DOG KIDNEY MICROPUNCTURE AND ELECTROPHYSIOLOGICAL STUDIES.

by John F. Seely, M.D.

Renal & Electrolyte Division

Royal Victoria Hospital

Montreal, Que.

MICROPONCTION ET ETUDES ELECTROPHYSIOLOGIQUES DU REIN CHEZ LE CHIEN.

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RESUME

Le recouvrement par microponction des tubules proximaux et distaux du rein du chien, a été pratiqué durant une infusion hypertonique au mannitol, durant charge saline seule, ainsi qu'avec l'administration de furosemide. Des augmentations dans le taux d'excrétion urinaire de sel et d'eau, furent hautement corrélatives avec une augmentation du débit au néphron distal et, réflétèrent l'activité de l'anse de Henlé. Une dissociation marquée, existait entre l'étendue de l'absorption proximale, et l'excrétion urinaire.

La résistance électrique, ainsi que la différence du potentiel des tubules proximaux et distaux chez le chien, furent mesurés et, la perméabilité ionique relative fut évaluée dans le tubule proximal. La faible résistance spécifique, ainsi qu'un manque relatif de sélectivité à l'infiltration ionique, indiquèrent une importante voie paracellulaire dans le tubule proximal.

MICROPUNCTURE AND ELECTROPHYSIOLOGICAL STUDIES OF THE PROXIMAL AND DISTAL TUBULES OF THE DOG KIDNEY

A) Micropuncture assessment of nephron function during altered sodium excretion: evidence for loop effects

B) Electrophysiological assessment of nephron function: evidence for a paracellular transport path

by

John F. Seely, M.D.

This thesis is submitted to the Faculty of Graduate Studies and Research, McGill University, in partial fulfillment of the requirements for the doctoral degree in physiology

Renal and Electrolyte Division
Royal Victoria Hospital
McGill University Clinic
Montreal 112, Quebec

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Ph.D. Renal and Electrolyte Division

John F. Seely, M.D.

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Abstract

Recollection micropuncture of proximal and distal tubules of the dog kidney was performed during hypertonic mannitol infusion, saline loading alone and with furosemide administration. Increases in urinary excretion of salt and water were highly correlated with increased delivery to the distal nephron and reflected activity of the loop of Henle. Marked dissociation existed between extent of proximal inhibition and urinary excretion.

The electrical resistance and potential difference of the dog proximal and distal tubules was measured and relative ionic permeability assessed in the proximal tubule. The low specific resistance and relative lack of selectivity to ion permeation indicated an important paracellular path in the proximal tubule.

Preface

It is a pleasure to acknowledge the debt I owe to Dr. John H. Dirks with whose supervision, direction and assistance a large part of the work reported in part A was performed and in whose laboratory many of the micropuncture and microanalytical techniques were acquired. I am also very grateful to Dr. Gerhard Giebisch, Chairman of the Department of Physiology at Yale University Medical School, and especially to Dr. Emile Boulpaep of the same department who assisted in and directed my work there, which is reported in part B of the thesis. To these three the author is deeply appreciative for their help and advice on countless matters. I would also like to acknowledge Mrs. Jacqueline Fraser, Mrs. Jean Kanter, Miss Laura Guillermo, Mr. James Pettit (all of McGill) and Mrs. Francoise Diezi (Yale) who gave valuable technical assistance during various phases of the work. I am most grateful to the Medical Research Council of Canada who supported me throughout the period of time these studies were performed with a Centennial Fellowship.

General Introduction

The experimental work that forms the basis of this thesis falls naturally into two major subdivisions. The first half of the work which was performed in the Department of Experimental Medicine, McGill University, deals with a micropuncture assessment of segmental nephron function in the dog under a variety of experimental diuretic conditions. The second half was performed in the Department of Physiology at Yale University Medical School and details the results of a study of the electrophysiological properties of the proximal and distal tubules of the dog kidney. Because of the obvious differences in the objectives, methods and conclusions to be drawn the thesis has been correspondingly divided into two parts, A and B. A single bibliography is provided at the end.

Part A

Introduction

The control of salt and water excretion by the kidney is still imperfectly understood, despite more than a century of investigation, in large part because of the complexity of factors involved in this important homeostatic function. The present series of experiments were designed to provide a descriptive analysis of the alterations in water and electrolyte reabsorption

that occur at different sites within the nephron of kidneys during several experimentally induced changes in sodium excretion. Such a descriptive study is a necessary and complementary counterpart to studies of a more mechanistic approach that deal with events at a cellular level or at a given segment of the nephron, since the latter may neglect important interaction effects between the various parts of the entire nephron.

The conditions that were examined in this study included strong osmotic diuresis evoked by the administration of hypertonic mannitol solutions, extracellular volume expansion with isotonic saline infusions ("saline diuresis") and the superimposition of a potent diuretic drug, furosemide, upon a "saline diuresis". By these means it was possible to dissect the functional response within the nephron during conditions in which sodium excretion increased from less than 1% to over 30% of the filtered sodium load.

Mannitol was first introduced into the study of renal function by Homer Smith in 1940 (Smith, Finkelstein and Smith, 1940) in the search for additional substances that fulfilled the specifications of "glomerular excretion without tubular participation". It was thought that the finding of such substances, whose clearance equalled that of inulin or creatinine, would lend further support for the use of inulin as a measure of the glomerular filtration rate. Smith et al. found that the clearance of mannitol along

with sorbitol, sorbitan and dulcitol was essentially equal to the inulin or creatinine clearance, although later investigations showed that, in fact, mannitol is reabsorbed by the renal tubule to a slight extent (Berger, Farber and Earle, 1947; Corcoran and Page, 1947).

The osmotic properties of this agent were used first to great advantage in the study of renal tubular function by Wesson and Anslow in 1948 (Wesson and Anslow, 1948; Wesson, Anslow and Smith, 1948). These authors reasoned that since a large fraction of the filtrate may be excreted during strong osmotic diuresis, and since (they presumed) 85% of the filtrate was reabsorbed in the proximal tubule, therefore, "most if not all of this increased excretion must be a result of decreased reabsorption in the proximal system. Under conditions of moderate to extreme diuresis, the contributions of the distal tubule in modifying the composition of the urine are overwhelmed in the flood of proximal diuresis, so that the composition of the urine approaches that of the composition of the proximal urine" (Wesson and Anslow, 1948). This conclusion was supported by their finding that they were able to achieve rates of urine flow greater than 50% of the filtration rate and by the fact that urine osmolarity tended to approach that of plasma. It should be pointed out that the conditions used in this experiment were indeed extreme

since they infused a 25% mannitol solution at rates between 0.7 to 1.0 cc/kg body weight.min. They further noted that "it was usually necessary to terminate the experiment after about three-quarters of an hour of infusion of mannitol solution; a longer period of infusion at the rate and concentration of mannitol used here endangered the life of the animal". Nevertheless, several of their conclusions were remarkably sound. From their findings they surmised that sodium reabsorption was an active process independent of water reabsorption, that water was reabsorbed passively and secondarily to sodium reabsorption, and that the active process of proximal sodium reabsorption was limited during osmotic diuresis by the development of a critical or limiting concentration gradient for sodium across the tubular epithelium. They surmised that in the presence of a non-reabsorbable solute the concentration of sodium in the tubular fluid would fall progressively below that in the surrounding plasma until the point was reached at which no further transport of sodium could occur. The concentration gradient developed across the tubule at the point of no net transport was defined as the limiting concentration gradient. They also thought it likely that under normal conditions proximal tubular fluid would be slightly hypertonic to plasma, which thereby would provide the osmotic pressure gradient required for passive water movement. There were two major ramifications

to this work. The first related to the nature of sodium transport and the second related to the site and mechanism of inhibition of sodium transport during osmotic diuresis.

To consider first those aspects related to the nature of sodium transport, subsequent work has tended to support the original conclusions of Wesson and Anslow with few modifications. Although their view as to the presence of a limiting concentration gradient in the proximal tubule was at first disputed on the basis of other clearance data (Mudge, Foulks and Gilman, 1949; Thompson and Barrett, 1954), this was later substantiated by micropuncture studies under both free-flow (Windhager and Giebisch 1961; Ullrich et al., 1963) and stop flow conditions (Windhager et al. 1959; Kashgarian et al., 1963; Giebisch et al. 1964; Hierholzer et al. 1965; Malnic, Klose and Giebisch 1966; Ullrich 1967; Hayslett, Kashgarian and Epstein 1968). However, the extent of the gradient postulated by Wesson and Anslow (60-90 Meq/1) was somewhat in excess of what in fact has been found by direct micropuncture assessment ($\sim$ 35 mEq/1). These results confirmed the active nature of sodium transport in the proximal tubule and the passive nature of water reabsorption. Wesson and Anslow also felt that they had demonstrated the independence of sodium and water transport in contrast to the view advanced by Cushny (1926) that the proximal tubule reabsorbs fluid of a constant composition. However, it is apparent that this conclusion is

unwarranted in the presence of any passive back diffusion of water into the tubule. It is equally likely that reabsorption of a fluid whose sodium concentration was equal to that of plasma in the presence of a non-reabsorbable solute, would result in the lowering of the intratubular sodium concentration if water diffused back into the lumen down its osmotic gradient created by concentration of the non-reabsorbable solute.

The second aspect of Wesson and Anslow's work relates to the site of action where mannitol exerts its diuretic effects. This was presumed to be proximal for the obvious reasons outlined above. Distal sites were thought to play little or no significant role in mediating the overall diuresis. It is surprising, however, that little further direct work establishing the validity of their work was done. This was perhaps attributable to the confirmation of the fact that a limiting concentration gradient for sodium existed. The view, therefore, that the diuresis could be attributed to a proximal effect received wide acceptance in later studies of renal function (Rapoport et al. 1949; Rapoport and West 1950; West and Rapoport 1950; Wesson 1962). This fact (i.e. that a limiting concentration gradient for sodium exists in the proximal tubule), however, does not establish that this gradient is in fact reached during mannitol diuresis nor that the proximal tubule is the major site of inhibition, particularly when neither the lethal amounts of mannitol employed by Wesson and Anslow are used nor are the

urinary excretion rates as impressively high. The only previous studies that have examined this question were two micropuncture studies in the rat (Windhager and Giebisch 1961; Ullrich et al. 1963). While both these studies did show that tubule fluid to plasma (TF/P) sodium concentration ratios fell below unity during hypertonic mannitol diuresis, thereby supporting the limiting gradient concept, there was no convincing evidence in these same studies for large reductions in fractional fluid reabsorption in the proximal tubule nor did they indicate the extent to which more distal sites might be involved. In the study of Windhager and Giebisch (1961) there were no control data to allow any precise comparison. They estimated that a 20% reduction in net water reabsorption had resulted by comparing their result with data published some 20 years earlier (Walker and Oliver 1941). The data of Ullrich et al. (1963) were too limited to allow any conclusion with regard to net effect on fluid reabsorption within the proximal tubule. Both sets of data, however, established that TF/P sodium ratios in the early distal tubules were in fact lower during mannitol infusion than in control rats: it was thus possible that the actual load of sodium presented to the distal tubule might be only little or not at all greater than in control non diuretic state. If this were so, obviously the behavior of distal sites within the nephron

would have a decisive influence on the nature and extent of the actual diuresis.

An additional micropuncture study of the dog undergoing mannitol diuresis was reported by Dirks, Cirksena and Berliner (1966). They showed that proximal fractional fluid reabsorption, determined by measurement of TF/P inulin ratios, was readily inhibited by mannitol infusions when urine flow exceeded 5% of GFR; more modest loads failed to alter proximal reabsorption significantly. No tubule fluid electrolyte concentrations were measured, however, which thus precluded an assessment of fractional sodium handling, nor was there any measurement of distal tubule function in this study. The present studies were performed to allow a more direct estimate of both sites and extent of inhibition of sodium transport within the nephron than had previously been determined.

The second set of studies reported in part A dealt with the effects of extracellular volume expansion by means of saline infusions upon proximal and distal sodium reaborption. The central role of the kidney in the regulation of the extracellular fluid volume has long been recognized, at least since the time of Starling at the turn of the century. He wrote in 1909 that "in the kidney we find an organ whose function, broadly speaking, is the regulation of the amount and composition of the total fluid

of the body; a regulation which it is able to carry out in consequence of its sensitiveness to minute changes in composition and amount of blood circulating through its vessels". Starling was also aware that the kidney must be ultimately responsible for the accumulation of edema (i.e. an excess in the volume of extracellular body fluids) by virtue of undue salt and water retention. Peters (1935), also recognized the intimate relationship between body fluid volume and the renal regulation of salt balance and suggested that the volume of the circulating blood in some way could regulate sodium excretion. In view of the obvious importance of this subject for human physiology it is therefore surprising to find that little experimental effort was directed to this problem and, perhaps in consequence, that the concepts generally held were somewhat naive.

A small number of studies prior to 1960 had demonstrated that expansion of the extra-cellular fluid by means of isotonic saline infusions, is accompanied by an increase in the excretion of salt and water by the kidney, and it had been suggested from some of these studies that there must have been a reduction in the tubular reabsorption of sodium to account for the observed diuresis (Eggleton, Pappenheimer and Winton, 1940; Stamler et al., 1958; Keeler and Schnieden, 1958; Strauss et al. 1952; Crawford and Lindermann, 1951; Wiggins et al. 1951; Selkurt and Post, 1950; Wesson et al., 1950). Nevertheless, an authoritative review in 1957

by Wesson concluded that such changes could best be accounted for on the basis of small or immeasurable changes in the filtration rate. Since the tubules normally reabsorb almost all of the filtered sodium load, it obviously requires only a small increase in filtration rate to effect a very large change in sodium excretion if absolute sodium reabsorption remains constant. In retrospect this conclusion was seen to be a grossly oversimplified and naive view for the following reasons:

a) The exquisite regulation of sodium excretion would seem a priori to require the operation of many factors acting at both a tubular level as well as at the level of filtration rate.

b) It failed to deal adequately with some of the clearance experiments where changes in filtration rate bore no relationship to changes in salt excretion.

c) It assumed that changes in filtration rate alone would not be accompanied by proportional increases in tubular reabsorption and hence would result in a large diuresis. When this assumption was later directly tested in the dog by Lindheimer, Lalone and Levinsky (1967) it was shown that large increases in filtration rate, when not accompanied by extracellular volume expansion, gave only minimal increases in salt excretion.

d) Finally, evidence gathered by Epstein from experimental situations accompanied by reductions in salt excretion had led

him (1956), and also Homer Smith (1957) to suggest that there might be an antinatriuretic hormone released when there was a reduction in extracellular fluid volume or in the degree of filling of the arterial tree. If that were so then volume expansion would be expected to inhibit tubular sodium reabsorption either by the production of a natriuretic hormone or by inhibition of the anti-natriuretic system. Nevertheless it was not until the early 1960's that this problem was widely recognized and vigorously attacked by experimental methods. The experiments of de Wardener et al. (1961) in a simple and direct fashion showed conclusively that the diuresis resulting from saline infusions into dogs occurred despite actual reductions in filtration rate and despite maximal doses of mineralocorticoids. The exclusion of these two known factors in the control of sodium excretion led to the search for additional regulatory influences. Berliner has aptly summarized the contribution of de Wardener to this problem as follows: "The essence of what they did was to show that the diuresis that occurs when saline is infused in a dog cannot be explained by changes in any of the variables that, up to that time, had been considered to be important in mediating that response. Although in reality no single aspect of their studies was dramatically new, the simultaneous exclusion of most of the pertinent known variables brought renal physiologists to look at the problem squarely" (Berliner 1968).

Since that time there has been a flood of papers devoted to one or other aspect of this phenomena. We shall only consider here the development of those aspects relevant to the present work. The work of de Wardener et al. was rapidly confirmed by clearance experiments of Levinsky and Lalone (1963), Blythe and Welt (1963), and Rector et al. (1964). Moreover, the experiments of Rector et al. had shown that hypotonic saline loading led to increases in free water clearance, from which they suggested that the inhibition of sodium reabosrption had occurred in the proximal part of the nephron, i.e. proximal to the water impermeable segments of the nephron. By inference there must also have been increased reabsorption in the distal nephron. Their suggestion that saline loads inhibited proximal sodium reabsorption was proved directly the following year by Dirks, Cirksena and Berliner (1965). These authors used the recollection micropuncture technique by which the same tubule could be sampled two or more times under different experimental conditions, and showed that fractional reabsorption in the proximal tubule was inhibited by as much as 30% after saline infusions. Moreover, essentially identical results were seen when overall kidney filtration rate was reduced by aortic constriction, implying that both absolute as well as fractional reabsorption in this part of the nephron had been reduced. There was also a striking difference between the large inhibitory effect in the proximal tubule and the much smaller

natriuretic effects since urinary sodium averaged only 6-7% of the filtered load. This discrepancy gave further evidence for increased sodium and water reabsorption beyond the proximal tubule.

The findings of Dirks, Cirksena and Berliner (1965) have subsequently been confirmed by many micropuncture investigations in the rat as well as in the dog, both with respect to the large depression of fractional as well as absolute reabsorption when the latter is assessed by the split-droplet technique of Gertz (1965) or by microperfusion studies. Several micropuncture studies in the rat have also shown that sodium reabsorption increases in the distal parts of the nephron under the conditions of saline loading (Cortney et al. 1965; Giebisch, Klose and Windhager 1964; Landwehr, Klose and Giebisch 1967; Lassiter, Mylle and Gottschalk 1964). However, this has not yet been directly examined in the dog. The discrepancy between the extent of proximal inhibition and fractional urinary excretion rates that was observed by Dirks et al. has also been demonstrated in the dog in an even more dramatic fashion by the administration of hyperoncotic solutions which depress fractional reabsorption to the same extent as saline infusions but give even less of a diuretic response (Howards et al. 1968; Knox et al. 1968). The present experiments were therefore designed particularly to assess the distal handling

of salt and water in the dog under the conditions of a saline load, to gain further insight into the nature of this phenomenon.

These latter studies of saline loading were highlighted by the administration of a potent diuretic drug, furosemide, to animals undergoing a simultaneous saline diuresis. This was of interest not only for an examination of the effects of this agent within the nephron but also because it provided an additional comparison of the role of segmental effects in contributing to the diuresis under conditions of maximal inhibition of sodium reabsorption. Furosemide (4-chloro-N-(2-furyl-methyl)-5-sulfamoyl-O-anthranilic acid) is a recently discovered cogener of the thiazide group of drugs and is the most potent diuretic agent currently available. No satisfactory explanation has yet been forthcoming to account for the mechanism of its action on renal tubular sodium transport. Most experimental attention has been paid to a descriptive analysis of its site of action within the nephron by clearance as well as micropuncture techniques. It has been shown in the rat proximal tubule that this drug is capable of inhibiting both absolute and fractional rates of sodium reabsorption, though not to a proportionate extent since fractional reabsorption tends to be reduced to a lesser extent or in some studies not at all. Absolute reabsorption has repeatedly been shown to be reduced whether measured by the shrinking drop techniques of Gertz (1965; Rector et al., 1966), sodium efflux rates (Ullrich 1965; Ullrich et al., 1966) or by

single nephron microperfusion techniques (Morgan et al., 1970). The studies of Brenner et al. (1969), Meng (1967), Malnic, Vieira and Enokibara (1965), and Deetjen (1966) have shown that fractional reabsorption of sodium is also retarded, whereas Rector et al. did not observe any change in fractional reabsorption in their studies (1966, 1967). This lack of effect on net or fractional reabsorptive rate was attributed to a prolongation of tubular transit time. In the dog absolute reabsorption in the proximal tubule has also been shown to be reduced when measured by the shrinking drop method (Knox et al., 1969), while fractional reabsorption has been found to be unaffected in two studies (Dirks, Cirksena and Berliner, 1966, Knox et al., 1969).

Relatively few micropuncture studies have dealt with the effects of the drug in the distal nephron. Clearance studies have pointed to the loop of Henle as the major site at which furosemide exerts its inhibitory effects on sodium reabsorption in view of the marked impairment in urinary diluting and concentrating ability that follows its use. This has been substantiated by micropuncture of the distal convoluted tubule in the rat (Brenner et al., 1969, Malnic, Vieira and Enokibara 1965, and Meng 1967) and in the monkey (Bennett, Brenner and Berliner 1968) but has not yet been directly studied in the dog. These experiments thus afforded an opportunity to study the distal effects of this drug in the dog as well as providing an additional situation for the comparison of segmental effects with the extent of diuresis.

Methods

Acute experiments were performed in mongrel dogs weighing between 10 and 20 kg. The animals were anesthetized with intravenous pentobarbital 25 mg/kg and received small supplemented doses of pentothal as required during the experiment to maintain satisfactory anesthesia. A cuffed endotracheal tube was inserted and artificial ventilation with room air using a Harvard respirator was routinely performed. Polyethylene catheters were placed into a jugular foreleg vein for intravenous infusions and into femoral artery and vein for measurement of blood pressure and blood withdrawal respectively. The right ureter was catheterized close to its insertion into the urinary bladder by a midline suprapubic incision. The left kidney was then freed up on its vascular pedicle, cleared of surrounding adventitia via a subcostal flank incision and a 27 gauge needle placed in retrograde direction into the renal artery close to its takeoff from the abdominal aorta. The left ureter was catheterized close to the hilum of the kidney, and the kidney placed in a lucite holder for micropuncture.

Inulin was infused intravenously after a suitable priming injection in doses calculated to maintain plasma levels of approximately 100 mg/100 ml. Late proximal and distal convoluted tubules were identified on the surface of the kidney by means of intermittent injections of 0.1 to 0.2 ml of buffered lissamine green dye

solution into the renal artery. Selected tubules for micropuncture were marked with nigrosine dye to enable recollection of tubule fluid samples to be made from the same site during the same or subsequent phases of each experiment. Tubule fluid samples were collected using little or no aspiration into micropipettes which had been filled with heavy mineral oil, stained with Sudan black and previously equilibrated with water. The micropipette tips were sharpened to a fine bevel of 5-10 microns diameter. The kidney surface was illuminated via a quartz rod and observed under a Zeiss stereoscopic dissecting microscope at a power of 50-80 X magnification. The micropipettes were positioned in a de Fonbrune micromanipulator for the actual micropuncture. Prior to collection of tubule fluid a droplet of oil was injected into the tubule both to determine direction of tubule fluid flow and to prevent contamination of the sample by retrograde collection of tubule fluid from a point downstream to the site of puncture. Each sample was carefully checked under the microscope following collection and discarded if it contained any red blood cells.

Each experiment was carried out in two or three phases. An initial control phase (hydropenia), usually lasting 60-90 minutes, was performed during which time several proximal and or distal tubules were identified, marked and sampled. This

was then followed by an experimental phase, in which one of two protocols was followed.

Protocol 1: Mannitol: An intravenous infusion of 16% mannitol solution in modified Ringers ("saline") solution (composition Na 150 mEq/1; K 3.5 mEq/1; Cl 133.5 mEq/1 and HCO_3 20 mEq/1), was given at a rate of 12 ml/min to 22 dogs until a total volume of 400-600 cc had been infused. This was thereafter continued at 3 ml/min while additional saline was also given to match the rate of urine flow. Only when the rate of urine flow had stabilized at a new rate were recollections of tubule fluid made from as many of the previously sampled tubules as possible.

Protocol 2a: Saline: In 20 dogs saline was infused in amounts equal to 3 to 6% of body weight at a rate of 12 ml/min and thereafter at rates equal to the urine flow rates. Recollections were not begun until after stabilization of the urine flow.

Protocol 2B: In 12 of the saline loaded dogs after completion of recollections during the saline phase, furosemide was administered intravenously in a priming dose of 10 mg/kg and at 10 mg/kg/hr thereafter. As above, additional saline was given to match rates of urine flow.

Clearance periods of 15 minutes duration were performed from the micropuncture kidney during all phases concurrent with the micropuncture. Blood was drawn at the midpoint of each period for determination of the inulin clearance which was used as a measure of the glomerular filtration rate.

Urine was analyzed for sodium and potassium on an I.L. flame photometer (Model 143). Plasma osmolality was determined using an Advanced osmometer. Urine and plasma inulin concentrations were determined by the anthrone method of Führ, Kaczmarczyk and Krüttgen (1955). Inulin in tubule fluid samples was analyzed by the fluorometric method of Vurek and Pegram (1966) modified to increase the boiling time to 10 minutes. A set of 24 prepared inulin standards, handled as unknowns by this method, gave a mean recovery of 100.4% (± 2.3% SD). A set of twenty-four plasmas was also analyzed simultaneously by both anthrone and fluorometric methods and the fluorometric value expressed as a percentage of the anthrone result. The mean recovery was 98.7% (± 4.0% SD) which indicated satisfactory agreement between these two methods. Tubule fluid samples were analyzed for sodium and potassium with a helium glow photometer by the method of Vurek and Bowman (1965). Twenty-eight prepared electrolyte standards, handled as unknowns, gave mean recoveries for sodium of 98.8% (± 5.3% SD) and for potassium of 97.9% (± 9.1% SD). The addition

of mannitol in concentrations as high as 5 gm per 100 ml did not significantly affect these results. The plasma values for inulin, sodium, and potassium which corresponded to the individual tubule fluid samples were interpolated from the values obtained at the midpoint of each clearance period for the determination of TF/P inulin and electrolyte ratios. Arterial blood pressure was monitored from an indwelling femoral arterial catheter by a Statham pressure transducer connected to a Sanborn recorder. Fractional rejection of water, sodium and potassium was calculated in all tubule fluid samples from the following formula:

$$\text{Fractional rejection} = \frac{P}{TF} \quad X\ 100$$

Results

Control micropuncture recollections:

A) Proximal: The validity of the recollection technique was evaluated by means of recollection of tubule fluid samples from the same puncture site during a continuous hydropenic phase. In 13 dogs, 35 samples were recollected from previously marked proximal tubules. These results are illustrated in Fig. A-1. The TF/P inulin ratio obtained during the control period is plotted along the abscissa and the recollected value along the ordinate. The degree of deviation of points away from the line of identity in Fig. A-1 thus reflects the total cumulative error

involved in the experimental methods. The mean change in TF/P inulin ratio was +0.01. The mean ratio of the TF/P inulin during recollection to that in the control period was 1.01, SE ±0.02, which was not significantly different from unity.

B) Distal: The results of similar control recollections from the distal tubule are shown in Figs. A-2 and A-3 and summarized in Table A-1. The mean initial TF/P inulin ratio obtained from 34 distal samples during continuous hydropenia was 3.79 ± 0.21 which did not differ significantly from the recollected value of 3.56 ± 0.18. This change represents only a 2% decrease in functional fluid reabsorption at the site of puncture. Distal TF/P sodium and potassium ratio were also not significantly altered in paired samples. Figures A-2 and A-3 also include paired samples obtained during a continuous diuretic phase and as shown the recollected values are scattered randomly about the identity line.

I. Hypertonic Mannitol Infusion

A) Clearance Data: In the majority of experiments, paired tubule fluid samples were obtained from either proximal or distal convoluted tubules during the two phases. Since the protocol employed was identical in all cases, and since the clearance

data from those experiments in which only distal pairs were collected did not differ significantly from those that yielded only proximal data, the clearance results from all experiments have been pooled and analyzed together. In each animal, all consecutive clearance periods during which micropuncture was performed were averaged for the two phases. These results are summarized in Table A-2.

Striking increases in urine volume and electrolyte excretion occurred in all experiments. Mean urine flow rose from 0.40 ml/min to 6.61 ml/min, while sodium excretion rose from 56.5 μEq/min to 425.3 μEq/min. Potassium excretion increased significantly from 28.1 μEq/min to 59.0 μEq/min. The mean inulin clearance decreased by 27% (35.1 ml/min to 25.2 ml/min) which was highly significant ($p < 0.01$). Plasma osmolality was measured on alternate blood samples on all but the initial two experiments. The mean value during hydropenia was 304 mOs/kg H_2O and this rose to 364 mOs/kg H_2O during the mannitol phase. This was accompanied by a small but highly significant decrease in plasma sodium from 150.7 mEq/l to 144.7 mEq/l. The fractional excretion rates for sodium and potassium significantly increased from 1.06 to 12.6% and 23.8% to 63.9% respectively. The venous hematocrit was measured in 16 experiments and fell significantly from 37.8 to 29.1. The arterial blood pressure remained unchanged throughout the two phases of these experiments.

B) Micropuncture Data: The micropuncture results have been analyzed primarily in terms of the total number of paired tubule fluid samples that were obtained. This method essentially treats each tubule as a separate observation irrespective of the number of paired samples collected from each individual animal. These results are summarized in Table A-3. Table A-4 includes an analysis of the data treating each animal as a separate observation. In this case, the micropuncture results for each animal are averaged to give a single value for each of the two phases. This second method however may be unduly biased by the results of any one pair of tubule fluid samples in a series of animals, a number of which contribute only several pairs (as is unavoidable in distal recollection experiments in the dog). Since the number of observations is correspondingly reduced, it is also more difficult to demonstrate significant differences by this method. Therefore, the fact that these two methods yield virtually indistinguishable results lends added confidence to their evaluation.

