Kidney International, Vol. 7 (1975), p. 35–43

Lanthanum permeability of tight junctions along the collecting duct of the rat

C. CRAIG TISHER and WILLIAM E. YARGER

Division of Nephrology, Department of Medicine and the Department of Pathology, Duke University Medical Center, Durham, North Carolina

Lanthanum permeability of tight junctions along the collecting duct of the rat. The permeability of the tight junctions (*zonulae occludentes*) was evaluated along the entire length of the collecting duct of the rat using a lanthanum tracer technique. Nine rats with hereditary hypothalamic diabetes insipidus were studied using standard micropuncture and clearance techniques. Glomerular filtration rate (GFR) estimated from inulin clearance, urine and plasma osmolality (U/P_{osm}) and urine flow rate (V) were determined in eight of nine animals. During either sustained diuresis (five animals) or vasopressin-induced antidiuresis (four animals), individual surface convolutions of distal convoluted tubules or early cortical collecting ducts were preserved for ultrastructural examination by intraluminal microperfusion with a glutaraldehyde-formaldehyde fixative followed by a second microperfusion with a lanthanum tracer. Mean GFR during diuresis was $6.31 \pm$ SE 0.63 ml/min/kg of body wt and $V = 797 \pm$ SE 108 μl/min/kg or $13.6 \pm$ SE 2.2% of the filtered load of water. After administration of exogenous vasopressin, V fell to 311 ± 157 μl/min/kg or $5.2 \pm$ SE 3.8% of the filtered load of water and U/P_{osm} rose from $0.658 \pm$ SE 0.043 to 2.124 ± 0.454. Tight junctions of cortical and outer medullary segments of the collecting duct resisted lanthanum penetration. Tight junctions of the inner medullary and papillary segments of the collecting duct were freely permeable to lanthanum suggesting the presence of a paracellular shunt pathway for solute and water movement. The results were independent of the presence or absence of vasopressin. Physiological studies have previously demonstrated that cortical and outer medullary segments of the collecting duct have a low urea permeability while inner medullary and papillary segments of the collecting duct have a relatively high urea permeability. The possibility is suggested that urea movement across the inner medullary and papillary segments of the collecting duct may occur, at least in part, via a paracellular pathway formed by the nonoccluding tight junction and the lateral intercellular space.

Perméabilité au lanthane des jonctions serrées le long du canal collecteur du rat. La perméabilité des jonctions serrées (zonulae occludentes) a été évaluée tout au long du canal collecteur du rat, au moyen d'une technique utilisant le lanthane comme traceur. Neuf rats atteints de diabète insipide hypothalamique héréditaire ont été étudiés au moyen des techniques habituelles de microponction et de clearance. La filtration glomérulaire (GFR) mesurée par la clearance de l'inuline, le rapport des osmolarités urine/plasma (U/P_{osm}) et le débit urinaire (V) ont été obtenus chez huit des neuf animaux. Au cours de la diurèse entretenue (cinq animaux) ou de l'antidiurèse induite par la vasopressine (quatre animaux) des convolutions superficielles de tubes contournés distaux ou des canaux collecteurs précoces ont été préservés aux fins d'étude ultrastructurale par la microperfusion intraluminale d'un fixateur glutaraldéhyde-formaldéhyde suivie d'une deuxième microperfusion de lanthane. Le GFR moyen au cours de la diurèse était de $6,31 \pm$ SE 0,63 ml/min/kg poids corporel et $V = 797 \pm$ SE 108 μl/min/kg ou $13,6 \pm$ SE 2,2% de la charge d'eau filtrée. Après l'administration de vasopressine, V a diminué à 311 ± 157 μl/min/kg ou $5,2 \pm$ SE 3,8 % de la charge d'eau filtrée et U/P_{osm} est passé de $0,658 \pm$ SE 0,043 à $2,124 \pm 0,454$. Les jonctions serrées des segments corticaux et médullaire externe des canaux collecteurs ont résisté à la pénétration de lanthane. Les jonctions serrées des segments médullaire interne et papillaire des canaux collecteurs ont été librement perméables au lanthane ce qui suggère une voie de shunt paracellulaire pour les mouvements d'ezu et de substances dissoutes. Les résultats sont indépendants de la présence ou de l'absence de vasopressine. Les études physiologiques antérieures ont montré que les segments corticaux et médullaires externes des canaux collecteurs ont une perméabilité faible pour l'urée, cependant que les segments médullaires internes et papillaires ont une perméabilité élevée. Il est suggéré que des mouvements d'urée à travers les segments médullaires internes et papillaires des canaux collecteurs puissent avoir lieu, au moins en partie, par une voie paracellulaire formée par les jonctions serrées non occlusives et l'espace intercellulaire latéral.

Received for publication April 26, 1974;
and in revised form July 15, 1974.

