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# FACTORS AFFECTING THE RENAL EXCRETION OF ORGANIC BASES

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LOU ANN PILKINGTON

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FACTORS AFFECTING THE RENAL EXCRETION OF ORGANIC BASES

APPROVED BY

M. Jack Kyle

Alan K. Lawson

J. M. Dabney

Garman H. Dason

Paul W. Smith

DISSERTATION COMMITTEE

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# FACTORS AFFECTING THE RENAL EXCRETION OF ORGANIC BASES

## CHAPTER I

## INTRODUCTION

The biological actions of organic bases have long been recognized and have been the subject of many investigations. However, investigators focused relatively little attention on the mechanisms involved in the excretion of these compounds. In 1948 Sperber showed that $N^1$-methylnicotinamide (NMN) was excreted by filtration and tubular secretion. Since that time, the excretory processes for many specific organic bases have been studied; however, the specific factors affecting their excretion are far from being understood.

Organic bases are classified as either strong or weak bases. In the physiological pH ranges of plasma and urine, strong bases are totally dissociated while weak bases exist in both the dissociated and undissociated forms. In the work to be presented here, attention was focused on a) the excretion relationships of strong and weak organic bases, and b) the excretion relationships of organic cations and inorganic cations, particularly potassium.

In the intact animal, using the classical clearance technique (Smith 1951, 1956), substances are shown to be excreted by glomerular filtration alone, by filtration as well as tubular secretion, or by filtration together with partial tubular reabsorption. The evidence for tubular reabsorption or tubular secretion of a substance is obtained by comparison studies with a compound such as inulin or creatinine that has been shown to be neither secreted nor reabsorbed by tubular cells. Tubular secretion is shown to occur if the clearance of the substance (urinary concentration of the substance times the urinary volume divided by the plasma concentration) is greater than the simultaneously measured clearance of inulin or creatinine. Tubular reabsorption is shown to occur if the clearance of the substance is less than the simultaneously measured creatinine or inulin clearance. The clearance of inulin or creatinine is taken as a measure of the glomerular filtration rate of the kidney.

Tubular reabsorption or secretion of a substance may occur by passive diffusion and/or by an active transport mechanism operating within the tubular cells.

A system which transports a substance against a concentration gradient, either from tubular lumen to peritubular fluid, or from peritubular fluid to the tubular lumen, and which utilizes energy derived from cellular metabolism is defined as an active transport system (Tag-

gart, 1958). An active transport mechanism exhibits a) saturation of the mechanism and a fixed maximal rate of transfer (Wilbrandt, 1954), and b) competitive or non-competitive inhibition. Two compounds are believed to be transported by a "common" transport system when a single non-competitive inhibitor is shown to block the transport of both substances, and when the clearance of one compound is inhibited by the simultaneous infusion of a second compound.

There is conclusive evidence that strong organic bases are excreted by filtration and tubular secretion as shown in Table 1.

Furthermore, evidence has been presented which suggests that the tubular secretion of at least certain of these bases is by an active transport mechanism. The strongest evidence in support of active transport has been shown as a result of the mutual competitive inhibition in the tubular excretion of one organic base on another, and in the inhibition of tubular excretion by metabolic inhibitors. This evidence was secured in both in vivo and in vitro studies.

Kandel and Peters (1957) showed that Darstine produced inhibition of the tubular secretion of NMN in dogs. Further, they showed an inhibition of Darstine excretion when high NMN loads were simultaneously infused.

TABLE 1

TUBULAR SECRETION SHOWN FOR CERTAIN ORGANIC BASES IN THE MAMMALIAN OR AVIAN KIDNEY

| Base | Animal | Investigators |
|---|---|---|
| $N^1$-methyl-nicotinamide (NMN) | Chicken | Sperber (1948) |
| Guanidine | Chicken | Sperber (1948) |
| Piperidine | Chicken | Sperber (1948) |
| Tolazoline (Priscoline) | Dog | Orloff et al., (1953) |
| N-methyl-nicotinamide | Dog | Beyer et al., (1948) |
| Tetraethylammonium chloride (TEA) | Dog | Rennick et al., (1954) |
| Mepiperphenidol (Darstine) | Dog | Beyer et al., (1953) |
| Thiamine | Chicken | Rennick (1958) |
| Choline | Chicken | Rennick (1958) |
| Hexamethonium | Chicken | Rennick (1958) |

Sperber (1948) demonstrated that tubular secretion of piperidine in the chicken was inhibited by the administration of NMN. Other inhibitory relations have been shown in this species: Darstine inhibited the excretion of NMN; Priscoline inhibited NMN (Volle et al., 1959); Priscoline inhibited the excretion of histamine (Lindahl and Sperber, 1958), and thiamine depressed the excretion of choline (Rennick, 1958). Furthermore, both TEA and choline have been shown to inhibit NMN tubular excretion, while NMN has been shown to inhibit TEA excretion (Green et al.,

1959; Volle et al., 1959).

The in vitro technique used in studying renal tubular transport (Cross and Taggart, 1950) involves the measurement of the accumulation of the base in kidney slices following incubation of the slice in the Warburg apparatus. The accumulation in the slice is related to that concentration remaining in the medium after incubation, and is expressed as the slice/medium ratio (S/M ratio). An S/M ratio greater than one is considered indicative of active transport, i.e., movement from a lesser concentration to a greater concentration.

TEA (Farah and Rennick, 1956), Darstine (LeSher and Shidemen, 1956) and NMN (Farah et al., 1959) have been shown to accumulate in renal slices with S/M ratios exceeding 7. Priscoline, guanidine, and piperidine, when added simultaneously with TEA in the suspending medium, inhibited the accumulation of TEA in the slice (Farah et al., 1957). Similarly, Priscoline, Darstine, choline, and guanidine inhibited the uptake of NMN in renal slices (Farah et al., 1959).

2, 4-dinitrophenol (DNP), an inhibitor of cellular metabolism (Cross et al., 1949) has been shown to depress the tubular transport of TEA in the dog (Rennick and Farah, 1956), and to depress the accumulation of TEA (Farah and Rennick, 1956), NMN (Farah et al., 1959) and Darstine

(LeSher and Shideman, 1956) in renal slices.

These findings strongly suggest not only that strong bases are excreted by active tubular transport but also that they share, at least in some part, a common transport system.

The excretion of weak bases has been attributed primarily to filtration and tubular secretion or reabsorption as a result of passive diffusion of the undissociated base (Milne et al., 1958). The undissociated form of a substance penetrates cell membranes more rapidly than the ionized form (Ousterhout, 1925; Jacobs, 1940). The flux direction, i.e., secretion or reabsorption, for this undissociated molecule is dependent upon the pH gradient between urine and plasma. When the urine is alkaline, more of the base exists in the urine in the undissociated form than in the plasma and thus diffuses from tubule to plasma. The result is net tubular reabsorption of the base. When the urine is acid, the concentration gradient for the undissociated form of the base is from plasma to urine. This un-ionized form diffuses into the tubular lumen, where it dissociates into the ionic form as a result of the more acidic fluid. Thus the base is trapped and net tubular secretion results.

Table 2 is adapted from Milne (1958). The organic bases listed in this table have been shown to be reabsorbed when the urine was alkaline, and secreted when the urine was acid.

TABLE 2

WEAK BASES BELIEVED TO BE EXCRETED BY FILTRATION AND NON-IONIC DIFFUSION

| Base | Animal | Investigator |
|---|---|---|
| Nicotine | Man | Haag and Larsons (1942) |
| Quinine | Man | Haag et al., (1943) |
| Quinocrine (Atabrine) | Man | Jailer et al., (1947) |
| Chloroquine (Aralen) | Man | Jailer et al., (1947) |
| Ammonia | Rat | Ferguson (1951) |
| Ammonia | Dog | Orloff and Berliner (1956) |
| Procaine | Dog and rabbit | Terp (1951) |
| Mecamylamine (Inversine) | Dog | Baer et al., (1956)<br>Scribner et al., (1959) |

Implicit in the concept for non-ionic diffusion is that the direct movement of the substance from peritubular fluid into the tubular lumen, or from the tubular lumen into the peritubular fluid does not require energy from the cell, and that the movement of the substance is neither competitively nor non-competitively inhibited.

There is little direct evidence that active tubular transport of weak bases does or does not occur. In the aglomerular fish, however, tetraethylamine oxide was shown

to be actively secreted Forster (et al., 1958). The excretion of NMN in the chicken was shown to be inhibited rapidly when mecamylamine, quinine, or quinacrine were infused simultaneously (Volle et al., 1960; and Volle and Peters, 1959). However, a decisive active tubular transport in this species for the three weak bases could not be demonstrated.

The inorganic, monovalent cations, sodium and potassium, have been shown to be transported actively by the tubular cells of the kidney. The sodium retaining ability of the mammalian kidney is well documented (Smith, 1955, 1951).

Independent investigations by Mudge et al., (1948), and Berliner and Kennedy (1948) presented evidence that potassium is excreted by glomerular filtration and tubular secretion. Berliner hypothesized that the secretion of potassium was the result of an ion exchange involving potassium and sodium.

The secretion of potassium shows certain features associated with an active transport mechanism. It was shown to be competitively inhibited by the hydrogen ion (Berliner et al., 1951) and non-competitively inhibited by mercurial diuretics (Mudge et al., 1950).

Inorganic and organic cation tubular excretion relationships have been demonstrated for certain bases.

Kandel (1956) showed that, after a period of forced feeding of KCl in the dog, the simultaneous infusion of KCl with Darstine or NMN resulted in a reduction of the renal tubular excretion of these bases. A depression of potassium excretion when NMN, Darstine, and Priscoline were infused was shown in Diamox treated dogs (Kandel and Domer, 1957).

After a period of forced-feeding of Priscoline, NMN or Darstine to dogs, inhibition of potassium secretion occurred when these bases were simultaneously infused with potassium (Domer, 1960). Solomon and co-workers, (1960) presented evidence of increased reabsorption of sodium and potassium when choline was infused in the dog.

In summary, the evidence presented appears to indicate a) strong organic bases are excreted by filtration and active tubular excretion; b) some phase of the tubular movement of strong bases involves a common transport mechanism; c) weak bases are excreted by filtration and non-ionic diffusion; d) strong bases and the inorganic cations, sodium and potassium, may share a common base-excreting mechanism.

The hypothesis used in investigating organic and inorganic base relationships for this work resulted from preliminary studies in this laboratory on the excretion relationship of potassium and a weak base, mecamylamine.

Baer and co-workers, (1956) showed that the excretion of mecamylamine was profoundly influenced by the pH of

the urine; tubular secretion occurred as a result of a highly acidic urine, and reabsorption occurred in a highly alkaline urine. When urinary pH was maintained constant, secretion or reabsorption was not inhibited by probenecid (Benemid) or para-aminohippurate (PAH). No maximal rate of transfer occurred when plasma concentrations of mecamylamine were increased.

These investigators suggested that, although the results could be interpreted as excretion by passive diffusion, the secretion and reabsorption of mecamylamine could also be by an active bi-directional transport system, with the direction governed indirectly by the pH of the tubular urine. Lotspeich (1958) further proposed that mecamylamine may participate in a cation exchange transport system.

Subsequent studies on mecamylamine excretion by Scribner et al., (1959) confirmed the results presented by Baer. These workers, however, concluded that mecamylamine reabsorption and secretion was solely by non-ionic diffusion.

This laboratory corroborated the results of the previous workers but further showed that there was a definite inverse relationship between mecamylamine excretion and potassium excretion when the urine pH was changed from acidic to basic. This evidence suggested that mecamylamine could participate in a cation exchange mechanism.

The working hypothesis for this study, therefore, was that the tubular secretion of mecamylamine occurred primarily by a pH-dependent transport system presumably competing with potassium for cation exchange. As the excretion of Darstine, a strong base, had been shown to be inhibited by potassium administration, it was of interest to investigate the excretion of this compound under similar conditions used to study the excretion of mecamylamine.

The experiments in this study were designed to answer several specific questions.

1. Is the tubular excretion of mecamylamine related to a cation exchange transport system?

2. Is potassium the cation involved in the exchange system?

3. Is the tubular excretion of Darstine (a strong base) similar to mecamylamine (a weak base)?

4. Where in the tubule does the secretion and reabsorption of mecamylamine occur, and where does the secretion of Darstine occur?

5. Does the tubular excretion of mecamylamine occur by an active transport system?

Clearance studies were performed to answer the first three questions.

The generally accepted transport system for cation exchange in the renal tubules involves sodium reabsorption

with the potassium and hydrogen ion competing for secretion (Pitts, 1958). The hydrogen ion secretion is dependent upon the hydrogen ion concentration in plasma and the secretion is competitively inhibited by increased plasma potassium concentration.

The tubular excretion of potassium is inversely related to plasma hydrogen ion concentration. Increased excretion of potassium occurs with decreased hydrogen ion concentration and decreased excretion occurs with increased hydrogen ion concentration.

Therefore, to determine if mecamylamine participates in the cation exchange system, as well as to evaluate mecamylamine excretion by "non-ionic" diffusion, the excretion of the drug was studied not only at normal systemic pH valves, but also when plasma hydrogen ion concentrations were varied by the induction of systemic acidosis or alkalosis, with low or high urinary flow rates. Potassium excretion was routinely investigated throughout the experiments.

Furthermore, in order to evaluate if potassium is the cation involved in the exchange system, the effects of the acute administration of mecamylamine on potassium excretion, and the effects of the acute administration of mecamylamine on potassium excretion were studied.

In order to determine if the excretion of Darstine is similar to the excretion of mecamylamine, Darstine and

potassium tubular excretion were investigated with the same experimental procedures used for mecamylamine studies.

Sodium excretion was studied in five of the mecamylamine experiments and in three of the Darstine experiments.

Mecamylamine is a secondary amine and a ganglionic blocking drug (Stone et al., 1956); Darstine is a quaternary ammonium compound and an anticholinergic drug (Beyer et al., 1953). Mecamylamine is used clinically as a hypotensive drug; Darstine, although used clinically as a spasmolytic drug, has been shown to have hypotensive effects with high plasma concentrations. Thus, as either of these compounds could drastically change systemic blood pressure and thereby alter kidney function, it was important to monitor blood pressure throughout each experiment.

A technique for localization of renal tubular activity by stop-flow analysis in the intact animal was introduced in 1957 by Malvin and Wilde. Tubular secretion of TEA has been shown to occur in the proximal tubule of the dog (Rennick et al., 1959). The stop-flow analysis, therefore, was used to determine the site in the nephron of tubular secretion and reabsorption of mecamylamine, and the tubular secretion of Darstine.

In order to evaluate if the tubular secretion of mecamylamine is by an active transport system the accumulation of the compound in rat kidney slices was studied.

# CHAPTER II

## EXPERIMENTAL PROCEDURES AND METHODS

Three experimental procedures were used in this research: The standard clearance technique (Jollifee and Smith, 1931), the stop flow analysis (Malvin et al., 1957), and the in vitro accumulation studies in rat kidney slices (Cross and Taggart, 1950).

### Standard Clearance Technique

The utilization of this technique enabled the excretion studies to be evaluated in the intact, unoperated animal.

Renal clearances for all experiments were performed on post-absorptive mongrel female dogs weighing between 18-25 kg. Each animal was hydrated orally with 500 ml. tap water. Following hydration, if the animal was to be anesthetized, 30 mg/kg. of sodium pentobarbital was administered intravenously.

Following the administration of the anesthetic, the brachial vein was catheterized with a No. 90 polyethylene catheter. A priming solution of creatinine, mecamylamine or Darstine was given intravenously to establish the plasma

levels of these substances. In order to maintain the plasma levels constant an infusion solution was delivered at the rate of 5 ml/min. by a Sigmamotor constant infusion pump. Equilibration periods of 40-120 minutes were allowed from the beginning of the infusion to the beginning of the first collection period.

During this equilibration period, the femoral artery was catheterized with No. 90 polyethylene tubing. Clotting in the tubing was prevented by periodic injections of heparin. An indwelling Foley catheter was inserted into the bladder for urine collections.

Experimental procedure varied for each of the experiments. Each urinary collection period was timed. Three control periods were followed by three experimental periods, and in many experiments, three additional experimental urine collection periods were taken. The rate of urine flow at the beginning of each experiment determined the length of the collection periods. At high flow rates timed periods were ten minutes in duration, at low flow rates fifteen minutes in duration.

Arterial blood samples were taken at the mid-point in each timed urine collection period. Blood samples were collected under oil, and allowed to stand thirty minutes at room temperature for separation of serum. The samples were then centrifuged and the serum was transferred under oil to clean tubes.

Urine samples were also collected under oil into graduated cylinders. In those experiments with very low urine flows, the bladder was washed out with 10 ml. of distilled water and room air. The bladder was emptied by manual suprapubic pressure.

The exogenous creatinine clearance was used as the measure of glomerular filtration rate. Creatinine concentrations were determined by the methods of Bonsnes and Tassosky (1945). The method consists of adding alkaline picrate and reading samples in a colorimeter at 525 mμ.

Mecamylamine concentration in plasma and urine was determined by the method of Brodie and Undenfriend (1945). The method includes extraction of the base with ethylene dichloride at an alkaline pH and the subsequent formation of a highly colored acid salt with methyl orange. The concentration of the base is determined indirectly through measurement of the sulfonic acid salt photometrically. Recoveries of added base average 95 per cent of theoretical, with total quantities as low as five micrograms.

Darstine concentrations in urine and plasma were determined by the method of Tillison et al., (1954). This method is similar to that described for mecamylamine, in that the base is extracted into ethylene dichloride at an alkaline pH and the subsequent formation with methyl orange of a highly colored acid salt acidified with boric acid.

The concentration of the base is determined indirectly through measurement of the sulfonic acid salt photometrically.

Plasma and urine $K^+$ and $Na^+$ concentrations were analyzed with the Baird flame photometer (internal standard method).

The clearance of para-aminohippurate (PAH) was used as a measure of renal plasma flow. PAH concentrations in urine and plasma were determined by the method of Smith et al., (1945).

The pH of plasma and urine was determined under oil with a Beckman Model 76 Expanded Scale pH meter using a glass electrode (limit of accuracy 0.02 pH units), at room temperature and corrected to 37° C.

Total carbon dioxide content of plasma and urine was measured on a Van Slyke Magne-Matic apparatus using the technique of Peters and Van Slyke (1924). Plasma and urine bicarbonate concentration, partial pressure of carbon dioxide, and carbonic acid concentration were calculated from the Henderson-Hasselbalch equation.

Respiratory alkalosis was induced by hyperventilation with a Harvard Apparatus respiration pump, Model 1063. The rates of hyperventilation varied, and are recorded in appropriate tables in Chapter III.

Respiratory acidosis was induced by supplying the

animal with 10 per cent $CO_2$ and 90 per cent $O_2$ mixture. The mixture was inspired through a respiratory high velocity demand valve; expiration was into room air.

Blood pressure recordings were obtained through a Statham transducer connected to a 4-channel Grass polygraph. The transducer was attached to the No. 90 polyethylene catheter inserted into the femoral artery.

The ECG was recorded on the Grass polygraph simultaneously with blood pressure recordings.

## Stop Flow Analysis

Of special interest to renal physiologists was the introduction of this technique, designed to indicate the site of renal tubular secretion and tubular reabsorption of substances in the intact animal. This method in no way supersedes or supplants the more laborious and quantitative method of localization of tubular activity by direct micropuncture introduced by A. N. Richards (1929). Rather, it supplements the direct micropuncture technique, and indeed, final interpretation of results have largely rested on results derived from direct micropuncture studies of tubular contents.

Qualitative localization of tubular sites for secretion and reabsorption is accomplished in three ways. First, the tubular fluid pressure is raised by blocking the ureter until effective filtration pressure is exceeded.

Glomerular filtration ceases and movement of tubular fluid is halted. Renal blood flow continues as was shown by the introduction of $Na^{+}$ and $K^{+}$ isotopes during stop-flow studies (Malvin et al., 1958). Second, the static condition of the tubular fluid is maintained several minutes. This condition enables tubular activity along the nephrons to exaggerate concentrations of substances, by either more completely adding or more completely removing them, than can occur during free-flow. Third, glomerular filtration is reinstituted by unclamping the ureteral catheter and tubular fluid is collected in low volume consecutive samples. The urinary concentrations of solutes from these individual serial samples are plotted graphically together with free-flow urine samples prior to and following stop-flow samples. By comparing free-flow concentrations with stop-flow concentrations sites of tubular ability to reabsorb or secrete are inferred.

Free-flow urine is enhanced by the use of the osmotic diuretic, mannitol. During stop-flow, inulin is injected intravenously and thus entrance of new filtrate from the glomerulus after flow is reinstituted is indicated by the appearance of inulin in the tubular samples. Creatinine is infused with the mannitol solution, and its concentration in urine and plasma used as a measure of glomerular filtration rate during free-flow and is a measure of water reabsorption throughout the nephron in the serial samples.

## Method for Stop Flow Analysis

Postabsorptive mongrel dogs, male or female, weighing 10-18 kg. were hydrated with 500 ml. tap $H_2O$ p.o. 20 minutes prior to administration of 30 mg/kg. sodium pentobarbital anesthesia.

The jugular vein was catheterized with No. 60 polyethylene tubing which was closed with a three-way stop cock. Arterial blood samples were collected from the femoral artery which was catheterized with No. 60 polyethylene tubing. Coagulation was prevented by the periodic use of heparin.