Proximal Tubule

Thirty-nine paired tubule fluid samples were collected from the proximal tubule in twelve experiments and analyzed for sodium concentration. Owing to technical problems, potassium

analysis was not performed on seven paired samples from the first two experiments, which left only thirty-two paired TF/P potassium ratios. Because of the larger volume of tubule fluid required for inulin analysis, it was not possible to analyze inulin concentration ratios in nine pairs. A deliberate attempt was made to select late proximal tubule sites for micropuncture, by means of lissamine green dye injection, in as many instances as possible. The mean TF/P inulin ratio obtained during hydropenia was 1.63 and this fell significantly during mannitol diuresis to 1.45 ($p < 0.01$). These results are illustrated in Fig. A-4. The results of TF/P electrolyte ratios are shown in Fig. A-5 (sodium) and Fig. A-6 (potassium) plotted against their respective inulin concentration ratios as a measure of increasing amounts of fractional fluid reabsorption. The TF/P sodium ratios were clustered around unity during hydropenia with a mean of 0.97. There was a highly significant decrease during the mannitol phase (0.93, $p < 0.01$) which is not so readily apparent when the data are presented in an unpaired fashion as in Fig. A-5. Potassium concentration ratios showed greater scatter, although the results were qualitatively similar to that for sodium. The mean hydropenia value significantly decreased ($p < 0.05$) from 1.05 to 0.98 during mannitol loading.

The mean values for the fractional rejection of water, sodium and potassium in the proximal tubule, during the two

phases, are shown in Table A-5. The fractional rejection of water in the proximal tubule increased to a small but significant degree from 63.7% to 70.8%. Since this value is based on all of the observations from the proximal tubule, a number of which were collected from early proximal sites, this value tends to underestimate the degree of fractional inhibition that would be manifested in the late proximal tubule. Assuming that fractional reabsorption at the late proximal site under hydropenic conditions approximates 50% of the filtrate, then extrapolation of the results that were obtained suggests that fractional rejection at the late proximal tubule during mannitol diuresis would have increased by approximately 10% (i.e. from 50% to 60%). Fractional rejection of both sodium and potassium was increased by approximately 4% which was not significant in either case. A similar calculation to that for water, shown above, would increase this value by only an additional 2% - 3%. It is apparent that the urinary fractional excretion rates for both water (29%) and sodium (13%), shown also in Table A-5, were considerably greater than the degree of fractional inhibition observed in the proximal tubule.

Distal Tubule

In contrast to the small effects observed in the proximal tubule, mannitol loading resulted in striking changes in distal

inulin and electrolyte ratios. These data have been summarized in Table A-3 and A-4. The results of sixteen paired distal TF/P inulin ratios obtained in eleven experiments are illustrated in Fig. A-7. A marked drop occurred in every instance. The mean value fell from 5.38 during hydropenia to 1.94 during the diuretic phase ($P < 0.01$). Distal electrolyte ratios were obtained from an additional seven puncture sites, making a total of twenty-three pairs. The TF/P sodium ratios, depicted in Fig. A-8, increased after mannitol in all but two instances. The mean value in the mannitol phase of 0.59 was significantly higher than the mean hydropenic value of 0.38 ($p < .01$). The distal sodium ratios during the mannitol phase were also significantly higher than the corresponding final urine to plasma sodium ratios (mean 0.40, $p < 0.01$). The results of distal TF/P potassium ratios indicated a considerable range during hydropenia (0.15 to 2.75). There was a noticeable tendency for these ratios to approach unity during the mannitol phase as illustrated in Fig. A-9, and the range was correspondingly reduced (0.56 to 1.82).

The mean values for the fractional rejection of water, sodium and potassium in the distal tubule are also summarized in Table A-5 along with the corresponding values from the proximal tubule and final urine. The latter values were derived from the clearance results of all experiments, while the proximal and distal figures were based on all paired tubule fluid samples.

The mean value for all the distal data was used to estimate the degree of fractional rejection that took place within the pars recta and loop of Henle and the collecting system, i.e. the segments of the nephron between the late proximal tubule, distal tubule and ureteral urine. This calculation thereby increases the estimated fractional reabsorption at the loop and collecting duct by a combined amount equal to the fraction reabsorbed along the length of the distal convoluted tubule. However, since the distal tubule of the dog, under normal hydropenic conditions, has been shown to reabsorb only a small proportion of the filtered water (∿8%) and sodium (∿3%) (Bennett, Clapp and Berliner 1967), and probably less during mannitol diuresis, it is likely that taking the mean of all the distal data introduces only a small error of the order of less than 5% for water and even less for sodium into these calculations. It will be noted that the fractional rejection of water increased by 32% at the distal tubule during the mannitol phase, while fractional rejection of both sodium and potassium was also increased to almost the same extent (∿26%). This indicates the large degree to which reabsorption of salt and water was retarded between the late proximal and mid-distal tubule. Fractional urinary excretion of water was augmented by approximately the same extent (27%) as in the distal tubule. Fractional reabsorption in the collecting

duct, therefore, is similar during the two phases. Fractional excretion of sodium in the urine increased by only 12%, which indicates that substantial further reabsorption of sodium occurs within the collecting duct. These changes in the fractional rejection of sodium and water at different nephron sites are illustrated graphically in Fig. A-10. Rejection of potassium at the distal tubule averaged 52% during the diuretic phase compared with a urinary rejection of 64%. Since the mean distal value probably includes both early and late distal sites, it is not possible to estimate whether additional secretion of potassium has taken place in the collecting duct or not.

II. Isotonic Saline ± Furosemide

A. Clearance Data

The results of a series of twenty dogs to which isotonic saline was administered are summarized in Tables A-6 and A-7 and depicted in the accompanying Figs. A-11 to A-15. The data collected from twelve of these dogs who received furosemide during a subsequent third phase are also included. The clearance data from the left (micropuncture) kidney only are shown. In every experiment, the clearance periods obtained during hydropenia and the successive experimental phases during which micropuncture was performed were averaged to express the results as a single value for each

of the various phases. These results are summarized for all animals in Table A-6. It is evident from Table A-6 that plasma sodium concentration increased to a small extent, while glomerular filtration rate measured by inulin clearance fell slightly, but not significantly, following isotonic saline infusion. Urine flow rose modestly after saline from 0.43 ml/min to 1.83 ml/min. This amounted to an increase of approximatley 5% in the fraction of filtered water excreted (0.5% to 6.4%). Urinary sodium and potassium excretion rose from 40.4 uEq/min to 171.5 uEq/min and from 25.0 uEq/min to 38.1 uEq/min, while fractional excretion rates increased from 0.9% to 3.9% and from 24.1% to 37.6% respectively. When furosemide was superimposed upon the saline diuresis, striking increases in urine flow and electrolyte excretion occurred. Mean rate of urine flow reached 12.2 ml/min, while sodium excretion increased to 1633.4 uEq/min. Fractional excretion of water and sodium increased to 40.3% and 34.9% respectively. Net secretion of potassium was observed in ten of these experiments, as evidenced by the mean fractional excretion rate of 117.9%.

B) Micropuncture Data

The corresponding micropuncture data from these experiments are summarized in Table A-7. The means of all samples collected from proximal and distal convoluted tubules in all three phases are shown. The values obtained during saline loading were

compared to their respective hydropenic controls, whereas those collected during furosemide administration were compared to the corresponding results collected from the same tubule site during the preceding saline phase. Tubule fluid to plasma (TF/P) sodium ratios did not differ significantly from unity in all three phases. The mean TF/P potassium ratio was 1.07 during hydropenia and this fell to unity after saline loading, which was barely significant ($p < .05$). There was no further change following furosemide.

The TF/P inulin data from the proximal tubule are depicted in Fig. 11. Saline loading, as expected, resulted in consistent and highly significant decreases in fractional reabsorption within the proximal tubule. Mean TF/P inulin ratio fell from 1.83 during hydropenia to 1.49 after extracellular volume expansion. The non-reabsorbed fraction of filtered sodium and water in the proximal tubule increased by 14% (from 57% to 71%) and thus was far in excess of the increase in urinary fractional excretion of water and sodium, which averaged only 5% and 3% respectively. After administration of furosemide, there was no significant alteration in proximal TF/P inulin ratios, while the mean rose only slighly. These results are illustrated on the right hand side of Fig. A-11. Despite the lack of any apparent further action on the proximal tubule, the urinary excretion of water and sodium exceeded one third of the filtered load in both instances.

The distal tubule micropuncture data are depicted in Figs. A-12 to A-15 and are also summarized in Table A-7. Saline loading led to a significant reduction in distal TF/P inulin ratios, illustrated in Fig. A-12. The mean hydropenic value fell from 4.78 to 3.60 after saline ($p < .01$). Distal transit times illustrated in Fig. A-13 were not significantly altered, averaging 110 seconds in hydropenia and 100 seconds after saline. The increase in the non-reabsorbed fraction of filtered water averaged only 7% (24% to 31%), indicating that at least half and probably more of the excess load delivered from the proximal tubule ($\sim$14%) had been reabsorbed in the loop of Henle. Distal TF/P sodium ratios showed a consistent tendency to increase after saline loading and are illustrated in the left hand side of Fig. A-14. The mean TF/P sodium ratio rose from 0.34 during hydropenia to 0.42 during the saline diuresis, which was highly significant ($p < .01$). The non-reabsorbed fraction of filtered sodium in the distal tubule, however, had increased to an even smaller degree than that of water, rising from 8% to 12%. It is also apparent that the increments in the non-reabsorbed fraction of filtered water (7%) and sodium (4%) in the distal tubule which resulted after saline loading were remarkably similar in magnitude to the increases in urinary fractional excretion of water (5%) and sodium (3%).

The administration of furosemide produced even more extensive depression of TF/P inulin ratios in the distal tubule, which is apparent in Fig. A-12. The mean TF/P inulin ratio fell to 2.03, which was significantly lower than the preceding saline phase ($p < .01$). This result was accompanied by almost complete abolition of the sodium gradient in the distal tubule as TF/P sodium ratios now rose to a mean of 0.91. These results are illustrated on the right hand side of Fig. A-14. The TF/P potassium ratios, depicted in Fig. A-15, were not significantly altered after saline loading but were, however, consistently higher after furosemide than their paired saline controls. All but one in this case exceeded unity. The non-reabsorbed fraction of filtered water and sodium remaining in the distal tubule after furosemide increased to 51% and 46% respectively, the increases in both cases exceeding the excess delivered from the proximal tubule. Moreover, the increase in distal sodium rejection (38%) was very similar to the increased fractional excretion of sodium in the urine, which averaged 34%. The increased fractional excretion of water (39%), however, was more than could be accounted for by the excess rejection in the distal tubule (27%). These changes have been plotted in Fig. A-16.

These data allow estimates to be made of the reabsorption of sodium and water within successive nephron segments as a

percentage of the load presented to each segment in turn. The proximal data have been extrapolated to the point of 50% reabsorption during hydropenia so as to estimate the fractional reabsorption in the late proximal tubule and a proportional correction was applied to the saline-furosemide phases. The non-reabsorbed fraction at this point was used as an estimate of the load to the middle segment between the late proximal and mid-distal tubule. Reabsorption within this segment, which is representative of the loop of Henle, was calculated from the difference between the non-reabsorbed fractions in the late proximal and mid-distal tubule. A similar calculation based on the difference between non-reabsorbed fractions in the distal tubule and ureteral urine allowed estimates of the reabsorbed fraction of the load presented to the collecting duct segment of the nephron. These results are depicted in Fig. A-17. It is apparent that sodium and water reabsorption in the loop of Henle increased in proportion to the increase in load to this segment. The reabsorbed fraction of the segmental load therefore remained approximately constant when compared to the results during hydropenia. This constancy of fractional reabsorption within the loop of Henle provides a striking contrast to the reductions in fractional reabsorption of segmental load that resulted after saline loading in both the proximal tubule and in the collecting duct segment. Furosemide,

which had no greater effect on the proximal tubule than could be attributed to the saline load, had large inhibitory effects on the two latter segments.

DISCUSSION

The present study has attempted to characterize reabsorptive changes in the various segments of the nephron by means of an analysis of events at the late proximal tubule, mid distal tubule and ureteral urine. It is therefore important to consider first several of the underlying assumptions that have been made. It is generally implicitly assumed that the accessible or superficial cortical nephrons are representative of the entire nephron population, i.e. the nephron mass is homogeneous. Recently, evidence has been presented in the rat by Horster and Thurau (1968) for the functional separation of two distinct groups of nephrons: cortical and juxtamedullary. They observed that juxtamedullary nephron filtration rates were significantly greater than in cortical nephrons in rats fed a low salt diet. The opposite situation, however, prevailed in rats fed a high salt diet. By comparing individual nephron filtration rates with the filtration rate for the entire kidney, they were able to estimate that cortical type nephrons accounted for over 75% of the nephron mass. It is not yet clear whether this difference is apparent or significant on a normal dietary salt intake. Therefore, even if it were

possible to extrapolate this data to the dog, it is unlikely that estimates based on superficial nephron function would be in large error.[1]

The remaining assumptions that have been made relate to the evaluation of fractional reabsorption at the two sites not directly sampled, i.e. the loop of Henle and the collecting system. In order to do this, it has been assumed that under normal hydropenic conditions fractional reabsorption in the late proximal tubule is approximately 50% of the filtrate. This value is derived both from the fact that TF/P inulin ratios rarely exceed 2.0 despite deliberate attempts to sample the most distal portion of the accessible proximal tubule, as well as from similar results of experiments where microdissection was used to localize the site of puncture (Bennett, Clapp & Berliner, 1967). It is generally thought that only a small amount of filtrate is reabsorbed from the pars recta. Burg and Orloff (1968), moreover, have shown in rabbit kidney tubules that fluid transport in the pars recta is only approximately a third as great as in the pars convoluta. Finally, it was also assumed that the fractional amount of water and sodium reabsorbed along the course of the distal convoluted tubule is small and of such a magnitude that it may be ignored in comparison with the much larger effects that occurred proximal to and beyond the distal tubule. Bennett, Clapp and Berliner

[1]See Footnote p. 106.

(1967) have shown that the distal convoluted tubule of the dog reabsorbs only a small proportion of the filtered water (∿8%) and sodium (∿3%) under hydropenic conditions. Therefore using the mean value of all distal samples will overestimate loop changes to the slight extent that fractional reabsorption takes place in the pars recta and early distal tubule.

The small extent of proximal inhibition that was observed with hypertonic mannitol in this study is of considerable interest. The decrease in fractional water reabsorption, though significant, was much less than that seen when extracellular fluid volume was expanded by saline infusions. It is also apparent that the fractional inhibition within the proximal tubule after mannitol loading was less than the increased fractional excretion of sodium and water in the urine, in contrast to the effects of saline loading where far less of the proximal rejection fraction appears in the final urine. This difference speaks strongly for the role of nephron segments beyond the proximal tubule in determining over-all diuretic effects. It is evident that expressing the results of mannitol in terms of fractional changes masks the large decrease in absolute sodium reabsorption that did occur, since there was a 30% decrease in the filtered sodium load. Koch et al. (1967) have demonstrated in the rat that the reduction in filtration rate during a mannitol diuresis results from an

increase in the intratubular pressure which leads to a fall in effective filtration pressure. The drop in filtration rate thus produced may thereby limit the decrease in fractional reabsorption that might otherwise have occurred.

It is reasonable to ascribe the inhibition of salt and water transport within the proximal tubule to the osmotic effects of mannitol within the tubular lumen, as originally suggested by Wesson and Anslow (1948). The small extent of the transtubular sodium gradient that developed, due to the retention of relatively nonreabsorbable solute, may initially seem surprising in view of the previously cited studies in the rat (Windhager and Giebisch, 1961; Ullrich et al., 1963). However, it is likely that this results from the smaller degree of fractional reabsorption normally found in the dog proximal tubule than in the rat. If one assumes that mannitol contributed 70 mOsm/kg H_2O to the total plasma osmolality and a mid-proximal TF/P inulin ratio of 1.45, then the maximum transtubular mannitol gradient would be 31 mOsm/kg H_2O. The small but finite permeability of the tubular epithelium to mannitol might reduce this gradient to 20-25 mOsm/kg H_2O. The maximum transtubular sodium gradient that could be created while maintaining isosmotic conditions across the tubule would be 10-12 mEq/liter which is in agreement with the observed gradient (10.4 mEq/liter).

The possibility that retrograde collection of tubule fluid occurred during the diuretic phase due to high intratubular pressure should be considered. This would contaminate tubule fluid samples with fluid downstream to the collecting pipette and thus result in a smaller reduction of fractional reabsorption at the point of collection. The internal consistency of the inulin and sodium ratios referred to above, together with the fact that TF/P inulin ratios fell in almost all instances, makes this improbable. While the proximal inhibition could clearly be ascribed to the osmotic effects of mannitol within the tubular lumen, it is also possible that additional factors related to the pronounced expansion of extracellular volume occasioned by the mannitol infusions may also play inhibitory roles in view of the profound effects of volume expansion on proximal reabsorption (Dirks, Cirksena and Berliner, 1965; Cortney et al., 1965; Hayslett, Kashgarian and Epstein, 1967; Landwehr, Klose and Giebisch, 1967; Rector et al. 1967; Watson 1966).

Analysis of the distal inulin data indicated that a profound depression of water reabsorption occurs between the late proximal and distal tubule during mannitol diuresis. During hydropenia, approximately 25% of the filtrate is reabsorbed between these two sites. As yet it is not possible in the dog to estimate directly the extent of fractional reabsorption within each limb of the loop of Henle, whereas evidence from the rat has been

conflicting (Horster and Thurau, 1968; Jamison, 1969). In view of the lesser permeability to water in the ascending limb, it is unlikely that a large part of loop water reabsorption occurs there. During the hypertonic mannitol diuresis, virtually little or no water was reabsorbed beyond the late proximal tubule (mean fractional rejection of water in the distal tubule was 55%). This result may be ascribed to both the osmotic effect of mannitol within the lumen of the descending limb and to dissipation of the medullary osmotic gradient (Malvin and Wilde 1959; Goodman and Levitin 1964; Goldberg and Ramirez 1967).

The distal electrolyte data indicate that sodium reabsorption within the loop was also markedly retarded. It is of note that TF/P sodium ratios were significantly higher after mannitol, compared to their hydropenic values. Distal sodium ratios under normal hydropenic conditions are probably close to the value of the limiting gradient (Bennett, Clapp and Berliner, 1967). The addition of non-reabsorbable solute should therefore, of itself, produce no change in this electrolyte ratio or lower the ratio if initially higher than the limiting gradient. The latter situation appears to occur in the rat, where early distal sodium ratios are lower after mannitol loading than under hydropenic conditions (Windhager and Giebisch, 1961; Ullrich et al., 1963). This difference suggests a greater impermeability to water of

the dog distal tubule than that of the rat. This is also supported by the finding that tubule fluid remains hypotonic throughout the distal tubule of the dog (Bennett, Clapp and Berliner, 1967). The fact that sodium ratios were actually significantly higher after mannitol loading in the dog indicates an additional effect of mannitol infusions on sodium transport apart from its osmotic action within the tubule lumen.

The relevance of the present micropuncture study to such clearance studies of mannitol infusions should be considered. A number of studies in the dog and man (Goldberg and Ramirez 1967; Early, Kahn and Orloff 1961; Goldsmith et al. 1961; Porush and Abramson 1965; Stein et al. 1967; Raisz, Au and Scheer 1959; Zak, Brun and Smith 1954; Porusch et al. 1961; Goldstein et al. 1961) have shown that large increases in both free water clearance (CH_2O) and reabsorption (TCH_2O) occur during mannitol administration, and such data have been used to infer that increased sodium reabsorption occurs within the loop of Henle in response to a large increase in delivery of filtrate from the proximal tubule. These studies, therefore, appear to be at variance with the present data which indicate that the predominant site of inhibition is located within the loop of Henle. It is certainly possible that differences in juxtamedullary and cortical nephrons exist which could account for discrepancies when analysis of cortical nephrons is applied to over-all kidney clearance measurements.

There are a number of reasons, however, which support the view that this conflict is probably more one of interpretation than a reflection of a real difference in experimental results. It must be noted that the experimental conditions of free water clearance studies are totally different from those of the present study and therefore extrapolation to such studies is unwarranted. Even so, these data are not inconsistent with large increases in free water excretion, since the major inhibitory effects are exerted before the diluting segment. A large increase in load to the ascending limb, as a result of inhibition of both proximal tubule and descending limb, will readily lead to large increases in free water excretion despite a small limitation of the transtubular sodium gradient.

The conditions of the present study more closely resemble those used for TcH_2O studies. Free water reabsorption, as conventionally measured (osmolar clearance minus urine volume) is a valid estimate of the amount of water reabsorbed in the collecting system, and thus an indirect assessment of sodium transport in the loop, only in so far as two conditions are met: (a) tubule fluid reaching the collecting duct is isosmotic and (b) osmolar reabsorption in the collecting duct is negligible. Neither of these conditions, however, prevail in the dog. Clapp and Robinson (1966) have shown that tubule fluid remains uniformly hypotonic to plasma throughout the length of the distal convoluted tubule, and this

was subsequently confirmed by Bennett, Brenner, and Berliner in the Rhesus monkey (1968). Moreover, the studies of Bennett, Clapp and Berliner (1967) as well as the present study, have indicated that a considerable fraction of the filtered sodium load is reabsorbed in the collecting duct. Both these factors lead to a gross underestimate of collecting duct reabsorption of water by the TcH_2O formula. During osmotic diuresis, it is likely that the errors thus introduced become less as tubule fluid osmolality rises and the fraction of the filtered solute entering the collecting duct that is reabsorbed there decreases. Measured TcH_2O may thus rise appreciably even though absolute volume reabsorbed may not have changed or even decreased. Certainly, on the basis of present micropuncture evidence in the dog, it is hazarous to draw any definite conclusions about the function of the loop of Henle from measured changes in TcH_2O. The present data, which suggest that large inhibitory effects occur within the loop, moreover, seem more consistent with the finding that strong osmotic diuresis leads to almost total dissipation of the osmotic gradient in the papilla (Malvin and Wilde, 1959; Goodman and Levitin, 1964; Goldberg and Ramirez, 1967; Atherton, Hai and Thomas, 1968). Clearly, further studies specifically designed to correlate intratubular events with over-all measurements of CH_2O or TcH_2O under the appropriate experimental conditions are needed.

Turning to the results of the saline infusion experiments, large reductions in fractional fluid reabsorption were seen in the proximal tubule after isotonic saline infusions, which agrees with earlier published work in the dog (Dirks, Cirksena and Berliner, 1965) and in the rat (Cortney et al. 1965; Landwehr, Klose and Giebisch, 1967; Rector et al., 1967).

The administration of furosemide, in addition to an isotonic saline load, led to no further inhibition of proximal reabsorption. Despite the fact that the absolute rate of sodium reabsorption is inhibited in the dog and rat when measured by the shrinking drop technique and by single nephron microperfusion experiments (Morgan et al., 1970; Rector et al., 1966, 1967; Brenner et al., 1969; Knox et al., 1969), several observers have failed to show any significant action of furosemide on fractional reabsorption in the proximal tubule of the dog (Dirks, Cirksena and Berliner, 1966; Knox et al., 1969), or in the Rhesus monkey (Bennett, Brenner and Berliner, 1968), whereas evidence in the rat has been more conflicting (Brenner et al., 1969; Deetjen, 1966; Rector et al., 1966, 1967; Meng 1967; Malnic et al. 1969). It is not surprising, therefore, in these experiments that furosemide had no further effect on the proximal tubule, particularly after a saline load which had already inhibited fractional reabsorption in this segment.

The response of the loop of Henle to the increase in load resulting from proximal inhibition was of major interest in this study. The distal micropuncture data after saline loading indicated that a large part of the extra load delivered from the proximal tubule after saline infusion had been reabsorbed in the intervening segment, which is presumed to reflect largely the activity of the loop of Henle. This view confirms the more indirect studies of loop transport by clearance techniques (Buckalew, Ramirez and Goldberg 1967; Eknoyan et al., 1967; Goldberg, McCurdy and Ramirez 1965; Goldberg and Ramirez 1967; Gottschalk and Mylle 1959; and Porusch et al., 1961) and also agrees with micropuncture experiments in the rat which also slow increased sodium reabsorption in the loop following volume expansion (Cortney et al. 1965; Giebisch, Klose and Windhager 1964; Landwehr, Klose and Giebisch, 1967; Lassiter, Mylle and Gottaschalk, 1964). This ability of the loop of Henle to increase its rate of reabsorption in response to increases in delivery has the effect of buffering the distal nephron from large changes in end proximal load. The same phenomenon can also be demonstrated when single loops are perfused from a late proximal tubule and the effluent collected from an early distal puncture site in the same nephron (Morgan and Berliner 1969). This demonstrated that the response of the loop is not necessarily dependent on alteration of any other factors, intrinsic or extrinsic to the kidney, than load entering the loop. This is in contrast to the proximal tubule ,

where load-dependent changes in reabsorption are not seen in the absence of changes in the extra-tubular environment (Burg and Orloff, 1968). The greater part of water reabsorption within the loop clearly must occur within the descending limb as a result of osmotic equilibration with the hypertonic medullary interstitium. Because of the high permeability to water in this segment, an increase in the load to the loop will result in a correspondingly higher absolute reabsorption as long as equilibration of descending limb fluid takes place. In contrast to water handling, all sodium reabsorption within the loop must occur within the ascending limb of the loop for obvious reasons. Sodium reabsorption presumably occurs throughout the length of the ascending limb and continues until the intraluminal sodium concentration is reduced to the point at which passive back leak equals the rate of active efflux. In the antidiuretic state, the TF/P sodium concentration ratio within the fluid leaving the ascending limb is at or close to the value of the limiting concentration ratio. This can probably account for the ability of the loop to increase the absolute rate of sodium reabsorption at higher loads, since as load and flow rate are raised the limiting concentration gradient in the ascending limb is not reached. The sodium concentration in the fluid leaving the ascending limb therefore rises and thus permits absolute reabsorption to continue. When flow rates are reduced below normal, the rate of absolute sodium reabsorption exhibits a corresponding reduction

which paradoxically is accompanied by a small elevation in the intraluminal sodium concentration in the fluid emerging from the ascending limb (Morgan and Berliner 1969). The explanation for this phenomenon is not apparent at the present time. It is possible that changes in intratubular pressure or possibly increased entry of sodium into the descending limb at low flow rates, thereby giving an erroneous estimation of sodium reabsorption from analysis of early distal fluid, are responsible.

The distal TF/P sodium ratios were found to be consistently significantly higher than their paired hydropenic controls after isotonic saline administration similar to the results after mannitol infusions. From these results alone it is not possible to say whether this change represented a flow-dependent phenomenon as seen in microperfusion experiments (discussed above) or whether in fact saline loading resulted in a limitation of the distal tubule equilibrium concentration ratio. The mannitol results suggest the latter as discussed earlier. Landwehr, Klose and Giebisch (1967) measured the distal sodium gradient using stationary microperfusion techniques in saline loaded rats and considered their value to lie within the normal range. However, this comparison suffers from a lack of precise controls and their mean value is higher than the figure reported for either the early or late distal tubule by Malnic, Klose and Giebisch (1966). Direct measurement of the limiting concentration gradient for sodium in the dog distal tubule

will obviously be needed under these various experimental conditions to determine whether in fact the higher free flow sodium ratios reflect a limitation of active sodium efflux or perhaps increased passive influx of sodium.

It is clear that furosemide exerted its predominant effects within the loop of Henle, in agreement with previously published work (Bennett, Clapp and Berliner, 1967; Meng, 1967; Bennett, Brenner and Berliner, 1968; Clapp and Robinson, 1968). Despite almost complete loss of the distal sodium gradient, however, sodium and water reabsorption in the loop were not totally abolished. The present study also confirms earlier observations by Bennett, Clapp and Berliner (1967) and Bennett, Brenner and Berliner (1968) that TF/P potassium ratios in the distal tubule generally exceed unity after administration of furosemide. This may have resulted from either an increased transtubular electrical potential or from an inhibition of active potassium transport at the luminal membrane. It is possible that the high intratubular concentration of sodium might depolarize the luminal membrane, thereby increasing the transepithelial electro potential difference and hence the passive influx of potassium into the lumen (Giebisch, Klose and Malnic, 1967). Bennett and Clapp and Berliner (1967) have considered this unlikely since sodium reabsorption is also inhibited, and favor the view that furosemide inhibits active luminal potassium transport. Moreover this hypothesis is supported by the observation of Malnic et al. (1969) that distal transtubular potentials were not significantly altered by furosemide in the rat.

The estimates of reabsorption in the late distal tubule and collecting duct under the various experimental conditions studied suggest a limited capacity for sodium reabsorption in this segment, since most of the excess load delivered to the distal tubule was excreted in the final urine. This, too, agrees with previous estimates of collecting duct function from micropuncture studies in the rat (Cortney et al. 1965; Giebisch, Klose and Windhager 1964; Landwehr, Klose and Giebisch 1967; Landwehr et al. 1968; Lassiter, Mylle and Gottschalk 1964). The additional effect on water reabsorption seen after furosemide in this segment is readily attributable to dissipation of the hypertonic medullary gradient due to its primary effect on sodium transport in the ascending limb of Henle's loop.