Electrophysiological measurements in a wide variety of transporting epithelia suggest that certain of these epithelia may possess a physiologically significant paracellular shunt pathway for ion and water movement. The anatomic locus of this shunt pathway has not been absolutely defined but current available evidence suggests that the *zonula occludens* or "tight" junction located on the apical cell surface together with the lateral intercellular space may represent the morphological correlate of this "electrical" paracellular shunt pathway. With the use of electron dense tracers such as lanthanum, which are largely confined to the extracellular space under optimum conditions of

tissue preservation, it has been possible to evaluate the permeability characteristics of the *zonulae occludentes* of several epithelia in order to establish morphologic evidence for the presence of anatomic shunt pathways between adjacent cells. Where both electrophysiological and morphological data are currently available, it has been observed that epithelia which have tight junctions that are truly occluding in nature, such as those in the cortical collecting tubule of the rat [1, 2] and in frog skin [4], also have a high transepithelial electrical resistance [4, 5] indicating the apparent absence of a functional paracellular shunt pathway. Conversely, it has been observed that epithelia with tight junctions that are readily penetrated by lanthanum (nonoccluding in type) such as are found in the proximal tubules of the rat [1, 2] and toad kidney [6] and in the rabbit gall bladder [7] possess a low transepithelial electrical resistance [7, 8] suggesting the presence of a functional shunt pathway.

Although attention has been directed primarily toward the role of the shunt pathway in the transepithelial movement of water and electrolytes, it is quite possible that certain nonelectrolytes may also move through these channels when they exist. For example, Baldamus et al have provided data to suggest that urea may move across the proximal tubule via a paracellular pathway [9]. Whether this is also true in other segments of the mammalian nephron, particularly the collecting duct, which is intimately involved in the handling of urea, is presently unknown. Therefore, the present study was initiated to determine whether known differences in the permeability characteristics of urea in the various anatomical segments of the mammalian collecting duct could be related to possible differences in permeability characteristics of their respective *zonulae occludentes* or tight junctions.

In the Brattleboro strain of Long-Evans hooded rat with congenital hypothalamic diabetes insipidus (DI) [10], it was observed that *zonulae occludentes* of the cortical and outer medullary segments of the collecting ducts resisted lanthanum penetration. Conversely, the *zonulae occludentes* of the inner medullary and papillary segments of the collecting duct were freely permeable to lanthanum. The results were not influenced by the presence or absence of vasopressin. These findings are compatible with the thesis that urea may move from the lumen of the inner medullary and papillary collecting ducts into the interstitium, at least in part, via a paracellular pathway formed by the nonoccluding tight junction and the lateral intercellular space which is not influenced by vasopressin. However, the results do not exclude the presence of a transcellular pathway for urea movement which may be influenced by vasopressin.

Methods

Nine rats with hereditary hypothalamic diabetes insipidus (DI) of both sexes weighing from 150 to 260 g were utilized in the present study. In the initial phase of the investigation, the animals were studied after an overnight fast during which free access to water was permitted. On the morning of the experimental day, a water diuresis was initiated by the administration of two separate water loads via gastric gavage at 30-min intervals (each water load was equivalent to 3% of body wt). The animals were then anesthetized via the i.p. administration of sodium pentobarbital (35 mg/kg of body wt), a tracheostomy was performed and polyethylene catheters were inserted into the carotid or femoral artery and the jugular or femoral vein. The animals were placed on a thermostatically controlled warming table and the left kidney was mobilized through an abdominal incision and prepared for micropuncture as previously described [11]. A polyethylene (P.E. 160) catheter was inserted into the urinary bladder via a suprapubic incision. Estimated surgical blood loss was replaced with isotonic saline (approximately 2 ml). The water diuresis was sustained by the constant i.v. infusion of a hypotonic sodium chloride solution (110 mM NaCl; 25 mM $NaHCO_3$; osmolality = 260 mOsm/kg of H_2O) at a rate of 0.2 ml/min. Mean arterial pressure was monitored throughout the entire procedure with a pressure transducer (Sanborn 267 BC) connected to a recorder (Sanborn, Hewlett-Packard Co., Waltham Div., Waltham, MA).

During the initial hour of the experimental period, inulin (100 mg/ml) added to 0.85% saline solution was infused i.v. in eight of the nine animals at a rate of 0.02 ml/min. After a 60-min equilibration period, three 20-min urine specimens and mid-point blood samples (200 μl/sample) were collected for the determination of baseline inulin clearance, urine flow and urine osmolality. After completion of the baseline clearance measurements, the hypotonic infusion was gradually increased until a sustained water diuresis of at least 100 μl/min/kg of body wt had been achieved. In five of the nine animals, the hypotonic infusion was maintained for an additional 60 to 90 min and collection of blood and urine samples for sequential clearance periods were obtained. During this period of sustained diuresis, surface convolutions of four to six distal tubules were identified following the i.v. injection of a 20 μl bolus of a 10% solution of lissamine green in 0.85% saline. A small-tipped micropipette was inserted into the tubule lumen and the individual tubules were perfused antegrade for 10 to 15 min with a glutaraldehyde-formaldehyde fixative solution containing 0.5% lissamine green (osmolality: 960 mOsm/kg of H_2O). The fixation technique was designed to

preserve an entire collecting duct beginning at the site of puncture and extending to the papillary tip. After perfusion fixation, the micropipette was withdrawn and a second micropipette containing a lanthanum solution (prepared according to the method of Revel and Karnovsky [12]) was introduced at the same puncture site, and the tubule was again perfused in an antegrade fashion with lanthanum for five to ten minutes. Distention and retrograde filling of the tubule lumen were carefully avoided during all perfusions. In some animals a third micropipette filled with latex (General Biological Supply House, Chicago, IL) was used to enter the same puncture site. The latex was perfused along the entire length of the collecting duct from cortex to papillary tip to aid in its subsequent identification [13]. Following completion of the microinjections, each kidney was removed and hemisected to reveal the entire papilla and inner and outer medulla. One-mm^3 blocks of tissue containing one or more of the fixed tubules were excised from all levels of the medulla beginning at the papillary tip and extending to the cortico-medullary junction. Blocks containing the surface convolutions of the distal convoluted tubule and initial collecting duct and segments of deeper cortical collecting duct were also dissected free from the adjacent unfixed tissue. All tissue was then postfixed for two hours in 1% osmium tetroxide buffered in *s*-collidine (osmolality: 190 mOsm/kg of H_2O) before routine dehydration in a graded series of alcohols and infiltration and embedding in epoxy resin (Epon) [14] for routine light and electron microscopy. No lanthanum was added to either the osmium tetroxide solution during postfixation or the alcohols that were used for dehydration.

