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Water, urea, sodium, chloride, and potassium transport in the in vitro isolated perfused papillary collecting duct

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The collecting ducts are formed in the renal cortex by the connection of several nephrons. They descend within the medullary rays of the cortex, penetrate the outer medulla, and in the inner medulla successively fuse together. Based on these topographical considerations, we can recognize three segments of the collecting duct system: the cortical collecting tubule, the outer medullary collecting duct, and the papillary collecting duct. In this review, we call papillary collecting duct (PCD) that part of the collecting duct system that extends from the junction of outer medulla and inner medulla to the area cribosa. The collecting ducts in the outer medullary zone rarely have branches; however, such branches present in the inner zone have hampered study by micropuncture and microcatheterization techniques. The cortical collecting tubule (CCT) contains two types of cells, principal and intercalated cells, whereas in most animals the PCD contains only principal cells [1]. The general ultrastructure of the PCD cell seems simpler than that of the cortical collecting tubule, suggesting that the PCD is less specialized and less metabolically active than other nephron segments [2]. However, the accumulated data reveal a remarkable reabsorptive capacity for water and sodium by the PCD [3–6], thus indicating that this final part of the nephron plays an important role in the regulation of salt and water balance.

Before the development of the in vitro isolated perfused tubule technique, the method of directly studying transepithelial transport in individual nephron segments was in vivo micropuncture. However, this technique was limited to the examination of those nephron segments accessible from either the cortical surface or the tip of the papilla. In studying PCD of the rat kidney, two methods have been used: The first consists of micropuncture of terminal duct segments lying at the surface of the exposed papilla tip, and the second, that of microcatheterization, consists of advancing fine polyethylene catheters into the collecting duct via the exposed tip of the papilla. In antidiuretic conditions, both techniques showed net reabsorption of salt, urea, and water in the PCD. During extracellular fluid volume expansion, however, the results obtained with both methods were opposed diametrically [7]. Technical problems encountered in these methods have been reviewed recently [7, 8].

In this article, our objective is to analyze transepithelial transport of sodium, chloride, potassium, urea, and water in the PCD and to describe some studies done in our laboratory illustrating the application of renal tubule perfusion in vitro to the examination of transtubular transport. Several adaptations of the method of isolated renal tubule perfusion described originally by Burg et al have been made to allow microperfusion of the isolated PCD in vitro [9]. PCD segments are dissected from a section of inner medulla of freshly killed animals without the use of enzymatic procedures to aid in the dissection. Because the CCT of the rabbit has been the easiest to dissect among the collecting duct segments, it has been the most widely studied collecting duct segment. PCD's are quite difficult to isolate, but in the middle part of the terminal papilla, dissection is facilitated by the fact that the tubular lumen is open; this helps one to visualize the branches and thus to separate short segments (0.8 mm) between branches or larger segments having two or more branches. This improvement of technique made it possible for us to perfuse in vitro the PCD not only of rabbits, but also of rats, guinea pigs, and humans. The in vitro perfusion of rabbit PCD allows the investigator to compare ion and water transport in this segment with that observed in the CCT using the same technique.

Water and urea transport in the PCD. The main function attributed to the PCD is osmotic equilibration between the hypertonic interstitium and tubular fluid in the presence of ADH, thus generating a hypertonic urine. A relationship between concentrating ability and characteristics of urea and water transport in the PCD could be demonstrated by measuring urea and water transport in the in vitro isolated perfused PCD [10] of animals with different urinary concentrating capacities: rats with a maximum urine osmolality of 3,500 mOsm/kg H_2O, and rabbits and guinea pigs with a maximum determined urine osmolality of about 1,200 mOsm/kg H_2O. As shown in Table 1, the diffusional water permeability (P_{Dw}), measured by the disappearance of ^3H_2O added to the perfusate in the presence of ADH, was twice in the rat as that measured in the rabbit and guinea pig. Moreover, the osmotic water permeability (P_{Osm}) in the rat PCD was tenfold higher than that of the rabbit and guinea pig. These results suggest that osmotic equilibration between the tubular fluid of PCD and the medullary interstitium must be obtained faster in the rat than in the rabbit and guinea pig.