In conclusion these studies have attempted to dissect out the contribution of various nephron segments to the net diuretic response under three different conditions a) an osmotic diuresis evoked by hypertonic mannitol infusions, b) extracellular volume expansion and c) the administration of the potent diuretic furosemide. The mechanisms whereby each of these experimental conditions inhibits tubular sodium reabsorption are undoubtedly quite different, nevertheless all studies alike emphasize the role of the loop of Henle in establishing the magnitude of diuretic effects. It is apparent from the saline study that inhibition of the proximal tubule alone is insufficient in itself to cause large diuretic effects since the loop is capable

of large increases in salt and water reabsorption when the entering load is increased. Moreover since the distal nephron, beyond the loop is incapable of increasing the rate of reabsorption to a great extent, then the increments in non reabsorbed salt and water reaching this segment will largely determine the extent of the diuresis. For the reasons given above this is primarily determined by the extent of inhibition established in the loop itself. It is also apparent that inhibition of sodium transport in the loop of Henle will necessarily affect the extent of passive water reabsorption in the collecting duct by virtue of a decrease in the hypertonicity of the renal medullary interstitium. This was particularly marked with furosemide since this agent almost totally abolished hypertonic sodium transport within the ascending limb.

INTRODUCTION

Electrophysiological studies of the renal tubule have only recently attracted much attention by renal physiologists but have provided a powerful tool for the study of epithelial transport processes which give information which cannot be easily obtained with other techniques. Such methods are not only essential to a precise assessment of the total electrochemical driving force for ion transport, but can also provide estimates of the relative permeabilities to various ions across cell membranes. Moreover, an analysis of the electrical characteristics of the epithelium has given new insights into the mechanisms of salt and water transport in the renal tubule.

Initially such studies focused on the existence and magnitude of electrical potential differences across various segments of the nephron. It is obvious that a knowledge of the potential difference is crucial to any analysis of the driving forces acting on a particular charged ion species and helps to distinguish active from passive transport processes. Beginning with the initial studies of Wilbrandt (1938), a large number of investigators subsequently reported the finding that the luminal side of the nephron was invariably negative with respect to the outside or peritubular surface. Many groups found values of approximately -20 mV in the proximal tubule of rat (Bank 1962; Bloomer, Rector and Seldin 1963; Clapp, Rector and Seldin 1962; Kashgarian et al. 1963;

Malnic, Klose and Giebisch, 1964, 1966; Marsh and Solomon 1964; Solomon 1957; Vieira and Malnic 1968) and Necturus (Giebisch 1958, 1961; Whittembury 1963; Whittembury and Windhager 1961). It was thought that this figure was likely to underestimate the true value since the possibility of damage by the recording electrode could not be excluded (see Windhager and Giebisch 1961). It was subsequently shown by Frömter and his associates (1966, 1967) that in the proximal tubule of the rat no potential difference could be detected when rigorous methods were used to prove the intraluminal localization of the recording electrode. They demonstrated that although insertion of a microelectrode into a proximal tubule initially might give a large negative potential, ejection of fluid from the tip of the electrode, thereby ensuring that the electrode was actually within the tubule lumen, led to disappearance of the previously observed potential. The implication of their work was that previous measurements had been artifactual and arose from the tip of the electrode being still in contact with the cell lining the tubule. Maude (1969) has also subsequently reported the absence of a transepithelial potential in proximal tubules of rat kidney slices.

There had previously been only one study in a second mammalian species, the dog, that dealt with the measurements of electrical potential differences. In 1964 Watson, Clapp and Berliner reported

a mean value of -22 mV in the dog proximal tubule which was then in agreement with values found by others in the rat. The paucity of any other electrophysiologic data in the dog and the doubt thrown on the validity of these data by the subsequent work of Frömter et al. therefore led to the present studies, which were initially designed to investigate the existence of an electrical potential difference in this species. Since this work of Frömter et al. (1966, 1967) was reported the finding of a significant negative potential (-15 mV) across the proximal tubules of Necturus has been reconfirmed (Boulpaep 1967). Although initial studies in the in vitro perfused rabbit proximal tubule indicated no potential (Burg et al. 1968), improved insulation techniques subsequently made it possible to demonstrate the existence of a small negative potential (-4mV) (Burg 1969). De Mello and Malnic (1970) have recently reported similar values in the rat proximal tubule. No further independent studies have yet been reported in the rat.

These present studies also afforded an opportunity to examine several other aspects related to the electrical properties of the proximal tubule which had never previously been investigated in the dog kidney. These dealt principally with an assessment of relative ionic permeabilities across the proximal tubule and the measurement of transepithelial resistance under a variety of experimental conditions. Measurement of the electrical resistance

of the rat proximal tubule was first reported by Hegel, Frömter and Wick (1967), although preliminary work on cable properties of the rat proximal tubule had previously been reported by Windhager and Giebisch (1961). They have subsequently reported on the behaviour of the transepithelial resistance during osmotically induced bulk flow only in abstract publication. These authors however did not even attempt to establish or speculate as to the nature of the resistance paths across the tubule or the role such paths might play in regulation of salt and water transport.

Ussing and Windhager in an extremely important paper in 1964 had suggested on the basis of resistance measurements in the frog skin, the existence of parallel extracellular "shunt" paths across the epithelium in addition to a purely transcellular route. Several years previously theoretical considerations arising from studies of transport across intestinal epithelia had led Curran (1960) to propose the existence of a third space within such epithelia and the morphological counterpart was later assigned to the intracellular channels chiefly as a result of the work of Diamond (1962) in gall bladder. These concepts were subsequently incorporated into the schematic framework of renal tubule transport largely by the work of Windhager, Boulpaep and Giebisch (1966). These authors first reported to the third International Congress of Physiology in 1966 that large changes in the transepithelial resistance occurred

in Necturus under conditions of varying osmolality, whereas transmembrane resistances were not significantly altered. The estimated value for the combined resistance of the two cell membranes in series, luminal and peritubular, was far less than that found across the entire epithelium. Boulpaep (1967) subsequently proposed an equivalent circuit for the proximal tubule cell incorporating an extracellular shunt resistance based on interaction phenomena between luminal and peritubular membrane following changes in ionic concentrations bathing one or other membrane. Boulpaep has also subsequently (1969) shown that changes in the extracellular shunt resistance may play an important role in the regulation of net rate of sodium and water transport in the proximal tubule, since increases in shunt conductance were observed after saline loading in Necturus while net sodium transport was depressed. He then proposed that the shunt path might modulate net rate of transport via changes in the passive rate of back leak from interstitium to lumen. It was therefore of importance to extend resistance measurements to another mammalian kidney and to attempt to relate measurements of transepithelial resistance to the behaviour of the extracellular shunt path.

Initial experiments in the dog encountered the considerable problem of pulsatile and respiratory movements of the kidney surface, which had previously been commented upon by Watson, Clapp and

Berliner (1964). It was therefore necessary to develop and employ a system of isolated isobaric auto-perfusion of the dog kidney which abolished all pulsatile movements.

Isolated perfused kidney preparations have attracted the attention of numerous investigators in the past, particularly because of the ease of direct experimental approach they offer to problems of hemodynamic interest and to the effects of changes in perfusate composition. Despite intensive recent work in this area, pump-perfused kidneys still show significant functional impairment within several hours of perfusion (Bahlmann et al. 1967; Berkowitz, Miller and Itskovitz 1967; Nizet et al. 1967; Rosenfeld, Sellers and Katz 1959; Waugh and Kubo 1969). The necessity of using such a system of perfusing the isolated dog kidney arose from the fact that pulsatile and respiratory movements of the kidney in this species make it extremely difficult, if not impossible, to apply certain micropuncture and electrophysiological techniques to the study of surface nephrons. Since the excursion at the surface with each pulse is often as much as several tubule diameters and the proximal tubule epithelium offers little resistance to the small tip size of glass microelectrodes ($<1.0 \times 10^{-4}$cm), it is obviously impossible to maintain a microelectrode in a stable position for any length of time. This becomes even more difficult when attempting to place and maintain a combination of two or

more microelectrodes and micropipettes in adjacent areas of the kidney at the same time. Single micropipettes that are used for tubule fluid collection are less of a problem, since their larger tip size (5-8 10^{-4} cm) presents greater resistance to displacement and aids in stabilizing the punctured tubule. An auto-perfused kidney preparation was therefore used by which the pulse pressure in the arterial flow to the perfused kidney could be totally abolished.

Auto-perfusion offers a number of advantages over a pump perfused preparation and moreover provides that control observations may be made from the non auto-perfused intact kidney for a functional comparison. Although somewhat similar preparations have been used previously, functional studies in such kidneys have generally been lacking or incomplete (Goodyer and Glenn 1951; Hardin, Scott and Haddy 1960; Langston, Guyton and Gillespie 1959). Furthermore such systems of auto-perfused kidneys have not previously been used for micropuncture studies. It is apparent that adequate functional assessment is an important adjunct to the electrophysiological investigations on the tubules of such kidneys. The results support the view that renal function is well maintained for a considerable period of time (≈ 5 hr) after onset of perfusion.

METHODS

Acute experiments were performed on mongrel dogs ranging in weight from 12 to 26 kg. Food was withheld for 16 to 20 hr but free access to water was permitted before each experiment. Animals were anesthetized with Pentobarbital (30 mg/kg), and supplementary doses of 1 to 2 ml Thiopental (25 mg/ml) were administered intravenously as required, to maintain satisfactory levels of anesthesia. A cuffed endotracheal tube was inserted and the animals were allowed to breathe room air spontaneously. Polyethylene catheters were placed into a femoral vein for blood withdrawal, into a femoral artery for measurement of mean arterial pressure and into a foreleg vein for intravenous infusion. The left common carotid artery was catheterized by a stainless steel cannula (4.5 mm id, 5 mm od), which was attached to Tygon tubing (4.5 mm id, 6.5 mm od). The same Tygon tubing was used to catheterize the left external jugular vein directly. A polyethylene catheter was advanced up the ureter into the pelvis of the right kidney via a suprapubic incision. The left kidney was then exposed by a flank incision and stripped of its perirenal fat. The hilar vessels were carefully dissected as far proximally as possible and the left ureter catheterized with polyethylene tubing.

Hemostasis was secured by careful electrocoagulation of all bleeding points. Heparin was administered intravenously in a dose of 400 units/kg body weight along with 200 ml of Tyrode solution

(composition in mM: Na 149.1, Cl 144.2, HCO_3 11.9, H_2PO_4 0.3, K 2.7, Ca 1.8, and Mg 0.5) prior to onset of perfusion, and 100 units Heparin/kg body weight was given by IV route each succeeding hour.

Method of autoperfusion: The system of auto-perfusion is illustrated in Fig. B.1. The chamber and tubing were siliconized prior to use and primed with Tyrode solution. Blood flow from the left common carotid catheter was passed through a glass depulsator chamber, kept at 37 C by a continuous flow of water from a constant temperature bath through an outer jacket. The volume of blood in the chamber was kept between 30 - 60 cc during perfusion while the total volume of blood in the entire circuit was approximately 150 cc. The chamber communicated with a large reservoir of air (1 liter). The pressure in this circuit was recorded on a mercury manometer and could easily be changed so as to keep it close to the level of the mean arterial pressure, which was continuously recorded from the femoral artery by a Statham strain gauge (P 23AA) connected to a Gilson MP5 recorder. A small vibrostaltic pump (CRC) was used occasionally to boost perfusion pressure above 90 mm Hg when systemic pressure had fallen below this level.

Catheterization of the renal vessels usually required less than 30 sec for each vessel. The renal artery was transected between two ligatures, which were tied as close as possible to

its origin. The artery could then be exteriorized and catheterized by a stainless steel cannula (3.5 mm id, 4 mm od) connected to the outflow of the depulsator chamber by a short length of Tygon tubing. This proved easier and quicker than attempting to catheterize the artery in situ. In several experiments it was impossible to catheterize the main renal artery because of its bifurcation close to the aorta. In these instances it was possible to catheterize the aorta below the level of the left renal artery and then ligate and remove the involved portion of the aorta, which proved equally satisfactory. This had the advantage of a shorter ischemic time but occasionally led to the loss of the right kidney. The renal vein was catheterized using Tygon tubing (4.5 mm id, 6 mm od) and was transected directly in situ. Renal venous blood was returned to the left external jugular through a rotameter (Fischer and Porter Flowrator tube 2F-1/4-16-5/36), which allowed continuous measurement of renal blood flow (R.B.F.). A T tube in the return circuit permitted direct measurement of timed venous effluent, and was used to calibrate the flowmeter. Total ischemic time for the perfused kidney was invariably one minute or less. No transfusion was given to compensate for the loss of the blood volume in the circuit. The fall in temperature in the complete circuit was estimated from the difference between body temperature and venous effluent temperature; it was found to average 1 to 1.5 C.

The kidney, freed of its attachement to the abdominal cavity, was then placed in a lucite chamber measuring 10 cm by 5 cm by 5 cm. Part of one side and the bottom of the chamber could be removed to facilitate placement of the kidney and allowed the pedicle to be brought out through an opening in the bottom. The chamber was placed about 10 cm above the heart level of the animal and level with the depulsator chamber. A small area on the upper surface of the kidney (≈ 1 cm^2) was stripped of its capsule and Tyrode-agar (1.5%) solution was poured, after boiling and then cooling to 38 C, around the kidney so as to form a shallow well surrounding the decapsulated area. The agar gel afforded thermal insulation as well as providing mechanical stability for the kidney. Tyrode solution heated to 37 C was continuously dripped on to the kidney surface within the agar well and a constant level maintained <1 mm above the kidney by means of continuous suction through a small pipette. It was thus possible to keep the microscopic field relatively free of any blood which tended to ooze from the decapsulated area, as well as to allow for rapid changes to be made in the composition of the bathing solution. In experiments in which tubule fluid samples were collected by micropuncture, heavy mineral oil was allowed to drip onto the kidney surface and to run off freely without the constraints of the agar well. The surface of the kidney was illuminated by means of a fiber-optic light source (Dolan Jenner).

In experiments in which clearances were measured a priming infusion of 4 ml/kg of a 5% solution of inulin in physiologic saline (0.9%) was then given, followed by a sustaining infusion of 4.8 ml/kg/hr; two to four consecutive clearance periods of 10 to 15 min were performed at varying intervals after onset of perfusion in 11 dogs. No clearances were performed after more than four hours of perfusion; the majority were performed during the second and third hour after onset of perfusion.

Transit times were measured by injection of 0.3 ml of 5% Lissamine green dye solution (in 5 mM phosphate buffer brought to pH 7.4 by addition of NaOH) into the arterial circuit close the renal artery. Proximal transit time was taken to be the time from the initial vascular flush of dye to the point when the last wave of dye had cleared all the proximal convolutions in the area under observation. The first appearance of dye in distal convolutions was also recorded. Tubule radii (r) were measured by ocular micrometer. Free flow micropuncture collections were performed in three experiments for the purposes of measuring fractional water and chloride reabsorption from randomly selected proximal convoluted tubules. Collections were made spontaneously after the distal deposition of a stained mineral oil droplet to prevent contamination from retrograde flow. All samples were carefully checked, and rejected if they contained any red blood cells. Tubule fluid inulin concentrations (TF_{inulin}) were measured in duplicate by the method of Vurek and Pegram (1966)

modified to increase heating time to 10 min, and tubule fluid chloride (TF_{Cl}) and plasma chloride $(Cl)_p$ concentrations were measured in triplicate by the second electrometric method of Ramsay, Brown and Croghan (1955). Plasma and urine inulin concentrations were determined by a modification of the fluorometric method. Urine was diluted to bring inulin concentrations into the appropriate range. Plasma proteins were precipitated with 3% TCA and the supernatant analyzed for inulin. Plasma and urine blanks failed to show any fluorescence with the Dimedone reagent. Plasma chloride and inulin values were routinely corrected for 94% water concentration. Urine was periodically collected from both kidneys throughout each experiment and was analyzed for total osmolality (Precision osmometer, model 2007) and sodium and potassium concentrations by flame photometer (Instrumentation Laboratories model 143). Hemotocrit (Hct) was measured by microhematocrit tubes. Renal plasma flow (TPF) was calculated from the equation: RPF = RBF (1 - Hct).

Reabsorptive capacity (K) and net rate of water flux (J_{H_2O}) were assessed from the following equations (Gertz et al. 1965):

$$K = \frac{\ln TF/P \text{ inulin}}{\text{transit time}} \qquad (1)$$

$$J_{H_2O} = K \cdot \frac{r}{2} \qquad (2)$$

Chloride concentration in the reabsorbate $(Cl)_r$ was estimated from the following equation:

$$(Cl)_r = \frac{TF/P_{inulin} - TF/P_{Cl}}{TF/P_{inulin} - 1} \cdot (Cl)_p \qquad (3)$$

Plasma chloride was corrected for plasma water of 94% and a Donnan factor of 1.05 in this equation. Net chloride flux (J_{Cl}) was measured as follows:

$$J_{Cl} = J_{H_2O} \cdot (Cl)_r \qquad (4)$$

Electrophysiological Methods

Single and double barrelled microperfusion pipettes were pulled from glass pyrex tubing, 0.8 mm OD by 0.53 mm ID, and were sharpened to a tip size of 7 to 10 10-4 cm before use. Single (M_1) and double barrelled (M_2) glass microelectrodes were prepared from pyrex glass capillary tubing, 1.2 mm OD by 0.6 mm ID and 1.0 mm OD by 0.70 mm ID, respectively. The electrodes were filled with 3 M KCl according to the method of Tasaki, Polley and Orrego (1954) and stored in 3 M KCl at 4 C prior to use. The electrodes were connected to a Ag/AgCl electrode and potentials from M_1 electrodes recorded on a Keithley electrometer (model 602) connected to a Gilson recorder (model M P 5). Potentials from one barrel of an M_2 electrode were recorded on a differential amplifier (Grass P 6 - 12) connected to a Brush recorder (model mark 220) and simultaneously displayed on an oscilloscope (Tekronix 502 A). The return lead to ground was made through a Ag/AgCl electrode, which made contact with the normal Tyrode solution surrounding the kidney through a 3 M KCl agar bridge. The total asymmetry in the circuit was

never more than ±2 mV and was balanced out in the recording equipment. Any additional asymmetry introduced by the tip of the microelectrode was always checked in the bath before and after each measurement of transepithelial potential; M1 electrodes were discarded if the tip potential was greater than ±5 mV. The mean tip potential was -1.1 ±0.6 mV. The electrode resistance of M_1 electrodes averaged 27.6 ±1.6 10^6 ohm, and the tip resistance and coupling resistance of M_2 electrodes averaged 15.2 ±1.8 10^6 ohm and 32.0 ±2.6 10^3 ohm, respectively.

The composition of the solutions used is shown in Table B-1. A series of test solutions were prepared in which sodium or chloride were in large part replaced by one or other test ion. All solutions were prepared from reagent grade chemicals with the exception of cyclamate, which was obtained from Abbott labs. Sodium acetylglycinate was prepared by equimolar addition of acetylglycine and sodium hydroxide. Solutions from which both sodium and chloride were removed were made isosmotic with normal Tyrode's solution by addition of sucrose, and hypertonic solutions prepared by adding sucrose to normal Tyrode solution. No pre-equilibration with gases was undertaken.

Procedures

Randomly selected proximal convoluted tubules were used for the measurement of transepithelial electrical potential. The

technique that was employed is illustrated in Fig. B-2a. A double barrelled perfusion pipette was inserted into a proximal tubule and coloured Tyrode solution (0.05% FD&C Green, Keystone Aniline & Chemical Co.) injected to outline the surface convolutions of the same tubule. A M_1 electrode was then inserted into the lumen of the same tubule and the resting potentail recorded. At that point, perfusion of the lumen was performed with a test solution, and the resting potential accepted as being of transepithelial origin only when and if the potential changed reversibly with perfusion by the test solution. If the electrode tip had remained within a damaged cell, one would expect either no response from the M_1 electrode or the opposite response to that predicted if the solution were to leak out of the tubule and thereby affect the peritubular membrane potential. For example, perfusion with choline Tyrode's would be expected to depolarize (i.e. make more positive) the transepithelial potential, but should hyperpolarize (i.e. make more negative) the peritubular membrane potential if choline is less permeant than sodium. This procedure avoided any further manipulation of the exploring electrode that might inflict additional damage on the tubule under study (such as might be produced by the injection of concentrated KCl solution from the electrode tip). The response to the various ionic substitutions also afforded an opportunity to estimate the relative ionic

permeability coefficients of sodium and chloride to the substituent test cation or anion.

Streaming potentials in the proximal tubule were measured using the technique outlined in Fig. B-2b. Bulk flow of water into the lumen was induced by perfusion with hypertonic solutions. Reversal of flow was accomplished by perfusion of peritubular capillaries with hypertonic solution. Single barrelled pipettes were used to perfuse the peritubular capillaries and adequacy of perfusion checked by visual inspection of blanching in the area surrounding the tubule being studied.

The transepithelial specific resistance was measured by cable analysis experiments, performed according to the method illustrated in Fig. B-2c. A double barrelled perfusion pipette was inserted into a proximal tubule as in Fig. B-2a and M_2 and M_1 electrodes inserted some distance apart into the same tubule. The position of both electrodes was checked by perfusion with choline Tyrode solution. The input resistance (R_{input}) of the M_2 electrode was measured by passing hyperpolarizing current from a constant current source (square wave pulses 1 to 5 x 10^{-7} A for 1 sec duration) through one barrel and recording the voltage deflection from the second barrel after the appropriate correction for the coupling resistance in the bath has been made. The corresponding voltage deflection at M_1 was recorded and the distance measured by a filar micrometer. The M_1 electrode was then withdrawn and reinserted close to or further from the M_2 electrode and the procedure repeated.

Fig. B-2d illustrates the technique used for comparison of R_{input} when the electrolyte composition of the fluids bathing the membrane was changed. Perfusion of test solutions through a double barrelled perfusion pipette in the tubule lumen was coupled with simultaneous perfusion of the peritubular capillaries surrounding the tubule with the same solution, which thus allowed rapid changes to be made in the solution on both sides of the epithelium. The bathing solution was simultaneously changed as well during the perfusion in these experiments. R_{input} was obtained as described for Fig. 1c, but current pulses of smaller magnitude (1×10^{-7}A) were used.

Proof of the intraluminal position of the recording electrode was therefore secured by several methods. First, in all instances microperfusion with test solutions was used as described above. Second, in the cable analysis experiments, the degree of coupling between the M_2 and M_1 electrodes confirmed the intraluminal localization of both tips. If the tip of one of the electrodes had been lodged in a cell or had become plugged by cell fragments, a smaller electrotonic potential would be picked up for a given distance separating it from the current injection site. A third method of localization was provided by estimates of input resistance, since the transepithelial value of input resistance is invariably smaller than the input resistance across the peritubular cell membrane.

Distal tubules were identified by lissamine green transit time and the transepithelial potential recorded by direct puncture. No additional proof of localization was attempted or thought necessary since the p.d. was invariably much larger and considerably more stable than in the proximal tubule, often persisting without decline for periods of up to ten or more minutes. Nor was any attempt made to localize puncture site along the length of the distal tubule. Occasionally it was possible to recognize distal tubules on the surface from the short segments and their more opaque appearance.

The conductivity of certain solutions (see below) was measured with a Radiometer conductivity meter (type CDM 2d).

RESULTS

Pulsatile movement of the kidney was totally abolished by the method of perfusion that has been described and it was possible to obtain sustained recording from an intraluminally placed glass microelectrode for periods of 5 to 10 minutes. Prior attempts to stabilize the surface of kidneys prepared for in vivo micropuncture by means of an external agar gel together with a concentric ring on the surface, were inadequate to achieve a satisfactory degree of damping. The auto-perfused kidney occasionally showed slow phasic movements that varied with respiration and presumably reflected small changes in central venous pressure. Less frequently there were movements that could be correlated with ureteral peristalsis.

Experiments were terminated either because of progressive deterioration of the animal with a fall in systemic blood pressure, or because of the development after 6 to 7 hr of perfusion of small ischemic areas on the surface of the kidney.

Hemodynamic Data

Total renal blood flow to the perfused kidney is shown against elapsed perfusion time for 22 dogs in Fig. B-3. Flow is expressed in ml/min gm kidney weight. Although flow could not be measured prior to the onset of perfusion, the initial values in the first hour were consistently well within the normal range for anesthetized dogs (3-5 ml/min gm k.w.) (Kirchheim and Gross 1970; Liebau, Levine and Thurau 1968: Selkurt 1962; Waugh and Kubo 1969). There was a slow but progressive decline in total renal blood flow, however, which did not fall below the lower limits of normal until at least four hours of perfusion had elapsed. Renal vascular resistance was calculated from the renal blood flow corrected for kidney weight and the arterial pressure measured in the depulsator chamber. While this does not take into account the renal venous pressure or the pressure drop from chamber to kidney, these two factors are small and relatively constant in comparison with the perfusion pressure in the chamber. This calculation therefore allows a meaningful assessment of relative changes in the intrarenal vascular resistance. Both the perfusion pressure, measured in the chamber, and the

calculated renal vascular resistance have also been plotted in Fig. B-3. It is evident from this plot that while perfusion pressure was maintained relatively constant throughout the period of observation, resistance rose concomitantly with the fall in renal blood flow. There was a mean gradient of 19 ±3 mm Hg between the systemic arterial pressure and that of the depulsator chamber. In two experiments the gradient was over 50 mm Hg, which undoubtedly resulted from partial obstruction of the arterial catheter. If these two observations are excluded the mean gradient was 15 mmHg. This is in part due to the hydrostatic pressure difference between animal and chamber and in part due to frictional losses in the circuit. The average hematocrit for all animals was 44.8 ±1 and did not change significantly or in any consistent direction throughout the duration of these experiments.

Excretory Data

Sequential urine collections from the two kidneys for 22 dogs were performed and analyzed for osmolarity and electrolyte concentrations. Since there was no consistent trend for the urine volume or osmolarity to change with time, these results have been expressed as a pooled average for each kidney for all animals and are summarized in Table B-2. None of the differences between perfused and non-perfused kidneys were significant, although there

was considerable variation between animals. Urine volume, sodium and potassium concentration all tended to be slightly lower on the perfused side but there is considerable overlap in the two groups. The urine osmolarity tended to be consistently greater than that of plasma throughout the experiments.

Inulin clearances were performed in 11 of these animals and the results are shown in detail with the corresponding hemodynamic data in Table B-3. Filtration rate for the perfused kidney was invariably lower than the non-perfused kidney and averaged 22.6 ml/min compared to 30.9 ml/min for the control kidney ($p < 0.01$). Filtration rate for the perfused kidney expressed per gm kidney weight averaged 0.47 ml/min.gm k.w., which was at the lower limits for normal dogs (Smith 1951). There was no significant correlation of filtration rate with time within the first 200 minutes. Urine volume was reduced to approximately the same extent of the autoperfused side but failed to reach significance. Fractional water excretion was thus comparable in the two groups and averaged 2.19% for the auto-perfused kidney and 2.43% for the control kidney. Urine to plasma sodium concentration ratios were also significantly lower in the auto-perfused kidney than the control kidney. Fractional sodium excretion averaged 1.37% from the auto-perfused kidney and was not significantly lower than the non auto-perfused side (1.58%). Mean pressure in the depulsator chamber for these animals

was 109 mmHg which was significantly lower than the mean arterial pressure by 13 mm Hg. Renal blood flow to the auto-perfused kidney averaged approximately 200 ml/min. Calculated filtration fraction was 0.24 for the auto-perfused kidney which reflects the fact that filtration rate was depressed to a greater extent than renal blood flow.

Micropuncture Data

The microscopic appearance of the kidney surface was uniformly healthy following onset of auto-perfusion and remained so at times for up to 6 to 7 hr of perfusion until deterioration of the animal's condition precluded further experimentation. Peritubular capillary blood flow was vigorous, and although oozing of blood from surface capillaries was encountered this could generally be flushed away from the surface by the continuous flow of Tyrode's solution through the agar well.

Proximal transit times were measured in 13 experiments and averaged 31.1 sec. In three experiments the transit time was markedly prolonged for no apparent reason (46, 56, and 57 secs), while the remaining transit times were 33 sec or less. These values are clearly prolonged in comparison to transit times measured in the *in vivo* kidney. In four animals prepared for *in vivo*

micropuncture, a mean value of 18.2 ±1.9 sec was obtained using identical criteria.

Transit times to the early distal tubule averaged 79.8 ±5 sec compared with 48 ±3.3 sec in the in vivo kidney. This difference is more than can be accounted for by the prolongation of the proximal transit time and indicates slower mean velocity of flow in the loops of the surface nephrons. Proximal tubule diameters were measured in 68 instances during four experiments and averaged $34.8 \pm 0.9 \times 10^{-4}$ cm.

