Title
Renal excretion of pseudoephedrine in the rat

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Publication Date
1979

Peer reviewed|Thesis/dissertation

RENAL EXCRETION OF PSEUDOEPHEDRINE IN THE RAT

by

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DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

DOCTOR OF PHILOSOPHY

in

PHARMACEUTICAL CHEMISTRY

in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA

San Francisco

[redacted]

Date University Librarian

Degree Conferred: APR 1 1979

Abstract

RENAL EXCRETION OF PSEUDOEPHEDRINE IN THE RAT

Alice E. Till

Pseudoephedrine is an organic base used in the treatment of upper respiratory tract disorders. Its excretion has been shown to be influenced by urinary pH. It is also likely that it is secreted in the renal tubule by means of the base transport system. Because of episodes of toxicity in children with renal tubular acidosis who were administered pseudoephedrine, we chose to study this basic drug so as to define its renal mechanisms of excretion.

Surgical techniques and experimental procedures were developed for the study of renal mechanisms in the rat. The ability to accurately measure renal clearance and to demonstrate renal secretion by a carrier-mediated transport system was verified by comparison of results for N^1-methylnicotinamide (NMN) excretion studies with literature results. After infusion of solutions of NMN and inulin, the renal clearance of each was calculated. As steady-state plasma levels of NMN increased, clearance ratios of NMN to inulin decreased demonstrating saturation of the secretory process. Clearance ratios of NMN decreased 45% when mepiperphenidol was added to the infusion.

A microanalytical procedure was developed using gas chromatography-electron capture which is capable of detecting 2 ng of pseudoephedrine in 20 µl of plasma. The ability to assay such small plasma samples permitted a sufficient number of measurements to be made to adequately

study the pharmacokinetics and renal excretion of pseudoephedrine in a single rat.

The pharmacokinetics of pseudoephedrine were studied in three rats following intravenous administration of a bolus dose of the drug. Plasma levels of pseudoephedrine showed a biphasic decline; the plasma concentration-time data was analyzed according to a two compartment body model. The derived pharmacokinetic parameters exhibited little inter-animal variation. Average values of α and β were 0.152 and 0.0200 min^{-1} respectively. The average terminal half-life was 35 minutes. Calculated renal clearances were greater than the reported glomerular filtration rate in the rat. The fraction of the dose excreted as unchanged pseudoephedrine was 0.45. Initial clearance studies with pseudoephedrine showed renal clearance to be three times that of inulin, indicating that renal tubular secretion plays a significant role in the excretion of pseudoephedrine.

Following administration of increasing doses of pseudoephedrine, steady-state plasma levels increased from 0.16 mcg/ml to 1.5 mcg/ml. As plasma levels of pseudoephedrine increased, transport rate increased in a linear fashion; clearance of pseudoephedrine and clearance ratios of pseudoephedrine to inulin remained essentially constant. These results indicated that secretion of pseudoephedrine was not saturable in the range of plasma levels studied.

Renal excretion of pseudoephedrine was found to be sensitive to urinary pH in the range of 6.3 to 7.2. These results are consistent

with reported effects of urinary pH on ephedrine excretion and suggest a passive reabsorption by non-ionic diffusion for pseudoephedrine.

In the presence of mepiperphenidol, clearance ratios of pseudoephedrine to inulin decreased 20 - 50 % depending on the dosing schedule for mepiperphenidol. The corresponding decrease in the ratio of the rate of pseudoephedrine transport (corrected for glomerular filtration rate) to plasma concentration in the presence of mepiperphenidol was 18 - 66%. The demonstration of inhibition of pseudoephedrine transport by mepiperphenidol indicates that pseudoephedrine is transported by the organic base transport system.

In the presence of procainamide, the average decrease in the pseudoephedrine/inulin clearance was 30%; decrease in the ratio of pseudoephedrine transport (corrected for glomerular filtration rate) to plasma concentration was 40%.

In three out of four rats, neither the clearance ratio of pseudoephedrine to inulin nor pseudoephedrine transport decreased in the presence of ethambutol; in the fourth rat there was a dramatic decrease in these parameters. From the limited data available, it was impossible to determine whether or not ethambutol had an effect on the renal secretion of pseudoephedrine.

To
my parents
Franklin and Regina
my sisters
Bettie, Joane and Grace
my brother
George

Past failures very little mean
When once success is won,
None cares how rough the road
has been
When the long journey's done.

Edgar A. Guest

Acknowledgements

I would like to express my gratitude to:

Professor Leslie Z. Benet for encouraging me to enter the Ph.D. program in Pharmaceutical Chemistry at UCSF and for continued encouragement, guidance, support and friendship throughout the years in this program,

Professor C. Anthony Hunt and Professor Floyd C. Rector for their helpful suggestions during the course of my research and in the preparation of this dissertation,

Dr. Emil T. Lin for his help with the pseudoephedrine assay, American Foundation for Pharmaceutical Education for more than four years of financial support as an H. A. B. Dunning Memorial Fellow and the Earl C. Anthony fund of the University of California for partial research funding,

Merck Sharp and Dohme Research Laboratories for providing the mepiperphenidol,

Dr. King Chiu Kwan for his long distance encouragement and Karen M. Schultheis for her sincere friendship and constant moral support.

Alice E. Till
February, 1979

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Statement of Purpose

Pseudoephedrine is an organic base used in the treatment of upper respiratory tract disorders. Toxicity following administration of pseudoephedrine has been observed in children with renal tubular acidosis. It is therefore clinically relevant to define the mechanisms of pseudoephedrine renal excretion.

The objectives of this work are to develop appropriate surgical and experimental techniques for studying the renal excretion of pseudoephedrine in the rat; to determine the net excretion of pseudoephedrine, specifically looking for the presence of secretion; to look at the effect of urinary pH on the net excretion; to determine if secretion of pseudoephedrine is saturable and subject to competitive inhibition and thus carrier-mediated.

CHAPTER 1

INTRODUCTION

Each mammalian kidney is composed of many units or nephrons. A nephron consists of an individual renal tubule and its glomerulus. The glomerulus is formed by the invagination of a tuft of capillaries into the dilated blind end of the nephron (Bowman's capsule). The capillaries are supplied by an afferent arteriole and drained by an efferent arteriole. The renal tubule consists of the proximal convoluted tubule (pars convoluta) which drains into the straight portion of the proximal tubule (pars recta), which forms the first part of the loop of Henle. The thick ascending limb of the loop of Henle reaches the glomerulus of the nephron from which the tubule arose and passes close to its afferent arteriole. The final portion of the tubule is the distal convoluted tubule. Distal tubules coalesce to form collecting ducts which pass through the renal cortex and medulla to empty into the pelvis of the kidney. The vascular supply of the tubules is essentially a portal one in that the blood that perfuses the peritubular capillaries has initially traversed the glomerular capillaries (1-4).

A. Renal Excretion Mechanisms

The nephrons carry out the vital functions of the kidney essentially via three major processes: glomerular filtration, tubular reabsorption of water and filtered substances from the lumen of the tubule into the plasma, and tubular secretion of substances from the plasma

across the tubular membrane into the tubular lumen (2-6). The processes of tubular reabsorption and secretion may be governed either by passive mechanisms (simple diffusion) or carrier-mediated mechanisms (facilitated diffusion and active transport). While the renal processes primarily serve to maintain extracellular fluid volume and osmolarity, to conserve important solutes, and to help regulate acid-base balance, they are also important in controlling the excretion of exogenous compounds such as drugs.

1. Glomerular filtration

A fluid conforming closely to that of an ideal ultrafiltrate of plasma moves across the glomerular membrane. It has nearly the same composition as plasma with respect to water and low molecular weight solutes. During the process of glomerular filtration gross particulate matter and colloidal materials such as proteins and protein bound lipids are removed from solution. Factors which govern filtration across the glomerular capillaries include the size of the capillary bed, the permeability of the capillaries, and the hydrostatic and osmotic pressure gradients across the capillary wall. Variations in these factors have predictable effects on glomerular filtration rate (GFR). A decrease in the total area of the capillary bed resulting from diseases which destroy glomeruli with or without destruction of the tubules (glomerulonephritis) and partial nephrectomy will decrease GFR. Although many capillaries are completely blocked in glomerulonephritis, those not blocked often permit increased filtration of protein. Changes in glomerular capillary hydrostatic pressure, brought about by changes in systemic blood

pressure and afferent or efferent arteriolar constriction, will affect GFR; when efferent arteriolar constriction is greater than afferent constriction GFR tends to be maintained, but either constriction decreases tubular blood flow. Changes in the hydrostatic pressure in Bowman's capsule caused by ureteral obstruction and edema of the kidney will decrease GFR. Dehydration and hypoproteinuria will change the concentration of plasma proteins and in turn alter glomerular filtration. Increased GFR can result from the increase in glomerular plasma flow accompanying increased renal blood flow (as occurs over the first eight months of pregnancy); a decrease in glomerular plasma flow decreases GFR. In grave emergencies such as fright, pain, syncope, severe exercise, deep anesthesia, hemorrhage, GFR and renal blood flow are sacrificed in defense of the blood perfusion of organs more immediately vital to existence, e.g., heart and brain (2-4, 7). Many of the factors which affect GFR will also affect the tubular processes to be discussed next e.g. changes in renal blood flow, afferent or efferent arteriolar constriction, diseases which destroy the tubules as well as the glomeruli, partial nephrectomy etc. At their onset, renal diseases may affect primarily the function of the glomerular capillary tuft or the activities of one or more segments of the renal tubules. When damage to one element of the nephron leads to its destruction, however, other elements of the same nephron atrophy and the functional mass of the kidney is reduced (3).

2. Passive transport (simple diffusion) across the renal tubule

Passive transport of exogenous compounds, such as drugs, in the renal

tubule involves simple diffusion along a concentration (or more properly, chemical potential) gradient (4,6,8-11). The concentration gradient between urine and plasma is the driving force for diffusion, while the rate of diffusion is also governed by the diffusivity of the molecule through the tubular membrane, the membrane/aqueous phase partition coefficient for the molecule, the thickness of the membrane at the site of diffusion and the area of the membrane through which the molecule passes (12). It is generally assumed that biological membranes, being lipid in nature, are more permeable to lipid soluble substances and that transmembrane diffusion depends in part on the lipid solubility of the diffusing compound (4,12). In the case of acids and bases, the unionized species exhibits greater lipid solubility than the ionized species and is thus believed to be either the sole diffusing species or the more rapidly diffusing species (4,6,8,10-12). The concentration gradient of the diffusing species of an acid or base is governed by the pKa of that acid or base and the pH gradient across the membrane (12). In the specific case of passive diffusion across the renal tubular membrane, the concentration gradient is dependent on urinary pH (assuming intracellular and blood pH to be constant) (4,10-12). Since the concentration of drug in the urine is a function of urinary flow rate, diffusion across the tubular membrane may also be affected by changes in urinary flow rates (10). There has been some evidence of urinary flow dependent excretion of ionized substances (e.g., N^1-methylnicotinamide) which suggests that ionized substances as well as unionized substances may indeed be subject to simple diffusion across renal tubular membranes (10). Although diffusion across the tubular

membrane can theoretically occur in either direction, passive diffusion from blood to tubular lumen is highly improbable for organic acids but may occur for a few organic bases as has been discussed in detail by Weiner (10).

The amphetamines and barbiturates are classic examples of drugs which undergo passive reabsorption in the renal tubule. Asatoor et al (13) studied the excretion of dexamphetamine (a weak base) and its derivatives in man and in the rat when urinary pH was varied. The excretion of amphetamine (pKa 9.77) was increased in acid urine and decreased in alkaline urine. Output of hydroxyamphetamine, the main metabolite of amphetamine in the rat was unaffected by variation in urinary pH. Hydroxyamphetamine (pKa 10.7) is a stronger base than amphetamine and the lipid solubility of its unionized fraction is reduced due to the addition of the polar hydroxy group. Beckett and Rowland studied the urinary excretion kinetics of amphetamine and methylamphetamine (pKa 9.87) in man (14,15). Fluctuations in excretion rate of both amines occurred and were associated with changes in urinary pH. The total amount of both amines excreted was lower under alkaline urine conditions. Phenobarbital is a weak acid with a pKa of 7.2. Waddell and Butler (16) found that the renal clearance of phenobarbital increased with increasing rate of urine flow. At any given rate of flow, the clearance was much higher when the urine was alkaline than when it was acid. The clearance of barbital (pKa 7.9) is affected to a lesser extent by changes in urinary pH than phenobarbital (10,16). This difference in behavior of the two drugs has been largely attributed to the

difference in pKa (10). In general, the effect of changing urinary pH on the renal excretion of acids and bases has been examined in a qualitative manner, i.e., for a given acid or base consistent changes in urinary excretion are seen as urinary pH is varied. Beckett et al (17,18) describe a method involving the use of an analog computer for determining a quantitative relationship between measured urinary pH and renal tubular reabsorption. Their method is based on the use of drug excretion versus time profiles under normal conditions and of absorption, metabolism and excretion rate constants, determined under conditions of controlled urinary pH, i.e., acidic urine for basic drugs. Using this method a mathematical relationship between percentage excretion of amphetamine and urinary pH was established and the relationship was used to predict the quantitative excretion of the drug with fluctuations in urinary pH. In studies in this laboratory, Melmon, Benet, Morris, and Brater (19) proposed a method for quantitatively predicting urinary excretion of pH sensitive drugs using equations described by Wagner and Sedman (20) for the excretion rate and renal clearance of drugs which are passively reabsorbed in the kidney tubule. This method involves determining renal clearances of drugs under controlled minimum, maximum and intermediate urinary pH values and developing predictive models from the resulting renal clearance vs pH profiles.

3. Carrier-mediated transport across the renal tubule

Carrier-mediated transport is a term used to describe transfer across a biological membrane which is at a higher rate than could be attributed to diffusion alone and which shows many of the

properties of enzyme kinetics (21). In the kidney this transport has been thought to involve binding of the solute or drug molecule to a component (carrier protein) of the renal tubular membrane and translocation of this carrier-solute complex from one side of the membrane to the other where it dissociates and discharges the solute molecule (4,12,22). Current studies show that while this model is essentially correct, the actual transport across the tubular membrane is complex and although the overall process shows carrier-mediated transport characteristics, one or more mediated and diffusion processes may be involved (23,24).

Two types of carrier-mediated transport can be distinguished. Facilitated diffusion is the term applied to a carrier mediated transport system that operates along a concentration gradient of the permeating solute. At equilibrium the solute attains the same concentration on either side of the membrane as in simple diffusion. No apparent energy expenditure is involved. There has been no clear demonstration of facilitated diffusion playing an important role in renal transmembrane movement of drug molecules (12). The term active transport is generally applied only to those systems in which a substance is transported across a biological membrane against a concentration gradient at the expense of energy derived from the metabolism of the cell. Active transport processes are important in the membrane permeation of drug molecules and figure prominantly in the renal excretion of many drugs and their metabolites (9,12). However, since it is often difficult to experimentally distinguish between facilitated diffusion and active transport, the general term,

carrier-mediated transport, is appropriate to distinguish simple diffusion from those renal tubular processes (reabsorptive or secretive) having the characteristics of 1.) susceptibility to interference by metabolic or competitive inhibitors and, 2.) a maximal capacity of the transporting mechanism (4,10). Specific examples of carrier-mediated transport of drugs will be presented in a later section.

It is possible for a given compound to be subject to all of these renal excretion processes, the direction of net transfer depending on the prevailing conditions. As noted previously, however, passive blood to urine transport (simple diffusion) across the renal tubule is improbable. A large number of organic acids and bases are thought to be excreted by a three-component mechanism: glomerular filtration, active secretion and passive reabsorption (25,26). An expression for the excretion rate of a drug which is excreted by this three-component mechanism has been described by Wagner and Sedman (20):

$$\frac{dAu}{dt} = \sigma k_1 V_d C_p + \frac{T_M C_p}{K_M + C_p} + K_{app} V_T C_u \qquad \text{(eq. 1)}$$

where dAu/dt is the excretion rate of the drug (mass/time), σ is the fraction of drug in the plasma at the total concentration (C_p) which is non-protein bound, k_1 is a first-order rate constant for glomerular filtration ($time^{-1}$), V_d is the appropriate volume of distribution for glomerular filtration (equivalent to the central compartment volume in a pharmacokinetic model), T_M is the transport maximum (mass/time), K_M is the "Michaelis constant" of the transport mechanism (mass/

volume), and K_{app} is a derived permeability constant. This permeability constant includes a rate constant for transport of the diffusing species out of the membrane, the fraction of total drug in the tubular lumen which is undissociated, and the intrinsic partition coefficient of the diffusing species between the membrane and lumenal fluid. V_T is the effective volume of tubule fluid from which reabsorption occurs, and C_u is the concentration of drug in the urine.

The amount of a substance excreted by the kidney is equal to the amount filtered by the glomerulus plus the net amount transferred by the tubules. The renal clearance of a substance is defined as the number of milliliters of plasma completely cleared of that substance by the kidney in one minute of time (3). An expression for renal clearance can be obtained by dividing the excretion rate equation by total plasma concentration. Observed renal clearance essentially consists of two components - glomerular filtration and net tubular transport. If a particular compound is not bound to macromolecules in the plasma its renal clearance will: equal glomular filtration (GFR) if there is no net tubular secretion or reabsorption; exceed GFR if there is net tubular secretion; be less than GFR if there is net tubular reabsorption. Various techniques have been used to further separate the tubular component of excretion into reabsorptive, secretive, passive or carrier-mediated processes.

B. Methods for Studying Renal Excretion

A number of techniques have been employed for studying renal processes both in vitro and in vivo. In vitro techniques used to examine renal tubular transport mechanisms include renal slices, suspensions of renal tubules, perfused isolated renal tubules and perfused isolated kidneys (27-31). In vivo methods for studying renal excretion processes include clearance techniques, the stop-flow technique, micropuncture and microperfusion (32-36). Clearance techniques have the advantage of being technically easy, enabling one to quantify the function of the kidney as a whole, and being applicable to man. The limitations of clearance techniques include the inability to separate reabsorption from secretion for substances that undergo both processes and the inability to define transport mechanisms or localize function to specific nephron segments (32). Stop-flow, micropuncture and microperfusion techniques enable localization and quantitation of transport processes in the nephron and provide information on mechanisms of transport. However, these methods are technically difficult, require considerable alteration of physiological state and are not applicable to man (33,35).

The most frequent use of clearance techniques is in the estimation of glomerular filtration rate. Knowledge of the rate of filtration is important in evaluating renal function and in interpreting the mechanism by which any substance is excreted (32). Methods for estimating GFR require a reference material that is freely filterable at the glomerulus and is neither reabsorbed nor secreted in the tubules. In addition the substance should be physiologically

inert and nontoxic, neither destroyed, synthesized or stored within the kidney and should have a constant clearance over a wide range of plasma concentrations (3,32,37). Inulin (a fructose polymer of molecular weight 5,200) apparently meets all criteria and is a valid index of GFR. Clearances are usually measured during constant intravenous infusion of inulin. Urine is collected over a specified period of time and a plasma sample is taken at an appropriate time during the urine collection period. Most investigators use the midpoint plasma concentration for clearance calculations (32). Other materials which have been successfully used as indices of GFR include polyfructosan, a synthetic fructose polymer, creatinine (a valid measure of GFR in those species in which there is no tubular secretion of this compound), and contrast media such as iothalamate-^{131}I which have clearances close enough to inulin to be acceptable as clinical measures of GFR (32). When using clearance techniques to study the renal excretion of a compound an appropriate marker for GFR is infused simultaneously with this compound. Blood and urine samples are collected and analyzed for the reference material and the compound being studied; renal clearances of each are then calculated. Useful information can be obtained from comparison of these clearances. When the ratio of the clearance of the compound being studied to that of the reference is greater than one, net secretion is indicated; when the ratio is less than one (after correcting for plasma protein binding) net reabsorption is indicated. A change in the ratio with a change in urinary pH implies some passive transport as does a change in ratio with changing urinary flow rates. A change in the ratio with increasing plasma

concentration of the study compound, or in the presence of a competitive or metabolic inhibitor of transport indicates the presence of a carrier-mediated transport process.

C. Carrier-mediated Secretion of Organic Acids and Bases

Using a number of the in vitro and in vivo techniques described above, the renal transport of many solutes has been studied. Although there is evidence that some organic ions undergo simultaneous active bidirectional transport i.e. carrier-mediated secretion and reabsorption (10,34,38-40), the predominant transport of most organic ions in the renal tubule is in the secretory direction (39). The existence of renal tubule transport systems for the secretion of organic acids and bases has been well documented. These systems appear to be separate and independent mechanisms which are not subject to inhibition by the same competitive inhibitors (6,9,10,39, 41).

1. Organic anion (acid) transport system

Phenol red was the first anion for which tubular secretion was demonstrated (10, 41). It was shown in the dog that the total rate of excretion of this compound was composed of a linear element and a saturable element with a defined maximal rate of secretion (41,42). Although in recent years PAH (p-aminohippurate) has been shown to undergo bidirectional transport (10,30,40), it was originally thought to be handled by the kidney entirely by filtration and secretion (10) and throughout the years has been used as the

prototype for the acid secretory system (10,40,43). Consequently the organic acid system is often referred to as the hippurate or p-aminohippurate system (26). Secretion of PAH was found to occur in the proximal convoluted and proximal pars recta portions of the renal tubule, with the pars recta being the major site of net secretion (29,30). A wide variety of organic acids have been shown to be actively transported by the proximal tubule. Table I is a partial list of these acids as taken from Cafruny (44) and Prescott (45).

Table I. Acids Secreted by Active Tubular Transport

p-aminohippurate	salicyclic acid	probenecid
phenylbutazone	indomethacin	phenol red
diodrast	penicillin	sulphonamides
chlorothiazide	acetazolamide	chlorpropamide
methotrexate	furosemide	ethacrynic acid
cephaloridine	spironolactone	oxalic acid
glucuronides	etheral sulfates	hydrochlorothiazide

The diversity of chemical structures in this group of transported acids makes definition of structural requirements for the acid system difficult although attempts have been made to do so (9, 10, 43,44). Probenecid was synthesized in the early 1950's as an inhibitor of renal tubular transport mechanisms. It was found to inhibit the renal tubular secretion of the penicillins, PAH and phenol red and was thought to act by inhibiting an enzyme that requires a source of high phosphate bond energy for the completion of one reaction involved in secretion (46). It was subsequently shown that probenecid itself is secreted by the organic acid mechanism and it was postulated that it inhibits secretion of other organic acids by competing for that secretion (10). Probenecid has been used as the

classical inhibitor of the acid transport system and its ability to inhibit secretion of a compound is taken as evidence for secretion of that compound via the acid transport system. In addition to the fact that the secretion of a wide variety or organic acids exhibits saturation and inhibition characteristics, studies in newborn animals indicate that the carrier can also be induced when the animal is pretreated with a substance known to be transported by that carrier (47). All of these observations support the hypothesis that the secretion of organic acids is mediated by protein or a protein-like carrier (22). Because of the large structural differences in the acids shown to be secreted and the failure of the active secretion of some organic acids such as oxalate to be inhibited by probenecid (10,40) the term "organic acid secretory system" may actually encompass more than one transport system.

2. Organic cation (base) transport system

The secretion of organic bases was demonstrated in the late 1940's. Initial work was done by Rennick et al (48) who demonstrated the tubular secretion of tetraethylammonium ion, and Sperber (49) who developed the Sperber chicken preparation for looking at active tubular secretion. Sperber used the chicken to demonstrate secretion of such bases as guanidine, N^1-methylnicotinamide, piperidine and methylguanidine (41). According to Weiner (10), organic bases are generally much more "pharmacologically active" than organic acids. This places restrictions on the number and type of compounds that can be studied using in vivo clearance experiments. It is often difficult to reach plasma concentrations sufficient to demonstrate

saturation of the system without reaching toxic levels and, consequently, a good deal of the work involving secretion of organic bases has been done using *in vitro* preparations and in the Sperber chicken preparation. Table II, taken from Weiner (10), lists some bases which are actively secreted by the renal tubule. Of the bases listed, few are of clinical significance today, although many currently therapeutically useful drugs are organic bases which are likely to be secreted by the renal tubule via a carrier-mediated process.

Table II. Bases Secreted by Active Tubular Transport

choline	morphine	guanidine
dihydromorphine	neostigmine	hexamethonium
dopamine	pempidine	histamine
hydrazine	mecamylamine	mepiperphenidol
methylguanidine	N^1-methylnicotinamide	monomethylhydrazine
piperidine	procaine	quinine
serotonin	tetrabutylammonium	tetraethylammonium
thiamine	tolazoline	

It is interesting to note that all bases for which secretion has been demonstrated are nitrogen-containing compounds. There is some doubt, however, concerning the requirement of the nitrogen atom for transport by the base system. Smith *et al* (50) demonstrated inhibition of the renal tubular transport of tetraethylammonium and N^1-methylnicotinamide by non-nitrogenous onium compounds. If the inhibition is competitive, it is probable that the positive charge on the molecule and not the nitrogen atom is the requirement for the base transport system (10,50). Stop-flow analysis of mepiperphenidol and mecamylamine in the dog showed that these organic bases are secreted in the proximal part of the nephron (51). As will be discussed

later, mepiperphenidol has been shown to inhibit the transport of other organic bases and thus, it is probable that the location for secretion of organic bases, in general, is the proximal tubule which is analogous to the system for acid transport.

N^1-methylnicotinamide is the classic example of an organic base which is actively transported by the renal tubule. It was one of the earliest bases for which secretion was demonstrated and it has been used throughout the years to aid in defining the characteristics of the organic cation transport system. N^1-methylnicotinamide (NMN) is a quaternary ammonium compound, ionized at all pH's. It is the principle metabolic product of nicotinic acid (52). Sperber (49) first observed that NMN appeared to be secreted by the kidney of the chicken. This observation prompted Beyer et al (53) to evaluate the excretory components of NMN elimination in the mammalian kidney. Clearance experiments in female dogs were used in these studies. The results were as follows: 1.) As the plasma concentration of NMN was elevated, its renal clearance decreased to approach that of glomerular filtration rate (creatinine) as a limit; 2.) NMN had no effect on the renal clearance and tubular secretory capacity for p-aminohippurate; 3.) PAH did not inhibit the clearance or the clearance ratio of NMN/creatinine; 4.) Carinamide did not inhibit the clearance of NMN when administered under the same conditions in which this compound completely suppressed the tubular secretion of penicillin and markedly depressed the clearance of PAH. The authors concluded that ". . . the renal elimination of N^1-methylnicotinamide is brought about, in addition to glomerular filtration, by a tubular

secretory transport mechanism that is distinct, functionally, from that responsible for the secretion of hippurates, penicillins, and certain pyridones."

Beyer et al (54) next decided to explore the role of renal tubular secretion in the "physiological economy" of mepiperphenidol, a visceral anticholinergic compound which is also a quaternary ammonium compound. The results showed that the renal clearance of mepiperphenidol markedly exceeded glomerular filtration rate (creatinine clearance in the dog) at low plasma concentrations; when PAH was co-administered with mepiperphenidol there was no depression of mepiperphenidol clearance or clearance ratio; probenecid did not decrease mepiperphenidol clearance at plasma concentrations that profoundly depressed the secretion of PAH and penicillin-G. In in vitro studies Beyer et al (54) found no depression of the renal secretion of mepiperphenidol by NMN and suggested that mepiperphenidol and NMN were secreted by two different mechanisms. LeSher and Shideman (55) were also unable to demonstrate inhibition of NMN with the accumulation of mepiperphenidol in rabbit kidney slices and they too suggested that mepiperphenidol was secreted by a mechanism different from that of NMN. It was later shown by other workers (56), however, that when NMN and mepiperphenidol were simultaneously infused into dogs, mepiperphenidol was a potent competitive inhibitor of NMN transport. It is probable therefore that NMN and mepiperphenidol are secreted by the same transport mechanism and that failure to demonstrate in vitro inhibition of mepiperphenidol secretion by NMN is due to differences in the affinity of the compounds for the

carrier-protein involved in the transport process. Mepiperphenidol was also shown to be excreted by the renal tubules of the chicken (57), and its inhibition of NMN transport was demonstrated in this species.

NMN transport in the chicken was also shown to be inhibited by mecamylamine, quinine, quinidine, quinacrine (58), cyanine #863 and a number of bisquaternary compounds (59). Since the renal excretion of mecamylamine, quinine, and quinacrine show considerable dependence on urinary pH, it was originally thought that these compounds (and all weak bases or amines in general) were secreted by passive, nonionic diffusion (8,25). However, in addition to inhibition of NMN transport in the chicken by mecamylamine and quinine (58), Smith et al (60) showed that quinine and mecamylamine also inhibit tubular excretion of NMN in the dog. They also showed that cyanine #863 and mepiperphenidol decrease renal excretion of mecamylamine in the dog. Pilkington and Keyl (51) demonstrated the secretion of mecamylamine in the proximal tubule and reabsorption in the distal tubule of the dog. These studies not only support the secretion of mecamylamine, quinine and quinacrine by a transport system, but support the concept of at least a three-component excretory system for bases, namely, glomerular filtration, carrier-mediated secretion, and passive reabsorption. Although bidirectional transport has been demonstrated for organic acids, there has as yet been no demonstration of active or carrier-mediated transport in a reabsorptive direction for bases (4).