The apparent urea permeability, measured from the disappearance of ^{14}C-urea added to the perfusate, was high in the PCD of rats and was increased threefold by the addition of

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Table 1. Papillary collecting duct ^{14}C-urea permeability, osmotic (P_{Osm}) and diffusional ^3H_2O water (P_{Dw}) permeabilities[a]

		Rat	Rabbit	Guinea pig
$P_{Urea} \times 10^{-5}$ cm · sec^{-1}	Control	13.8 ± 2.5[c]	2.0 ± 0.2	1.8 ± 0.3
	ADH	36.2 ± 5.4[b,c]	2.0 ± 0.3	2.6 ± 0.6
$P_{Osm} \times 10^{-6}$ cm · at^{-1} sec^{-1}	ADH	48.0 ± 8.6[b,c]	5.0 ± 1.2	5.4 ± 1.7
$P_{Dw} \times 10^{-5}$ cm · sec^{-1}	Control	70 ± 11[c]	22 ± 5	24 ± 2
	ADH	113 ± 19[b,c]	54 ± 8[b]	48 ± 7[b]
Reflection coefficient for urea	ADH	0.35 ± 0.03[c]	0.74 ± 0.05	—
Reflection coefficient for NaCl	ADH	1.00 ± 0.05	1.00 ± 0.03	—

[a] The values are expressed as mean ± SEM. ADH is in presence of vasopressin (2 mU/ml) in the bath.
[b] $P < 0.05$ ADH vs. control.
[c] $P < 0.05$ rat vs. rabbit or rat vs. rabbit and guinea pig.

vasopressin to the bath. These results are similar to those observed in the PCD of excised rat papillae studied by a micropuncture technique in vitro [11]. In contrast, the PCD of the rabbit and guinea pig showed a much lower urea permeability than the rat, and this value was unchanged by the addition of vasopressin to the bath. These properties are summarized in Table 1 [10, 12].

During antidiuresis, ADH-enhanced water abstraction from urea-impermeable cortical and outer medullary collecting tubules results in the accumulation of urea in fluid entering the PCD [12, 13]. Since the latter is urea permeable, passive urea transport down a chemical gradient from tubular fluid to medullary interstitium contributes to medullary hypertonicity. There is also evidence for recycling urea from medullary interstitium into pars recta lumen by secretion, ultimately to reach PCD lumen again. The high urea permeability in the rat PCD creates conditions to generate, in hydropenia, a greater urea concentration in the medulla, and thus urea recycling might be prominent in this species. The low urea permeability in the PCD of rabbits and guinea pigs, on the other hand, indicates that urea recycling may be less significant in these animals. Thus, these urea transport differences between rat and rabbit PCD could explain the observation that urea administration produces an important enhancement of concentrating ability in rats but a lesser effect in rabbits [14].

In Figure 1 the water and urea permeabilities of isolated in vitro perfused PCD of normal rats, guinea pigs, rabbits, newborn rats, and rats with chronic renal failure are plotted against their maximal urine osmolalities [10, 15, 16]. The high correlation obtained indicates that urinary concentrating capacity is related quantitatively to the osmotic and urea permeabilities of PCD. In these measurements there was also a close correlation between the magnitude of osmotic and urea permeabilities.

The relationship between water and solute transtubular transport can be estimated in the in vitro PCD by measuring the reflection coefficients of various solutes. A comparison of net water absorption driven by a urea or sodium chloride gradient, as opposed to the net absorption generated by an osmotic gradient of raffinose, gave a urea reflection coefficient of 0.35 and 0.74 in the rat and rabbit, respectively; the reflection coefficient for sodium chloride was 1.0 in both animals [10, 12]. Since the main solutes in the papillary interstitium are urea and sodium chloride, we can conclude from these results: first, the urea efflux in the PCD should be followed in the same direction by water movement, and second, sodium chloride in the

