance of furosemide in pulmonary edema patients (2.3) was higher than previously reported in normal subjects (mean 1.08, Cutler et al 1974, and mean 1.4, Andreasen et al 1977). The fact that in pulmonary edema patients the ratio is higher suggests changes in disposition of furosemide in the disease. The fact that the highest ratio was found in 3 patients with severe pulmonary edema suggests that in this disease state nonrenal elimination of furosemide plays an important role.

Renal Clearance of Furosemide During the Alpha and Beta Phases of Furosemide Disposition

The renal clearance of furosemide was higher during the alpha than the beta phase of furosemide disposition. At the same time, renal sodium clearance was higher during the alpha than the beta phase of furosemide disposition. If the action of furosemide can be quantified for the amount of sodium, chloride and urine excreted, then these data suggest that the pharmacodynamic action of this drug occurs mainly during the alpha phase of furosemide disposition. Therefore, when comparison between the ratio of renal sodium clearance to renal furosemide clearance is made, it results in a higher ratio during the alpha than during the beta phase of furosemide disposition. The early effect of furosemide has been noted by several investigators (Calesnick et al 1966, Kelly et al 1977, Branch et al 1977), but no attempt has been made to relate this diuretic effect with the alpha or beta phase of furosemide disposition. Data from this study in pulmonary edema patients include more elements simultaneously with

respect to explain how this drug acts in man.

Excretion of Furosemide and its Metabolites

The excretion of unchanged furosemide in patients with pulmonary edema was found to be less than previously reported for normal subjects (Calesnick et al 1966, Beermann et al 1975, Andreasen et al 1977). The mean renal excretion of 80% of the administered dose of unchanged furosemide reported for normal subjects by Calesnick et al (1966) and Beermann et al (1977) is greater than the median renal excretion of 56% of the administered dose of unchanged furosemide in pulmonary edema patients. This suggests that the excretion of unchanged furosemide is impaired in patients with pulmonary edema. These results are closer to the mean value of 63% excretion of unchanged furosemide found by Andreasen et al (1977) in patients with heart failure. There was a large interindividual variation in the excretion of unchanged furosemide among the patients with pulmonary edema with approximately the same creatinine clearance. Therefore no significant relationship was found between these two parameters. Excretion of unchanged furosemide was found to be less in patients with than without myocardial infarction. At the same time, patients with myocardial infarction tended to excrete more of both furosemide metabolites in urine than patients without myocardial infarction. The fact that this relationship was not found to be statistically significant may be due to the small population studied. The severity of acute pulmonary edema appears not to be a determinant in the excretion of furosemide metabolites. This is not surprising since assessment of the severity of acute pulmonary edema was based

mainly on the chest X-ray film, and in clinical practice it is accepted that radiological signs do not always correspond well with clinical manifestations of the disease.

Urinary Excretion of Furosemide Glucuronide

The excretion of the glucuronide conjugate of furosemide in normal subjects and in patients with renal or heart failure has been reported by Beermann et al (1975) and Andreasen et al (1977). After treatment of urine samples with β-glucuronidase, they reported less furosemide glucuronide in normal subjects than in patients with renal or heart failure. Andreasen et al (1977) suggested that long treatment with furosemide might stimulate the metabolic pathway. The present study shows that this metabolite is found in greater amounts in patients with pulmonary edema than has been previously reported for normal subjects. The possibility of the relationship between long treatment with furosemide and the amount of glucuronide metabolite found in urine could not be confirmed in this study, since it was not part of the experimental design. These pulmonary edema patients were treated with furosemide for different periods of time before their arrival at the hospital. The fact that a significant relationship between creatinine clearance and the formation of furosemide glucuronide was found suggests that the production of this metabolite increases as renal function decreases. The fact that patient number 8 with renal failure excreted very little of this metabolite could be explained by the severity of pulmonary edema, and the probability of increased fecal excretion. Increased excretion of radioactivity in feces of patients with renal failure compared to normal subjects (Beermann et al 1977) support

this interpretation. The fact that patient number 4, who received multiple doses of furosemide over a short period of time, excreted a greater proportion of the glucuronide conjugate of furosemide suggested the possibility of a dose dependent relationship for the formation of this metabolite. Analysis of the extent of furosemide conjugated with glucuronic acid versus dose showed no dose dependent relationship. This may be due to interindividual variation in the excretion of furosemide glucuronide in patients receiving the same dose. A more comprehensive study of this relationship is required in order to arrive at a final conclusion.

