A Functional Comparison of the Cortical Collecting Tubule and the Distal Convoluted Tubule

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ABSTRACT Electrical and permeability features of the distal convoluted tubule (DCT) and the cortical collecting tubule (CCT) were examined using the technique in which isolated segments of rabbit tubules were perfused in vitro. When rabbits were given a regular diet and tubules were perfused and bathed in artificial solutions simulating plasma ultrafiltrate, the potential difference (PD) was +3.7±1.9 mV in the CCT and −40.4±2.8 mV in the DCT. When rabbits were given a low sodium, high potassium diet plus i.m. deoxycorticosterone acetate (DOCA) (1 mg/kg per day), the PD in both the CCT (−30.8±3.9 mV) and the DCT (−33.8±5.5 mV) was negative. The PD in the CCT was quantitatively similar to that of diet plus DOCA when animals were given DOCA alone. The PD in both segments was inhibited by ouabain (10^{-5} M) in the bath or by amiloride (10^{-5} M) in the perfusate. Addition of vasopressin (200 μU/ml) to the bath caused a gradual decline of PD to zero in the CCT but failed to produce a potential response in the DCT. Osmotic water permeability was essentially zero in both segments in the absence of vasopressin. After addition of the vasopressin to the bath, osmotic water permeability in the DCT remained zero but increased to 71.9±25.5 × 10^{-7} cm/s per atm in the CCT. We conclude that both segments are similar in that each possesses an electrogenic transport process but that these segments differ in that: (*a*) the CCT requires either exogenous or endogenous mineralocorticoid to maintain a maximal negative PD, whereas the PD in the DCT appears to be independent of mineralocorticoid effect; and (b) the CCT responds to vasopressin with a marked rise in water permeability, whereas the DCT is impermeable to water before and after addition of vasopressin.

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INTRODUCTION

Previous direct information relating to the discrete functional characteristics of the distal convoluted tubule (DCT)[1] has been derived from micropuncture studies (1–19). In addition, indirect information concerning the characteristics of the DCT has been obtained from clearance (20–26) and from toad bladder experiments (27–30). However, the recent observations of Woodhall and Tisher raise serious questions about the interpretation of previous micropuncture studies in relation to functional characterization of the DCT (31). These authors found a significant anatomic heterogeneity of surface tubules available for distal puncture when the standard micropuncture definition of the distal tubule was used. The epithelium lining tubules just before the junction of one "distal" segment with another, usually referred to in micropuncture studies as the "late distal tubule," was found in all cases to consist of epithelium of the cortical collecting tubule (CCT).[2]

In the Wistar rat, 48.3% of available tubules were of true distal epithelial type, 45% were collecting tubules, and 6.7% were transition zones (31). Thus, it is clear that many of the previous micropuncture results have

[1] *Abbreviations used in this paper:* ADH, antidiuretic hormone; CCT, cortical collecting tubule; DCT, distal convoluted tubule; DOCA, deoxycorticosterone acetate; PD, potential difference.

[2] The anatomic criteria used by Woodhall and Tisher for defining the DCT and CCT were those established in previous morphologic studies (32–34). The DCT is lined with cells that are more nearly columnar than cuboidal and that contain many mitochondria as well as cytoplasmic vesicles along the luminal border. The lateral and basilar surfaces contain many invaginations lined by mitochondria; these invaginations are so numerous and closely apposed on the basilar surface that it has a striated appearance. The CCT is lined by cells that are more nearly cuboidal in shape and contain fewer organelles and have less extensive lateral and basilar striations. In addition, a second cell type, the "dark cell" or intercalated cell, which has numerous cytoplasmic organelles, is found in a sparse distribution throughout the CCT.

been obtained from heterogeneous populations of tubules, and in all likelihood, using standard morphologic criteria for the DCT and CCT, a significant fraction of the results probably represents the function of the CCT rather than the DCT.

Although there have been several studies of the collecting duct utilizing the in vitro perfusion of isolated tubules (35–41), this technique has not previously been employed to study the DCT. Therefore the purpose of the present study was to utilize the in vitro microperfusion technique to characterize and compare certain basic physiologic features of each segment.

METHODS

Segments of CCT and DCT were perfused in vitro by the same technique described for other segments of the nephron (42). Female New Zealand rabbits weighing 1.5–2.5 kg were used in all experiments.

Two different types of diet were used: (*a*) standard laboratory diet containing approximately 140 meq/kg Na^+ and 390 meq/kg K^+ and (*b*) a low sodium, high potassium diet containing approximately 2 meq/kg Na^+ and 1,750 meq/kg K^+. In addition, animals on the low sodium, high potassium diet were given i.m. deoxycorticosterone acetate (DOCA), 1 mg/kg per day, and these animals were sacrificed only after a minimum period of 4 days on this regimen. All animals had free access to tap water before guillotine decapitation.

After 10 days on either the regular or the low sodium, high potassium diet, plasma aldosterone determinations were done on several animals from each dietary group (43).

The kidney was quickly removed and cut into 1–2-mm slices. A segment of either CCT or DCT was dissected out in a chilled dish of Ringer's-bicarbonate solution to which fetal calf serum, 5% by volume, had been added. The solution was kept at pH 7.4 by continuous bubbling with 95% O_2-5% CO_2. CCT, 0.5–2.0 mm in length, were dissected free and transferred to a Lucite perfusion chamber. The DCT was identified by dissecting free individual glomeruli and identifying that convoluted tubule which is clearly distinct from the proximal convoluted tubule (both the appearance and diameter of the DCT is markedly different from that of the proximal convoluted tubule under a dissecting microscope at × 40), and by identifying its attachment to the glomerulus (i.e., macula dense) (Fig. 1A and C). A small segment (0.3–0.8 mm) was isolated and transferred (usually with the glomerulus still attached) to the bath chamber. All studies were conducted at 37°C. Tubules were perfused with an artificial solution of the following composition: NaCl 105 mM, KCl 5 mM, $NaHCO_3$ 25 mM, Na_2HPO_4 4 mM, Na acetate 10 mM, $MgSO_4$ 1 mM, $CaCl_2$ 1.8 mM, glucose 8.3 mM, alanine 5 mM, total osmolality 298 mosmol/kg H_2O. The bath solution was identical except for the addition of 5% fetal calf serum to maintain tubular viability.

The transtubular electrical potential difference (PD) was measured by techniques previously reported (44). Equivalent bridges of 300 mosmol/liter Ringer's solution in 4% agarose were connected to the end of the perfusion pipette and to the bath. The other ends of the bridges were submerged in saturated KCl solution that contained Beckman (Beckman Instruments, Inc., Fullerton, Calif.) calomel half-cells. Both ends of the tubule were sealed from electrical and hydraulic leaks by first coating and then placing Sylgard 184 (Dow Corning Corp., Midland, Mich.) in the tips of the holding pipettes. Ouabain (2×10^{-5} M) was added to the bath, and amiloride (10^{-5} M) was added to the perfusate in those experiments in which the effect of inhibitors on the transtubular PD was studied.