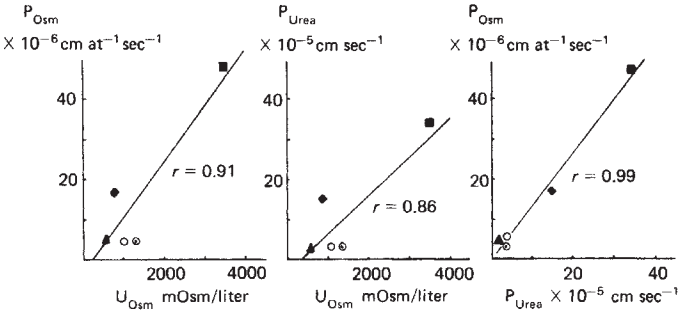

Fig. 1. *Relationship of maximum urine osmolality (U_{Osm}) to osmotic water permeability* (P_{Osm}; right panel) *and urea permeability* (P_{Urea}, central panel). The correlation between osmotic water permeability and urea permeability is plotted in the left *panel*. Each *point* is the mean of all observations in the perfused papillary collecting duct isolated from normal rats (■), newborn rats (▲), chronic renal failure rats (◆), rabbits (⊙), and guinea pigs (○).

interstitium is the principal driving force for water abstraction in the PCD, and hence, for increases in urine osmolality.

The in vitro experiments described above indicate the presence of urea efflux in the PCD; however, several questions concerning the nature of this transport remain to be answered. When animals are maintained on a regular diet, the urea concentration in the interstitial medulla is much lower than in the urine. This suggests that urea leaves the PCD by simple diffusion from a higher to a lower concentration [17, 18]. However, carrier-mediated urea transport cannot be excluded, nor can the participation of such passive processes as single file and exchange diffusion.

Several studies have demonstrated that in antidiuretic sheep and rats fed a low protein diet, the urea concentration in the inner medulla was higher than in the urine; urea reabsorption in this condition proceeds against a chemical gradient [17–21]. These data have been interpreted as suggesting active transport of urea across the PCD. Studies utilizing the in vitro microperfusion technique were designed to examine the possibility of active urea transport in the PCD of rats maintained on a normal diet [10]. In these experiments, isotopic unidirectional urea fluxes, measured sequentially in the same tubule, showed a significant net urea absorption in the absence of net water transport or of a chemical or osmotic gradient, suggesting the presence of active urea absorption in the rat PCD. Figure 2

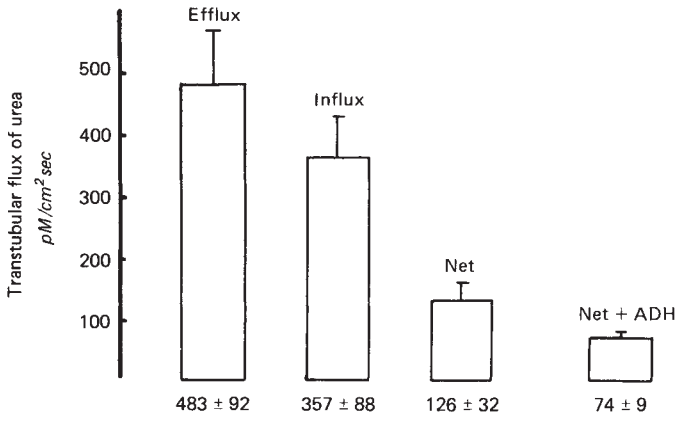

Fig. 2. *Unidirectional urea fluxes measured in the isolated in vitro perfused papillary collecting duct obtained from rat kidney.* The perfusion and bath fluid was isotonic Ringer/HCO_3 solution plus urea 50 mg%. Experiments were performed at 37°C. Net urea absorption did not change significantly by the addition of vasopressin to the bath.

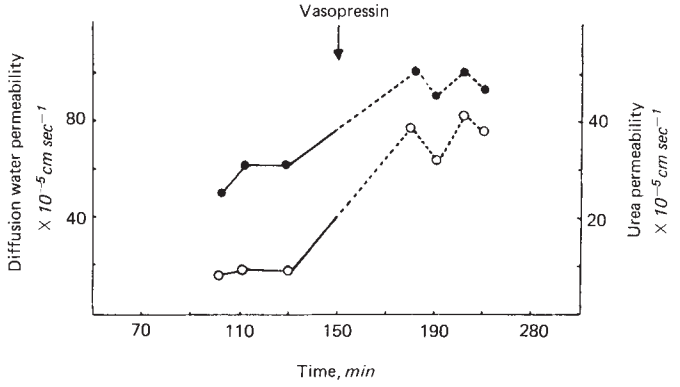

Fig. 3. *Human papillary collecting duct.* Effect of vasopressin (2 mU/ml) on diffusional water permeability (●) (P_{Dw}) and urea permeability (○) measured by the disappearance of ^3H_2O and ^{14}C-urea from the perfusate. The perfusion and bath fluids were isoosmotic Ringer's/HCO_3 solutions.