Fractional water and chloride reabsorption were assessed by micropuncture of randomly selected proximal tubules and the results are summarized in Table B-4. The mean TF/P inulin ratio from 26 tubules (3 dogs) was 1.61 ±0.07 and ranged from 1.22 to 2.69. The mean fractional reabsorption of filtered water was 35.2%, which is very similar to micropuncture results for the in vivo kidney (Part A). In 20 of these samples (2 dogs) TF/P chloride ratios were also measured and averaged 1.13 ±0.015 (range 1.03 to 1.31). This value is significantly greater than unity and is reflected in the fact that fractional chloride reabsorption (32.6%) was significantly less than fractional water reabsorption (36.9%) in the corresponding samples ($p < 0.001$). It will be noted that the TF/P chloride value given in Table B-4 has not been corrected for a Donnan distribution. The reason for this is the uncertainty

of the estimation of the chloride concentration in the interstitial fluid bathing the peritubular side of the epithelial cell. This uncertainty is the result of a lack of knowledge of a) the concentration of chloride in the peritubular capillary blood, and b) the protein concentrations in the peritubular capillary blood and the peritubular interstitial fluid. Since bicarbonate appears to be preferentially reabsorbed in comparison to chloride, as evidenced by the fact that TF/P chloride ratios are greater than unity, this will have the effect of reducing the chloride concentration in the peritubular interstitial fluid compared to that in the plasma. Moreover since there is a small concentration of protein in the interstitial fluid, which may range anywhere between 30 to 80% of the plasma protein concentration (Henry, Keyl and Bell 1969; Lebrie 1968; Vogel, Ulbrich and Gärtner 1969), it is clearly unwarranted to correct the plasma chloride value for the full Donnan effect of the plasma proteins or to apply a correction based on ultrafiltration of plasma through an artificial membrane. Since these two corrections are in the opposite direction and probably similar in the extent of their correction and because of the imprecision of either correction, the TF/P chloride ratios have been corrected only for the plasma water concentration as a reasonable estimate of the actual chloride concentration ratio across the tubule epithelium. It is apparent that had a Donnan correction of 1.05 been applied, this would not have altered the significant difference between TF/P chloride

ratios and unity nor the conclusion with regard to preferential bicarbonate reabsorption. This argument obviously does not apply to the calculation of the fractional chloride reabsorption, which must take into account the Donnan distribution and which was applied as shown in Table B-4.

Tubule fluid chloride ratios have been plotted against TF/P inulin values in Fig. B-4. TF/P inulin ratios have been used in this plot to indicate the extent of fractional fluid reabsorption and they probably also reflect distance along the proximal tubule. This figure reveals that there was a significant positive correlation between these two variables and that the intercept on the Y axis equals a TF/P chloride of 1.04. This value is very close to the expected value in the glomerular filtrate for a Donnan distribution. Since proximal fluid remains isosmotic to plasma throughout its length and since the two major anions in the filtrate are chloride and bicarbonate, these results suggest that bicarbonate ratios should be less than unity and show a small decline with length or with inreasing fractional fluid reabsorption along the proximal tubule.

Table B-5 summarizes and compares the results for transit time, tubular radius, estimates of reabsorptive capacity and net rates of water and chloride flux with previously reported values from *in vivo* micropuncture studies for the dog proximal tubule.

It will be seen that the tubular radius obtained in this study is slightly larger than that reported by three other groups during free-flow micropuncture conditions (Knox et al. 1969; Levine et al. 1968; Wright et al. 1969). It must be noted that in the studies of Knox et al. (1969) and that of Wright et al. (1969) (in both of which the radius was approximately 12.5 10^{-4} cm), the kidney surface was exposed to mineral oil at room temperature, whereas the observations of Levine et al. (1968) were made on kidneys bathed with mineral oil at 37 C. Since the present observations were also made at 37 C, it is significant that the values of Levine et al. are very similar suggesting that these differences may in part be attributable to differences in ambient temperature.

Reabsorptive capacity was assessed in 11 instances where simultaneous transit times to the point of puncture and corresponding TF/P inulin ratios were available. The mean result was 4.07 10^{-2} sec^{-1}. A comparison with similar estimates in the dog from published <u>in vivo</u> micropuncture work based on transit time and TF/P inulin data or upon the shrinking droplet half time, reveals good agreement (range 3.53 to 6.28 10^{-2} sec^{-1}). Estimates for net rates of water flux show similar agreement. The mean value for the autoperfused kidney was 3.54 ±0.64 10^{-5} $cm^3 cm^{-2} sec^{-1}$ (equation 2), whereas estimates for the <u>in vivo</u> kidneys ranged from 2.7 to 4.96 10^{-5} cm^3 cm^{-2} sec^{-1}. The chloride concentration in the reabsorbate

was calculated to be 111 mM (equation 3, plasma Cl corrected for plasma water was 120 mM) and the net rate of chloride flux was found to be $3.93 \pm 0.72\ 10^{-9}$Eq cm^{-2} sec^{-1} (equation 4).

Electrophysiological Data

A) Proximal tubule

1) Transepithelial potential. The transepithelial p.d. was recorded from 135 separate proximal tubular impalements, in which the criteria for localization previously mentioned were satisfied. The mean of all measurements was -2.0 ± 0.2 mV, which differed significantly from zero ($p < 0.001$). The frequency distribution is displayed in Fig. B-5 and bears further comment. It will be seen that the distribution is skewed towards the negative side with the mode at zero, and median at -6 mV. If the true p.d. were indeed zero, one would expect to find readings symmetrically distributed about zero allowing for small random errors on either side of the zero. The skewed nature of the distribution suggests rather that the "true" resting p.d. may be negative to a small degree and in many cases may have been shunted to zero or partially decreased as the result of damage associated with penetration of the electrode. This also fits with the observations that

the proximal p.d. often slowly declined in a given tubule over the course of observation. It is unlikely that insertion of the double barrelled perfusion pipette accounted for the low p.d. since it was invariably placed more than several length constants away from the Ml electrode (see below). It is possible that the experimental procedures associated with isobaric auto-perfusion may have affected the resting p.d. This possibility seems unlikely since 24 impalements were made in several experiments in proximal tubules of dogs whose kidneys were prepared for micropuncture in a conventional fashion. The procedures used for localization of the electrode were otherwise identical. Proximal p.d.'s in these experiments ranged from +1 to -5 mV with a mean value of -1.7 mV, which was not significantly different from the results obtained in the auto-perfused kidney preparation. Since both intratubular and external solutions have similar compositions, the small observed potential is unlikely to be due to systematic changes in tip potential of the microelectrode.

2) Relative ionic permeability coefficients (P). The relative permeability of various ions across the proximal epithelium was estimated from the extent to which the transepithelial potential was altered by perfusion of the lumen with one or other test solutions.

i) Transepithelial salt gradients: P_{Na}/P_{Cl} was estimated from the potential change (WV) that resulted by imposing a concentration

gradient for both sodium and chloride across the epithelium. Luminal perfusion was performed with two solutions from which sodium and chloride were reduced in approximately the same proportion but to a varying extent and which were made isosmotic by the addition of sucrose. In the first instance the perfusion fluid contained 12.2 mM sodium and 10 mM chloride. This resulted in a positive deflection of the transepithelial potential which averaged +10.3 ±0.8 mV (n=31). The tracing from one such experiment is displayed in Fig. B-6. The second series of experiments with a solution containing 42 mM sodium and 37.3 mM chloride, resulted in a smaller positive deflection. The mean of nine observations was +4.4 ±0.56 mV. These results clearly establish a preferential permeability of sodium over chloride in a qualitative way. It will be noted that the concentration gradient for sodium was slightly different from that for chloride if the values in the luminal perfusate are compared to the respective concetrations in normal Tyrode's. In each instance the concentration gradient was slightly greater for chloride than for sodium so that the voltage changes might have been even greater had the gradients been precisely the same. A quantitative estimate of P_{Na}/P_{Cl} was gained by treating the voltage deflection as a liquid junction potential and solving for the transference numbers T_{Na}/T_{Cl}. The ratio T_{Na}/T_{Cl} may be equated with the relative permeability ratio P_{Na}/P_{Cl} for a given membrane when T_{Na} and T_{Cl} are determined simultaneously under

the same experimental conditions. These results are summarized in Fig. B-7, which shows a plot of voltage change as a function of the NaCl concentration gradient. There was a significant correlation between ΔV and the log of the concentration gradient which is fitted by the following regression equation:

$$\Delta V = 0.07 + 9.44 \log \frac{(NaCl)_{bath}}{(NaCl)_{lumen}}$$

$$r = 0.87$$

$$p < 0.001$$

Since $\Delta V = \frac{RT}{F}(T_{Na} - T_{Cl}) \ln \frac{(NaCl)_{bath}}{(NaCl)_{lumen}}$

Therefore, the regression slope ($b = 9.44$) corresponds to $61\ (T_{Na} - T_{Cl})$.

and $T_{Na} = 0.58$

$T_{Cl} = 0.42$

$$\frac{T_{Na}}{T_{Cl}} = \frac{P_{Na}}{P_{Cl}} = 1.38$$

This approach neglects the contribution of any other ion to the observed potential change, which is not unreasonable since sodium and chloride were the only two ions altered by the perfusion. Moreover, there was no concentration gradient for the other ions, which are presumably less permeant than sodium or chloride and in much smaller concentraton. It also assumes strictly equal concentration gradients for both sodium and chloride. Since the actual concentration gradient for sodium was used in this plot

the results would tend to slightly underestimate the difference in sodium permeability compared to chloride. Finally there is the possibility that these results may be influenced by effects of bulk flow on the membrane. It is likely that influx of water occurred along with sodium and chloride in these experiments. Such bulk flow may be associated with the development of streaming potentials that could contribute to the observed potential. This was thought unlikely on an a priori basis in view of the fact that streaming potentials in a variety of biologic membranes are generally of a much smaller magnitude (Diamond and Harrison 1966; Smyth and Wright 1966; Wright and Diamond 1968; Wright and Prather 1970). The possibility was also excluded by two series of experiments. First the lumen was perfused with a hypotonic solution containing 12 mM sodium and 10 mM chloride. In this instance only 70% of the osmotic pressure of the sodium chloride removed from normal Tyrode's was replaced with sucrose (assuming a reflection coefficient for sodium chloride of approximately 0.7). This resulted in similar positive trans-epithelial potentials (range +5 to +10 mV, n=6). Second, the magnitude of streaming potentials in the proximal tubule was measured directly and shown to be of too small an extent to contribute to the potential in the salt gradient experiments (see below). Comparable values to the present study have previously been reported for P_{Na}/P_{Cl} in the rat proximal tubule (Frömter

1969; Fromter, Müller and Knauf 1968). The greater permeability of the membrane to sodium than to chloride is in striking contrast to their opposite behavior in free solution.

ii) Single ion substitutions: The magnitude of the potential change (ΔV) induced by the various test solutions also afforded an estimate of the relative permeability of a test cation (I^+) to sodium or of a test anion (I^-) to chloride for which they substituted. This was done by the use of the following form of the constant field equation:

$$\Delta V = \frac{RT}{F} \ln \frac{P_{Na}(Na)^1 + P_{Cl}(Cl)^P + P_{I^+}(I^+)^1}{P_{Na}(Na)^P + P_{Cl}(Cl)^1 + P_{I^-}(I^-)^1}$$

This treatment makes no assumption about the origin of the small resting p.d. but considers that the ionic gradients superimpose their effect on the control value. The equation was solved (using the previously determined value for P_{Na}/P_{Cl}) for substituent cations in terms of P_{I^+}/P_{Na} and for substituent anions in terms of P_{I^-}/P_{Cl}. The use of the equation in this form neglects the contribution of other ions, which was thought to be insignificant in view of their smaller concentration and lesser degree of permeability.

Tracings from two representative experiments, one in which sodium was replaced by choline and the other in which chloride was replaced by isethionate are shown in Figs. B-8 and 9. The mean results of the voltage deflections for each of the various test solutions are summarized in Table B-6. The relative cation

permeability (P_{I^+}/P_{Na}) compared to sodium is given in the third column. The cation substitutions revealed P_K to be only slightly greater than P_{Na}, while considerably less permeant than sodium. The relative anion permeabilities P_{I^-}/P_{Cl} given in the fourth column revealed all of the anions tested to be less permeant than chloride. Using the value of P_{Na}/P_{Cl} obtained previously the cation permeabilities were also expressed in terms of chloride. This allowed all ions studied to be ranked in order of decreasing permeability as follows:

$$P_{K^+} > P_{Na^+} > P_{Cl^-} > P_{choline^+} > P_{HCO_3^-} > P_{Propionate^-} > P_{Isethionate^-} > P_{Acetylglycinate^-} > P_{Cyclamate^-}$$

Table B-6 also includes the relative permeabilities of these ions to chloride in free solution. These values were obtained from the limiting equivalent conductivity of each ion previously published for K, Na, Cl, and proprionate (Robinson and Stokes 1965), and estimated from conductivity measurements of dilute solutions of the following salts: choline chloride, sodium isethionate, sodium acetyglycinate and sodium cyclamate. With the exception of sodium acetylglycinate the ranking in either the cation or anion series agrees with that predicted on the basis of their behavior in free solution. The striking finding was the preferential permeability for cations in general over anions, in contrast to their behaviour in free solution. Similar values for $PHCO_3/PCl$ and $P_{Cyclamate}/P_{Cl}$ have been previously reported for the rat (Frömter and Müller 1969;

Müller and Fromter 1970). These results suggest that transepithelial ionic permeability is in large part determined by charge as well as by size of the hydrated molecule. A comparison of the experimentally determined permeability ratios with the expected ones in free solution shows that sieving effects need not be postulated except for the largest anion cyclamate.

3) Osmotic flow and transepithelial potential. The effect of bulk flow upon the transepithelial potential produced by osmotic gradients was tested by means of luminal perfusions with normal Tyrode's solution to which sufficient sucrose had been added to create a +300 or a +600 mOsm gradient. The effect of flow in the reverse direction was tested by peritubular capillary perfusions using the Tyrode's containing the additional 600 mOsm of sucrose. Hyperosmotic perfusions of the lumen led to a positive deflection in the transepithelial potential (+3 mV) whereas reversal of the gradient gave the opposite result (-1.7 mV) suggesting that bulk flow occurs through negatively charged pores thereby giving greater mobility to cations. This result is therefore in agreement with that based on salt gradient experiments.

4) Resistance measurements. The mean R_{input} obtained with a double-barrelled electrode in 46 proximal impalements was 29.0 $\pm 2.0 \times 10^3$ ohms. The specific resistance of the proximal epithelium

was estimated by cable analysis of sixteen experiments using the method illustrated in Fig. B-2c. A representative experiment is displayed in Fig. B-10 and shows a continuous recording from a M_2 electrode as well as two successive impalements of a M_1 electrode at varying distances from the M_2 electrode. On the tracing of M_2 are shown initially the deflections due to the coupling resistance with the microelectrode in the bath. This is followed by subsequent impalement of a proximal tubule, indicated by the negative deflection. At this point M_1 was also introduced into the tubular lumen at 103×10^{-4} cm away from M_2. The position of both electrodes was then verified by a positive response to perfusion of the tubule with choline Tyrode's. Application of current pulses through M_2 resulted in simultaneous voltage deflections at both M_1 and M_2. M1 was withdrawn and reinserted 28×10^{-4} cm away from M_2 and the procedure repeated. In these experiments the sequence of impalement of the electrode M_1 with regard to the distance from M_2 was randomly varied and did not appear to affect the results, nor did the sequential impalements affect R_{input} measured with the M_2 electrode. The results in all 16 proximal tubules are displayed in Fig. B-11. The change in voltage observed at M_1 is plotted as a decimal log function of the interelectrode distance between M_2 and the subsequent M_1 impalements, and the lines join corresponding values for two impalements in each tubule. The mean slope (b) for all 16 pairs of observations was found to be -0.00464. The length constant (λ) is given by the equation:

$$\lambda = \frac{\log e}{b} = \frac{0.434}{0.00464} = 93.6 \ 10^{-4} \text{ cm.}$$

R_{input} determined in these same 16 proximal tubules was $27.5 \pm 4.7 \times 10^3$ ohms.

The specific resistance for the proximal epithelium (R_m) was then determined from the following equation (Hodgkin and Rushton 1946), using the previously obtained values for R_{input} (27.5×10^3 ohms), λ (93.6×10^{-4} cm) and the specific resistance of the fluid within the lumen (R_l), which was determined directly for normal Tyrode's solution at 37 C to be 55 ohm cm:

$$R_m = 4 \lambda \Pi R_{input} \sqrt{\frac{R_l \lambda}{2 R_{input} \Pi}}$$

$$= 5.58 \text{ ohm cm}^2$$

The electrical radius calculated from these results was 17.26×10^{-4} cm, which agreed exceptionally well with that determined directly by optical methods (17.4×10^{-4} cm). These results are summarized in Table B-7.

R_{input} was also assessed in a separate series of experiments (Fig. B-2d) in which chloride was substituted by cyclamate in the lumen, peritubular capillary and surrounding bath. In these experiments R_{input} was measured in a control situation (luminal perfusion of normal Tyrode's) before and after chloride substitution. R_{input} significantly increased from $33.4 \pm 3.4 \times 10^3$ ohm during

control (R_{input}^T) to 49.3 ±4.7 x 10^3 ohm during cyclamate replacement (R_{input}^C) (n=17, p <0.001). The relative change in the specific membrane resistance during cyclamate (R_m^C) to that during control situation (R_m^T) was estimated from the following equation:

$$\frac{R_m^T}{R_m^C} = \frac{(R_{input}^T)^2}{R_i^T} \cdot \frac{R_i^C}{(R_{input}^C)^2} = 0.712$$

The specific resistivity of the cyclamate Tyrode's solution (R_i^C) was measured directly at 37 C and found to be 85.5 ohm cm. Specific resistance of the epithelium thus increased by 40% during substitution of chloride by cyclamate, as expected for replacement of chloride by a less permeant anion. The specific membrane conductance G_m is given by the reciprocal of R_m. The fraction of the membrane conductance due to chloride (G_{Cl}/G_m) was derived in the following way:

$$\frac{R_m^T}{R_m^C} = \frac{G_m^C}{G_m^T} = \frac{(G_m^T - G_{Cl}) + G_{Cyclamate}}{G_m^T} = 0.712$$

and from Table B-6, $G_{Cyclamate} = 0.26\ G_{Cl}$

therefore: $G_m^T - G_{Cl} + 0.26\ G_{Cl} = 0.712\ G_m^T$

$$\frac{G_{Cl}}{G_m^T} = 0.39 = T_{Cl}$$

This estimate is remarkably close to the previous estimate of T_{Cl} derived from the salt gradient experiments, which was found

to be 0.42, and supports the view that chloride is less permeant than sodium in the approximate ratio of 2/3. The partial chloride conductance (G_{Cl}) could therefore be calculated in two ways, which are summarized in Table B-8. The mean of these two estimates for G_{Cl} was 72.5 x 10^{-3} ohm^{-1} cm^{-2}.

Changes in R_{input} were also assessed by similar methods during hypertonic luminal or peritubular capillary perfusions in an attempt to correlate changes in R_m with previously observed streaming potentials. In these experiments the solution containing the additional 600 mOsm sucrose was used. R_{input} increased significantly from 26.1 ±2.3 x 10^3 ohms to 45.0 ±4.6 x 10^3 ohm during luminal perfusion (n = 9, p <001) when net water flow was directly into the lumen; R_{input} fell significantly from 26.0 ±1.9 x 10^3 ohm to 21.25 ±0.9 x 10^3 ohm during peritubular capillary perfusion when net water flow was directed outwards (n=8, p <0.05). Specific resistance of the Tyrode's + 600 mOsm solution was 90.7 ohm cm. The relative change in the specific membrane resistance under the two conditions, was estimated from the effects on input resistance using an equation similar to that shown for the cyclamate substitution experiments. The results indicated an increase of R_m during inwardly directed bulk flow of 80%, whereas R_m fell by 37% when bulk flow occurred from lumen to peritubular side of the membrane. Specific membrane conductance therefore averaged 99.5 and 286 x 10^{-3} ohm^{-1} cm^{-2}

during influx and efflux, respectively. Specific membrane resistance has also been previously shown to vary, depending on the direction of bulk flow and in the manner indicated by the present studies in the proximal tubule of the rat (Frömter and Luer 1969).

B) Distal Tubule

1) Transepithelial potential. Transepithelial potentials were recorded from distal tubules using both M_1 and M_2 electrodes. The mean p.d. from 25 distal impalements with M_1 electrodes was -40.8 ± 2.9 mV, while the mean of 20 impalements with M_2 electrodes was -45.0 ±4.0 mV. The mean of all measurements was -42.7 ±2.3 mV. The frequency distribution for all values is shown in Fig. B-12.

2) Resistance measurements. In 17 impalements R_{input} was measured with double barrelled electrodes as previously noted for the proximal tubule and was found to average 528 ±61 x 10^3 ohm. Using this volume, an estimate of the specific resistance of the distal tubular fluid R_i = 124 ohm.cm) and assuming a radius of 15 ±2 x 10^{-4} cm, the specific transepithelial resistance of the distal tubule can be calculated to be approximately 600 ohm cm^2 and lie within a range of 400 to 900 ohm cm^2.

DISCUSSION

The initial results of this study described the functional assessment of an auto-perfused dog kidney preparation in terms of various hemodynamic, excretory and micropuncture parameters. Although significant alterations in function were observed as compared to the non auto-perfused kidney the results did not reveal any major defects in the tubular handling of sodium, chloride or water.

Renal blood flow exhibited a slow, progressive fall associated with a corresponding increase in the renal vascular resistance. Glomerular filtration rate appeared to be depressed to a greater extent, resulting in a significantly lower value for the calculated filtration fraction (0.24) than that normally found in the dog (0.31) (Selkurt 1962). Paired observations with the non auto-perfused control side also demonstrated significantly lower values for filtration rate in the autoperfused kidney.

The reasons for these changes in renal blood flow and glomerular filtration rate are probably multiple. Undoubtedly the modest hemorrhage that resulted from filling the perfusion circuit at the start of the experiment, in addition to any further blood loss at the operative site owing to systemic heparinization, would tend to increase renal vascular resistance as a result of various reactive mechanisms. This would not account, however, for the differences in function between left and right kidneys. Two

major factors may account for these observed differences. First, there is the effect of passing blood through the perfusion system on the vasoconstrictor properties of the blood. Nizet has shown that simple stagnation of blood in a foreign vessel rapidly leads to release of vasoconstrictor substances chiefly from red blood cells (Nizet et al. 1967). This effect is increased by agitation of the blood but does not seem to be a function of contact with glass since increasing the surface area of glass exposed to blood did not increase vasoconstrictor activity, nor did the use of non-wetting agents decrease the effect. Others have implicated the release of vasoconstrictor substances from platelets during perfusion (Bracco and Curti 1954; Lustinec 1965; Zucker 1944). Whatever the exact mechanism it is clear that conditions were such in our perfusion as to promote the release of these substances. The fact that function was as good as it was and deteriorated at a very slow rate can be attributed to the small volume of blood in the chamber at any one moment and to the presence of the whole animal in the circuit, since the liver, lungs and spleen all appear to remove vasoconstrictor substances released during perfusion (Bing 1941; Nizet et al. 1957).

Second, it is clear that the pressure head to the auto-perfused kidney was appreciably less than to the control kidney. The average pressure drop measured in the depulsator chamber was 15 to 20 mmHg, to which must be added a small additional pressure drop

in the tubing connecting the depulsating chamber to the renal artery. Not only was the mean pressure lower, but in addition the pressure in the auto-perfused system was constant in contrast to the pulsatile nature of the pressure to the control kidney. Several groups have recently emphasized that the total effective pressure in a pulsatile system is greater than in a nonpulsatile system and results in higher flow than a nonpulsatile system at equal mean pressure (Agishi, Peirce and Kent 1969; Giron et al. 1966; Shephard, Simpson and Sharp 1966). Thus while mean pressure in the auto-perfused kidney was well within the normal autoregulatory range, this cannot be applied without reservation to the isobaric system. It is apparent from the excretory data that the response of the autoperfused kidney resembled that seen when the renal artery is partially constricted - i.e. a fall in urine volume and urinary sodium concentration together with a high urine to plasma osmolarity ratio.

The effect of depulsation per se on renal function, apart from whatever effects are due to a lower effective perfusion pressure, has been a source of debate (Wilkens, Regelson and Hoffmeister 1962) for a considerable period of time among renal physiologists and the issue has never been adequately settled, primarily because of the difficulty in establishing an adequate control whereby the effects of depulsation can be separated from the effects of the perfusion system which is used to produce depulsation.

Although initial work suggested a deleterious effect of depulsation (Gesell 1913) later and better controlled experiments failed to confirm these effects (Selkurt 1951; Wolf, Dluhy and Lauler 1969). Skinner, McCubbin and Page (1964) have also shown that renin release is not stimulated by a reduction in pulse pressure per se contrary to earlier reports. A recent study of Wolf, Dluhy and Lauler (1969) compared the function of a pump-perfused in situ kidney with the contralateral control kidney and showed that when the difference in perfusion pressure was within 15 mmHg on the two sides there was neither a significant difference in inulin or PAH clearance nor in absolute or fractional urinary electrolyte excretion rates. Although pulse pressure was markedly reduced by their pump it was not abolished and ranged between 10 - 15 mmHg. A second possible drawback to this study arose from the fact that a modest diuresis was produced by hypotonic mannitol and saline infusions, which might have obscured any subtle differences in function between the two sides existing under hydropenic conditons. Many et al. (1967) recently showed, using a system of total depulsation in studies of one or both kidneys, that urine volume was significantly reduced and urine osmolarity significantly increased by this procedure, despite the absence of any significant change in renal blood flow or creatinine clearance. These authors also suggested on the basis of angiographic studies that depulsation might cause a redistribution of blood flow from cortex to medulla.

These experiments were obviously not designed to examine the effects of depulsation on renal function specifically, but the results support the view that depulsation of itself is probably not a major factor in compromising renal function. No gross swelling of the kidney was ever observed during the course of the experiments, which has been suggested to be a possible consequence of depulsation and which has been reported for pump-perfused kidneys (Nizet et al. 1967; Waugh and Kubo 1969). Furthermore in six experiments the contralateral kidney was also weighed and there was no significant difference between the two sides (mean kidney weights: left (auto-perfused) 41.4 ±4.2g; right 41.3g).

It is unlikely that the short ischemic period required to catheterize both renal vessels contributed to subsequent deterioration of the kidney. Selkurt (1945) has shown that periods of ischemia lasting up to 5 min are well tolerated by the kidney with full return to normal function in the ensuring 30 - 60 min. It has also been shown that such short periods of clamping are often followed by a transient increase in renal blood flow (Feezor and Boyce 1965; Spencer 1956). These factors, however, would not be operative by the time experiments were first begun on the autoperfused kidney, which was at least 60 min after catheterization.

It is possible that with the system of depulsation that was used, micro air embolism may have occurred and contributed to the increased renal resistance and fall in filtration rate.

Other systems of depulsation have not interrupted the blood stream but have passed a continuous flow of blood through some sort of expansion chamber under pressure, thus avoiding the risk of air embolism. In preliminary experiments with this sort of system it was found that the pulse pressure was not satisfactorily damped and therefore a system was adopted whereby the column of blood is completely interrupted. It has been suggested that fat globulemia produced by the effects of either heparin or a blood gas interface may contribute to a fall in filtration rate (Waugh and Kubo 1969), however, no formation of a fatty layer was observed, at least grossly in the perfusion chamber.

The micropuncture observations also support the view that individual tubule function was not significantly altered. Fractional and absolute rates of fluid reabsorption appear to be within the normal range established for the dog both by these and other's reports under in vivo conditions. Tubule diameters were only slightly larger than in studies by Levine et al. (1968) also performed on tubules bathed with fluid at body temperature, though they were appreciably larger than values reported by Knox et al. (1969) and Wright et al. (1969) for tubules of kidneys bathed with mineral oil at room temperature. It is possible that other factors associated with this preparation, such as denervation or depulsation, are responsible for a certain degree of tubular dilatation. Despite

these differences in tubule diameters, the reabsorptive capacity of the proximal tubule appears to be similar to these other studies and supports the view that tubule diameter is not an important factor in determining intrinsic reabsorptive capacity (Schnermann, Levine and Horster 1969; Wahl et al. 1968).

The tubule fluid to plasma chloride ratios are of additional interest. The only previous observations of TF/P chloride ratios in the dog were made by Clapp, Watson and Berliner (1963), who found a mean value of 1.04 (the exact correction used for plasma water and Donnan distribution was not given). This is quite similar to the present results considering that the TF/P ratios were not corrected for a Donnan ratio. Clapp found in the same study (1963) that TF/P bicarbonate ratios were slightly less than unity, although a subsequent study in the dog (Bernstein and Clapp 1968) failed to show a significant bicarbonate gradient under hydropenic conditions. However, the quinhydrone method used in these latter studies to measure tubule fluid bicarbonate concentrations may slightly overestimate the true bicarbonate if there is any loss of CO_2 from the sample, and hence may tend to underestimate the bicarbonate gradient. Numerous studies in the rat proximal tubule have shown that TF/P chloride ratios range from 1.15 to 1.29 (Danielson, Persson and Ulfendahl 1970; Gottschalk 1963; Kashgarian et al. 1963, 1965; Kunau et al. 1968; Malnic et al. 1969; Warren et al. 1970;

Weinstein 1968). The present results suggest that chloride concentration rises continuously along the proximal tubule, and does not reach a plateau as suggested by previous observations in Necturus and the rat (Bott 1962; Litchfield and Bott 1962; Walker et al. 1941; Warren et al. 1970), although in the absence of localization of puncture sites, this remains speculative. This may be related to the fact that TF/P_{Cl} values are not so high in the dog as in these other species.