Bladder urine was collected through a Foley catheter inserted into female dogs or through a No. 60 polyethylene catheter in male dogs.

The left ureter was exposed by a small flank incision. The ureter was catheterized with No. 60 polyethylene tubing which was passed well up into the renal pelvis and then tied securely. The catheter was secured to the edge of the dog table prior to collecting serial samples.

A mixture of 10 per cent mannitol, 0.9 per cent NaCl, 0.2 per cent creatinine, 0.05 per cent PAH, and the test substance was infused by a constant infusion pump through a No. 90 polyethylene catheter inserted into a brachial vein at 10 ml/min. A priming solution of 20 ml of 6 per cent creatinine, 0.25 ml. of 20 per cent PAH, and the test substance was given simultaneously with the start of the infusion mixture.

When urine flow from the left catheter had stabilized at 7-10 ml/min., timed free-flow urine collections were taken. Midway between the collection periods, blood samples were obtained. At the end of the second collection period, the catheter in the left ureter was clamped with a hemostat and urine flow from that kidney halted. The catheter was clamped either 5 or 6 minutes. Two minutes prior to releasing the clamp, a third blood sample was taken. One minute before releasing the clamp, 1.0 gm. inulin in 100 ml. distilled water was given into the jugular vein.

Immediately upon releasing the clamp serial urine samples were collected. These samples, from 0.5 - 0.6 ml/sample, were collected into 3 ml. glass tubes. The tubes were passed rapidly beneath the left ureteral catheter, and 30 samples collected in a time interval of 2 1/2 - 4 minutes.

A post-occlusion timed urine collection of six minutes, with a mid-point blood collection, was then taken. The volume of each serial sample was accurately measured with a delivery pipette and this volume diluted with glass-distilled water to a final volume of 10 ml. Determinations of concentrations of substances in each sample were then made.

The methods for creatinine, PAH, and Darstine were those described under standard clearance technique. $Na^+$ and $K^+$ analyses were made on the Baird flame photometer (direct standard). Inulin concentration in urine and plasma

was determined by the method of Levine and Becker (1959).

Pre-occlusion and postocclusion urine samples and blood samples were collected under oil where indicated in results. Total $CO_2$, $H_2CO_3$, $HCO_3^-$ and pH measurements were conducted as described under clearance techniques.

## Rat Kidney Slices

The flux of a substance between tubular urine and peritubular plasma is investigated with *in vivo* renal studies. The specific role of the tubular cell in this movement can be studied by an *in vitro* system, such as the kidney slices.

These experiments were carried out in a Warburg manometric apparatus (Umbreit *et al.*, 1957).

Male rats weighing 300-400 gm. each were exsanguinated and the kidneys were quickly removed and chilled in ice-cold isotonic NaCl. Slices of 0.4 to 0.5 mm. thickness were cut with the Stadie-Riggs micrometer (Stadie and Riggs, 1944) and placed in the cold NaCl solution. The tissue slices were then blotted on filter paper and 200-300 mg. of slices were placed in a Warburg flask containing 0.87 ml of 0.3 M NaCl, 0.2 ml of 0.1 M sodium phosphate buffer (pH 7.4), 0.36 ml of 0.3 M KCl, 0.1 ml of 0.02 M $CaCl_2$, 0.2 ml of appropriate molarity of Darstine or mecamylamine and brought to final volume of 2.8 ml with distilled water.

The center well of each flask contained 0.2 ml 6N NaOH on fluted filter paper as $CO_2$ absorber. The flasks were shaken at the rate of 100 cycles/min. at 37° C. All flasks were flushed with $O_2$ and after a 15 minute equilibration period appropriate taps were closed and oxygen consumption was recorded for two hours.

Immediately following the incubation period, the vessels were again chilled in cracked ice. Kidney slices from each vessel were quickly blotted, weighed to the nearest tenth of a mg. on a torsion balance and transferred to a graduated centrifuge tube. Each tube contained 1 ml. of 0.1 N HCl in 15 per cent trichloroscotic acid. Water was added to a total volume of 3 ml. (Le Sher and Shideman, 1956). After removal of the slices from the vessels, 2 ml. of medium were added to 1 ml. of 15 per cent trichloroacetic acid for a final volume of 3 ml.

The slices were pulverized with a glass rod and both the medium samples and slice samples were allowed to stand overnight. Samples were then centrifuged and the supernatant from the slices or the medium were analyzed for the organic base by methods previously described.

Tubular transport in renal slices is based on a comparison of medium and slice concentration of a substance. If a substance moves by diffusion alone from the medium into the slice, at the end of the incubation period the ratio

of the concentration of the compound in the slice to the concentration in the medium should be unity. If, however, this ratio exceeds unity, it is indicative of movement of the substance from a lower concentration to a higher concentration by active transport. Results are expressed as slice/medium ratio (S/M ratio).

## CHAPTER III

## EXPERIMENTS AND RESULTS

### Clearance Studies

Results obtained from clearance studies on the excretion of mecamylamine and Darstine are represented in Tables 3 - 21.

The headings of the columns for these tables have the following definitions:

| | |
|---|---|
| $C_x$ | clearance of creatinine ($C_{Cr}$), PAH ($C_{PAH}$), bicarbonate ($C_{HCO_3^-}$), mecamylamine ($C_M$), Darstine ($C_D$), potassium ($C_K$), sodium ($C_{Na}$), ml/min. |
| $P_x$ | plasma concentration of mecamylamine ($P_M$), Darstine ($P_D$) mg/ml; and potassium ($P_K$) or sodium ($P_{Na}$), mEq/L. |
| $C_{Cr}P_x$ | clearance of creatinine times plasma concentration of mecamylamine or Darstine equals amount of substance filtered, mg/min. |
| $C_x/C_{Cr}$ | clearance of substance, mecamylamine or Darstine, divided by creatinine clearance. |
| V | volume of urine, ml/min. |
| $U_x$ | urine concentration of mecamylamine ($U_M$), Darstine ($U_D$), mg/ml., potassium ($U_K$), sodium ($U_{Na}$), mEq/ml. |
| $U_xV$ | urine concentration of a substance times the urine volume equals the amount of substance excreted. Includes amount of substance in urine contributed by filtration and tubular movement. |

| | |
|---|---|
| $U_xV-C_{Cr}P_x$ | amount of substance excreted minus the amount of substance filtered mg/min. (+) denotes amount of substance added to urine by tubular secretion over the amount filtered, (-) denotes amount of substance removed from urine as a result of tubular reabsorption. |
| $U_{pH}$ | urinary pH |
| $P_{pH}$ | plasma pH |
| $C_{PAH}$ | clearance of para-aminohippurate, ml/min. |
| $U_{TCO_2}$ | total $CO_2$ in urine, mM/L. |
| $P_{TCO_2}$ | total $CO_2$ in plasma, mM/L. |
| $U_{pCO_2}$ | partial pressure of $CO_2$ in urine, mmHg. |
| $P_{pCO_2}$ | partial pressure of $CO_2$ in plasma, mmHg. |
| $U_{HCO_3^-}$ | bicarbonate concentration in urine, mM/L. |
| $P_{HCO_3^-}$ | bicarbonate concentration in plasma, mM/L. |
| $C_{HCO_3^-}$ | clearance of bicarbonate, ml/min. |
| $U_{H_2CO_3}$ | carbonic acid concentration in urine, mM/L. |
| $P_{H_2CO_3}$ | carbonic acid concentration in plasma, mM/L. |

The concentrations of mecamylamine in plasma are reported as total concentrations uncorrected for that bound to plasma protein (Baer et al., 1956). Thus, the amount of mecamylamine filtered is the value if all of the drug were completely filterable. Corrections for plasma binding were not made as the direction of mecamylamine clearance changes for the controls and experimental periods would be the same as those reported when secretion of mecamylamine occurs. Where mecamylamine is shown to be reabsorbed, calculations to include the correction for plasma binding were executed; in

all cases, mecamylamine is shown to be reabsorbed as the data presented indicate.

Darstine has been shown to be freely filterable at the glomerulus (Le Sher and Shideman, 1956).

TABLE 3

EFFECTS OF ACUTE METABOLIC ACIDOSIS ON MECAMYLAMINE AND POTASSIUM EXCRETION DURING MANNITOL DIURESIS

| Period | Time | $C_{Cr}$ | $P_M$ | $C_{Cr} P_M$ | V | $U_M$ | $U_M V$ | $U_M V - C_{Cr} P_M$ | $C_M$ | $C_M / C_{Cr}$ | $U_{pH}$ | $P_{pH}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | min | ml/min | mg/ml | mg/min | ml/min | mg/ml | mg/min | mg/min | ml/min | | | |
| | -125 | 500 ml. $H_2O$ p.o. | | | | | | | | | | |
| | -105 | 30 mg/kg. sodium pentobarbital, I.V. | | | | | | | | | | |
| | - 60 | Prime I: 20 ml. 6% creatinine + 2.5 mg/kg. mecamylamine | | | | | | | | | | |
| | | Infusion I: .38% creatinine, 2.0 mg/kg/hr. mecamylamine in 5% mannitol, I.V. @ 5 ml/min. | | | | | | | | | | |
| 1 | 10 | 29.93 | .0019 | .0569 | 4.9 | .0228 | .1120 | +.0551 | 58.80 | 1.96 | 6.66 | 7.43 |
| 2 | 20 | 29.22 | .0020 | .0584 | 5.2 | .0226 | .1180 | +.0596 | 58.76 | 2.01 | 6.66 | 7.45 |
| 3 | 30 | 28.90 | .0023 | .0665 | 5.5 | .0210 | .1160 | +.0495 | 50.22 | 1.74 | 6.66 | 7.44 |
| | 30 | Add 25 ml. .25 N HCl to infusion | | | | | | | | | | |
| 4 | 60 | 30.41 | .0025 | .0760 | 6.60 | .0356 | .2350 | +.1590 | 93.98 | 3.09 | 6.40 | 7.34 |
| 5 | 70 | 30.32 | .0027 | .0819 | 6.80 | .0414 | .2820 | +.2001 | 104.24 | 3.44 | 6.36 | 7.29 |
| 6 | 80 | 29.67 | .0029 | .0860 | 6.82 | .0428 | .2910 | +.2050 | 100.65 | 3.39 | 6.30 | 7.19 |
| | 80 | Add KCl to infusion to give .05 N KCl | | | | | | | | | | |
| 7 | 110 | 32.74 | .0040 | .1310 | 10.40 | .0350 | .3640 | +.2330 | 91.00 | 2.80 | 5.99 | 7.14 |
| 8 | 120 | 31.02 | .0046 | .1427 | 9.62 | .0384 | .3690 | +.2263 | 80.31 | 2.59 | 5.98 | 7.12 |

| Period | $U_{TCO_2}$ | $P_{TCO_2}$ | $U_{pCO_2}$ | $P_{pCO_2}$ | $U_{HCO_3^-}$ | $P_{HCO_3^-}$ | $C_{HCO_3^-}$ | $U_{H_2CO_3}$ | $P_{H_2CO_3}$ | $P_K$ | $U_K$ | $U_K V$ | $C_K$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mM/L | | mm Hg | | mM/L | | ml/min | mM/L | | mEq/L | | mEq/min | ml/min |
| 1 | 12.53 | 26.33 | 87 | 40 | 9.84 | 25.33 | 1.90 | 2.69 | 1.20 | 3.95 | 11.75 | 57.58 | 14.58 |
| 2 | 12.06 | 26.80 | 84 | 40 | 9.47 | 25.60 | 1.92 | 2.59 | 1.20 | 3.95 | 11.70 | 60.84 | 15.40 |
| 3 | 11.48 | 26.75 | 80 | 40 | 9.01 | 25.55 | 1.94 | 2.47 | 1.20 | 4.00 | 11.70 | 64.35 | 16.08 |
| 4 | 10.21 | 20.88 | 110 | 39 | 6.81 | 19.70 | 2.28 | 3.40 | 1.18 | 3.95 | 13.20 | 87.12 | 22.05 |
| 5 | 9.28 | 18.24 | 107 | 38 | 5.97 | 17.10 | 2.37 | 3.31 | 1.14 | 3.92 | 13.10 | 89.08 | 22.72 |
| 6 | 8.53 | 16.36 | 107 | 42 | 5.22 | 15.24 | 2.34 | 3.31 | 1.12 | 4.05 | 12.95 | 88.32 | 21.81 |
| 7 | 5.55 | 9.36 | 79 | 27 | 3.11 | 8.75 | 3.70 | 2.44 | .61 | 8.10 | 18.00 | 187.20 | 23.11 |
| 8 | 5.74 | 8.31 | 80 | 26 | 3.27 | 7.70 | 4.09 | 2.47 | .61 | 8.75 | 20.40 | 196.25 | 22.43 |

## Experiment 1

The data in Table 3 show the effects of acute metabolic acidosis on mecamylamine and potassium excretion. Hydrochloric acid was added to the standard infusion after control values for mecamylamine and potassium had been established.

This experiment was outlined (1) to show a definite inverse excretion relationship between mecamylamine and potassium when the urine was acidified, (2) to indicate if potassium administration would inhibit the high mecamylamine secretion into the acid urine.

In earlier experiments on mecamylamine excretion, urinary flow rates averaged 0.10 -0.30 ml/min. with isotonic NaCl infusion. In order to increase these urinary flows mannitol as an osmotic diuretic was added to the infusion solution in this and in other experiments where indicated.

The acidosis produced as the beginning of the first experimental periods (periods 4, 5 and 6) did cause a two-fold increase in mecamylamine clearance ($C_M$). However, potassium clearance ($C_K$) increased as well (60%). As was to be expected from the acidosis, urinary and plasma pH decreased, as did total $CO_2$ and urinary plasma bicarbonate. Bicarbonate clearance increased ($C_{HCO_3^-}$) and urinary $pCO_2$ and $H_2CO_3$ concentrations increased.

Scribner et al., (1959) had argued that if a base

were excreted by filtration and non-ionic diffusion, the clearance of the base should increase with increased urinary flow, as well as with a decreased urinary pH. However, the administration of potassium chloride after metabolic acidosis had been established resulted in a 15-20% depression of mecamylamine clearance, concomitant with a continued decrease in urinary pH and increased urine flow rate.

Results from this experiment were interpreted as evidence that (1) with the 15-20% depression of mecamylamine clearance following KCl administration superimposed on metabolic acidosis, mecamylamine excretion was related to a tubular cation exchange system, (2) potassium was the cation involved in the exchange system, (3) with the two-fold increase in mecamylamine clearance following the induction of metabolic acidosis, mecamylamine secretion occurred in part by non-ionic diffusion.

TABLE 4

EFFECTS OF KCl ADMINISTRATION ON MECAMYLAMINE EXCRETION DURING MANNITOL DIURESIS

| Period | Time | $C_{Cr}$ | $P_M$ | $C_{Cr}P_M$ | V | $U_M$ | $U_MV$ | $U_MV-C_{Cr}P$ | $C_M$ | $C_M/C_{Cr}$ | $U_{pH}$ | $P_{pH}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | min | ml/min | mg/ml | mg/min | ml/min | mg/ml | mg/min | mg/min | ml/min | | | |
| | -125 | 500 ml. $H_2O$ p.o. | | | | | | | | | | |
| | -105 | 30 mg/kg. sodium pentobarbital I.V. | | | | | | | | | | |
| | - 60 | Prime I: 20 ml. 6% creatinine + 2.5 mg/kg. mecamylamine I.V. | | | | | | | | | | |
| | | Infusion I: .38% creatinine, 2.0 mg/kg/hr. mecamylamine in 5% mannitol I.V. @ 5 ml/min. | | | | | | | | | | |
| 1 | 10 | 35.11 | .0021 | .0737 | 6.29 | .0200 | .1258 | +.0521 | 59.90 | 1.71 | 6.90 | 7.37 |
| 2 | 20 | 31.68 | .0022 | .0697 | 5.21 | .0292 | .1521 | +.0824 | 69.15 | 2.18 | 6.87 | 7.36 |
| 3 | 30 | 31.20 | .0023 | .0717 | 5.46 | .0286 | .1562 | +.0845 | 67.89 | 2.15 | 6.79 | 7.39 |
| | 30 | Add KCl to infusion to give .05 N KCl | | | | | | | | | | |
| 4 | 70 | 36.61 | .0024 | .0879 | 6.90 | .0192 | .1325 | +.0446 | 55.20 | 1.51 | 6.77 | 7.30 |
| 5 | 80 | 37.29 | .0025 | .0932 | 8.00 | .0158 | .1264 | +.0332 | 50.56 | 1.35 | 6.81 | 7.30 |
| 6 | 90 | 38.02 | .0026 | .0989 | 8.32 | .0176 | .1464 | +.0475 | 56.32 | 1.48 | 6.82 | 7.19 |
| 7 | 100 | 38.03 | .0027 | .1027 | 8.66 | .0170 | .1472 | +.0445 | 54.53 | 1.43 | 6.76 | 7.20 |

| Period | $U_{TCO_2}$ | $P_{TCO_2}$ | $U_{PCO_2}$ | $P_{PCO_2}$ | $U_{HCO_3^-}$ | $P_{HCO_3^-}$ | $C_{HCO_3^-}$ | $U_{H_2CO_3}$ | $P_{H_2CO_3}$ | $P_K$ | $U_K$ | $U_KV$ | $C_K$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mM/L | | mm Hg | | mM/l | | ml/min | mM/L | | mEq/L | | uEq/min | ml/min |
| 1 | 13.90 | 23.43 | 62 | 40 | 11.98 | 22.23 | 3.39 | 1.92 | 1.20 | 3.93 | 10.50 | 66.05 | 16.81 |
| 2 | 15.94 | 23.55 | 75 | 41 | 13.62 | 22.32 | 3.18 | 2.32 | 1.23 | 3.75 | 12.25 | 63.82 | 17.02 |
| 3 | 15.74 | 23.32 | 86 | 38 | 13.08 | 22.18 | 3.21 | 2.66 | 1.14 | 3.63 | 12.90 | 70.43 | 19.40 |
| 4 | 14.85 | 22.52 | 85 | 44 | 12.22 | 21.20 | 3.98 | 2.63 | 1.32 | 5.73 | 21.50 | 148.35 | 25.89 |
| 5 | 16.06 | 22.40 | 86 | 44 | 13.40 | 21.08 | 5.08 | 2.66 | 1.32 | 5.50 | 22.40 | 179.20 | 32.58 |
| 6 | 15.89 | 22.37 | 82 | 56 | 13.36 | 20.70 | 5.37 | 2.53 | 1.67 | 5.38 | 22.30 | 185.54 | 34.49 |
| 7 | 15.89 | 22.52 | 92 | 55 | 13.05 | 20.86 | 5.42 | 2.84 | 1.66 | 5.60 | 23.90 | 206.97 | 36.96 |

## Experiment 2

The experiment reported in Table 4 was designed to investigate the effects of the acute administration of KCl on mecamylamine excretion. Mannitol was infused to produce an osmotic diuresis.

The data show that mecamylamine clearance decreased 20% when potassium chloride was added to the infusion solution. This depression occurred again together with an increased urinary flow rate. Urine pH did not change significantly. The data show also that the urinary concentration of mecamylamine ($U_M$) decreased and, further, that the amount of mecamylamine excreted ($U_MV$) as well as the amount of mecamylamine in the urine contributed by tubular secretion ($U_MV - C_{Cr}P_M$) was depressed.

It is to be noted that, with those substances which show both net tubular secretion and net tubular reabsorption in clearance studies, a decrease in net tubular secretion could occur as the result of enhancement of reabsorption, or an inhibition of tubular secretion.

The results from this experiment were interpreted as evidence that mecamylamine could participate in the tubular cation exchange system.