Four of the original nine DI rats were treated in an identical manner except that after the water diuresis had been achieved, an i.v. infusion of aqueous vasopressin (Pitressin, Parke, Davis and Co., Detroit, MI) was initiated at the rate of 0.15 mU/min. Microperfusion fixation and lanthanum exposure of the distal tubule and entire collecting duct was then initiated within five minutes after the development of antidiuresis. The i.v. infusion of hypotonic saline was decreased to 0.05 ml/min after the animals became fully antidiuretic to avoid severe volume overload. Subsequent tissue processing was conducted in a manner identical to that already described herein.

Sections for light microscopy were cut 1 μ in thickness on a Porter-Blum ultramicrotome and stained with toluidine blue [15]. Thin sections for electron microscopy were cut with diamond knives, doubly stained with uranyl acetate [16] and lead citrate [17] or left unstained and examined and photographed with an electron microscope (AEI 6B, AEI Scientific Apparatus, Inc., Elmsford, NY).

Inulin concentrations in plasma and urine were measured by the anthrone method of Führ, Kaczmarczyk and Krüttgen [18]. The osmolality of urine was measured cryoscopically with an osmometer (Advanced Instruments, Inc. Needham Heights, MA).

Numerical data were analyzed by Student's *t* test. Data are expressed as mean ± SE and *P* values greater than 0.05 are considered to be nonsignificant (NS).

Results

Physiological observations. The results of the sequential clearance observations performed in the DI rats during diuresis and antidiuresis are shown in Table 1. In the eight rats in which physiological measurements were obtained, the average rate of glomerular filtration (GFR) was 6.31 ± SE 0.63 ml/min/kg of body wt. In

Table 1. Clearance data[a]

Animal No.	Vasopressin	V	GFR	V/GFR × 100	U/P_{osm}
1	−	0.612	3.44	17.7	0.632
2	−	0.529	7.93	6.7	0.816
3	−	0.763	5.45	14.0	0.863
4	−	0.592	6.18	9.6	0.632
5	−	0.557	8.71	6.4	0.628
	+	0.081	7.07	1.1	2.695
6	−	0.991	4.59	21.6	0.509
	+	0.103	6.15	1.7	3.044
7	−	0.906	7.51	12.0	0.537
	+	0.301	—	—	1.688
8	−	1.427	6.64	21.5	0.655
	+	0.758	5.92	12.7	1.070

[a] V = urine flow rate in μl/min/kg of body wt; GFR = glomerular filtration rate in ml/min/kg of body wt; V/GFR × 100 = % fractional water excretion and U/P_{osm} = ratio of urine to plasma osmolality.

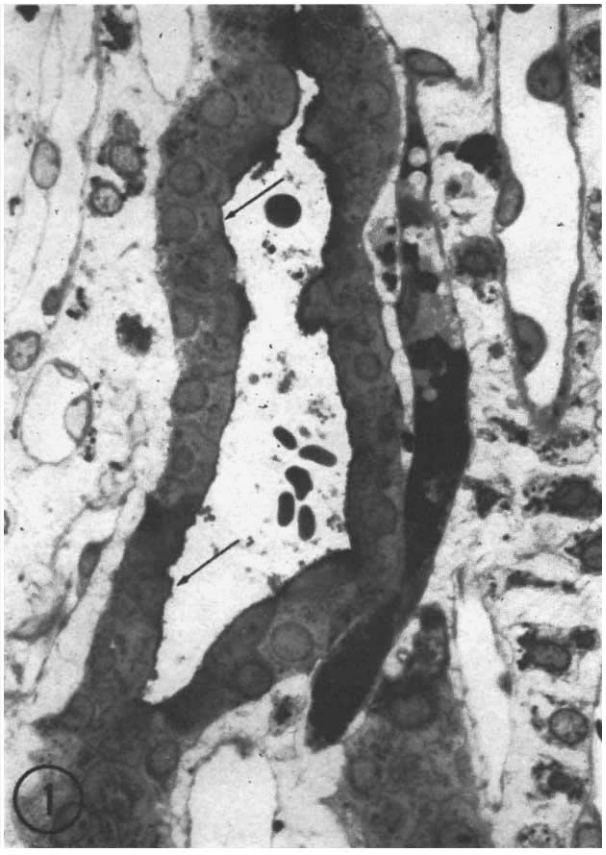


Fig. 1. *Photomicrograph of a rat collecting duct near the papillary tip in which lanthanum is discernible along the luminal cell borders* (arrows). (Epoxy resin [Epon] section stained with toluidine blue × 600.)

the absence of exogenous vasopressin these rats excreted water at a rate of 797 ± SE 108 μl/min/kg which represented 13.6 ± SE 2.2% of the filtered water load. When exogenous vasopressin was administered to these rats, the urine flow rate decreased to 211 ± SE 158 μl/min/kg or 5.2 ± 3.8% of the filtered load. Correspondingly, the U/P osmolar ratio rose from 0.658 ± SE 0.043 before vasopressin to 2.124 ± SE 0.454 after vasopressin administration was begun.