Until recently, most of the in vivo experiments demonstrating organic base transport have been done in the chicken or in the dog. In 1975, Ross et al (61) studied the renal excretion of NMN in the rat. Renal clearance experiments demonstrated that: 1.) NMN is secreted; 2.) A maximum secretory capacity could be reached; 3.) NMN secretion is inhibited by mepiperphenidol. Free flow micropuncture experiments indicated that NMN is secreted in the proximal tubule and is not secreted or reabsorbed in the distal tubule. When intratubular microinjections of (^{14}C) NMN were made into diuretic animals (diuresis was induced by infusing 2.5% NaCl at rates sufficient to produce urine flows of 0.1-0.2 ml/min) approximately 10% of the NMN injected into early proximal tubules was reabsorbed. Non-diuretic animals (infused with 0.9% NaCl) showed no significant reabsorption of NMN. The authors concluded that NMN transport is a carrier-mediated process and that reabsorption, if it occurs, plays only a minor role.

Secretion of NMN by the chicken kidney has been used to define the energy requirements for the cation transport system. Nechay and Pardee (62) showed that the secretion of NMN is reduced by natriuretic doses of ouabain. Ouabain is a cardiac steroid which inhibits Na^+ plus K^+ dependent adenosine triphosphatase, an enzyme found in the membrane fraction of kidney homogenates. Earlier workers (63) found that 2,4-dinitrophenol, which uncouples oxidative phosphorylation, interferes with NMN uptake in dog renal slices. This influence of ouabain and 2,4-dinitrophenol on the transport of NMN suggests that the energy for transport of NMN (and presumably other bases) is derived from adenosine triphosphate (62).

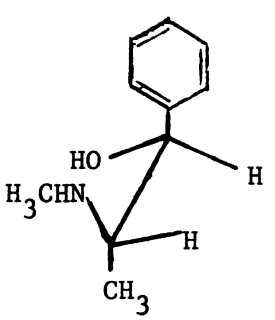
In the past decade, there has been substantial work attempting to identify and isolate the proteins involved in the transport of organic bases. Much of this work has been done using NMN. Ross et al (64) fractionated renal tissue and were able to show that the protein fraction was implicated in or possibly actually contained the renal carrier of NMN. These studies were extended by Magour et al (65) who were able to solubilize and partially purify a carrier-like protein for organic bases from dog kidney. In 1975, Holohan et al (22) measured the binding of NMN and PAH to particulate matter obtained from dog renal cortex tissue. Binding of NMN and PAH was found to be tissue specific. In addition, binding was pH, time, temperature, protein-concentration and ligand-concentration dependent. The studies showed that NMN and PAH binding share many features in common but that the two processes were independent of each other. These in vitro studies along with the foregoing information concerning the organic acid and organic base transport systems clearly indicate that these two major transport systems are analagous, but separate and independent of each other.

Most all the acids and bases studied to date, transported by the renal tubule, appear to be handled exclusively by the anionic or cationic system, respectively. There are, however, a few compounds which are not handled entirely by one system or the other. Catecholamines appear to belong to this class of compounds. Rennick and Pryor (66) showed that the renal transport of norepinephrine is sensitive to both the organic anion transport inhibitor probenecid and the organic cation transport inhibitor cyanine #863. Quebbemann

et al (67) showed that the transport of dopamine, tyramine, phenylethylamine and 3,4-dimethoxyphenylethylamine were inhibited by probenecid and that epinephrine transport was inhibited by quinine as well as by cyanine #863, both inhibitors of organic base transport. Previously it had been shown that epinephrine transport was inhibited by probenecid (68). The most likely explanation of this dual transport mechanism for catecholamine is that there are two species being transported simultaneously, the original amine and acid metabolites formed by the kidney (39).

D. Project Objectives

The overall purpose of this work is to more clearly define the renal mechanisms involved in the excretion of pseudoephedrine. Pseudoephedrine is an organic base, currently used clinically for the treatment of upper respiratory tract disorders. It is frequently used in pediatric practice because of its lack of cardiovascular stimulation. Toxiciy of pseudoephedrine in normal children is infrequently seen and consists of mild central nervous system stimulation and occasional increases in blood pressure in hypertensive individuals (69). Pseudoephedrine is a stereoisomer of ephedrine (d-ψ-ephedrine). The commercially available form is L(+)-pseudoephedrine:



L(+)-pseudoephedrine

Lefler et al (70) report a pKa for ephedrine of 9.58. The pKa for pseudoephedrine has not been reported. Wilkinson and Beckett (71) studied the influence of urinary pH and urine volume output on the absorption, metabolism and excretion of the ephedrines in man. Their results were based solely on urinary measurements. It was shown that both urinary excretion and relative metabolism of the ephedrines was pH dependent. However, this dependency varied, increasing as the amino group was successively methylated. It was postulated that the net excretion of ephedrines is the summation of glomerular filtration, possibly active secretion in the proximal tubule and passive reabsorption in the distal tubule. Although these workers did see variations in urinary excretion of ephedrine with changes in urinary pH leading to postulation of a passive reabsorption, they in fact had no data on which to base the postulate of possible active secretion. Pickup et al (72) studied the pharmacokinetics of ephedrine after oral dosing in asthmatics. In this study, both plasma and urine levels of ephedrine were measured and it was therefore possible to estimate renal clearances from their data. The average estimated renal clearance for six subjects was greater than two times the normal glomerular filtration rate for man (125 ml/min) indicating net secretion of ephedrine for these subjects. Urinary pH for these subjects ranged from 5.3 - 6.4. The influence of urinary pH on the plasma half-life of pseudoephedrine in man and dog was studied (73). When urinary pH was increased to 8.0 in three male volunteers, the plasma half-life of pseudoephedrine was almost double that found in normal subjects. When urinary pH in these same volunteers was decreased to about 5.2, the plasma half-life of pseudoephedrine was decreased.

Similar results were found in the dog. It is thus evident that urinary pH has an effect both on ephedrine and pseudoephedrine, indicating involvement of a passive tubular process (reabsorption) in the renal excretion of these compounds. Based on the fact that ephedrine shows a net renal secretion, it is highly likely that tubular secretion is also involved in the excretion of pseudoephedrine.

Children with renal tubular acidosis and persistent alkalinuria have manifested symptoms of toxic psychoses secondary to administration of pseudoephedrine (74). In view of the observed urinary pH dependent excretion of ephedrines in general and pseudoephedrine in particular, it is highly likely that the toxic effects seen in these children with alkaline urine is due to an increase in the passive reabsorption of pseudoephedrine resulting in increased plasma levels of drug. It is important, however, to look at all the possible mechanisms for renal excretion of pseudoephedrine and consider the ultimate effects of increased reabsorption and increased plasma levels on these processes. It is clinically relevant therefore to elucidate the processes involved in the renal excretion of pseudoephedrine and to characterize these processes with respect to maximum capacity (saturation of) if applicable, competition for the processes and effect of urinary pH on the processes. By necessity this work must be carried out in a convenient laboratory animal with the understanding that the results will not necessarily be extrapolatable to man and should be used only as an indicator of what might be happening in man and as an aid in designing appropriate clinical studies.

The rat has been chosen as the animal model for these studies. There is a broad base of information available on important renal physiological parameters in the rat; the rat has been successfully used to demonstrate the secretion of organic bases (61); the rat is an inexpensive model and therefore many animals can be used to generate a large data base.

Specific Objectives

1.) To develop appropriate surgical and experimental techniques for studying the renal excretion of pseudoephedrine in the rat;

2.) To validate the techniques developed by reproducing the results of Ross *et al* (61) showing secretion of N^1-methylnicotinamide, saturation of this secretion, and inhibition of the secretion with mepiperphenidol;

3.) To modify existing assays for pseudoephedrine in plasma and urine samples to accomodate the small sample volumes of blood drawn from the rat;

4.) To elucidate the pharmacokinetics of pseudoephedrine in the rat following intravenous administration of pseudoephedrine hydrochloride;

5.) To determine the direction of net excretion of pseudoephedrine in the rat, specifically looking for the presence of secretion;

6.) To determine the effect of increasing plasma concentrations of pseudoephedrine on the net excretion; attempt to saturate any possible carrier-mediated processes;

7.) To determine the effect of a classical competitive

inhibitor of the organic base transport system, such as mepiperphenidol, on the excretion of pseudoephedrine;

8.) To examine the effect of other organic bases which might clinically be administered along with pseudoephedrine and for which competition for transport processes is possible;

9.) To examine the effect of urinary pH on the net excretion of pseudoephedrine.

CHAPTER 2

EXPERIMENTAL

A. Animal Set-up

Various surgical techniques have been employed for continuously following renal clearance in the rat. Harvey and Malvin (75) cannulated the carotid artery or femoral artery for the collection of blood samples and the jugular or femoral vein for the infusion of test solution; the ureter was catheterized for urine collection. Kau et al (76) cannulated the femoral vein and artery and collected urine by means of a plastic tube fixed below the penis of the rat. Ross et al (61) collected blood samples from the carotid artery and catheterized the bladder for urine collection in their studies on the renal excretion of N^1-methylnicotinamide in the rat. The following surgical preparation is a modification of these techniques. [Subsequent to the development of the preparation detailed below, Brennan et al (77) described essentially the same set-up for studying the effect of dopamine and saluretics on glomerular filtration rate in the rat.]

1. Surgical preparation

Male Sprague-Dawley rats weighing 220 gm to 330 gm were used. The animals were allowed food (Purina Rat Chow, Ralston Purina Company, St. Louis, Missouri) and water ad libitum until the time of experimentation. In preparation for surgery, the animals were anesthetized with sodium pentobarbital (Nembutal^R Sodium for veterinary use, 60 mg/ml; Abbott Laboratories, North Chicago, Illinois) 48 mg/Kg

intraperitoneally.

a. vascular catheterization

i. materials

Polyethylene tubing (Intramedic^{R} Non-Radiopaque Polyethylene Tubing, PE-50, I. D. 0.023", O. D. 0.038"; Clay Adams, Parsippany, New Jersey); disposable syringes (Monoject Sterile Disposable Syringe, 6 cc; Sherwood Medical Industries Inc., Deland, Florida); disposable needles (Monoject Disposable Hypodermic Needles 23 gauge, 1 inch; Sherwood Medical Industries Inc., Deland, Florida); three-way stopcock (Pharmaseal, Inc., Toa Alta, Puerto Rico); 4-0 cotton suture (Ethicon Inc., Somerville, New Jersey); sodium heparin injection (Liquaemin^{R} Sodium 1,000 USP units/ml; Organon, West Orange, New Jersey); normal saline solution (0.9% sodium chloride prepared extemporaneously).

ii. methods

Following induction of anesthesia, the rats were securely fastened to a temperature-controlled small animal operating table. Vascular catheters were prepared as follows: Approximately 0.5 ml of sodium heparin solution was drawn into each of two 6 cc syringes equipped with 23 gauge needles; the syringes were "rinsed" several times with the heparin and the excess heparin solution expelled; the syringes were then filled with normal saline; the needle of the venous cannula was placed directly on the hub of the syringe and a three-way stopcock was attached to the arterial syringe. From 12 to 15 inches of PE 50 tubing was fitted directly onto the needle of each syringe; the end of the tubing was beveled to facilitate insertion into the vessel and the tubing was flushed with the solution contained in the syringes (tubing was thus "heparinized" and clotting of blood in the tubing during the

experimental run prevented). A small right groin incision (1.5 - 2 cm) was made with scissors in the skin only; the femoral vein and artery were exposed by rending the fat with forceps to minimize bleeding. The remaining fascia was then carefully removed from the vessels with curved blunt forceps and the vessels separated. Venous cannulation: A ligature was tied tightly around the distal end of the exposed vein; the ends of the ligature were taped firmly to the surgical table, keeping the vessel taut. A second ligature was positioned at the proximal end of the vessel. A small cut was made in the vein with iris-scissors between the two ligatures and the catheter introduced into this opening and eased into the vein until it was approximately one centimeter past the proximal ligature. Both ligatures were then tied firmly around the vessel and the catheter. Arterial cannulation: Ligatures were placed at the distal and proximal ends of the artery as with the vein. In addition, the artery was clamped off just beyond the proximal ligature with a small artery forceps. Before cannulating, the stopcock on the syringe was placed in the closed position to create a back pressure to counteract the arterial blood pressure. As with the vein, a small incision was made in the vessel, the catheter inserted and moved into the vessel past the proximal ligature to the artery forceps. At this point the artery forceps was removed, the catheter eased another 0.5 cm into the artery and both ligatures tied securely around the artery and catheter. The exposed vessels were then covered with a saline-soaked gauze pad.

b. bladder cannulation

The procedure for cannulating the bladder is a modification of that

used by Kates (78) in his Ph.D. dissertation on the inhibition of biliary secretion of methotrexate.

i. materials

Polyethylene tubing (Intramedic^{R} Non-Radiopaque Polyethylene Tubing, PE 90, I. D. 0.034", O. D. 0.050"; Clay Adams, Parsippany, New Jersey); 1/2 circle taper point needle (Ferguson Needle, 1/2 circle, taper point; The Torrington Co., Torrington, Connecticut); disposable syringe (Monoject Sterile Disposable Tuberculin Syringe, 1 cc; Sherwood Medical Industries Inc., Deland, Florida); disposable needle (Monoject Disposable Hypodermic Needle, 25 gauge, 5/8"; Sherwood Medical Industries Inc., Deland, Florida).

ii. methods

A midline incision was made in the abdomen of the rat and the bladder exposed. Prior to cannulation, the bladder was drained of urine using a 1 cc disposable syringe with a 25 gauge needle. A purse-string suture was made approximately 0.5 cm from the apex of the bladder using a 1/2 circle taper point needle and 4-0 cotton suture. A small cut was made in the center of the sutured area with iris-scissors. A 10-cm section of PE 90 tubing, flanged at one end, was inserted in the bladder and the suture pulled tight and tied. The flange in the tubing helped to prevent the cannula from slipping out of the bladder. The bladder was covered with a gauze pad kept moist with saline.

c. tracheotomy

A tracheotomy was routinely performed to insure a patent airway throughout the duration of the experiment. An incision was made in the skin of the neck with scissors; the fascia was separated with

a mosquite clamp and the trachea exposed. A small incision was made in the trachea and a 6-cm piece of PE 205 tubing (Intramedic^{R} Non-Radiopaque Polyethylene Tubing, PE 205, I. D. 0.062", O. D. 0.082"; Clay Adams, Parsippany, New Jersey) inserted and tied in place with cotton suture.

2. Maintenance

The rat was kept on the operating table throughout the experiment. Body temperature was monitored by means of a rectal probe (YSI Model 402 Small Flexible Vinyl Rectal Probe, Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio) connected to a temperature control unit (YSI Model 73ATD Indicating Controller, Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio). This unit was also connected to the heating element of the operating table. In this manner, the body temperature of the rat was maintained at 37°$\pm$ 0.5°C throughout the experiment. The rat was kept anesthetized by injecting 0.1 ml (6mg) sodium pentobarbital intraperitoneally every hour or as needed. Blood pressure of the animal was monitored periodically during some experiments with a mercury manometer which was constructed to facilitate direct hook-up with the femoral artery cannula. Immediately upon completion of the experiment the animal was sacrificed by injection of 0.4 ml euthanasia solution (Somlethol^{R}, sodium pentobarbital, 6 gr/ml in alcohol-propylene glycol base with water; Med. Tech. Inc., Elwood, Kansas) in the venous catheter. Figure 1 is a schematic representation of the animal preparation, figure 2 is a photograph of the entire set-up.

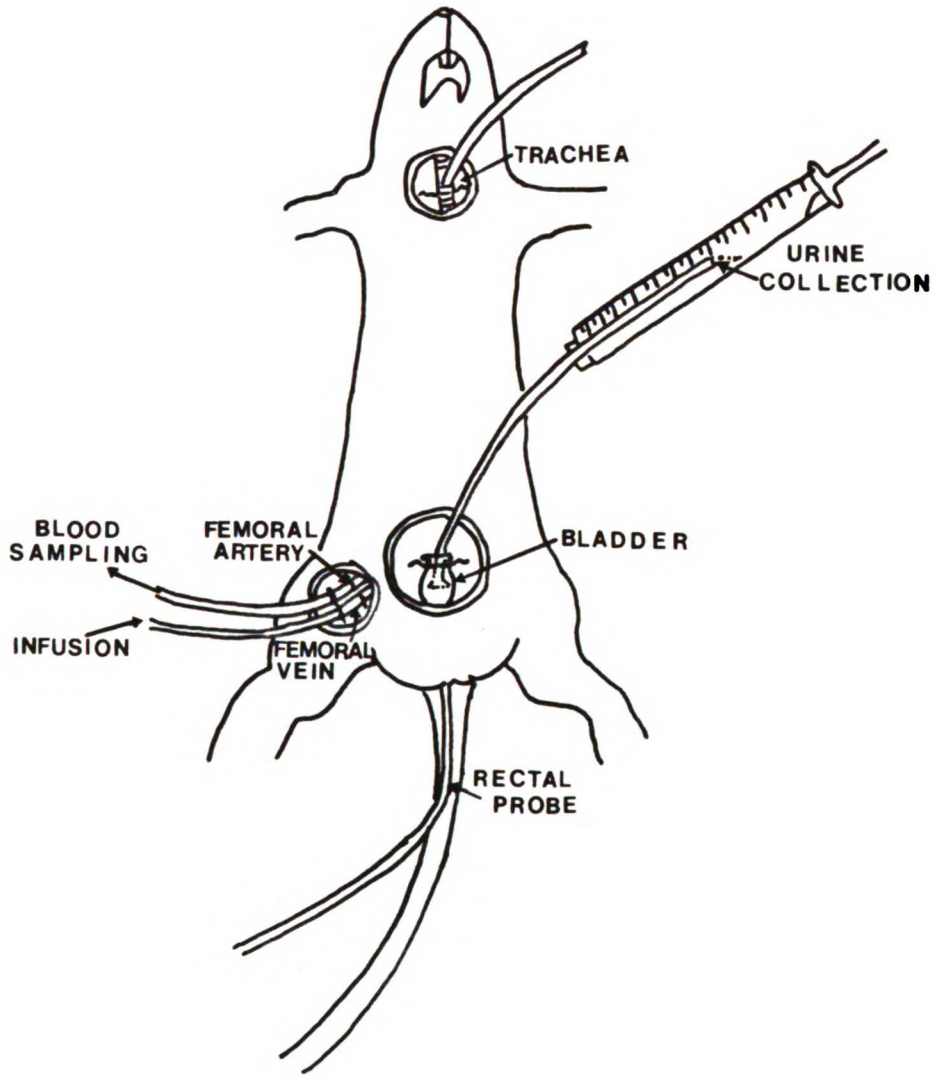


Figure 1. Schematic representation of rat preparation.

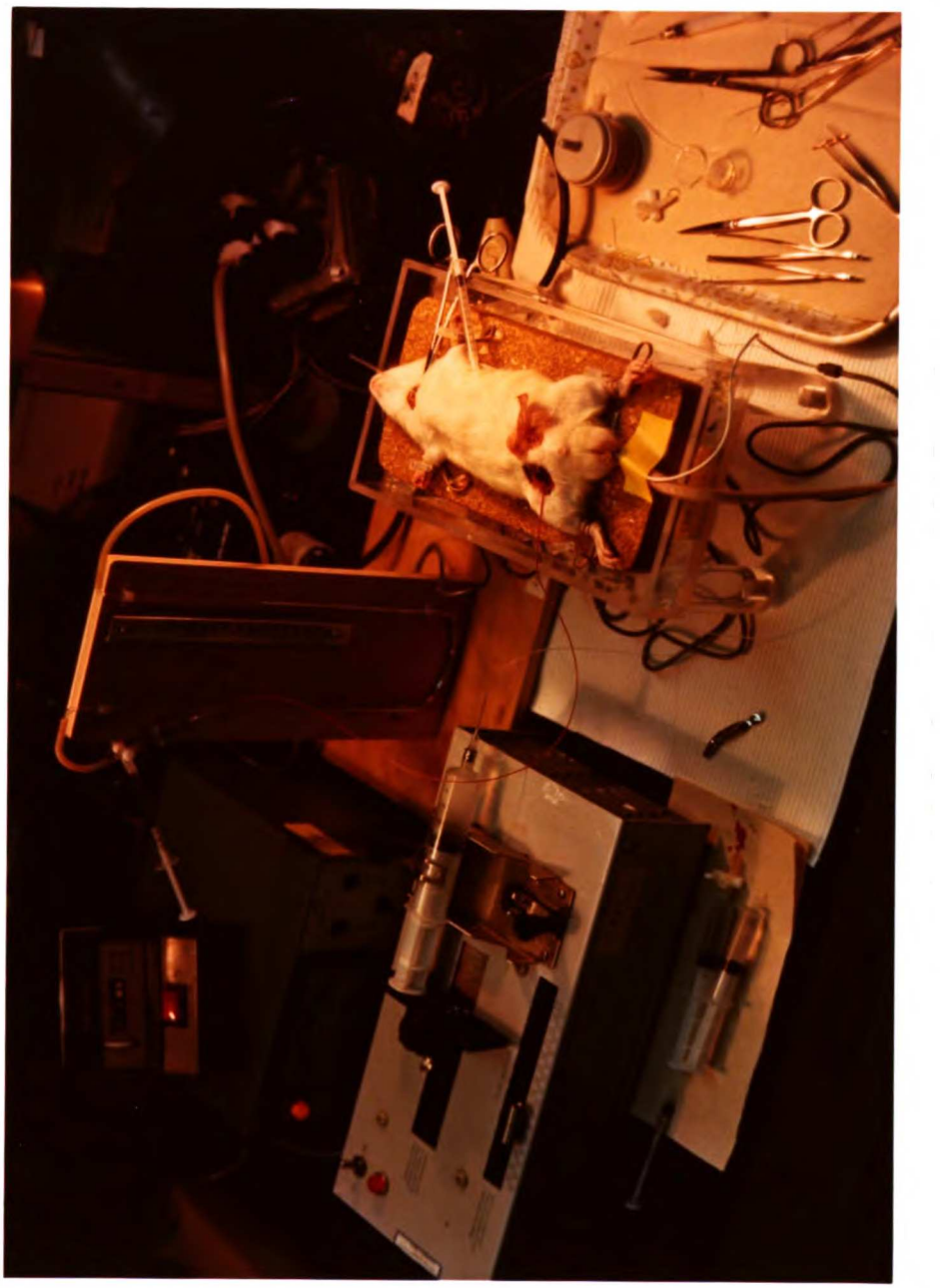


Figure 2. Photograph of experimental set-up.

B. General Procedures

The general procedures of Ross et al (61) were followed for the initial clearance experiments, saturation experiments and inhibition experiments.

1. Clearance experiments

a. materials

Mannitol (Mannitol N. F., powder; Mallinckrodt Chemical Works, St. Louis, Missouri); inulin (Inulin from Dahlia Tubers; Sigma Chemical Company, St. Louis Missouri); polyethylene micro test tubes, 0.5 ml and 1.5 ml (Bolab Incorporated, Derry, New Hampsire); Harvard Apparatus Compact Infusion Pump (Harvard Apparatus, Millis, Massachusetts); Beckman Spinco 150 Microfuge (Beckman Instruments, Inc., Fullerton, California); Beckman Research pH Meter (Beckman Instruments, Inc., Fullerton, California); Miramark^{R} Combination electrode (Markson Science Inc., Del Mar, California).

b. methods

Immediately following surgery, blank blood (0.4 ml) and urine samples were taken. An intravenous priming dose of a 0.9% sodium chloride solution containing 5% mannitol, 4% inulin and the desired concentration of drug to be studied was administered via the femoral vein. After administration of the priming dose, the animals were continuously infused with the same solution (drug concentration differing in some cases) at a rate of 50 µl/min throughout the experiment using a Harvard Apparatus Compact Infusion Pump. The 5% mannitol was included in the solution to insure adequate urine flow and to maintain the pH of the urine in an acid range for the pseudoephedrine studies. Inulin

was included as the marker for glomerular filtration rate. The animals were equilibrated for 90 minutes to insure the attainment of steady-state plasma levels of both inulin and drug. Urine collected during this period was discarded. Following equilibration, clearance was measured during four 20-minute periods. Urine was collected in a 1 cc disposable syringe which enabled accurate measurement of the volume and also limited exposure to air. Urine was collected during the 90-110 min, 110-130 min, 130-150 min, and 150-170 min periods following the start of infusion. Blood samples of 0.3 - 0.4 ml were collected from the femoral artery at the midpoint of each urine collection i.e., 100 min, 120 min, 140 min, and 160 min. Blood samples were collected directly in heparinized 0.5 ml polyethylene micro test tubes, immediately centrifuged in a Beckman Spinco 150 Microfuge and the plasma removed and frozen. The urine was transferred from the syringe to a 1.5 ml polyethylene micro test tube and the pH immediately measured using a Beckman Research pH meter with a Miramark^{R} combination electrode. Appropriate aliquots of the urine were taken and diluted for the drug and inulin assays and the remainder frozen.

2. Saturation experiments

Saturation experiments were run exactly as the clearance experiments. The concentration of the drug administered was varied between rats such that a wide range of steady-state plasma levels was attained. In this manner, the relationship between renal clearance of drug and plasma concentration was studied.

3. Inhibition experiments

The inhibition experiments were run in a manner similar to clearance experiments. After the second clearance period (110-130 min) a bolus of the inhibitor (5 mg/Kg) in 0.9% sodium chloride was administered. The animals were then equilibrated for 10 minutes or one hour and samples were collected during two more 20-minute clearance periods i.e. either 140-160 min and 160-180 min or 190-210 min and 210-230 min. Figure 3 is a graphical summary of the procedures for clearance, saturation and inhibition experiments.

4. Inulin assay

Ross et al (61) used a fluorometric method developed by Vurek and Pegram (79) for the determination of inulin in plasma and urine samples. This method was developed for nanogram quantities of inulin that might be expected in micropuncture studies. The method is involved and requires special apparatus. Davidson and Sackner (80) determined inulin in plasma and urine samples using a modification of the classical anthrone method. Their method requires 2 ml of plasma. Dirks et al (81) describe a modified anthrone method for the microanalysis of inulin in 0.028 µl of tubule fluid. The following method for inulin determination is an anthrone assay similar to those described above, but is modified to accomodate plasma volumes of 75 µl.

a. materials

Anthrone (Anthrone 'Baker Analyzed' Reagent; J. T. Baker Chemical Co., Phillipsburg, New Jersey); sulfuric acid (Sulfuric Acid, Analytical Reagent; Mallinckrodt Inc., Paris, Kentucky); trichloroacetic acid (Trichloroacetic Acid Practical; Matheson Coleman and Bell, Norwood,

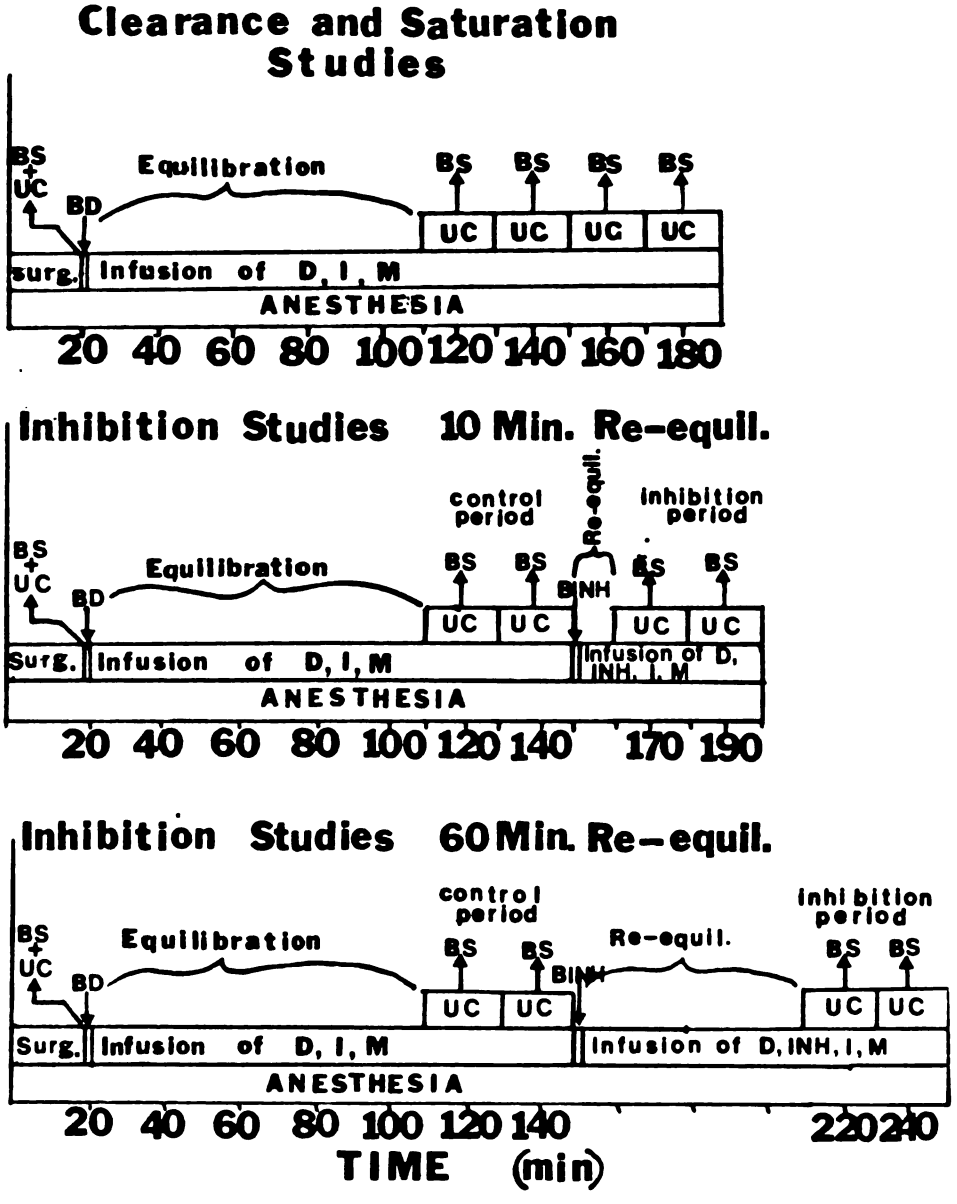


Figure 3. Graphical representation of experimental procedures. Surg = surgery; D = drug; I = inulin; M = mannitol; INH = inhibitor; BD = bolus drug; BINH = bolus inhibitor; BS = blood sample; UC = urine collection.