TABLE 5

EFFECTS OF MECAMYLAMINE ADMINISTRATION ON POTASSIUM EXCRETION DURING ISOTONIC NaCl DIURESIS

| Period | Time | $C_{Cr}$ | $P_M$ | $C_{Cr}P_M$ | V | $U_M$ | $U_M V$ | $U_M V - C_{Cr} P_M$ | $C_M$ | $C_M/C_{Cr}$ | $U_{pH}$ | $P_{pH}$ | $C_{PAH}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | min | ml/min | mg/ml | mg/min | ml/min | mg/ml | mg/min | mg/min | mg/ml | | | | mg/ml |
| | -125 | 500 ml. $H_2O$ p.o. | | | | | | | | | | | |
| | -105 | 30 mg/kg. sodium pentobarbital I.V. | | | | | | | | | | | |
| | - 60 | Prime I: 20 ml. 6% creatinine + .25 ml. 20% PAH I.V. | | | | | | | | | | | |
| | | Infusion I: .38% creatinine, .1% PAH in .9% NaCl, I.V. @ 5 ml/min. | | | | | | | | | | | |
| 1 | 15 | 25.78 | | | 2.93 | | | | | | 5.95 | 7.38 | 113.73 |
| 2 | 30 | 20.81 | | | 1.93 | | | | | | 6.19 | 7.41 | 112.80 |
| | 30 | Prime II: 2.5 mg/kg. mecamylamine in 20 ml. $H_2O$ I.V. | | | | | | | | | | | |
| | | Add 2.0 mg/kg/hr. mecamylamine to infusion | | | | | | | | | | | |
| 4 | 105 | 46.77 | .0022 | .1029 | 4.49 | .0118 | .053 | -.0499 | 24.08 | .515 | 7.16 | 7.51 | 123.48 |
| 5 | 120 | 42.29 | .0024 | .1015 | 3.70 | .0119 | .044 | -.0575 | 18.35 | .434 | 7.15 | 7.51 | |
| 6 | 135 | 43.70 | .0026 | .1136 | 3.25 | .0121 | .039 | -.0746 | 15.13 | .346 | 7.22 | 7.51 | 129.38 |
| 7 | 150 | 45.86 | .0028 | .1284 | 3.55 | .0122 | .043 | -.0854 | 15.47 | .337 | 7.20 | 7.49 | 138.05 |

| Period | $U_{TCO_2}$ | $P_{TCO_2}$ | $U_{pCO_2}$ | $P_{pCO_2}$ | $U_{HCO_3}$ | $P_{HCO_3}$ | $C_{HCO_3}$ | $U_{H_2CO_3}$ | $P_{H_2CO_3}$ | $P_K$ | $U_K$ | $U_K V$ | $C_K$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mM/L | | mm Hg | | mM/L | | ml/min | mM/L | | mEq/L | | uEq/min | ml/min |
| 1 | 2.077 | 23.35 | 36 | 51 | .965 | 26.80 | .106 | 1.11 | 1.53 | 4.25 | 7.56 | 22.15 | 5.21 |
| 2 | 2.654 | 29.03 | 39 | 45 | 1.454 | 27.50 | .102 | 1.20 | 1.53 | 4.13 | 7.90 | 16.25 | 3.69 |
| 3 | | | | | | | | | | | | | |
| 4 | 27.15 | 28.27 | 70 | 35 | 24.99 | 27.21 | 4.12 | 2.16 | 1.06 | 4.03 | 24.50 | 110.00 | 27.30 |
| 5 | 29.30 | 27.82 | 78 | 35 | 26.89 | 26.78 | 3.72 | 2.41 | 1.04 | 4.03 | 25.70 | 95.09 | 23.60 |
| 6 | 29.57 | 23.10 | 67 | 29 | 27.50 | 22.11 | 4.04 | 2.07 | .99 | 3.80 | 26.90 | 87.43 | 23.01 |
| 7 | 28.30 | 22.53 | 67 | 30 | 26.23 | 21.70 | 4.29 | 2.07 | .83 | 3.68 | 21.80 | 77.89 | 21.02 |

Experiment 3

The data presented in Table 5 represent results from an experiment designed to show the effects of the acute administration of mecamylamine on potassium excretion during isotonic NaCl infusion.

Two control periods (periods 1 and 2) were established prior to the infusion of mecamylamine. The changes noted after mecamylamine administration were a fourfold increase in potassium clearance, increase in urinary total $CO_2$, urinary bicarbonate ($U_{HCO_3^-}$), clearance of bicarbonate ($C_{HCO_3^-}$), urinary volume and pH. These changes closely resembled results from earlier experiments in which the carbonic anhydrase inhibitor, acetazolamide (Diamox$^R$) was administered.

These data are therefore suggestive of a carbonic anhydrase inhibitor action of mecamylamine. Unexplainable is the twofold increase seen in GFR ($C_{Cr}$) following mecamylamine administration.

TABLE 6

EFFECTS OF KCl ADMINISTRATION ON MECAMYLAMINE EXCRETION DURING ACUTE METABOLIC ALKALOSIS

| Period | Time | $C_{Cr}$ | $P_M$ | $C_{Cr}P_M$ | V | $U_M$ | $U_MV$ | $U_MV-C_{Cr}P$ | $C_M$ | $C_M/C_{Cr}$ | $U_{pH}$ | $P_{pH}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | min | ml/min | mg/ml | mg/min | ml/min | mg/ml | mg/min | mg/min | ml/min | | | |
| | -125 | 500 ml. $H_2O$ p.o. | | | | | | | | | | |
| | -105 | 30 mg/kg. sodium pentobarbital I.V. | | | | | | | | | | |
| | - 60 | Prime I: 20 ml. 6% creatinine + 2.5 mg/kg. mecamylamine I.V. | | | | | | | | | | |
| | | Infusion I: .38% creatinine, 2.0 mg/kg/hr. mecamylamine in 7.5% $NaHCO_3$ I.V. @ 5 ml/min. | | | | | | | | | | |
| 1 | 10 | 45.90 | .0009 | .0413 | 12.00 | .00132 | .0158 | -.0255 | 17.60 | .383 | 7.62 | 7.45 |
| 2 | 20 | 46.27 | .0011 | .0509 | 12.80 | .00140 | .0180 | -.0329 | 16.37 | .354 | 7.67 | 7.46 |
| 3 | 30 | 40.71 | .0010 | .0407 | 11.40 | .00120 | .0143 | -.0264 | 14.25 | .351 | 7.71 | 7.46 |
| | 30 | Add KCl to infusion to give .05 N KCl | | | | | | | | | | |
| 4 | 60 | 38.24 | .0012 | .0459 | 12.00 | .00135 | .0162 | -.0297 | 13.51 | .353 | 7.73 | 7.49 |
| 5 | 70 | 33.80 | .0017 | .0575 | 10.35 | .00165 | .0171 | -.0404 | 10.05 | .297 | 7.73 | 7.51 |
| 6 | 80 | 42.87 | .0016 | .0686 | 12.86 | .00150 | .0193 | -.0493 | 12.06 | .281 | 7.64 | 7.53 |

| Period | $U_{TCO_2}$ | $P_{TCO_2}$ | $U_{pCO_2}$ | $P_{pCO_2}$ | $U_{HCO_3^-}$ | $P_{HCO_3^-}$ | $C_{HCO_3^-}$ | $U_{H_2CO_3}$ | $P_{H_2CO_3}$ | $P_K$ | $U_K$ | $U_KV$ | $C_K$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mM/L | | mm Hg | | mM/L | | ml/min | mM/L | | mEq/L | | mEq/min | ml/min |
| 1 | 180.92 | 76.90 | 172 | 108 | 175.60 | 73.65 | 28.84 | 5.32 | 3.25 | 3.19 | 12.50 | 150.00 | 47.02 |
| 2 | 174.91 | 77.04 | 148 | 107 | 170.34 | 73.82 | 29.54 | 4.57 | 3.22 | 3.18 | 13.10 | 167.68 | 52.73 |
| 3 | 188.00 | 79.18 | 149 | 109 | 183.40 | 75.90 | 27.56 | 4.60 | 3.28 | 3.10 | 13.20 | 150.48 | 48.54 |
| 4 | 207.91 | 90.30 | 154 | 117 | 203.15 | 86.78 | 28.09 | 4.76 | 3.52 | 4.15 | 16.60 | 199.20 | 48.00 |
| 5 | 215.88 | 89.73 | 160 | 112 | 210.94 | 86.36 | 25.29 | 4.94 | 3.37 | 4.26 | 17.80 | 184.23 | 43.25 |
| 6 | 224.41 | 92.57 | 204 | 110 | 218.11 | 89.26 | 31.42 | 6.30 | 3.31 | 4.41 | 18.90 | 243.05 | 55.11 |

## Experiment 4

Earlier experiments had shown that net tubular reabsorption of mecamylamine occurred when the urine was alkalinized. The experiment represented by the data presented in Table 6 was designed to study the effects of the administration of potassium chloride on mecamylamine excretion during metabolic alkalosis.

The control values in periods 1, 2 and 3 show tubular reabsorption of mecamylamine, with the clearance ratios for mecamylamine to creatinine clearance ($C_M/C_{Cr}$) of less than one. Urinary pH was well within the alkaline ranges. The potassium clearance indicated active tubular secretion of potassium as the clearance of potassium was higher than the simultaneously measured clearance of creatinine.

Following potassium chloride infusion (periods 4, 5 and 6) the clearance of mecamylamine ($C_M$) continued to decrease. The amount of mecamylamine reabsorbed increased 25% and the $C_M/C_{Cr}$ was reduced further. Urine volume and urine pH remained constant. Potassium clearance continued to indicate active tubular secretion of potassium.

The results from this experiment indicate further evidence of potassium and mecamylamine participation in a cation exchange system. Potassium loading resulted in increased mecamylamine reabsorption with no change in urinary

pH. This effect is difficult to explain with the concept of excretion by filtration and non-ionic diffusion.

TABLE 7

EFFECTS OF MECAMYLAMINE ADMINISTRATION ON POTASSIUM EXCRETION DURING ACUTE METABOLIC ACIDOSIS

| Period | Time | $C_{Cr}$ | $P_M$ | $C_{Cr}P_M$ | V | $U_M$ | $U_MV$ | $U_MV—C_{Cr}P$ | $C_M$ | $C_M/C_{Cr}$ | $U_{pH}$ | $P_{pH}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | min | ml/min | mg/ml | mg/min | ml/min | mg/ml | mg/min | mg/min | ml/min | | | |
| | - 95 | 500 ml. $H_2O$ p.o. | | | | | | | | | | |
| | - 75 | 30 mg/kg. sodium pentobarbital I.V. | | | | | | | | | | |
| | - 30 | Prime I: 20 ml. 6% creatinine I.V. | | | | | | | | | | |
| | | Infusion I: .38% creatinine in .9% NaCl + 25 ml. .25 N HCl, I.V., @ 5 ml/min. | | | | | | | | | | |
| 1 | 15 | 43.45 | | | .600 | | | | | | 5.50 | 7.21 |
| 2 | 30 | 43.21 | | | .600 | | | | | | 5.32 | 7.20 |
| 3 | 45 | 43.89 | | | .607 | | | | | | 5.20 | 7.21 |
| | 45 | Prime II: 8 mg/kg. mecamylamine in 20 ml. $H_2O$ | | | | | | | | | | |
| | | Add 10 mg/kg/hr. mecamylamine to infusion | | | | | | | | | | |
| 4 | 120 | 45.43 | .0039 | .1771 | 1.577 | .264 | .416 | +.2389 | 106.67 | 2.35 | 5.27 | 7.01 |
| 5 | 135 | 36.13 | .0055 | .1987 | 1.673 | .447 | .748 | +.5493 | 136.00 | 3.76 | 5.28 | 6.94 |
| 6 | 150 | 26.11 | .0067 | .1749 | 1.960 | .442 | .866 | +.6911 | 129.25 | 4.95 | 5.27 | 6.82 |

| Period | $U_{TCO_2}$ | $P_{TCO_2}$ | $U_{pCO_2}$ | $P_{pCO_2}$ | $U_{HCO_3^-}$ | $P_{HCO_3^-}$ | $C_{HCO_3^-}$ | $U_{H_2CO_3}$ | $P_{H_2CO_3}$ | $P_K$ | $U_K$ | $U_KV$ | $C_K$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mM/L | | mm Hg | | mM/L | | ml/min | mM/L | | mEq/L | | uEq/min | ml/min |
| 1 | 2.264 | 17.43 | 15.0 | 41 | 1.804 | 16.19 | .668 | .460 | 1.244 | 4.35 | 29.03 | 17.42 | 4.00 |
| 2 | .977 | 15.51 | 4.5 | 37 | .838 | 14.40 | .349 | .139 | 1.112 | 4.38 | 32.83 | 19.70 | 4.50 |
| 3 | 1.043 | 13.09 | 3.8 | 31 | .927 | 12.16 | .463 | .116 | .931 | 4.38 | 37.70 | 22.88 | 5.22 |
| 4 | .606 | 7.09 | 2.5 | 26 | .528 | 6.46 | .129 | .078 | .632 | 4.43 | 40.90 | 64.50 | 14.56 |
| 5 | .627 | 6.40 | 2.7 | 27 | .544 | 5.59 | .163 | .083 | .810 | 4.88 | 41.94 | 70.17 | 14.40 |
| 6 | .627 | 4.23 | 2.6 | 22 | .546 | 3.57 | .300 | .081 | .660 | 5.43 | 42.34 | 82.98 | 15.28 |

Experiment 5

The secretion of potassium has been shown to be minimal during metabolic acidosis, as the tubular sodium exchange is primarily with the hydrogen ion (Berliner et al., 1951). Data shown in Table 7 is from an experiment designed to show if the administration of mecamylamine would enhance potassium excretion during metabolic acidosis. An isotonic NaCl solution was infused.

With the addition of mecamylamine to the infusion solution, there was a threefold increase in the clearance of potassium. Concurrently, urine flow doubled. Values for both urine and plasma $H_2CO_3$, $pCO_2$, $HCO_3^-$ and total $CO_2$ decreased from controls. These depressed urine and plasma $CO_2$ and related values in periods 3, 4 and 5 are indicative of increased $HCO_3^-$ reabsorption with sustained metabolic acidosis.

The increase in potassium excretion following mecamylamine administration was interpreted as further evidence of a cation tubular exchange relationship between mecamylamine and potassium.

TABLE 8

EFFECTS OF MECAMYLAMINE ADMINISTRATION ON POTASSIUM EXCRETION DURING ACUTE RESPIRATORY ALKALOSIS

| Period | Time | $C_{Cr}$ | $P_M$ | $C_{Cr}P_M$ | V | $U_M$ | $U_MV$ | $U_MV—C_{Cr}P$ | $C_M$ | $C_M/C_{Cr}$ | $U_{pH}$ | $P_{pH}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | min | ml/min | mg/ml | mg/min | ml/min | mg/ml | mg/min | mg/min | ml/min | | | |
| | -125 | 500 ml. $H_2O$ p.o. | | | | | | | | | | |
| | -105 | 30 mg/kg. sodium pentobarbital I.V. | | | | | | | | | | |
| | - 60 | Prime I: 20 ml. 6% creatinine I.V. | | | | | | | | | | |
| | | Infusion I: .38% creatinine in .9% NaCl I.V. @ 5 ml/min. | | | | | | | | | | |
| | - 40 | Hyperventilate 30 strokes/min. 275 cc/stroke | | | | | | | | | | |
| 1 | 15 | 35.82 | | | .325 | | | | | | 6.10 | 7.42 |
| 2 | 30 | 30.74 | | | .207 | | | | | | 6.15 | 7.42 |
| | 30 | Prime II: 8 mg/kg mecamylamine in 20 ml. $H_2O$ I.V. | | | | | | | | | | |
| | | Add 10 mg/kg/hr. mecamylamine to infusion | | | | | | | | | | |
| 4 | 75 | 41.17 | .0036 | .148 | 2.32 | .154 | .357 | +.209 | 99.16 | 2.41 | 6.75 | 7.41 |
| 5 | 90 | 39.20 | .0040 | .156 | 2.32 | .171 | .398 | +.242 | 99.50 | 2.54 | 6.80 | 7.43 |
| 6 | 105 | 37.17 | .0050 | .185 | 1.85 | .208 | .385 | +.200 | 77.00 | 2.07 | 6.76 | 7.50 |

| Period | $U_{TCO_2}$ | $P_{TCO_2}$ | $U_{pCO_2}$ | $P_{pCO_2}$ | $U_{HCO_3^-}$ | $P_{HCO_3^-}$ | $C_{HCO_3^-}$ | $U_{H_2CO_3}$ | $P_{H_2CO_3}$ | $P_K$ | $U_K$ | $U_KV$ | $C_K$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mM/L | | mm Hg | | mM/L | | ml/min | mM/L | | mEq/L | | uEq/min | ml/min |
| 1 | .625 | 14.79 | 20 | 22 | .025 | 14.13 | .058 | .600 | .66 | 4.12 | 56.65 | 18.41 | 4.47 |
| 2 | .566 | 14.40 | 17 | 22 | .045 | 13.74 | .062 | .525 | .66 | 4.07 | 92.84 | 19.22 | 4.72 |
| 4 | 3.85 | 11.90 | 23 | 18 | 3.14 | 11.36 | .641 | .712 | .54 | 3.40 | 18.30 | 42.46 | 12.49 |
| 5 | 4.20 | 11.67 | 22 | 17 | 3.51 | 11.16 | .732 | .680 | .51 | 3.25 | 18.40 | 42.69 | 13.14 |
| 6 | 4.20 | 11.56 | 24 | 15 | 3.46 | 11.11 | .576 | .740 | .45 | 3.17 | 22.44 | 30.52 | 9.27 |

## Experiment 6

The results shown in Table 8 are from an experiment designed to indicate the effect of the acute administration of mecamylamine on potassium excretion during respiratory alkalosis. Mecamylamine was added to the infusion solution following the establishment of control values for potassium (periods 1 and 2).

In respiratory alkalosis $P_{T_{CO_2}}$, $P_{HCO_3^-}$ and $P_pCO_2$ are decreased. The renal tubular reaction to the increased alkalinity of the plasma is a decreased hydrogen ion secretion as a result of less $CO_2$ available for hydration within the tubular cells. This decreased hydrogen ion secretion results in an increased secretion of potassium ions in exchange for the sodium ions which are continually being reabsorbed (Elkington and Danowski, 1956). Therefore, if mecamylamine secretion does inhibit potassium secretion, there should be evidence of this inhibition with mecamylamine infusion.

The results of the mecamylamine infusion on potassium excretion showed no tubular inhibition of potassium, but rather potassium clearance increased threefold. (The increase in potassium clearance could be as a result of the inhibition of potassium tubular reabsorption). Concurrently with the enhanced potassium clearance, there was seen an increased urine flow, an increased $U_{T_{CO_2}}$, $U_{HCO_3^-}$ and $C_{HCO_3^-}$, as well as an increased UpH.

A control experiment was conducted to determine the effects of respiratory alkalosis alone on the excretion of potassium. During the six periods, potassium clearance remained relatively constant, although potassium concentration in the urine varied inversely with urine flow. Therefore, the data from this experiment were interpreted as evidence that mecamylamine administration enhanced potassium secretion.

TABLE 9

EFFECTS OF INCREASED PLASMA LEVELS OF MECAMYLAMINE ON POTASSIUM EXCRETION DURING MANNITOL DIURESIS

| Period | Time | $C_{Cr}$ | $P_M$ | $C_{Cr} P_M$ | V | $U_M$ | $U_M V$ | $U_M V - C_{Cr} P_M$ | $C_M$ | $C_M / C_{Cr}$ | $U_{pH}$ | $P_{pH}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | min | ml/min | mg/ml | mg/min | ml/min | mg/ml | mg/min | mg/min | ml/min | | | |
| | -105 | 500 ml. $H_2O$ p.o. | | | | | | | | | | |
| | - 90 | 30 mg/kg. sodium pentobarbital I.V. | | | | | | | | | | |
| | - 45 | Prime I: 20 ml. 6% creatinine I.V. | | | | | | | | | | |
| | | Infusion I: .38% creatinine in 5% mannitol, I.V. @ 5 ml./min. | | | | | | | | | | |
| 1 | 10 | 26.86 | | | 6.6 | | | | | | 6.88 | 7.25 |
| 2 | 20 | 27.44 | | | 6.8 | | | | | | 6.84 | 7.32 |
| 3 | 30 | 28.56 | | | 7.4 | | | | | | 6.83 | 7.30 |
| | 30 | Prime II: 2.5 mg/kg. mecamylamine in 20 ml. $H_2O$ I.V. | | | | | | | | | | |
| | | Add 5 mg/kg/hr. mecamylamine to infusion | | | | | | | | | | |
| 4 | 60 | 24.57 | .0024 | .0590 | 6.27 | .0120 | .0752 | +.0162 | 31.35 | 1.28 | 6.80 | 7.40 |
| 5 | 70 | 23.60 | .0024 | .0566 | 6.49 | .0114 | .0740 | +.0174 | 30.83 | 1.31 | 6.79 | 7.41 |
| 6 | 80 | 24.49 | .0024 | .0588 | 6.80 | .0150 | .1020 | +.0432 | 42.50 | 1.74 | 6.72 | 7.46 |
| | 80 | Prime III: 1.2 mg/kg. mecamylamine in 20 ml. $H_2O$ I.V. | | | | | | | | | | |
| | | Add 10 mg/kg/hr. mecamylamine to infusion | | | | | | | | | | |
| 7 | 110 | 19.73 | .0040 | .0789 | 5.85 | .033 | .1931 | +.1142 | 48.26 | 2.45 | 6.71 | 7.42 |
| 8 | 120 | 16.40 | .0054 | .0886 | 4.85 | .042 | .2037 | +.1151 | 37.72 | 2.30 | 6.70 | 7.32 |
| 9 | 130 | 16.37 | .0055 | .0900 | 5.05 | .044 | .2222 | +.1322 | 40.40 | 2.47 | 6.65 | |

| Period | $U_{TCO_2}$ | $P_{TCO_2}$ | $U_{pCO_2}$ | $P_{pCO_2}$ | $U_{HCO_3^-}$ | $P_{HCO_3^-}$ | $C_{HCO_3^-}$ | $U_{H_2CO_3}$ | $P_{H_2CO_3}$ | $P_K$ | $U_K$ | $U_K V$ | $C_K$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mM/L | | mm Hg | | mM/L | | ml/min | mM/L | | mEq/L | | mEq/min | ml/min |
| 1 | 15.16 | 21.51 | 70 | 48 | 13.00 | 20.07 | 4.27 | 2.16 | 1.44 | 4.60 | 6.05 | 39.93 | 8.68 |
| 2 | 14.40 | 20.83 | 72 | 41 | 12.27 | 19.60 | 4.25 | 2.22 | 1.23 | 4.65 | 6.35 | 43.13 | 9.29 |
| 3 | 14.29 | 21.96 | 73 | 44 | 12.03 | 20.64 | 4.32 | 2.25 | 1.32 | 4.60 | 6.85 | 50.69 | 11.10 |
| 4 | 13.49 | | 73 | | 11.23 | | | 2.26 | | 4.50 | 6.50 | 40.76 | 9.06 |
| 5 | 13.03 | 21.39 | 72 | 34 | 10.80 | 20.37 | 3.45 | 2.22 | 1.02 | 4.65 | 6.70 | 43.48 | 9.35 |
| 6 | 12.92 | | 72 | | 10.69 | | | 2.22 | | 4.68 | 7.05 | 47.94 | 10.24 |
| 7 | 12.00 | | 71 | | 9.62 | | | 2.38 | | 4.30 | 6.35 | 37.15 | 8.64 |
| 8 | 11.27 | 17.07 | 73 | 33 | 9.01 | 16.06 | 2.72 | 2.26 | .99 | 4.30 | 5.75 | 27.89 | 6.49 |
| 9 | 10.36 | | 74 | | 8.07 | | | 2.29 | | 4.35 | 5.85 | 29.54 | 6.79 |

Experiment 7

This experiment (Table 9) and the following experiment (Table 10) were conducted to assess the effect of increased plasma levels of mecamylamine on potassium excretion during mannitol diuresis. Three control periods (1, 2 and 3) were followed by two additional experiment collection periods (4, 5 and 6; 7, 8 and 9) between which mecamylamine plasma levels were increased.