Water flux experiments. The hydraulic conductivity of water was determined by measuring net fluid movement in response to an osmotic gradient. [^{125}I]Iothalamate (Glofil-125, Abbott Laboratories, North Chicago, Ill.) was used as a volume marker. Preliminary experiments measuring isotope leakage into the bath solution indicated that this substance does not penetrate either the CCT or DCT. A bath-to-lumen osmotic gradient was established by addition of either NaCl or raffinose to the bath. In the present experiments either a 150–200-mosmol/liter raffinose or a 60-mosmol/liter NaCl gradient was used. An initial equilibration period of at least 120 min from decapitation to start of experiment was allowed, as previous work has shown that water permeability in the CCT progressively declines and reaches a stable base line during this period (41). Subsequently, three consecutive control collections were made, a maximal dose of 200 μU/ml vasopressin (Pitressin, Parke, Davis & Company, Detroit, Mich.) was added to the bath, and a minimum of 45 min was then allowed for development of a full antidiuretic hormone (ADH) response (35, 41). Three experimental collections were then made. Perfusion rate was kept constant by means of a Sage model 255-3 variable-speed microsyringe pump (Sage Instruments, Div., Orion Research Inc., Cambridge, Mass.). Tubular fluid was collected in a pipette of constant volume. The constant volume pipette was calibrated at the conclusion of each experiment. Timed collections were made, and the rate of volume collection (V_o) was calculated by dividing the volume of the pipette by the collection time. Perfusion rates (V_i) were calculated from the known concentration of volume marker in the initial perfusion fluid and the measured amount of volume marker recovered in the collected fluid. The net volume efflux (J_v) was computed from the difference between perfusion and collection rates. The osmolality of the perfusate, bath, and collected fluid was measured by techniques previously described (42). In these experiments the perfusion rate was kept sufficiently high in relation to tubular length to prevent osmotic equilibration of tubular fluid with that of the bath. Under these circumstances L_p can be expressed as:

$$L_p = \frac{J_v}{\sigma_i \Delta\pi}, \tag{1}$$

where J_v (cm^3/cm^2 per s) is the net water flow, σ_i is the reflection coefficient of the *i*th solute (used to generate the osmotic gradient), and $\Delta\pi$ is the logarithmic mean osmotic gradient between bath and collected fluid. It was assumed that σ_i for raffinose is unity, since it does not permeate most biologic membranes. It has been previously shown that σ_i for NaCl in the rabbit CCT is unity (39). Therefore, Eq. 1 can be reduced to:

$$L_p = \frac{J_v}{\Delta\pi}, \tag{2}$$

where $\Delta\pi$ is given by:

$$\Delta\pi = \sqrt{(\pi_b - \pi_p)(\pi_b - \pi_c)},$$

where π_b is the osmolality of the bath, π_p is the osmolality of the perfusate, and π_c is the osmolality of the collected

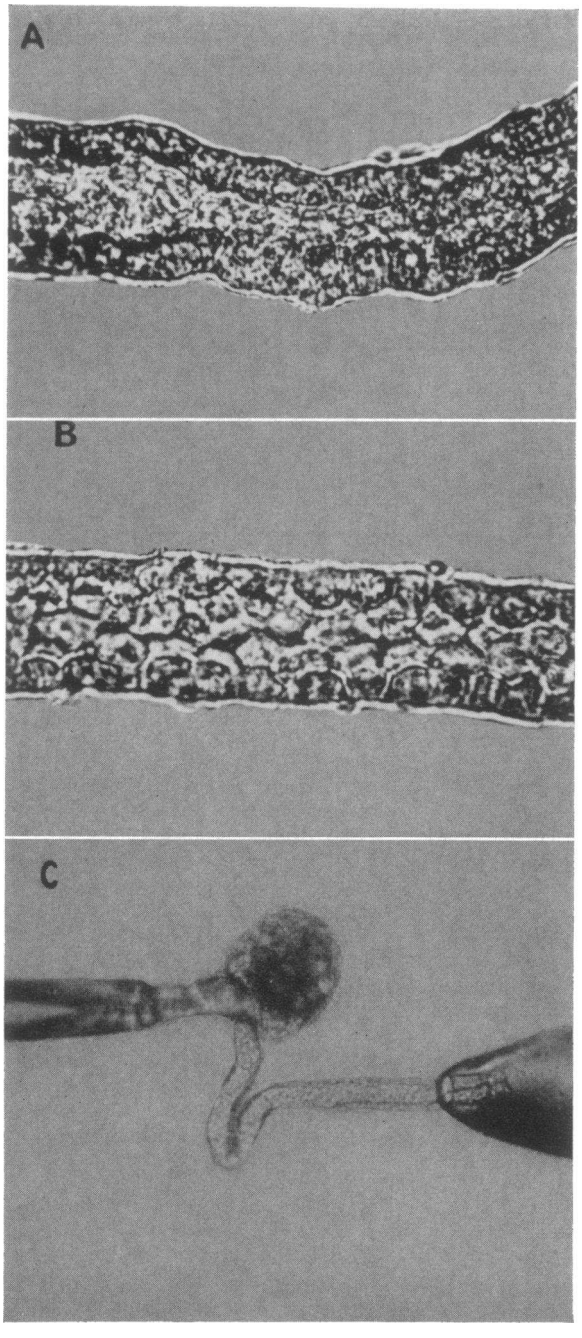

FIGURE 1 Morphology of the DCT and CCT. The typical morphologic appearance of the DCT is shown in the upper panel (A; ×400). There is marked variation in luminal diameter and the lining cells tend to be somewhat columnar in appearance. The middle panel (B; ×400) depicts the typical appearance of the CCT, with more nearly cuboidal cells, giving a somewhat cobblestone appearance. The lower panel (C; ×200) shows a perfused DCT with an attached glomerulus.

fluid. L_p (cm/s per atm) can be expressed as:

$$L_p = \frac{J_v}{\sqrt{(\pi_b - \pi_p)(\pi_b - \pi_c)}}, \quad (3)$$

and was used in the calculation of the final values.

The radioactivity of [^{125}I]iothalamate was measured by a Packard model 3365 three-channel gamma spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.). The osmolality of artificial solutions was measured in an osmometer (Advanced Instruments, Inc., Needham Heights, Mass.). The osmolality of collected fluid, perfusate, and bath was determined by a modified (42) Clifton nanoliter osmometer (Clifton Technical Physics, Hartford, N. Y.).

The data for each tubule were obtained as means of two to four collection periods. The results in turn are expressed as mean±SE of number of tubules (n) studied. The statistics were calculated as a nonpaired t test analysis.