The electrical characteristics of proximal and distal tubules in the dog are in many respects similar to those recently reported for the rat, which suggests that the same may apply to other mammalian species. The previous finding of Watson, Clapp, and Berliner (1964) of a large negative p.d. in the proximal tubule of the dog was not confirmed when strict criteria for intraluminal localization were employed. In a number of instances it was possible after impalement to obtain larger p.d.s that did not change with luminal perfusion; presumably, such instances represent artifacts due to the tip being lodged within a partially damaged cell. These results support the findings in the rat to the extent that the early reports of a large negative potential in the proximal tubule (= -20 mV) were also artifactual. (Frömter and Hegel 1966; Frömter, Wick and Hegel 1967). The reasons for thinking that the small negative p.d. which were observed in a majority of punctures is

probably a slight underestimate of the true resting p.d. have already been given. This view is supported by the recent reports of a mean p.d. of -3.8 mV in the perfused isolated rabbit tubule (Burg 1969). There is no ready explanation for the absence of a p.d. in the rat reported by Frömter et al. (1966, 1967) and Maude (1969) compared to the findings of Burg (1969) in the rabbit and those in the present study. Whether this represents a species difference or whether the procedures used in the rat are possibly associated with sufficient damage to the area of the tubule immediately adjacent to the electrode so as to cancel any resting p.d. remains for further work to decide. In view of the low electrical resistance of the proximal tubule it is likely that a slight amount of trauma might be sufficient to reduce or destroy any resting p.d.

These studies provide little evidence for the mechanism responsible for the origin of the proximal p.d. It is not a diffusion potential for sodium or potassium since there is no detectable gradient for these ions across the tubule, while that for chloride is in the wrong direction. Whereas the bicarbonate gradient is such that it could conceivably cause a small negative diffusion potential, the results with varying bicarbonate perfusions exclude this possibility since the corresponding effects of a reciprocal chloride gradient ($P_{Cl} > P_{HCO_3}$) would clearly overshadow any small effect of bicarbonate. Similarly a hydrogen ion gradient as a possible mechanism is also

excluded by the same experiments. Electrokinetic effects due to bulk flow as a consequence of active sodium transport were excluded, since such effects could account at most for -0.5 mV. The remaining possibilities include a double membrane potential with differing permeabilities at the two sides of the cell, an electrogenic pump, or an additional electromotive force at a paracellular site. This latter possibility has recently been proposed to account for a small positive p.d. across the rabbit gall bladder (Machen and Diamond 1969), however, the direction of the observed p.d. is opposite to that found in the kidney tubule. These various possibilities cannot be decided on the basis of present data.

The studies of relative permeability ratios established two salient features: 1) the relatively greater permeability across the epithelium of cations over anions than that seen in free solution, and 2) the discrimination within either cation or anion series considered separately, was not more selective than that expected in free solution on the basis of ionic mobility. The range of hydrated radii (calculated from the corrected Stokes formula (Robinson and Stokes 1965) was from 2.4 Å to 3.9 Å for the anions, and from 2.4 Å to 3.7 Å for the cations. Steric hindrance effects need not be invoked to explain the observed permeability sequence, with the possible exception of cyclamate.

The existence of streaming potentials suggests that water and electrolytes share a similar route through the epithelium.

Thus three lines of evidence - 1) $T_{Na} > T_{Cl}$ from salt gradients experiments, 2) the sequence of permeability ratios from single ion substitutions, and 3) the existence of positive streaming potentials in the direction of bulk flow - all indicate that electrolyte permeation across the epithelium takes place through channels with negative fixed charges and hence more mobile cations in the channel. Several other epithelial membranes have been shown to behave in a similar fashion with respect to preferential cationic permeation and to streaming potentials of similar magnitude and sign (Brown, Vick and Jacobson 1970; Clarkson 1967; Curran and Solomon 1957; Diamond 1962; Diamond and Harrison 1966; Smyth and Wright 1966; Wright and Diamond 1968; Wright and Prather 1970) as well as the proximal tubule epithelium in the rat (Frömter 1969; Fromter, Müller and Knauf 1968). A lesser permeability for anions would tend to favour the maintenance of anionic gradients across the membrane.

The measurement of R_{input} with double barrelled electrodes in the dog was almost identical with values reported in the rat (Hegel, Frömter and Wick 1967) in which slightly different methods were used. In the rat, the use of double barrelled electrodes was found to give unduly high values. The internal consistency of estimates of R_m obtained from calculations using either R_{input} or the length constant separately give confidence to the results

obtained by this method in the present study. This is also supported by the good agreement between the electrical and experimentally determined values of the radius, as well as for R_i determined directly and R_i calculated from the length constant, radius and R_{input}. The length constant was found to be in the same range as that measured earlier in the rat (Hegel, Frömter and Wick 1967; Windhager and Giebisch 1961). The low value for the specific resistance of the proximal tubule is also comparable to the results of the rat (Hegel, Frömter and Wick 1967), where somewhat different methods were also employed. The mammalian proximal tubules appear to have a conductance one order of magnitude higher than in an amphibian species, Necturus, where R_m was found to be ≈70 ohm cm^2 (Boulpaep 1970). This fits with the observation of higher ionic permeability coefficients across the mammalian proximal epithelium (Baumann, Frömter and Ullrich 1967; Ullrich 1967) than in the amphibian (Oken and Solomon 1963; Oken et al. 1963). In contrast, more distal parts of the nephron (distal tubule and collecting duct) are characterized by transepithelial conductance at least two orders of magnitude less than that in the proximal tubule (Burg et al. 1968; Giebisch and Malnic 1968; Malnic, Mello Aires and Giebisch 1968).

These results allow some tentative conclusions to be drawn with respect to the morphologic basis of the transepithelial transport paths. The following lines of evidence favor the view that the

major route of passive permeation does not cross individual cell membranes but bypasses the cell through paracellular channels: 1) The very low values for R_m itself suggests a paracellular path rather than a transcellular path through two membranes in series, since the specific resistance of biological membranes is two to three orders of magnitude greater than the total transepithelial resistance. 2) The ranking of relative ionic permeability coefficients and the small range of their differences is at variance with that observed for individual cell membranes. 3) The change in conductance on both sides of the epithelium elicited by extracellular substitution of chloride for cyclamate, compares well with that predicted from an estimate of T_{Cl} derived from salt gradient experiments which treated the epithelium as a single barrier. Recent studies in Necturus (Boulpaep 1967, 1970; Windhager, Boulpaep and Giebisch 1966) have shown conclusively that the transepithelial resistance is at least two orders of magnitude smaller than the sum of the series resistances of peritubular and luminal cell membranes alone. It had previously been found necessary (Boulpaep 1967) to incorporate a paracellular low resistance pathway into an equivalent circuit of the proximal

epithelium in order to account for electrical interaction between the peritubular and luminal membrane potentials. Hoshi and Sakai (1967) have also adduced evidence for a paracellular low resistance pathway in the proximal tubule of the newt kidney. A physiological role for such pathways in the regulation of net sodium and water transport has subsequently been demonstrated in Necturus (Boulpaep 1969, 1972). It is likely that the morphological counterpart of such paracellular paths can be assigned to the area of the tight junction and intercellular space between each cell. A correlation between size of intercellular spaces and fluid transport has also been found in several other transporting epithelia (Grantham et al. 1969; Kaye et al. 1966; Schmidt-Nielsen and Davis 1968; Tormey and Diamond 1967). Finally, saline loading in Necturus, which leads to a decreases in the transepithelial resistance in this species, was also accompanied by an increase in the permeability to a large non-electrolyte which is presumably confined to the extracellular space (Boulpaep 1972).

The electrical characteristics of the membrane allow an evaluation of the nature of chloride transport across the proximal epithelium. The net chloride flux that could be predicted on the basis of the available passive forces was estimated from the following equation:

$$J_{Cl} = \frac{G_{Cl} (V - E_{Cl})}{zF}$$

The electrochemical driving force ($V-E_{Cl}$) was determined from the difference between the resting p.d. ($V = -1.97$ mV) and the equilibrium potential for chloride ($E_{Cl} = \frac{RT}{zF} \text{ In } \frac{1}{(TF/P)_{Cl}} = +3.24$ mV). Mean G_{Cl} was taken from the results given in Table B-8. The predicted value for J_{Cl} was found to be $3.91\ 10^{-9}$ Eq cm^{-2} sec^{-1}, which agrees remarkably well with determinations of actual net chloride flow derived from micropuncture data (see B-5) and summarized in Table B-9. These results suggest that passive forces can account for net chloride reabsorption in the proximal tubule.

The results of the transepithelial potential obtained in the distal tubule of the dog agree well with previous reports of other mammalian and amphibian species (Clapp, Rector and Seldin 1962; Frömter and Hegel 1966; Giebisch et al. 1966; Kashgarian et al. 1963; Malnic et al. 1964, 1966, 1969, 1970; Maude, Shehadeh and Solomon 1966; Rector and Clapp 1962; Solomon 1957; Vieira and Malnic 1968). R_{input} measurements were also comparable to those obtained in the rat (Hegel, Frömter and Wick 1967; Wright 1971) as was the estimate of R_m (Giebisch and Malnic 1968; Malnic, Mello Aires and Giebisch 1968).

To summarize the salient features, the proximal tubule can be characterized by a low resting p.d., low resistance and relative lack of selectivity. At the same time, net transport of salt and water in this structure is large, and occurs against little

or no concentration gradient but in the presence of important undirectional fluxes. The amphibian proximal tubule is characterized by a higher resistance and higher resting p.d., which fits with both smaller net flux and undirectional fluxes. Both species show similar qualitative differences between proximal and distal tubules. The latter shows a higher transepithelial p.d., higher specific resistance and, conversely, transports much smaller quantities of ions against steep concentration gradients. It would thus seem most likely that paracellular pathways must play a much smaller role in this part of the nephron.

[1] The validity of the recollection micropuncture technique under conditions of saline loading has recently been questioned in view of the recent finding that this led to spuriously high measurements of single nephron filtration rates. However tubule fluid on plasm inulin ratios are not altered by recollection per se and thus estimates of fractional reabsorption based on inulin concentration ratios can still be used as a valid reflection of proximal handling of salt and water (see Wright and Giebisch 1972 for full discussion). Furthermore recent evidence cited in the same paper also suggests that although a small difference exists in filtration rate between cortical and juxtamedullary nephrons, saline loading of the magnitude employed in the present study, does not alter distribution of filtration among the nephron population.

CLAIMS TO ORIGINALITY

The findings presented in this thesis are believed to constitute an original contribution to scientific knowledge in the following areas:

a) This was the first extensive quantitative study of segmental nephron function by micropuncture techniques during strong osmotic diuresis in the mammalian kidney, and the first such study of the effects of saline loading and furosemide administration in the dog. The results have emphasized the importance of the loop of Henle in determining the ultimate extent of urinary electrolyte excretion and have enlarged present understanding of the physiologic interaction between the various segments of the nephron under diuretic conditions.

b) The electrophysiologic studies reported in Part B were the first such studies in the dog kidney and have helped to consolidate current knowledge of mammalian kidney electrophysiology. In particular they have established that the electrical potential difference in the proximal tubule is far less than the previously reported value of approximately 20 mV, lumen negative, in this species as in the rat, although the exact magnitude (thought to be -5 mV or less) could not be resolved by the methods used. The response to ionic substitutions established a relative preference for cationic permeability over anionic permeability whereas the selectivity among either cations or anions studied was not greater than that to be expected in free solution. The specific resistance of the proximal epithelium was determined by cable analysis. The low value for the specific

resistance and the relative lack of selectivity to ion permeation in the proximal tubule was felt to indicate an important paracellular path in addition to a transcellular path for transepithelial transport. The results are consistent with the notion that chloride is largely reabsorbed passively in the proximal tubule.

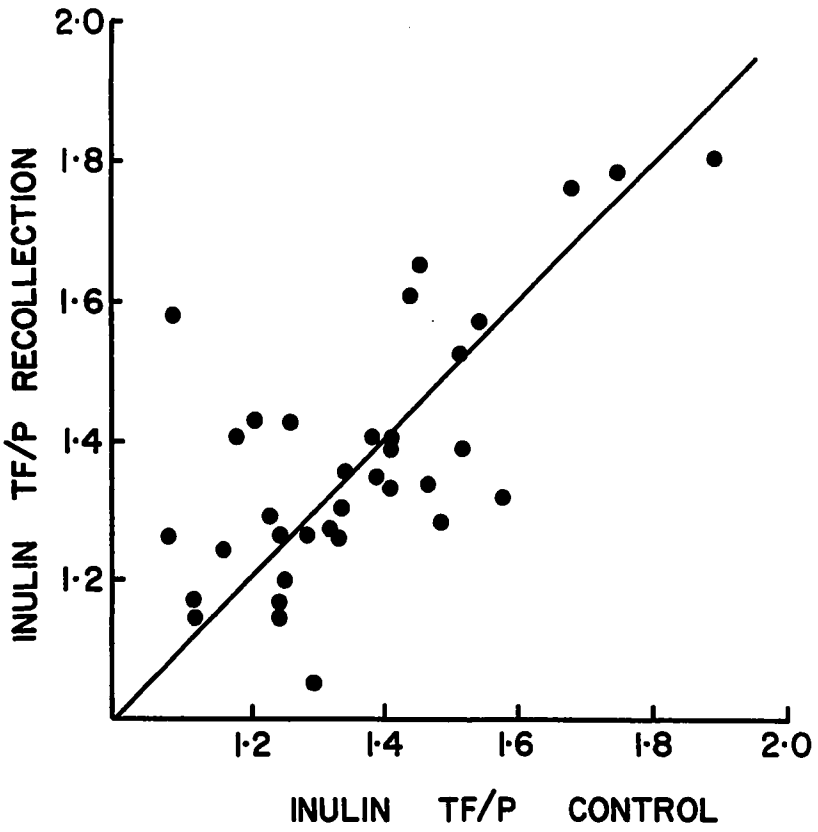

Figure A-1: Proximal tubule fluid to plasma (TF/P) inulin ratios. The values obtained during the recollection phase in hydropenia are plotted along the ordinate against their respective value along the abscissa obtained from the same tubule puncture site during the initial control phase.

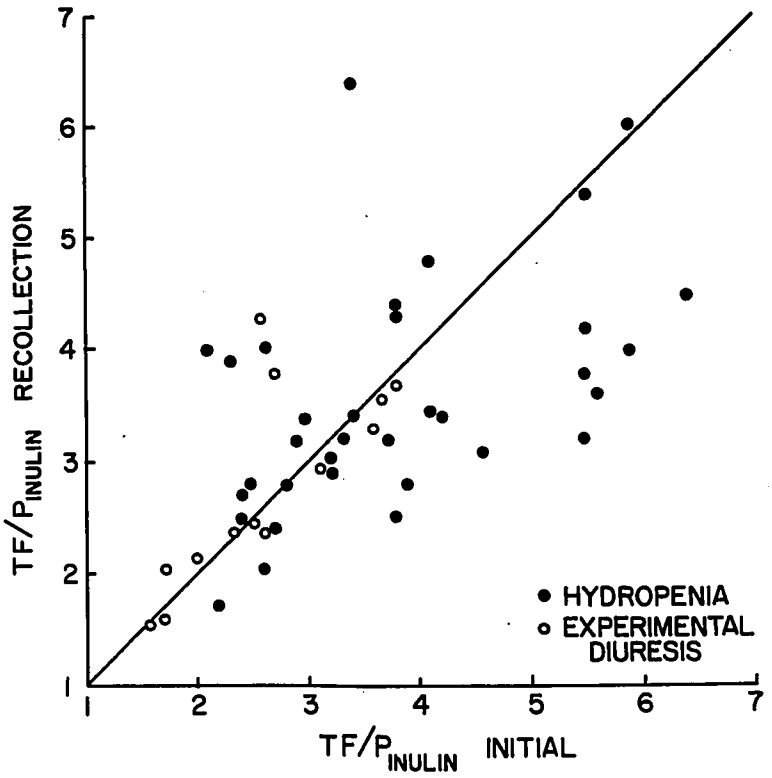

Figure A-2: Distal tubule fluid to plasma (TF/P) inulin ratios. The values obtained during the recollection phase in either continued hydropenia (closed circles) or during a continuous diuretic phase (open circles) are plotted along the ordinate against their respective paired values along the abscissa obtained from the same tubule puncture site during the initial phase.

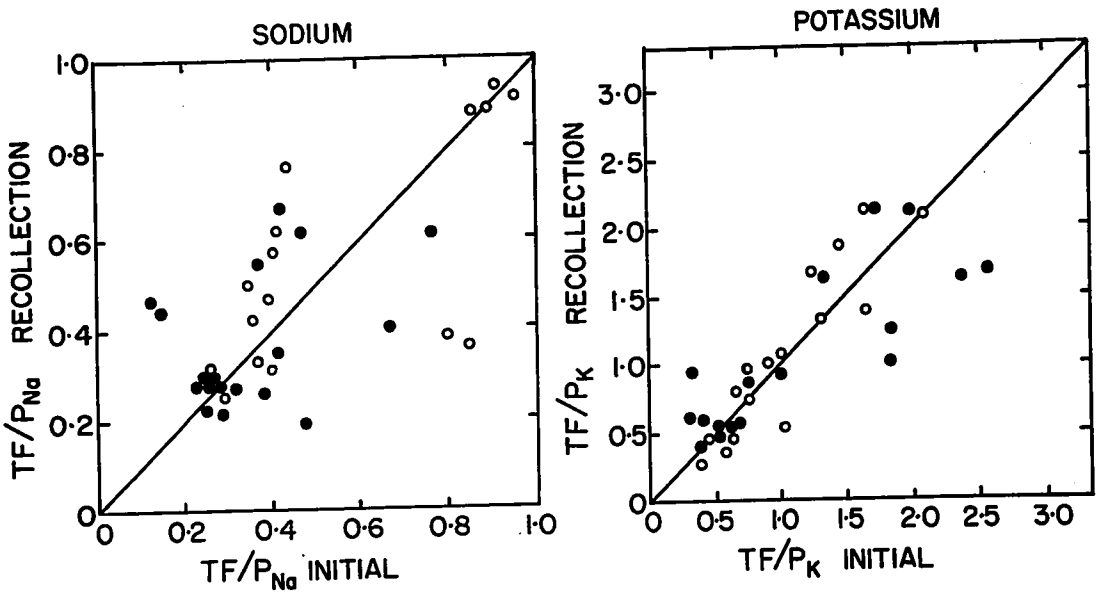

Figure A-3: Distal tubule fluid to plasma (TF/P) sodium (Na) and potassium (K) ratios. The values obtained during the recollection phase in either continued hydropenia (closed circles) or during a continuous diuretic phase (open circles) are plotted along the ordinate against their respective paired values along the abscissa obtained from the same tubule puncture site during the initial phase.

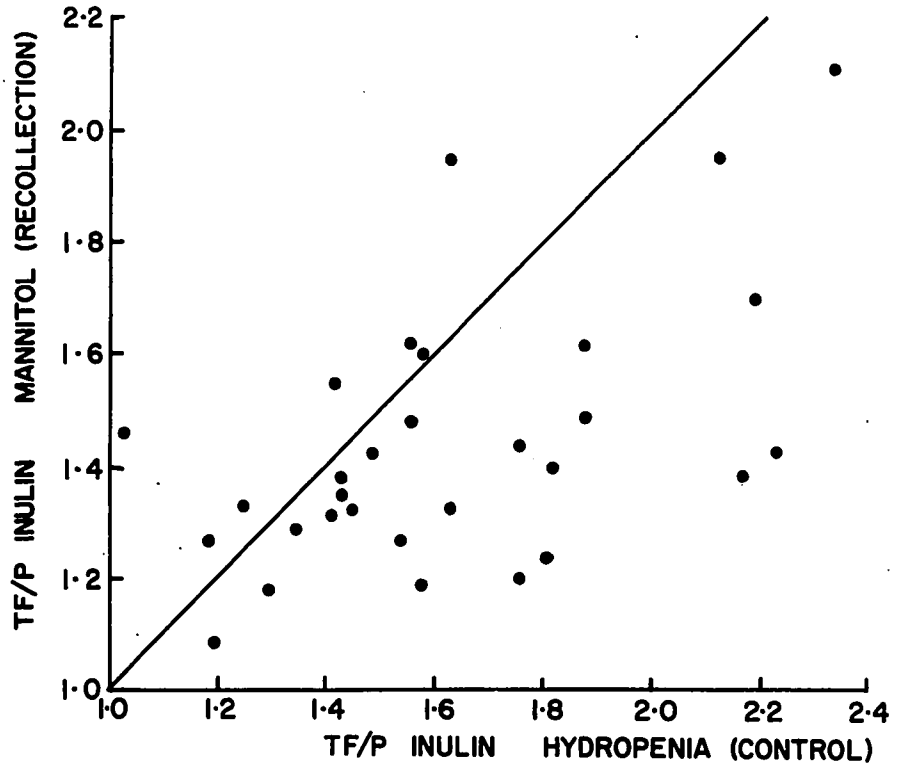

Figure A-4: Paired proximal tubule fluid to plasma (TF/P) inulin ratios. The results obtained during mannitol diuresis are plotted along the ordinate against the corresponding value obtained during the hydropenic control phase along the abscissa for each tubule sampled.

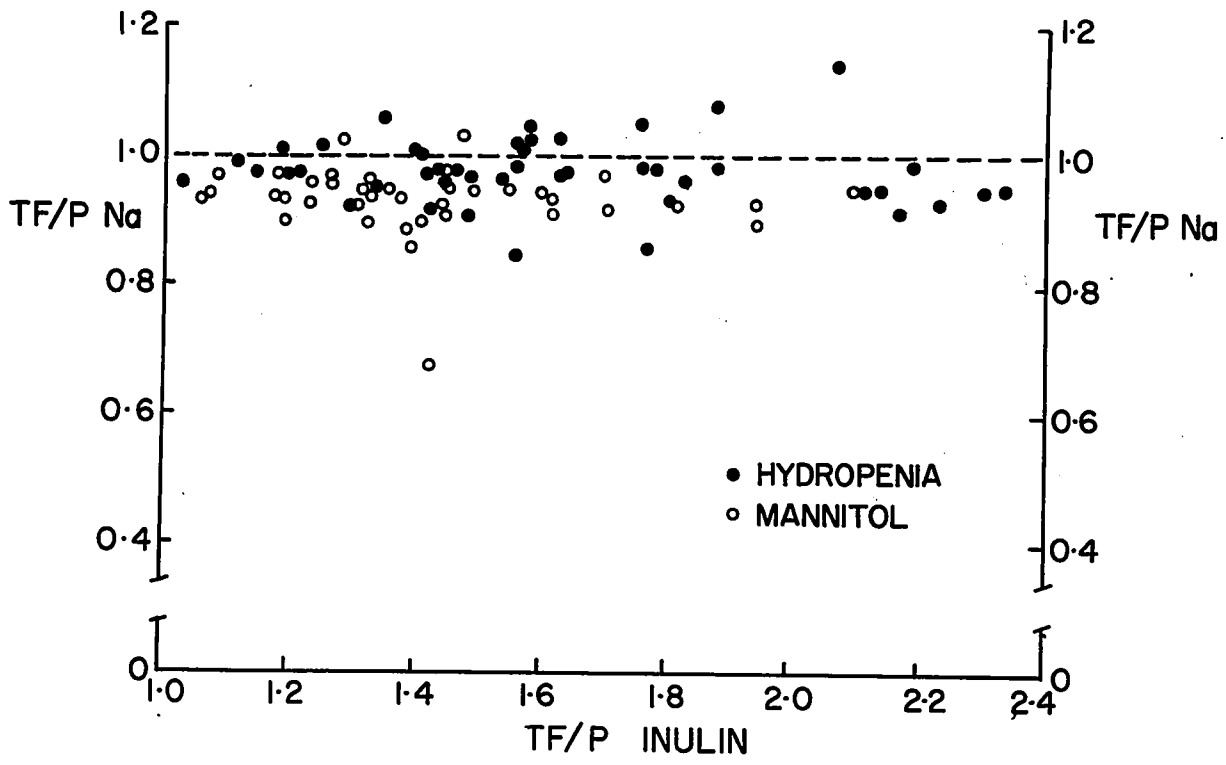

Figure A-5: Proximal tubule fluid to plasma (TF/P) sodium ratios. The values obtained during mannitol diuresis (shown by the open circles) and hydropenia (closed circles) are plotted against the corresponding tubule fluid to plasma (TF/P) inulin ratio determined in the same sample.

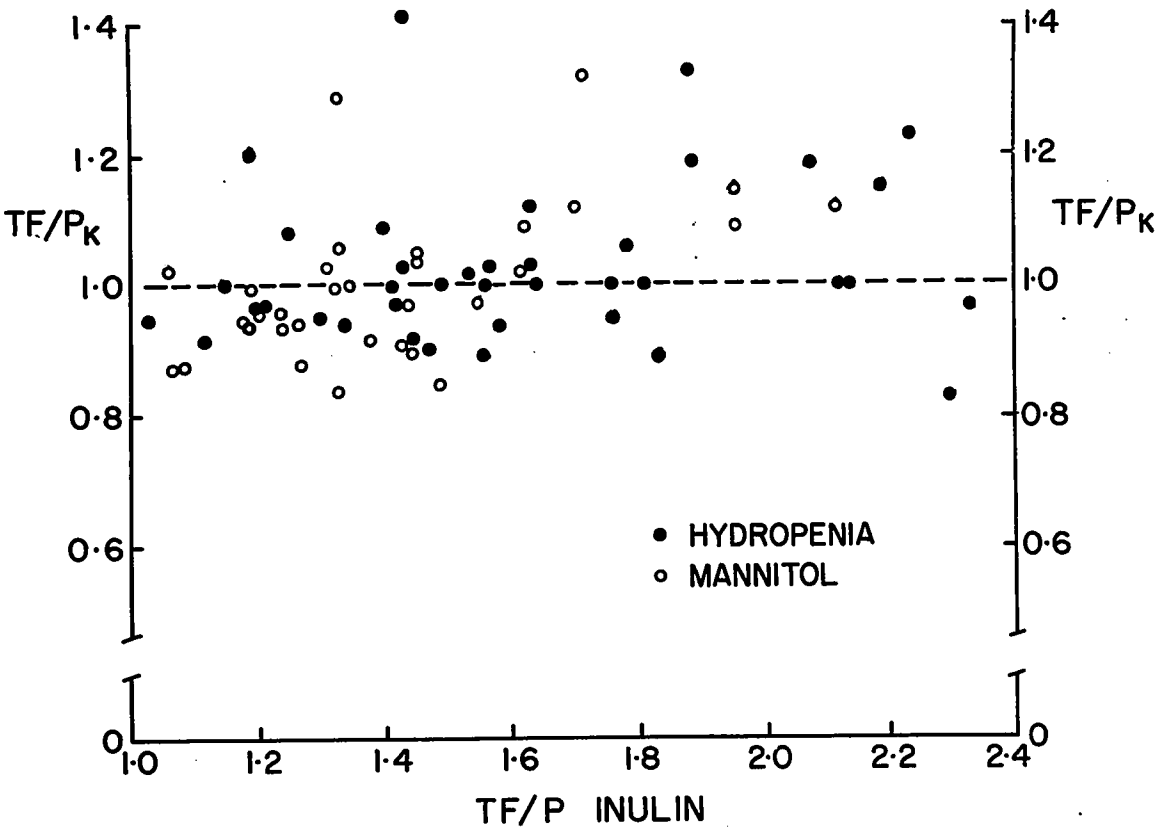

Figure A-6: Proximal tubule fluid to plasma (TF/P) potassium ratios. The values obtained during mannitol diuresis (open circles) and hydropenia (closed circles) are plotted against the corresponding tubule fluid to plasma (TF/P) inulin ratios determined in the same sample.