Ohio); Beckman DB Spectrophotometer (Beckman Scientific and Process Instruments Division, Fullerton, California); Cary 15 Recording Spectrophotometer (Cary Instruments, Monrovia, California).

b. methods

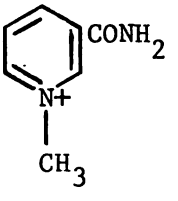
Preparation of reagents: 1.) A 9.3% solution of trichloroacetic acid in water was prepared fresh on the day of assay. 2.) Anthrone reagent: 70 ml of concentrated sulfuric acid, reagent grade, was slowly added to 30 ml of water. The mixture was allowed to cool, and then 0.2 gm of anthrone was dissolved in it. The reagent was prepared no longer than 24 hours before use and stored in the refrigerator. 3.) Inulin standard solution: A stock solution of inulin, 100 mg per cent, in water was prepared with gentle heating on a water bath. Dilutions of this solution (1, 2, 4, 8, 16, 20 mg %) were prepared as standards for the inulin standard curve.

Assay procedure: Protein-free plasma filtrates were prepared by adding 1.5 ml water and 0.75 ml of the 9.3% trichloroacetic acid solution to 0.075 ml plasma. The mixture was vortexed (Vari-Whirl Mixer; VWR Scientific, San Francisco, California) for a few seconds, centrifuged, and 0.2 ml of the protein-free supernatant was transferred to a disposable culture tube. Urine samples were diluted 1 - 500 at time of collection; 0.2 ml of the diluted sample was transferred to a culture tube. Each of the standard inulin solutions (0.2 ml) and a water blank were placed into clean culture tubes. To each of the plasma, urine and standard solutions was added 2 ml anthrone reagent. The samples were mixed rapidly with a vortex mixer and incubated in a water bath at 37°C for 50 minutes. Absorbance of the samples was

determined in cuvettes on either a Beckman DB spectrophotometer or on a Cary 15 Recording spectrophotometer at 620 nm.

Possible interference of mannitol: Davidson and Sackner (80) found that a plasma or urine mannitol level of 100 mg per cent depresses average inulin recoveries to 85 per cent and that a mannitol level of 50 mg per cent depresses average inulin recoveries to 94 per cent. An experiment was performed to determine if infusing 5% mannitol would yield levels of mannitol in plasma and urine sufficient to depress inulin recovery. Plasma and urine samples were taken from a rat that had been infused with 5% mannitol in 0.9% sodium chloride for 90 minutes and from an untreated rat. Protein-free plasma filtrates were prepared by adding 0.75 ml water and 0.350 ml 9.3% trichloroacetic acid to 0.075 ml plasma, shaking and centrifuging. To 0.1 ml of the filtrate was added 0.1 ml of an inulin standard solution. Urine samples were diluted 1 - 250 and 0.1 ml of this dilution was added to 0.1 ml of inulin standard. Samples were then assayed as described above and the results of samples containing mannitol compared with those not containing mannitol.

C. N^1-methylnicotinamide (NMN) Excretion Studies



N^1-methylnicotinamide

1. NMN assay

Huff (82) showed that when N^1-methylnicotinamide was treated with acetone and alkali in the cold, followed by heating in the presence of acid, a highly fluorescent and stable compound was formed. Huff and Perlzweig (83) used this observation to develop a fluorometric method for the determination of NMN in urine. Peters et al (84) modified the procedure of Huff and Perlzweig and adapted it for determination of plasma NMN as well as urine NMN. The modified procedure of Peters et al was used by Ross et al (61) in their studies with NMN in the rat. The assay procedures for the present work were essentially those employed by Peters et al (84) with appropriate modifications to accomodate sample volume differences.

a. materials

N^1-methylnicotinamide chloride (Sigma Chemical Company, St. Louis, Missouri); sodium hydroxide (Sodium Hydroxide USP Pellets; Mallinckrodt, Inc., St. Louis, Missouri); potassium phospate, monobasic ('Baker Analyzed' Reagent; J. T. Baker Chemical Co., Phillipsburg, New Jersey); hydrochloric acid, analytical reagent (Mallinckrodt Inc., St. Louis, Missouri); manganous chloride, 4-hydrate ('Baker Analyzed' Reagent; J. T. Baker Chemical Co., Phillipsburg, New Jersey); methylethyl ketone ('Photrex'^{TM}Reagent; J. T. Baker Chemical Co., Phillipsburg, New Jersey); Perkin-Elmer Model 203 Spectrofluorometer (Perkin-Elmer, Norwalk, Connecticut).

b. methods

Preparation of reagents: 1.) Sodium hydroxide, saturated solution: 85 gm of NaOH USP pellets in 100 ml water; stir on magnetic stirrer several hours; allow undissolved NaOH to settle. 2.) Potassium

phosphate, monobasic (KH_2PO_4): 20% solution in water. 3.) Trichloroacetic acid: 5% TCA in water, prepared fresh for each assay. 4.) Hydrochloric acid solutions (HCl): 0.01 N HCl, 0.02 N HCl, 0.5 N HCl. 5.) Manganous chloride ($MnCl_2 \cdot 4H_2O$); 0.005 M solution $MnCl_2$ in water. 6.) $MnCl_2$-NaOH reagent: 2 ml saturated solution NaOH, 36 ml water, 0.18 ml 0.005 M $MnCl_2$ prepared fresh for each assay. 7.) N^1-methylnicotinamide chloride: stock solution containing 152.5 mcg/ml NMN; dilutions of NMN stock solution in concentration range of 0.76 mcg/ml to 114.41 mcg/ml NMN. All solutions were prepared in doubly distilled water. Assay procedure for plasma NMN: 1.) 20 µl of plasma sample (or blank), 10 µl of water (or NMN stock solution) and 10 µl of 0.02 N HCl were put in a 0.5 ml polyethylene micro test tube. 2.) 200 µl 5% TCA was added and the samples were centrifuged in a Beckman Spinco 150 Microfuge. 3.) 20 µl of the supernatant was transferred to a culture tube. 4.) 20 µl of methylethyl ketone was added to the supernatant and the mixture shaken. 5.) 50 µl $MnCl_2$-NaOH reagent was added and the mixture again shaken. 6.) 500 µl 0.5 N HCl was added and mixture shaken a final time. 7.) The tubes were placed in a boiling water bath for 5 minutes and then allowed to cool to room temperature. 8.) 500 µl 20% KH_2PO_4 was added, samples were shaken and read on a Perkin-Elmer Model 203 Spectrofluorometer at an excitation wave length of 360 nm and an emission wavelength of 460 nm.

Assay procedure for urine NMN: 1.) urine samples were diluted 1 - 100 with 0.01 N HCl. 2.) 10 µl of the diluted urine sample (or urine blank) and 10 µl of water (or NMN stock) were placed in a culture tube. The sample was then treated as per plasma assay steps 4 - 8.

2. Clearance experiments

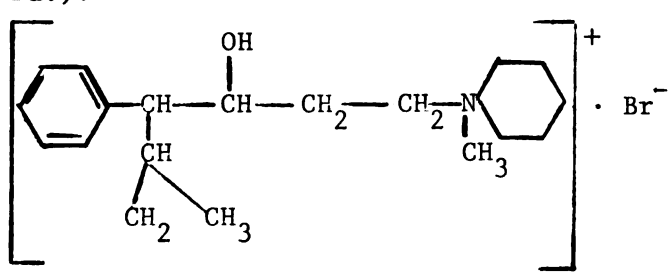
Clearance experiments with NMN were carried out as described in section B, General Procedures. A priming injection and infusion of 0.2% NMN, 4% inulin and 5% mannitol in 0.9% saline was administered to six rats. After a 90 minute equilibration period, four 20-minute clearance periods were followed. Plasma and urine samples were assayed for NMN and inulin.

3. Saturation experiments

In order to demonstrate saturation of NMN renal transport, concentrations of NMN ranging from 0.2 - 1.2% were infused. In addition to the six rats infused with 0.2% in the initial clearance experiments, two more rats were run at 0.2%, 3 at 0.4%, 3 at 0.6%, 1 at 0.8%, 3 at 1.0%, and 4 at 1.2%. Each rat was equilibrated for 90 minutes and then four clearance periods were followed.

4. Inhibition experiments

Competitive inhibition studies were carried out with mepiperphenidol (Darstine^{R}, mepiperphenidol bromide, donated by Pharmaceutical Research and Development, Merck Sharp and Dohme Research Laboratories, West Point, Pa.):



Mepiperphenidol Bromide

Two rats were given a priming dose and infusion of 0.2% NMN, 4% inulin and 5% mannitol. After the 90 minute equilibration period, two 20-minute clearance periods served as the control periods. At the end of the second clearance period a priming injection of mepiperphenidol, 5 mg/Kg, was administered and an infusion of 0.1% NMN, 0.6% mepiperphenidol, 4% inulin and 5% mannitol began. After ten minutes, two 20-minute clearance periods were followed. These later clearance periods served as the inhibition periods. Ross et al (61) reduced the concentration of NMN from 0.2% in the control periods to 0.1% in the inhibition periods in order to maintain a stable NMN plasma level in the presence of mepiperphenidol. It was appropriate to follow their procedure here. All plasma and urine samples were assayed for NMN and inulin. Levels of mepiperphenidol were not determined.

D. Pseudoephedrine Assay

Various methods have been used in the determination of pseudoephedrine in biological fluids. Cummins and Fourier (85) describe a gas-liquid chromatographic method for the determination of pseudoephedrine and related ephedrines in plasma. Their method involves formation of the heptafluorobutyryl derivative and detection of the derivatives by electron capture. Kuntzman et al (73) made an acetyl derivative of pseudoephedrine in plasma with tritiated acetic anhydride and quantified the pseudoephedrine by scintillation counting. Bye et al (86) used a nitrogen-sensitive detector to determine plasma pseudoephedrine by gas-liquid chromatography. Recently, Lin et al (87)

modified the procedure of Cummins and Fourier (85) to allow determination of pseudoephedrine and its metabolite, norpseudoephedrine, in human plasma and urine. Their assay requires 0.2 ml of plasma and 0.1 ml of urine. Renal excretion studies of pseudoephedrine in the rat require analysis of plasma and urine samples for inulin as well as for pseudoephedrine. Since the inulin assay requires 75 µl of plasma it was desirable to use as small a sample of plasma as possible for the assay of pseudoephedrine. With appropriate modifications of the Lin et al procedure, a micro-analytical method was developed for measurement of pseudoephedrine in 20 µl of plasma.

1. Materials

Ephedrine sulfate (Ephedrine Sulfate Inj., USP, 50 mg/ml; Abbott Laboratories, North Chicago, Illinois); pseudoephedrine hydrochloride (d-ψ-Ephedrine Hydrochloride; Sigma Chemical Company, St. Louis, Missouri); norpseudoephedrine (DL-Norpseudoephedrine; Pfaltz and Bauer, Inc., Flushing, New York); benzene nanograde quality (Mallinckrodt Inc., St. Louis, Missouri); pyridine (Pyridine Sequential Grade; Pierce Chemical Co., Rockford, Illinois); heptafluorobutyric anhydride (Pierce Chemical Co., Rockford, Illinois); Varian Aerograph Model 1400 Gas Chromatograph (Varian Instrument Division, Palo Alto, California); Scandium tritide electron capture detector (Varian Instrument Division, Palo Alto, California); 3% OV-17 on 100-200 mesh Gas Chrom-Q (Applied Science Laboratories, State College, Pennsylvania); Hewlett Packard Series 3370B Integrator (Hewlett Packard, Avondale, Pennsylvania).

2. Methods

Preparation of solutions: 1.) Ephedrine sulfate (ES): 3.5 mg/L. 2.) Sodium hydroxide (NaOH): 4 N NaOH. 3.) Pyridine: 10% pyridine in benzene. 4.) Pseudoephedrine hydrochloride (PS·HCl): stock solution containing 8.06 mg/L PS; dilutions of PS stock solution ranging from concentrations of 0.1 mcg/ml to 1.61 mcg/ml PS. 5.) norpseudoephedrine hydrochloride (NPS·HCl): stock solution containing 8.6 mg/L NPS; dilution of NPS stock solution to yield a concentration of 1.72 mcg/ml NPS. Microanalysis of pseudoephedrine in plasma: 1.) to each 0.02 ml plasma sample was added 0.01 ml ES solution (3.5 mg/L) as the internal standard, 0.1 ml water, 0.1 ml 4 N NaOH, and 1 ml benzene. 2.) The samples were then placed on a rotor for 45 minutes for extraction. 3.) After extraction, the samples were centrifuged for 5 minutes at 1000 x g and the aqueous layer discarded. 4.) The benzene layer was shaken with 0.2 ml water and centrifuged. 5.) Approximately 0.7 ml of the benzene layer was transferred to a clean test tube; 0.05 ml of 10% pyridine in benzene and 0.02 ml of heptafluorobutyric anhydride, the derivatizing agent, were added. 6.) The samples were mixed on a vortex mixer for 20 seconds and allowed to stand overnight at room temperature. 7.) The samples were then washed 2 times with 1 ml and once with 0.5 ml 0.01 N HCl. 8.) Three microliters of the benzene fraction was injected into a Varian Aerograph Model 1400 gas chromatograph equipped with a scandium tritide electron capture detector. The chromatographic column was 1/8 in. o. d. x 6 ft. glass, packed with 3% OV-17 on 100-120 mesh Gas-Chrom Q. Nitrogen was used as the carrier gas at a flow rate of 6 ml/min. The injector and detector temperatures were 200° and 210° respectively. Column

temperature was maintained at 140°.

Analysis of pseudoephedrine in urine: Urine samples were assayed for pseudoephedrine according to the procedure of Lin et al (87) using 0.1 ml of ephedrine sulfate stock solution, 3.5 mg/L, for the internal standard in place of 1-(1)-methyl-benzylamine. The gas chromatograph conditions were as described above for the plasma assay.

Preparation of standard curves: Standard curves for pseudoephedrine were prepared from assays of duplicate plasma samples in the concentration ranges of 0.10 to 0.81 mcg/ml and 0.20 to 1.61 mcg/ml and from assays of duplicate urine samples in the concentration range of 0.20 to 1.61 mcg/ml. The standard curves were constructed using the ratio of the area or height of the PS peak to that of the ES (internal standard peak). Peak areas were obtained using a Hewlett Packard Integrator.

Specificity of assay: A series of samples were prepared in water to check the ability of the assay procedure to adequately distinguish between the internal standard (ES), pseudoephedrine (PS) and norpseudoephedrine (NPS), the major metabolite of pseudoephedrine in humans. The metabolism of pseudoephedrine has not been studied in the rat. However, metabolites of ephedrine in the rat have been reported to include norephedrine (88) and it is likely that norpseudoephedrine would be a metabolite of pseudoephedrine in the rat. Ephedrine is also metabolized to p-hydroxynorephedrine in the rat, but the corresponding pseudoephedrine metabolite was not available for study. The following samples were prepared and assayed according to the above procedure for urine samples:

A 0.3 ml water

B 0.2 ml water + 0.1 ml ES (3.5 mg/L)

C 0.1 ml water + 0.1 ml ES (3.5 mg/L) + 0.1 ml PS (0.8 mcg/ml)

D 0.1 ml water + 0.1 ml ES (3.5 mg/L) + 0.1 ml NPS (1.72 mcg/ml)

E 0.1 ml NPS (1.72 mcg/ml) + 0.1 ml ES (3.5 mg/L) + 0.1 ml PS (0.8 mcg/ml)

E. Pseudoephedrine Pharmacokinetics

1. Intravenous bolus doses of pseudoephedrine

In order to calculate appropriate pseudoephedrine infusion rates for the renal clearance studies it was first necessary to elucidate the pharmacokinetics of pseudoephedrine in the rat. Rats were dosed on a mg/Kg basis at doses of pseudoephedrine equivalent to that which Kuntzman et al (73) administered to dogs. Three male Sprague-Dawley rats were anesthetized with sodium pentobarbital (48 mg/Kg i.p.). Rat A weighed 245 gm, B weighed 244 gm, and C weighed 273 gm. The animal set-up was as described previously. Cannulae were inserted in the femoral vein and artery for administration of drug solution and collection of urine samples. Intravenous boluses of pseudoephedrine in doses of 2.5 mg/Kg, 3.0 mg/Kg and 2.5 mg/Kg were administered to rats A, B, and C respectively. Immediately following administration of pseudoephedrine an infusion of 5% mannitol in 0.9% sodium chloride (50 µl/min) was begun and continued throughout the experiment to insure adequate urine flow. Blood samples (0.2 ml) were drawn at 0, 5, 10, 20, 30 and 60 minutes from all three rats. Additional samples were drawn at 45, 75 and 90 minutes from rat C and at 120 minutes from rat B. Urine samples were collected at time 0, 10 - 30 minutes and 30 - 60

minutes following drug administration from rats A and B; time 0, 30 - 60 minutes and 90 - 120 minutes from C. Urinary pH was determined immediately upon collection of the urine samples. Blood samples were centrifuged immediately upon collection. Plasma and urine samples were frozen and later assayed for pseudoephedrine as described above. Pseudoephedrine plasma concentration versus time curves and urinary excretion data were analyzed pharmacokinetically.

2. Intravenous infusion of pseudoephedrine

Based on the pharmacokinetic parameters derived from the intravenous bolus studies, priming doses and infusion rates were calculated to yield pseudoephedrine steady-state plasma levels at the lower level of assay detection. Four rats were dosed accordingly to see if the desired steady-state levels could indeed be attained in the ninety minute equilibration period to be used in renal clearance studies.

Preparation of solutions: 1.) Pseudoephedrine·HCl stock solution: 1 mg/ml pseudoephedrine·HCl (0.82 mg/ml pseudoephedrine) in distilled water. 2.) Priming solution: 3.66 ml pseudoephedrine·HCl stock solution, 0.5 gm mannitol, qs ad 10 ml 0.9% sodium chloride. 3.) Infusion solution: 3.26 ml pseudoephedrine·HCl stock solution, 2.5 gm mannitol, qs ad 50 ml 0.9% sodium chloride. Each of four rats received a priming dose of 150 mcg pseudoephedrine (0.5 ml priming solution) followed by an infusion of 2.67 mcg pseudoephedrine per minute (50 µl/min infusion solution). The rats were equilibrated for 90 minutes. Blood samples (0.2 ml) were taken at 100 minutes, 120 minutes, 140 minutes and 160 minutes. Plasma samples were

assayed for pseudoephedrine.

F. Pseudoephedrine Protein Binding Studies

Plasma protein binding of pseudoephedrine was determined by ultracentrifugation. Because of the large volume of plasma required for this procedure, human plasma was used rather than rat plasma. A stock solution containing 0.5 mg/ml pseudoephedrine in water was prepared. Aliquots of this solution were added to 5 ml samples of fresh human plasma (from one individual) to yield plasma concentrations of 0.2 to 1.8 mcg/ml pseudoephedrine. The samples were incubated at 37°C for one hour. Approximately 0.5 ml was taken from each sample and refrigerated. The remaining samples were then centrifuged at 52,000 rpm (278,000 times gravity) for 18 hours. Temperature was maintained at 37°C. Both the plasma withdrawn before centrifugation and the supernatant of the centrifuged samples were assayed for pseudoephedrine.

G. Red Blood Cell Partitioning of Pseudoephedrine

The partitioning of pseudoephedrine into red blood cells was studied in rat blood. Aliquots from a stock solution of pseudoephedrine (0.10 mg/ml) were added to one ml samples of heparinized whole blood to yield blood concentrations of 0.30 and 0.60 mcg/ml. The samples were incubated at 37°C for 120 minutes. Samples were then centrifuged and the plasma assayed for pseudoephedrine. The hematocrit was determined on a blood sample drawn from the same rat.

H. Pseudoephedrine Excretion Studies

1. Clearance experiments

Clearance experiments were run in three rats using the same priming dose and infusion rate of pseudoephedrine as was administered in the preliminary infusion studies. Preparation of solutions: 1.) Priming solution: 3.66 ml pseudoephedrine stock soltuion (0.82 mg/ml), 0.5 gm mannitol, 0.4 gm inulin qs ad 10 ml 0.9% sodium chloride. 2.) Infusion solution: 3.26 ml pseudoephedrine stock solution, 2.5 gm mannitol, 2.0 gm inulin qs ad 50 ml 0.9% sodium chloride solution. After 0.5 ml of the priming solution (150 mcg), the infusion was begun and the experiment run as described in General Procedures. Plasma and urine samples were assayed for pseudoephedrine and inulin.

2. Saturation experiments

Clearance experiments were run with increasing doses of pseudoephedrine to determine if the renal clearance of pseudoephedrine is saturable. The dosing regimen was designed to yield a wide range of pseudoephedrine steady-state plasma levels which would encompass all levels which might be encountered in a clinical situation. The following doses were administered:

	Priming Dose (mcg)	Infusion (mcg/min)	No. of Rats
A	200	3.56	4
B	300	5.35	6
C	600	10.68	2
D	1200	21.36	4

Solutions for priming dose and infusion were prepared as described for the clearance experiments.

3. Effect of urinary pH on pseudoephedrine renal excretion

It has been shown that urinary pH influences the plasma half-life of pseudoephedrine in man and dog (73). This influence indicates the presence of a passive renal tubular component (reabsorption) of the renal excretion of pseudoephedrine. The effect of urinary pH on the excretion of pseudoephedrine in the rat was therefore studied.

Preparation of solutions: 1.) Pseudoephedrine priming solution: 300 mcg/ml pseudoephedrine, 2.5% mannitol, 4% inulin in 0.9% sodium chloride. 2.) Acid priming solution: 1.61% sodium sulfate anhydrous (Mallinckrodt Chemical Works, St. Louis, Missouri) in 0.9% sodium chloride. 3.) Base priming solution: 1.4% sodium bicarbonate (Merck and Co., Inc., Rahway, New Jersey) in 0.9% sodium chloride. 4.) Acid infusion: 53.4 mcg/ml pseudoephedrine, 1.61% sodium sulfate, 2.5% mannitol, 4% inulin in 0.9% sodium chloride. 5.) Base infusion: 53.4 mcg/ml pseudoephedrine, 1.4% sodium bicarbonate, 2.5% mannitol, 4% inulin in 0.9% sodium chloride.

Procedure for studies: These experiments were carried out following procedures similar to that for the inhibition experiments. The rat first received a pseudoephedrine priming dose of 0.5 ml as well as a 0.5 ml priming dose of either acid or base. Either an acid or base infusion (50 µl/ml) was then begun. The rat was equilibrated for ninety minutes, then two 20-minute clearance periods were followed.

A base or acid priming dose of 0.5 ml was next administered. The rat was re-equilibrated for sixty minutes with a base or acid infusion (50 µl/min) and two more 20-minute clearance periods were followed. The results of the first two clearance periods, acid or base, were compared with the results of the second two clearance periods, base or acid. Three rats received acid priming doses and infusions followed by base priming doses and infusions; two rats received base priming doses and infusions followed by acid priming doses and infusions.

4. Inhibition experiments: mepiperphenidol

Inhibition experiments with mepiperphenidol were performed essentially as described in General Procedures. Appropriate modifications in the procedure were periodically introduced when necessary to resolve questions arising from results of preceding experiments. A priming dose and infusion of pseudoephedrine was administered. After a ninety minute equilibration period, two 20-minute clearance periods were followed. A priming dose of mepiperphenidol was then administered and an infusion begun containing pseudoephedrine and mepiperphenidol. After a ten minute (or 60 minute) re-equilibration period, two more clearance periods were followed. The first two clearance periods served as controls. The last two clearance periods were the experimental or inhibition periods. Plasma and urine samples were analyzed for pseudoephedrine and inulin. Table III summarizes the dosing schedules followed.

Check for assay interference: Possible interference of mepiperphenidol in the pseudoephedrine assay was checked in two ways. 1.) Water

Table III

Dosing Schedule for Pseudoephedrine (PS) and Mepiperphenidol (Mepi) in Inhibition Studies

Schedule[1]	PS Priming Dose[2]	PS Infusion[3]	Mepi Priming Dose	Mepi Infusion[3]	No. of Rats
A	300 mcg/ml PS 4% inulin 5% mannitol in 0.9 NaCl	53.4 mcg/ml PS 4% inulin 5% mannitol in 0.9% NaCl	5 mg/Kg as 2 mg/ml Mepi in 0.9% NaCl	53.4 mcg/ml PS 0.6% Mepi 4% inulin 5% mannitol in 0.9% NaCl	2
B	"	106.8 mcg/ml PS 8% inulin 10% mannitol in 0.9% NaCl	"	"	2
C	"	"	"	53.4 mcg/ml PS 0.3% Mepi 4% inulin 7.5% mannitol in 0.9% NaCl	3
D	"	"	"	53.4 mcg/ml PS 0.3% Mepi 4% inulin 5% mannitol in 0.9% NaCl	
E	"	"	2.5 mg/Kg as 2 mg/ml Mepi in 0.9% NaCl	"	2

Table III (Continued)

Schedule[1]	PS Priming Dose[2]	PS Infusion[3]	Mepi Priming Dose	Mepi Infusion[3]	No. of Rats
F	300 mcg/ml PS 4% inulin 4% Na_2SO_4 in 0.9% NaCl	53.4 mcg/ml PS 4% inulin 4% Na_2SO_4 in 0.9% NaCl	2.5 mg/Kg as 2 mg/ml Mepi in 0.9% NaCl	53.4 mcg/ml PS 0.3% Mepi 4% inulin 4% Na_2SO_4 in 0.9% NaCl	2
G	as A	as A	"	as C	2

1. A, B, C, D, E re-equilibrated for 10 minutes, F, G re-equilibrated for 60 minutes
2. Administered 0.5 ml
3. Infused at a rate of 50 µl/min

samples were spiked with mepiperphenidol and/or pseudoephedrine and assayed for pseudoephedrine. 2.) A rat was infused with mepiperphenidol and plasma and urine samples were assayed for "pseudoephedrine". Method 2 also provided a check for possible interference of unknown metabolites of mepiperphenidol. Method 1 - a stock solution containing 100 mcg/ml mepiperphenidol (Mepi) in water was prepared. Pseudoephedrine (PS) and ephedrine sulfate (ES) stock solutions were identical to those prepared for the standard curves described in the section on pseudoephedrine assay. The following samples were prepared and assayed according to the procedure for pseudophedrine in urine.

A 0.2 ml water

B 0.2 ml water + 0.1 ml ES (3.5 mg/L)

C 0.1 ml water + 0.1 ml ES (3.5 mg/L) + 0.1 ml PS (0.8 mcg/ml)

D 0.2 ml water + 0.05 ml Mepi (100 mcg/ml)

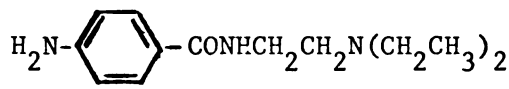
E 0.2 ml water + 0.1 ml ES (3.5 mg/L) + 0.05 ml Mepi (100 mcg/ml)

F 0.1 ml water + 0.1 ml ES (3.5 mg/L) + 0.1 ml PS (0.8 mcg/ml) + 0.05 ml Mepi (100 mcg/ml)

Method 2 - A rat was surgically prepared as if for a clearance experiment. An intravenous bolus of mepiperphenidol, 5 mg/Kg, was administered and an infusion containing 0.6% mepiperphenidol and 5% mannitol in 0.9% sodium chloride was begun at a rate of 50 µl/min. After ninety minutes, four 20-minute clearance periods were followed with appropriate collection of blood and urine samples. Plasma and urine was assayed according to the procedures described for pseudoephedrine.