Morphological observations. Initial light microscopic examination was performed on a total of 96 separate specimens of tissue obtained from the nine DI rats. Detailed electron microscopic evaluation was then conducted in 37 of the 96 specimens in which collecting ducts containing lanthanum tracer were identifiable. Lanthanum tracer was often visible by light microscopy along the luminal cell surface of the collecting ducts, including those in the inner medulla and papilla, following the antegrade perfusion of the tracer into accessible surface convolutions of late distal convoluted tubules or early cortical collecting ducts (Fig. 1). In the collecting duct the tight junction or *zonula occludens* is approximately 0.3μ in depth and forms a continuous belt-like structure around the entire cell. Ultrastructural examination of the tight junctions in the cortical and outer medullary segments of the collecting duct consistently demonstrated their resistance to lanthanum penetration (Figs. 2 and 3). The results were identical in the presence and absence of exogenous vasopressin.

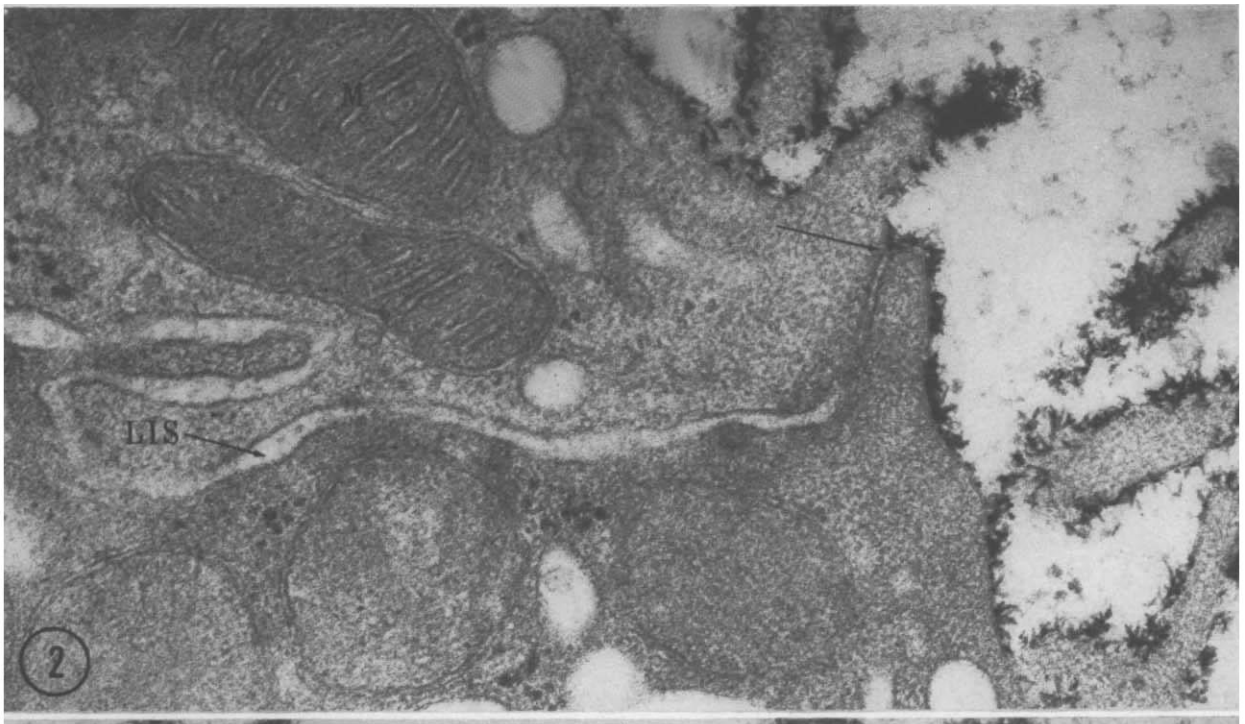
In striking contrast, the majority of the tight junctions in the inner medullary and papillary segments of the collecting duct were freely permeable to lanthanum. Again, the results were independent of the presence or absence of exogenous vasopressin (Figs. 4 through 6). The lanthanum was observed to penetrate the entire depth of the tight junction and frequently extended into the region of the intermediate junction or *zonula adhaerens* (Fig. 6). In general, the presence of the lanthanum in the tight junctions in these segments of the collecting duct appeared to correlate best with the total amount of lanthanum that was present within the tubule lumen. The transition from occluding to non-occluding tight junctions in the collecting duct generally occurred at the boundary between the outer and inner zones of the medulla, although the point of transition was not always precise so that a certain amount of overlap did occur.