shows that vasopressin added to the bath (2 mU/ml), in the absence of an osmotic gradient, has no effect on net urea absorption. Also, measurements of bidirectional fluxes illustrated in Figure 2 allowed a quantitative estimation of both passive and active transport. In the absence of ADH, urea efflux was approximately four times greater than the net urea absorption. The high urea influx raises the possibility of secretion in the PCD as a function of the urea concentration gradient between the papillary interstitium and tubular fluid. Urea secretion by PCD was described recently by the microcatheterization technique during water and mannitol diuresis [22].

The differences in water and urea transport observed between PCD of the rat and rabbit invite inferences about these characteristics in the human PCD. For example, recent studies on CCT's dissected from human kidneys have shown that the increase in osmotic water permeability in response to vasopressin is of the same order of magnitude as has been described in the rabbit CCT [23, 24]. However, preliminary experiments carried out in our laboratory showed that the in vitro isolated perfused human PCD, derived from kidneys harvested for transplantation, responds to vasopressin by increasing not only the diffusional water permeability but also the urea permeability (Rocha, Kudo, and Magaldi, unpublished observations). As illustrated in Figure 3, the urea permeability was high in human PCD just before the addition of ADH to the bath. These preliminary results indicate that the human PCD has characteristics of transtubular flux of urea more similar to the rat than to the rabbit.

Transepithelial potential difference. Several in vivo measurements of potential difference (PD) in the PCD have been reported in the rat and gold hamster using microcatheterization and transmural puncture [25–28]. Substantial differences in recorded values have been reported by these techniques. The major problems involved in in vivo recording of PD in the PCD include: *(1)* damage of the electrode tip; *(2)* localization of the electrode tip; *(3)* damage of the tubular epithelium during impailment; *(4)* diffusion potentials between the tubule and the site of the reference electrode; *(5)* liquid-junction potentials at the tip of the electrode between the solution in the electrode and the tubular fluid; *(6)* wide variations in the composition of collecting duct and interstitial fluid; and *(7)* the possibility of electrical leaks at the site of puncture.

All these technical problems can be avoided when the PD is determined in the isolated perfused PCD in vitro using agar-Ringer bridges connected to the bath and perfusion solution [29]. Junction potentials are avoided by the use of identical NaCl/Ringer solutions in the perfusate, bath and agar-bridges. The histogram of Figure 4 shows the values of PD obtained in the PCD and CCT with this technique [30]. The mean PD recorded in segments dissected from the inner medulla of DOCA-treated rabbits was not different from zero (0.7 ± 0.2 mV), whereas the CCT obtained from the same animal displayed a significant lumen-negative PD (−20 ± 3 mV). It is important to point out that this low PD was seen in PCD of both rats and rabbits. The influence of mineralocorticoids on transtubular PD of PCD examined in isolated perfused segments showed that treatment with DOCA for 5 days (5 mg/day) did not change the PD from zero in rats or rabbits. In contrast, in the rabbit CCT the potential difference increased significantly from −1.0 ± 0.1 mV to −21.9 ± 3.0 mV after DOCA administration. Another characteristic of PCD was the absence of an effect of ouabain on PD, as illustrated in Figure 5 [30].

In summary, in vitro perfusion studies demonstrate an electrical heterogeneity along the mammalian collecting duct system. The CCT has a large PD (lumen negative) which is sensitive to aldosterone and ouabain [31–33]; this is followed by the outer medullary collecting duct which has a small PD (lumen positive) [34]; the collecting duct terminates in the PCD in which the PD is zero and which is insensitive to DOCA and ouabain.