5. Inhibition experiments: procainamide

Competitive inhibition studies were carried out with procainamide (Procainamide HCl, E. R. Squibb and Sons, Inc., Princeton, New Jersey). Procainamide is an organic base with a pKa of 9.4 (89) used clinically as an effective agent in the treatment of cardiac arrhythmias (90). Its structure is:



Koch-Weser and Klein (90) found that renal clearance of procainamide varied from 179 - 309 ml/min in patients. Since the normal glomerular flitration rate in man is 120 - 125 ml/min, these workers concluded that about one-half of the amount of procainamide cleared by the kidneys is actively secreted by the tubules. Weily and Genton (89) found that in dogs, at urinary pH 6 - 7, procainamide renal clearance was greater than that of inulin (4.82 ml/Kg·min and 3.28 ml/Kg·min respectively). Since procainamide is secreted by the renal tubule, it is probable that it is transported by the organic base transport system and therefore is a possible inhibitor of other bases transported by this system.

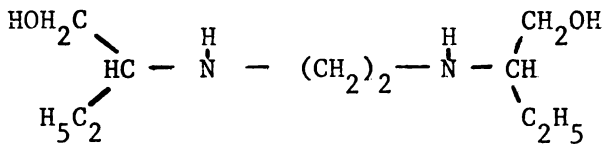
Check for assay interference: Possible interference of procainamide and its metabolites in the plasma and urine assays for pseudoephedrine was checked by means of method 2 described in the preceding section on mepiperphenidol inhibition. Solutions for infusion were prepared containing 2 mg/ml procainamide, 5% mannitol and 4 mg/ml procainamide, 5% mannitol in 0.9% sodium chloride. Following a bolus dose of 5% mannitol in 0.9% sodium chloride, one rat received an infusion

of 2 mg/ml procainamide at a rate of 50 µl/min, another received an infusion of 4 mg/ml procainamide at the rate of 50 µl/min. Sample collection was begun after ninety minutes. Plasma and urine samples were assayed for "pseudoephedrine".

Procedure for inhibition studies: The following solutions were prepared for the priming doses and infusions: 1.) Pseudoephedrine priming solution - 300 mcg/ml pseudoephedrine, 5% mannitol, 4% inulin in 0.9% sodium chloride. 2.) Pseudoephedrine infusion - 53.4 mcg/ml pseudoephedrine, 5% mannitol, 4% inulin in 0.9% sodium chloride. 3.) Procainamide priming solution - 2 mg/ml procainamide in 0.9% sodium chloride. 4.) Pseudoephedrine, procainamide infusion - 53.4 mcg/ml pseudoephedrine, 4 mg/ml procainamide, 5% mannitol, 4% inulin in 0.9% sodium chloride. Two rats each received 0.5 ml of the pseudoephedrine priming solution followed by the pseudoephedrine infusion at a rate of 50 µl/min. After ninety minutes, two 20-minute clearance periods were followed. The procainamide priming solution was then administered at a dose of 5 mg/Kg and the pseudoephedrine, procainamide infusion begun. The rats were re-equilibrated for 60 minutes before following two more clearance periods. Plasma and urine samples were assayed for pseudoephedrine and inulin.

6. Inhibition experiments: ethambutol

Competitive inhibition studies were also carried out with ethambutol (Myambutol^{R}, 10% solution; Lederle Laboratories, Pearl River, New York). Ethambutol is an organic base, pKa's 6.6 and 9.5 (91), useful in the treatment of tuberculosis. Its structure is:



Lee (91) reports a renal clearance of 8.24 ml/min·Kg for ethambutol in the rhesus monkey after parenteral administration. The average glomerular filtration rate in the rhesus monkey is 3 ml/min·Kg. When normal human subjects were administered ethambutol, orally, the average renal clearance for ethambutol was 6.75 ml/min·Kg. Average body weight of the subjects was 58.8 Kg. Thus renal clearance of ethambutol is greater than glomerular filtration rate in monkeys and humans, indicating secretion of this base, probably mediated by the organic base transport system. Ethambutol is another possible inhibitor of bases transported by this system.

Check for assay interference: Possible interference of ethambutol and its metabolites in the plasma and urine assays was checked in one rat following the same procedure as described for procainamide. The solution for infusion contained 1.2 mg/ml ethambutol, 5% mannitol in 0.9% sodium chloride.

Procedure for inhibition studies: Pseudoephedrine priming and infusion solutions were prepared as described for procainamide. Ethambutol priming solution contained 2 mg/ml ethambutol in 0.9% sodium chloride. Pseudoephedrine, ethambutol infusions contained 53.4 mcg/ml pseudoephedrine, 1.2 mg/ml or 2.4 mg/ml ethambutol, 5% mannitol, 4% inulin in 0.9% sodium chloride. Experimental procedure for

these studies was as described for procainamide. Two rats received the infusion containing 1.2 mg/ml ethambutol; two rats received the infusion containing 2.4 mg/ml ethambutol.

CHAPTER 3

RESULTS

A. General Procedures

1. Data treatment for clearance, saturation and inhibition experiments. For each clearance period the following parameters were calculated:

$\dot{V}$	urinary flow rate in ml/min·Kg
U_D	urinary drug concentration in mcg/ml (or mM)
U_I	urinary inulin concentration in gm per cent
P_D	drug plasma concentration in mcg/ml (or μM)
P_I	inulin plasma concentration in gm per cent
CL_D	renal clearance of drug in ml/min·Kg
T_D	net tubular transport of drug in mcg/min·Kg (or μmol/min·Kg)

Clearance of drug or inulin was calculated from equation 2.

$$CL_D \text{ or } CL_I = \frac{U_{(D \text{ or } I)} \cdot \dot{V}}{P_{(D \text{ or } I)}} \qquad \text{(eq. 2)}$$

Net tubular transport of drug was calculated from equation 3.

$$T_D = U_D \cdot \dot{V} - \alpha P_D \cdot CL_I \qquad \text{(eq. 3)}$$

where $U_D \cdot \dot{V}$ = total urinary excretion of drug, $\alpha P_D \cdot CL_I$ = that part of urinary excretion which is due to glomerular filtration and, α = the fraction of drug in the plasma which is not bound to protein.

When evaluating saturation experiments, the relationships CL_D/CL_I vs P_D and T_D vs P_D were considered. Assuming that there is no tubular reabsorption or passive diffusion of drug from plasma to tubular lumen, T_D can be evaluated in terms of Michaelis-Menten type kinetics.

$$T_D = \frac{T_{M_D} \cdot P_D}{K_M + P_D} \qquad \text{(eq. 4)}$$

where T_{M_D} is the maximum transport rate and K_M is a "Michaelis" type constant. When P_D is negligible with respect to K_M, T_D increases linearly with increasing P_D. When K_M is negligible with respect to P_D (i.e. high plasma concentrations of drug), $T_D = T_{M_D}$ and thus, transport rate is a constant with increasing P_D. $K_M \cong P_D$ leads to an intermediate situation. Figure 4 is a graphical representation of these situations.

Rearranging equation 3, dividing both sides by P_D and CL_I, and then combining with equation 2, yields equation 5.

$$\frac{CL_D}{CL_I} = \frac{CL_I + T_{M_D}/(K_M + P_D)}{CL_I} \qquad \text{(eq. 5)}$$

When P_D is negligible with respect to K_M, CL_D/CL_I is constant as P_D increases. When $K_M \cong P_D$, CL_D/CL_I decreases with increasing P_D. When K_M is negligible with respect to P_D, CL_D/CL_I continues to decrease, approaching a limit of 1 as P_D increases towards infinity i.e. T_{M_D}/P_D becomes infinitely small. These situations are represented in figure 5.

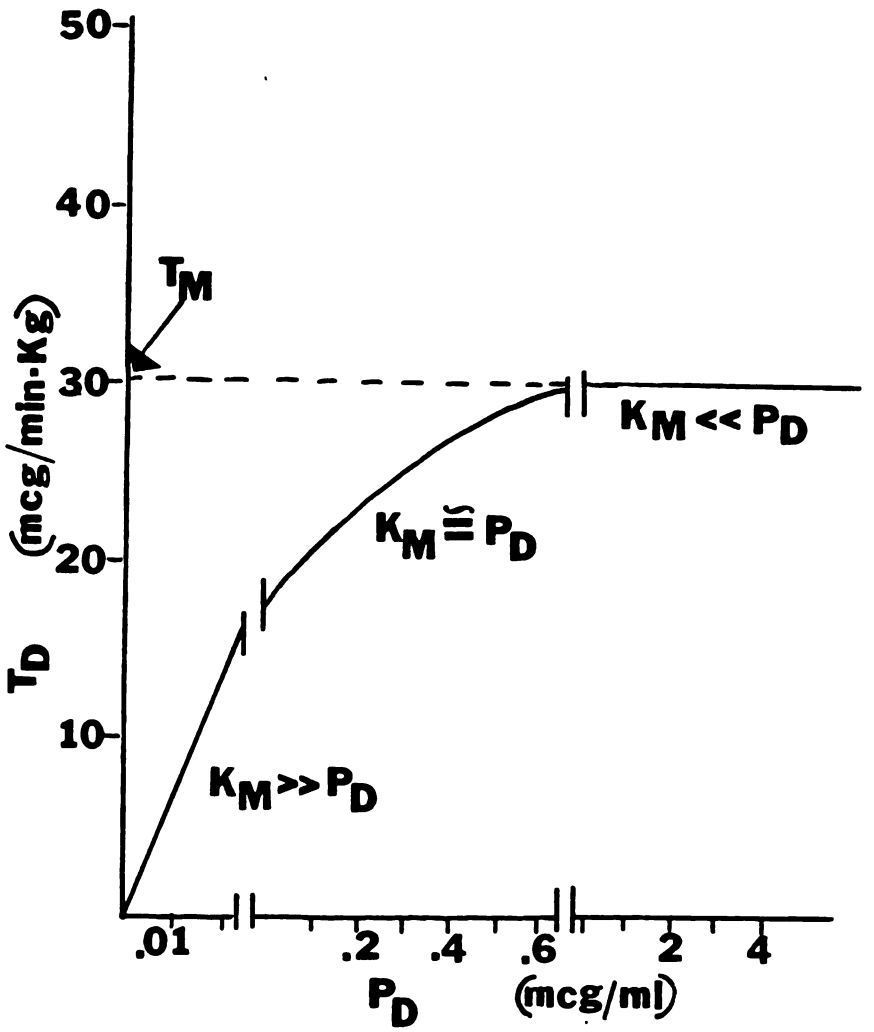


Figure 4. Theoretical plot for T_D vs P_D when transport is saturable.

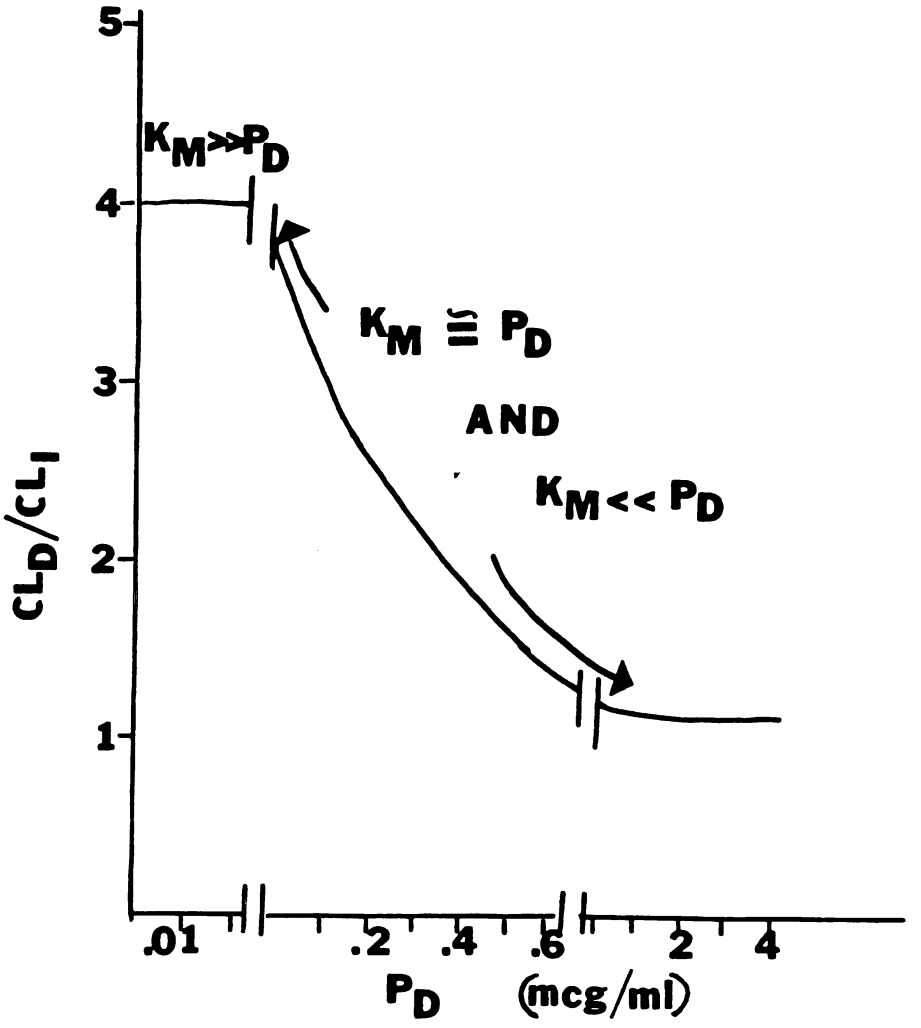


Figure 5. Theoretical plot for CL_D/CL_I vs P_D when CL_D has a saturable transport component.

The results of the inhibition experiments were evaluated by comparing the control periods and inhibition periods with respect to 1.) CL_D; 2.) CL_D/CL_I; 3.) T_D.

2. Inulin assay

a. standard curve

The standard curve for inulin was linear over a concentration range of 1 mg per cent to 20 mg per cent inulin. Blank urine and plasma samples gave negligible readings indicating that there was little or no interference of endogenous compounds in the anthrone reaction. Although standard curves were quite reproducible from assay to assay, a new standard curve was constructed for each assay. Figure 6 depicts a typical inulin standard curve. The linear equation for this set of data is:

$$y = 0.02 + 0.0575 \; x \qquad r^2 = 0.9982$$

b. effect of mannitol in inulin assay

Table IV contains the results of the study designed to check the possibility of depression of the anthrone reaction by plasma and urine mannitol. As can be seen, the absorbance readings for urine and plasma samples with and without mannitol are either the same as those for inulin standards in water or deviate from the standards in a random fashion.

E. N^1-methylnicotinamide (NMN) Excretion Studies

1. NMN assay

Linear plasma standard curves were obtained over an NMN concentration

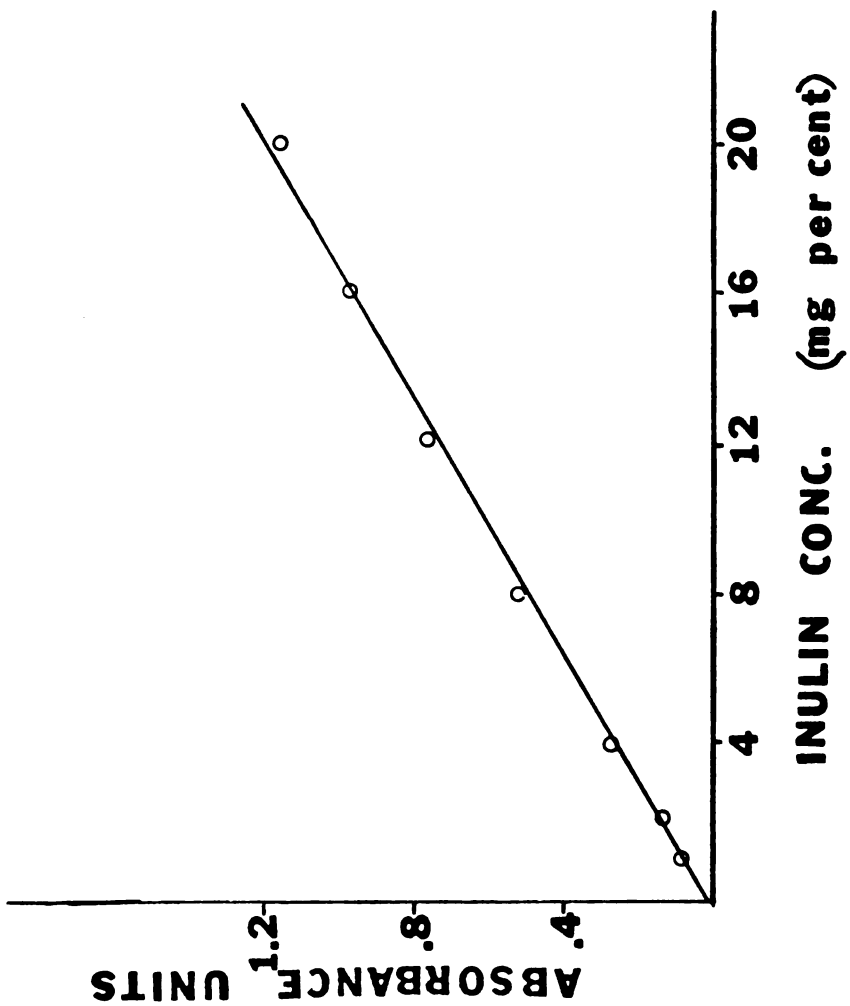


Figure 6. Standard curve for the spectrophotometric measurement of inulin in water.

Table IV

Effect of Mannitol on Inulin Assay

Inulin conc. (mg %)	Inulin Standards in water	Urine Samples without mannitol	Urine Samples with mannitol	Plasma Samples without mannitol	Plasma Samples with mannitol
	Absorbance Units				
0	---	-0.005	-0.005	-0.005	0.000
2	0.125	0.125	0.125	0.140	0.125
4	0.250	0.240	0.270	0.260	0.270
8	0.500	0.490	0.460	0.480	0.500

range of 1.5 to 76.0 mcg/ml. Linear urine standard curves were obtained over an NMN concentration range of 1.5 to 152.0 mcg/ml. Figure 7 is a typical plasma standard curve. The linear equation for this curve is:

$$y = 1.455 + 0.638\ x$$

$$r^2 = 0.9956$$

Figure 8 is a typical urine standard curve. The linear equation is:

$$y = -0.740 + 0.656\ x$$

$$r^2 = 0.9988$$

2. Clearance experiments

Table V contains the results of a single clearance experiment. The rat was infused with 0.2% NMN. The results for each of the four clearance periods and the average of the four periods are given. Urine flow ($\dot{V}$) was relatively constant for the four periods and averaged 0.166 ml/min·Kg. There was some random fluctuation in NMN urine and plasma concentrations, but a steady-state plasma concentration was essentially attained. Average renal clearance of NMN was 35.8 ml/min·Kg. Steady-state plasma levels were also reached with respect to inulin. Variation in these levels over the four clearance periods was random. Average inulin clearance, or GFR, was 6.79 ml/min·Kg. The ratio of NMN renal clearance to inulin clearance (CL_{NMN}/CL_I) was 5.28, indicating considerable net secretion. Average transport was 1.67 μmol/min·Kg. Table VI contains the average results of the six experiments in which rats received 0.2% NMN. The four clearance periods were averaged for each rat to obtain a single value for each parameter. These values were then averaged for the six rats and the mean and standard deviation reported. Included in the table are the average results of seven

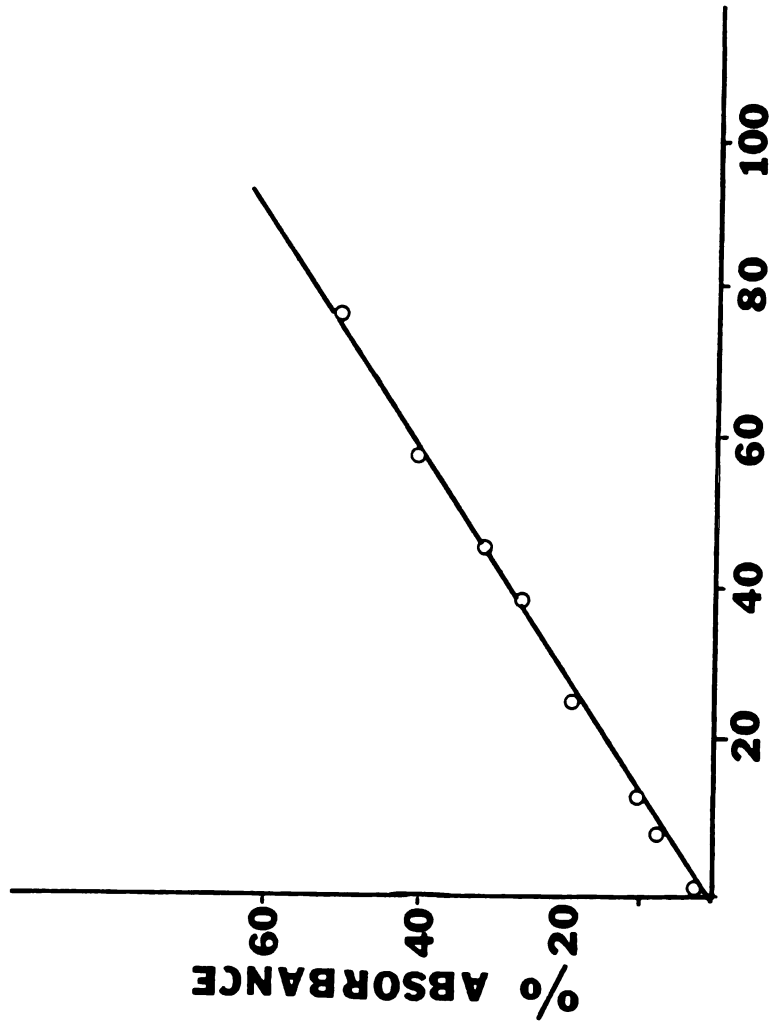


Figure 7. Standard curve for the spectrofluorometric measurement of NMN in plasma.

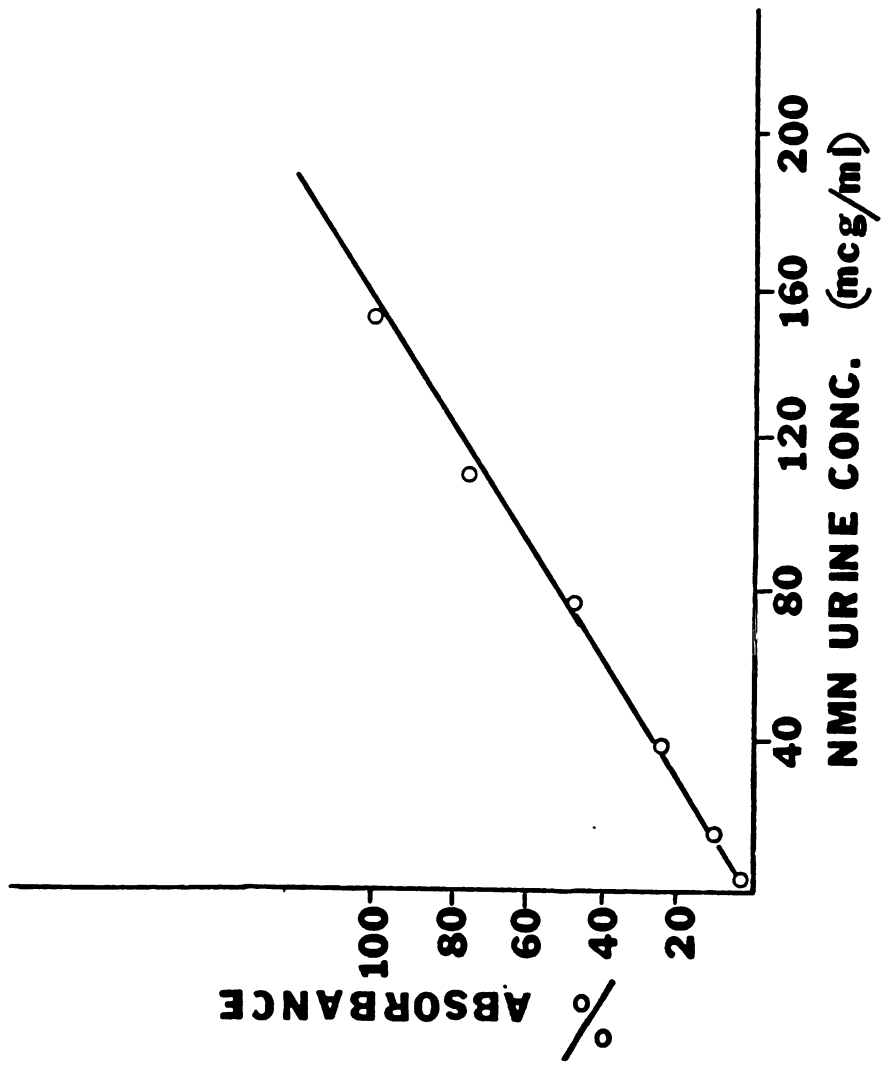


Figure 8. Standard curve for the spectrofluorometric measurement of NMN in urine.

Table V

Excretion Parameters for Four Clearance Periods Following an I.V. Infusion of 0.2% NMN in a Rat[a]

Period	$\dot{V}$ (ml/min·kg)	U_{NMN} (mM)	P_{NMN} (μM)	CL_{NMN} (ml/min·kg)	U_I (gm %)	P_I (gm%)	CL_I (ml/min·kg)	$\frac{CL_{NMN}}{CL_I}$	T_{NMN} (μmol/min·kg)
1	0.145	12.4	62.0	29.0	4.90	0.108	6.58	4.41	1.39
2	0.172	13.1	54.7	41.3	4.50	0.112	6.91	5.96	1.88
3	0.169	11.7	54.7	36.1	4.75	0.127	6.32	5.70	1.63
4	0.179	12.4	58.3	38.0	3.70	0.090	7.36	5.17	1.79
Mean	0.166	12.4	57.4	35.9[b]	4.46	0.109	6.79[b]	5.29[b]	1.67[b]
S.D. ±	0.015	0.6	3.5	---	0.53	0.015	---	---	---

[a]Priming injection and infusion of 0.2% NMN, 4% inulin, 5% mannitol

[b]Calculated from mean parameters

Table VI

Comparison of NMN Clearance Parameters[a]
Obtained Experimentally with Those of Ross et al (61)

		$\dot{V}$ (ml/min·kg)	U_{NMN} (mM)	P_{NMN} (μM)	CL_{NMN} (ml/min·kg)	CL_I (ml/min·kg)	$\frac{CL_{NMN}}{CL_I}$	T_{NMN} (μmol/min·kg)
Mean								
	E[b]	0.120	14.7	85.9	23.8	6.55	3.68	1.25
	L[c]	0.280	9.53	79.9	28.4	9.37	3.05	1.48
S.D.								
	E	±0.030	±1.7	±23.7	±10.6	±2.09	±1.23	±0.47
	L	±0.150	±3.80	±13.1	±7.6	±1.36	±0.91	±0.51

[a]Priming injection and infusion of 0.2% NMN, 4% inulin, 5% mannitol

[b] E = Experimental (6 runs)

[c] L = Literature (7 runs)

experiments performed by Ross et al (61). These values were obtained by averaging the two control clearance periods for each of seven inhibition experiments in which rats were infused with 0.2% NMN.

3. Saturation experiments

The results of the studies where saturation of transport was tested by increasing the infusion rate of NMN are depicted graphically in figures 9 and 10. NMN was infused at concentrations of 0.2 - 1.2%. Average NMN steady-state plasma levels attained ranged from 57.4 μM to 769 μM. The clearance ratio of NMN to inulin (CL_{NMN}/CL_I) vs NMN steady-state plasma level is plotted in figure 9. Each experimental data point represents one rat, or the average of four clearance periods. The data of Ross et al (61) is included for comparison. Transport rate of NMN (T_{NMN}) vs P_{NMN} is plotted in figure 10. Again, Ross and coworkers results are included for comparison.

4. Inhibition experiments

Inhibition of NMN renal clearance by mepiperphenidol was studied in two rats. Two control periods (administration of NMN only) and two inhibition periods (administration of NMN and mepiperphenidol) were averaged for each rat. The control and inhibition periods were then compared with respect to CL_{NMN}, CL_{NMN}/CL_I and T_{NMN}. Table VII summarizes the average results for the two rats and includes a summary of results obtained by Ross et al (61) for seven rats.