RESULTS

Morphology. Anatomically, it is not difficult to distinguish between the CCT and DCT. The typical morphologic appearances of the CCT and DCT are shown in Fig. 1. Although the tubular diameter is somewhat less in the DCT as compared with the CCT, the lining cells of the DCT are more nearly columnar, giving the impression of a somewhat thicker tubular wall. The cells of the CCT are somewhat smaller and more flattened than the cells of the DCT. There is also a greater

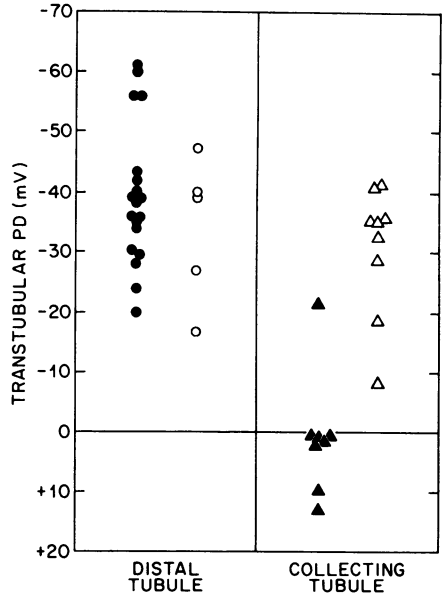

FIGURE 2 Effect of diet on the PD in the DCT and CCT. The PD in the DCT was negative and not significantly different when tubules were obtained from animals on a low sodium, high potassium diet plus DOCA (open circles) or from animals on standard lab chow (closed circles). In the CCT, however, the PD was small and usually positive when animals were fed a regular diet (closed triangles), and became strongly negative in animals receiving the low sodium, high potassium diet plus DOCA (open triangles).

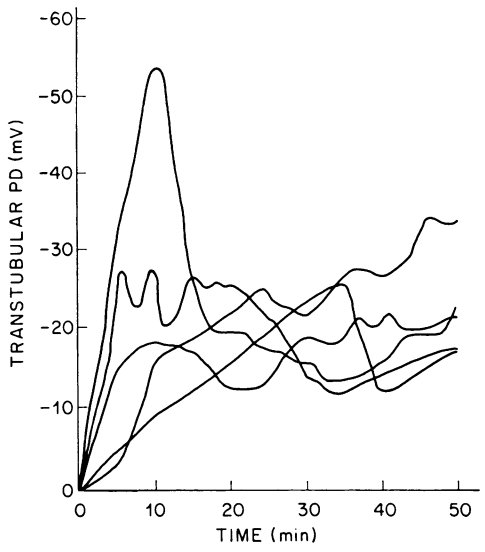

FIGURE 3 The influence of DOCA on the PD in the CCT. The PD in the CCT was both quantitatively and qualitatively similar, in animals given DOCA alone, to that measured in animals receiving a low sodium, high potassium diet plus DOCA.

variability in the diameter of the tubular lumen in the DCT. In addition, the course of the DCT is convoluted, whereas the CCT is without convolutions.

Effect of diet on the transtubular PD. When tubules were perfused at 37°C there was a marked difference between the DCT and CCT in the response of the transtubular PD to previous dietary maneuvers. As shown in Fig. 2, regardless of previous treatment regimen, the PD in the DCT was always negative (with respect to the lumen) and, after stabilization, reached a mean value of -40.4 ± 2.8 mV ($n = 19$) for animals given a regular diet and -33.8 ± 5.5 mV ($n = 5$) for animals maintained on a low sodium, high potassium diet plus i.m. DOCA (1 mg/kg per day). These two values are not statistically different ($P > 0.25$) when analyzed by the nonpaired t test. When animals were given a standard diet, the PD was $+3.7\pm1.9$ mV ($n = 8$) in the CCT. After the low Na, high K diet plus DOCA, the PD averaged -30.8 ± 3.9 mV ($n = 9$), $P < 0.001$. This dependency of the CCT on diet is similar to that previously mentioned by Frindt and Burg (40). As shown in Fig. 3, when animals were given a regular diet and also treated with i.m. DOCA (2 mg/kg per day), the PD in the CCT was similar to that of animals given a low Na^+, high K^+ diet plus DOCA (Figs. 2 and 4) as well as that of animals given a low Na^+, high K^+ diet alone (40). The mean plasma aldosterone value for the animals on the regular diet was 4.6 ± 1.3 ng/ml ($n = 6$). Animals given the low sodium, high potassium diet had a mean plasma aldosterone value of 239 ± 54 ng/ml ($n = 8$).

It therefore appears that the PD response of the CCT to the low Na^+, high K^+ diet is a mineralocorticoid effect, endogenous mineralocorticoid having been stimulated both by the low sodium intake and by the potassium load, inasmuch as this PD could be elicited with DOCA alone. In contrast, the DCT did not exhibit any dependency of the PD on either diet or exogenous DOCA.

Time course of the PD. There was also a different pattern in the time course of the transtubular PD in the two segments after initiation of perfusion. As shown in the upper panel of Fig. 4, the PD in the CCT showed a gradual rise in the low Na^+, high K^+ group of animals after initiation of perfusion, with stabilization

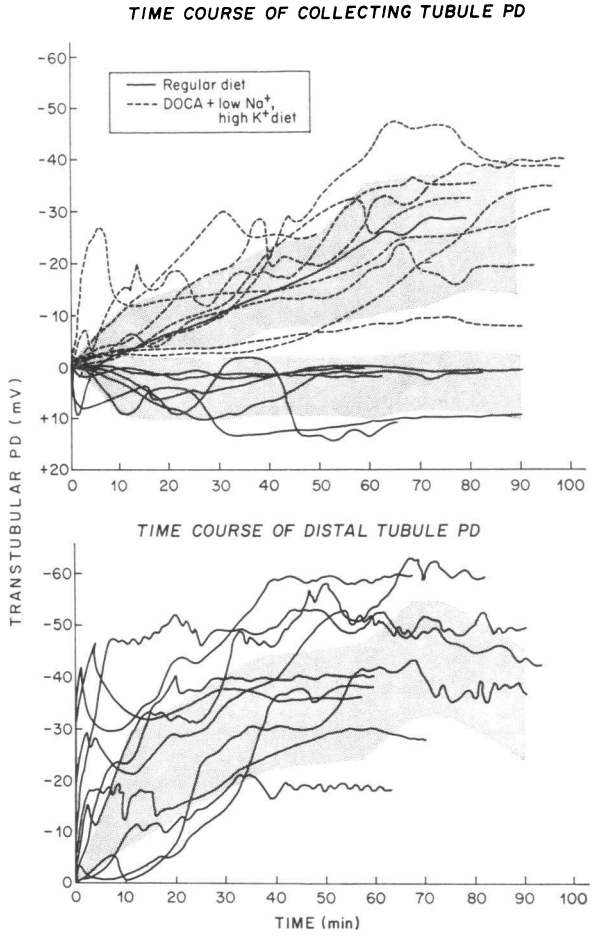

FIGURE 4 Time course of the PD in the CCT and DCT. The course of the PD in the CCT after initiation of perfusion (upper panel) was much slower than that found in the DCT (lower panel), requiring 80–90 min to reach a stable, maximum value. At 30 min, the PD in the DCT was not statistically different from its steady-state value at 90 min, whereas the PD in the CCT at 30 min was only 46% of its steady-state value at 90 min ($P < 0.05$). Shaded areas represent mean values of all experiments ±SD. All tubules were perfused at 37°C.