Sodium and chloride transport. In spite of the discrepant results concerning volume expansion, the in vivo microcatheter method and the direct puncture technique clearly showed that, in salt-deprived, hydropenic rats, an important fraction of sodium chloride is reabsorbed in the PCD [4–7, 25]. However, neither method was useful for evaluating sodium and chloride transepithelial transport mechanisms in the PCD.

The in vitro perfusion of isolated segments of rabbit PCD allowed us to measure the bidirectional isotopic flux of both

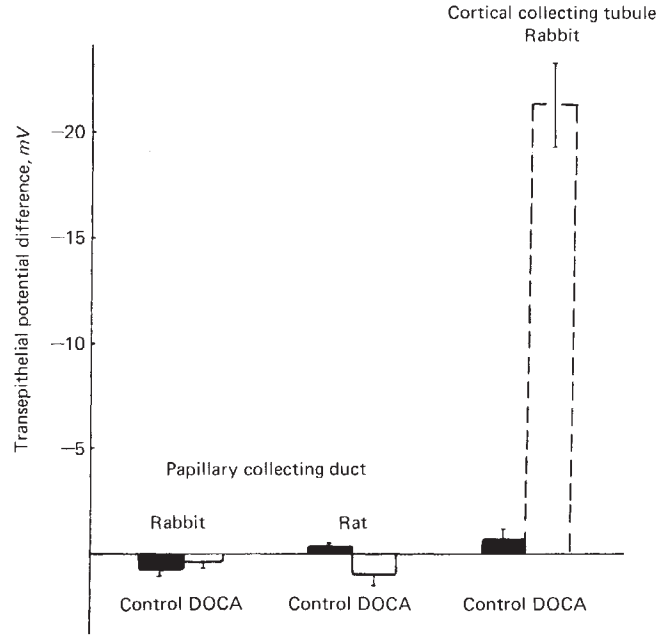

Fig. 4. *Electrical potential difference across cortical and papillary collecting tubules dissected from normal and DOCA-treated (5 mg/day) rabbits and rats.* All values represent the mean ± SEM.

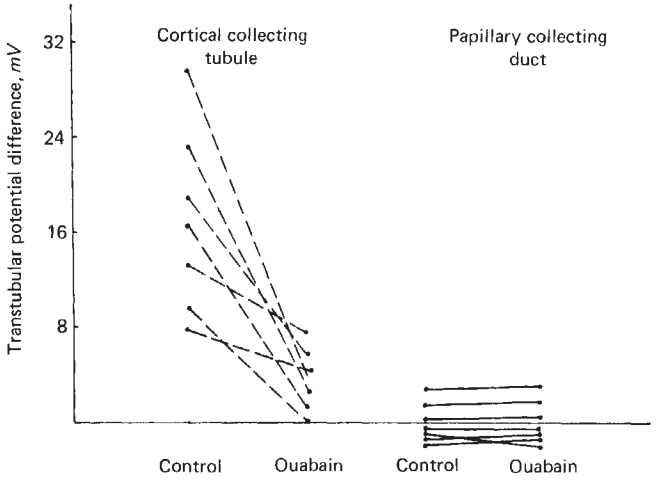

Fig. 5. *The ouabain effect (10^{-4}M) on the transtubular potential difference in cortical and papillary collecting tubules obtained from DOCA-treated rabbits.*

sodium and chloride with simultaneous recording of transtubular PD [30]. In the absence of chemical and electrical gradients, and without net water reabsorption, the efflux of both sodium and chloride was twice the influx, as presented in Table 2. These results indicate that the mechanism of sodium and chloride transport in the PCD is more complex than just simple diffusion down an electrical potential gradient. The sodium and chloride absorption could not be explained by passive mechanisms. It is important to point out that the magnitude of sodium absorption calculated per area of PCD is similar to that described by identical techniques in the CCT and pars recta [33, 36].