C. Pseudoephedrine Assay

Microanalysis of pseudoephedrine in plasma: Figure 11 includes sample

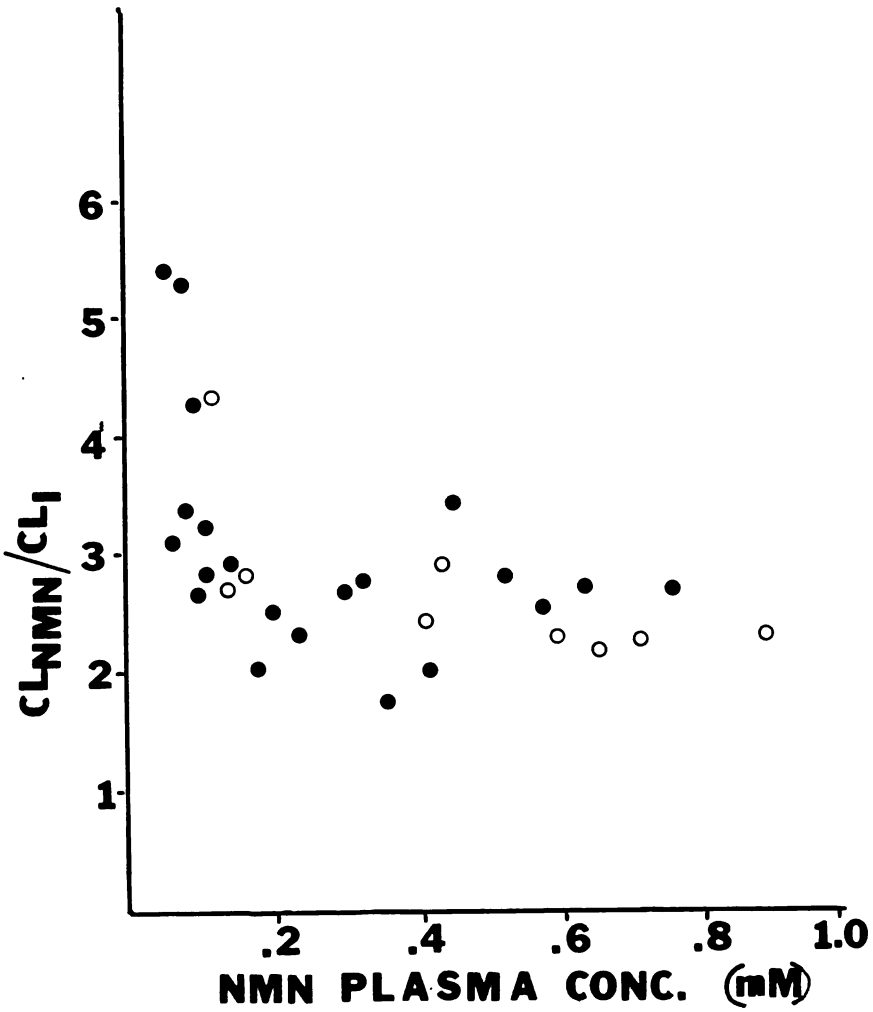


Figure 9. Ratio of NMN to inulin clearance (CL_{NMN}/CL_I) as a function of NMN plasma concentration for values determined experimentally (●) and as reported in the literature (o) by Ross et al (61).

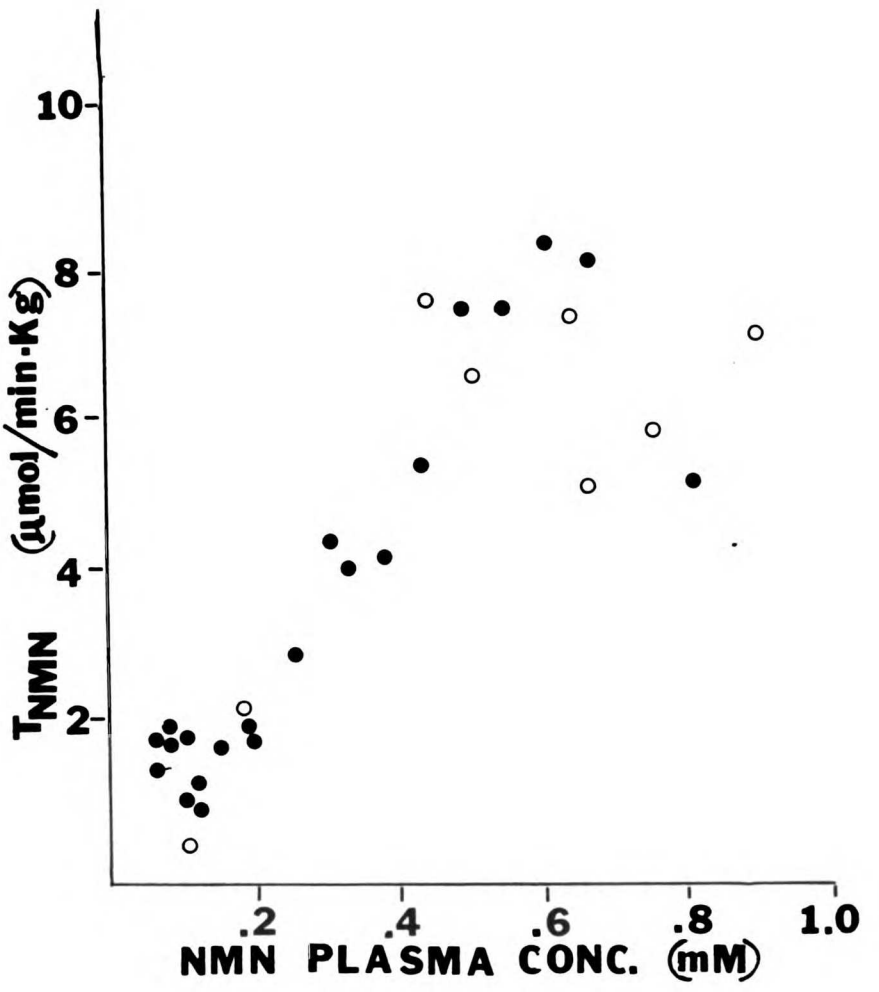


Figure 10. Transport rate of NMN (T_{NMN}) plotted as a function of NMN plasma concentration for values determined experimentally (•) and as reported in the literature (o) by Ross et al (61).

Table VII

Inhibition of NMN Renal Clearance by Mepiperphenidol
as Compared to Literature Results

	CL_{NMN} (ml/min·kg)		$\frac{CL_{NMN}}{CL_I}$		T_{NMN} (μmol/min·kg)	
	Control	Inhibition	Control	Inhibition	Control	Inhibition
E[a]	38.4	13.2	3.70	2.03	1.45	0.480
S.D.	±4.2	±7.4	±0.06	±0.51	±0.04	±0.180
L[b]	28.4	16.0	3.05	1.76	1.46	0.600
S.D.	±7.6	±8.7	±0.91	±0.64	±0.63	±0.160

[a] E = experimental; average of 2 rats

[b] L = Literature [Ross *et al* (61)]; average of 7 rats

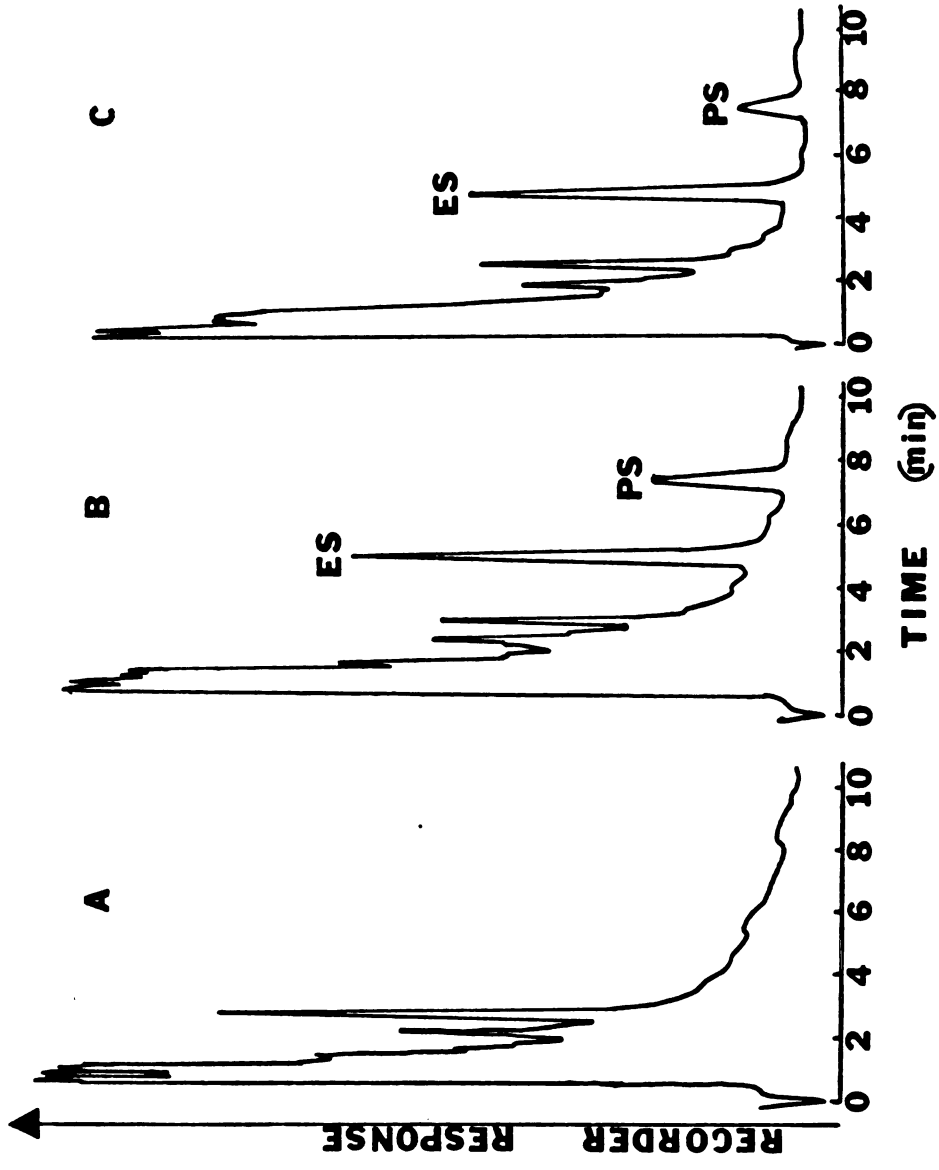


Figure 11. Chromatograms of pseudoephedrine in plasma. A: plasma blank; B: pseudoephedrine (PS) and internal standard (ES) in plasma; C: plasma sample from rat infused with PS. Instrument attenuation was set at 32.

chromatograms for pseudoephedrine in plasma. The chromatogram at the far left (A) represents a blank plasma sample. Chromatogram B represents a plasma sample spiked with ephedrine sulfate (ES), the internal standard, and pseudoephedrine (PS); concentration of PS was equivalent to 0.40 mcg/ml or 8 ng in a 20 µl plasma sample. The chromatogram on the far right (C) represents a plasma sample taken from a rat which had been infused with pseudoephedrine (2.67 mcg/min after a bolus of 150 mcg); PS concentration was 0.22 mcg/ml. Retention times for the internal standard (ES) and PS were 4.9 minutes and 7.3 minutes, respectively.

Figure 12 is a calibration curve for pseudoephedrine in plasma. Peak area ratio, PS/ES, is plotted against PS plasma concentration. The linear equation for the plot and the regression coefficient are:

$$\text{Peak area ratio PS/ES} = -0.054 + 1.301 \text{ PS conc. (mcg/ml)}$$

$$r^2 = 0.9966$$

Standard curves for PS over both concentration ranges of 0.1 mcg/ml and 0.2 mcg/ml to 1.61 mcg/ml are included in this plot. These curves were prepared on different days. Seven repeat analysis of a plasma sample containing 1.61 mcg/ml PS gave a mean concentration of 1.66 mcg/ml and a standard deviation of 0.076.

Assay of pseudoephedrine in urine: Chromatograms for pseudoephedrine in urine are shown in figure 13. Chromatogram A represents a blank urine sample; B represents a urine sample spiked with ephedrine sulfate and pseudoephedrine; C represents a urine sample taken from a rat which had been infused with pseudoephedrine.

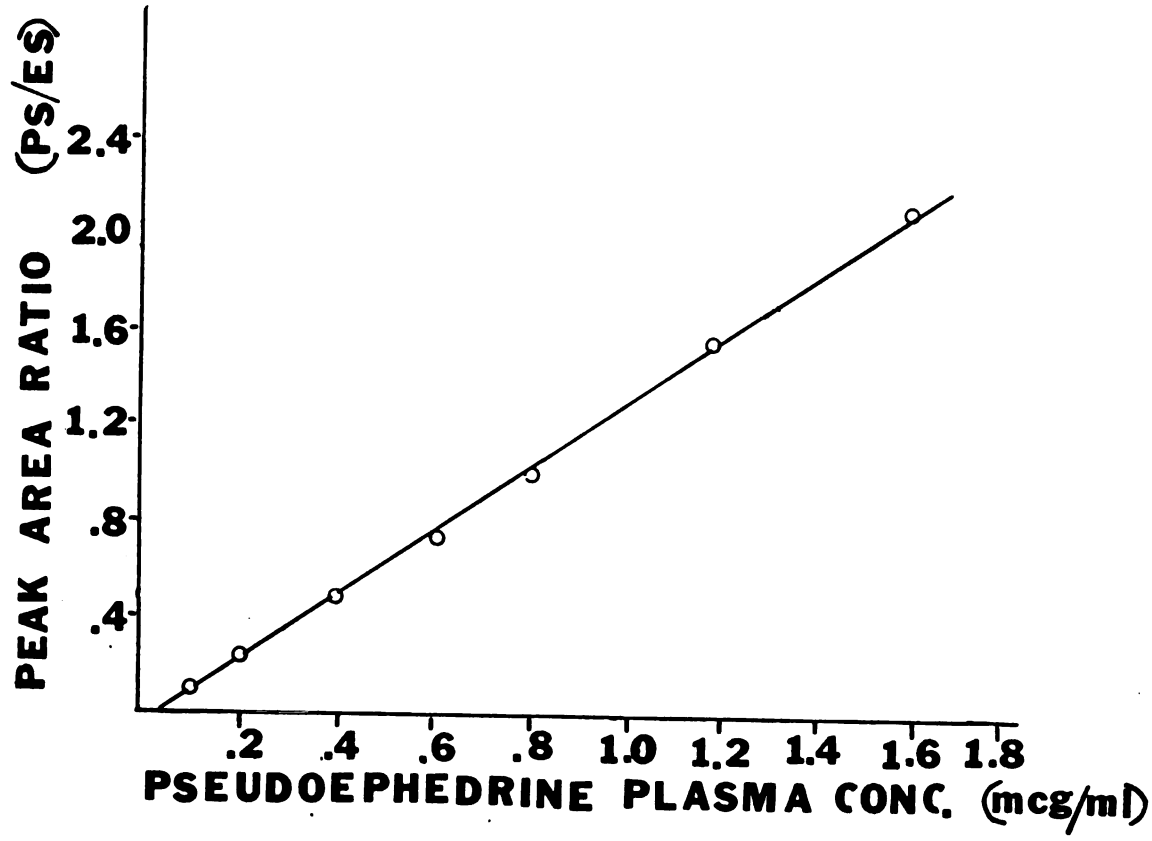


Figure 12. Calibration curve relating peak area ratio of pseudoephedrine to internal standard (PS/ES) vs plasma concentration of PS.

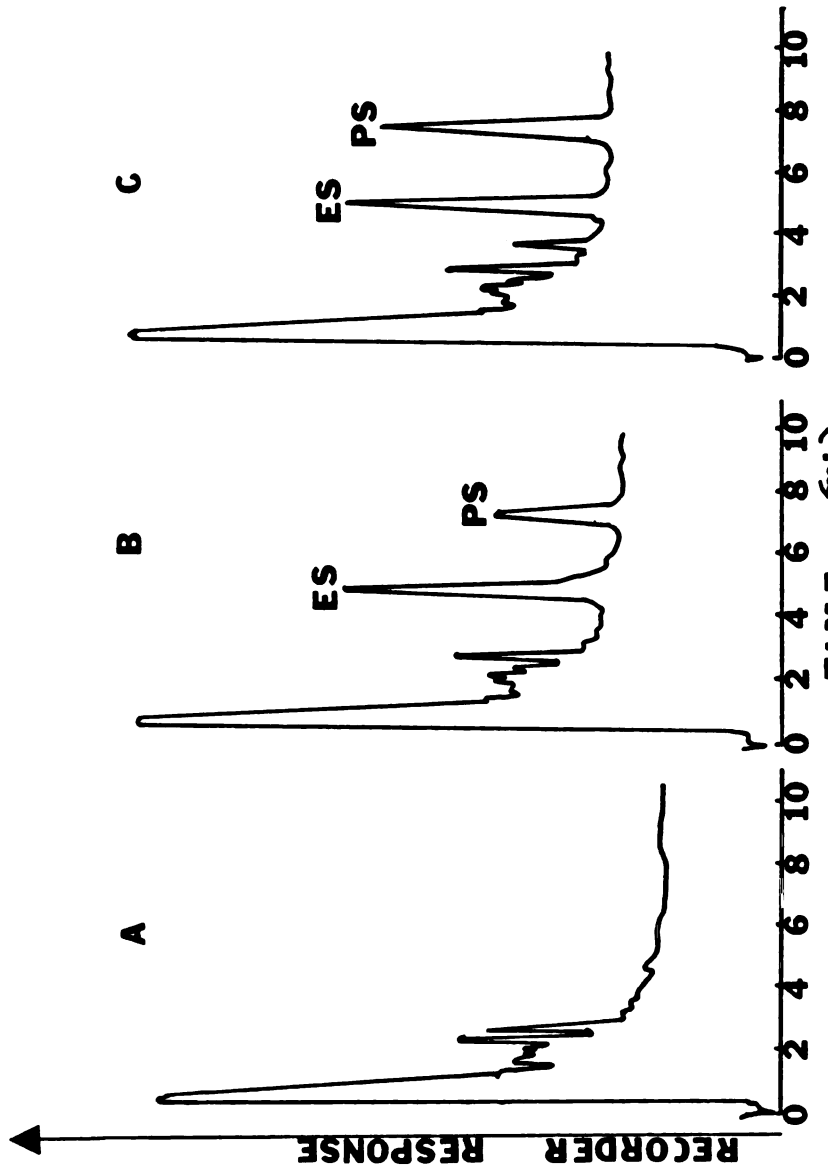


Figure 13. Chromatograms of pseudoephedrine in urine. A: urine blank; B: pseudoephedrine (PS) and internal standard (ES) in urine; C: urine sample from rat infused with PS. Instrument attenuation was set at 64.

Figure 14 is a standard curve for pseudoephedrine in urine. Peak area ratio, PS/ES, is plotted against PS urine concentration. The linear equation for the plot and the regression coefficient are:

$$\text{Peak area ratio PS/ES} = -0.0108 + 0.672 \text{ PS conc. (mcg/ml)}$$

$$r^2 = 0.9965$$

Assay specificity: When water samples spiked with norpseudoephedrine (NPS) were assayed according to the procedure for pseudoephedrine in urine, the retention time for NPS was 3.5 minutes. Figure 15 is a chromatogram of a water sample spiked with NPS, ES and PS.

D. Pseudoephedrine Pharmacokinetics

1. Administration of intravenous bolus doses of pseudoephedrine Following the intravenous administration of pseudoephedrine to three rats, plasma levels declined biexponentially. The plasma concentration (P_{PS}) - time data can be described by the following equation:

$$P_{PS} = Ae^{-\alpha t} + Be^{-\beta t} \qquad \text{(eq. 6)}$$

where $A = \dfrac{(k_{21} - \alpha)\ \text{Dose}}{(\beta - \alpha)\ V_1}$; $B = \dfrac{(k_{21} - \beta)\ \text{Dose}}{(\alpha - \beta)\ V_1}$

$$\begin{matrix}\alpha \\ \beta\end{matrix} = \frac{(k_{12} + k_{21} + k_{13}) \pm \left[(k_{12} + k_{21} + k_{13})^2 - 4\, k_{13} \cdot k_{21}\right]^{1/2}}{2}$$

V_1 = volume of the central compartment

k_{12}, k_{21} = intercompartmental rate constants describing transfer from compartment 1 to 2 and vice versa

k_{13} = rate constant describing elimination of unchanged drug from compartment 1.

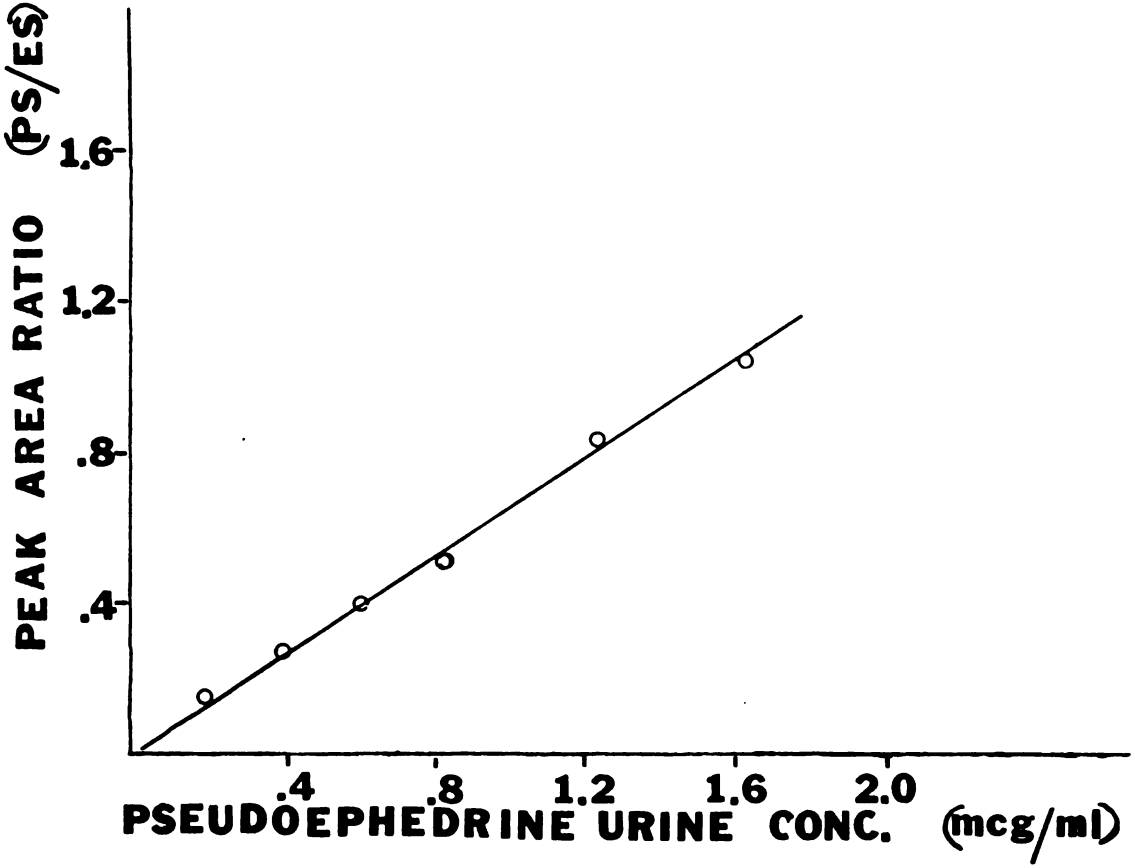


Figure 14. Calibration curve relating peak area ratio of pseudoephedrine (PS) to internal standard (ES) vs urine concentration of PS.

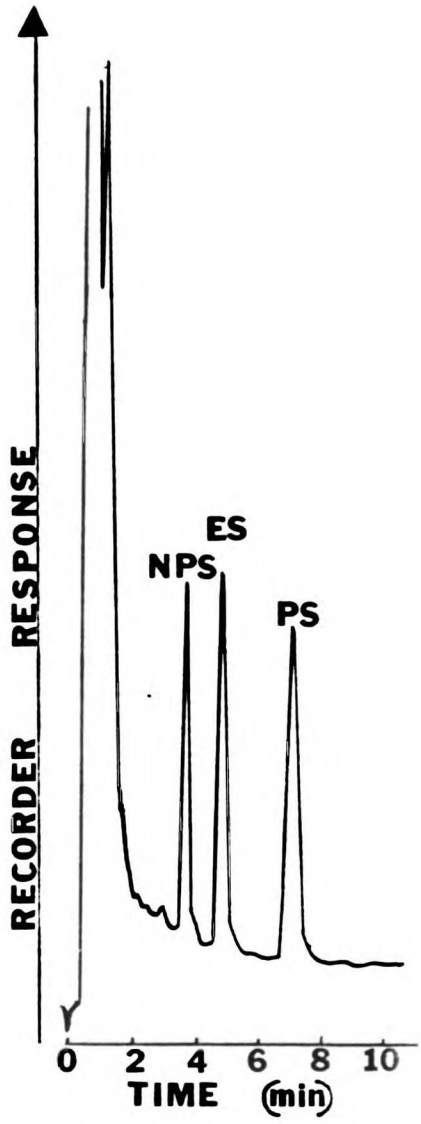


Figure 15. Chromatogram of norpseudoephedrine (NPS), ephedrine sulfate (ES) and pseudophedrine (PS) in water. Instrument attenuation was set at 64.

Pharmacokinetic parameters derived from PS plasma concentration-time curves (figure 16) are found in Table VIII for each of the three rats. Average parameters are α = 0.152 min^{-1}, β = 0.020 min^{-1}, k_{13} = 0.048 min^{-1}, k_{12} = 0.060 min^{-1}, k_{21} = 0.063 min^{-1}, V_1 = 378 ml. Average plasma clearance calculated model independently from dose divided by area under the plasma concentration time curve (AUC) and model dependently from the product k_{13} x V_1 were 67.9 ml/min·Kg and 70.2 ml/min·Kg respectively.

Urinary excretion data is found in Table IX. Renal clearance was calculated by:

$$CL_{PS} = \frac{\text{amt PS excreted in urine}_t^{t'}}{AUC_t^{t'}} \qquad \text{(eq. 7)}$$

where t is the beginning of urine collection and t' is the end of urine collection. An estimate of the fraction of the dose excreted as unchanged pseudoephedrine was calculated from the ratios of the average renal clearance value for each rat to the model independently determined clearance for that rat. The average fraction excreted unchanged for the three rats was 0.45.

2. Intravenous infusion of pseudoephedrine

Based on the pharmacokinetic parameters from the intravenous bolus studies, priming doses and infusion rates of pseudoephedrine were calculated to yield steady-state plasma levels of 0.15 mcg/ml pseudoephedrine. Doses were calculated such that the desired steady-state level would be closely approximated within 30 minutes. The following method was used to calculate these doses:

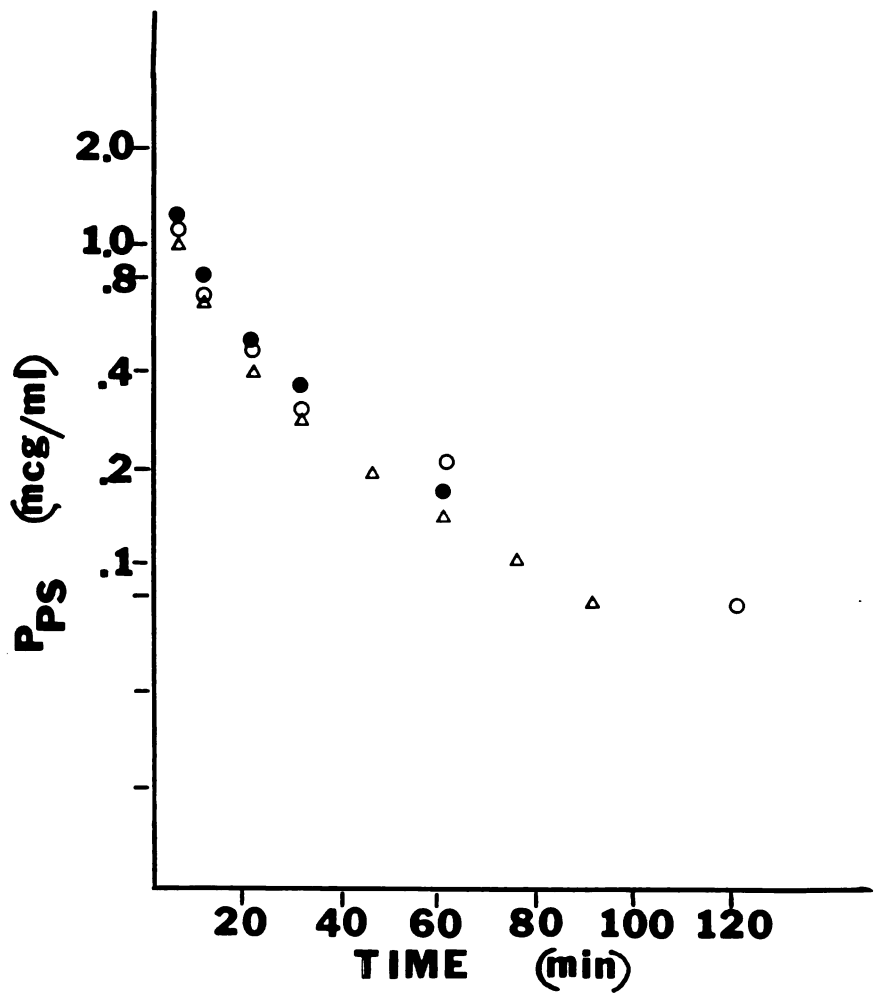


Figure 16. Semilogarithmic plot of pseudoephedrine plasma concentration (P_{PS}) versus time following intravenous bolus doses of PS to three rats. Rat A (●) received 0.61 mg PS; Rat B (o) received 0.73 mg PS; Rat C (Δ) received 0.68 mg PS.