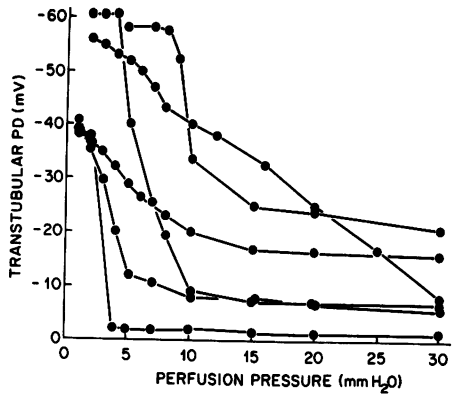

FIGURE 5 Effect of hydrostatic pressure. The transtubular PD in the DCT shows an inverse correlation with transtubular hydrostatic pressure. An essentially identical relationship holds also for the CCT (not shown, see text).

of the PD near a maximal value after approximately 80–90 min. The PD in the DCT (lower panel of Fig. 4) showed a more rapid rise, stabilizing after approximately 40–60 min.

Effect of perfusion pressure on transtubular PD. When the perfusion column was raised or lowered to vary perfusion pressure and thus transtubular hydrostatic pressure, the DCT showed a marked sensitivity to changes in perfusion pressure (Fig. 5), with the magnitude of the PD showing an inverse relationship to perfusion pressure. A similar response was noted in the CCT, as has been described previously (36). Since perfusion flow rate varies in direct proportion to perfusion pressure, this response of the PD could reflect either the influence of changes in flow rate per se or changes in hydrostatic pressure. However, for any given perfusion rate, when flow was stopped by occluding the tubular lumen at the site of attachment to the collecting pipette, the PD fell immediately in both the DCT and CCT. Since PD increased as both flow rate and perfusion pressure decreased, the fall in PD from a maximal value at low flow rates when flow was stopped by tubular occlusion indicates that the PD response is dictated primarily by changes in transtubular hydrostatic pressure. The possibility of a separate but quantitatively much smaller influence of flow rate that is masked by the large response to hydrostatic pressure cannot, of course, be excluded by these data.

It is not clear how the effect of hydrostatic pressure on the transtubular PD is mediated. However, changes in transtubular hydrostatic pressure in vivo may have an influence on the excretion of electrolytes which is mediated by similar changes in the transtubular PD.

Effect of inhibitors on the transtubular PD. Fig. 6 shows the response of the PD in the DCT to the addition of 2×10^{-5} M ouabain to the bath solution. There was a prompt fall in the transtubular PD toward zero, with recovery of the PD after the bath solution was changed. A similar response to ouabain was observed in the CCT, as previously described (36). This response supports the concept that the negative PD in both segments is an active transport PD, since no passive source of PD exists in these experiments, where the fluids on the two sides of the membrane are identical. The previous study by Grantham, Burg, and Orloff (38) of the isolated perfused CCT also provided strong evidence for active electrogenic sodium transport in this segment. Addition of 10^{-5} M amiloride to

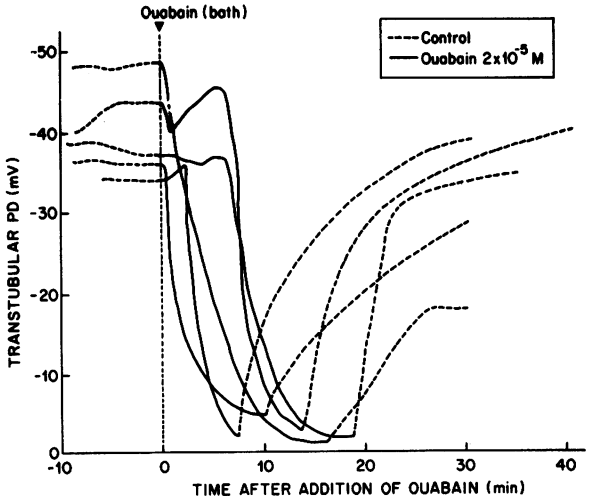

FIGURE 6 Effect of ouabain on the PD in the DCT. Addition of 2×10^{-5} M ouabain, to the bath solution resulted in a prompt and reversible inhibition of the PD in the DCT. The same findings were also observed in the CCT (not shown, see text).

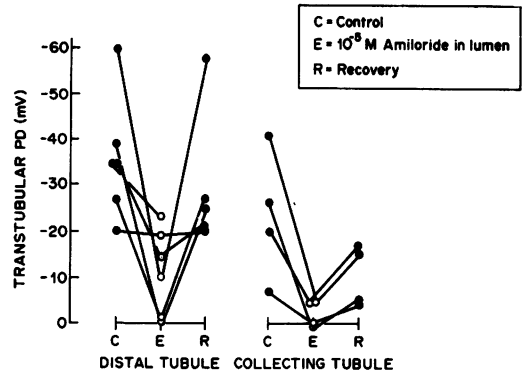

FIGURE 7 Effect of amiloride on the PD in the CCT and DCT. Addition of amiloride, 10^{-5} M, to the perfusing solution, resulted in a rapid fall in the transtubular PD towards zero, in both the CCT and the DCT, with rapid recovery after reperfusion with the original solution. Animals were on a low sodium, high potassium diet plus DOCA in the CCT experiments.

the perfusion solution resulted in a prompt drop in the PD to zero both in the CCT and in the DCT (Fig. 7); this response was also reversible when the perfusate was changed back to the original solution. This finding would also support the concept that the transtubular PD in both segments is primarily due to outward transport of sodium from the lumen. Evidence from toad bladder experiments indicates that amiloride decreases the flux of sodium across the mucosal membrane, apparently by decreasing the mucosal permeability to this ion (45, 46).

Response of the PD to vasopressin. As depicted in the upper and lower panels of Fig. 8, there was a marked difference between the DCT and CCT in the electrical response to vasopressin. When 20 μU/ml vasopressin was added to the bath solution, the CCT showed a transient initial increase in the magnitude of the PD, followed by a gradual decline toward zero, with gradual recovery after washing the agent from the bath solution. In contrast, addition of vasopressin had no influence on the PD in the DCT.