Table 2. Sodium and chloride bidirectional fluxes in the rat papillary collecting duct

			$Ø_{LB}$	$Ø_{BL}$	$Ø_{NET}$	PD *mV*
Sodium[a]	Mean	±	2538	1389	1149	+1.3
	SEM		290	325	250	0.3
Chloride[b]	Mean	±	3625	1419	2216	+0.6
	SEM		666	392	276	0.3

Abbreviations: $Ø_{LB}$, outflux; $Ø_{BL}$, influx; $Ø_{NET}$, net absorption.
[a] Isotopic sodium fluxes (pEq · $cm^{-2}sec^{-1}$) were measured by adding ^{22}Na to the perfusate and ^{24}Na to the bath.
[b] Isotopic chloride fluxes (pEq · $cm^{-2}sec^{-1}$) were determined by adding ^{35}Cl to the perfusate and bath successively.

The apparent passive permeabilities for sodium and chloride calculated from the isotopic bath to lumen flux were 0.92 ± 0.21 × 10^{-5} cm sec^{-1} and 1.20 ± 0.25 × 10^{-5} cm sec^{-1}, respectively. These low permeabilities are in agreement with the concept that, at least under certain circumstances, the PCD epithelium must be quite impermeable to sodium and chloride so as to maintain the final urinary concentrations as low as 1 mM/liter. The low sodium and chloride permeabilities are also in accord with the large electrical resistance observed in the hamster PCD [27]. However, undetermined is the passive permeability of the PCD in other conditions, such as volume expansion and postobstructive natriuresis, where the described sodium chloride secretion could be associated with passive backleak into the PCD [7, 37].

Sodium and chloride absorption in the absence of a transepithelial PD is in agreement with the presence of neutral sodium chloride transport or counter balancing electrogenic processes. Inhibitor and ion replacement studies will be required to evaluate this issue further.

Mineralocorticoids seem to be major determinants of both PD and net sodium flux in the CCT, but this is not the case in the outer medullary collecting duct [31, 33, 34]. Microcatheterization techniques applied to the study of PCD did not find a difference of net sodium absorption between normal and adrenalectomized rats [38]. On the contrary, the shrinking droplet method, as well as simultaneous perfusion of the peritubular capillaries used in the measurements of net sodium absorption in the PCD, showed that sodium transport in that nephron segment is controlled by mineralocorticoids [5]. The PD in the in vitro isolated perfused PCD obtained from rat and rabbit kidneys was unchanged by chronic DOCA administration, as shown in Figure 4. In addition, the efflux of ^{22}Na did not change upon the addition of spirolactone to the bath solution [30]. Therefore, the effect of aldosterone on sodium and chloride transport in the PCD requires further investigation.

Potassium transport. Micropuncture studies of superficial nephrons have demonstrated that the distal tubule is the principal site of regulation of urinary potassium excretion [39]. However, comparison of the end distal micropuncture site and the final urine indicates that the collecting duct segments also have an important role in the maintenance of potassium balance [40]. In vitro microperfusion studies of CCT have shown that this segment is able to secrete potassium under several condi-

tions [41], in contrast to the absence of potassium transport described recently with the same technique in the outer medullary collecting duct [34]. Little is known about potassium transport in the PCD, where both papillary micropuncture and microcatheterization failed to demonstrate net potassium transport under normal antidiuretic conditions. Even in rats on a high potassium diet or following unilateral nephrectomy, no significant potassium secretion was found [7, 35]. However, potassium secretion was described by microcatheterization of PCD in vivo during massive hypervolemia [42]. The large variability of data from both techniques in different duct systems at the same level of the papilla makes a final conclusion about potassium transport in the PCD difficult. As previously mentioned, recent micropuncture studies proposed that potassium is reabsorbed by the medullary collecting tubule, trapped in the medullary interstitium, and secreted in the pars recta [43, 44]. This potassium recycling in the renal medulla could be suppressed by potassium deprivation or increased by a high potassium diet [44, 45].