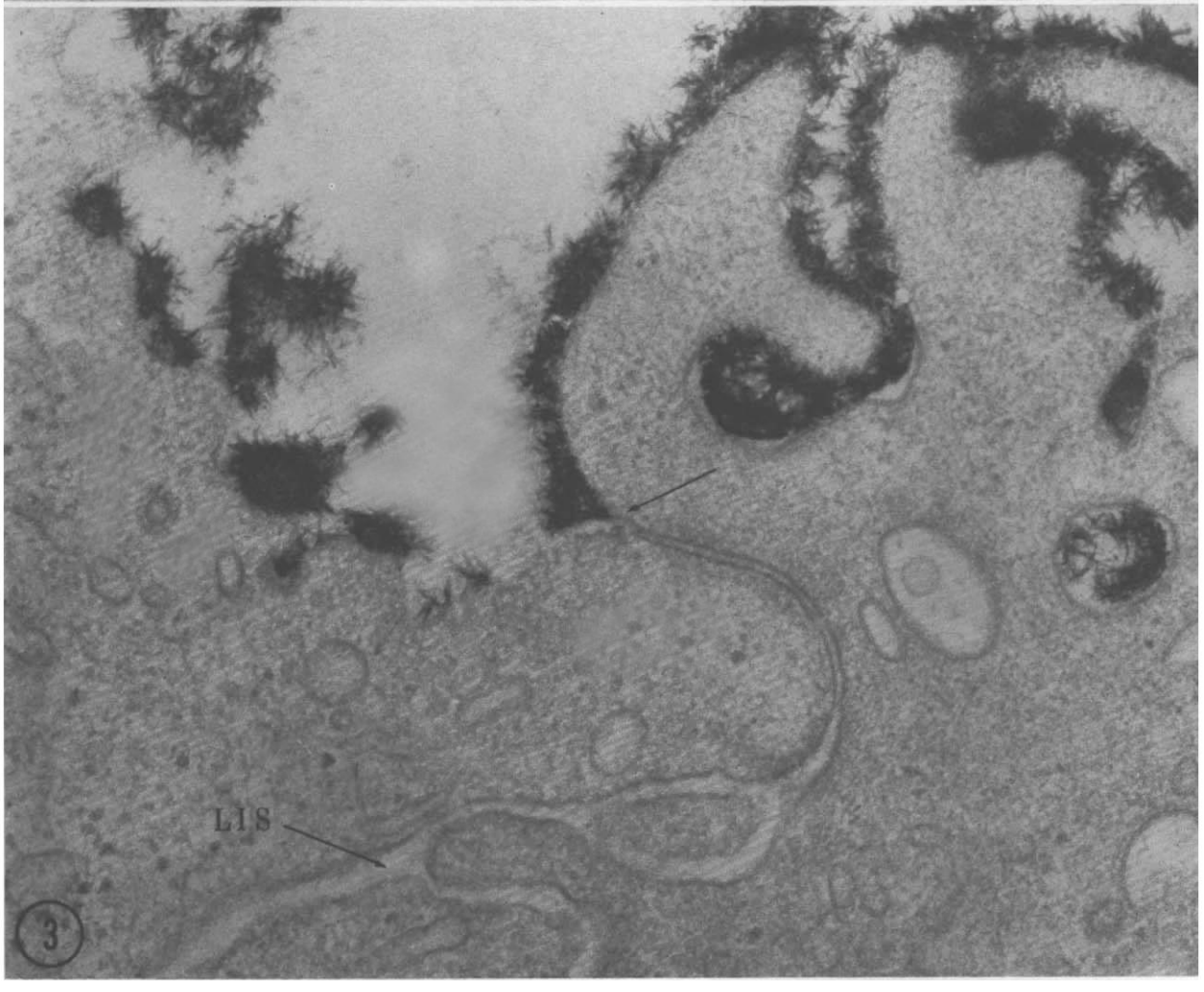
Discussion

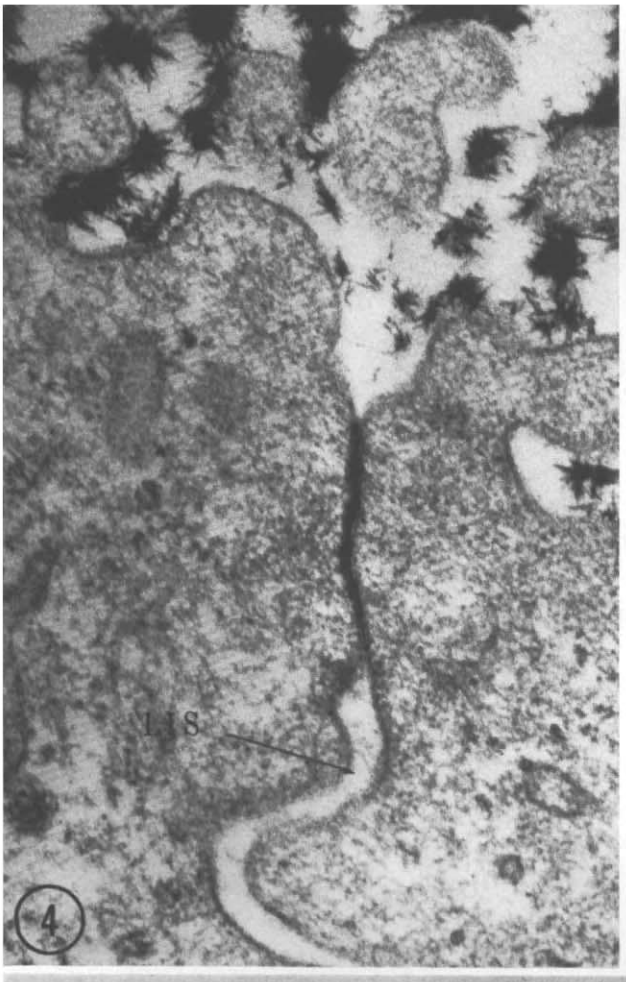
The present morphological study demonstrates for the first time a marked difference in the ability of lanthanum, employed as an extracellular tracer, to penetrate the *zonulae occludentes* or tight junctions in the various anatomical segments of the collecting duct of the rat. Earlier observations in the collecting duct of the Sprague-Dawley rat were limited exclusively to the cortical segment where it was also demonstrated that the tight junctions resisted lanthanum penetration [1]. Those morphologic findings were interpreted as being