The results from the experiment presented in Table 9 show that mecamylamine infusion had no significant effect on potassium excretion during periods 4, 5 and 6. During periods 7, 8 and 9, with the increased plasma levels of mecamylamine, potassium clearance was inhibited 20% from the previous experimental periods. This decrease in clearance occurred, however, with a concomitant decrease in the glomerular filtration rate ($C_{Cr}$). Therefore, the amount of potassium reabsorbed ($C_{Cr}P_K - U_KV$) was calculated for the nine periods. Reabsorption of potassium decreased by 12% when mecamylamine was first infused, and decreased by 40% upon further raising the plasma levels of mecamylamine, from control values.

Data from this experiment, therefore, were interpreted as evidence that mecamylamine inhibited the tubular reabsorption of potassium.

TABLE 10

EFFECTS OF INCREASED PLASMA LEVELS OF MECAMYLAMINE ON POTASSIUM EXCRETION DURING MANNITOL DIURESIS

| Period | Time | $C_{Cr}$ | $P_M$ | $C_{Cr}P_M$ | V | $U_M$ | $U_MV$ | $U_MV - C_{Cr}P$ | $C_M$ | $C_M/C_{Cr}$ | $U_{pH}$ | $P_{pH}$ | $C_{PAH}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | min | ml/min | mg/ml | mg/min | ml/min | mg/ml | mg/min | mg/min | ml/min | | | | |
| | -125 | 500 ml. $H_2O$ p.o. | | | | | | | | | | | |
| | -105 | 30 mg/kg. sodium pentobarbital I.V. | | | | | | | | | | | |
| | - 60 | Prime I: 20 ml. 6% creatinine + .25 ml. 20% PAH, I.V. | | | | | | | | | | | |
| | | Infusion I: .38% creatinine, .1% PAH in 5% mannitol, I.V. @ 5 ml/min. | | | | | | | | | | | |
| 1 | 10 | 33.16 | | | 7.10 | | | | | | 6.67 | 7.30 | 104.35 |
| 2 | 20 | 34.44 | | | 8.07 | | | | | | 6.65 | 7.36 | 120.66 |
| 3 | 30 | 34.78 | | | 8.60 | | | | | | 6.64 | 7.35 | 96.28 |
| | 30 | Prime II: 3.0 mg/kg. mecamylamine in 20 ml. $H_2O$, I.V. | | | | | | | | | | | |
| | | Add 6 mg/kg/hr. mecamylamine to infusion | | | | | | | | | | | |
| 4 | 65 | 37.83 | .0021 | .079 | 11.16 | .0192 | .214 | +.135 | 102.03 | 2.70 | 6.69 | 7.29 | |
| 5 | 75 | | .0023 | | 9.25 | .0212 | .196 | | 85.14 | | 6.72 | 7.32 | 115.59 |
| 6 | 85 | 43.92 | .0024 | .105 | 14.41 | .0224 | .323 | +.218 | 134.49 | 3.06 | 6.75 | 7.33 | |
| | 85 | Prime III: 1.5 mg/kg mecamylamine in 20 ml. $H_2O$, I.V. | | | | | | | | | | | |
| | | Add 12 mg/kg/hr. mecamylamine to infusion | | | | | | | | | | | |
| 7 | 120 | 33.10 | .0057 | .189 | 10.21 | .0508 | .517 | +.328 | 90.99 | 2.70 | 7.00 | 7.31 | 181.00 |
| 8 | 130 | 38.67 | .0063 | .244 | 12.00 | .0588 | .706 | +.462 | 112.00 | 2.90 | 7.06 | 7.34 | 181.00 |
| 9 | 140 | 30.81 | .0080 | .256 | 9.11 | .0684 | .623 | +.367 | 77.89 | 2.52 | 7.02 | 7.30 | 178.00 |

| Period | $U_{TCO_2}$ | $P_{TCO_2}$ | $U_{pCO_2}$ | $P_{pCO_2}$ | $U_{HCO_3^-}$ | $P_{HCO_3^-}$ | $C_{HCO_3^-}$ | $U_{H_2CO_3}$ | $P_{H_2CO_3}$ | $P_K$ | $U_K$ | $U_KV$ | $C_K$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mM/L | | mm Hg | | mM/L | | ml/min | mM/L | | mEq/L | | mEq/min | ml/min |
| 1 | 9.14 | 21.87 | 63 | 44 | 7.19 | 20.57 | 2.48 | 1.95 | 1.30 | 4.50 | 7.10 | 50.41 | 11.20 |
| 2 | 8.46 | 21.53 | 60 | 38 | 6.61 | 20.39 | 2.62 | 1.85 | 1.14 | 4.35 | 7.30 | 58.91 | 13.54 |
| 3 | 8.34 | 21.19 | 60 | 39 | 6.49 | 20.00 | 2.79 | 1.85 | 1.19 | 4.35 | 8.10 | 69.66 | 16.01 |
| 4 | 11.05 | 21.36 | 61 | 44 | 9.16 | 20.02 | 5.11 | 1.89 | 1.34 | 4.56 | 10.60 | 118.30 | 25.94 |
| 5 | 11.55 | 20.92 | 72 | 41 | 9.32 | 19.68 | 4.38 | 2.23 | 1.24 | 4.18 | 11.35 | 104.99 | 25.17 |
| 6 | 11.78 | | 70 | | 9.62 | | | 2.16 | | 4.23 | 12.25 | 176.52 | 41.78 |
| 7 | 13.82 | | 60 | | 11.97 | | | 1.85 | | 5.33 | 15.55 | 158.77 | 29.78 |
| 8 | 14.72 | 19.69 | 57 | 37 | 12.96 | 18.60 | 8.36 | 1.76 | 1.09 | 5.33 | | | |
| 9 | 15.18 | 19.80 | 63 | 40 | 13.23 | 18.80 | 6.41 | 1.95 | 1.00 | 5.75 | 16.65 | 151.68 | 26.38 |

## Experiment 8

As was described in the previous experiment, Table 10 also indicates the effects of increased mecamylamine plasma levels on potassium excretion during mannitol diuresis. The clearance of para-aminohippurate ($C_{PAH}$) as a measure of renal plasma flow was included in this and all subsequent experiments.

The results in this experiment show that, after the addition of mecamylamine to the infusion solution, potassium clearance ($C_K$) increased 70% over control values and increased 10% further when plasma levels of mecamylamine were raised. This change in potassium excretion occurred with no significant change in the amount of potassium filtered.

The infusion of a mannitol solution has been shown to result in a graded increase in the rate of the excretion of potassium and bicarbonate. Urine pH, $U_{T_{CO_2}}$, as well as urine flow also increase. Although these changes are found to occur in these data and simultaneously with mecamylamine administration, the change in potassium clearance from control values was sharp and sudden, not graded.

The results from this experiment have been interpreted as further evidence that mecamylamine either increases potassium secretion or inhibits potassium reabsorption.

TABLE 11

EFFECTS OF MECAMYLAMINE AND KCl ADMINISTRATION ON POTASSIUM AND MECAMYLAMINE EXCRETION DURING MANNITOL DIURESIS

| Period | Time | $C_{Cr}$ | $P_M$ | $C_{Cr}P_M$ | V | $U_M$ | $U_MV$ | $U_MV-C_{Cr}P_M$ | $C_M$ | $C_M/C_{Cr}$ | $U_{pH}$ | $P_{pH}$ | $C_{PAH}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | min | ml/min | mg/ml | mg/min | ml/min | mg/ml | ml/min | mg/min | ml/min | | | | ml/min |
| | -110 | 500 ml. $H_2O$ p. o. | | | | | | | | | | | |
| | - 90 | 30 mg/kg. sodium pentobarbital I.V. | | | | | | | | | | | |
| | - 45 | Prime I: 20 ml. 6% creatinine + .25 ml. 20% PAH I.V. | | | | | | | | | | | |
| | | Infusion I: .38% creatinine, .1% PAH in 5% mannitol I.V. @ 5 ml/min. | | | | | | | | | | | |
| 1 | 10 | 40.66 | | | 3.945 | | | | | | 6.77 | 7.42 | 109.44 |
| 2 | 20 | 46.05 | | | 4.700 | | | | | | 6.85 | 7.42 | 120.93 |
| 3 | 30 | 40.56 | | | 4.470 | | | | | | 6.73 | 7.41 | 110.85 |
| | 30 | Prime II: 2.0 mg/kg. mecamylamine in 20 ml. $H_2O$ I.V. | | | | | | | | | | | |
| | | Add 3.0 mg/kg/hr. mecamylamine to infusion | | | | | | | | | | | |
| 4 | 70 | 42.14 | .0016 | .0674 | 6.70 | .0254 | .1702 | +.1028 | 106.37 | 2.52 | 6.63 | 7.40 | 164.73 |
| 5 | 80 | 44.30 | .0019 | .0842 | 7.10 | .0304 | .2158 | +.1316 | 113.57 | 2.56 | 6.61 | 7.41 | 177.95 |
| 6 | 90 | 46.55 | .0020 | .0931 | 7.48 | .0332 | .2483 | +.1552 | 124.15 | 2.67 | 6.69 | 7.43 | 185.11 |
| | 90 | Add KCl to infusion to give .05 N KCl | | | | | | | | | | | |
| 7 | 130 | 42.85 | .0026 | .1114 | 7.70 | .0352 | .2710 | +.1596 | 104.23 | 2.43 | 6.66 | 7.32 | 162.62 |
| 8 | 140 | 47.90 | .0027 | .1293 | 8.47 | .0466 | .3947 | +.2654 | 146.18 | 3.05 | 6.61 | 7.32 | 181.34 |
| 9 | 150 | 51.17 | .0028 | .1433 | 9.40 | .0414 | .3892 | +.2459 | 139.00 | 2.72 | 6.61 | 7.38 | 192.27 |

| Period | $U_{TCO_2}$ | $P_{TCO_2}$ | $U_{pCO_2}$ | $P_{pCO_2}$ | $U_{HCO_3^-}$ | $P_{HCO_3^-}$ | $C_{HCO_3^-}$ | $U_{H_2CO_3}$ | $P_{H_2CO_3}$ | $P_K$ | $U_K$ | $U_KV$ | $C_K$ | $P_{Na}$ | $U_{Na}$ | $U_{Na}V$ | $C_{Na}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mM/L | | mm Hg | | mM/L | | ml/min | mM/L | | mEq/L | | mEq/min | ml/min | mEq/L | | mEq/min | ml/min |
| 1 | 21.03 | 22.42 | 120 | 34 | 17.32 | 21.40 | 3.16 | 3.71 | 1.02 | 3.95 | 7.40 | 29.19 | 7.39 | 104 | 20.4 | 80.46 | .77 |
| 2 | 19.89 | 21.74 | 98 | 33 | 16.86 | 20.75 | 3.82 | 3.03 | .99 | 3.95 | 6.05 | 28.44 | 7.20 | 105 | 12.6 | 57.96 | .55 |
| 3 | 19.12 | 21.51 | 118 | 33 | 15.47 | 20.58 | 3.36 | 3.65 | .99 | 3.95 | 4.95 | 22.13 | 5.60 | 102 | 10.0 | 44.70 | .44 |
| 4 | 18.66 | 21.96 | 138 | 35 | 14.40 | 20.91 | 4.62 | 4.26 | 1.05 | 4.10 | 5.80 | 38.86 | 9.48 | 104 | 24.0 | 160.80 | 1.55 |
| 5 | 19.00 | 22.93 | 145 | 36 | 14.52 | 21.85 | 4.72 | 4.48 | 1.08 | 4.00 | 5.60 | 41.18 | 10.30 | 101 | 23.9 | 169.69 | 1.68 |
| 6 | 19.12 | 22.63 | 127 | 34 | 15.20 | 22.61 | 5.04 | 3.92 | 1.02 | 4.40 | 15.60 | 41.89 | 9.50 | 100 | 21.5 | 160.82 | 1.60 |
| 7 | 19.80 | 22.40 | 138 | 42 | 15.54 | 21.14 | 5.66 | 4.26 | 1.26 | 5.80 | 12.60 | 97.02 | 16.73 | | 17.6 | 135.52 | |
| 8 | 20.03 | 22.06 | 153 | 42 | 15.31 | 20.80 | 6.23 | 4.72 | 1.26 | 6.20 | 15.70 | 132.98 | 21.45 | 100 | 16.0 | 135.52 | 1.35 |
| 9 | 20.48 | 21.37 | 157 | 35 | 15.63 | 21.32 | 6.88 | 4.85 | 1.05 | 6.65 | 19.60 | 184.24 | 27.71 | 97 | 14.5 | 136.30 | 1.41 |

## Experiment 9

The data in Tables 11 and 12 represent results from experiments designed to assess the mutual effects of both mecamylamine and potassium administration on potassium and mecamylamine excretion during a mannitol diuresis. Table 11 shows data on mecamylamine-potassium excretion relationships in the anesthetized dog. Table 12 shows these relationships in the unanesthetized dog. Sodium concentrations were determined in experiments 9 and 10 (Tables 11 and 12).

In Table 11, control values are indicated in periods 1, 2 and 3. Mecamylamine was then added to the infusion solution. Following three collection periods (4, 5 and 6) potassium chloride was then included in the infusion mixture. The effects of potassium administration on mecamylamine excretion are shown in periods 7, 8 and 9.

Mecamylamine administration resulted in a 30% increase in the clearance of potassium and a threefold increase in sodium clearance. Total urinary $CO_2$ and $U_{HCO_3^-}$ did not change significantly. Urine flow rate changed from 4.5 cc/min. to 7.0 cc/min. The clearance of PAH ($C_{PAH}$) was enhanced 30%, but creatinine clearance was not significantly changed.

Potassium loading prior to periods 7, 8 and 9 did not result in a depression of mecamylamine clearance ($C_M$) (except in period 7).

The data from this experiment were interpreted as further evidence that mecamylamine excretion is in part by non-ionic diffusion, for the clearance increased as the urinary flow rate increased. Evidence for a tubular cation exchange relationship between sodium and mecamylamine was indicated with the threefold increase in sodium clearance following mecamylamine administration.

TABLE 12

EFFECTS OF MECAMYLAMINE AND KCl ADMINISTRATION ON POTASSIUM AND MECAMYLAMINE EXCRETION DURING MANNITOL DIURESIS IN THE UNANESTHETIZED DOG

| Period | Time | $C_{Cr}$ | $P_M$ | $C_{Cr}P_M$ | V | $U_M$ | $U_MV$ | $U_MV-C_{Cr}P_M$ | $C_M$ | $C_M/C_{Cr}$ | $U_{pH}$ | $P_{pH}$ | $C_{PAH}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | min | ml/min | mg/ml | mg/min | ml/min | mg/ml | ml/min | mg/min | ml/min | | | | ml/min |
| | - 85 | 500 ml. $H_2O$ p.o. | | | | | | | | | | | |
| | - 30 | Prime I: 20 ml. 6% creatinine + .25 ml. 20% PAH I.V. | | | | | | | | | | | |
| | | Infusion I: .38% creatinine, .1% PAH in 5% mannitol I.V. @ 5 ml/min. | | | | | | | | | | | |
| 1 | 10 | 29.65 | | | 5.53 | | | | | | 5.75 | 7.30 | 108.18 |
| 2 | 20 | 34.30 | | | 5.22 | | | | | | 5.76 | 7.32 | 100.05 |
| 3 | 30 | 37.64 | | | 5.50 | | | | | | 5.81 | 7.31 | 112.20 |
| | 30 | Prime II: 3 mg/kg. mecamylamine in 20 ml. $H_2O$ I.V. | | | | | | | | | | | |
| | | Add 8 mg/kg/hr. mecamylamine to infusion | | | | | | | | | | | |
| 4 | 70 | 45.47 | .0018 | .0818 | 4.79 | .0386 | .1897 | +.1079 | 105.39 | 2.31 | 6.25 | 7.34 | 112.91 |
| 5 | 80 | 42.32 | .0020 | .0846 | 6.11 | .0404 | .2468 | +.1622 | 123.40 | 2.91 | 6.38 | 7.34 | 132.73 |
| 6 | 90 | 34.27 | .0022 | .0754 | 5.35 | .0426 | .2279 | +.1525 | 103.50 | 3.02 | 6.45 | 7.34 | 114.64 |
| | 90 | Add KCl to infusion (without mecamylamine) to give .05 N KCl | | | | | | | | | | | |
| 7 | 130 | 36.68 | .0015 | .0536 | 7.11 | .0272 | .1934 | +.1398 | 132.47 | 3.61 | 6.65 | 7.35 | 127.49 |
| 8 | 140 | 39.75 | .0015 | .0572 | 8.05 | .0260 | .2093 | +.1521 | 145.35 | 3.65 | 6.65 | 7.36 | 143.75 |

| Period | $U_{TCO_2}$ | $P_{TCO_2}$ | $U_{pCO_2}$ | $P_{pCO_2}$ | $U_{HCO_3^-}$ | $P_{HCO_3^-}$ | $C_{HCO_3^-}$ | $U_{H_2CO_3}$ | $P_{H_2CO_3}$ | $P_K$ | $U_K$ | $U_KV$ | $C_K$ | $P_{Na}$ | $U_{Na}$ | $U_{Na}V$ | $C_{Na}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mM/L | | mm Hg | | mM/L | | ml/min | mM/L | | mEq/L | | uEq/min | ml/min | mEq/L | | uEq/min | ml/min |
| 1 | 1.61 | 19.43 | 16 | 39 | 1.13 | 18.29 | .34 | .48 | 1.14 | 5.08 | 8.05 | 44.52 | 8.76 | 137.50 | 20.16 | 105.23 | .77 |
| 2 | 1.26 | 18.97 | 12 | 36 | .90 | 17.89 | .23 | .36 | 1.08 | 4.68 | 9.80 | 51.16 | 10.93 | 141.50 | 47.50 | 247.95 | 1.75 |
| 3 | 1.03 | 18.97 | 11 | 37 | .70 | 17.86 | .21 | .33 | 1.11 | 4.60 | 12.30 | 67.65 | 14.71 | 135.00 | 67.00 | 368.50 | 2.73 |
| 4 | 3.45 | 20.12 | 47 | 36 | 2.04 | 19.04 | .51 | 1.41 | 1.08 | 4.63 | 22.83 | 106.00 | 22.89 | 136.67 | 154.28 | 739.00 | 5.41 |
| 5 | 4.25 | 19.89 | 47 | 36 | 2.84 | 18.81 | .92 | 1.41 | 1.08 | 4.63 | 20.20 | 123.42 | 26.57 | 135.00 | 235.61 | 1439.58 | 10.66 |
| 6 | 4.94 | 19.09 | 49 | 34 | 3.47 | 18.07 | 1.03 | 1.47 | 1.02 | 4.63 | 19.07 | 102.02 | 22.03 | 136.00 | 296.88 | 1588.31 | 11.68 |
| 7 | 6.32 | 20.23 | 45 | 36 | 4.97 | 19.15 | 1.80 | 1.35 | 1.08 | 5.30 | 19.40 | 137.93 | 26.02 | 135.00 | 201.00 | 1429.11 | 11.77 |
| 8 | 6.54 | 19.77 | 46 | 34 | 5.16 | 18.75 | 2.21 | 1.38 | 1.02 | 5.52 | 21.70 | 174.69 | 31.52 | 135.00 | 200.50 | 1614.02 | 10.59 |

## Experiment 10

As indicated in the previous experiment, data from Table 12 show mecamylamine and potassium tubular relationships in the unanesthetized dog. Again, mannitol was used in the infusion solution. It should be noted that mecamylamine was added to the infusion solution only during the equilibration period following the controls periods. Hence, in periods 7 and 8 mecamylamine was not infused simultaneously with potassium.

The clearance of potassium increased 80% following mecamylamine administration and sodium excretion was enhanced threefold. The urinary total $CO_2$, $UpCO_2$, $U_{HCO_3^-}$ and $C_{HCO_3^-}$ increased as well. These changes were reflected by a urine pH increase.

The clearance of mecamylamine increased 20% following potassium loading. However, the amount of mecamylamine contributed by tubular secretion ($U_M V - C_{Cr} P_M$) decreased 10%. Thus the clearance change reflected the lowered plasma levels of mecamylamine in periods 7 and 8 rather than lack of potassium inhibition.