Osmotic water flow in response to vasopressin. The hydraulic conductivity was used as an index of water permeability and was measured in both segments, before and after vasopressin, in response to an induced osmotic gradient. As depicted in Table I, net water flow was so low and variable in both segments in the absence of vasopressin that it could not be distinguished from zero. After addition of 200 μU/ml vasopressin to the bath, water flow (J_v) increased in the CCT, and the mean calculated value for Lp increased to 71.9± 25.5×10^{-7} cm/s per atm ($n = 6$). In the DCT there was no increase in the net water flux in response to vasopressin and L_p remained essentially zero. These data, along with the difference in electrical response to ADH, provide direct physiologic support for lack of response of the DCT to ADH in contrast with the clear-cut response of the CCT to the hormone. Moreover, in the present studies, the cells of the CCT showed definite swelling within 30 min after addition of vasopressin, as previously reported in in vitro perfusion studies (35, 41). There was no change in the cellular appearance of the DCT after addition of ADH.

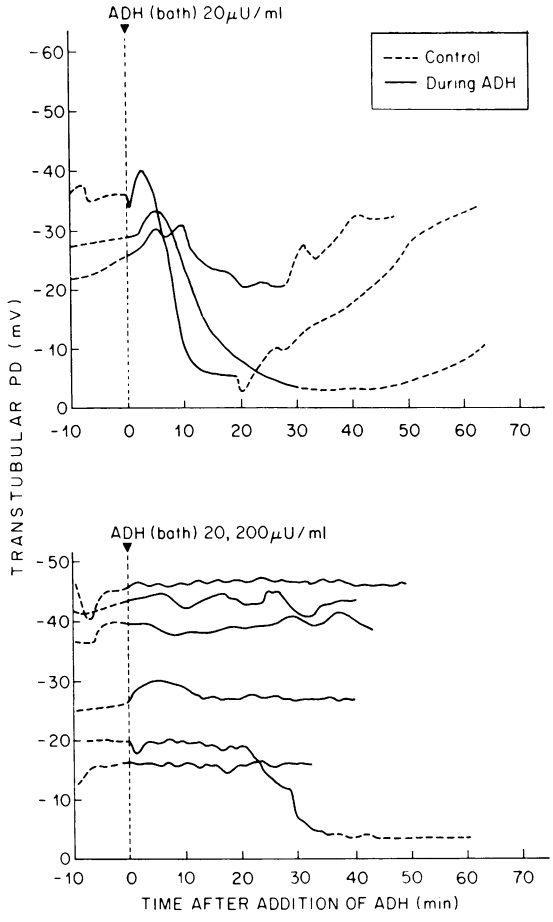

FIGURE 8 Effect of ADH on the PD in the CCT and DCT. In the CCT (upper panel) there was an initial increase, followed by a gradual decline in the PD after ADH, with slow recovery after washing ADH from the bath solution. When ADH was added to the bath solution, there was no change in the transtubular PD in the DCT (lower panel). Animals were on a low sodium, high potassium diet plus DOCA in the CCT experiments.

DISCUSSION

The present in vitro microperfusion studies provide evidence for discrete physiologic differences in the epithelium of the CCT and the DCT. These include the time course of the transtubular PD in each segment as well as the response of the PD to dietary manipulations and/or exogenous mineralocorticoid. In addition, there is a clear-cut distinction between the response of each segment to vasopressin, both in terms of electrical and hydraulic criteria. However, there are also certain similarities in that both segments appear to demonstrate electrogenic active transport. Evidence for the latter includes the electronegative PD in the absence of an ionic gradient for passive diffusion and the inhibition of the PD by inhibitors of active transport.

Previous micropuncture studies of the distal tubule have indicated that this segment maintains a negative PD, although the magnitude of the potential varies with different physiologic circumstances (6, 8, 9, 11, 12, 18). Previous studies have suggested that cationic diffusion across the luminal membrane into the cell plays a major role in the genesis of the negative transtubular PD in the distal tubule (8). Replacement of sodium by choline in the luminal perfusing solution results in a reduction and even a reversal of the polarity of the transtubular potential (8). More re-

TABLE I

*Hydraulic Conductivity in the CCT and DCT**

Type of tubule	Perfusion rate: Control	Perfusion rate: Experimental	Net water flux before ADH	Change in net water‡ flux after ADH (200 μU/ml)	Hydraulic‡ conductivity (L_p) after ADH 10^{-7}	n
	nl/min	*nl/min*	*nl/mm/min*	*nl/mm/min*	*cm/s/atm*	
Collecting tubule	17.2	13.0	−0.12§	+0.49¶	71.9	6
SEM	5.4	5.7	0.08	0.06	25.5	—
Distal tubule	5.8	7.8	−0.01§	−0.08‖	−15.2	5
SEM	3.1	2.4	0.33	0.08	11.1	—

* Experiments were done in the presence of either a 60-mosmol/kg H_2O NaCl or a 150–200-mosmol/kg H_2O raffinose bath-to-lumen gradient.

‡ The negative mean values for net water flux, and thus for L_p, in the collecting tubule experiments without ADH, and in the distal tubule experiments, in the presence or absence of ADH, are due to random variation of net water flux around zero.

§ Not statistically different from zero ($P > 0.30$).

‖ Not statistically different from control ($P > 0.30$).

¶ Statistically different from control ($P < 0.01$).

cently similar findings for the rabbit collecting duct have been reported by Grantham et al. (38), supporting the importance of diffusion of sodium from the lumen to cell in the genesis of the transtubular PD. Partial removal of sodium from the perfusate, thus limiting the amount available for diffusion across the luminal membrane and into the cell, resulted in a fall in the transtubular potential (38). Although diffusion of sodium across the luminal membrane is down an electrochemical gradient, extrusion of this ion from the cell to the peritubular fluid occurs against an electrochemical gradient. Thus the net overall transtubular movement of sodium appears to involve an active component. Evidence supporting the latter was also given by these authors in that (*a*) there was a marked inhibition of the PD by ouabain and (*b*) the steady-state mean value of E_{Na} (footnote 3) differed markedly from the measured PD.

The bulk of the available evidence would therefore support the view that the transtubular potential in both the CCT and DCT is the result of the passive efflux of sodium from the lumen into the cell. The low intracellular sodium concentration in turn is maintained by an active sodium efflux pump at the antiluminal border of the cell.