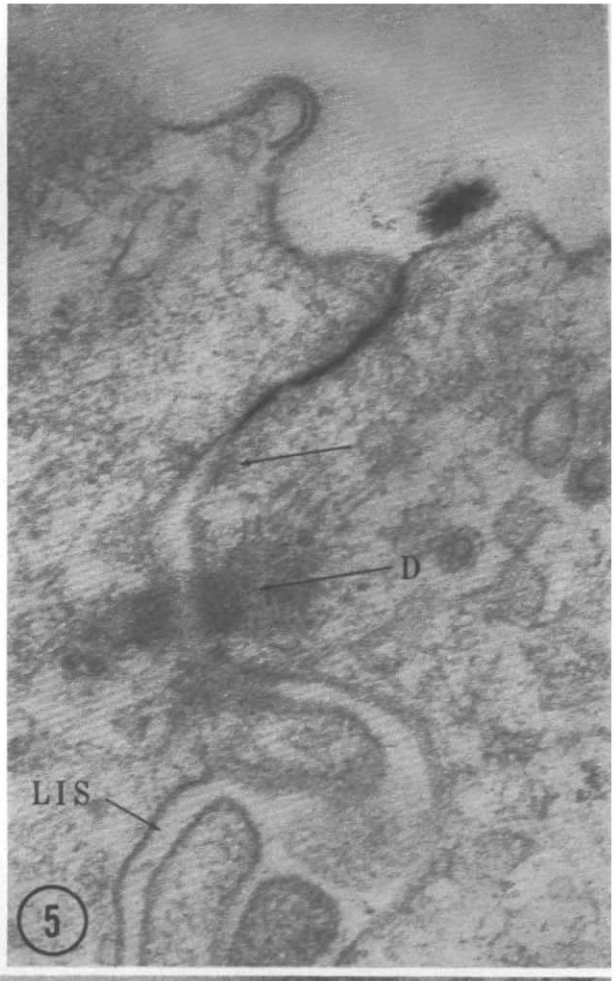
Fig. 2. *Electron micrograph of the apical region of a cortical collecting duct from a DI rat preserved for histologic examination during vasopressin-induced antidiuresis.* The tubule lumen is *at the right.* Electron dense lanthanum precipitate is adherent to the apical plasma membrane but fails to penetrate the tight junction or *zonula occludens* (*arrow*). LIS, lateral intercellular space; M, mitochondrion. (Section doubly stained with uranyl acetate and lead citrate, × 82,800.)

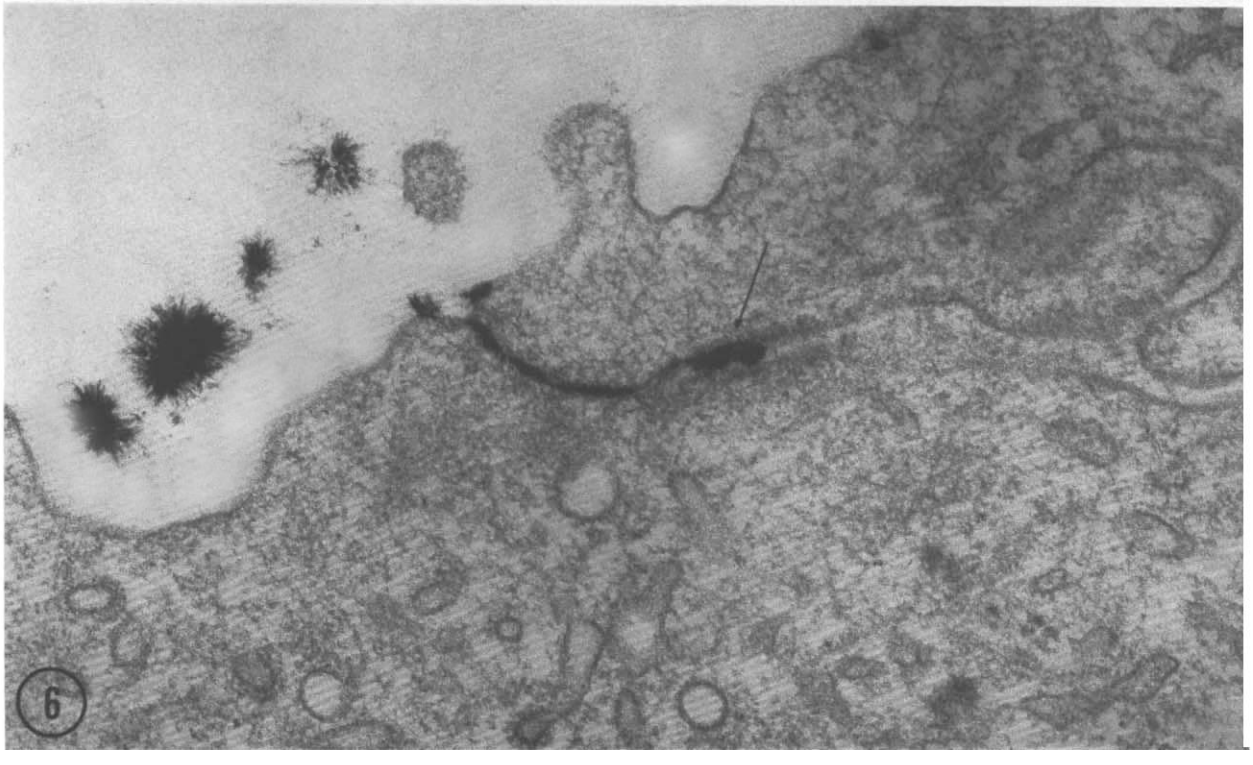
Fig. 3. *Electron micrograph depicting the apical region of the outer medullary segment of a collecting duct from a DI rat preserved for histological evaluation during vasopressin-induced antidiuresis.* The tight junction or *zonula occludens* (*arrow*) resists penetration by lanthanum. LIS, lateral intercellular space. (Section doubly stained with uranyl acetate and lead citrate, × 91,200.)