The results from this experiment were interpreted as additional evidence that mecamylamine and potassium were involved in a cation exchange transport mechanism.

TABLE 13

EFFECTS OF DARSTINE AND KCl ADMINISTRATION ON POTASSIUM AND DARSTINE EXCRETION DURING MANNITOL DIURESIS IN THE UNANESTHETIZED DOG

| Period | Time | $C_{Cr}$ | $P_D$ | $C_{Cr}P_D$ | V | $U_D$ | $U_D V$ | $U_D V - C_{Cr}P_D$ | $C_D$ | $C_D/C_{Cr}$ | $U_{pH}$ | $P_{pH}$ | $C_{PAH}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | min | ml/min | mg/ml | mg/min | ml/min | mg/ml | mg/min | mg/min | ml/min | | | | |
| | - 85 | 500 ml. $H_2O$ p.o. | | | | | | | | | | | |
| | - 30 | Prime I: 20 ml. 6% creatinine + .25 ml. 20% PAH I.V. | | | | | | | | | | | |
| | | Infusion I: .38% creatinine, .1% PAH in 5% mannitol I.V. @ 5 ml/min. | | | | | | | | | | | |
| 1 | 10 | 49.48 | | | 4.28 | | | | | | 5.78 | 7.41 | 150.73 |
| 2 | 20 | 51.12 | | | 4.85 | | | | | | 5.72 | 7.42 | 158.15 |
| 3 | 30 | 53.25 | | | 5.40 | | | | | | 5.76 | 7.41 | 164.35 |
| | 30 | Prime II: 3.0 mg/kg. Darstine in 20 ml. $H_2O$ I.V. | | | | | | | | | | | |
| | | Add 4.0 mg/kg/hr. Darstine to infusion | | | | | | | | | | | |
| 4 | 70 | 53.62 | .0050 | .268 | 7.12 | .070 | .498 | +.230 | 99.60 | 1.86 | 6.30 | 7.41 | 179.70 |
| 5 | 80 | 56.95 | .0047 | .268 | 8.06 | .058 | .467 | +.199 | 99.36 | 1.74 | 6.42 | 7.40 | 193.82 |
| 6 | 90 | 54.55 | .0043 | .235 | 7.91 | .054 | .427 | +.192 | 99.30 | 1.82 | 6.52 | 7.41 | 180.80 |
| | 90 | Add KCl to infusion to give .05 N KCl | | | | | | | | | | | |
| 7 | 130 | 58.98 | .0047 | .277 | 9.52 | .033 | .314 | +.037 | 66.81 | 1.13 | 6.78 | 7.42 | 203.25 |
| 8 | 140 | 59.37 | .0043 | .255 | 10.11 | .032 | .324 | +.039 | 75.34 | 1.27 | 6.81 | 7.42 | 216.59 |
| 9 | 150 | 64.18 | .0043 | .302 | 11.03 | .028 | .309 | +.007 | 65.74 | 1.02 | 6.83 | 7.42 | 230.05 |

| Period | $U_{TCO_2}$ | $P_{TCO_2}$ | $U_{pCO_2}$ | $P_{pCO_2}$ | $U_{HCO_3^-}$ | $P_{HCO_3^-}$ | $C_{HCO_3^-}$ | $U_{H_2CO_3}$ | $P_{H_2CO_3}$ | $P_K$ | $U_K$ | $U_K V$ | $C_K$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mM/L | | mm Hg | | mM/L | | ml/min | mM/L | | mEq/L | | mEq/min | ml/min |
| 1 | 2.08 | 21.49 | 22 | 33 | 1.40 | 20.50 | .292 | .68 | .99 | 4.25 | 14.35 | 61.42 | 14.45 |
| 2 | 1.85 | 21.71 | 18 | 33 | 1.31 | 20.72 | .306 | .54 | .99 | 4.43 | 13.15 | 63.78 | 14.40 |
| 3 | 2.08 | 21.83 | 21 | 34 | 1.43 | 20.81 | .371 | .65 | 1.02 | 4.35 | 14.00 | 75.60 | 17.38 |
| 4 | 6.20 | 23.90 | 77 | 37 | 3.82 | 22.79 | 1.19 | 2.38 | 1.11 | 4.23 | 8.50 | 60.52 | 14.31 |
| 5 | 4.83 | 22.06 | 50 | 34 | 3.28 | 21.04 | 1.26 | 1.55 | 1.02 | 4.40 | 8.50 | 68.51 | 15.57 |
| 6 | 5.97 | 22.64 | 57 | 35 | 4.18 | 21.59 | 1.52 | 1.79 | 1.05 | 4.48 | 8.30 | 65.65 | 14.65 |
| 7 | 9.19 | 21.71 | 51 | 33 | 7.61 | 20.72 | 3.50 | 1.58 | .99 | 5.33 | 14.80 | 140.90 | 26.43 |
| 8 | 10.57 | 21.60 | 55 | 32 | 8.88 | 20.64 | 4.35 | 1.69 | .96 | 5.50 | 17.60 | 177.94 | 32.35 |
| 9 | 11.49 | 21.71 | 58 | 33 | 9.67 | 20.72 | 5.15 | 1.82 | .99 | 5.68 | 20.60 | 227.22 | 40.00 |

TABLE 14

EFFECTS OF MECAMYLAMINE AND KCl ADMINISTRATION ON POTASSIUM AND MECAMYLAMINE EXCRETION DURING ISOTONIC NaCl DIURESIS

| Period | Time | $C_{Cr}$ | $P_M$ | $C_{Cr}P_M$ | V | $U_M$ | $U_MV$ | $U_MV-C_{Cr}P_M$ | $C_M$ | $C_M/C_{Cr}$ | $U_{pH}$ | $P_{pH}$ | $C_{PAH}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | min | ml/min | mg/ml | mg/min | ml/min | mg/ml | ml/min | mg/min | ml/min | | | | ml/min |
| | -185 | 500 ml. $H_2O$ p.o. | | | | | | | | | | | |
| | -165 | 30 mg/kg. sodium pentobarbital I.V. | | | | | | | | | | | |
| | -120 | Prime I: 20 ml. 6% creatinine + .25 ml. 20% PAH I.V. | | | | | | | | | | | |
| | | Infusion I: .38% creatinine, .1% PAH in .9% NaCl I.V. @ 5 ml/min. | | | | | | | | | | | |
| 1 | 15 | 29.58 | | | 3.76 | | | | | | 6.83 | 7.32 | 92.00 |
| 2 | 30 | 32.89 | | | 3.53 | | | | | | 6.82 | 7.31 | 104.09 |
| 3 | 45 | 32.52 | | | 3.40 | | | | | | 6.79 | 7.32 | 101.12 |
| | 45 | Prime II: 5 mg/kg. mecamylamine in 20 ml. $H_2O$ I.V. | | | | | | | | | | | |
| | | Add 10 mg/kg/hr. mecamylamine to infusion | | | | | | | | | | | |
| 4 | 85 | 29.33 | .0043 | .126 | 2.74 | .128 | .351 | +.225 | 82.00 | 2.79 | 6.56 | 7.38 | 86.66 |
| 5 | 100 | 30.75 | .0036 | .111 | 3.17 | .103 | .328 | +.217 | 91.11 | 2.95 | 6.63 | 7.37 | 81.77 |
| 6 | 115 | 29.67 | .0037 | .110 | 3.73 | .098 | .366 | +.256 | 98.91 | 3.33 | 6.76 | 7.37 | 78.57 |
| | 115 | Add KCl to infusion to give .05 N KCl | | | | | | | | | | | |
| 7 | 155 | 33.59 | .0053 | .178 | 4.95 | .087 | .431 | +.253 | 81.32 | 2.42 | 6.82 | 7.42 | 80.82 |
| 8 | 170 | 35.38 | .0057 | .202 | 4.97 | .088 | .437 | +.235 | 76.67 | 2.16 | 6.82 | 7.42 | 83.17 |

| Period | $U_{TCO_2}$ | $P_{TCO_2}$ | $U_{pCO_2}$ | $P_{pCO_2}$ | $U_{HCO_3^-}$ | $P_{HCO_3^-}$ | $C_{HCO_3^-}$ | $U_{H_2CO_3}$ | $P_{H_2CO_3}$ | $P_K$ | $U_K$ | $U_KV$ | $C_K$ | $P_{Na}$ | $U_{Na}$ | $U_{Na}V$ | $C_{Na}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mM/L | | mm Hg | | mM/L | | ml/min | mM/L | | mEq/L | | mEq/min | ml/min | mEq/L | | mEq/min | ml/min |
| 1 | 16.39 | 22.87 | 83 | 43 | 13.82 | 21.58 | 2.41 | 2.57 | 1.29 | 3.19 | 12.40 | 46.62 | 14.62 | 148.0 | 143 | 537.0 | 3.63 |
| 2 | 15.82 | 22.00 | 81 | 42 | 13.32 | 20.74 | 2.27 | 2.50 | 1.26 | 3.40 | 13.75 | 48.54 | 14.28 | 148.0 | 150 | 529.0 | 3.58 |
| 3 | 13.66 | 21.83 | 75 | 41 | 11.35 | 20.60 | 1.87 | 2.31 | 1.23 | 3.50 | 16.35 | 55.59 | 15.88 | 148.0 | 150 | 510.0 | 3.45 |
| 4 | 7.74 | 19.88 | 64 | 33 | 5.76 | 18.89 | .83 | 1.98 | .99 | 3.97 | 18.50 | 50.69 | 12.77 | 146.0 | 107 | 293.0 | 2.08 |
| 5 | 9.33 | 19.66 | 68 | 33 | 7.23 | 18.67 | 1.23 | 2.10 | .99 | 4.16 | 21.00 | 66.57 | 16.00 | 146.0 | 109 | 345.0 | 2.37 |
| 6 | 11.27 | 19.77 | 65 | 33 | 9.26 | 18.78 | 1.84 | 2.01 | .99 | 4.25 | 21.00 | 78.33 | 18.43 | 146.0 | 108 | 402.0 | 2.76 |
| 7 | 13.02 | 18.73 | 67 | 28 | 10.95 | 17.89 | 3.02 | 2.07 | .84 | 5.20 | 25.50 | 126.23 | 24.27 | 145.0 | 122 | 603.9 | 4.16 |
| 8 | 12.87 | 18.38 | 66 | 28 | 10.83 | 17.54 | 3.06 | 2.04 | .84 | 5.30 | 29.75 | 147.86 | 27.90 | 145.0 | 136 | 675.9 | 4.66 |

## Experiment 12

Data presented in Tables 14 and 15 are those from experiments designed to evaluate the excretion changes of mecamylamine and potassium, and Darstine and potassium with isotonic NaCl as the infusion medium.

The experiments were designed as follows: The isotonic NaCl solution was infused two hours prior to determination of control values (periods 1, 2 and 3) in order to establish stable urine flow rates (Wessen et al., 1950). Hence, it was inferred that with the constant urine flow, any changes in values noted following the administration of mecamylamine, Darstine and potassium would more nearly reflect the true effects of these substances.

Mecamylamine and potassium relationships are seen in Table 14. The administration of mecamylamine resulted in a slight increase in the clearance of potassium (8%). This increase was reflected in an enhanced potassium urinary concentration ($U_KV$). Mecamylamine loading also resulted in a 50% depression of sodium clearance. Urinary flow rate was depressed temporarily. Total urinary $CO_2$ ($U_{T_{CO_2}}$), urine bicarbonate concentration ($U_{HCO_3^-}$) and the clearance of bicarbonate ($C_{HCO_3^-}$) were depressed following mecamylamine infusion.

When potassium chloride was added to the infusion mixture, the clearance of mecamylamine decreased 20%. This

decrease occurred even in the presence of a 30% increase in urine flow.

Results from this experiment were interpreted as further evidence of a cation exchange relationship between mecamylamine and potassium. The results also indicate that sodium, as well as potassium, may be involved with mecamylamine in the exchange system.

TABLE 15

EFFECTS OF DARSTINE AND KCl ADMINISTRATION ON POTASSIUM AND DARSTINE EXCRETION DURING ISOTONIC NaCl DIURESIS

| Period | Time | $C_{Cr}$ | $P_D$ | $C_{Cr}P_D$ | V | $U_D$ | $U_D V$ | $U_D V - C_{Cr}P$ | $C_D$ | $C_D/C_{Cr}$ | $U_{pH}$ | $P_{pH}$ | $C_{PAH}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | min | ml/min | mg/ml | mg/min | ml/min | mg/ml | ml/min | mg/min | ml/min | | | | ml/min |
| | -185 | 500 ml. $H_2O$ p.o. | | | | | | | | | | | |
| | -165 | 30 mg/kg. sodium pentobarbital I.V. | | | | | | | | | | | |
| | -120 | Prime I: 20 ml. 6% creatinine +.25 ml. 20% PAH I.V. | | | | | | | | | | | |
| | | Infusion I: .36% creatinine, .1% PAH in .9% NaCl I.V. @ 5 ml/min. | | | | | | | | | | | |
| 1 | 15 | 39.92 | | | 2.98 | | | | | | 6.82 | | 182.42 |
| 2 | 30 | 47.43 | | | 2.33 | | | | | | 6.98 | | 194.98 |
| 3 | 45 | 49.44 | | | 2.00 | | | | | | 6.97 | | 191.18 |
| | 45 | Prime II: 4 mg/kg. Darstine in 20 ml. $H_2O$ I.V. | | | | | | | | | | | |
| | | Add 5 mg/kg/hr. Darstine to infusion | | | | | | | | | | | |
| 4 | 85 | 50.67 | .0120 | .608 | 3.77 | .257 | .969 | +.361 | 80.74 | 1.59 | 6.74 | 7.40 | 174.27 |
| 5 | 100 | 49.80 | .0120 | .598 | 4.20 | .216 | .907 | +.309 | 75.60 | 1.52 | 6.70 | 7.41 | 169.58 |
| 6 | 115 | 51.58 | .0125 | .645 | 3.90 | .230 | .897 | +.252 | 71.76 | 1.40 | 6.73 | 7.42 | 176.60 |
| | 115 | Add KCl to infusion to give .05 N KCl | | | | | | | | | | | |
| 7 | 155 | 56.74 | .0130 | .738 | 4.04 | .237 | .957 | +.219 | 73.65 | 1.30 | 6.86 | 7.40 | 199.00 |
| 8 | 170 | 51.00 | .0133 | .678 | 3.91 | .233 | .911 | +.233 | 68.50 | 1.34 | 6.86 | 7.40 | 188.76 |
| 9 | 185 | 50.14 | .0138 | .692 | 3.97 | .224 | .889 | +.197 | 64.44 | 1.29 | 6.86 | 7.41 | 197.49 |

| Period | $U_{TCO_2}$ | $P_{TCO_2}$ | $U_{pCO_2}$ | $P_{pCO_2}$ | $U_{HCO_3^-}$ | $P_{HCO_3^-}$ | $C_{HCO_3^-}$ | $U_{H_2CO_3}$ | $P_{H_2CO_3}$ | $P_K$ | $U_K$ | $U_K V$ | $C_K$ | $P_{Na}$ | $U_{Na}$ | $U_{Na}V$ | $C_{Na}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mM/L | | mm Hg | | mM/L | | ml/min | mM/L | | mEq/L | | mEq/min | ml/min | mEq/L | | mEq/min | ml/min |
| 1 | 11.27 | | 58 | | 9.48 | | | 1.79 | | 3.50 | 17.50 | 52.06 | 14.86 | 146.00 | 57.50 | 171.7 | 1.17 |
| 2 | 17.64 | | 66 | | 15.60 | | | 2.04 | | 3.30 | 24.00 | 55.99 | 16.97 | 146.00 | 97.00 | 226.3 | 1.55 |
| 3 | 19.35 | | 74 | | 17.07 | | | 2.28 | | 3.40 | 27.00 | 54.00 | 15.88 | 145.00 | 116.50 | 232.0 | 1.60 |
| 4 | 9.52 | | 57 | | 7.76 | | | 1.76 | | 3.45 | 13.00 | 49.01 | 14.21 | 145.00 | 62.00 | 233.7 | 1.61 |
| 5 | 7.70 | | 51 | | 6.15 | | | 1.55 | | 3.45 | 13.75 | 57.75 | 16.74 | 145.00 | 60.50 | 254.1 | 1.75 |
| 6 | 10.08 | | 62 | | 8.16 | | | 1.92 | | 3.45 | 17.50 | 66.50 | 19.28 | 146.00 | 65.00 | 253.5 | 1.74 |
| 7 | 13.26 | | 64 | | 11.34 | | | 1.92 | | 4.15 | 32.00 | 129.28 | 31.15 | 148.00 | 89.50 | 253.50 | 2.44 |
| 8 | 13.82 | | 66 | | 11.78 | | | 2.04 | | 4.35 | 32.15 | 125.71 | 28.90 | 149.00 | 105.00 | 361.58 | 2.76 |
| 9 | 13.14 | | 63 | | 11.19 | | | 1.95 | | 4.45 | 37.00 | 146.89 | 33.00 | 148.00 | 117.00 | 410.55 | 3.14 |

Experiment 13

The excretion relationships of Darstine and potassium were investigated in Experiment 13, Table 15, during isotonic NaCl infusion. Control values were established in periods 1, 2 and 3.

After Darstine had been added to the infusion solution, the data in periods 4, 5 and 6 show that there was no apparent change in the clearance of potassium or sodium. However, the urine concentration of potassium ($U_K$) and sodium ($U_{Na}$) decreased by 40%. When the amount of sodium and potassium reabsorbed was calculated, sodium reabsorption increased 5-10%, while potassium reabsorption did not change significantly. Darstine loading also resulted in a decrease in urine pH, urinary total $CO_2$ ($U_{T_{CO_2}}$), $UpCO_2$, $U_{HCO_3^-}$, and $U_{H_2CO_3}$. It should be noted that these changes parallel those seen after the administration of mecamylamine in Experiment 12 (Table 14).

The administration of potassium chloride resulted in a 5-10% reduction in Darstine clearance. This effect of potassium was reflected in the change in Darstine/creatinine clearance ratio ($C_D/C_{Cr}$) from 1.52 to 1.32.

The results from this experiment show evidence that (1) Darstine tubular secretion is involved in the cation exchange system; (2) sodium and potassium both are the cations involved in the exchange system; (3) Darstine and mecamylamine tubular secretion are related.