Excretion of the Acid Metabolite of Furosemide

Excretion of 2-amino-4-chloro-5-sulfamoylanthranilic acid as the only metabolite of furosemide has been reported by Hadju and Haussler (1964) and Haussler and Wicha (1965), while others found no evidence of it (Calesnick et al 1966, Beermann et al 1975). Recently, Andreasen et al (1978) have reported the acid metabolite in both normal subjects and anephric patients. This study showed that the acid metabolite is present in urine of patients with pulmonary edema and increased when renal function decreased. The acid metabolite accounted for a lesser proportion of the dose in patients with pulmonary edema than the glucuronide metabolite of furosemide. These data suggest that in patients with pulmonary edema, the main metabolite is the glucuronide conjugate of furosemide and not the acid metabolite.

Relationship Between the Pharmacokinetic and Pharmacodynamic Disposition of Furosemide

The 24 hour urine volume and excretion of electrolytes in some

of the patients with pulmonary edema were noted to be lower than values for normal 24 hour urine excretion. This could be explained by the fact that they were chronically ill with restricted intake of salt and water. This decrease in excretion of urine volume and electrolytes has been reported by Branch et al (1977) in his kinetic study of furosemide disposition in normal subjects with restricted intake of salt.

Urine Osmolality

The minimum urinary osmolality was always found to occur during the alpha phase. It was coincident with the peak of diuresis and was always found within 80 minutes after the dose. The minimum urine osmolality is similar to that reported by Cutler et al (1974). This is coincident with the rapid onset of furosemide action and with its main diuretic action during the alpha phase of furosemide disposition. This effect appears to be similar for patients with different degrees of renal function, with or without myocardial infarction, and regardless of the severity of pulmonary edema.

Urinary Furosemide Excretion and Effect on Renal Function

The rates of renal sodium clearance to furosemide clearance was larger during the alpha phase than the beta phase of furosemide disposition. If the action of furosemide was constant during the alpha and beta phases, one would expect that the ratio between the renal clearance of sodium to the renal clearance of furosemide would be similar during the two phases. However, this ratio is greater during the alpha than during the beta phase, consistent with the clinical observation that the diuretic effect of furosemide is largely over by

the end of the alpha phase. However, clearance of furosemide by the kidney is still continuing during the beta phase of serum furosemide disposition. Since drug effect is said to be related to its concentration at the site of action, and since it has been reported by Burg et al (1973) in isolated nephron segments that access to the luminal rather than the plasma membrane appears to be necessary for the activity of furosemide, a comparison between the excretion of sodium, chloride and urine volume, and the excretion of furosemide was done. This resulted in a positive and linear relationship for all patients analyzed for all these comparisons. Therefore the excretion of urine volume, sodium and chloride increased linearly with the excretion of furosemide.

Effects of Furosemide on the Excretion of Other Electrolytes

The excretion of calcium was found to increase linearly with the excretion of furosemide. The increased excretion of calcium after a dose of furosemide has been reported by several investigators (Walser et al 1963, Toft et al 1970, Gall et al 1971, Suki et al 1970, and Walser et al 1971). This increase in calcium excretion with increasing doses of furosemide has been reported without quantitation. The results from this study show that furosemide can increase the excretion of calcium linearly. This suggests that the excretion of calcium should be greater with higher doses of furosemide. Careful attention must be paid to the administration of furosemide in older patients with osteoporosis in whom furosemide could accelerate this process.

Disposition of Furosemide and its Pharmacological Effects

It has been postulated that the action of furosemide in the ascending limb of the loop of Henle is due to the inhibition of chloride

reabsorption. In order to obtain this effect, access to the luminal membrane rather than the plasma membrane of the nephron by furosemide appears to be necessary (Burg et al 1973). Huang et al (1974) and Cutler et al (1974) did not find a correlation between plasma concentration of furosemide and the excretion of sodium or urine volume in normal subjects or in patients with renal failure. Rose et al (1976) found a good relationship between the excretion of furosemide and the excretion of sodium in azotemic dogs. They reported no correlation between plasma concentration of furosemide and urinary sodium excretion. Branch et al (1977) reported a significant relationship between the plasma concentration of furosemide and the rate of excretion of sodium in normal subjects. They reported that the urine concentration of furosemide did not have any consistent relationship with its diuretic response. Lawrence et al (1978) reported a linear correlction between furosemide excretion rate and sodium or potassium excretion rate in urine from normal subjects.