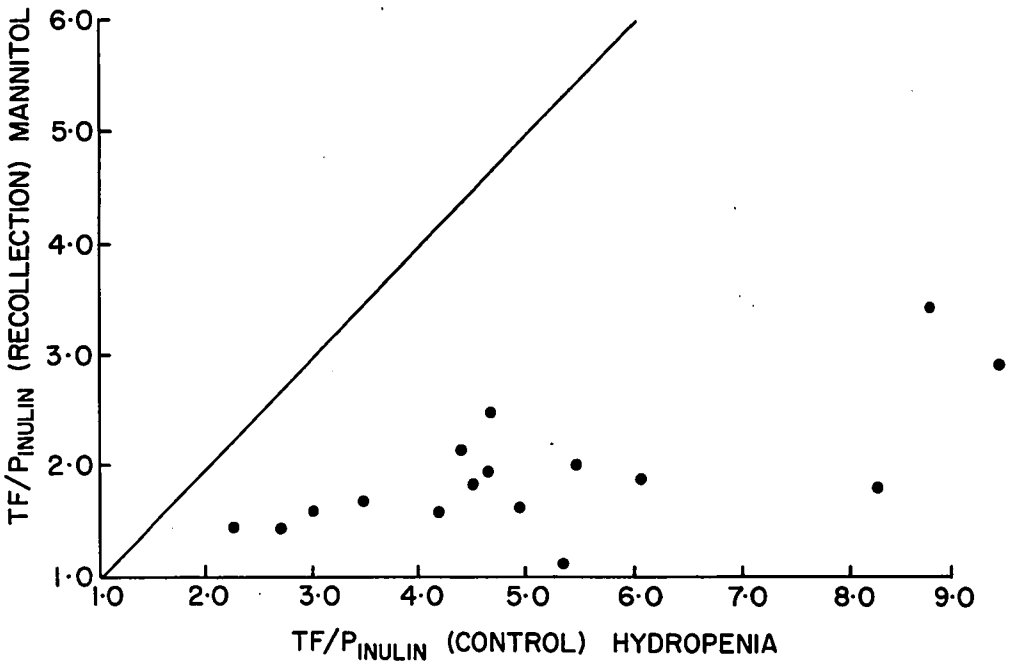

Figure A-7: Paired distal tubule fluid to plasma (TF/P) inulin ratios. Values obtained during mannitol diuresis are plotted along the ordinate against the corresponding value during hydropenia along the abscissa for each tubule sampled.

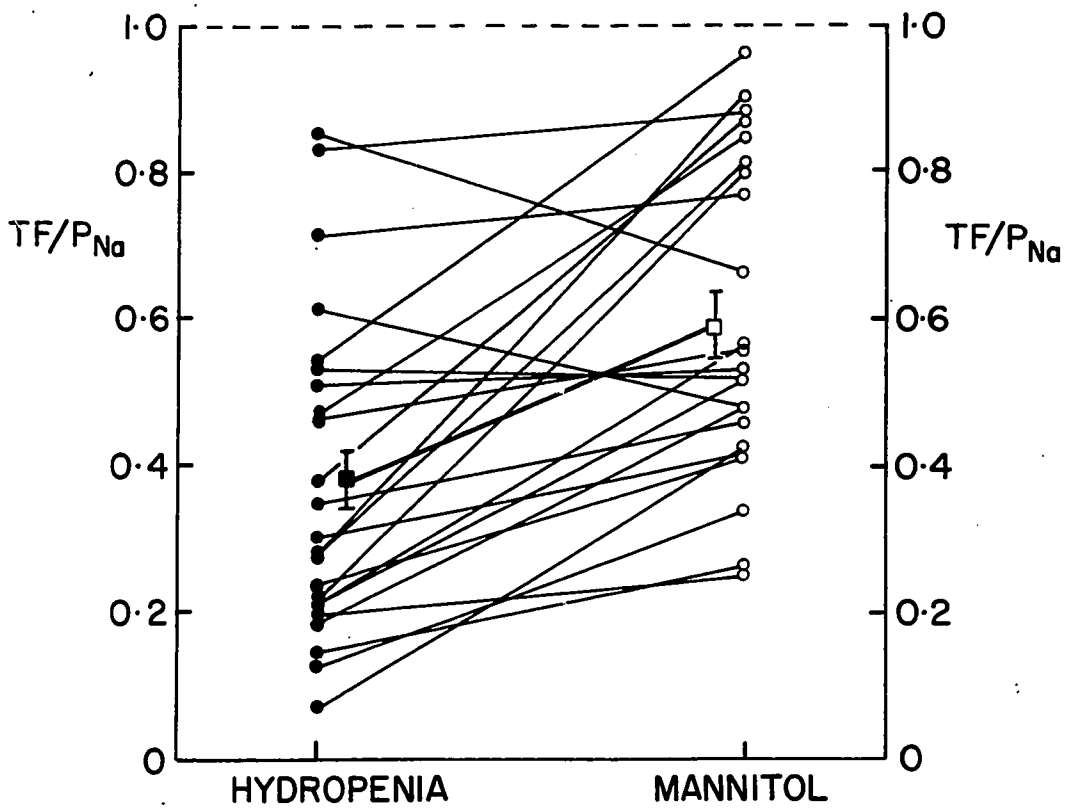

Figure A-8: Paired distal tubule fluid to plasma (TF/P) sodium ratios. The values obtained during hydropenia (shown on the left in closed circles) are joined to the corresponding value during mannitol diuresis (shown on the right by open circles) for each tubule sampled. The means ± 1 SEM are shown by the open and closed squares joined by the dark line.

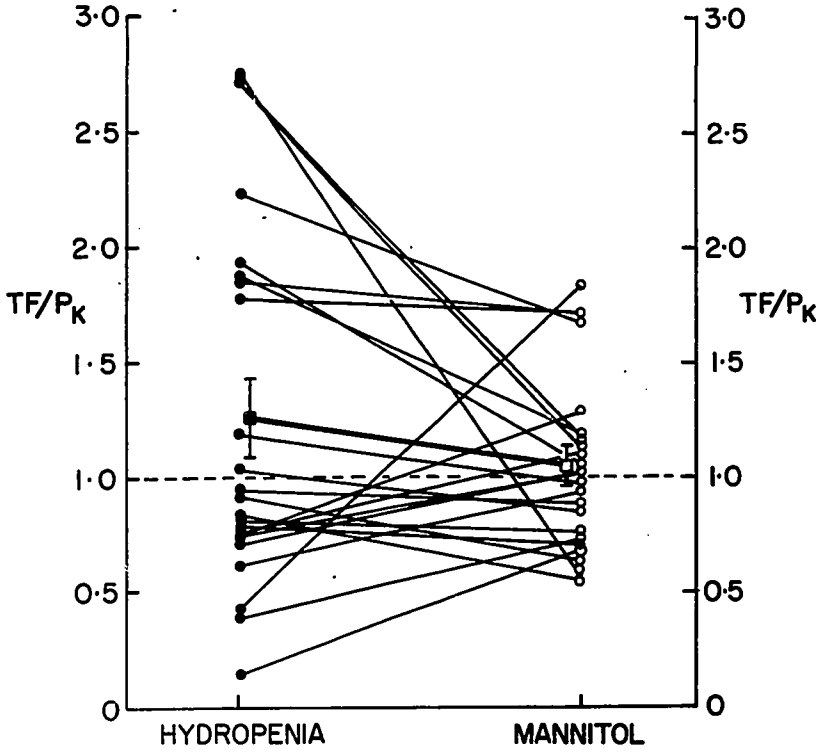

Figure A-9: Paired distal tubule fluid to plasma (TF/P) potassium ratios. The values obtained during hydropenia (shown on the left in closed circles) are joined to the corresponding value during mannitol diuresis (shown on the right by open circles) for each tubule sampled. The means ±1 SEM are shown by the open and closed squares joined by the dark line.

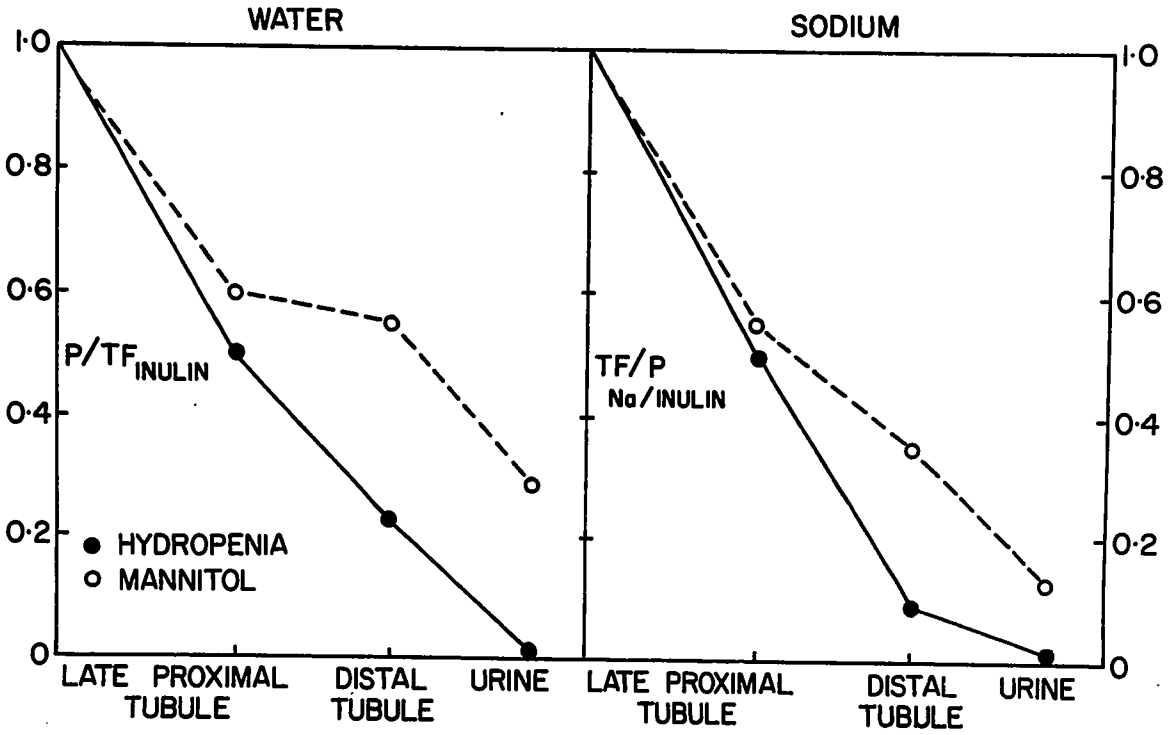

Figure A-10: Fractional rejection of water and sodium at successive nephron sites during hydropenia (solid line) and mannitol diuresis (dashed line). The values shown for the proximal tubule are estimated values for the late proximal tubule (see text). Values shown for distal tubule are the means of all distal tubule fluid samples; those for the urine were determined from the mean clearance data from the micropuncture kidney in all experiments.

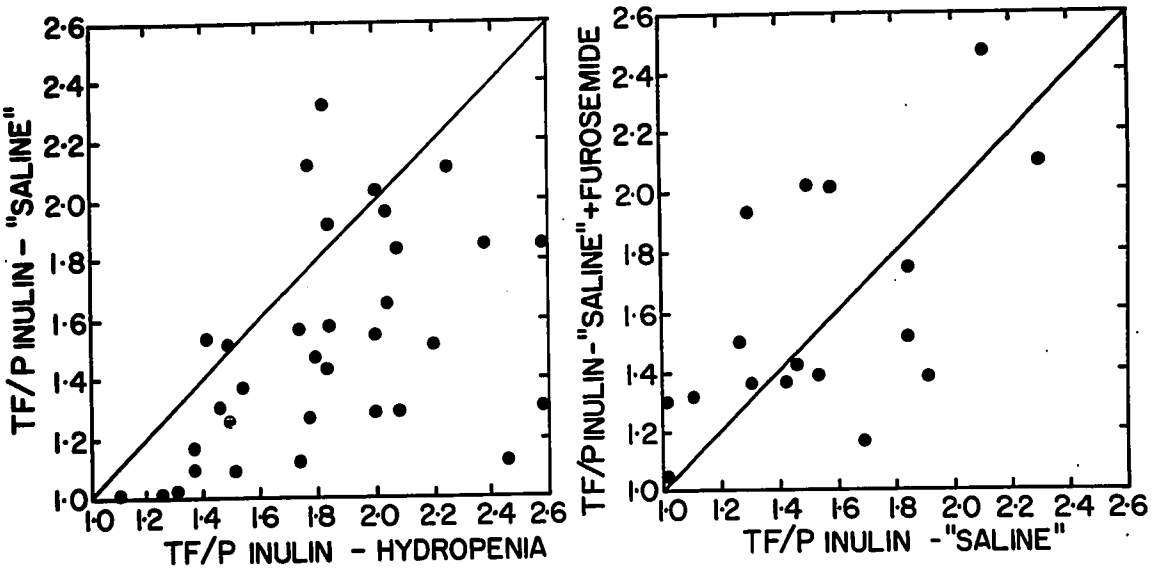

Figure A-11: Proximal TF/P inulin ratios. Results of isotonic saline loading are plotted along the ordinate against their hydropenic controls, shown along the abscissa in left-hand figure. Effects of furosemide are plotted along ordinate against their respective paired values during isotonic saline loading, shown along abscissa in right-hand figure.

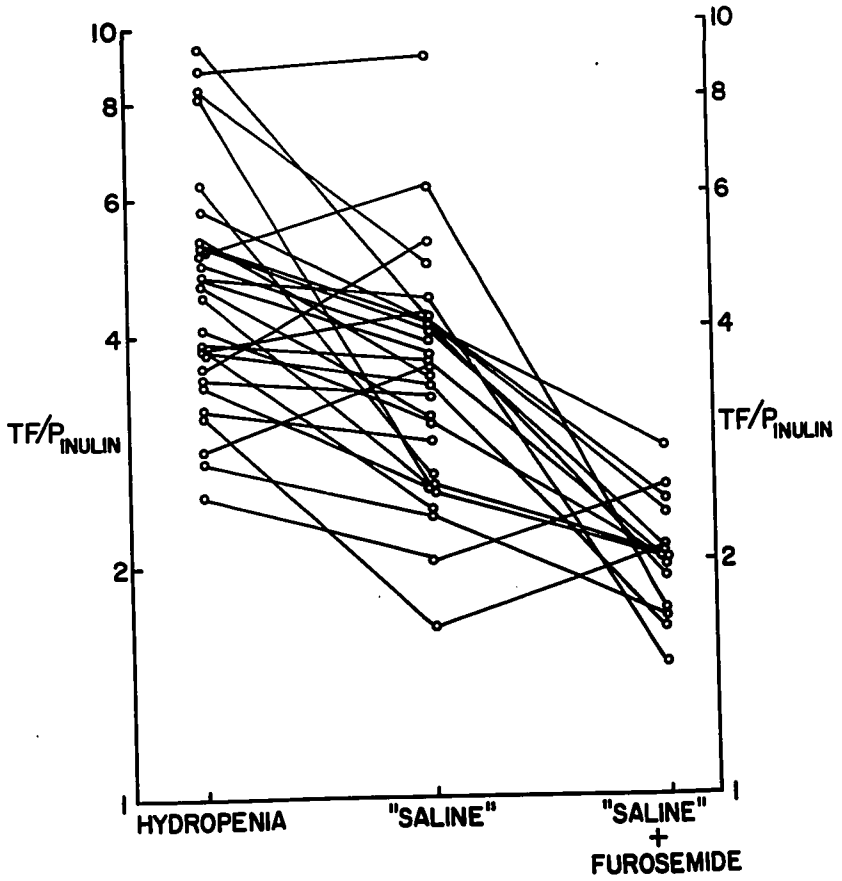

Figure A-12: Distal tubule fluid to plasma (TF/P) inulin ratios. The lines join corresponding values obtained from the same tubule puncture site during the initial control phase in hydropenia, during "saline" loading and following the addition of furosemide to a saline diuresis.

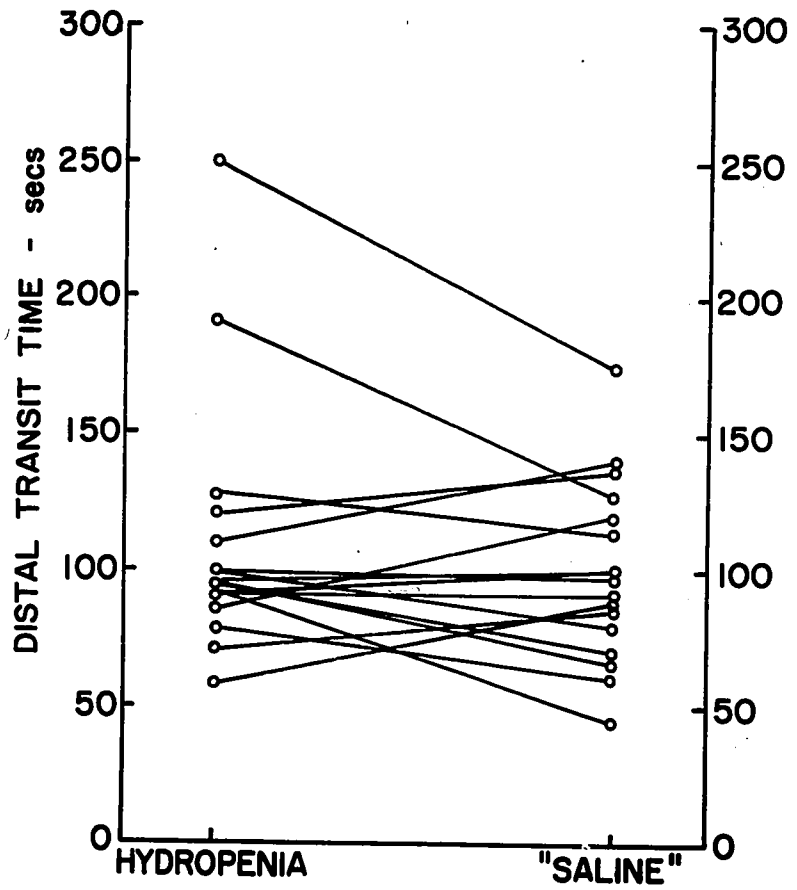

Figure A-13: Distal tubule transit times. The lines join corresponding observations from the same tubule during the initial control phase in hydropenia and following "saline" loading.

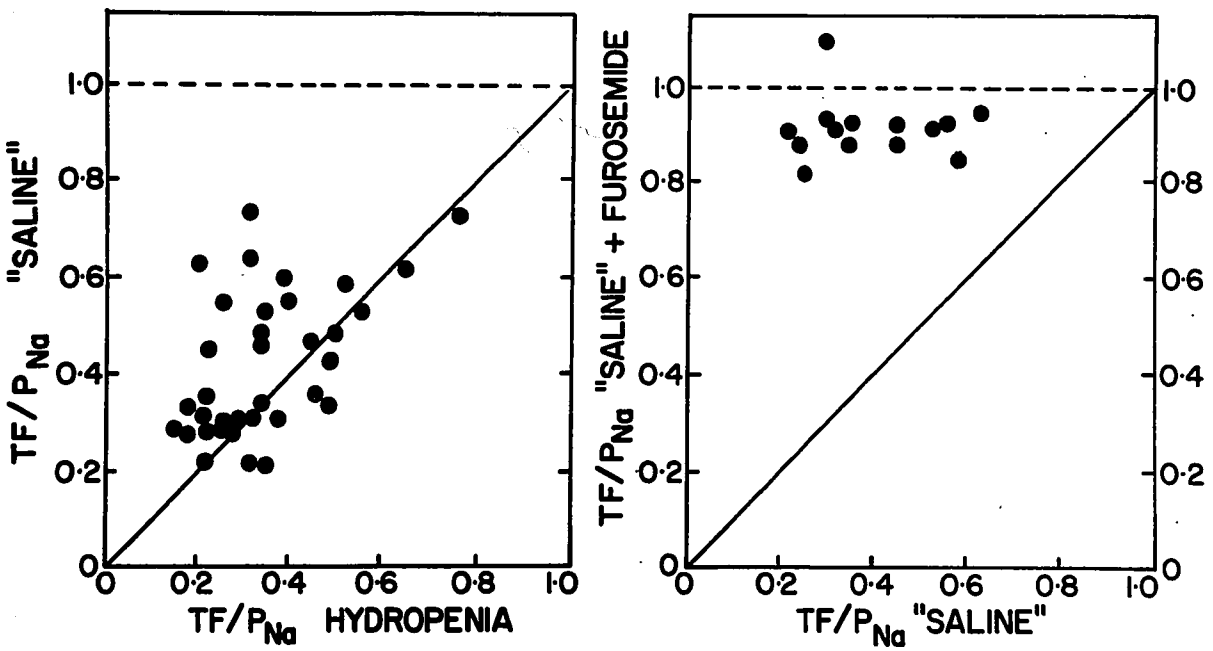

Figure A-14: Distal TF/P sodium ratios. Results of isotonic saline loading are plotted along ordinate against their paired hydropenic controls, shown along abscissa in left-hand figure. Results of furosemide are plotted along ordinate against their respective paired values during isotonic saline loading, shown along abscissa in right-hand figure.

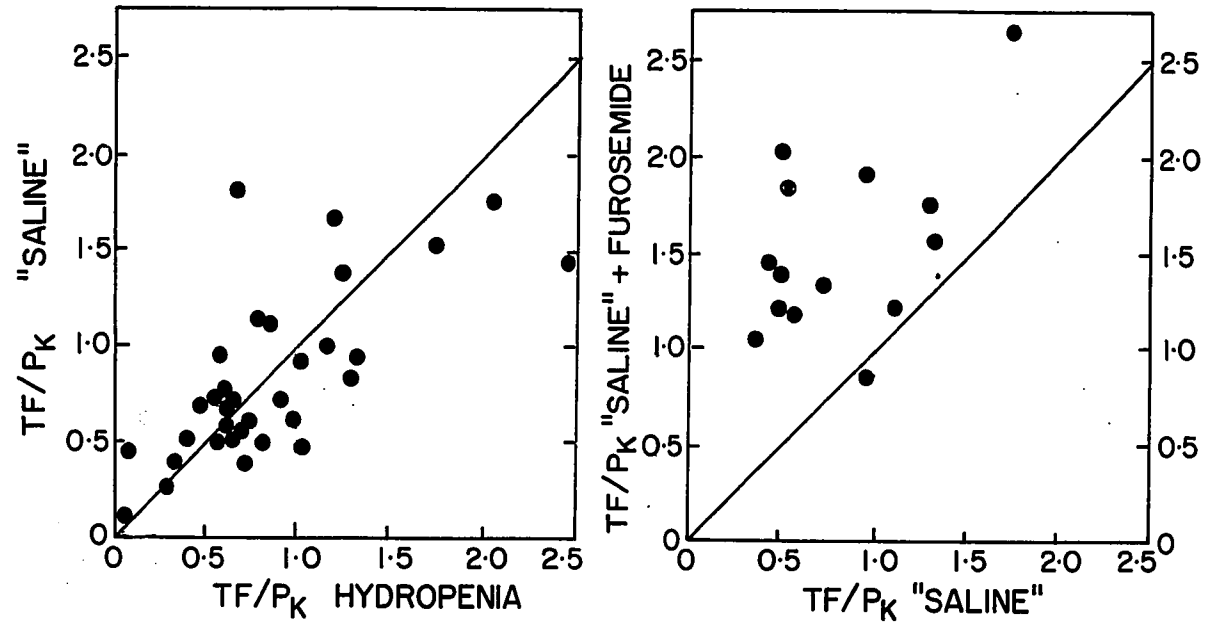

Figure A-15: Distal TF/P potassium ratios. Results of isotonic saline loading are plotted along ordinate against their paired hydropenic controls, shown along abscissa in left-hand figure. Results of furosemide are plotted along ordinate against their respective paired values during isotonic saline loading, shown along abscissa in right-hand figure.

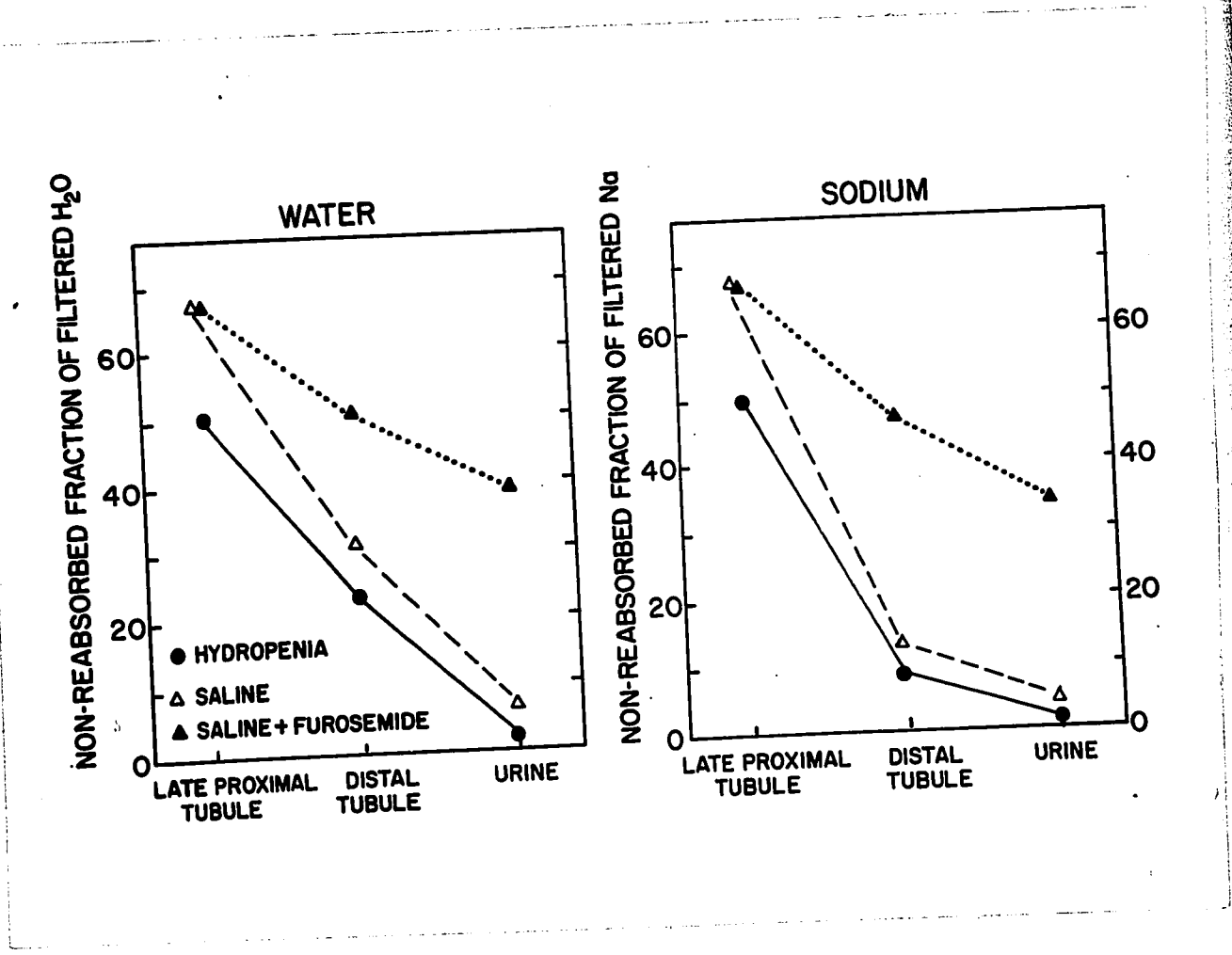

Figure A-16: Fractional rejection of water and sodium at successive nephron sites during hydropenia (solid line), saline loading (dashed line) and saline loading plus furosemide (dotted line). Values shown for the late proximal tubule were extrapolated (see text) while those shown for the distal tubule and urine were derived from the mean of all distal tubule and urine data respectively.

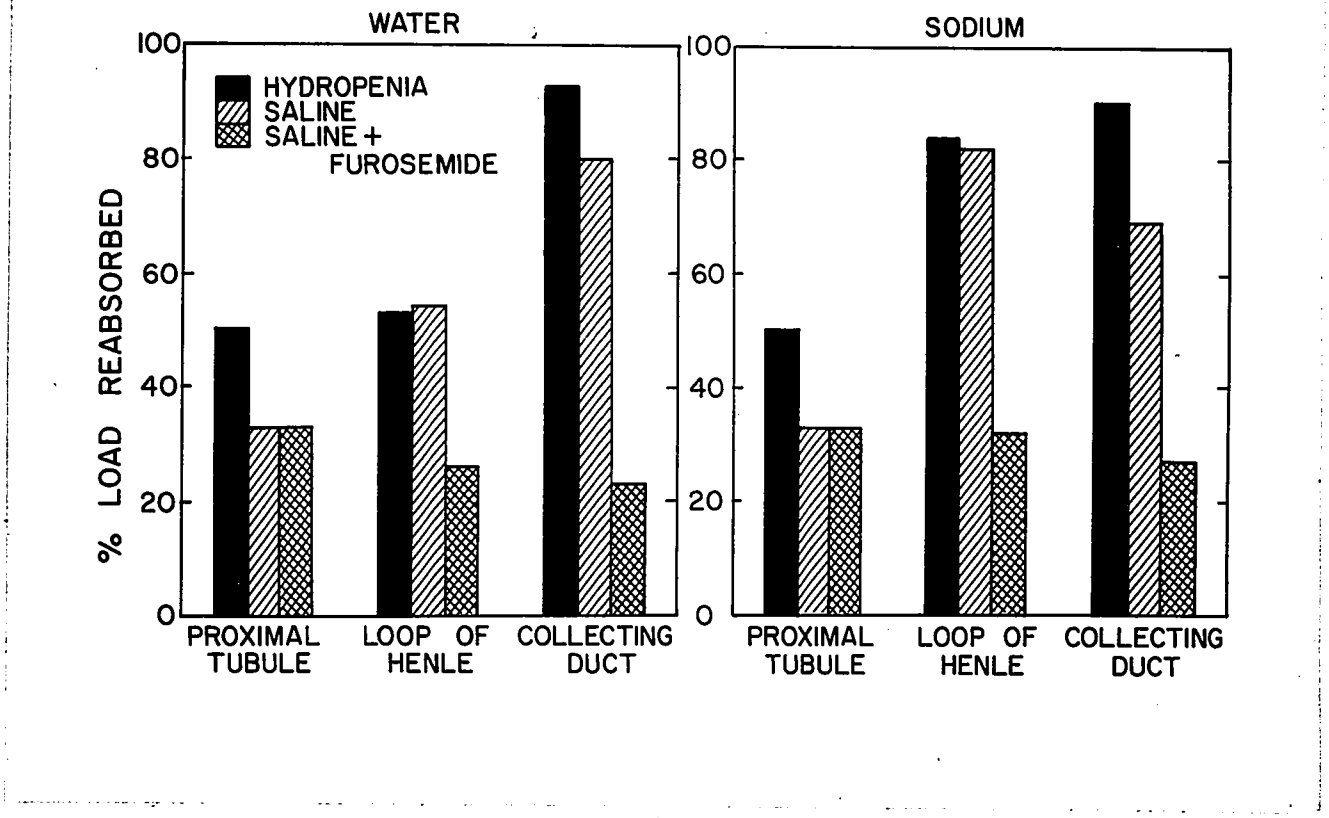

Figure A-17: Fractional reabsorption of segmental load of water (left-hand figure) and sodium (right-hand figure) during hydropenia, isotonic saline loading, and addition of furosemide to an isotonic saline load. (See text for explanation).