Although previous micropuncture studies have indicated the overall pattern of transport processes and of the transtubular potential in the distal segments of the nephron, the discrete characteristics of the DCT and the CCT have not been clearly separated by the previous micropuncture studies (31). The finding by Woodhall and Tisher (31) of a marked heterogeneity in the cellular composition of the subcapsular tubules available for distal micropuncture makes the interpretation of previous studies problematic. The inclusion of collecting-duct epithelium within tubules chosen for "late distal" micropuncture may have resulted in the attribution to the distal tubule of certain functional characteristics that are actually those of the CCT. Moreover, the marked species variation within the rat in the percentages of histologic types of tubules available for distal micropuncture further compounds the problem (31). Previous clearance studies were not capable of making clear-cut distinctions between various segments of the distal nephron, and the exact analogy of the anuran toad bladder to the mammalian nephron has not been established.

The present studies provide the first direct effort to examine the discrete physiologic aspects of the DCT and CCT, utilizing the in vitro microperfusion technique. To avoid anatomic heterogeneity in the segments studied, the following anatomic criteria were utilized. The CCT was always dissected free from its attachment, along its longitudinal course, to either the pars recta or the cortical thick ascending limb of Henle's loop. When perfused, it had the characteristic histologic appearance described by Grantham, Ganote, Burg, and Orloff (45) and by Schafer and Andreoli (41). The DCT was isolated by first identifying single glomeruli and subsequently dissecting free that convoluted segment which clearly differed in appearance from surrounding proximal convoluted tubules. The shortest possible segment attached to a glomerulus was isolated to avoid inclusion of any transition zone or segment of

[3] The equilibrium potential for sodium, given by $E_{Na} = (RT/ZF)\ln([Na]_o/[Na]_t)$. $[Na]_o$ and $[Na]_t$ are the bath and collected fluid concentrations of sodium, respectively.

CCT. The characteristic appearances of both portions of the nephron during perfusion are depicted in Fig. 1.

The present studies support the view that the PD in both segments is an active cation transport potential, most likely representing active sodium transport. The presence of a negative potential suggests active cation transport, in the absence of a transtubular ionic gradient for diffusion. Inhibition of the PD by ouabain is in agreement with the previous findings of Grantham et al. (38) in the CCT and provides additional evidence for an active transport process in both segments. The addition of amiloride also completely inhibited the transtubular potential in both segments. This agent has been found to inhibit the distal transtubular PD in micropuncture studies, and evidence from toad bladder experiments indicated that it decreases the mucosal-to-serosal sodium flux by decreasing the mucosal permeability to this ion (45, 46). Recently Stoner, Burg, and Orloff have reported that in the isolated, perfused rabbit collecting tubule, amiloride decreases membrane permeability, as indicated by the increase in transtubular electrical resistance, decreases the net sodium and potassium fluxes, and completely inhibits the negative transtubular PD (47). Therefore, the fall of the potential to zero in both the CCT and DCT in these experiments strongly supports the view that the diffusion of sodium from lumen into the cell is primarily responsible for the transtubular PD. In this context, ouabain may reduce the transtubular potential by inhibiting active sodium extrusion from the tubular cell, resulting in depolarization of the cell and a rise in intracellular sodium concentration, both of which would reduce the electrochemical gradient for diffusion of this ion out of the lumen.

The influence of dietary manipulations on the PD in the CCT appears to be a function of mineralocorticoid effect. When animals are given a standard diet (sodium content 140 meq/kg; potassium content 390 meq/kg), the PD is essentially zero or slightly positive (Fig. 2). Under these circumstances the sodium intake of the animals is quite high (average daily sodium intake approximately 25–30 meq for a 2-kg rabbit; average potassium intake 60–75 meq/day); this would be expected to suppress endogenous mineralocorticoid secretion. Indeed, the mean plasma aldosterone value for this group of animals was 4.6±1.3 ng/ml. On the low sodium, high potassium diet (sodium content 2 meq/kg, potassium content 1,750 meq/kg) maximal stimulation of endogenous mineralocorticoid secretion would be expected (average daily sodium intake 0.4 meq; average potassium intake 350 meq/day), and the mean plasma aldosterone value for these animals was 239±54 ng/ml. Thus, variations in endogenous mineralocorticoid secretion seem the likely explanation for the differences in transtubular potential observed with diets of different sodium and potassium content described by Frindt and Burg (40). The similarity of response of the PD to exogenous DOCA with that of diet alone or diet and DOCA, as in the present studies, would further support this interpretation. In micropuncture studies both the steady-state transtubular sodium concentration gradient and the net outward flux of sodium in the collecting duct of the rat were markedly augmented by aldosterone (48, 49).

The apparent independence of the potential in the DCT with respect to diet or exogenous mineralocorticoid is open to several interpretations. Since tubules are perfused in vitro in artificial solutions shortly after nephrectomy, the maintenance of a negative potential in the DCT in animals on a regular diet alone, in contrast to the CCT, could represent a difference in aldosterone binding affinity to tubular epithelium in the two segments or a difference in the half-life of an aldosterone-stimulated transport protein. It is also conceivable, though unlikely, that aldosterone exerts an electrogenic effect in the CCT, as has been shown in toad bladder (28, 29), but has a nonelectrogenic effect in the DCT. Finally, sodium transport may be independent of aldosterone in the latter segment, the collecting duct being the portion of the distal nephron responding to this hormone. In micropuncture studies, the distal tubular sodium concentration during both free-flow and stop-flow conditions has been found to be higher, and net sodium flux has been found to be lower in adrenalectomized rats as compared with controls (9, 10). The distal transtubular PD was the same in both groups, however. These results are difficult to interpret because of the previously mentioned heterogeneity of the so called "distal tubule" in micropuncture experiments.

The mechanism of potassium secretion by the distal nephron and the identification of factors that modulate this process have been the subject of numerous investigations (8, 9, 17, 19, 50). Most evidence favors the view that net secretion of potassium occurs by purely passive mechanisms, namely, diffusion down an electrochemical gradient from the tubular cell into luminal fluid (8, 17). The recent finding by Wright of a parallel and progressive rise in the electronegative PD and the luminal potassium concentration along the course of the distal tubule in the rat is keeping with this hypothesis (17). The magnitude of the transtubular potential can fully account for potassium secretion by a purely diffusional process along the entire distal tubule.

The present studies are the first to examine variations in the transtubular PD in the CCT in concert with changes in dietary sodium and potassium intake and thus to examine the possibility of a modulating influ-

ence of variations in the PD on ion excretion. In this regard, the ability of this segment of the nephron to vary the potential over a wide range with respect to spontaneous changes in dietary sodium and potassium intake may be a major factor in determining overall potassium balance. Indeed, a recent micropuncture study of potassium excretion by the remnant kidney indicated that changes in potassium excretion attending variations in potassium intake were mediated exclusively by the collecting duct (19). Although Grantham, Burg, and Orloff in contrast to most other investigators, found some evidence for a possible active component of potassium excretion in the collecting tubule, in that the calculated E_K was higher than the measured transtubular potential during equilibrium conditions (38), these data should not be interpreted to indicate that the PD is not a major determinant of transcellular potassium movement.