An attempt to determine the most satisfactory relationship between the disposition of furosemide and its pharmacological effect on renal handling of salt and water in patients with pulmonary edema was made. A comparison between the renal clearance of sodium and chloride to the renal clearance of furosemide resulted in a completely unsatisfactory explanation for the pharmacological effects of this drug, since a poor correlation was found for all the patients analyzed. The renal clearance of sodium and chloride were compared to the serum concentration of furosemide on the basis that the pharmacological effect might be related to the plasma concentration. This relationship was an improvement over

that observed between the renal clearance of sodium and furosemide. However, this relationship was unsatisfactory for half of the patients analyzed. Therefore the plasma concentration of furosemide did not have a consistent relationship with the diuretic response to this drug. Finally, a comparison of the renal excretion of sodium and chloride to the urinary excretion of furosemide correlated most satisfactorily with furosemide disposition and its diuretic effect. This comparison was found to be satisfactory for all patients analyzed.

CONCLUSIONS

Analysis of the data from the present study suggest that:

1. Furosemide disposition is altered in patients with pulmonary edema. This alteration is defined as a prolongation in the alpha and beta half-lives of furosemide, increased furosemide biotransformation, and a decrease in excretion of unchanged drug in urine.

2. Alteration of furosemide metabolism in pulmonary edema patients does not prevent its diuretic action.

3. The diuretic response to furosemide is associated with the distribution of drug outside of the plasma to a compartment which is in rapid equilibrium with it. The response to furosemide is determined by the concentration of drug in this tissue compartment.

4. The excretion of furosemide into tubular urine is required for its diuretic effect in patients with pulmonary edema.

5. The excretion of sodium, chloride, calcium and urine volume increase linearly with the excretion of unchanged furosemide in urine.

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Appendix 1 Approval of the Ethics Committee of the Montreal General Hospital for the investigation of Pharmacokinetics and pharmacodynamics of furosemide in patients with acute pulmonary edema.

Appendix 2 Summary of pharmacokinetic data for disposition of furosemide in man.

TABLE XVIII. Summary of the serum furosemide half-lives in man. Mean and range of values are reported.

Reference	Assay	Subject	alpha $t_{1/2}$ (min)	beta $t_{1/2}$ (min)
Calesnick et al 1966	^{35}S	Normal	7	70
Cutler et al 1974	Fluoro-metric	Normal		29.5 18.5-40.4
Beermann et al 1975	^{35}S	Normal		47-53
Beermann et al 1977	GLC	Normal		47.4 29.4-72
Andreasen et al 1977	Fluoro-metric	Normal	9.5	71.8
Branch et al 1977	Fluoro-metric	Normal		50
Homeida et al 1977	Fluoro-metric	Normal		38.4
Lawrence et al 1978	Fluoro-metric	Normal		36.0
Huang et al 1974	Fluoro-metric	Renal failure		582 216-1200
Cutler et al 1974	Fluoro-metric	Renal failure		80.7 40.1-121.3
Kelly et al 1977	^{35}S	Hepatic and renal failure		42-98
Beermann et al 1977	GLC	Renal failure		69.0-1475
Benet et al 1976	Fluoro-metric	Heart failure		76.7
Andreasen et al 1977	Fluoro-metric	Heart failure	18.9	134.1
Perez, 1978*	GLC	Pulmonary edema	29.4 15-79	283 127-1190

*Median and range

TABLE XIX. Summary of furosemide clearance rates and excretion as a percent of the administered dose. Mean and range of values are reported.