The determination of isotopic bidirectional potassium fluxes in the in vitro isolated perfused PCD shows that this final collecting duct segment is able to absorb potassium in the absence of electrical and chemical gradients, and in the absence of water absorption (Kudo and Rocha, unpublished observations). Figures 6 and 7 show that both the net absorption and potassium efflux increase as a function of the potassium perfusion load. The increase of potassium absorption seems to be higher in the PCD obtained from potassium-deprived rats as opposed to DOCA-treated animals and rats on a high potassium diet. The apparent potassium permeability, calculated from bath to lumen isotopic flux, showed a mean value of $4.0 \pm 1.0 \times 10^{-5}$ cm sec^{-1}, which is four times the sodium and chloride permeabilities observed in the rabbit PCD with the same technique. This relatively high potassium permeability makes potassium secretion possible in the PCD when the potassium concentration gradient from medullary interstitium to tubular lumen is greater than one. This is demonstrated in the experiments plotted in Figure 8. Therefore, the findings obtained recently with the in vitro microperfusion technique suggest that the PCD has the capacity to absorb potassium actively, and also to secrete it by a passive transport process.

Summary. The practical use of in vitro microperfusion of isolated nephron segments has made possible the direct examination of ion, water, and solute transport in the papillary collecting duct (PCD) under varying physiological conditions. Moreover, the improvement of dissection technique brought with it the ability to analyze the PCD isolated from rabbit, rat, guinea pig, and human kidneys, and thus to make comparative studies. The osmotic and diffusional water permeabilities of rat PCD were, respectively, ten times and twice those observed in the rabbit and guinea pig. In the rat PCD, vasopressin produced an additional increase in the high urea permeability measured during the control period; in contrast, the rabbit PCD had a low permeability to urea and was vasopressin resistant. A comparative study of several mammals showed a high correlation between both osmotic and urea permeabilities and maximum urinary concentrating capacity. Isotopic unidirectional fluxes in the rat PCD showed significant net urea absorption in the absence of chemical and osmotic gradients, suggesting active urea transport in the rat PCD. The human PCD exhibited an

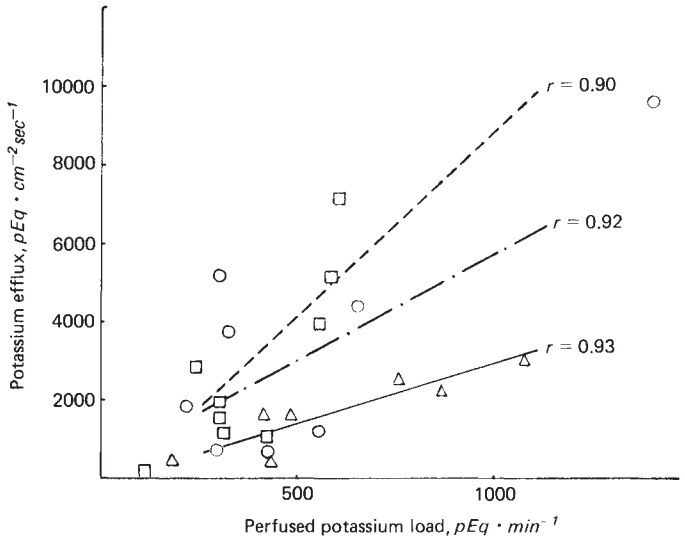

Fig. 6. *Relationship of potassium efflux to the potassium perfusion load in the absence of a potassium chemical gradient.* Potassium efflux was computed from the difference between the rates of potassium perfusion and collection using ^{42}K as a marker. The perfusion and bath fluid was an identical Ringer's/HCO_3 solution. The potassium perfusion load was increased by the simultaneous addition of potassium to the perfusate and bath. The *lines* depict the mean regression lines. Symbols: (□ ----), potassium deprivation; (○ — · — · —), potassium load; (△ ———), DOCA-treated (5 mg/day) rats.