Table VIII

Pharmacokinetic Parameters Obtained from Plasma Concentration vs Time Plots for Pseudoephedrine following an I.V. Bolus Dose of Pseudoephedrine

	Rat A (Dose = 0.61 mg)	Rat B (Dose = 0.73 mg)	Rat C (Dose = 0.68 mg)	Mean ± S.D.
A (mcg/ml)	1.40	1.00	1.30	1.23 ± 0.21
B (mcg/ml)	0.75	0.56	0.48	0.60 ± 0.14
α (min^{-1})	0.187	0.131	0.138	0.152 ± 0.030
$t_{1/2\alpha}$ (min)	3.7	5.3	5.0	4.7 ± 0.8
β (min^{-1})	0.0231	0.0165	0.0204	0.0200 ± 0.0033
$t_{1/2\beta}$ (min)	30	42	34	35 ± 6
V_1 (ml)	284	468	382	378 ± 92
k_{12} (min^{-1})	0.0760	0.0524	0.0523	0.0602 ± 0.0136
k_{21} (min^{-1})	0.0830	0.0576	0.0521	0.0633 ± 0.0150
k_{13} (min^{-1})	0.0538	0.0375	0.0540	0.0484 ± 0.0095
AUC $\int_0^\infty$ (mcg-min/ml)	41.0	43.2	34.4	39.5 ± 4.6

Table IX

Urinary Excretion Parameters Following an I.V. Bolus Dose of Pseudoephedrine (PS)

Rat	Time Interval (min)	$\dot{V}$ (ml/min·kg)	Urinary pH	U_{PS} (mcg/ml)	CL_{PS} (ml/min·kg)	f_e[a]
A	10-30	0.0776	6.2	213.2	27.56	
	30-60	0.0857	6.0	91.02	28.20	0.46
B	30-60	0.0697	6.0	98.40	24.67	0.36
C	30-60	0.0796	6.2	99.22	33.92	
	60-90	0.0806	6.2	61.50	42.08	0.52

[a]Estimated fraction of dose excreted in the urine unchanged

At steady-state

$$R^{o} = (k_{13} \cdot V_1) \cdot P_{ss} \qquad \text{(eq. 8)}$$

where R^{o} = infusion rate in amt/time and P_{ss} = steady-state plasma concentration of drug. After determining the rate of infusion necessary to reach the desired steady-state plasma level, the plasma level at anytime (P_t), when infusing drug at this rate, was calculated from equation 9.

$$P_t = k_{13} \cdot P_{ss} \frac{(k_{21} - \alpha)\ (e^{-\alpha t} - 1)}{-\alpha(\beta - \alpha)} + \frac{(k_{21} - \beta)\ (e^{-\beta t} - 1)}{-\beta(\alpha - \beta)} \qquad \text{(eq. 9)}$$

Subtracting P_t from P_{ss} gave the plasma level needed from a priming dose at time, t = 30 minutes. The priming dose was then calculated from a re-arrangement of equation 6.

$$(P_{ss} - P_t) = \frac{\text{Dose}}{V_1} \frac{(k_{21} - \alpha)}{(\beta - \alpha)} e^{-\alpha t} + \frac{(k_{21} - \beta)}{(\alpha - \beta)} e^{-\beta t} \qquad \text{(eq. 10)}$$

Steady-state PS plasma levels in four rats were 0.15 mcg/ml, 0.22 mcg/ml, 0.24 mcg/ml and 0.13 mcg/ml following priming doses of 150 mcg PS and infusion rates of 2.67 mcg PS/min. The desired plasma level was 0.15 mcg/ml.

E. Pseudoephedrine Protein Binding Studies

The protein binding of pseudoephedrine was measured in fresh human plasma at pseudoephedrine plasma concentrations of 0.2 to 1.8 mcg/ml. The average per cent free (per cent of total concentration not bound to plasma protein) for 10 measurements was 79.4 $\pm$ 7.3. There was a tendency for the percent free or unbound fraction to increase somewhat

at the higher pseudoephedrine concentrations.

F. Red Blood Cell Partitioning of Pseudoephedrine

Red blood cell (RBC) partitioning was measured in rat whole blood at pseudoephedrine (PS) concentrations of 0.30 mcg/ml and 0.60 mcg/ml. The results are reported as RBC PS concentration divided by plasma PS concentration. The average RBC partitioning for 7 measurements was 2.14 $\pm$ 0.15. Hematocrit was 0.41.

G. Pseudoephedrine Excretion Studies

1. Clearance experiments

Table X contains the results of a single clearance experiment. The rat was given a priming dose of 150 mcg PS and an infusion of 2.67 mcg PS/min. The results for each of the four clearance periods and the average for the four periods are reported. A steady-state PS plasma level of 0.21 mcg/ml was reached. Average renal clearance of PS was 22.94 ml/min·Kg. The ratio of PS renal clearance to inulin clearance (CL_{PS}/CL_I) was 3.07. Average transport rate was 3.57 mcg/min·Kg. Table XI contains the results for all 3 rats dosed at this level. Each value is the average of four clearance periods.

2. Saturation experiments

The results of the studies where saturation of transport was tested by increasing the plasma concentration of PS are summarized in Table XII. Pseudoephedrine was infused at rates ranging from 3.56 mcg/min to 21.36 mcg/min; corresponding priming doses were 200 mcg to 1200 mcg PS.

Table X

Excretion Parameters for Four Clearance Periods
Following an I.V. Infusion of Pseudoephedrine (PS) in a Rat[a]

Period	$\dot{V}$ (ml/min·kg)	U_{PS} (mcg/ml)	P_{PS} (mcg/ml)	CL_{PS} (ml/min·kg)	Urinary pH	CL_I (ml/min·kg)	$\frac{CL_{PS}}{CL_I}$	T_{PS} (mcg/min·kg)
1	0.103	51.04	0.20	26.33	6.33	7.92	3.32	4.00
2	0.099	46.94	0.21	21.84	6.31	6.52	3.35	3.56
3	0.103	48.79	0.22	23.41	6.32	8.65	2.71	3.51
4	0.095	45.92	0.20	21.87	6.30	7.16	3.05	3.22
Mean	0.100	48.17	0.21	22.94[b]	6.31	7.48[b]	3.07[b]	3.57[b]
S.D. ±	0.004	2.25	0.01	----	0.01	----	----	----

[a] Dose = 150 mcg PS bolus, 2.67 mcg PS/min infusion (4% inulin, 5% mannitol in 0.9% NaCl)

[b] Calculated from mean parameter values

Table XI

Average Excretion Parameters[a] for Three Rats Receiving Pseudoephedrine (PS) As a 150 mcg Priming Dose[b] and 2.67 mcg/min Infusion[b]

Rat No.	$\dot{V}$ (ml/min·kg)	U_{PS} (mcg/ml)	P_{PS} (mcg/ml)	CL_{PS} (ml/min·kg)	Urinary pH	CL_{PS} (ml/min·kg)	$\frac{CL_{PS}}{CL_I}$	T_{PS} (mcg/min·kg)
1	0.100	48.17	0.21	22.94	6.32	7.48	3.07	3.57
2	0.142	45.94	0.16	40.77	6.18	8.45	4.82	5.45
3	0.231	28.14	0.27	24.08	6.02	8.39	2.87	4.70
Mean	0.157	40.75	0.21	29.26	6.17	8.11	3.59	4.57
S.D. $\pm$	0.067	10.95	0.06	9.98	0.15	0.54	1.07	0.95

[a]Values for each rat are the average of four clearance periods

[b]Priming dose and infusion contained 4% inulin and 5% mannitol

Table XII

Renal Clearance Parameters Obtained Following I.V. Infusion of Pseudoephedrine (PS) in 19 Rats[a]

Rat No.	$\dot{V}$ (ml/min·kg)	U_{PS} (mcg/ml)	P_{PS} (mcg/ml)	CL_{PS} (ml/min·kg)	Urinary pH	CL_I (ml/min·kg)	$\frac{CL_{PS}}{CL_I}$	T_{PS} (mcg/min·kg)
1[A']	0.100	48.17	0.21	22.94	6.32	7.48	3.07	3.57
2[A']	0.142	45.94	0.16	40.77	6.18	8.45	4.82	5.45
3[A']	0.231	28.14	0.27	24.08	6.02	8.39	2.87	4.70
4[A]	0.092	69.84	0.32	20.08	6.14	6.59	3.05	4.75
5[A]	0.147	54.70	0.31	26.00	---	8.90	2.92	5.85
6[A]	0.114	81.75	0.26	35.84	6.13	8.64	4.15	7.54
7[A]	0.091	82.50	0.24	31.28	6.37	7.26	4.31	6.12
8[B]	0.103	119.00	0.35	35.02	6.26	6.23	5.62	10.52
9[B]	0.160	93.12	0.36	41.09	6.00	8.69	4.76	12.42
10[B]	0.206	60.51	0.32	38.95	6.01	10.50	3.71	9.80
11[B]	0.166	67.26	0.28	39.88	6.02	10.83	3.68	8.76
12[B]	0.223	54.00	0.36	33.45	5.92	8.84	3.79	9.52
13[B]	0.168	78.12	0.52	25.24	5.95	8.31	3.04	9.69
14[C]	0.116	194.82	0.77	29.10	6.94	7.73	3.76	17.87
15[C]	0.132	211.65	0.80	34.92	6.30	7.52	4.62	23.16
16[D]	0.111	382.50	1.50	28.30	6.80	6.80	4.16	34.34
17[D]	0.075	330.00	1.20	20.62	6.76	5.30	3.89	19.70

Table XII (Continued)

Rat No.	$\dot{V}$ (ml/min·kg)	U_{PS} (mcg/ml)	P_{PS} (mcg/ml)	CL_{PS} (ml/min·kg)	Urinary pH	CL_I (ml/min·kg)	$\frac{CL_{PS}}{CL_I}$	T_{PS} (mcg/min·kg)
18^D	0.106	405.00	1.40	30.66	6.46	6.87	4.46	35.29
19^D	0.100	335.00	1.22	27.46	6.28	6.90	3.98	26.82

a All priming doses and infusions contained 4% inulin (I) and 5% mannitol

A' Dose = 150 mcg bolus, 2.67 mcg/min infusion

A Dose = 200 mcg bolus, 3.56 mcg/min infusion

B Dose = 300 mcg bolus, 5.35 mcg/min infusion

C Dose = 600 mcg bolus, 10.68 mcg/min infusion

D Dose = 1200 mcg bolus, 21.36 mcg/min infusion

Included in the table for comparison purposes are the results of the 3 rats infused with 2.67 mcg/min. Steady-state PS plasma levels ranged from 0.16 mcg/ml to 1.5 mcg/ml. There was no apparent systematic change in CL_{PS} or CL_{PS}/CL_I with increasing plasma concentration; T_{PS} increased with increasing PS plasma concentration. The clearance ratio of PS to inulin (CL_{PS}/CL_I) vs P_{PS} is plotted in figure 17; T_{PS} vs P_{PS} is plotted in figure 18. A partial correlation and multivariate regression analysis (BMDP6R, Health Sciences Computing Facility, University of California, Los Angeles) was performed to determine the relationship between the independent variables P_{PS}, $\dot{V}$, urinary pH and the dependent variables CL_{PS}/CL_I and CL_{PS}. A t-statistic was calculated for each independent variable for the significance of that independent variable given the other independent variables. There was no significant partial correlation between P_{PS}, $\dot{V}$, urinary pH and CL_{PS}/CL_I, CL_{PS} at the 0.05 level.

3. Effect of urinary pH on pseudoephedrine renal excretion

The effect of urinary pH on PS clearance parameters was studied in 5 rats. The results of these experiments are summarized in Table XIII. The two clearance periods for acid conditions and the two periods for basic conditions were averaged to give single values for each condition. In rats 1, 2 and 3, CL_{PS}, CL_{PS}/CL_I and T_{PS} decreased as urinary pH increased; in rats 4 and 5, CL_{PS}, CL_{PS}/CL_I and T_{PS} increased as urinary pH decreased. Figure 19 is a graphical interpretation of the effect of urinary pH on the clearance ratio of PS to inulin (CL_{PS}/CL_I).

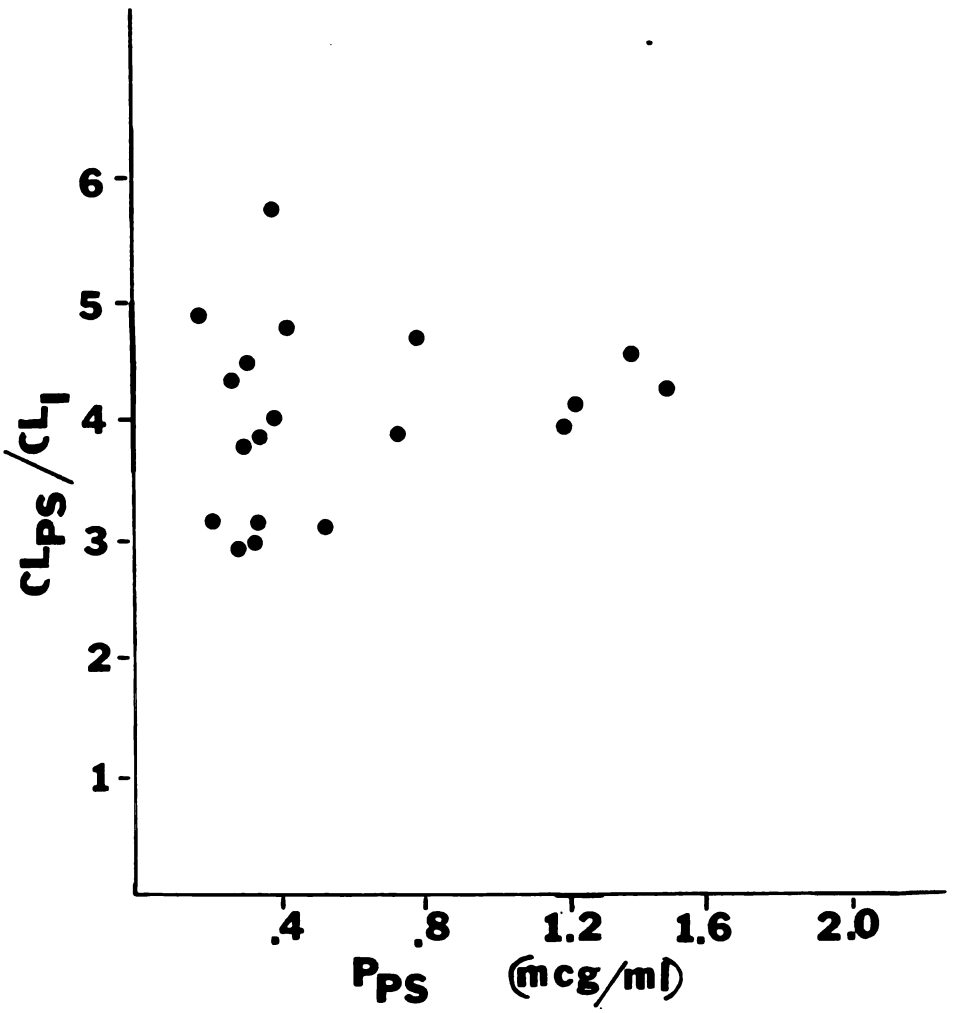


Figure 17. Ratio of pseudoephedrine (PS) to inulin clearance (CL_{PS}/CL_I) as a function of PS plasma concentration.

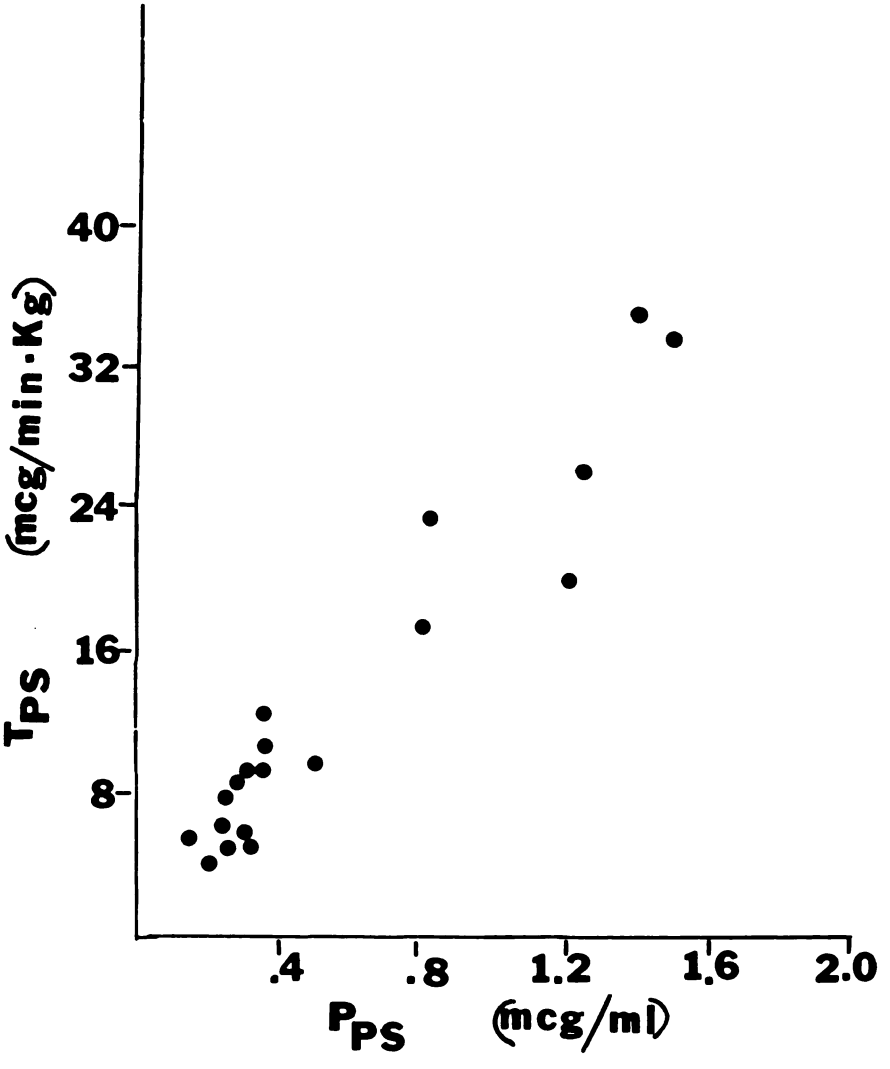


Figure 18. Transport rate of pseudoephedrine (T_{PS}) plotted as a function of pseudoephedrine plasma concentration (P_{PS}).

Table XIII

Renal Clearance Parameters Following Infusion of Pseudoephedrine (PS) Under Acidic[a] and Basic[b] Conditions

Rat No.		$\dot{V}$ (ml/min·kg)	U_{PS} (mcg/ml)	P_{PS} (mcg/ml)	CL_{PS} (ml/min·kg)	Urinary pH	CL_I (ml/min·kg)	$\frac{CL_{PS}}{CL_I}$	T_{PS} (mcg/min·kg)
1	Acid	0.693	9.25	0.16	40.05	5.81	12.01	3.33	4.88
	Base	0.433	3.75	0.22	7.38	7.17	10.13	0.73	-0.15
2	Acid	0.345	13.50	0.11	42.36	6.69	10.64	3.98	3.73
	Base	0.335	9.50	0.12	26.52	6.99	11.69	2.27	2.06
3	Acid	0.139	33.50	0.13	35.82	5.94	10.29	3.48	3.59
	Base	0.180	14.25	0.16	16.08	7.17	9.76	1.65	1.33
4	Base	0.326	8.50	0.12	23.12	7.71	13.68	1.69	1.47
	Acid	0.253	13.88	0.15	23.39	6.66	11.84	1.98	2.10
5	Base	0.248	10.25	0.21	12.11	7.68	11.73	1.03	0.59
	Acid	0.204	16.38	0.16	20.85	6.65	8.96	2.33	2.20

a 1.61% Na_2SO_4 added to infusion solutions (and also given as priming dose)

b 1.40% $NaHCO_3$ added to infusion solutions (and also given as priming dose)

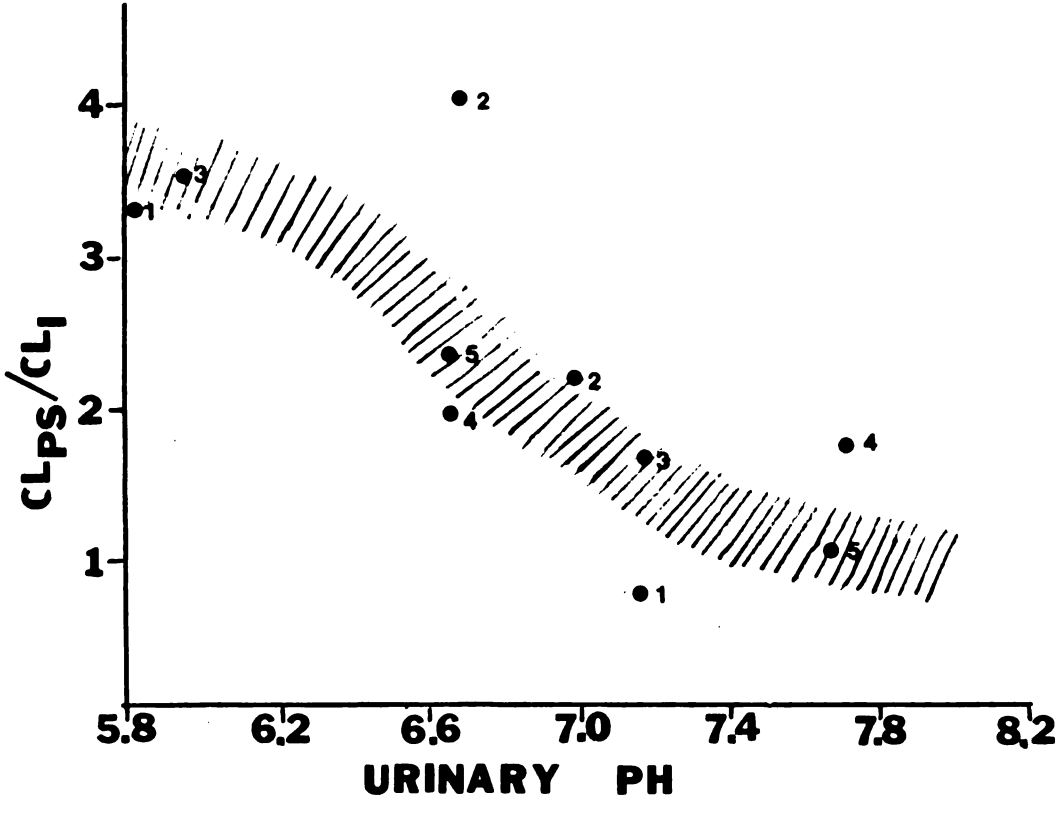


Figure 19. Relationship between CL_{PS}/CL_I and urinary pH. Numbers next to data points refer to rat nos. in Table XII. Hatched line represents an approximate fit of the data.

4. Inhibition experiments: mepiperphenidol

Check for assay interference: When a water sample spiked with mepiperphenidol was assayed for pseudoephedrine, the chromatogram was not different from that of a blank water sample; the chromatogram for a sample spiked with ephedrine sulfate (ES) and mepiperphenidol showed only one peak with a retention time of 4.9 minutes; the chromatogram spiked with ES, PS and mepiperphenidol showed two peaks corresponding to ES and PS (retention times 4.9 and 7.3 minutes respectively). Figure 20, panels A and B are plasma and urine chromatograms of the samples taken from a rat infused with mepiperphenidol. The samples were spiked with ES and assayed for "pseudoephedrine". Only one peak, corresponding to ES can be seen. No peaks are seen at or near the point where the PS peak would appear.

Inhibition studies: The results of a single inhibition experiment in one rat are found in Table XIV. During the control periods, the rat was infused with 2.67 mcg PS/min. After a bolus of 5 mg Mepi/Kg, an infusion containing both PS and Mepi (0.6%) at a rate of 50 µl/min was begun and the rat was re-equilibrated for 10 minutes. Two inhibition clearance periods were then followed. On the average, in the presence of Mepi, P_{PS} increased, CL_{PS}, CL_{PS}/CL_I and T_{PS} decreased. The results for all inhibition studies with mepiperphenidol are summarized in Table XV. The results are reported as the average of two control periods and average of two inhibition periods for each rat. The dosing schedules are as described in Table III in Chapter 2, Experimental. In most cases P_{PS} increased and, CL_{PS} and CL_{PS}/CL_I decreased during the inhibition period. Rat 8 had a decrease in P_{PS} and

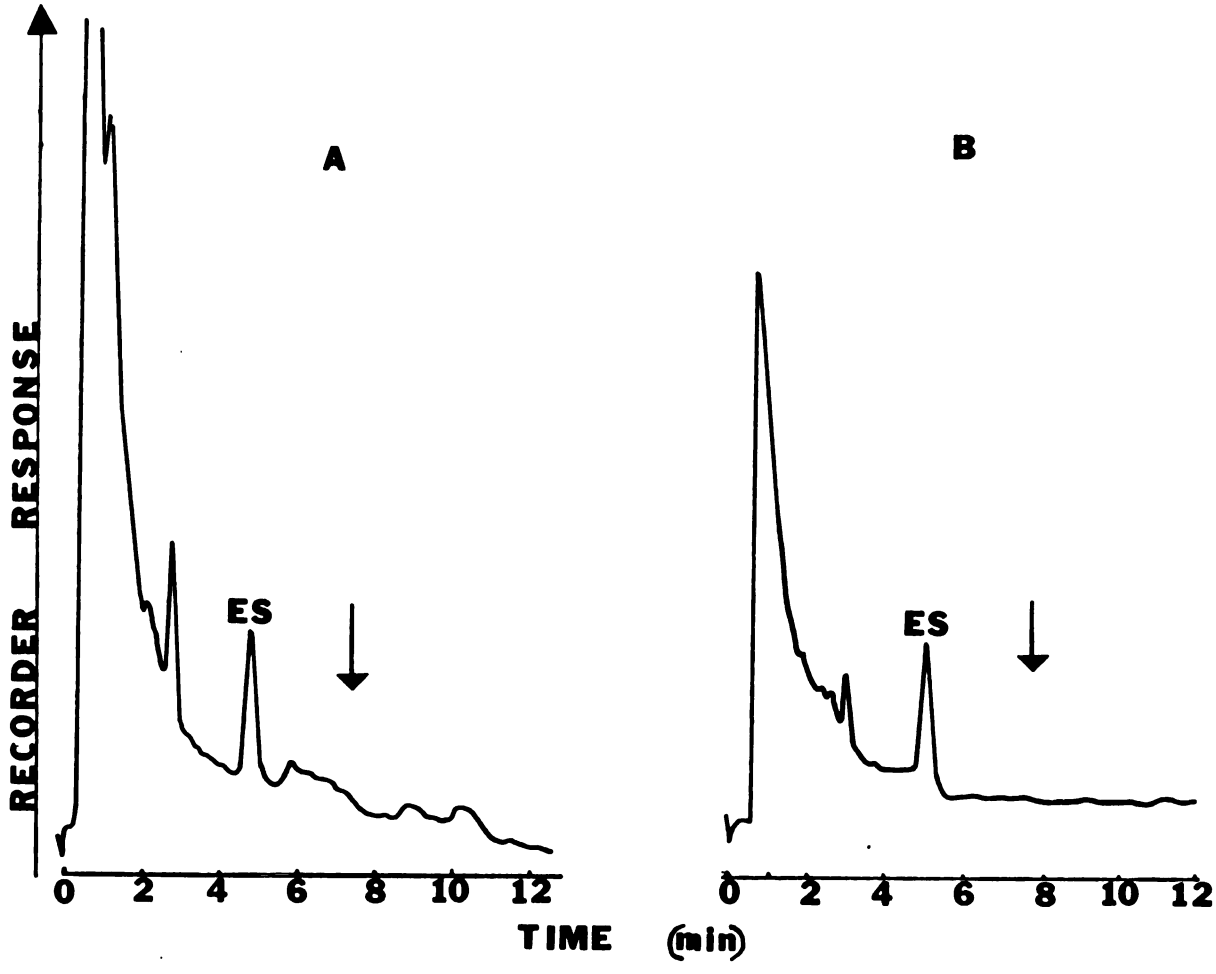


Figure 20. Chromatograms of rat plasma (A) and urine (B) following infusion of mepiperphenidol. ↓ = point where pseudoephedrine would be expected. ES = internal standard, ephedrine sulfate. Instrument attenuation was set at 16 for plasma, 32 for urine.