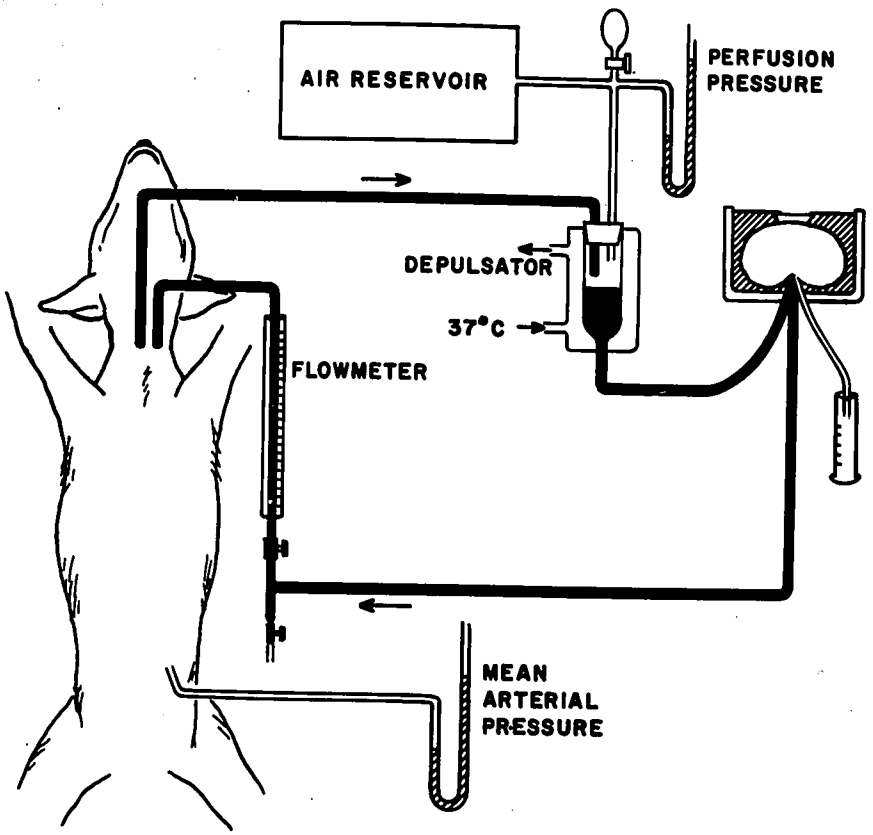

Figure B-1: Diagram of circuit for isobaric auto-perfusion of the dog kidney. Arterial blood from the carotid passes through a depulsator at 37 C connected to a damping reservoir maintaining a constant perfusion pressure. The isolated kidney is embedded in agar, leaving a small area on the surface accessible for micropuncture. Renal venous blood returns through a rotameter to the jugular vein.

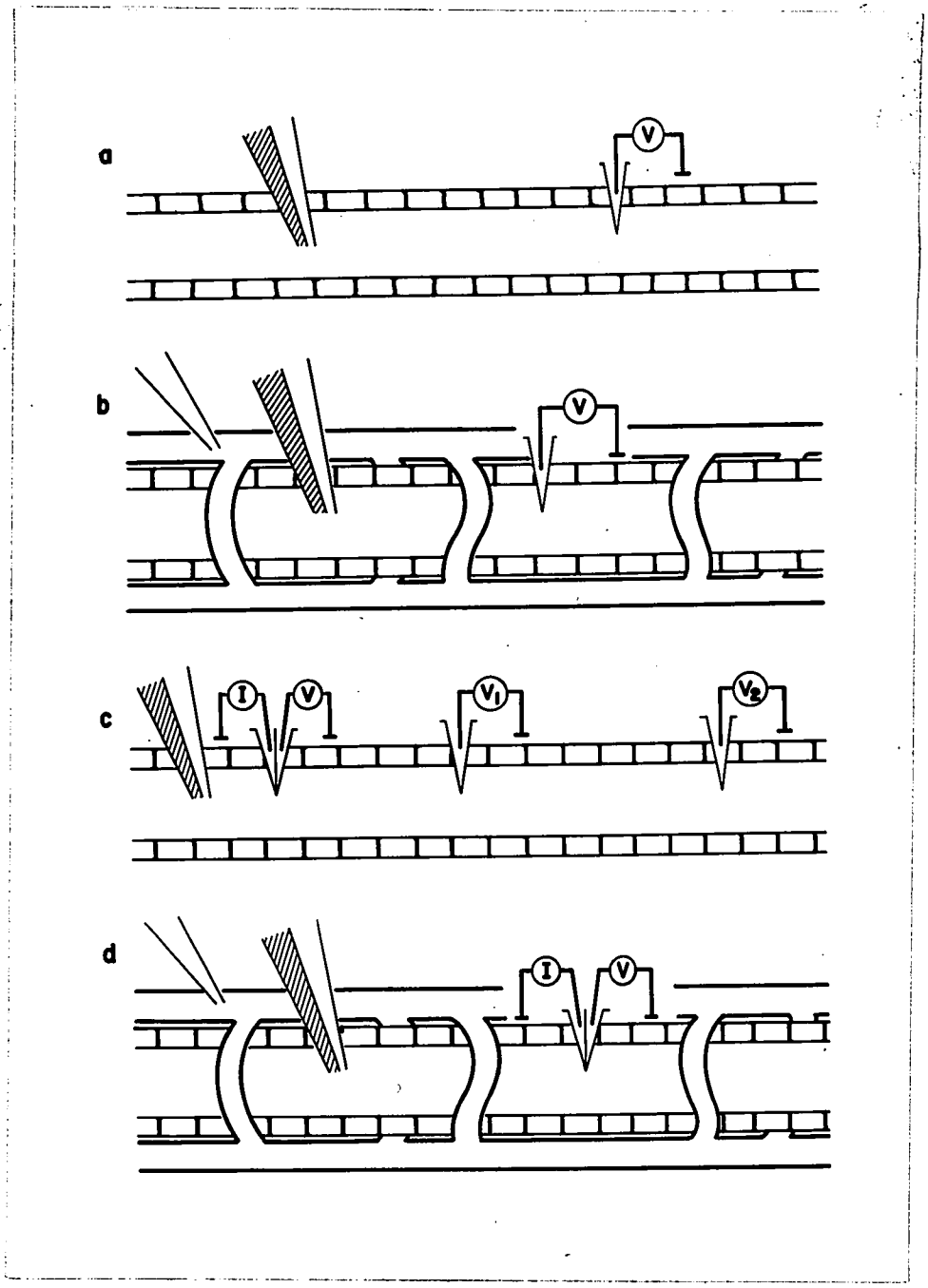

Figure B-2: Methods used for microperfusion of proximal tubules and peritubular capillaries, and for measurement of transepithelial potential differences and resistances. (See text for explanation).

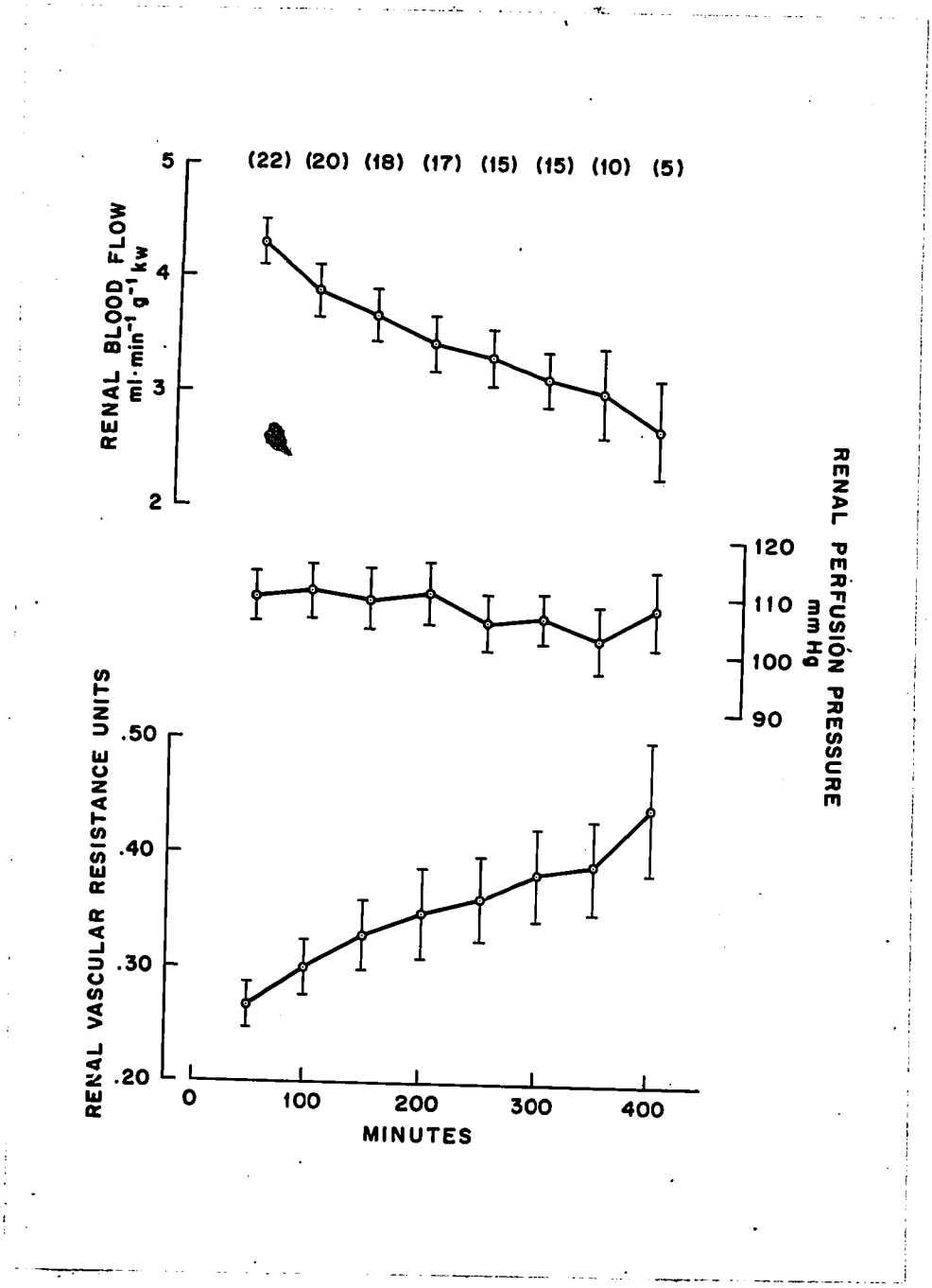

Figure B-3: Plot of total renal blood flow, perfusion pressure and vascular resistance of the isobaric auto-perfused kidney against elapsed perfusion time.

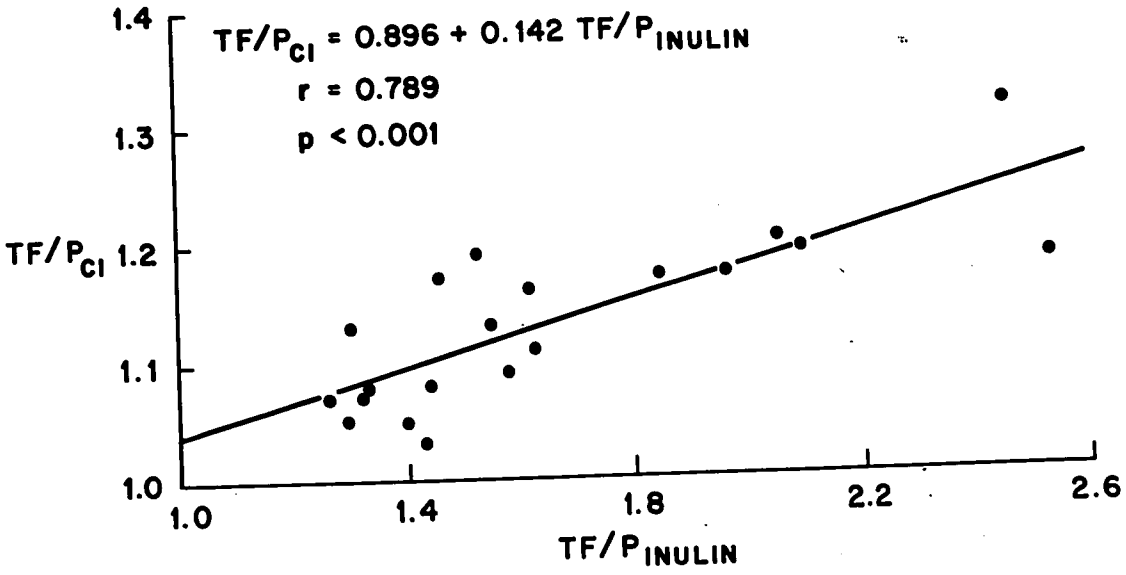

Figure B-4: Values of TF/P_{Cl} in proximal tubule fluid samples obtained from the isobaric auto-perfused kidney plotted against the corresponding TF/P_{inulin} ratios for the same samples.

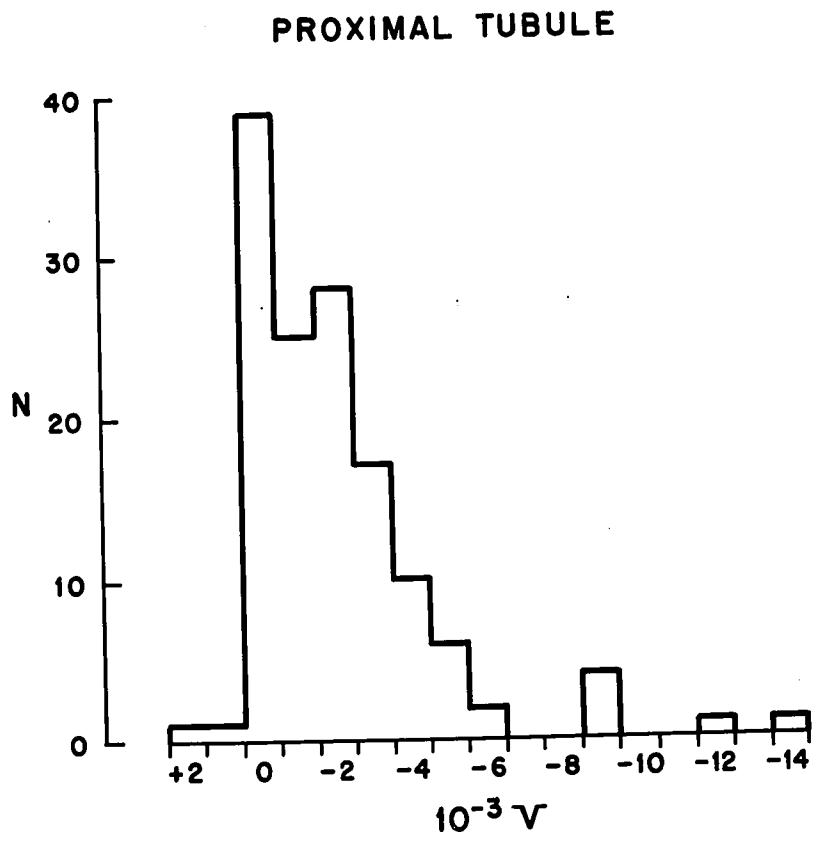

Figure B-5: Frequency distribution of transepithelial potential differences across the proximal tubules of the dog kidney.

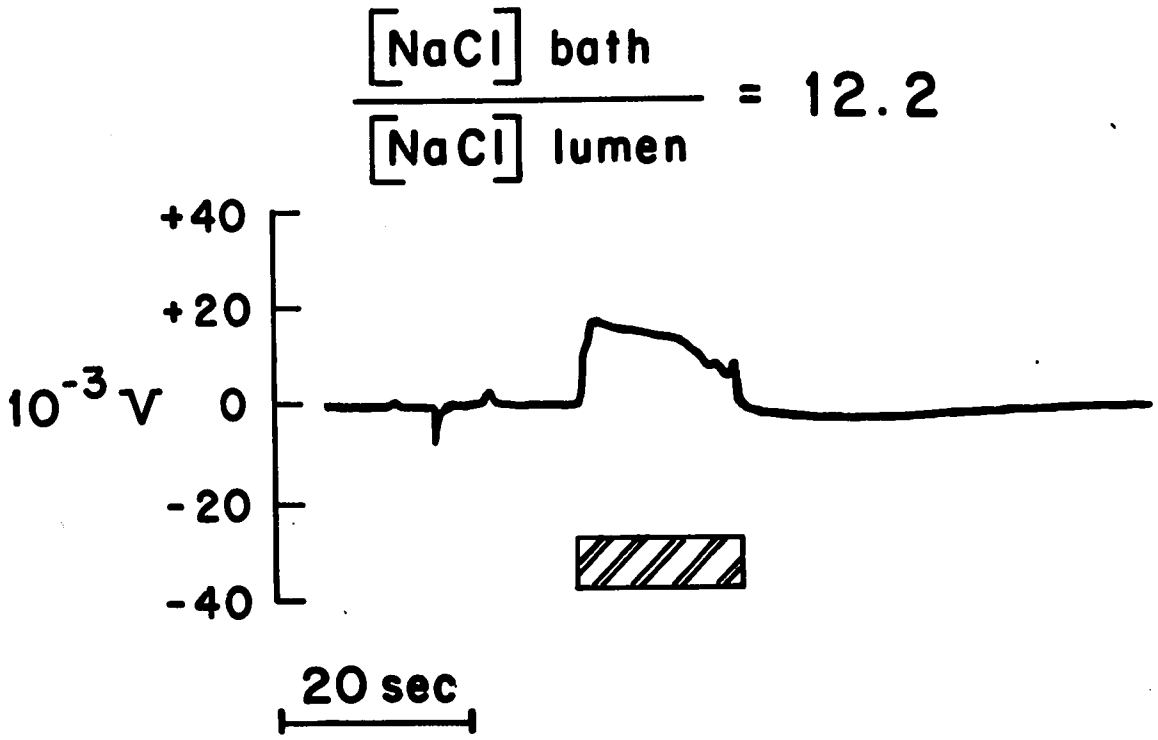

Figure B-6: Tracing from a representative salt gradient experiment. Penetration into the lumen is indicated by the initial downward deflection. The lumen was perfused for an interval corresponding to the hatched bars with a test solution in which NaCl was partly substituted by sucrose.

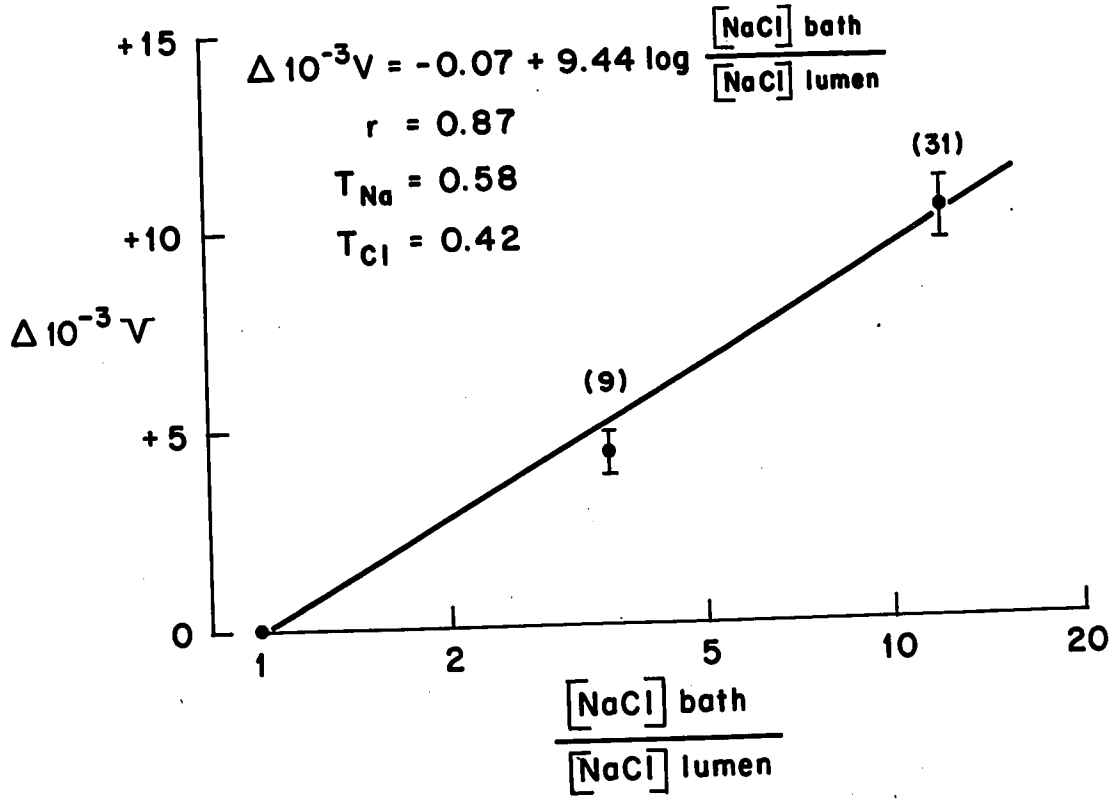

Figure B-7: Relationship between the magnitude of the transepithelial potential change during salt gradient experiments to the logarithm of the imposed concentration gradient. Mean changes ±1 S.E. are shown for the two solutions used and the line was drawn according to the regression equation shown. The number of observations are shown in brackets.

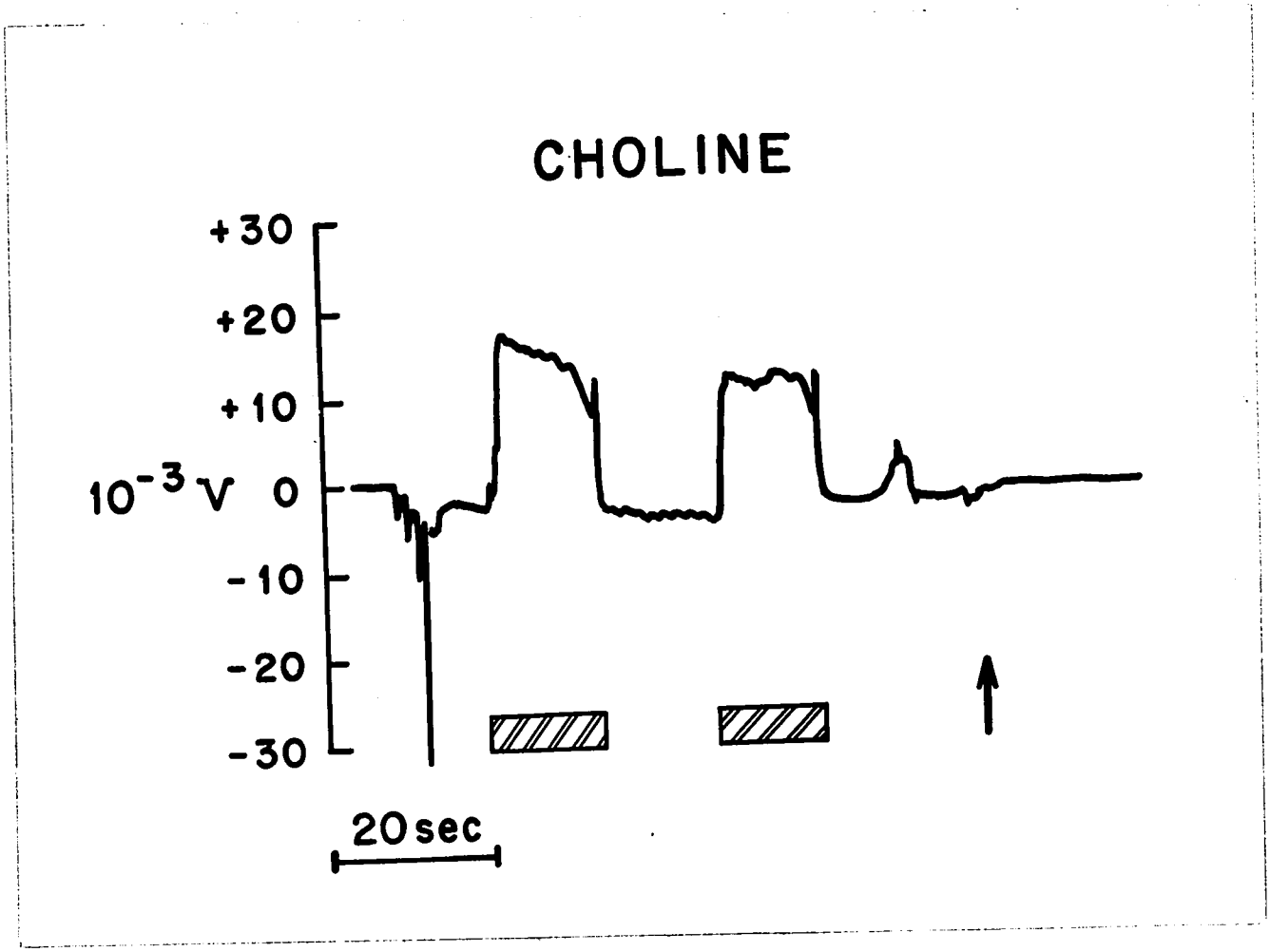

Figure B-8: Tracing from a representative cation substitution experiment. The lumen was perfused with choline Tyrode's for the intervals corresponding to the hatched bars. The arrow indicates withdrawal of the microelectrode from the lumen.

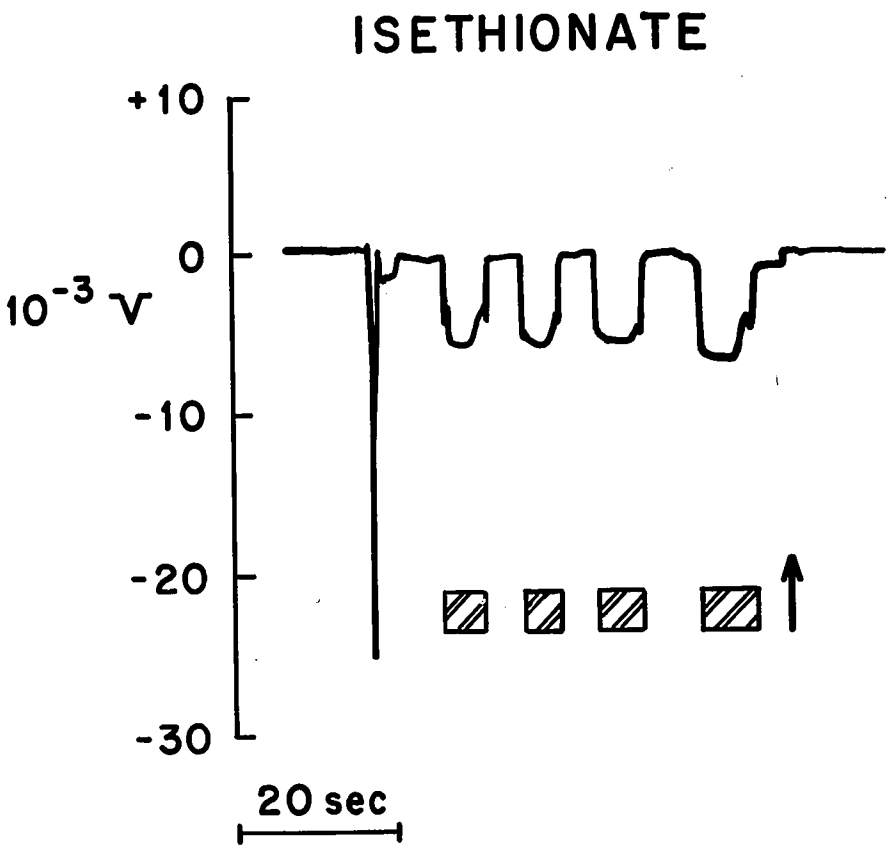

Figure B-9: Tracing from a representative anion substitution experiment. The lumen was perfused with isethionate Tyrode's for the intervals corresponding to the hatched bars.