M
LIS
2


LIS
3


LIS
4


D
LIS
5


6

consistent with the absence of a significant paracellular shunt pathway for ion and water movement in the cortical segment of the collecting duct. The current study extends the earlier observations in the cortical collecting duct by demonstrating that these permeability characteristics of the tight junction, as determined morphologically, were not altered by the presence of exogenous vasopressin in amounts sufficient to convert DI rats from a state of water diuresis to antidiuresis. These morphologic results correlate well with measurements of transepithelial electrical resistance that have been reported previously in this segment of the mammalian collecting duct. Helman, Grantham and Burg [4], working with isolated segments of rabbit cortical collecting tubules, found a relatively high transepithelial electrical resistance of approximately 867 $\Omega-cm^2$. Although these same workers found that the transepithelial potential difference (PD) was transiently increased after the addition of vasopressin to the bathing media in a concentration sufficient to induce osmotic water flow, the high transepithelial electrical resistance was not significantly effected. Thus, the available morphologic and electrophysiologic data are in agreement and fail to provide evidence of a functional paracellular shunt pathway for ion and water movement in this segment of the collecting duct. Furthermore, the results suggest that the tight junction does not represent a major pathway for vasopressin-induced osmotic water flow within the cortical collecting duct.

In the outer medullary segment of the collecting duct, as in the cortical collecting duct, no morphologic evidence was found for the existence of a paracellular shunt pathway. Again, the results were not influenced by the presence or absence of vasopressin. Measurements of transepithelial electrical resistance are not currently available from this segment of the renal tubule to relate to the morphologic findings, but based on the permeability characteristics of the tight junction, it would be predicted that the transepithelial electrical resistance in the outer medullary collecting duct should also be quite high, again reflecting the probable absence of a functional paracellular shunt pathway.

Only within the inner medullary and papillary segments of the collecting duct was there morphologic evidence of a paracellular shunt pathway. Here, too, no apparent influence of vasopressin was noted on the permeability characteristics of the tight junctions since the morphologic findings were identical in the presence and absence of the hormone. Again, measurements of transepithelial electrical resistance which would permit correlation with the morphologic evidence suggesting the presence of a paracellular shunt pathway are not currently available in these segments of the collecting duct. However, if the same relationship between transepithelial electrical resistance and lanthanum permeability of the tight junction pertains here as in other transporting epithelia, it would be anticipated that values for transepithelial electrical resistance should be low in the inner medullary and papillary segments of the collecting duct.

The functional significance of these morphologic findings remains largely speculative at this time. However, an increasing body of evidence is accumulating to suggest that low electrical resistance paracellular shunt pathways may play a dominant role in the passive movement of ions and water across certain epithelia [1, 2, 6–8, 19, 20]. Results of recent electrophysiologic studies by Frömter utilizing a voltage scanning microelectrode strongly suggest that the anatomic locus for this electrical paracellular shunt is located in the region of the junction between cells [19]. Where morphologic data are available, it has been shown that epithelia with electrical shunt pathways have nonoccluding tight junctions that can be easily penetrated by extracellular markers such as lanthanum [1, 2, 6–8], while high resistance tissues without significant electrical shunt pathways have tight junctions which resist lanthanum penetration [1, 2, 4, 5].

Recently, more attention has been devoted to the potential role of these paracellular shunt pathways in the movement of nonelectrolytes. At present the most

---

Fig. 4. *Electron micrograph demonstrating lanthanum penetration of the tight junction between adjacent cells of a collecting duct from the inner medulla.* Tissue preservation was accomplished during vasopressin-induced antidiuresis. LIS, lateral intercellular space. (Section doubly stained with uranyl acetate and lead citrate, ×98,400.)

Fig. 5. *Electron micrograph depicting lanthanum penetrating the tight junction (zonula occludens) between two cells from the papillary segment of a collecting duct.* Tissue preservation was performed during water diuresis in the absence of vasopressin. Note the paucity of lanthanum along the apical cell surface, yet the tight junction contains the extracellular tracer. LIS, lateral intercellular space; *arrow*, intermediate junction or *zonula adhaerens;* D, desmosome or *macula adhaerens.* (Section doubly stained with uranyl acetate and lead citrate, ×94,000.)