The value obtained in these studies for the L_p in the CCT after ADH (Table I), is similar to that reported by Schafer and Andreoli (41) of $136 \pm 28 \times 10^{-7}$ cm/s per atm and to those obtained by Grantham and Burg (35) and Grantham and Orloff (37) of $115–135 \times 10^{-7}$ cm/s per atm. The reason for the somewhat lower value reported in the present studies is not clear but probably relates to small differences in experimental technique.

Woodhall and Tisher (31) suggested that the apparent response of the distal tubule to vasopressin as reported in micropuncture studies was probably due to the inclusion of collecting-duct epithelium within the puncture segment. They found cellular swelling and dilatation of the lateral and basal interspaces, evidence of transtubular water flow in response to ADH, only in those tubules containing cells of collecting-duct morphology; tubules that were strictly of distal tubular epithelial type showed no changes (31). The present study provides the first direct physiologic demonstration of a clear-cut difference in ADH responsiveness in these two segments and would further support the concept of anatomic heterogeneity of the distal tubule. We would agree with the suggestion of Woodhall and Tisher that the reported rise in TF/P inulin along the course of the distal tubule in micropuncture studies (1) reflects the inclusion of initial collecting duct in the later portions of the distal tubule. As a hypotonic fluid from the more proximal, water-impermeable true distal tubule enters this water-permeable segment, osmotic equilibration with the surrounding interstitium occurs, and TF/P inulin rises.

There are different embryological origins of the mammalian DCT and the CCT, and it is not surprising that distinct functional differences were found between these two segments in the present studies. The CCT is formed as a result of the outgrowth and repeated bifurcation of the ureteric bud, which itself is derived from the mesonephric duct (34). In contrast, the DCT and all segments proximal to it, as well as the glomerulus, are derived from the growth and differentiation of the metanephric blastema (34). Thus, the distinct physiological differences between these two nephron segments in the present studies are corroborated not only by anatomic differences but also by their disparate embryological origins.

It should be emphasized that caution should be utilized in extrapolating the results obtained in this study of the rabbit to other mammalian species. The heterogeneity of transition from distal tubule to collecting duct among different species of the rats has already been mentioned (31). In addition, some evidence now exists that the positive transtubular PD in the cortical thick ascending limb of Henle's loop may extend in some strains of the rat beyond the macula densa (51). It is clear from the present study that this is not the case in the rabbit DCT, since a positive potential was never observed even though the macula densa was included in the perfused segment. Nevertheless, the results of the present study clearly indicate that there are multiple discrete differences in the physiologic profiles of the CCT and DCT.

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REFERENCES

1. Gottschalk, C. W. 1961. Micropuncture studies of tubular function in the mammalian kidney. *Physiologist.* **4**: 35–55.
2. Wirz, H. 1956. Der osmotische Druck in den corticalen tubuli der Rattenniere. *Helv. Physiol. Pharmacol. Acta.* **14**: 353–362.
3. Ullrich, K., G. Rumfich, and G. Fuchs. 1964. Wasser permeabilität and transtubulärer Wasserfluss corticaler Nephronabschnitte bei verschiedenen Diuresezuständen. *Arch. Gesamte Physiol. Mens. Tiere (Pfluegers).* **280**: 99–119.
4. Gertz, K. H. 1963. Transtubulare Natrium chlorid flusse und Permeabilitat fur nichtelektrolyte im proximalen und distalen konvolut der Rattenniere. *Arch. Gesamte Physiol. Mens. Tiere (Pfluegers).* **276**: 336–356.
5. Giebisch, G., and E. E. Windhager. 1964. Renal transfer of sodium, chloride, and potassium. *Am. J. Med.* **36**: 643–669.
6. Malnic, G., R. Klose, and G. Giebisch. 1966. Micropuncture study of distal tubular potassium and sodium transport in rat nephron. *Am. J. Physiol.* **211**: 529–547.