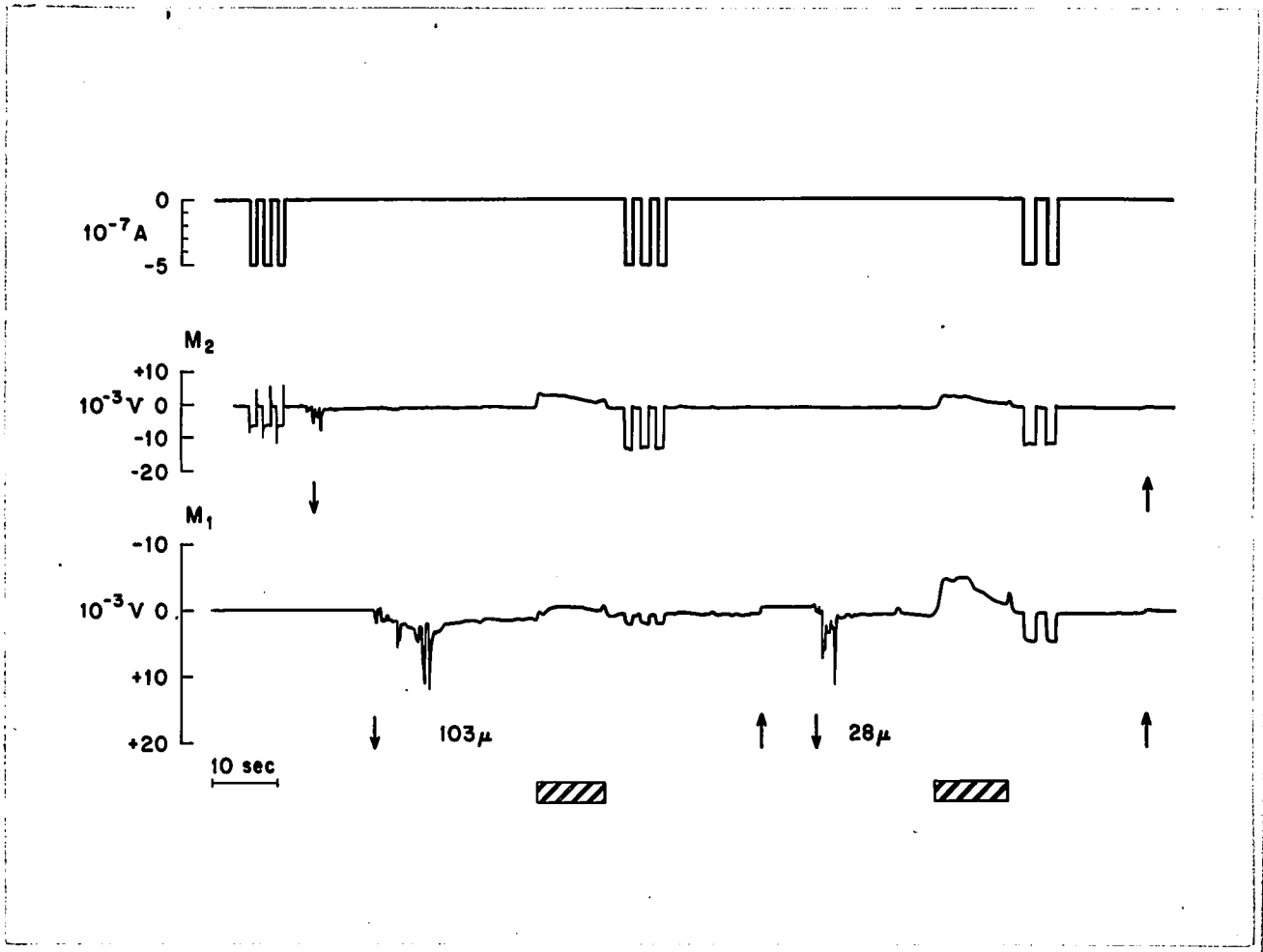

Figure B-10: Representative tracing from a cable analysis experiment. Top: Hyperpolarising current pulses applied to M_2. Middle: Electrical potential recorded continuously from M_2. Bottom: Electrical potential recorded from two successive impalements of M_1 at different distances from M_2 in the same tubule. The downward and upward pointing arrows indicate insertion and withdrawal of the microelectrodes respectively. Perfusion of the lumen with choline Tyrode's is indicated by the hatched bars.

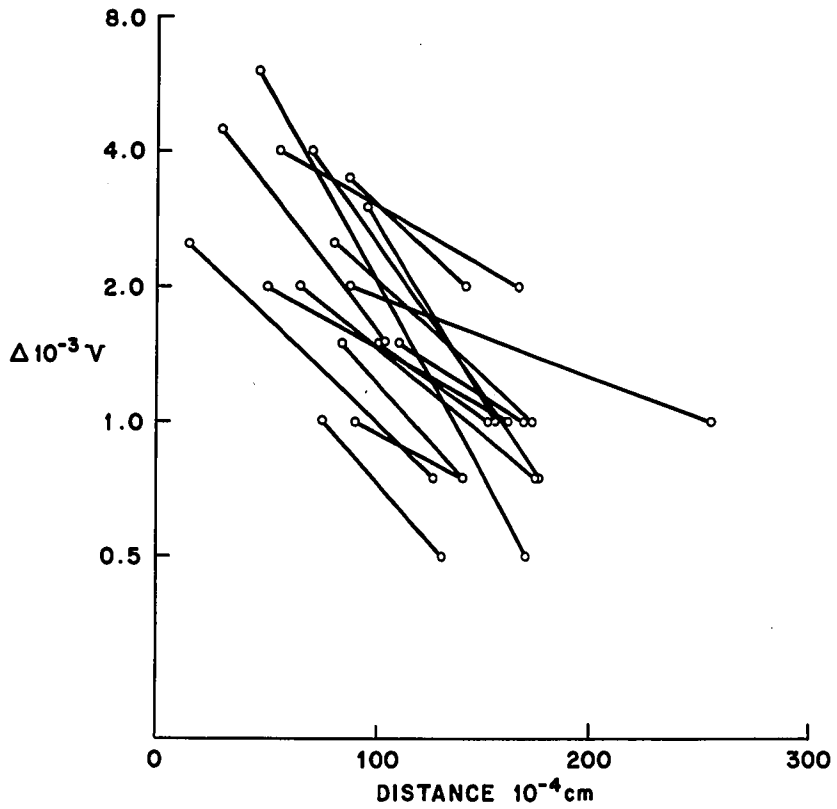

Figure B-11: Results of transepithelial cable analysis experiments. Plot of the logarithm of the voltage changes at M_1 subsequent to current injection at M_2 against the interelectrode distance between M_2 and two subsequent impalements of M_1 for each tubule.

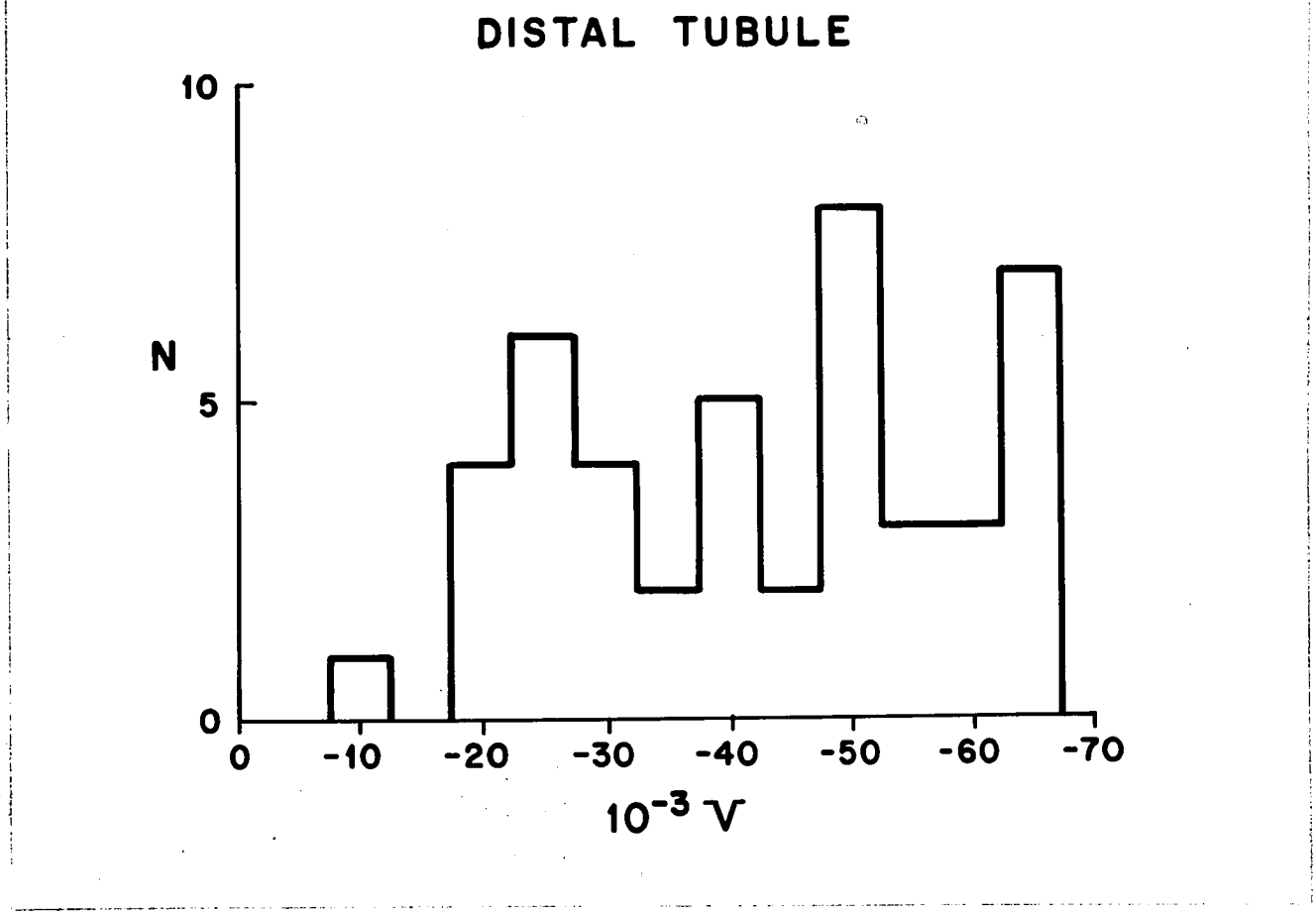

Figure B-12: Frequency distribution of transepithelial potential differences across the distal tubule of the dog kidney.

Table A-1. Mean Initial and Recollected Distal Tubule Fluid to Plasma (TF/P) Inulin, Sodium and Potassium Concentration Ratios during Continued Hydropenia

	n	Initial	Recollected
Distal TF/P inulin	34	3.79 ± .21	3.56 ± .18
Distal TF/P Sodium	18	0.35 ± .04	0.37 ± .04
Distal TF/P Potassium	17	1.15 ± .19	1.05 ± .14

Table A-2. Summary of Clearance Data, Plasma Composition and Urinary Electrolyte Excretion for all Animals during Hydropenia and Hypertonic Mannitol Diuresis*

		Hydropenia	Mannitol	P
Inulin clearance	ml/min	35.1 +2.8	25.2 +2.0	<.01
Plasma sodium	mEq/l	150.7 +1.2	144.7 +1.1	<.01
Plasma osmolality	mOs/kg H_2O	304 ±2	364 ±5	<.01
Urine flow	ml/min	.40 ±.05	6.61 ±.30	<.01
Urinary sodium excretion	uEq/min	56.5 ±9.8	425.3 ±46.5	<.01
Urinary potassium excretion	uEq/min	28.1 ±2.2	59.0 ±6.8	<.01

* Values shown are means ± 1 SEM

Table A-3. Analysis of Micropuncture Data Based on all Paired Tubule Fluid Samples*

	Proximal Tubule				Distal Tubule			
	n	Hydropenia	Mannitol	P	n	Hydropenia	Mannitol	P
TF/P inulin	30	1.63 ±.06	1.45 ±.04	<0.01	16	5.38 ±.70	1.94 ±.15	<0.01
TF/P sodium	39	0.97 ±.01	0.93 ±.01	<0.01	23	0.38 ±.05	0.59 ±.05	<0.01
TF/P potassium	32	1.05 ±.02	0.98 ±.02	<0.05	23	1.25 ±.17	1.05 ±.08	NS

Table A-4. Analysis of Micropuncture Data Based on Mean Values for the Two Phases from each Animal*

	Proximal Tubule				Distal Tubule			
	n	Hydropenia	Mannitol	P	n	Hydropenia	Mannitol	P
TF/P inulin	9	1.60 ±.08	1.42 ±.06	<0.02	10	5.67 ±.69	2.00 ±.18	<0.01
TF/P sodium	12	0.98 ±.01	0.94 ±.01	<0.01	11	0.40 ±.07	0.57 ±.06	<0.02
TF/P potassium	10	1.06 ±.04	0.96 ±.04	NS	11	1.38 ±.22	1.17 ±.11	NS

* Values shown are means ± SEM, NS = not significant.

Table A-5. Fractional Rejection (%) of Water, Sodium and Potassium in the Proximal Tubule, Distal Tubule and Final Urine during Hydropenia and Hypertonic Mannitol Diuresis*

	Proximal Tubule			Distal Tubule			Urine		
	Hydropenia	Mannitol	P	HYdropenia	Mannitol	P	Hydropenia	Mannitol	P
Water	63.7 ±2.4	70.8 ±1.9	<.01	22.6 ±2.4	55.3 ±3.5	<.01	1.28 ±.19	28.7 ±2.0	<.01
Sodium	62.3 ±2.4	65.9 ±2.0	NS	9.3 ±2.1	35.2 ±4.7	<.01	1.06 ±.15	12.6 ±1.1	<.01
Potassium	67.3 ±3.1	70.3 ±2.1	NS	26.8 ±4.0	52.4 ±6.1	<.01	23.8 ±2.6	63.9 ±5.3	<.01

* Values shown are mean ± SEM, NS = not significant

Table A-6. Summary of Plasma Composition, Inulin Clearance, Urine Flow and Electrolyte Excretion during Hydropenia, Isotonic Saline Infusion and Furosemide Administration

	Hydropenia (20)	Saline (20)	Furosemide (12)
P_{Na} mEq/l	150.1 ±1.0	153.1 ±.9	154.3 ±1.3
P_K mEq/l	3.36 ±.07	3.36 ±.10	3.08 ±.10
C_{IN} ml/min	32.1 ±2.3	30.1 ±1.8	29.9 ±2.1
$U_{Na}V$ uEq/min	40.4 ±5.9	171.5 ±15.3	1633.4 ±249.5
U_KV uEq/min	25.0 ±1.8	38.1 ±3.2	106.6 ±7.6
V ml/min	0.43 ±.05	1.83 ±.16	12.2 ±1.5

Values shown are means ± 1 SEM:
(n) = number of animals
Abbreviations: P_{Na} and P_K = plasma sodium and potassium concentrations;
C_{IN} = inulin clearance;
$U_{Na}V$ and U_KV = urinary excretion of sodium and potassium;
V = urine flow

Table A-7. Summary of Tubule Fluid to Plasma (TF/P) Inulin, Sodium and Potassium Ratios during Hydropenia, Isotonic Saline Infusion and Furosemide Administration

	Proximal Tubule			Distal Tubule		
	TF/P_{IN}	TF/P_{Na}	TF/P_{K}	TF/P_{IN}	TF/P_{Na}	TF/P_{K}
Hydropenia	1.83 ±.07 (33)	1.01 ±.01 (25)	1.07 ±.02 (19)	4.78 ±.34 (40)	.34 ±.02 (36)	.92 ±.11 (35)
Saline	1.49** ±.06 (33)	1.01 ±.01 (25)	1.00* ±.03 (19)	3.60** ±.26 (40)	.42** ±.03 (36)	.91 ±.12 (35)
Furosemide	1.61 ±.10 (16)	1.01 ±.01 (9)	.99 ±.05 (7)	2.03** ±.08 (18)	.91** ±.02 (15)	1.53** ±.13 (14)

Values shown are means ± 1 SEM. (n) = number of observations.

* = significantly different from paired values of preceding phase, $p < .05$;

** = $p < .01$

Table B-1. Composition of Solutions (in mM)

	Solution	Na^+	K^+	Cl^-	HCO_3^-	$H_2PO_4^-$	Ca^{++}	Mg^{++}	Substituent ion (italics)	Sucrose
1)	Normal Tyrode	149.1	2.7	144.2	11.9	0.3	1.8	0.5	-	--
2)	42 mM Na^+	42.2	2.7	37.3	11.9	0.3	1.8	0.5	-	194.5
3)	12 mM Na^+	12.2	5.4	10.0	11.9	0.3	1.8	0.5	-	244
4)	Hypotonic 12 mM Na^+	12.2	5.4	10.0	11.9	0.3	1.8	0.5	-	171
5)	KCl	12.2	139.6	144.2	11.9	0.3	1.8	0.5	-	--
6)	Choline chloride	12.2	2.7	150.5	11.9	0.3	1.8	0.5	143.2	-
7)	0 mM HCO_3^-	149.1	2.7	156.1	0	0.3	1.8	0.5	-	-
8)	25 mM HCO_3^-	149.1	2.7	131.1	25.0	0.3	1.8	0.5	--	-
9)	75 mM HCO_3^-	149.1	2.7	81.1	75.0	0.3	1.8	0.5	--	-
10)	Na propionate	149.1	2.7	7.3	11.9	0.3	1.8	0.5	136.9	-
11)	Na isethionate	149.1	2.7	7.3	11.9	0.3	1.8	0.5	136.9	-
12)	Na acetylglycinate	149.1	2.7	7.3	11.9	0.3	1.8	0.5	136.9	-
13)	Na cyclamate	149.1	2.7	7.3	11.9	0.3	1.8	0.5	136.9	-
14)	Na sulfate	149.1	2.7	0	11.9	0.3	1.0*	0.5	71.3	91.7
15)	Tyrode + 300 mOsm/l	149.1	2.7	144.2	11.9	0.3	1.8	0.5	-	292
16)	Tyrode + 600 mOsm/l	149.1	2.7	144.2	11.9	0.3	1.8	0.5	-	584

* Obtained from dissociation of highest concentration of $CaSO_4$ soluble in water, about 8 mM/liter.

Table B-2. Summary of Excretory Data for the Perfused (Left) and Control (Right) Kidney in 22 Dogs

	Left	Right	
Urine volume (ml min^{-1})	0.45 ± 0.06	0.57 ± 0.09	N.S.
Urine [Na] (mEq l^{-1})	82.5 ± 9.8	98.4 ± 10.9	N.S.
Urine [K] (mEq l^{-1})	84.0 ± 11.5	90.2 ± 12.4	N.S.
Urine osmolarity (mOsm l^{-1})	631.8 ± 61.5	673.3 ± 62.9	N.S.
$U_{Na}V$(μEq min^{-1})	42.3 ± 7.9	56.2 ± 10.1	N.S.
$U_{K}V$(μEq min^{-1})	28.4 ± 2.9	34.1 ± 3.3	N.S.

Values are means ± S.E.M.
Abbreviations: N.S. = difference statistically not significant
$U_{Na}V$ = urinary sodium excretion rate
$U_{K}V$ = urinary potassium excretion rate.

Table B-3. Summary of clearance and renal hemodynamic data for the perfused (L) and control (R) kidney in 11 dogs.

Dog#	B.W. Kg	Hct. %		K.W. g.	GFR $ml.min^{-1}$	V $ml.min^{-1}$	U/P_{osm}	U/P_{Na}	U/P_K	V/GFR %	$F.E._{Na}$ %	P.P. mm.Hg	M.A.P. mm.Hg.	R.B.F. $ml.min^{-1}$	R.P.F. $ml.min^{-1}$	F.F.
1	19.8	-	L	47	19.7	1.15	1.02	0.80	10.4	5.82	4.17	125		237	-	-
			R		34.1	1.66	1.14	1.29	13.1	4.86	3.64		140			
2	20.4	46	L	46	28.2	1.19	1.57	0.86	13.6	4.33	3.76	121		171	92	0.311
			R		38.9	0.63	2.98	1.23	26.3	1.68	2.16		127			
3	17.9	44	L	39	28.8	0.47	1.55	0.54	16.3	1.60	0.86	100		135	76	0.379
			R		33.8	0.78	1.23	0.96	11.9	2.30	2.20		135			
4.	15.9	46	L	55	16.0	0.38	0.67	0.15	11.7	2.40	0.37	90		90	49	0.327
			R		16.8	0.73	0.52	0.10	9.3	4.30	0.51		100			
5	13.6	37.5	L	30	22.8	0.23	2.56	0.97	19.3	1.00	0.95	100		115	72	0.317
			R		27.8	0.29	2.76	1.39	17.3	1.06	1.47		90			
6	24.5	43.5	L	69	22.1	0.54	1.23	0.49	14.7	2.59	1.31	139		202	114	0.196
			R		35.1	1.53	0.89	0.39	8.5	4.59	1.87		146			
7	18.2	41.2	L	42	18.9	0.23	2.59	0.76	26.2	1.24	.96	135		132	78	0.242
			R		31.2	0.57	2.64	1.30	16.5	1.84	2.08		150			
8	19.1	41	L	49	19.0	0.13	3.10	0.15	31.8	.69	.11	75		230	136	0.140
			R		27.8	0.32	2.77	0.67	31.3	1.70	.83		100			
9	25.9	42.5	L	65	33.7	0.52	1.77	0.35	16.8	1.53	.59	100		264	152	0.224
			R		42.4	0.89	1.49	0.48	13.6	2.09	1.18		110			
10	21.4	43	L	66.5	20.4	0.13	3.58	0.06	7.6	.64	.03	100		197	112	0.182
			R		22.9	0.16	2.13	0.19	9.7	.68	.15		112			
11	21.4	44	L	59	19.1	0.43	2.21	0.87	22.5	2.26	2.00	115		323	181	0.105
			R		29.1	0.46	2.47	0.82	25.0	1.65	1.33		130			
mean	19.8	42.9	L mean	51.5	22.6	0.49	1.99	0.55	17.35	2.19	1.37	109		191	106	0.243
S.E.	±1.1	±0.8	S.E.	±3.7	±1.6	±0.11	±0.27	±0.10	±2.16	±0.48	±0.42	±5.9		±21.2	±12.9	±0.028
			R mean		30.9	0.73	1.91	0.80	16.59	2.43	1.58		122			
			S.E.		±2.2	±0.14	±0.27	±0.14	±2.32	±0.44	±0.29		±6.2			
					P<0.01	N.S.	N.S.	P<0.01	N.S.	N.S.	N.S.		P<0.01			

B.W. = body weight. Hct = hematocrit. K.W. = kidney weight. GFR. = glomerular filtration rate. V = urine flow. U/P = urine to plasma concentration ratio. F.E. = fractional excretion. P.P. = perfusion pressure. M.A.P. = mean arterial pressure. R.B.F. = renal blood flow. R.P.F. = renal plasma flow. F.F. = filtration fraction. N.S. = Difference statistically not significant in paired t test at the level of P.0.05.

Table B-4. Summary of Micropuncture Data

	Mean	Standard Error	N.
TF/P Inulin	1.61	± 0.07	26
(1 - P/TF Inulin) x 100*	35.2	± 2.4	26
TF/P Cl*	1.13	± 0.02	20
$(1 - \frac{\text{TF/P Cl}}{\text{TF/P Inulin}}) \times 100$*†	32.6	± 2.4	20

Abbreviations: TF = tubular fluid concentration
P = plasma concentration

* plasma concentrations were corrected for plasma water concentration of 94%

† plasma chloride concentration also corrected for Donnan factor (1.05)

Table B-5. Comparison of transit times, proximal tubular radii and estimated proximal water and chloride fluxes for the perfused and intact dog kidney

	Perfused	Intact	
		Free flow	Stop flow
Proximal transit time (sec)	31.07 ± 3.8 (13)	18.2 ± 1.9 (4)	
Distal transit time (sec)	70.8 ± 5.0 (13)	48.0 ± 3.3 (4)	
Proximal tubular radius (10^{-4}cm)	17.4 ± 0.6 (68)	15.8 ± 3.1* 12.6** 12.3 ± 0.5 (11)†	16.6** 17.8 ± 0.41 (11)†
Reabsorptive capacity ($10^{-2}sec^{-1}$)	$\frac{In(TF/P)\ Inulin}{T.T.}$	$\frac{In(TF/P)\ Inulin}{T.T.}$	$\frac{0.693}{t\ 1/2}$
	4.07 ± 0.74 (11)	6.28* 3.75 ± 0.24 (8)** 3.53 ± 0.21 (16)†	4.23 ± 0.29 (8)** 3.98 ± 0.25 (11)†
J_{H_2O} ($10^{-5}cm^3cm^{-2}sec^{-1}$)	3.54 ± 0.64 (11)	4.96* 3.08 ± 0.16 (8)** 2.7 ± 0.13 (16)†	3.47 ± 0.17 (8)** 3.55 ± 0.23 (11)†
J_{Cl} (10^{-9}Eq $cm^{-2}sec^{-1}$)	3.93 ± 0.72 (11)		

Values are means ± standard error of the mean, followed by the number of observations in brackets.
* from Levine et al. (1968).
**calculated from data of Wright et al. (1969).
† calculated from data of Knox et al. (1969).

Table B-6. Relative ionic permeabilities derived from potential changes

Substituent solution	$\Delta 10^{-3}$ V.*	P_{I^+}/P_{Na}**	P_{I^+}/P_{Cl}**	P_{I^+}/P_{Cl} in free solution†
KCl	-1.4 ± 0.2 (8)	1.10	1.52	0.97
Choline Cl	+8.2 ± 0.4 (69)	0.49	0.68	0.51
$NaHCO_3$ 75 mM	-1.8 ± 0.1 (9)		0.63	0.58
Na propionate	-5.5 ± 0.4 (10)		0.53	0.47
Na isethionate	-6.2 ± 0.3 (12)		0.47	0.46
Na acetylglycinate	-6.3 ± 0.6 (13)		0.46	0.51
Na cyclamate	-9.3 ± 0.6 (10)		0.26	0.41
Na sulfate	-4.5 ± 0.2 (37)			

* All differences significant to the level $P < 0.001$
** P_I permeability coefficient for the substituent cation or anion
† Calculated from limiting equivalent conductivities at 25 C.

Table B-7. Cable Analysis on 16 Proximal Tubules

R_{input} (V_o/I)	27.5 ± 4.7 (16)	10^3 ohm
Length constant (λ)	93.6 ± 9.2 (16)	10^{-4} cm
Specific internal resistance (R_i)	55.0	ohm cm
Specific membrane resistance (R_m)	5.58	ohm cm^2
Electrical radius (a)	17.3	10^{-4}cm

Table B-8. Comparison of Estimates of Partial Chloride Conductance

1) From transference numbers obtained in NaCl salt gradient:

$$G_{Cl} = T_{Cl}\, G_m = T_{Cl}\, \frac{1}{R_m} = 0.42\ (17.9) = 75.18\ 10^{-3}\ ohm^{-1}\ cm^{-2}$$

2) From relative conductance changes in absence of chloride:

$$\frac{G_m^{\,C}}{G_m^{\,T}} = \frac{G_m^{\,T} - G_{Cl} + G_C}{G_m^{\,T}} = \frac{(R_{input}^{\,T})^2 R_i^{\,C}}{(R_{input}^{\,C})^2 R_i^{\,T}} = 0.71$$

$$G_{Cyclamate} = 0.26\ G_{Cl}$$

$$G_{Cl} = \frac{0.29}{0.74}\, G_m^{\,T} = 0.39\ (17.9) = 69.81\ \ 10^{-3}\ ohm^{-1}\ cm^{-2}$$

Mean partial chloride conductance:

$$G_{Cl} = 72.5\ \ 10^{-3}\ ohm^{-1}\ cm^{-2}.$$

Superscripts "C" and "T" are used to indicate whether the measurement was made in Cyclamate Tyrode's (in absence of Cl) or in normal Tyrode solution respectively.

TABLE B-9. Comparison of Transepithelial Chloride Fluxes

Predicted net flux from passive forces:

$$J_{Cl} = \frac{G_{Cl}\,(V-E_{Cl})}{z\,F} = 3.91\ 10^{-9}\ \text{Eq cm}^{-2}\ \text{sec}^{-1}$$

$$G_{Cl} = 72.5\ 10^{-3}\ \text{ohm}^{-1}\ \text{cm}^{-2}$$

$$V = -\ 2\ 10^{-3}\ \text{V}$$

$$E_{Cl} = \frac{RT}{zF}\ \text{In}\ (TF/P)_{Cl} = +3.24\ 10^{-3}\ \text{V}$$

$$(TF/P)_{Cl} = 1.13^{*}$$

Observed net flux:*

$$J_{Cl} = J_{H_2O}\ (Cl)_r = 3.93\ \ 10^{-9}\ \text{Eq cm}^{-2}\ \text{sec}^{-1}$$

* Taken from Table B-5.

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