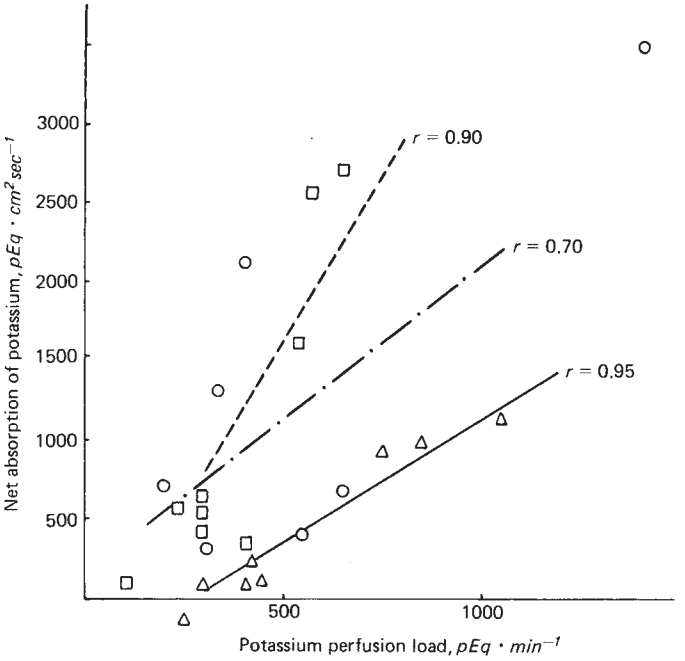

Fig. 7. *Correlation between net potassium absorption and potassium perfusion load in the absence of a chemical gradient.* Net potassium absorption was calculated from the difference between the rates of perfusion and collection using ^{42}K as a potassium marker. Potassium perfusion load was increased by a potassium addition to the perfusate and bath without generation of a chemical gradient. The *lines* depict the mean regression lines. Symbols: (□ ----), rats with potassium deprivation; (○ — · — · —), potassium load diet; (△ ———), chronic DOCA-treated rats.

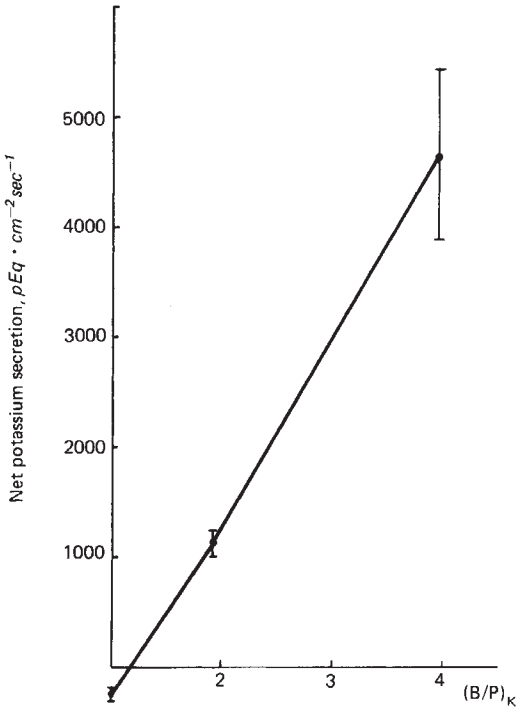

Fig. 8. *Linear correlation between net potassium secretion and bath-to-lumen (B/P) potassium concentration ratio.* The bath-to-lumen gradient was increased by adding potassium to the bath. ^{42}K was used as a potassium marker. In the absence of a potassium chemical gradient, there was significant net potassium absorption.

increase of water and urea permeabilities after the addition of vasopressin to the bath, as has been previously well described in the rat PCD. The electrical potential difference (PD) measured in both rat and rabbit PCD was not significantly different from zero in both DOCA-treated and untreated animals, which contrasts that observed in the rabbit cortical collecting tubule of DOCA-treated animals, where the PD was -20 ± 3 mV (lumen negative). The bidirectional isotopic flux of sodium and chloride measured in the PCD of DOCA-treated rabbits in the absence of an electrochemical gradient showed an efflux twice the influx. The magnitude of net sodium absorption was similar to that described in the cortical collecting duct and pars recta of the proximal tubule. The sodium and chloride passive permeabilities calculated from the ion backflow were very low, in agreement with the PCD's capacity to maintain high transtubular concentration gradients. The analysis of isotopic bidirectional potassium fluxes showed that the rat PCD is able to absorb potassium in the absence of electrical and chemical gradients. It was also demonstrated that net potassium absorption has a linear correlation with perfusion load and is increased in potassium-deprived rats. Furthermore, a capacity for passive potassium secretion by rat PCD was shown in experiments in which the potassium concentration in the bath was increased. The PCD demonstrated a relatively high potassium permeability calculated from the potassium influx.

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