7. Malnic, G., R. M. Klose, and G. Giebisch. 1966. Microperfusion study of distal tubular potassium and sodium transfer in rat kidney. *Am. J. Physiol.* **211**: 548–559.
8. Giebisch, G., G. Malnic, R. M. Klose, and E. E. Windhager. 1966. Effect of ionic substitutions on distal potential differences in rat kidney. *Am. J. Physiol.* **211**: 560–568.
9. Hierholzer, K., M. Wiederholt, J. Holzgreve, G. Giebisch, R. M. Klose, and E. E. Windhager. 1965. Micropuncture study of renal transtubular concentration gradients of sodium and potassium in adrenalectomized rats. *Arch. Gesamte Physiol. Mens. Tiere (Pfluegers).* **285**: 193–210.
10. Hierholzer, K., M. Wiederholt, and H. Stolte. 1966. Hemmung der Natrium resorption im proximalen und distalen Konvolut adrenalektomierter Ratten. *Arch. Gesamte. Physiol. Mens. Tiere (Pfluegers).* **291**: 43–62.
11. Rector, F. C., Jr., and J. R. Clapp. 1962. Evidence for active chloride reabsorption in the distal tubule of the rat. *J. Clin. Invest.* **41**: 101–107.
12. Clapp, J. R., F. C. Rector, Jr., and D. W. Seldin. 1962. Effect of unreabsorbed anions on proximal and distal transtubular potentials in rats. *Am. J. Physiol.* **202**: 781–786.
13. Gottschalk, C. W., and M. Mylle. 1959. Micropuncture study of the mammalian urinary concentrating mechanism: evidence for the countercurrent hypothesis. *Am. J. Physiol.* **196**: 927–936.
14. Rector, F. C., Jr., N. W. Carter, and D. W. Seldin. 1965. The mechanism of bicarbonate reabsorption in the proximal and distal tubules of the kidney. *J. Clin. Invest.* **44**: 278–290.
15. Rector, F. C., Jr. 1971. Renal secretion of hydrogen. *In* The Kidney, C. Rouiller and A. F. Muller, editors. Academic Press, Inc., New York. **3**: 209–252.
16. Morgan, T., and R. W. Berliner. 1970. A study by continuous microperfusion of water and electrolyte movements in the loop of Henle and distal tubule of the rat. *Nephron.* **6**: 388–405.
17. Wright, F. S. 1971. Increasing magnitude of electrical potential along the renal distal tubule. *Am. J. Physiol.* **220**: 624–638.
18. Boulpaep, E. L., and J. F. Seely. 1971. Electrophysiology of proximal and distal tubules in the auto-perfused dog kidney. *Am. J. Physiol.* **221**: 1084–1096.
19. Bank, N., and H. S. Aynedjian. 1973. A micropuncture study of potassium excretion by the remnant kidney. *J. Clin. Invest.* **52**: 1480–1490.
20. Berliner, R. W. 1961. Renal mechanism for potassium excretion. *Harvey Lect.* **55**: 141–171.
21. Orloff, J., H. N. Wagner, Jr., and D. G. Davidson. 1958. The effect of variations in solute excretion and vasopressin dosage on the excretion of water in the dog. *J. Clin. Invest.* **37**: 458–464.
22. Berliner, R. W., and D. G. Davidson. 1957. Production of hypertonic urine in the absence of pituitary antidiuretic hormone. *J. Clin. Invest.* **36**: 1416–1427.
23. Giebisch, G., M. B. MacLeod, and R. F. Pitts. 1955. Effect of adrenal steroids on renal tubular reabsorption or bicarbonate. *Am. J. Physiol.* **183**: 377–386.
24. Pitts, R. F., and R. S. Alexander. 1945. The nature of the renal tubular mechanism for acidifying the urine. *Am. J. Physiol.* **144**: 239–254.
25. Law, R. 1973. The effects of 11-deoxycorticosterone and antidiuretic hormone (Pitressin) on fluid exchange and electrolyte excretion by normal and starved polyuric-polydipsic rabbits. *Pfluegers Arch. Eur. J. Physiol.* **345**: 249–263.
26. Seldin, D. W., G. Eknoyan, W. N. Suki, and F. C. Rector, Jr. 1966. Localization of diuretic action from the pattern of water and electrolyte excretion. *Ann. N. Y. Acad. Sci.* **139**: 328-343.
27. Hays, R. M., and A. Leaf. 1962. Studies on the movement of water through the isolated toad bladder and its modification by vasopressin. *J. Gen. Physiol.* **45**: 905–919.
28. Sharp, G. W. G., and A. Leaf. 1964. Biological action of aldosterone *in vitro*. *Nature (Lond.).* **202**: 1185–1188.
29. Sharp, G. W. G., C. H. Coggins, N. S. Lichtenstein, and A. Leaf. 1966. Evidence for a mucosal effect of aldosterone on sodium transport in the toad bladder. *J. Clin. Invest.* **45**: 1640–1647.
30. Handler, J. S., A. S. Preston, and J. Orloff. 1969. Effect of adrenal steroid hormones on the response of the toad's urinary bladder to vasopressin. *J. Clin. Invest.* **48**: 823–833.
31. Woodhall, P. B., and C. C. Tisher. 1973. Response of the distal tubule and cortical collecting tubule to vasopressin in the rat. *J. Clin. Invest.* **52**: 3095–3108.
32. Bloom, W., and D. W. Fawcett. 1968. The urinary system. *In* A Textbook of Histology. W. B. Saunders Company, Philadelphia. 9th edition. 652-684.
33. Trump, B. F., and R. E. Bulger. 1968. The morphology of the kidney. *In* The Structural Basis of Renal Disease. E. L. Becker and J. Ellis, editors. Hoeber Medical Division of Harper & Row, Publishers, New York. 1–92.
34. Huber, G. C. 1909–1910. The morphology and structure of the mammalian renal tubule. *Harvey Lect.* **5**: 100–149.
35. Grantham, J. J., and M. B. Burg. 1966. Effect of vasopressin and cyclic AMP on permeability of isolated collecting tubules. *Am. J. Physiol.* **211**: 255–259.
36. Burg, M. B., L. Isaacson, J. J. Grantham, and J. Orloff. 1968. Electrical properties of isolated perfused rabbit renal tubules. *Am. J. Physiol.* **215**: 788–794.
37. Grantham, J. S., and J. Orloff. 1968. Effect of prostaglandin E_1 on the permeability response of the isolated collecting tubule to vasopressin, adenosine 3',5'-monophosphate, and theophylline. *J. Clin. Invest.* **47**: 1154–1161.
38. Grantham, J. J., M. B. Burg, and J. Orloff. 1970. The nature of transtubular sodium and potassium transport in isolated rabbit renal collecting tubules. *J. Clin. Invest.* **49**: 1815–1826.
39. Schafer, J. A., and T. E. Andreoli. 1970. Vasopressin-dependent reflection coefficients in isolated collecting tubules. *Physiologist.* **13**: 302. (Abstr.)
40. Frindt, G., and M. B. Burg. 1972. Effect of vasopressin on sodium transport in renal collecting tubules. *Kidney Int.* **1**: 224–231.
41. Schafer, J. A., and T. E. Andreoli. 1972. Cellular constraints to diffusion. The effect of antidiuretic hormone on water flow in isolated mammalian collecting tubules. *J. Clin. Invest.* **51**: 1264–1278.
42. Kokko, J. P. 1970. Sodium chloride and water transport in the descending limb of Henle. *J. Clin. Invest.* **49**: 1838–1846.
43. Gomez-Sanchez, C., D. C. Kem, and N. M. Kaplan. 1973. A radioimmunoassay for plasma aldosterone by immunologic purification. *J. Clin. Endocrinol. Metab.* **36**: 795–798.

44. Kokko, J. P. 1973. Proximal tubule potential difference. Dependence on glucose, HCO_3 and amino acids. *J. Clin. Invest.* **52**: 1362–1367.
45. Grantham, J. J., C. E. Ganote, M. D. Burg, and J. Orloff. 1969. Paths of transtubular water flow in isolated renal collecting tubules. *J. Cell Biol.* **41**: 562–576.
46. Eigler, J., and J. Crabbe. 1969. Effect of diuretics on active Na transport in amphibian membranes. *In* Renal Transport and Diuretics. K. Thurau and J. Jarhmarker, editors. Berlin, Springer-Verlag KG. 195–207.
47. Stoner, L. C., M. B. Burg, and J. Orloff. 1974. Ion transport in cortical collecting tubule; effect of amiloride. *Am. J. Physiol.* **227**: 453–459.
48. Uhlich, E., C. A. Baldamus, and K. J. Ullrich. 1969. Einfluss von aldosteron auf der natriumtransport in den sammelrohren der Sausetierniere. *Pfluegers Arch. Eur. J. Physiol.* **308**: 111–126.
49. Uhlich, E., R. Halbach, and K. J. Ullrich. 1970. Einfluss von Aldosteron auf den ausstrom markierten natriums aus den Sammelrohren der Ratte. *Pfluegers Arch. Eur. J. Physiol.* **320**: 261–264.
50. Hierholzer, K. 1961. Secretion of potassium and acidification in collecting duets of mammalian kidney. *Am. J. Physiol.* **201**: 318–324.
51. Barratt, L. J., F. C. Rector, Jr., J. P. Kokko, C. C. Tisher, and D. W. Seldin. 1973. Transepithelial potential difference profile of the distal tubule of the rat kidney. *Am. Soc. Nephrol.* **6**: 7. (Abstr.)