Table XIV

Results from a Single Pseudoephedrine (PS)-Mepiperphenidol (Mepi) Inhibition Study in One Rat

	$\dot{V}$ (ml/min·kg)	U_{PS} (mcg/ml)	P_{PS} (mcg/ml)	CL_{PS} (ml/min·kg)	Urinary pH	CL_I (ml/min·kg)	$\frac{CL_{PS}}{CL_I}$	T_{PS} (mcg/min·kg)
Control[a]								
1	0.078	33.62	0.18	14.51	6.40	3.86	3.76	2.07
2	0.105	35.26	0.20	18.46	6.40	5.52	3.34	2.82
Mean	0.092	34.44	0.19	16.48	6.40	4.69	3.55	2.45
Inhibition[b]								
1	0.094	36.08	0.22	15.51	6.20	5.93	2.62	2.36
2	0.081	35.26	0.28	10.21	6.10	4.35	2.35	1.89
Mean	0.088	35.67	0.25	12.86	6.15	5.14	2.48	2.12

[a] Infusion = 2.67 mcg PS/min, 4% inulin, 5% mannitol

[b] Infusion = 2.67 mcg PS/min, 0.6% Mepi, 4% inulin, 5% mannitol

Table XV

Results from Pseudoephedrine (PS)-Mepiperphenidol Inhibition Studies in 18 Rats

Dosing Schedule	Rat No.	Period	$\dot{V}$ (ml/min·kg)	U_{PS} (mcg/ml)	P_{PS} (mcg/ml)	CL_{PS} (ml/min·kg)	Urinary pH	CL_I (ml/min·kg)	$\frac{CL_{PS}}{CL_I}$	T_{PS} (mcg/min·kg)
A	1	C	0.091	34.44	0.19	16.49	6.40	4.67	3.53	2.98
		I	0.088	35.67	0.25	12.56	6.10	5.09	2.47	2.13
	2	C	0.091	86.50	0.58	13.57	5.96	5.62	2.41	5.28
		I	0.056	50.00	0.56	5.00	6.28	2.59	1.93	1.65
B	3	C	0.352	36.50	0.34	37.79	6.35	9.53	3.96	10.28
		I	0.158	24.00	0.31	12.23	6.23	7.17	1.70	2.03
	4	C	0.383	38.40	0.42	35.02	6.24	10.84	3.23	11.09
		I	0.166	28.50	0.38	12.45	6.34	6.88	1.81	2.66
C	5	C	0.142	38.50	0.24	22.78	5.90	9.62	2.37	3.63
		I	0.189	27.00	0.38	13.43	5.83	8.28	1.62	2.60
	6	C	0.087	57.00	0.20	24.80	6.34	9.12	2.72	3.51
		I	0.129	44.50	0.26	22.08	6.12	10.70	2.06	3.53
	7	C	0.098	59.75	0.22	26.60	6.22	10.48	2.54	4.02
		I	0.205	31.00	0.31	20.50	5.73	10.73	1.91	3.71

C = Average of two control periods; I = Average of two inhibition periods

Table XV (Continued)

Dosing Schedule	Rat No.	Period	$\dot{V}$ (ml/min·kg)	U_{PS} (mcg/ml)	P_{PS} (mcg/ml)	CL_{PS} (ml/min·kg)	Urinary pH	CL_I (ml/min·kg)	$\frac{CL_{PS}}{CL_I}$	T_{PS} (mcg/min·kg)
D	8	C	0.091	50.50	0.16	28.72	6.55	7.87	3.65	3.60
		I	0.089	53.00	0.14	33.69	6.36	7.93	4.25	3.84
	9	C	0.105	42.75	0.14	32.06	6.28	9.57	3.35	3.42
		I	0.157	39.37	0.28	22.08	5.84	9.93	2.22	3.97
	10	C	0.096	51.00	0.22	22.25	6.10	8.76	2.54	3.36
		I	0.136	33.00	0.27	16.62	6.14	10.29	1.62	2.28
	11	C	0.086	42.00	0.11	32.95	6.64	8.55	3.85	2.86
		I	0.126	38.00	0.20	23.92	6.57	11.70	2.04	2.93
	12	C	0.169	35.00	0.10	59.22	5.86	13.41	4.42	4.85
		I	0.331	21.00	0.17	40.86	6.06	12.48	3.27	5.27
E	13	C	0.142	22.50	0.16	20.80	5.35	10.66	1.93	1.84
		I	0.217	20.50	0.22	19.40	5.12	12.84	1.52	2.20
	14	C	0.192	31.75	0.15	40.42	5.99	12.92	3.21	4.56
		I	0.213	21.75	0.21	21.86	5.66	11.14	1.96	2.78

Table XV (Continued)

Dosing Schedule	Rat No.	Period	$\dot{V}$ (ml/min·kg)	U_{PS} (mcg/ml)	P_{PS} (mcg/ml)	CL_{PS} (ml/min·kg)	Urinary pH	CL_I (ml/min·kg)	$\frac{CL_{PS}}{CL_I}$	T_{PS} (mcg/min·kg)
F	15	C	0.275	24.25	0.14	47.60	6.15	12.27	3.88	5.30
		I	0.305	24.25	0.18	41.14	6.05	10.59	3.88	5.88
	16	C	0.241	24.75	0.12	49.75	5.70	12.33	4.03	4.79
		I	0.264	25.25	0.20	33.38	5.80	10.39	3.21	5.02
G	17	C	0.313	21.00	0.14	45.29	6.03	12.26	3.69	5.21
		I	0.246	24.25	0.22	27.08	5.67	7.57	3.58	4.64
	18	C	0.127	42.50	0.12	43.11	6.03	10.18	4.23	4.43
		I	0.146	49.00	0.22	32.60	5.46	10.53	3.10	5.31

an increase in CL_{PS}, CL_{PS}/CL_I and T_{PS} in the inhibition period. Several rats had an apparent increase in T_{PS} in the inhibition period; rat 15 had a slight increase in T_{PS} and CL_{PS}/CL_I stayed the same. The increase in P_{PS} and decrease in CL_{PS} and CL_{PS}/CL_I in the presence of Mepi were found to be significant ($p<0.001$) using a paired t-test.

5. Inhibition experiments: Procainamide

Check for assay interference: Figure 21, panels A and B are chromatograms of plasma and urine samples from a rat infused with procainamide which were assayed for "pseudoephedrine". The samples were spiked with ephedrine sulfate (ES), the internal standard. There were no peaks corresponding to pseudoephedrine (7.3 minutes).

Inhibition studies: Inhibition studies were run in two rats. The average results for the control periods and inhibition periods for each rat are given in Table XVI. In both rats, P_{PS} increased and CL_{PS} and CL_{PS}/CL_I decreased during the inhibition period. In rat 1, T_{PS} also decreased during inhibition, but in rat 2, there was an increase in T_{PS}.

6. Inhibition experiments: ethambutol

Check for assay interference: Figure 22, panels A and B are chromatograms of plasma and urine samples from a rat infused with ethambutol. The samples were spiked with ephedrine sulfate (ES) and assayed for "pseudoedphedrine". No peaks corresponding to pseudoephedrine were found in either chromatogram.

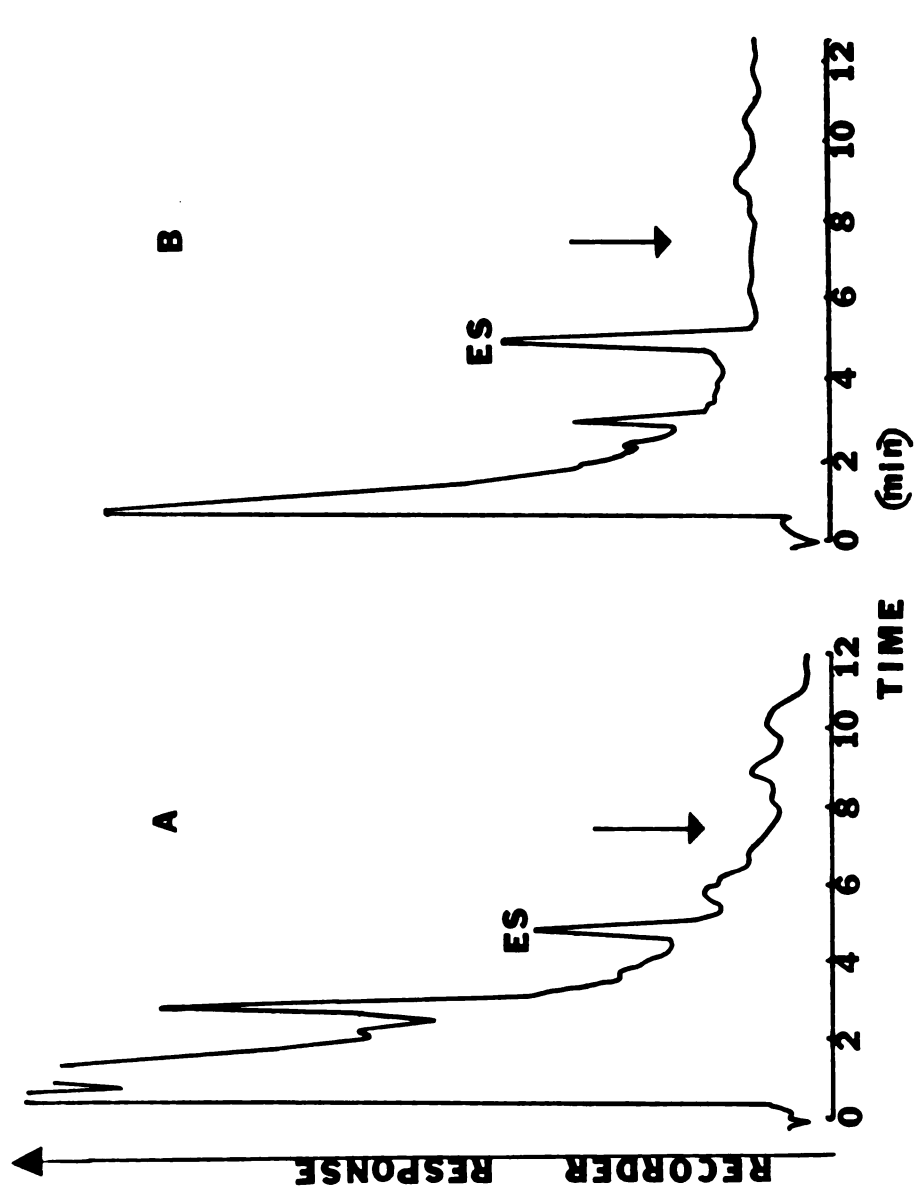


Figure 21. Chromatograms of rat plasma (A) and urine (B) following infusion of procainamide. ↓ = point where pseudoephedrine peak would be expected. ES = internal standard, ephedrine sulfate. Instrument attenuation was set at 16 for plasma, 32 for urine.

Table XVI

Results from Pseudoephedrine (PS)-Procainamide[a] Inhibition Studies in 2 Rats

Rat No.	Period	$\dot{V}$ (ml/min·kg)	U_{PS} (mcg/ml)	P_{PS} (mcg/ml)	CL_{PS} (ml/min·kg)	Urinary pH	CL_I (ml/min·kg)	$\frac{CL_{PS}}{CL_I}$	T_{PS} (mcg/min·kg)
1	C	0.137	39.00	0.14	38.11	5.47	11.19	3.40	4.10
	I	0.154	31.25	0.23	20.88	5.27	10.42	2.00	2.91
2	C	0.069	55.00	0.13	29.19	6.38	10.04	2.91	2.76
	I	0.104	49.25	0.21	24.51	6.08	9.98	2.45	3.46

[a] Procainamide infused at a rate of 200 mcg/min

C = Average of two control periods; I = Average of two inhibition periods

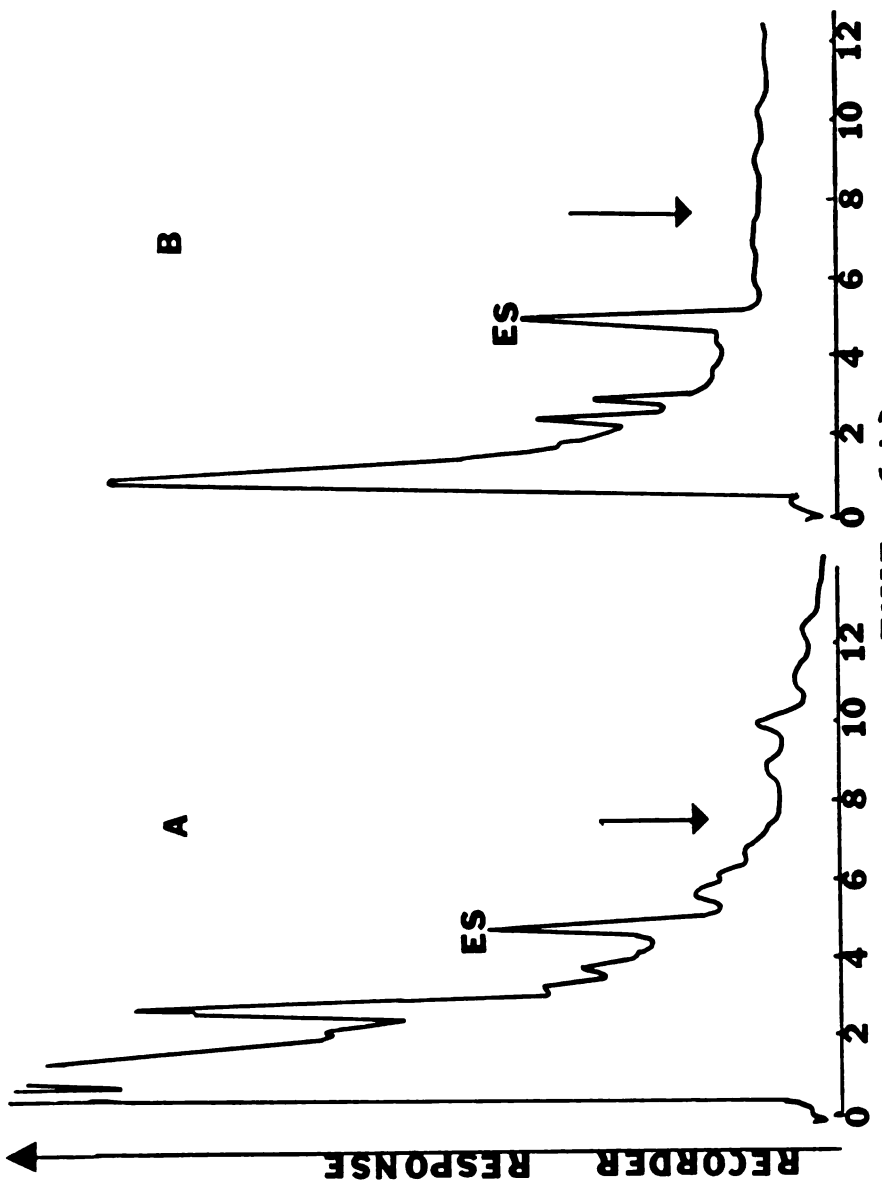


Figure 22. Chromatograms of rat plasma (A) and urine (B) following infusion of ethambutol. ↓ = point where pseudoephedrine peak would be expected. ES = internal standard, ephedrine sulfate. Instrument attenuation was set at 16 for plasma, 32 for urine.

Inhibition studies: Inhibition studies were run in 4 rats at two different dosage levels of ethambutol. The average results for the control periods and inhibition periods for each rat are given in Table XVII. In 3 rats P_{PS} increased slightly, CL_{PS}, CL_{PS}/CL_I and T_{PS} also increased. In the fourth rat, there was a large increase in P_{PS} and a corresponding large decrease in CL_{PS}, CL_{PS}/CL_I and T_{PS}.

Table XVII

Results from Pseudoephedrine (PS)-Ethambutol Inhibition Studies in 4 Rats

Rat No.	Period	$\dot{V}$ (ml/min·kg)	U_{PS} (mcg/ml)	P_{PS} (mcg/ml)	CL_{PS} (ml/min·kg)	Urinary pH	CL_I (ml/min·kg)	$\frac{CL_{PS}}{CL_I}$	T_{PS} (mcg/min·kg)
1[a]	C	0.086	45.25	0.15	25.85	6.58	11.77	2.20	2.48
	I	0.129	35.75	0.17	27.04	6.30	11.66	2.32	3.04
2[a]	C	0.133	28.00	0.11	33.75	6.01	11.11	3.04	2.75
	I	0.163	25.78	0.12	35.03	6.00	7.96	4.40	3.44
3[b]	C	0.210	21.50	0.14	32.19	6.58	10.70	3.01	3.32
	I	0.206	26.25	0.16	33.91	6.46	9.00	3.77	4.26
4[b]	C	0.300	18.50	0.16	34.69	5.29	9.76	3.55	4.31
	I	0.149	23.50	0.25	14.01	5.62	8.83	1.59	1.75

[a] Ethambutol infused at a rate of 60 mcg/min

[b] Ethambutol infused at a rate of 120 mcg/min

C = average of two control periods; I = average of two inhibition periods

CHAPTER 4

DISCUSSION

A. Animal Set-up

The animal model, surgical techniques and experimental procedures employed proved to be adequate for measurement of renal clearance and demonstration of saturation and inhibition of tubular transport.

Surgical techniques were performed so as to cause a minimal amount of trauma. Time for surgery was generally 15 minutes or less. There has been some question as to the appropriateness of sodium pentobarbital as the anesthetic agent in clearance experiments. Kau et al (76) claimed that pentobarbital caused a depression of renal function due to the lowering of blood pressure. These authors used ketamine as an anesthetic agent. After an initial i.p. injection of inulin, inulin was infused continuously and renal clearance was followed at twenty minute intervals for 140 minutes. Glomerular filtration rate (GFR) remained steady at 11.0 - 11.7 ml/min·Kg. Guignard and Peters (92) used sodium pentobarbital as the anesthetic agent when looking at the effects of diuretics on urinary acidification and potassium excretion in the rat. Experiments were stopped whenever mean blood pressure of the anesthetized animal fell below 90 mg Hg (less than 10% of the rats in each group). The authors found an average GFR of 8.5 ml/min·Kg in rats infused with 0.15 M NaCl and inulin. Brennan et al (77) used 25% urethane as the anesthetic when studying renal clearance in the rat. Average GFR obtained for controls in two studies was 9.2 ml/min·Kg and 11.5

ml/min·Kg. Glomerular filtration rate as measured by inulin clearance in the current work using sodium pentobarbital as the anesthetic agent ranged from an average of 6.6 ml/min·Kg in the NMN experiments (Table VI) to 7.9 ml/min·Kg in the PS experiments (Table XII), 9.6 ml/min·Kg in PS - Mepi inhibition experiments (Table XV) and an average value of 11.1 ml/min·Kg in studies of the effect of urinary pH on PS clearance (Table XIII). Although there was quite a bit of variation in GFR between rats, GFR (reported as CL_I) was relatively constant within a rat over four clearance periods (Tables V and X). Smith et al (93) report that when dogs were anesthetized with sodium pentobarbital (30 mg/Kg i.p.) there was no consistent change in inulin and diodrast clearances or in the T_M for diodrast. They also reported that pentobarbital did not depress the extraction ratio of diodrast and concluded that the renal plasma flow was not changed during anesthesia.

In the clearance experiments it was important to maintain an adequate urine flow for several reasons: 1.) To insure complete flushing of the bladder and to minimize the effect of any residual urine which might remain in the bladder following a collection period; 2.) To give a sufficient volume of urine for accurate pH measurement and for drug and inulin assay; 3.) To minimize the effect of urinary flow on pH dependent reabsorption of drug from tubule lumen to plasma; 4.) To eliminate the possibility of flow effects on inulin clearance. Barber and Bourne (95) found that in rats weighing 320 - 420 gm, inulin clearance was independent of urine flow when flow rates were greater than 0.03 ml/min (0.071 - 0.094 ml/min·Kg), but at

lower flow rates inulin clearance was reduced and appeared to correlate with the rate of flow. Infusion of 5% mannitol produced adequate urine flow rates. In only a few instances were rates less than 0.03 ml/min in the present work and when calculated on a per Kg basis, they were always greater than 0.07 ml/min·Kg. No correlation was observed between inulin clearances and urinary flow rate. In some of the PS-Mepi inhibition studies there did appear to be a relationship between urinary flow and the apparent transport. This will be discussed later.

An important problem often encountered in these studies was respiratory distress in the rat. This was at first thought to be a result of anesthesia, surgical techniques, drug toxicities, etc. Often kidney function declined drastically and the results of an experiment were discarded. In a number of instances death resulted. It was eventually concluded that the cause of the problem was a pre-existing respiratory problem prevalent among rats purchased from Simonson (Gilroy, California). Charles River rats (Charles River, Massachusetts) were used in all experiments on the effect of urinary pH on PS excretion, procainamide and ethambutol - PS inhibition studies and in several PS-Mepi inhibition studies (rats 11-18). None of these rats exhibited any signs of respiratory distress throughout the experiments. In general inulin clearances were higher than those found for the Simonson rats.

When using the rat as an experimental model it is important to be aware of variables which when uncontrolled might account for some of

the variation in the data. Housing, handling, feeding, diurnal rhythms are a few such variables. Although these variables were controlled to a limited extent in our experiments, they may still have contributed to the observed inter-animal variation in NMN and pseudoephedrine renal excretion parameters.

B. Inulin Assay

The conditions of the inulin assay allowed for formation of a stable reproducible color in standards and samples. Interference of glucose in plasma and urine samples was negligible as evidenced by low or zero absorbance readings for blanks.

Plasma and urine levels of mannitol obtained after continuous infusion of 5% mannitol in a rat had no effect on the production of the color formed during the inulin-anthrone reaction. This assay was therefore suitable for the determination of plasma and urine inulin levels obtained under the described experimental procedures.

C. N^1-methylnicotinamide (NMN) Excretion Studies

1. Clearance experiments

When 0.2% NMN was infused in six rats the average NMN plasma level and renal clearance were essentially the same as those obtained by Ross et al (61) for seven rats (Table VI). Average inulin clearance (GFR) was slightly lower than that found by Ross et al and therefore the ratio of NMN to inulin clearance (CL_{NMN}/CL_I) obtained experimentally was higher than that reported by Ross. However, clearance

ratios in both our studies and those of Ross et al were considerably greater than one and indicated a large secretory component for NMN. Transport of NMN (T_{NMN}) was calculated using equation 3. Ross et al (61) showed that there was no plasma protein binding of NMN and therefore α was equal to one. These workers were also unable to demonstrate any significant reabsorption of NMN in nondiuretic rats. In diuretic rats, after tubular microinjection, they observed only 10% reabsorption of NMN in the proximal tubules with no reabsorption beyond the late proximal tubule. T_{NMN} therefore can be assumed to be free of a reabsorptive component and to be representative of secretion of NMN from plasma to urine. The average transport rate for NMN obtained experimentally agreed closely with that reported by Ross and coworkers.

2. Saturation experiments

After infusion of NMN at concentrations of 0.2 - 1.2%, at a rate of 50 µl/min, NMN steady-state plasma levels ranged from 57 µM to 770 µM in 21 rats as compared to a range of 100 µM to 900 µM found by Ross et al (61) in 9 rats. Clearance ratios of NMN to inulin (CL_{NMN}/CL_I) and transport of NMN (T_{NMN}) were plotted against NMN plasma concentration (P_{NMN}) (figures 9 and 10). Both the data from these experiments and that of Ross et al (61) exhibited a considerable amount of scatter. Figure 9 does, however, show a trend towards decreasing CL_{NMN}/CL_I with increasing P_{NMN}. Comparing figure 9 to figure 5, the theoretical curve for the relationship when drug clearance contains a saturable transport component, one can see a rough similarity particularly at low P_{NMN}. The data of Ross in

figure 10 shows a trend towards saturation of transport. Their data roughly approximates the theoretical curve in figure 4; they reported an average apparent T_M for NMN of 7 μmol/min·Kg. The experimental data less clearly defines transport saturation. Thus, although both figures indicate the possibility of saturable transport and therefore the presence of a carrier-mediated transport, demonstration of inhibition is necessary in order to conclude that carrier-mediated transport is operable.

3. Inhibition experiments

The results of inhibition experiments (Table VII) show that mepiperphenidol definitely inhibits the renal tubular secretion of NMN. Experimental results show a 45% decrease in CL_{NMN}/CL_I and a 67% decrease in T_{NMN} when mepiperphenidol was added to the NMN infusion. Ross et al (61) demonstrated a 42% decrease in CL_{NMN}/CL_I and a 59% decrease in T_{NMN}.

The results of the NMN clearance experiments, saturation experiments and inhibition experiments show that NMN is secreted by the renal tubule in the rat and that this secretion occurs via a carrier-mediated transport system. The results also demonstrate that the animal model, surgical techniques and experimental procedures used here are appropriate for studying the renal clearance of other organic bases and are capable of demonstrating the characteristics of carrier-mediated transport i.e. saturation and competitive inhibition for these compounds under the appropriate experimental conditions.

D. Pseudoephedrine Assay

Microanalysis of pseudoephedrine (PS) in plasma: The assay for PS in plasma permitted the use of plasma sample volumes as small as 20 µl. A sensitive assay requiring very small sample volumes is important when using small animals such as the rat to study the pharmacokinetic parameters and excretion of a drug. It enables one to make a sufficient number of measurements in an animal without compromising the blood supply of that animal. The ability to measure PS in small volumes of plasma might also be useful in assessing the toxicity problems of pseudoephedrine in children. The amount of information that can be obtained from children is often limited by the volume of blood which must be drawn to obtain this information. The superimposibility of standard curves for PS in plasma (figure 12) prepared on different days is indicative of the reproducibility of the assay. There are no interfering peaks in rat plasma (figure 11-A). Only peaks relating to PS and the internal standard, ephedrine sulfate (ES), appear in the sample taken from a rat infused with PS (figure 11-C), with no indication of detectable metabolite levels.

Assay of pseudoephedrine in urine: Comparing blank urine samples (figure 13-A) to samples spiked with PS and ES (figure 11-B) there are no compounds in urine which interfere with the measurement of PS. The chromatogram derived from a urine sample taken from a rat infused with PS (figure 13-C) shows well defined peaks corresponding to ES and PS. In addition there is a peak just before the ES peak which is not found in either the blank urine or the spiked urine. When figure 13-C is compared to figure 15, it can be seen that the

extra peak in the rat sample corresponds to the norpseudoephedrine (NPS) peak. It is thus likely that NPS is a major metabolite of PS in the rat as in humans. If PS is also metabolized to p-hydroxy-norpseudoephedrine as ephedrine is metabolized to p-hydroxyephedrine in the rat (88), it is possible that such a metabolite exists in detectable quantities. However, under the conditions of the present assay this metabolite should appear somewhere before the NPS peak (due to a greater potential for derivatization) and be lost in the solvent front.

E. Pseudoephedrine Pharmacokinetics

1. Administration of intravenous doses of pseudoephedrine (PS) Following administration of an i.v. bolus dose of PS in three rats, plasma levels declined biexponentially. PS plasma levels were obtained at the lower limit of assay detectability at 90 minutes in one rat and 120 minutes in another rat. The plasma concentration (P_{PS}) - time data was described by a two compartment model. Plasma clearance of PS calculated both model independently and model dependently was the same, indicating the appropriateness of the two compartment model. It was also possible to predict urinary excretion of pseudoephedrine using the pharmacokinetic parameters derived from the plasma data. This was done using the relationship,

$$U_t = k_{13} \cdot f_e \cdot \text{Dose} \quad \frac{(k_{21} - \alpha)\ (1 - e^{-\alpha t})}{\alpha(\beta - \alpha)} + \frac{(k_{21} - \beta)\ (1 - e^{-\beta t})}{\beta(\alpha - \beta)}$$

(eq. 11)

where U_t is the cumulative amount of PS in the urine up to time t; f_e is the fraction of the dose excreted in the urine as unchanged PS; α, β, k_{21}, k_{13} are as defined in Chapter 2, Experimental. Accurate prediction of urinary excretion is another test for the appropriateness of the model (96). Table XVIII shows the correspondence of the predicted and observed urinary excretion of PS for each urine collection period in each of the three rats.

The apparent central compartment volume of distribution (V_1) was quite large. Assuming that in the rat as well as man total body water is 60% of body weight, V_1 is 2 - 3 times total body water. Pickup et al (72) found that ephedrine in man had a volume of distribution which greatly exceeded total body water and which was comparable to values found for similar basic drugs.

The average fraction of the dose excreted in the urine as unchanged PS (f_e), 0.45, was similar to that found for ephedrine in rats (88). Following oral administration of 180 mg pseudoephedrine to humans (86), 121 - 130 mg of unchanged PS was excreted in the urine. Total urinary excretion of unchanged drug is equal to Dose$\cdot f_e \cdot F$, where F = the fraction of the administered dose available to the systemic circulation. If F = 1, f_e is 0.67 - 0.72; if F < 1, f_e would be greater. These values of f_e for pseudoephedrine are similar to those reported for ephedrine in humans (88). Although there is no literature information for pseudoephedrine in rats per se, based on the results obtained in the present studies and the literature results for ephedrine in the rat, it is likely that ephedrines in general are metabolized

Table XVIII

Urinary Excretion of Pseudoephedrine following an I.V. Bolus, Observed versus Predicted[a]

Rat	Time (min)	$U_{observed}$ (mcg)	$U_{predicted}$ (mcg)
A	10 - 30	81.0	74.7
	30 - 60	57.3	57.3
B	30 - 60	50.2	51.9
C	30 - 60	62.5	64.2
	60 - 90	40.6	34.0

[a]Calculated using equation 11

to a greater extent in the rat than in humans. Renal clearances of pseudoephedrine reported here are 3 - 4 times GFR values found in the literature indicating the presence of renal tubular secretion of pseudoephedrine.