TABLE 16

EFFECTS OF MECAMYLAMINE AND KCl ADMINISTRATION ON POTASSIUM AND MECAMYLAMINE EXCRETION DURING ISOTONIC NaCl DIURESIS WITH RESPIRATORY ALKALOSIS

| Period | Time | $C_{Cr}$ | $P_M$ | $C_{Cr}P_M$ | V | $U_M$ | $U_MV$ | $U_MV-C_{Cr}P_M$ | $C_M$ | $C_M/C_{Cr}$ | $U_{pH}$ | $P_{pH}$ | $C_{PAH}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | min | ml/min | mg/ml | mg/min | ml/min | mg/ml | ml/min | mg/min | ml/min | | | | ml/min |
| | -125 | 50 ml. $H_2O$ p.o. | | | | | | | | | | | |
| | -105 | 30 mg/kg. sodium pentobarbital I.V. | | | | | | | | | | | |
| | - 60 | Prime I: 20 ml. 6% creatinine + .25 ml. PAH I.V. | | | | | | | | | | | |
| | | Infusion I: .38% creatinine, .1% PAH in .9% NaCl I.V. @ 5 ml/min. | | | | | | | | | | | |
| | - 30 | Hyperventilate 15 strokes/min., 250 cc/stroke room air | | | | | | | | | | | |
| 1 | 15 | 36.23 | | | 4.12 | | | | | | 5.94 | 7.44 | 110.66 |
| 2 | 25 | 37.21 | | | 4.90 | | | | | | 5.93 | 7.44 | 111.51 |
| 3 | 35 | 38.45 | | | 4.80 | | | | | | 5.94 | 7.45 | 120.79 |
| | 35 | Prime II: 5 mg/kg. mecamylamine in 20 ml. $H_2O$ I.V. | | | | | | | | | | | |
| | | Add 10 mg/kg/hr. mecamylamine to infusion | | | | | | | | | | | |
| 4 | 80 | 31.86 | .0029 | .092 | 3.03 | .093 | .282 | +.190 | 97.24 | 3.05 | 6.80 | 7.46 | 120.07 |
| 5 | 90 | 32.90 | .0036 | .118 | 4.22 | .070 | .295 | +.177 | 81.94 | 2.49 | 6.87 | 7.46 | 127.02 |
| 6 | 100 | 32.17 | .0034 | .109 | 4.90 | .068 | .333 | +.224 | 97.94 | 3.04 | 6.88 | 7.46 | 120.88 |
| | 100 | Add KCl to infusion to give .05 N KCl | | | | | | | | | | | |
| 7 | 140 | | | | 4.29 | | | | | | 6.83 | 7.45 | |
| 8 | 150 | 31.07 | .0057 | .177 | 5.42 | .077 | .417 | +.240 | 73.16 | 2.35 | 6.82 | 7.44 | 99.21 |
| 9 | 160 | 32.96 | .0058 | .191 | 5.79 | .078 | .452 | +.261 | 77.93 | 2.36 | 6.81 | 7.43 | 96.77 |

| Period | $U_{TCO_2}$ | $P_{TCO_2}$ | $U_{pCO_2}$ | $P_{pCO_2}$ | $U_{HCO_3^-}$ | $P_{HCO_3^-}$ | $C_{HCO_3^-}$ | $U_{H_2CO_3}$ | $P_{H_2CO_3}$ | $P_K$ | $U_K$ | $U_KV$ | $C_K$ | $P_{Na}$ | $U_{Na}$ | $U_{Na}V$ | $C_{Na}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mM/L | | mm Hg | | mM/L | | ml/min | mM/L | | mEq/L | | uEq/min | ml/min | mEq/L | | uEq/min | ml/min |
| 1 | 1.96 | 19.60 | 13 | 28 | 1.56 | 18.76 | .34 | .40 | .84 | 3.80 | 5.00 | 20.60 | 5.42 | 148 | 26.50 | 109.12 | .737 |
| 2 | 1.96 | 20.42 | 13 | 30 | 1.56 | 19.52 | .39 | .40 | .90 | 3.90 | 6.00 | 29.40 | 7.54 | 149 | 32.00 | 156.80 | 1.058 |
| 3 | 2.07 | 19.16 | 14 | 27 | 1.64 | 18.35 | .42 | .43 | .81 | 4.00 | 10.00 | 48.00 | 12.00 | 145 | 36.00 | 172.80 | 1.191 |
| 4 | 8.62 | 19.27 | 46 | 27 | 7.20 | 18.46 | 1.18 | 1.42 | .81 | 3.75 | 20.25 | 61.36 | 16.36 | 145 | 52.00 | 157.60 | 1.086 |
| 5 | 8.62 | 18.96 | 41 | 26 | 7.35 | 18.18 | 1.71 | 1.27 | .78 | 3.75 | 17.50 | 73.85 | 19.69 | 145 | 45.00 | 189.90 | 1.310 |
| 6 | 7.45 | 18.84 | 34 | 26 | 6.40 | 18.06 | 1.73 | 1.05 | .78 | 3.70 | 15.50 | 75.95 | 20.53 | 142 | 42.00 | 205.80 | 1.450 |
| 7 | 6.97 | 18.72 | 35 | 27 | 5.89 | 17.91 | 1.41 | 1.08 | .81 | 4.90 | | | | | | | |
| 8 | 8.80 | 18.84 | 46 | 27 | 7.38 | 18.03 | 2.22 | 1.42 | .81 | 5.40 | 30.50 | 165.31 | 30.61 | 149 | 40.50 | 219.50 | 1.473 |
| 9 | 8.80 | 18.84 | 46 | 28 | 7.38 | 18.00 | 2.37 | 1.42 | .84 | 5.95 | 34.50 | 199.76 | 33.57 | 149 | 45.00 | 260.60 | 1.748 |

Experiment 14

The experimental procedure for the data presented in Tables 16 and 17 were identical. Respiratory alkalosis was instituted in dogs by hyperventilation at approximately the same time as the standard infusion solution of isotonic NaCl was started. This procedure resulted in the maintenance of a relatively constant urine flow, and a constant plasma $pCO_2$. Results from an experiment in which the excretion of mecamylamine and potassium were compared under such conditions are shown in Table 16.

The administration of mecamylamine after control values had been established resulted in a 50-100% increase in potassium clearance, and a 20% enhancement of sodium clearance. Paralleling the urinary potassium and sodium changes were the threefold increase in total urinary $CO_2$ ($U_{T_{CO_2}}$), $pCO_2$, $U_{HCO_3^-}$, $C_{HCO_3^-}$ and $H_2CO_3$. Mecamylamine loading caused very similar effects in the experiments listed in Tables 5, 8, 10 and 12.

With the addition of potassium chloride to the infusion solution a 20% reduction in mecamylamine clearance is seen. This reduction occurred even in the presence of a 20% increase in urinary flow while urine pH remained constant.

Results from this experiment were interpreted as further evidence that mecamylamine participates in a potassium exchange mechanism.

TABLE 17

EFFECTS OF DARSTINE AND KCl ADMINISTRATION ON POTASSIUM AND DARSTINE EXCRETION DURING ISOTONIC NaCl DIURESIS WITH RESPIRATORY ALKALOSIS

| Period | Time | $C_{Cr}$ | $P_D$ | $C_{Cr}P_D$ | V | $U_D$ | $U_D V$ | $U_D V - C_{Cr}P$ | $C_D$ | $C_D/C_{Cr}$ | $U_{pH}$ | $P_{pH}$ | $C_{PAH}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | min | ml/min | mg/ml | mg/min | ml/min | mg/ml | ml/min | mg/min | ml/min | | | | ml/min |
| | -125 | 50 ml. $H_2O$ p.o. | | | | | | | | | | | |
| | -105 | 30 mg/kg. sodium pentobarbital I.V. | | | | | | | | | | | |
| | - 60 | Prime I: 20 ml. 6% creatinine + .25 ml. PAH I.V. | | | | | | | | | | | |
| | | Infusion I: .38% creatinine, .1% PAH in .9% NaCl I.V. @ 5 ml/min. | | | | | | | | | | | |
| | - 55 | Hyperventilate 16 strokes/min. @ 300 cc/stroke room air | | | | | | | | | | | |
| 1 | 15 | 56.00 | | | .166 | | | | | | 6.98 | 7.45 | 148.78 |
| 2 | 30 | 61.00 | | | .317 | | | | | | 7.09 | 7.46 | 155.77 |
| 3 | 45 | 52.01 | | | .471 | | | | | | 7.29 | 7.47 | 142.80 |
| | 45 | Prime II: 4 mg/kg. Darstine in 20 ml. $H_2O$ I.V. | | | | | | | | | | | |
| | | Add 5 mg/kg/hr. Darstine to infusion | | | | | | | | | | | |
| 4 | 90 | 52.87 | .099 | .523 | .900 | .864 | .778 | +.255 | 78.59 | 1.49 | 7.46 | 7.47 | 165.18 |
| 5 | 105 | 49.00 | .010 | .490 | .722 | 1.194 | .862 | +.372 | 86.20 | 1.75 | 7.34 | 7.48 | 133.14 |
| 6 | 120 | 59.00 | .010 | .590 | .680 | 1.371 | .932 | +.342 | 93.28 | 1.58 | 7.24 | 7.48 | 144.29 |
| | 120 | Add KCl to infusion to give .06 N KCl | | | | | | | | | | | |
| 7 | 165 | 61.51 | .0105 | .646 | 1.517 | .694 | 1.053 | +.407 | 100.29 | 1.63 | 7.25 | 7.49 | 162.32 |
| 8 | 180 | 59.38 | .0107 | .635 | 1.953 | .574 | 1.121 | +.486 | 104.77 | 1.76 | 7.14 | 7.49 | 148.96 |
| 9 | 195 | 57.69 | .0108 | .623 | 1.286 | .470 | 1.074 | +.451 | 99.44 | 1.72 | 7.03 | 7.50 | 176.00 |

| Period | $U_{TCO_2}$ | $P_{TCO_2}$ | $U_{pCO_2}$ | $P_{pCO_2}$ | $U_{HCO_3^-}$ | $P_{HCO_3^-}$ | $C_{HCO_3^-}$ | $U_{H_2CO_3}$ | $P_{H_2CO_3}$ | $P_K$ | $U_K$ | $U_K V$ | $C_K$ | $P_{Na}$ | $U_{Na}$ | $U_{Na}V$ | $C_{Na}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mM/L | | mm Hg | | mM/L | | ml/min | mM/L | | mEq/L | | uEq/min | ml/min | mEq/L | | uEq/min | ml/min |
| 1 | 5.63 | 20.02 | 21 | 28 | 5.00 | 19.18 | .04 | .63 | .84 | 4.20 | 134.50 | 22.29 | 5.31 | 151.00 | 34.00 | 5.64 | .04 |
| 2 | 9.88 | 19.80 | 30 | 28 | 8.95 | 18.96 | .15 | .93 | .84 | 3.85 | 81.40 | 25.80 | 6.70 | 149.80 | 46.20 | 14.65 | .09 |
| 3 | 14.17 | 20.02 | 27 | 27 | 13.34 | 19.21 | .33 | .83 | .81 | 3.85 | 53.10 | 25.01 | 6.50 | 149.00 | 50.40 | 23.74 | .16 |
| 4 | 31.20 | 19.80 | 42 | 27 | 29.90 | 18.99 | 1.42 | 1.30 | .81 | 3.80 | 29.52 | 26.57 | 6.99 | 145.00 | 90.72 | 81.65 | .56 |
| 5 | 21.72 | 19.20 | 88 | 25 | 20.55 | 18.45 | .80 | 1.17 | .75 | 3.80 | 35.42 | 25.57 | 6.73 | 146.00 | 59.29 | 42.81 | .29 |
| 6 | 17.37 | 19.09 | 37 | 25 | 16.23 | 18.34 | .60 | 1.14 | .75 | 3.75 | 38.71 | 26.32 | 7.00 | 143.00 | 37.13 | 25.25 | .18 |
| 7 | 17.87 | 18.27 | 38 | 24 | 16.70 | 17.55 | 1.44 | 1.17 | .72 | 4.95 | 42.55 | 64.55 | 13.04 | 154.00 | 62.87 | 95.37 | .62 |
| 8 | 13.96 | 17.69 | 37 | 23 | 12.84 | 17.00 | 1.46 | 1.14 | .69 | 5.20 | 56.00 | 109.37 | 21.00 | 156.00 | 63.60 | 124.21 | .80 |
| 9 | 11.61 | 17.23 | 32 | 22 | 10.63 | 16.57 | 1.46 | .99 | .66 | 5.35 | 68.75 | 157.16 | 29.38 | 154.00 | 68.13 | 155.75 | 1.01 |

Experiment 15

The excretion of Darstine and potassium was studied in Experiment 15 (Table 17) during respiratory alkalosis and isotonic NaCl diuresis. The following changes from the similar experiment just described with mecamylamine are to be noted: the urine flow increased and the plasma $pCO_2$ decreased throughout the nine periods. Respiratory alkalosis is evident from the plasma pH values of 7.45-7.50, with the low plasma $pCO_2$ values (28-22 mmHg).

The administration of Darstine following the establishment of control values resulted in an increased sodium clearance ($C_{Na}$), increased $U_{Na}$ and $U_{Na}V$. Concurrent with urine sodium changes, urine total $CO_2$ ($U_{T_{CO_2}}$), urine $pCO_2$ ($UpCO_2$), bicarbonate ($U_{HCO_3^-}$), and $H_2CO_3$ increased 50 - 100%. Very similar results were seen when mecamylamine excretion was studied during respiratory alkalosis (Table 14). To be noted is that although potassium clearance did not increase significantly, the urinary concentration of potassium ($U_K$) decreased 50%. This $U_K$ decrease resulted in a constant amount of potassium excreted ($U_KV$) concurrent with the increased urine flow. The decrease in potassium concentration is accepted as evidence that Darstine does depress tubular secretion of potassium. This evidence is all the more significant in the presence of the respiratory alkalosis, when potassium excretion has been shown to increase.

With the addition of potassium chloride to the infusion solution, no significant change was seen in Darstine clearance. However, the urinary concentration of Darstine decreased 50%.

The results from this experiment show evidence that (1) as Darstine excretion did not show net tubular reabsorption when the urine was alkaline, as does the excretion of weak bases, the tubular secretion of Darstine is not in part by non-ionic diffusion; (2) potassium and Darstine are related in a tubular secretion cation exchange system.

TABLE 18

EFFECTS OF MECAMYLAMINE AND KCl ADMINISTRATION ON POTASSIUM AND MECAMYLAMINE EXCRETION DURING ISOTONIC NaCl DIURESIS WITH RESPIRATORY ACIDOSIS

| Period | Time | $C_{Cr}$ | $P_M$ | $C_{Cr}P_M$ | V | $U_M$ | $U_MV$ | $U_MV-C_{Cr}P_M$ | $C_M$ | $C_M/C_{Cr}$ | $U_{pH}$ | $P_{pH}$ | $C_{PAH}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | min | ml/min | mg/ml | mg/min | ml/min | mg/ml | ml/min | mg/min | ml/min | | | | ml/min |
| | -110 | 500 ml. $H_2O$ p.o. | | | | | | | | | | | |
| | - 90 | 30 mg/kg. sodium pentobarbital I.V. | | | | | | | | | | | |
| | - 45 | Prime I: 20 ml. 6% creatinine + .25 ml. PAH I.V. | | | | | | | | | | | |
| | | Infusion I: .38% creatinine, .1% PAH in .9% NaCl I.V. @ 5 ml/min. | | | | | | | | | | | |
| | - 35 | Animal breathing 10% $CO_2$ in 90% $O_2$ | | | | | | | | | | | |
| 1 | 10 | 75.43 | | | 1.172 | | | | | | 6.59 | 7.18 | 198.28 |
| 2 | 20 | 80.00 | | | .870 | | | | | | 6.63 | 7.18 | 225.63 |
| 3 | 30 | 81.00 | | | 1.265 | | | | | | 6.81 | 7.18 | 221.06 |
| | 30 | Prime II: 5 mg/kg. mecamylamine in 20 ml. $H_2O$ I.V. | | | | | | | | | | | |
| | | Add 10 mg/kg/hr. mecamylamine to infusion | | | | | | | | | | | |
| 4 | 70 | 71.70 | .0028 | .200 | 1.180 | .1385 | .163 | -.038 | 58.37 | .814 | 6.82 | 7.19 | 219.33 |
| 5 | 80 | 72.70 | .0033 | .240 | 1.555 | .1347 | .209 | -.031 | 63.47 | .873 | 6.80 | 7.15 | 210.55 |
| 6 | 90 | 74.87 | .0035 | .262 | 1.789 | .1294 | .231 | -.031 | 66.41 | .887 | 6.79 | 7.11 | 204.81 |
| | 90 | Add KCl to infusion to give .06 N KCl | | | | | | | | | | | |
| 7 | 130 | 77.60 | .0036 | .279 | 3.864 | .1325 | .512 | +.233 | 142.10 | 1.830 | 6.85 | 7.09 | 236.48 |
| 8 | 145 | 80.57 | .0040 | .322 | 4.615 | .1225 | .565 | +.243 | 141.32 | 1.754 | 6.82 | 7.10 | 235.22 |
| 9 | 150 | 80.42 | .0050 | .402 | 5.421 | .1000 | .542 | +.140 | 108.42 | 1.348 | 6.81 | 7.08 | 232.08 |

| Period | $U_{TCO_2}$ | $P_{TCO_2}$ | $U_{pCO_2}$ | $P_{pCO_2}$ | $U_{HCO_3^-}$ | $P_{HCO_3^-}$ | $C_{HCO_3^-}$ | $U_{H_2CO_3}$ | $P_{H_2CO_3}$ | $P_K$ | $U_K$ | $U_KV$ | $C_K$ | $P_{Na}$ | $U_{Na}$ | $U_{Na}V$ | $C_{Na}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mM/L | | mm Hg | | mM/L | | ml/min | mM/L | | mEq/L | | uEq/min | ml/min | mEq/L | | uEq/min | ml/min |
| 1 | 9.33 | 28.54 | 74 | 72 | 7.04 | 26.38 | .31 | 2.29 | 2.16 | 3.10 | 23.32 | 27.33 | 8.82 | 141.00 | 83.41 | 97.77 | .69 |
| 2 | 9.45 | 28.88 | 70 | 73 | 7.29 | 26.68 | .24 | 2.16 | 2.20 | 3.16 | 33.60 | 29.23 | 9.25 | 140.00 | 119.00 | 103.53 | .73 |
| 3 | 14.28 | 28.65 | 75 | 73 | 11.97 | 26.45 | .57 | 2.31 | 2.20 | 3.20 | 24.22 | 30.64 | 8.57 | 140.00 | 138.03 | 162.88 | 1.16 |
| 4 | 13.71 | 29.22 | 71 | 73 | 11.52 | 27.02 | .50 | 2.19 | 2.20 | 3.60 | 27.34 | 32.26 | 8.96 | 137.00 | 143.14 | 168.77 | 1.23 |
| 5 | 17.67 | 29.44 | 95 | 80 | 14.74 | 27.04 | .85 | 2.93 | 2.40 | 3.64 | 21.56 | 33.53 | 9.21 | 140.00 | 148.20 | 230.45 | 1.65 |
| 6 | 25.27 | 28.84 | 139 | 85 | 20.97 | 26.28 | 1.43 | 4.30 | 2.56 | 3.72 | 21.74 | 38.89 | 10.46 | 137.00 | 155.28 | 277.80 | 2.03 |
| 7 | 33.39 | 28.50 | 163 | 87 | 28.35 | 25.87 | 4.23 | 5.04 | 2.63 | 5.78 | 54.00 | 208.66 | 36.10 | 135.00 | 120.50 | 465.61 | 3.45 |
| 8 | 31.92 | 27.62 | 165 | 83 | 26.83 | 25.12 | 4.92 | 5.09 | 2.50 | 6.70 | 62.50 | 288.44 | 43.05 | 135.00 | 125.50 | 579.18 | 4.29 |
| 9 | 29.10 | 27.85 | 154 | 71 | 24.34 | 25.71 | 5.13 | 4.76 | 2.14 | 7.60 | 63.60 | 341.52 | 44.95 | 135.00 | 127.50 | 686.47 | 5.10 |

## Experiment 16

Mecamylamine and potassium and Darstine and potassium excretion relationships with sustained respiratory acidosis during isotonic NaCl diuresis are reported in Tables 18 and 19. Respiratory acidosis was induced very shortly following the start of the standard infusion mixture.

Respiratory acidosis has been shown to cause sodium retention, decreased potassium excretion, increased urine and plasma $pCO_2$ and decreased urine and plasma pH. These experiments were designed to show (1) whether mecamylamine or Darstine further depressed the excretion of potassium or sodium or whether these compounds caused an increase in potassium and sodium excretion, (2) whether potassium administration resulted in a depression of mecamylamine clearance in the presence of a highly acid urine, (3) whether Darstine clearance decreased with potassium loading or if the clearance increased in the presence of sustained systemic acidosis.

The data shown in Table 18 are results from an experiment in which mecamylamine and potassium excretion were compared. A systemic acidosis was apparent from the low plasma pH values (7.18-7.08) and the high plasma $pCO_2$ values (72-87 mm Hg).

Following the addition of mecamylamine to the infusion solution, no significant change is noted in the clearance of potassium. Sodium clearance was increased 10-80%.

It is seen that mecamylamine clearance showed net tubular reabsorption, and this reabsorption is reflected in the mecamylamine/creatinine clearance ratios ($C_M/C_{Cr}$) of less than one. This reabsorption in an acid urine (pH 6.80) is difficult to explain solely on the basis of excretion by filtration and non-ionic diffusion. Mecamylamine loading superimposed upon the respiratory acidosis resulted in a 10% decrease in GFR ($C_{Cr}$). The clearance of PAH ($C_{PAH}$) remained constant.

Following potassium chloride infusion, mecamylamine clearance increased twofold. The urinary concentration of mecamylamine decreased 5-20%. This decrease occurred concomitantly with a twofold to threefold increase in urine flow, and with a constant urine pH.

As the clearance of mecamylamine increased concomitantly with an increased urine flow and was not inhibited by potassium administration, results from this experiment were interpreted as further evidence of mecamylamine tubular secretion by non-ionic diffusion.