Reference	Assay	Subject	Cl_s (ml/min)	Cl_R (ml/min)	Urinary excretion of unchanged furosemide (%)	Fecal excretion (%)
Calesnick et al 1966	^{35}S	Normal			80 51-94	2.1
Cutler et al 1974	Fluorometric	Normal	162	149 83-201	92 67-99	
Kelly et al 1974	Fluorometric	Normal	138 104-197		30-50	
Beermann et al 1975	^{35}S	Normal			82-84	6-9
Kelly et al 1977	^{35}S	Normal	109-194	71-150		
Andreasen et al 1977	Fluorometric	Normal	166	116		
Branch et al 1977	Fluorometric	Normal	125	75	65	
Homeida et al 1977	Fluorometric	Normal	268	90		
Beermann et al 1977	GLC	Normal	194 172-235	95 71-130		
Lawrence et al 1978	Fluorometric	Normal	224	98		
Andreasen et al 1978	Fluorometric	Normal	219.3 166-306			
Cutler et al 1974	Fluorometric	Anephric	105 83-185			

TABLE XIX. - Continued

Reference	Assay	Subject	Cl_S (ml/min)	Cl_R (ml/min)	Urinary excretion of unchanged furosemide (%)	Fecal excretion (%)
Huang et al 1974	Fluorometric	Renal failure		3.29 0.93-11		
Kelly et al 1977	Fluorometric	Renal and hepatic railure	73-189	23-168		
Beermann et al 1977	GLC	Renal failure	80.3 26-124	15.6 1-50		60.4
Andreasen et al 1978	Fluorometric	Renal failure	52-80			
Andreasen et al 1977	Fluorometric	Heart failure	126.5-83.5	80	63	
Perez 1978*	GLC	Pulmonary edema	91.6 21-295	46.7 6.4-113	56	

*Median and range

TABLE XX. Summary of pharmacokinetic determinations of the apparent volume of distribution for furosemide in man. Mean and range of values are reported.

Reference	Assay	Subject	Vd	Vd_{area}	Vd_{ss}	V_1	V_2
Kelly et al 1974	Fluoro-metric	Normal	5.03 L			3.80L 3.36-3.66L	1.51L 2.11-2.65L
Kelly et al 1977	^{35}S	Normal	16.4-9.3L				
Beermann et al 1977	GLC	Normal	0.21L/kg 0.137-0.273 L/kg				
Andreasen et al 1977	Fluoro-metric	Normal	0.083L/kg	0.28 L/kg	0.181L/kg 0.136-0.266 L/kg		
Branch et al 1977	Fluoro-metric	Normal	11.9L				
Homeida et al 1977	Fluoro-metric	Normal	14.97L				
Lawrence et al 1978	Fluoro-metric	Normal	15.32L				
Huang et al 1974	Fluoro-metric	Renal failure				0.117 0.067-0.195	0.198L/kg 0.160-0.360 L/kg
Beermann et al 1977	GLC	Renal failure	0.141-0.497 L/kg				
Andreasen et al 1978	Fluoro-metric	Renal failure			0.197 0.156-0.226	0.105 0.035-0.156 L/kg	0.092

TABLE XX. - Continued

Reference	Assay	Subject	Vd	Vd_{area}	Vd_{ss}	V_1	V_2
Benet et al 1976	Fluoro-metric	Heart failure			11.4L	7.1L	4.3L
Andreasen et al 1977	Fluoro-metric	Heart failure		0.28 L/kg	0.198L/kg	0.100L/kg	0.098L/kg
Perez 1978*	GLC	Pulmonary edema		0.407 L/kg	0.317L/kg	0.144L/kg	0.210L/kg
			0.148-2.154 L/kg		0.085-1.67 L/kg	0.03-0.278 L/kg	0.05-1.47 L/kg

*Median and range

TABLE XXI. Summary of the rates of intercompartmental distribution of furosemide in man. Mean and range of values are reported.

Reference	Assay	Subject	k_{12} hr^{-1}	k_{21} hr^{-1}
Andreasen et al 1977	Fluorometric	Normal	0.924	3.2
Andreasen et al 1978	Fluorometric and TLC	Normal	2.58 0.60-5.75	2.35 0.66-4.08
Lawrence et al 1978	Fluorometric	Normal	2.91	4.67
Kelly et al 1974	Fluorometric	Renal failure	0.78 0.90-1.38	1.92 1.56-1.76
Huang et al 1974	Fluorometric	Renal failure	0.603 0.100-1.986	0.333 0.169-0.941
Andreasen et al 1978	Fluorometric and TLC	Renal failure	4.56 0.78-9.42	4.38 1.68-6.72
Andreasen et al 1977	fluorometric	Heart failure	0.432	3.95
Perez 1978*	GLC	Pulmonary edema	0.477 0.257-1.58	0.253 0.090-0.760

*Median and range