2. Intravenous infusion of pseudoephedrine

Average two compartment model parameters derived from the i.v. bolus studies were adequate to calculate the dosing regimen necessary to reach desired steady-state plasma levels of pseudoephedrine. The variation in steady-state pesudoephedrine plasma levels obtained following identical priming doses and infusion rates (Tables X - XII) reflects the variability between rats.

F. Pseudoephedrine Protein Binding Studies

The value for α (fraction of drug in the plasma which is not bound) obtained for pseudoephedrine in human plasma provides an estimate of the extent of binding which might occur in rat plasma. The plasma protein binding studies were performed in human plasma because of the large volumes of plasma needed for the ultracentrifugation technique. The accuracy of the value obtained for α was limited by the fact that radioactive pseudoephedrine was not available and the cold assay for the microanalysis of pseudoephedrine had to be used.

Calculations for α are quite sensitive to small fluctuations in reported PS plasma concentrations. For example if total plasma concentration of PS is 0.20 mcg/ml and concentration of unbound PS is 0.16 mcg/ml, α = 0.80; if unbound PS is 0.14 mcg/ml, α = 0.70; if unbound

PS is 0.18 mcg/ml, α = 0.90. Although α tends to increase somewhat with increasing total PS plasma concentrations in the range of 0.20 to 1.8 mcg/ml, lack of radioactive pseudoephedrine prevents further investigation of this observation. Estimates of protein binding for the drugs used as inhibitors are: mepiperphenidol, α = 0.80 (dog plasma) (54); procainamide, α = 0.85 (human plasma) (90); ethambutol, α = 0.7 - 0.8 (human plasma) (91). The estimate of α for pseudoephedrine in human plasma obtained in the present study is 0.794.

G. Pseudoephedrine Excretion Studies

1. Clearance experiments

Renal clearances calculated in the pharmacokinetic studies indicated that pseudoephedrine was secreted by the renal tubule. Clearance experiments verified this. The average renal clearance of pseudoephedrine was three times the clearance of inulin. Pseudoephedrine was found to be bound to plasma proteins to the extent of about 20%. Transport of pseudoephedrine (T_{PS}) was therefore calculated using equation 3 with α = 0.8. Only the filtration component of renal excretion is corrected for protein binding. Although protein binding reduces the amount of a drug that can be filtered, it usually does not alter the rate of tubular secretion of the drug (44). Unbound drug leaving the plasma and being transported across the tubule promotes the dissociation of drug from plasma protein. Assuming that the combination of drug with plasma protein is rapidly reversible, all drug in the plasma may dissociate and be transported across the tubule before the blood emerges from the peritubular capillaries (97). When a compound is

excreted by glomerular filtration with possible passive tubular reabsorption, the clearance should be calculated from the non-protein bound fraction in plasma. In the case of a substance with a clearance higher than GFR, i.e. involving tubular secretion, the total plasma concentration of the substance is used in the calculation of clearance (8). Transport rates calculated for pseudoephedrine are actually net transport rates and may not be a true measure of the rate of secretion. Depending upon the urinary pH, reabsorption of pseudoephedrine may or may not be negligible. If reabsorption is significant, the apparent rate of secretion as indicated by T_{PS} will be less than the actual rate of secretion. As urinary pH increases, this discrepancy between apparent and actual secretory transport rate will become greater. When urinary pH is such that reabsorption is significant, the net transport rate may also be influenced by urinary flow rates. For a given urinary pH, as urinary flow increases, reabsorption decreases and T_{PS} will become more reflective of secretion.

2. Saturation experiments

When Bye et al (86) administered 180 mg doses of pseudoephedrine hydrochloride orally to human subjects both as single doses and twice a day for 14 days, PS plasma levels were 0.60 mcg/ml or less at all times of measurement. Lin et al (87) reported a peak PS plasma concentration of about 1.1 mcg/ml following oral administration of PS at a dose of 5 mg/Kg. The normal therapeutic dose of pseudoephedrine is 60 mg three times a day. In the rat studies reported here steady-state plasma concentrations reached as pseudoephedrine doses were increased eight-fold covered a range of 0.16 mcg/ml to 1.50 mcg/ml

(Table XII). Thus, the range of plasma levels of PS obtained in the saturation studies in rats more than adequately covers the range of plasma levels which might be expected in a clinical situation.

Clearances of pseudoephedrine (CL_{PS}) ranged from 20.08 ml/min·Kg to 41.09 ml/min·Kg (rats 4 and 9, Table XII). Brennan et al (77) reported an average renal plasma flow in rats of 26.7 ml/min·Kg. Kau et al (76) found a PAH clearance (renal plasma flow) of 35 ml/min·Kg in rats. Clearances obtained in PS saturation studies are approximately equal to or greater than these reported renal plasma flows. It is likely that a portion of the excreted PS is derived from the red blood cell. These results are consistent with observed red blood cell partitioning (RBC/plasma ratio of 2.14).

Clearance ratios (CL_{PS}/CL_I) did not decrease with increasing pseudoephedrine plasma levels. Figure 17 does not show the characteristics of saturation which the theoretical curve for CL_D/CL_I versus P_D shows (figure 5). Transport of pseudoephedrine (T_{PS}) increased in a linear fashion with increasing P_{PS}. A maximum rate for transport was not reached. Based on these observations it can either be concluded that secretion of PS was not saturable over the range of plasma levels studied, or that the calculated T_{PS} was being influenced by variables which were masking saturation characteristics and that this T_{PS} was not really reflecting what is happening to secretion. One variable to be considered is the degree of plasma protein binding. If plasma protein binding of a drug decreases with increasing plasma concentrations of drug, using a constant α in equation 3 would give an increasing

overestimate of T_D as the drug plasma concentrations increased. However, since pseudoephedrine is bound to plasma proteins to such a small extent it is unlikely that correcting for a changing α when calculating T_{PS} would alter figures 17 and 18 significantly. Another variable which is much more likely to have a significant effect on the observed T_{PS} and on CL_{PS}/CL_I is the magnitude of reabsorption. Two factors having a potential influence on the reabsorption of pseudoephedrine are urinary pH and urinary flow rate. At higher pH and lower flow rate a greater degree of reabsorption might be expected. Reviewing Table XII, the higher urine pH's and lower flow rates tend to occur at the higher PS plasma concentrations. If reabsorption is a significant factor in these saturation studies, it would be expected to be greater at the higher PS plasma concentrations because of the favorable urine pH and flow conditions. Calculated T_{PS} would then be an underestimate of secretion. Consideration of this fact would reinforce the conclusion of non-saturation of secretion. Multivariate regression analysis of the data in Table XII showed, however, that there was no significant partial correlation between urinary flow, urinary pH and CL_{PS}/CL_I, T_{PS}.

3. Effect of urinary pH on pseudoephedrine renal excretion

When looking at the effect of urinary pH on the excretion of pseudoephedrine it was found that changing urinary pH in an individual rat caused a change in pseudoephedrine excretion (Table XIII). As pH increased CL_{PS}, CL_{PS}/CL_I and T_{PS} decreased; as pH decreased CL_{PS}, CL_{PS}/CL_I and T_{PS} increased. Urinary flow was relatively constant within each study and therefore did not influence the observed effects.

Figure 19 shows that the most dramatic effects of urinary pH on PS excretion occur over a pH range of 6.3 to 7.2. Below pH 6.3 and above pH 7.2, changing pH would be expected to have little influence on pseudoephedrine excretion. Pickup et al (72) found that small fluctuations in urinary pH had little effect on ephedrine plasma profiles implying little effect on ephedrine excretion. However, out of 29 subjects studied, 26 had a urine pH less than 6.4. Wilkinson and Beckett (71) found a profound effect of urinary pH on the excretion of ephedrine. In these studies urinary pH was either acidic (pH 5.0) or alkaline (pH 8.0).

The results of the studies on the effect of urinary pH on pseudoephedrine in individual rats (Table XIII) are consistent with the influence of urinary pH on ephedrine excretion. The negligible effect of urinary pH in the pseudoephedrine saturation studies, i.e. that there was no correlation between urinary pH and CL_{PS}/CL_I or T_{PS}, is also consistent with the above discussion since in 15 out of 19 rats urinary pH was 6.3 or less.

4. Inhibition experiments: mepiperphenidol

Inhibition of pseudoephedrine secretion by mepiperphenidol (Mepi) was studied in 18 rats under varying experimental conditions. Initially 0.6% Mepi was infused at a rate of 50 µl/min during the inhibition periods following a priming dose of 5 mg/Kg Mepi (dosing schedules A and B in Table III). This was the concentration of Mepi used by Ross et al (61) and used by us in the NMN studies reported here. Under this dosing schedule a number of rats appeared to have an increase in

respiratory distress during the inhibition periods and the possibility of Mepi toxicity was considered. Signs of toxicity in rats administered Mepi i.p. include tremors, dyspnea and death within one hour; the LD^{50} is 116 mg/Kg (98). The rats in the inhibition study received 5 mg/Kg bolus doses of Mepi plus approximately 72 mg/Kg more during an hour or so infusion. Although total dose of Mepi was below the LD^{50}, if the rats had respiratory problems prior to Mepi administration they most likely would have been more susceptible to its effects. The remainder of the experiments were run at a concentration of 0.3% Mepi (50 µl/min). In all dosing schedules except B, infused PS concentration was held constant for both control and inhibition periods; in dosing schedule B, PS concentration infused in the control experiments was twice that infused in the inhibition periods, following the procedure of the NMN studies. Dosing schedule E reduced the priming dose of Mepi (5 mg/Kg to 2.5 mg/Kg) to see if the initial concentration of Mepi was the important factor in inhibition rather than levels reached after equilibration. Schedules F and G extended the re-equilibration period from 10 minutes to 60 minutes to insure attainment of new steady-state PS plasma levels. In addition, schedule F used 4% Na_2SO_4 instead of 5% mannitol as the diuretic in an attempt to better maintain urinary pH in an acid range and thereby reduce or eliminate the complicating reabsorption factor.

Looking at the 18 rats under all experimental conditions (Table XV), in all but two instances (rats 8 and 15) the clearance ratio of pseudoephedrine to inulin, CL_{PS}/CL_I, decreased in the presence of Mepi. Percentage decrease in CL_{PS}/CL_I under each dosing schedule (excluding rats 8 and 15) was: A - 35%; B - 50%; C - 27%; D - 37%; E - 30%;

F - 20%; G - 27%. In many instances the calculated values for T_{PS}, however, either decreased by only a small percentage, stayed the same, or increased in the presence of Mepi; in only a few instances was the percentage decrease in T_{PS} equal to or greater than that seen with CL_{PS}/CL_I.

A number of factors could contribute to the apparently anomolous results for the transport of pseudoephedrine in the presence of mepiperphenidol: 1.) An increase in blood pressure and/or renal plasma flow during the inhibition period; 2.) Simultaneous inhibition of an active reabsorption process for pseudoephedrine; 3.) An increase in passive secretion of pseudoephedrine due to a favorable change in urinary pH and/or flow; 4.) A decrease in passive reabsorption of pseudoephedrine due to favorable changes in urinary pH and/or flow. Increases in blood pressure and renal plasma flow sufficient to increase tubular transport should also be reflected in increased glomerular filtration rate. Observed inulin clearances, however, indicate that GFR did not increase in the presence of mepiperphenidol. Although the possibility of active reabsorption (and inhibition thereof) for pseudoephedrine cannot be ruled out without extensive studies beyond the scope of our work, there has been no demonstration, as yet, of bidirectional carrier-mediated transport for organic bases (4). Our data, however, does suggest the possible influence of a passive tubular process on the observed T_{PS}. In most instances where T_{PS} decreased little or increased in the presence of mepiperphenidol there was a simultaneous increase in urinary flow and/or decrease in urinary pH. Since only a few bases have been found to be secreted by passive, non-ionic diffusion (10), the possible influence of changes in urinary flow and pH on T_{PS} will be discussed in terms of passive reabsorption.

Rearrangement of equations 4 and 5 is useful in analyzing the apparent inconsistency of the observation of an increasing T_{PS} in the presence of decreasing CL_{PS}/CL_I.

$$\frac{CL_D}{CL_I} = 1 + \frac{T_D}{P_D \cdot CL_I} \qquad \text{(eq. 12)}$$

where T_D is actually the difference between secretion rate and reabsorption rate for a drug such as pseudoephedrine which exhibits pH dependent excretion in a specified range of urinary pH (approximately 6.3 to 7.2). It follows from equation 12 that although the calculated T_D may increase under experimental conditions due to changes in urine flow and pH, a corresponding increase in P_D which is greater in magnitude than that of T_D could result in an overall decrease in CL_D/CL_I. If pH should decrease and/or urinary flow increase between control and inhibition periods, reabsorption would be expected to decrease. This could result in an increased T_D depending on the relative magnitude of the changes in reabsorption and secretion. If urinary flow and pH do not change between control and inhibition periods, reabsorption would be expected to remain relatively constant and T_D should decrease, reflecting inhibition of secretion. Of course if reabsorption should increase in the inhibition period (increasing urinary pH and/or decreasing flow) T_D might indicate a greater inhibition of secretion than has actually occurred. In order to minimize the effects of urinary pH and flow on the apparent T_{PS}, only rats whose urinary pH was 6.3 or less in both control and inhibition periods were considered for further analysis of the effect of mepiperphenidol on the transport of PS (resulting in the exclusion of rats 8 and 11). As discussed

earlier, excretion of PS was relatively insensitive to pH changes at pH values below 6.3. In this pH range small changes in urinary flow should also have little effect on PS excretion. Rats 1 and 2, receiving 0.6% Mepi infusions, were also eliminated from further analysis because of low inulin clearances.

The remaining rats were then grouped according to dose of Mepi received and time for re-equilibration. Group I included those rats receiving bolus doses of 5 mg/Kg Mepi, infusions of 0.3% Mepi with a 10 minute re-equilibration period; group II included those rats receiving bolus doses of 2.5 mg/Kg, 0.3% Mepi infusions with a 10 minute re-equilibration period; group III included those rats receiving bolus doses of 2.5 mg/Kg, 0.3% Mepi infusion with a 60 minute re-equilibration; group IV included rats receiving 5 mg/Kg bolus doses, 0.6% Mepi infusion with a 10 minute re-equilibration period. Table XIX summarizes the pseudoephedrine plasma levels (P_{PS}) and apparent transport rate normalized for GFR (T_{PS}/GFR) for each rat during control and inhibition periods. Six rats still had an apparent increase in pseudoephedrine transport during the inhibition period. The relationship between the average P_{PS} and T_{PS}/GFR for control and inhibition periods was then evaluated for each group. Since T_{PS} versus P_{PS} (figure 18) is linear over the observed PS plasma levels when no inhibitor is present, an increase in T_{PS} might be expected due to the increasing plasma levels that result from inhibition of secretion. It was therefore important to consider the relationship between T_{PS} and P_{PS} (or T_{PS}/GFR and P_{PS}) rather than T_{PS} (or T_{PS}/GFR) alone. If the increase in T_{PS}/GFR is less than what would be expected for the observed increase in P_{PS}, inhibition of

Table XIX

Inhibition of Pseudoephedrine (PS) Renal Transport
by Mepiperphenidol

		Control Period			Inhibition Period		
		P_{PS}	T_{PS}/GFR	$T_{PS}/GFR/P_{PS}$	P_{PS}	T_{PS}/GFR	$T_{PS}/GFR/P_{PS}$
Group I							
Rat No.	5	0.24	0.377	1.57	0.38	0.314	0.826
	6	0.20	0.385	1.92	0.26	0.330	1.27
	7	0.22	0.384	1.74	0.31	0.346	1.12
	9	0.14	0.357	2.55	0.28	0.400	1.43
	10	0.22	0.384	1.74	0.27	0.222	0.822
	12	0.10	0.362	3.62	0.17	0.423	2.49
Mean		0.19	0.375	2.19	0.28	0.339	1.33
S.D. $\pm$		0.05	0.012	0.78	0.07	0.071	0.62
Group II							
Rat No.	13	0.16	0.173	1.08	0.22	0.171	0.777
	14	0.15	0.353	2.35	0.21	0.250	1.19
Mean		0.16	0.263	1.72	0.22	0.210	0.984
S. D. $\pm$		0.01	0.127	0.90	0.01	0.056	0.292
Group III							
Rat No.	15	0.14	0.432	3.09	0.18	0.555	3.08
	16	0.12	0.388	3.23	0.20	0.483	2.42
	17	0.14	0.425	3.04	0.22	0.613	2.79
	18	0.12	0.435	3.62	0.22	0.504	2.29
Mean		0.13	0.420	3.24	0.20	0.539	2.64
S. D. $\pm$		0.01	0.22	0.27	0.02	0.058	0.36

Table XIX (Continued)

		Control Period			Inhibition Period		
		P_{PS}	T_{PS}/GFR	$T_{PS}/GFR/P_{PS}$	P_{PS}	T_{PS}/GFR	$T_{PS}/GFR/P_{PS}$
Group IV							
Rat No.	3	0.34	1.079	3.17	0.31	0.283	0.913
	4	0.42	1.023	2.44	0.38	0.387	1.02
Mean		0.38	1.051	2.80	0.34	0.335	0.966
S.D. $\pm$		0.06	0.040	0.52	0.05	0.074	0.074

transport is indicated. Figure 23 is a graphical interpretation of Table XIX.

Except for rat 15, all rats showed a decrease in $T_{PS}/GFR/P_{PS}$, suggesting that linearity of transport accounted for a significant part of the increase in T_{PS} in the presence of mepiperphenidol. The average percentage decrease in $T_{PS}/GFR/P_{PS}$ in the presence of mepiperphenidol was 43% for group I, 43% for group II, 18% for group III and 66% for group IV. Group I included a sufficient number of rats to perform a paired t-test on the ratio of $T_{PS}/GFR/P_{PS}$ for the control and inhibition periods. The decrease in this ratio in the inhibition period was found to be significant ($p<0.001$). The greatest inhibition of pseudoephedrine transport occurred when the re-equilibration period was only 10 minutes (groups I, II and IV) and was apparently independent of the bolus dose and infusion rate of Mepi. Group III had the lowest percentage inhibition. In this group the period of re-equilibration was 60 minutes. In addition, one rat, 15, showed no inhibition of PS transport. When this rat was excluded from the analysis, per cent inhibition of transport increased to 24% which was still considerably less than groups I, II and IV. In view of these results, it is probable that the plasma level of Mepi during the inhibition clearance periods influences the extent of inhibition of PS transport. Higher levels of Mepi might be expected at 10 minutes after the bolus than at 60 minutes. It is likely that the bolus dose overshoots the steady-state levels which would eventually be reached with the infusion of Mepi and that by 60 minutes most of the bolus dose has been eliminated whereas, at 10 minutes high levels of Mepi are still present from the bolus dose.

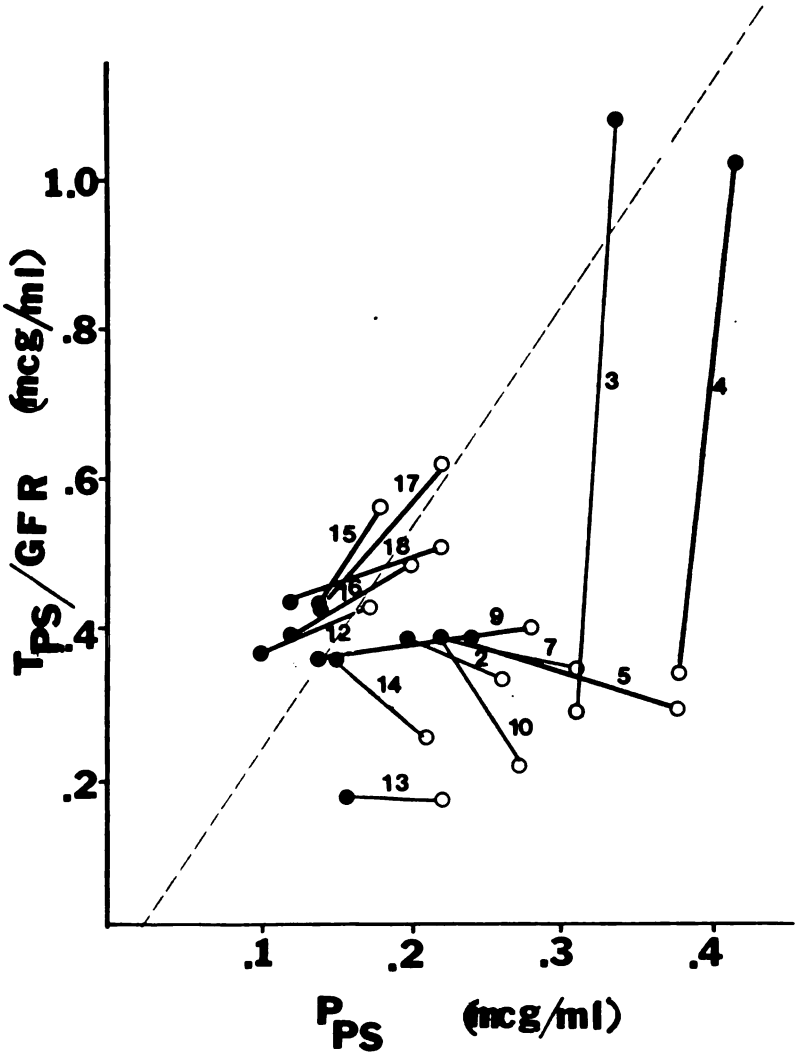


Figure 23. Pseudoephedrine transport, corrected for GFR (T_{PS}/GFR), versus plasma levels of pseudoephedrine (P_{PS}) for control periods (●) and inhibition periods (o) where mepiperphenidol was the inhibitor. Numbers refer to rat nos. in Table XIX. Rats 3 and 4 received one-half the dose of PS during the inhibition period as in the control period. The broken line is the linear regression line for saturation studies.

This situation would be analogous to the calculations for PS infusion where a bolus of PS was administered such that at 30 minutes after the dose plasma levels would approximate final steady-state levels; at any time before 30 minutes higher levels would exist. Furthermore, it is possible that for a given plasma level of pseudoephedrine there is a level of mepiperphenidol below which there is no inhibition of PS transport and a level above which there is no increase in PS transport inhibition. Mepiperphenidol plasma levels obtained 10 minutes after the bolus doses in these studies were probably well above levels needed to see a significant inhibition of PS transport.

Figure 23 is consistent with the results in Table XIX. In all but one instance the data shows an inhibition of PS transport by mepiperphenidol. Comparison of the slope of the line connecting the control and inhibition periods for an individual rat with the slope of the linear regression line for transport in the saturation studies permits evaluation of inhibition. A slope greater than or equal to that of the regression line indicates no inhibition of transport; a slope less than that of the regression line indicates inhibition. The relative magnitude of the deviation in a negative direction for the slope of the individual lines from that of the regression line reflects the relative extent of inhibition (except for group IV, rats 3 and 4, where the infusion rate of PS was reduced in the inhibition periods). Rat 15 has a slope equal to that of the regression line, indicating no inhibition of PS transport. Table XV shows that there was no decrease in CL_{PS}/CL_I for rat 15 in the presence of Mepi. For all other rats the slope was less than that of the regression line indicating inhibition

of PS transport.

Tables XV and XIX and figure 23 show that mepiperphenidol does inhibit the secretion of pseudoephedrine. Mepiperphenidol is itself transported by the organic base transport system and has been shown to inhibit the transport of other compounds handled by this system. The demonstration of inhibition of pseudoephedrine transport by mepiperphenidol indicates that pseudoephedrine is also transported by the organic base transport system.

5. Inhibition experiments: procainamide

Data from inhibition experiments with procainamide were analyzed as for the mepiperphenidol inhibition studies. This analysis (Table XX) indicates that procainamide also inhibits the renal secretion of pseudoephedrine. The average decrease in $T_{PS}/GFR/P_{PS}$ in the presence of procainamide was 40%. The average decrease in CL_{PS}/CL_I in the presence of procainamide (Table XVI) was 30%. Procainamide has been shown to exhibit net renal secretion (90), but its transport has not been specifically studied. In view of its ability to inhibit secretion of pseudoephedrine it is likely that procainamide is also secreted via the organic base transport system. Procainamide is used clinically and its administration concurrently with pseudoephedrine or other bases could lead to a drug interaction based on competition for tubular secretion.

6. Inhibition experiments: ethambutol

Table XVII shows that three out of four rats had a slight increase in

Table XX

Inhibition of Pseudoephedrine (PS) Renal Transport by Procainamide

	Control Period			Inhibition Period		
	P_{PS}	T_{PS}/GFR	$T_{PS}/GFR/P_{PS}$	P_{PS}	T_{PS}/GFR	$T_{PS}/GFR/P_{PS}$
Rat No.						
1	0.14	0.366	2.61	0.23	0.279	1.21
2	0.13	0.275	2.12	0.21	0.346	1.65
Mean	0.14	0.320	2.36	0.22	0.312	1.43
S.D.	0.01	0.064	0.35	0.01	0.047	0.31

CL_{PS}/CL_I and T_{PS} in the presence of ethambutol. The urine pH in rats 1 and 3 fell into the range in which pseudoephedrine excretion is sensitive to pH changes and it is possible that the observed increases in clearance ratio and PS transport are a reflection of decreased reabsorption. Rat 2 had a decrease in CL_I while PS excretion, renal clearance and plasma level stayed essentially the same. Rat 4, on the other hand, shows a dramatic decrease in CL_{PS}/CL_I and T_{PS} in the presence of ethambutol. From the limited data available it is impossible to determine whether ethambutol has an effect on PS secretion. Ethambutol has exhibited net renal secretion (91). Inability to conclusively demonstrate inhibition of pseudoephedrine with ethambutol does not preclude the possibility that ethambutol is also transported by the organic base transport system. Factors such as relative affinity of compounds for the transport system and relative plasma levels of these compounds can influence the ability to demonstrate competitive inhibition.

It is important to remember that the results of animal studies may not necessarily be extrapolated to humans. They can, however, be used as indicators of what might be expected to happen in humans and therefore can be useful in designing appropriate clinical studies. In examining the toxicity problems in children with renal tubular acidosis, for example, it is probable that the toxicity results in most part from urinary pH effects; saturation of transport would not be a problem unless psuedoephedrine plasma levels were much higher, due to extensive reabsorption, than those reached in the animal saturation studies. Interaction of pseudoephedrine with other bases at the level of tubular

secretion might be a potential problem, however. On one hand, the secretion of pseudoephedrine may be inhibited to such an extent that side effects and toxicities result from increased plasma levels. On the other hand, pseudoephedrine might inhibit the secretion of other bases leading to increased plasma levels of these bases. Although toxicity problems with pseudoephedrine are rare, other basic drugs have much lower therapeutic indices making unexpected increases in plasma levels dangerous.

CHAPTER 5

SUMMARY AND CONCLUSIONS

Pseudoephedrine is an organic base used in the treatment of upper respiratory tract disorders. Because of episodes of toxicity in children with renal tubular acidosis who were administered pseudoephedrine, this drug was studied so as to define its renal excretion mechanisms.

Surgical techniques and experimental procedures were developed for the study of renal mechanisms in the rat. The ability to accurately measure renal clearance and to demonstrate renal secretion by a carrier-mediated transport system was verified by comparing results from N^1-methylnicotinamide (NMN) excretion studies with literature results. NMN renal clearances were greater than inulin clearance, indicating the presence of tubular secretion. This secretion was shown to be saturable and was inhibited by mepiperphenidol to the same extent as that reported in the literature. Glomerular filtration rate as measured by inulin clearance was comparable to literature values and was used as an indicator of kidney function.

A microanalytical procedure was developed for the measurement of pseudoephedrine plasma levels using a gas liquid chromatograph with an electron capture detector. The procedure enabled detection of 2 mg of pseudoephedrine in 20 µl of plasma. Standard curves were reproducible and linear over a wide range of pseudoephedrine concentrations.

Pharmacokinetic studies showed that plasma levels of pseudoephedrine declined biphasically. Plasma concentration-time data was analyzed by a two compartment body model. Average values of α and β were 0.152 and 0.0200 min^{-1} respectively. The average terminal half-life was 35 minutes. Urinary excretion of pseudoephedrine was accurately predicted using the pharmacokinetic parameters derived from plasma data. The fraction of the dose excreted in the urine as unchanged pseudoephedrine was 0.45.

Pseudoephedrine was cleared by the kidney at a rate in excess of inulin and close to or possibly greater than renal plasma flow. In addition to filtration and secretion pseudoephedrine appeared to be subject to pH dependent passive reabsorption.

When the secretion of pseudoephedrine was studied in detail, it was found to be non-saturable for plasma levels of pseudoephedrine ranging from 0.16 mcg/ml to 1.5 mcg/ml. Secretion, however, was inhibited by mepiperphenidol and procainamide suggesting a carrier-mediated process.

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