TABLE 19

EFFECTS OF DARSTINE AND KCl ADMINISTRATION ON POTASSIUM AND DARSTINE EXCRETION DURING ISOTONIC NaCl DIURESIS WITH RESPIRATORY ACIDOSIS

| Period | Time | $C_{Cr}$ | $P_D$ | $C_{Cr}P_D$ | V | $U_D$ | $U_DV$ | $U_DV-C_{Cr}P$ | $C_D$ | $C_D/C_{Cr}$ | $U_{pH}$ | $P_{pH}$ | $C_{PAH}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | min | ml/min | mg/ml | mg/min | ml/min | mg/ml | ml/min | mg/min | ml/min | | | | ml/min |
| | -110 | 500 ml. $H_2O$ p.o. | | | | | | | | | | | |
| | - 90 | 30 mg/kg. sodium pentobarbital I.V. | | | | | | | | | | | |
| | - 45 | Prime I: 20 ml. 6% creatinine + .25 ml. PAH I.V. | | | | | | | | | | | |
| | | Infusion I: .36% creatinine, .1% PAH in .9% NaCl I.V. @ 5 ml/min. | | | | | | | | | | | |
| | | Animal breathing 10% $CO_2$ in 90% $O_2$ | | | | | | | | | | | |
| 1 | 15 | 53.59 | | | 1.279 | | | | | | 5.78 | 7.09 | 240.94 |
| 2 | 30 | 52.26 | | | 1.187 | | | | | | 5.78 | 7.08 | 215.88 |
| 3 | 45 | 54.62 | | | 1.507 | | | | | | 5.80 | 7.09 | 235.36 |
| | 45 | PrimeII: 4 mg/kg. Darstine in 20 ml. $H_2O$ I.V. | | | | | | | | | | | |
| | | Add 5 mg/kg/hr. Darstine to infusion | | | | | | | | | | | |
| 4 | 90 | 43.64 | .0132 | .576 | 1.381 | .6375 | .880 | +.304 | 66.70 | 1.53 | 5.94 | 7.05 | 154.21 |
| 5 | 105 | 39.93 | .0130 | .519 | 1.320 | .6424 | .848 | +.329 | 65.23 | 1.63 | 5.91 | 7.04 | 147.66 |
| 6 | 120 | 41.19 | .0135 | .556 | 1.311 | .7012 | .919 | +.363 | 68.09 | 1.65 | 5.90 | 7.03 | 136.80 |
| | 120 | Add KCl to infusion to give .06 N KCl | | | | | | | | | | | |
| 7 | 165 | | | | | | | | | | | | |
| 8 | 180 | 42.93 | .0160 | .687 | 2.971 | .3410 | 1.013 | +.326 | 63.31 | 1.47 | 6.08 | 7.01 | 153.67 |
| 9 | 195 | 42.32 | .0165 | .698 | 3.724 | .2800 | 1.014 | +.316 | 61.45 | 1.45 | 6.10 | 7.02 | 179.30 |

| Period | $U_{TCO_2}$ | $P_{TCO_2}$ | $U_{pCO_2}$ | $P_{pCO_2}$ | $U_{HCO_3^-}$ | $P_{HCO_3^-}$ | $C_{HCO_3^-}$ | $U_{H_2CO_3}$ | $P_{H_2CO_3}$ | $P_K$ | $U_K$ | $U_KV$ | $C_K$ | $P_{Na}$ | $U_{Na}$ | $U_{Na}V$ | $C_{Na}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mM/L | | mm Hg | | mM/L | | ml/min | mM/L | | mEq/L | | mEq/min | ml/min | mEq/L | | mEq/min | ml/min |
| 1 | 2.2 | 21.51 | 23 | 66 | 1.49 | 19.53 | .098 | .71 | 1.98 | 4.44 | 25.67 | 32.83 | 7.39 | 160.00 | 93.62 | 119.74 | .75 |
| 2 | 2.2 | 21.85 | 23 | 69 | 1.49 | 19.78 | .089 | .71 | 2.07 | 4.49 | 28.83 | 34.22 | 7.62 | 159.00 | 90.67 | 107.63 | .68 |
| 3 | 2.4 | 22.76 | 26 | 70 | 1.60 | 20.66 | .109 | .80 | 2.10 | 4.50 | 28.80 | 43.40 | 9.64 | 158.00 | 90.72 | 136.72 | .87 |
| 4 | 1.9 | 22.08 | 25 | 74 | 1.13 | 19.85 | .078 | .77 | 2.23 | 4.90 | 25.16 | 34.75 | 7.09 | 158.00 | 83.30 | 115.04 | .73 |
| 5 | 2.2 | 22.19 | 38 | 75 | 1.33 | 19.93 | .088 | .87 | 2.26 | 5.13 | 29.39 | 38.79 | 7.56 | 158.00 | 79.20 | 104.54 | .66 |
| 6 | 2.5 | 22.16 | 31 | 77 | 1.54 | 19.85 | .102 | .96 | 2.31 | 5.25 | 29.70 | 38.94 | 7.42 | 156.00 | 72.60 | 95.18 | .61 |
| 7 | | | | | | | | | | | | | | | | | |
| 8 | 6.8 | 21.75 | 20 | 79 | 6.18 | 19.37 | .958 | .62 | 2.38 | 9.50 | 43.50 | 129.24 | 13.60 | 151.00 | 89.50 | 265.90 | 1.76 |
| 9 | 7.0 | 21.18 | 23 | 76 | 6.29 | 18.89 | 1.240 | .71 | 2.29 | 10.25 | 45.50 | 169.44 | 16.53 | 156.00 | 98.00 | 364.95 | 2.34 |

Experiment 17

The excretion relationships of Darstine and potassium during respiratory acidosis and isotonic NaCl infusion are reported in this experiment (Table 19). Respiratory acidosis is evident from the plasma pH values (7.08-7.01) and the high plasma $pCO_2$ values (66-79 mm Hg).

The administration of Darstine (periods 4, 5 and 6) resulted in no significant change in the clearance of potassium or sodium. However, a 10-18% reduction in the urinary concentration of sodium occurred, and the amount of both sodium and potassium reabsorbed decreased. These potassium and sodium changes occurred concurrently with a 20% reduction in the GFR ($C_{Cr}$) and an 80% reduction in the clearance of PAH ($C_{PAH}$). Mecamylamine administration under similar experimental conditions has also been shown to depress the GFR (Table 18).

With the addition of potassium chloride to the infusion mixture, a 5-10% reduction in Darstine clearance occurred. This decrease was reflected in the change in the Darstine/creatinine clearance ratio ($C_D/C_{Cr}$) from 1.60 to 1.46.

The results from this experiment show further evidence of a tubular cation exchange relationship between Darstine and potassium. Sodium could also be involved in the cation exchange with Darstine.

## Blood Pressure and ECG Records

Systemic blood pressure determinations and ECG recordings were consistently obtained in the clearance studies. These records were secured prior to the infusion of the standard solution, and during the control and experimental collection periods.

No significant change was noted on the ECG recordings following the addition of either mecamylamine or Darstine to the infusion solution. ECG changes characteristic of hyperkalemia were seen only in these experiments where plasma potassium concentrations approached 6.5 - 7.0 mEq/L. during the infusion of potassium chloride.

Mean blood pressure values were calculated from the polygraph records for the control periods and the experimental periods. The change of mean blood pressure after the infusion of mecamylamine or Darstine from the control period was then expressed as a percentage change. This procedure was followed for the pressure change after the addition of potassium chloride; however, reference pressure values were those obtained during mecamylamine or Darstine infusion. Pulse pressure changes were calculated in like manner. The results which indicate the average per cent change in mean blood pressure and pulse pressure are shown in Table 20.

TABLE 20

AVERAGE PER CENT (%) CHANGE IN MEAN SYSTEMIC BLOOD PRESSURE AND PULSE PRESSURE

| Following Mecamylamine or Darstine Infusion | % Change |
|---|---|
| Systemic Pressure | |
| Anesthetized | -32 |
| Unanesthetized | - 9 |
| Pulse Pressure | |
| Anesthetized | -27 |
| Unanesthetized | -24 |
| Following Potassium Infusion | |
| Systemic Pressure | |
| Anesthetized | +21.0 |
| Unanesthetized | + .5 |
| Pulse Pressure | no change |

A significant decrease in systemic blood pressure and pulse pressure is noted with mecamylamine or Darstine infusion in the anesthetized animal. However, there was no correlation between the decrease in blood pressure, simultaneously measured glomerular filtration rates and PAH clearances, and tubular excretion changes which occurred with mecamylamine and Darstine infusion.

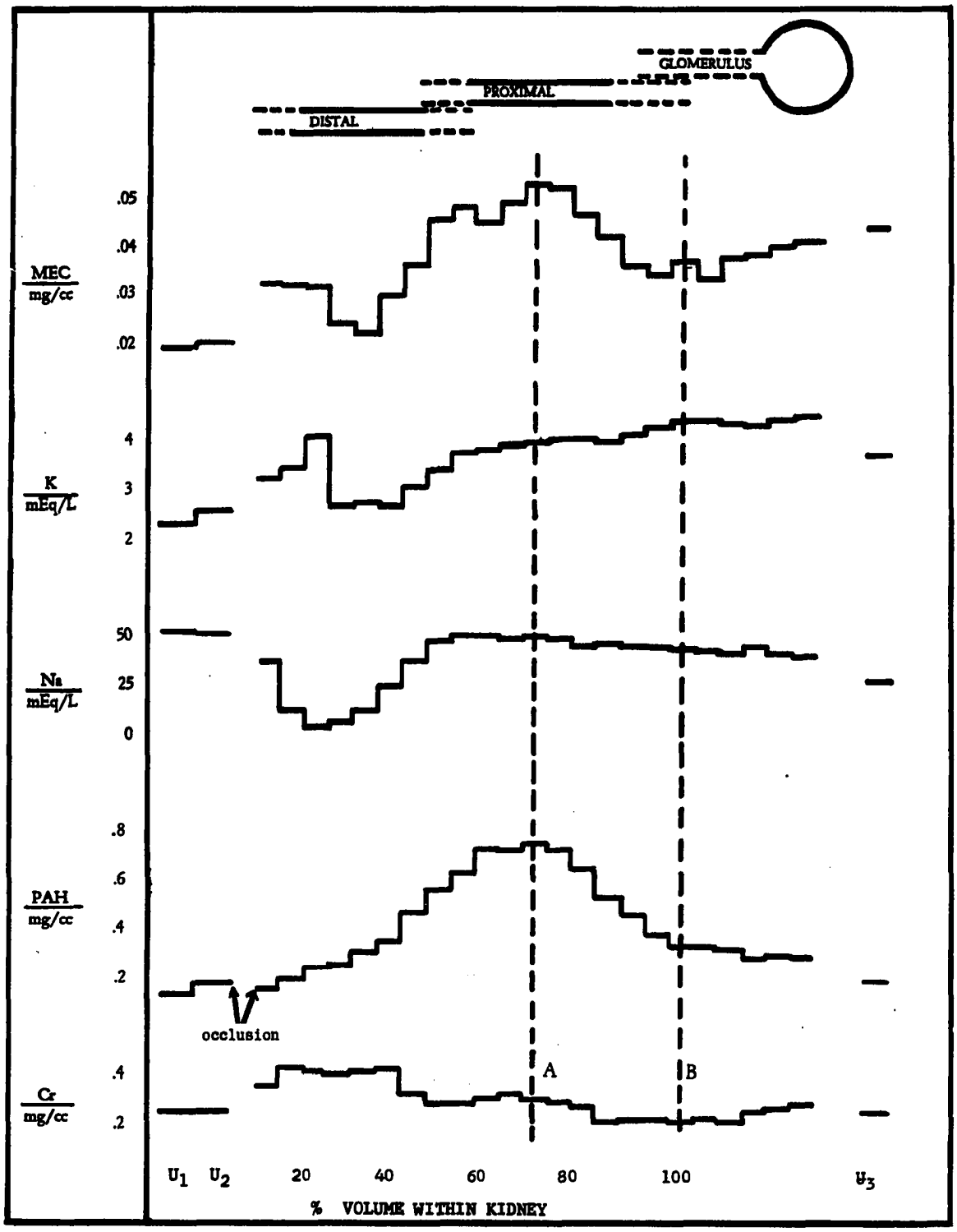


FIG. 1. Concentration pattern developed for mecamylamine, potassium, sodium, PAH and creatinine during stop flow in the dog. Maximal PAH concentration indicates proximal segment of the nephron marked by dotted line A. Minimal sodium concentration indicates distal segment. Dotted line B indicates total volume of urine trapped within kidney during the ureteral occlusion. Mecamylamine is secreted in the proximal segment.

## Stop-Flow Studies

### Figure 1

A control stop-flow concentration pattern for mecamylamine, sodium, potassium PAH and creatinine is shown in Figure 1.

Interpretation of concentration pattern: In Figure 1, concentrations of test substances in free-flow samples are plotted to the left of the first break in the curve indicated by $U_1$ and $U_2$. Concentrations in the stop-flow samples are plotted against the per cent total volume of fluid trapped within the tubules during the period of ureteral occlusion to the right of the first break. The second break at the right of the chart indicates cessation of serial sample collection. The post-occlusion free-flow sample concentration is indicated by $U_3$.

The total volume of fluid within the kidney is found by summing the stop-flow samples from tube 1 to that tube which contains 50% of the maximum concentration of inulin (Pitts et al., 1958). This tube is arbitrarily selected as marking the glomerular end of the proximal tubule, indicated by the dotted line B and the 100% volume at the bottom of the chart.

Peak maximum concentration of PAH, indicated by dotted line A, is used to mark the proximal segment. Proximal PAH secretion was demonstrated by Malvin and co-workers

(1958) by the simultaneous injection of the PAH and inulin one minute before the release of the clamp on the ureteral catheter. PAH appeared in stop-flow samples ahead of inulin and in high concentrations, indicating secretion from peritubular blood to the tubule lumen. Inulin remained at the glomerulus. The area of this peak concentration of PAH appearing in late sample collections was designated proximal segment.

The rise of PAH concentration from the early samples is interpreted as increased tubular secretion of PAH into the proximal lumen. The fall of the PAH concentration indicates new filtrate entrance into the tubule.

The terminal part of the nephron is designated distal segment with no attempt made to differentiate between distal convoluted tubule and the collecting ducts. It has been shown that sodium concentrations consistently tend to decrease to their lowest values in this distal segment and then rise to pre-occlusion values (Malvin et al., 1958). Hence, stop-flow samples with minimal sodium concentrations are used to mark the area of the distal segment.

Creatinine concentration changes reflect water movement throughout the nephron.

The concentration pattern for potassium shows a far distal peak, followed by a concentration decrease which occurs approximately in the area of minimal sodium concentra-

tion. This far distal peak represents a distal (or collecting duct) secreting mechanism for potassium (Pitts et al., 1958). The minimal concentration indicates reabsorption in this area.

In Figure 1, 5 mg/kg/hr. mecamylamine was added to the standard infusion solution containing mannitol, NaCl, creatinine and PAH. 4 mg/kg. mecamylamine was added to the priming solution.

Free-flow clearance determinations showed mecamylamine secretion, both pre- and post-occlusion. The stop-flow pattern developed for mecamylamine shows a slight fall in sample concentration in the distal segment. There then occurs a step-wise rise to a peak concentration in the proximal area followed by a step-wise decrease in concentration as the glomerulus is approached.

This pattern in the proximal segment closely resembles the pattern seen for PAH, with the peak concentration occurring simultaneously with PAH and likewise showing a fall in concentration toward free-flow values, indicating dilution by the addition of new filtrate. This pattern strongly suggests increased secretion of mecamylamine into the proximal tubule.

The fall in concentration in the distal tubular area suggests reabsorption of the mecamylamine. However, as the first stop-flow sample concentrations are increased over free-flow concentrations, $U_1$ and $U_2$, the fall in concentra-

tion is not decisive evidence that tubular reabosorption is occurring.

No true secretory peak concentration for mecamylamine is seen in the distal segment. This finding was surprising for Pitts and co-workers (1958), and Sullivan et al., (1960) had localized acidification in the nephron in the distal segment. Acidification is closely related to the cation exchange mechanism (Berliner et al., 1951). The two tubular mechanisms hypothesized for mecamylamine secretion are non-ionic diffusion into an acid urine, and a cation exchange system. As the free-flow urine samples showed tubular secretion, peak concentrations for mecamylamine could be expected in the area associated with acidification of urine.

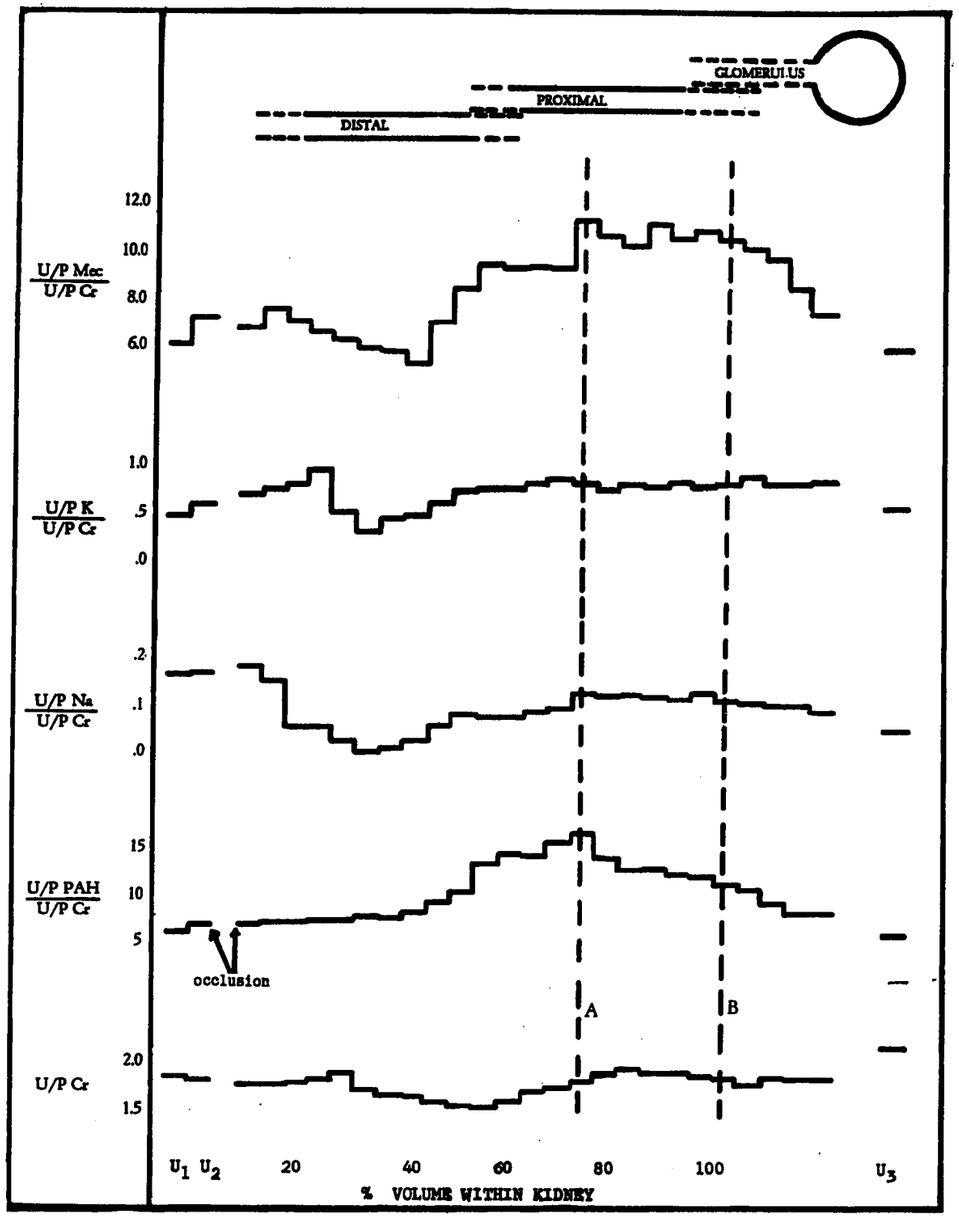


FIG. 2. Stop flow localization of mecamylamine secretion during metabolic acidosis. Secretion occurred in the proximal segment. $U/P_{Cr}$ indicates net movement of water out of the nephron. The proximal segment is marked by maximal $U/P_{PAH}/U/P_{Cr}$ ratios, the distal segment is marked by minimal $U/P_{Na}/U/P_{Cr}$ ratios. Hydrochloric acid was included in the infusion solution.



Figure 2

As mecamylamine has been shown to have a very high clearance rate when the urine was acidified, it was of interest to further investigate localization of tubular secretion under conditions of metabolic acidosis. Figure 2, therefore, is a stop-flow pattern developed for mecamylamine during metabolic acidosis.

The curves in Figure 2 are read as in Figure 1. However, the concentrations of test substances in the urine and plasma have been used to calculate the urine/plasma concentration ratios (U/P ratio) for each sample. These U/P ratios have been used to calculate the U/P ratio of test substance to the U/P ratio of creatinine. This $U/P_x$ / $U/P_{Cr}$ ratio therefore reflects concentrations corrected for water reabsorption throughout the nephron. A $U/P_x$ / $U/P_{Cr}$ ratio for a substance greater than unity therefore indicates tubular secretion; a $U/P_x$ / $U/P_{Cr}$ ratio for a substance less than unity indicates tubular reabsorption. Active tubular reabsorption for the substance is indicated only if the urine to plasma concentration ratios are less than unity, prior to calculation of the $U/P_x$ / $U/P_{Cr}$ ratio. Sodium can be shown very clearly to be reabsorbed by an active process in both stop-flow and clearance studies. It should be noted that the patterns along the nephron developed for potassium, sodium and PAH are identical to the pattern in Figure 1, where actual concentrations were plotted against per cent

volume in urine.

In Figure 2, standard clearance determinations in the timed free-flow urine collections showed net tubular secretion of mecamylamine. This secretion is reflected in the $U/P_M$ / $U/P_{Cr}$ ratio in $U_1$, $U_2$ and $U_3$ where these values approximate that of PAH.

The stop-flow pattern for mecamylamine closely resembles that pattern seen in Figure 1. After an initial slight peak in the far distal tubule the curve falls slightly toward the area of the proximal end of the distal segment and then rises in the proximal segment to show mecamylamine U/P/creatinine U/P ratios nearly twice that in free-flow samples. The curve for mecamylamine then appears to plateau, and finally falls toward post-occlusion ratio values.

The mecamylamine curve is interpreted as follows: The very slight peak in the far distal area could be an indication of increased tubular secretion in this area. This secretion peak is, however, certainly not the sharp peak that has been observed for potassium in the distal segment. The slight dip in the curve may be indicative of tubular reabsorption; however, this dip could as well be interpreted as less secretion in this area, for U/P mecamylamine / U/P creatinine ratios are still well over one. The rise of the curve in the area of the proximal tubule and the fall toward free-flow ratios is again an indication of increased tubular secretion of mecamylamine in the proximal segment.

The evidence for increased tubular secretion of mecamylamine in the distal area was not conclusively shown when the urine was acidified by metabolic acidosis.

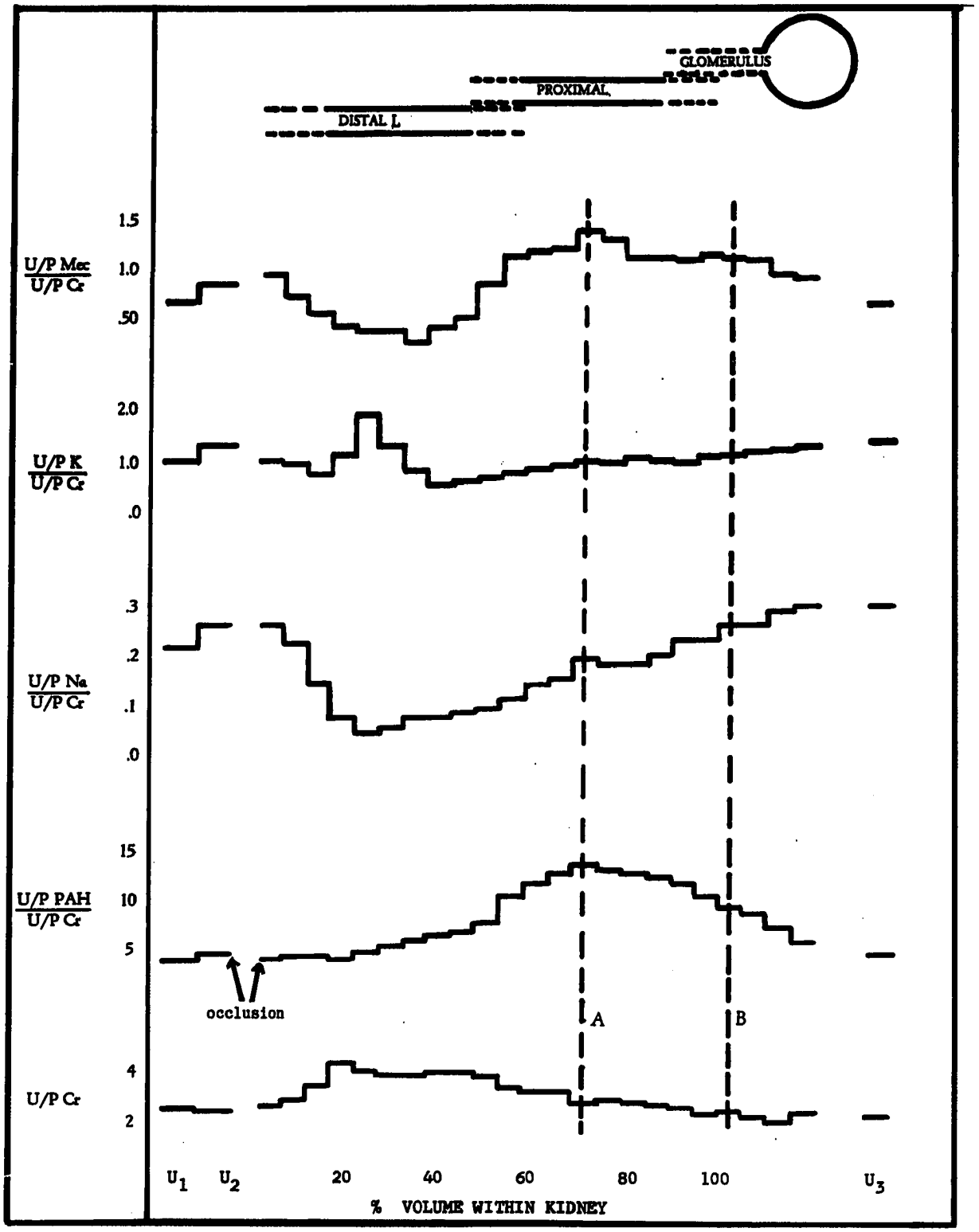


FIG. 3. Stop flow localization of mecamylamine reabsorption during metabolic alkalosis. Mecamylamine is reabsorbed in the distal segment. Sodium bicarbonate was included in the infusion solution.

Figure 3

The reabsorption of mecamylamine has been shown to occur in clearance studies when the urine was alkalinized with $NaHCO_3$ (Chapter 3; Scribner et al., 1959). Figure 3 represents a stop flow pattern designed to investigate the localization of tubular reabsorption of mecamylamine. $NaHCO_3$ added to the standard infusion solution.

Mecamylamine concentration in the infusion was 6 mg/kg/hr. Sodium bicarbonate was an 8% solution. A prime dose of 4 mg/kg mecamylamine was given.

The stop-flow pattern developed for mecamylamine is read as in Figure 2. The U/P ratios of test substances to the U/P ratios of creatinine are plotted against the per cent volume of samples.

Free-flow clearance determinations showed tubular reabsorption of mecamylamine, with mecamylamine to creatinine ratios less than unity. This reabsorption is reflected in the $U/P_M$ / $U/P_{Cr}$ ratios in the free flow samples $U_1$, $U_2$ and $U_3$; these ratios are shown to be less than 1.00.

The stop-flow pattern of mecamylamine shows a continual fall in the curve in the distal segment co-incident with that fall seen for sodium. The mecamylamine curve then rises to show a peak in the proximal segment, followed by a slight fall toward the post-occlusion free-flow sample.

The continual decrease in $U/P_M$ / $U/P_{Cr}$ ratio in the distal segment is evidence that tubular reabsorption of

mecamylamine occurs in this area of the nephron.

Clearance determinations from free flow samples showed potassium/creatinine ratios greater than unity, indicating active tubular secretion of potassium. A strong secretory peak for potassium is noted in the distal segment.

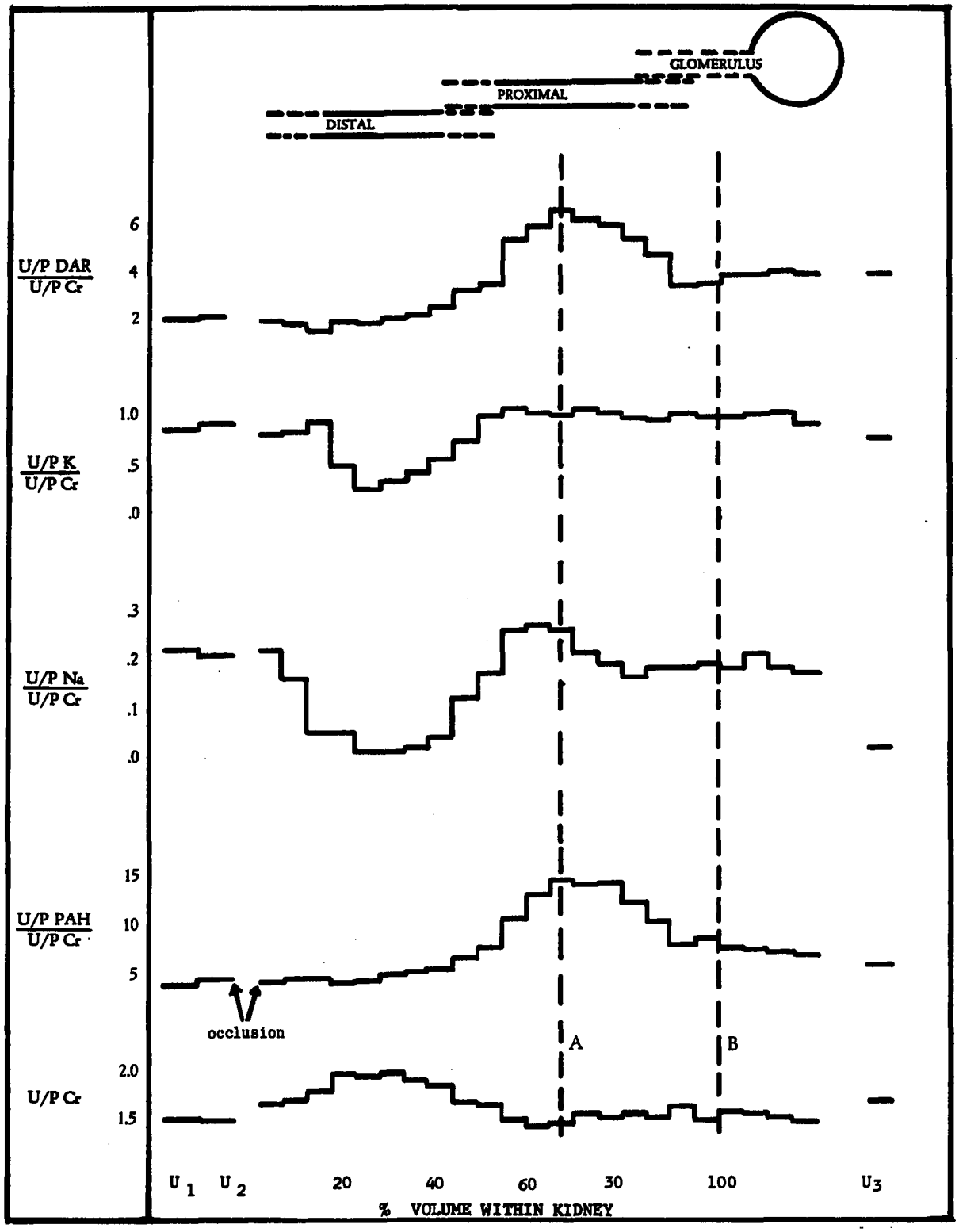


FIG. 4. Stop flow localization of Darstine secretion in the "proximal segment."

Figure 4

A control stop-flow pattern for Darstine is seen in Figure 4. The graph is read as in Figures 2 and 3.

Clearance determinations from free-flow samples $U_1$, $U_2$ and $U_3$ showed that Darstine was excreted by tubular secretion. The $U/P_{DAR}$ / $U/P_{Cr}$ ratios in $U_1$, $U_2$ and $U_3$ samples show values greater than one.

The Darstine stop-flow pattern closely resembles the pattern developed for PAH. There is little change in the curve until the distal part of the proximal segment is reached when the curve rises sharply to a peak in the proximal segment. The curve declines toward the free-flow post-occlusion sample.

This peak in $U/P_{DAR}$ / $U/P_{Cr}$ ratio in the proximal segment co-incident with the PAH is evidence of tubular secretion of Darstine in this segment of the nephron.

There is no indication that Darstine reabsorption occurs in the distal segment.

## Kidney Slice Studies

Darstine has been demonstrated by clearance studies to be actively secreted in both the dog and the chicken. Similarly, there is evidence that the kidney slices of rabbit and dog will accumulate Darstine under the conditions described in Chapter 2. The data from experiments in this work indicate that the kidneys of the rat will accumulate Darstine and also mecamylamine when suitably incubated.

The results from data on the uptake of mecamylamine and Darstine in the rat kidney slice are shown in Tables 21 and 22. Table 21 represents control studies on the two compounds. In the experiments with Darstine, 0.2 cc of 80 mg% was added consistently to the standard phosphate buffer of each vessel; 0.2 cc of 40 mg% mecamylamine was added to the medium in the accumulation studies of this agent.

TABLE 21

ACCUMULATION OF DARSTINE AND MECAMYLAMINE IN RAT KIDNEY SLICES

| | Number of Determinations | Mean S/M Ratio |
|---|---|---|
| Darstine | 6 | 3.90 ± .47 |
| | 5 | 5.62 ± .42 |
| | 5 | 4.53 ± .42 |
| Mecamylamine | 5 | 3.22 ± .32 |
| | 6 | 5.01 ± .62 |
| | 4 (dog kidney cortex) | 7.04 ± .90 |

Table 22 indicates the results when a known inhibitor of aerobic phosphorylation, 2, 4-dinitrophenol (DNP), was added to the medium simultaneously with the two bases.

TABLE 22

EFFECTS OF DNP ON DARSTINE AND MECAMYLAMINE ACCUMMULATION IN RAT KIDNEY SLICES

| | Mean S/M Ratio | |
|---|---|---|
| | Control | DNP |
| Darstine | 5.42 (3) | 4.36 (3) |
| Mecamylamine | 5.08 (3) | 3.82 (3) |

Oxygen consumption was measured for the slices in each vessel. Oxygen uptake did not differ measurably except for a significant increase in those slices where DNP was added to the medium.

## CHAPTER IV

## DISCUSSION

The results from the clearance studies a) substantiate the hypothesis that the tubular secretion of mecamylamine occurs at least in part by a cation exchange mechanism, b) strongly indicate that potassium is the cation involved in the exchange system, and c) provide further evidence that Darstine as well is related to this transport mechanism.

In seven of eight experiments the administration of potassium chloride resulted in a depression of mecamylamine excretion. In five of the seven experiments, during either mannitol or NaCl diuresis, the clearance of mecamylamine was depressed; in two of the experiments, the urinary concentration of mecamylamine decreased. Domer (1960) also showed an inhibition of mecamylamine clearance with KCl loading, but the inhibition occurred either in the presence of an increasing urinary pH or decreasing urinary flow. His data might suggest that the potassium effect could have been secondary to reabsorption by non-ionic diffusion. In the experiments reported here, depression of mecamylamine excretion occurred with either no change, or with an increased urinary flow rate;

urinary pH increased only slightly in two experiments, while in five no change was seen. Therefore, the depression of mecamylamine excretion by potassium could not be explained on the basis of a passive non-ionic diffusion but must be an inhibition of an active tubular process.

In nine of ten experiments reported to show the effects of the acute administration of mecamylamine on potassium excretion, either there was no change in the clearance of potassium, or the clearance increased twofold to threefold. In the tenth experiment the clearance of potassium decreased. This decrease occurred with a reduction in glomerular filtration rate. The urinary concentration of potassium, following mecamylamine loading, was depressed significantly in two of the ten experiments. In both of these experiments, urinary flow increased.

The acute administration of mecamylamine resulted in an increase in the clearance of sodium in four of five experiments in which sodium concentrations were determined. The results in the fifth experiment may be of some significance. In this experiment, the experimental conditions were such that neither the urine flow nor the glomerular filtration rate decreased by more than 10%, yet the clearance of sodium and the urinary concentration of sodium decreased by 33% following mecamylamine infusion.

Thus, although the data from the experiments presented show variable effects of mecamylamine loading, it is

tentatively suggested that mecamylamine enhances potassium excretion, and depresses sodium excretion.

The clearance of Darstine was shown to be inhibited by KCl administration in three of the four experiments where Darstine-potassium excretion relationships were studied. These results agree with the evidence presented by Kandel, (1956). In the fourth experiment, although Darstine clearance was not depressed, the urinary concentration of Darstine decreased 50%.

No significant change in the clearance of potassium was noted following the acute administration of Darstine in the four experiments. In two of these experiments, however, there was an 80% reduction in urinary concentration of potassium; the urine flow increased only 30 - 50%.

In the three experiments in which the excretion of sodium was studied simultaneously with the excretion of Darstine, the infusion of Darstine resulted in a) an increase in the clearance of sodium, b) no significant change in the clearance of sodium, and c) a reduction in the clearance. The increase in sodium clearance following Darstine loading occurred during respiratory alkalosis. This condition in itself has been shown to cause increased sodium excretion. Conversely, the reduction in sodium clearance following Darstine administration occurred during respiratory acidosis, with a decreased glomerular filtration rate. Both condi-

tions have been shown to cause a decrease in sodium excretion.

Two facts are of importance in evaluating the results in that experiment where there was no change in the clearance of sodium following Darstine administration. First, the urinary concentration of sodium decreased 80% while glomerular filtration rate remained constant, and urine flow increased. In the investigations of the effects of choline administration on urinary electrolyte excretion, Solomon et al., (1960) showed that the reabsorption of sodium was enhanced following choline administration. The evidence presented by these workers was based on decreased urinary sodium concentrations in experiments where the GFR remained constant or increased. The data also showed an increased excretion of sodium during diuresis in the presence of choline; however, the concentration of sodium in the urine remained depressed. Second, there was a marked similarity between the effects of Darstine administration on sodium excretion, and in the effects of mecamylamine administration on sodium excretion, (Tables 14 and 15).

Thus, the results indicate that potassium chloride loading administration inhibits Darstine excretion and Darstine administration enhances sodium reabsorption as well as potassium reabsorption.

Evidence which substantiates the hypothesis that

mecamylamine as well as Darstine can participate in the cation exchange system operating within the renal tubule is briefly outlined.

1. The reabsorption of sodium in the renal tubule is an active process.

2. At least part of the sodium from the lumen which is reabsorbed is exchanged for potassium or hydrogen from within the tubular cell.

3. Potassium and hydrogen compete for this exchange, this competition shown by a mutual reversable inhibition.

4. Mecamylamine and Darstine tubular secretion occurs by active transport.

5. Potassium chloride administration inhibits the excretion of Darstine and mecamylamine. This inhibition is not related to other factors.

6. Darstine administration inhibits the excretion of both potassium and sodium.

7. Mecamylamine administration enhances the excretion of potassium.

Although the effects on potassium excretion by Darstine and mecamylamine administration are reversed, the apparent inconsistency could be explained by relative affinities for the transport system, i.e., potassium, Darstine and mecamylamine in decreasing order. Peters (1960) presented evidence that strong bases demonstrated this phenom-

enon in relation to the "common" base secretory system. Further, Domer (1960) has compared potassium excretion during Darstine infusion before and after the force-feeding of Darstine. He found a decrease in potassium clearance; this would indicate some adaptive preference in the kidney for organic bases following the forced-feeding procedure.

The results of the stop-flow analyses on mecamylamine excretion showed that the tubular secretion of mecamylamine occurred in the proximal segment during both a control experiment and during metabolic acidosis. Reabsorption of mecamylamine occurred in the distal segment during metabolic alkalosis, when the urine was highly alkaline. These results from the stop-flow analyses demonstrate that mecamylamine (and perhaps other organic bases) may be secreted in one part of the tubule-and be reabsorbed at another site. Jailer (1947) had speculated on the possibility that this process could indeed occur within the renal tubule. It is highly probable that these two processes of secretion at one site and reabsorption at another, can occur simultaneously. These studies do not eliminate a possible distal site for mecamylamine secretion.

The stop-flow studies showed that the tubular secretion of Darstine occurred in the proximal tubule. No evidence either of increased tubular secretion or reabsorption in the distal tubule was apparent.

Potassium tubular secretion has been shown to occur in the distal segment; reabsorption may occur in this segment as well as in the proximal segment (Sullivan and co-workers, 1960).

It is therefore evident from the stop-flow studies that as a) the tubular secretion of potassium, Darstine and mecamylamine occur in different areas of the nephron; b) potassium and mecamylamine exhibit a bi-directional flux; and c) Darstine exhibits a uni-directional flux, and from the results of the clearance studies, a unifying theory for the excretion of these bases is impossible with available data.

There is evidence from this work that suggests that the tubular secretion of mecamylamine as well as Darstine could occur in part by the generally accepted organic base transport system. Briefly, this evidence is a) proximal tubular secretion of mecamylamine, b) the accumulation of mecamylamine in rat and dog kidney slices to show S/M ratios greater than one, and c) the inhibition of this uptake by 2, 4-dinitrophenol (DNP). The proposal is further substantiated from the data presented by Volle and co-workers which showed that the administration of mecamylamine inhibits NMN tubular transport in the chicken.

# CHAPTER V

## SUMMARY AND CONCLUSIONS

The renal excretion of two organic bases, mecamylamine (Inversine$^{R}$), a secondary amine and ganglionic blocking drug, and mepiperphenidol (Darstine$^{R}$), quarternary ammonium compound and cholinergic blocking drug were studied in the dog.

The excretion of mecamylamine has been shown to be primarily influenced by the pH of the urine. This evidence suggested the possibility that the tubular excretion of mecamylamine could be in part by the exchange mechanism which transports potassium. To test this hypothesis, the excretion of mecamylamine, potassium and sodium was studied following the acute administration of both mecamylamine and potassium chloride. Urinary pH changes were accomplished by systemic acidosis or alkalosis.

The renal tubular excretion of Darstine has been shown to be by an active transport system, and to be influenced by potassium chloride administration. It was, therefore, of interest to investigate the excretion of this quarternary ammonium compound under the experimental procedures

used for investigating the excretion of the secondary amine.

The classical clearance technique and the technique of localization of tubular activity by stop-flow analysis were used for the excretion studies in the intact animal. The accumulation of the compounds in rat kidney slices was used to evaluate the renal tubular excretion in vitro.

The findings in this work are:

1. Potassium chloride loading inhibits the tubular secretion of mecamylamine and Darstine in the intact animal. There was evidence that Darstine inhibits the tubular secretion of potassium. The inhibition of the compounds by potassium is discussed in relation to the mutual secretion of the organic and inorganic cations via the tubular exchange system.

2. Mecamylamine and Darstine accumulate within the rat kidney slice from the medium. The accumulation of these compounds in the slice is inhibited by 2, 4-dinitrophenol (DNP). These results are discussed in relation to evidence for active tubular transport of the organic bases.

3. The tubular secretion of mecamylamine occurs by non-ionic diffusion as well as by active transport. The tubular reabsorption of mecamylamine occurs by non-ionic diffusion, with no evidence of active transport.

4. Darstine excretion occurs by filtration and tubular secretion, as other workers had shown. No evidence

for Darstine reabsorption was noted.

5. As judged by stop-flow studies, the tubular secretion of both mecamylamine and Darstine occurs within the proximal segment of the nephron.

6. The tubular reabsorption of mecamylamine after alkalinization of the urine occurs in the distal segment of the nephron.

The results of this work are interpreted as evidence that a) the tubular secretion of mecamylamine and Darstine occurs in part by the transport system which involves inorganic cation exchange, b) the tubular secretion of mecamylamine occurs in part by the organic base mechanism which transports Darstine.

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