Fig. 6. *Electron micrograph demonstrating lanthanum penetration of the tight junction (zonula occludens) in the papillary segment of a collecting duct.* Tissue preservation was accomplished during water diuresis in the absence of vasopressin. The lanthanum has extended beyond the tight junction down to the level of the intermediate junction (*zonula adhaerens*) (*arrow*). (Section doubly stained with uranyl acetate and lead citrate, ×86,000.)

direct evidence in support of the movement of nonelectrolytes through paracellular shunt pathways formed by the tight junction and the lateral intercellular space has been obtained in the frog skin. Ordinarily this tissue has a high electrical resistance and is relatively impermeable to extracellular solutes such as sucrose [21]. However, when the solution bathing the outside of the frog skin is made hyperosmotic, the electrical resistance falls significantly and there is a marked increase in permeability to both electrolytes and nonelectrolytes such as sucrose [21, 22]. These results have been interpreted by Ussing [21, 22] as evidence that the hyperosmotic solutions caused the occluding tight junctions to open. Later studies by Martinez-Palomo, Erlij and Bracho [3] demonstrated that tight junctions of the frog skin were not penetrated by lanthanum when the epithelium was bathed with isotonic frog Ringer's solution, but when the external bathing solution was made hyperosmotic with urea, thus increasing its permeability to electrolytes and nonelectrolytes, lanthanum employed as an electron dense extracellular tracer easily penetrated the tight junctions [23]. Evidence is also available to suggest that urea may traverse certain epithelia via paracellular pathways. On the basis of data derived from stop-flow and continuous flow-through microperfusion experiments, Baldamus et al [9] have suggested that urea flux across the epithelium of the proximal tubule may occur primarily via a paracellular pathway. In other studies Mandel and Curran [24], working with frog skin, reported that urea movement, believed to occur through a paracellular shunt pathway, was increased as the conductance increased under conditions of skin depolarization.

The possibility is raised that the tight junctions in the inner medulla and papillary segments of the collecting duct along with the lateral intercellular spaces may form a functional paracellular shunt pathway. It is possible that such a shunt pathway could have considerable relevance to the mechanism of formation of a concentrated urine by the mammalian kidney, possibly by facilitating the movement of urea from the inner medullary and papillary segments of the collecting duct into the medullary interstitium. It is currently believed that urea recycling resulting in urea trapping in the inner medulla and papilla is necessary to form a maximally concentrated urine. To deliver urea in high concentration to the interstitium of the inner medulla and papilla, collecting ducts in the cortex and outer medulla should be relatively impermeable to urea but freely permeable to water in the presence of vasopressin. These permeability characteristics would facilitate abstraction of water from the tubular fluid and leave behind a fluid with an increased concentration of urea to enter the inner medulla. On the other hand, the inner medullary and papillary segments of the collecting duct should be permeable to urea in order to enhance movement of urea from the tubule lumen to the surrounding interstitium. It has been demonstrated that isolated segments of rabbit cortical and outer medullary collecting tubules are essentially impermeable to urea but permeable to water in the presence of vasopressin [25–28], while similar preparations of isolated papillary collecting ducts have been found to be permeable to both urea and water in the presence of vasopressin; however, contrary to the results of early studies [29–32], vasopressin was not found to enhance urea permeability in the papillary collecting duct [28]. If urea were to move principally via paracellular pathways, the apparent differences in lanthanum permeability of the tight junctions could serve to explain the differences in permeability characteristics to urea in the various isolated segments of the rabbit collecting duct. While there is generally good agreement that urea movement in both the cortical and outer medullary segments of the collecting duct is little affected by vasopressin, data regarding the effect of vasopressin on urea permeability of the papillary collecting duct is conflicting. In the rat, Gardner and Maffly [29] and Morgan and Berliner [30] found that vasopressin administration enhances urea permeability in the papillary collecting duct. Similar results were obtained by Jaenicke in the dog [31] and by Bowman and Foulkes [32] in the rabbit. On the other hand, Rocha and Kokko [28] have observed that the urea permeability of isolated segments of rabbit papillary collecting ducts is not enhanced by the addition of vasopressin to the preparation. Although the current morphologic observations are more in keeping with the physiologic observations of Rocha and Kokko [28], they cannot be used to resolve the apparent conflict in results with respect to the effect of vasopressin on urea permeability in the papillary collecting duct since the question of the presence of transcellular urea movement cannot be addressed by these morphologic techniques.

Acknowledgments

This work was supported in part by Public Health Service grants AM 13845, AM 17195 and HL 5848 and by project No. 9750–01 from the Veterans Administration Hospital and the Department of Medicine, Duke University Medical Center. Dr. Tisher is the recipient of Public Health Service Research Career Development Award HL 440074; Dr. Yarger is a Research and Education Associate of the Veterans Administration. A preliminary report of this work appeared in ab-

stract form (*Clin Res* 22:52a, 1974). Mrs. Helen Parks, Mrs. Kathy Blake and Mrs. Betty Waller provided technical assistance and Mrs. Jessie Calder prepared the illustrative material.

Reprint requests to Dr. C. Craig Tisher, Division of Nephrology, Box 3014, Duke University Medical Center, Durham, North Carolina 27710, U.S.A.

Addendum

Since submission and acceptance of the manuscript, Rau and Frömter (Rau, W.S., and Frömter, E. Electrical properties of the medullary collecting ducts of the Golden hamster kidney. II. The transepithelial resistance. *Pflügers Arch* 351:113–131, 1974) have reported measurements of transepithelial electrical resistance from what appear to represent inner medullary collecting ducts of the Golden hamster. Using several indirect techniques, these authors have estimated that the transepithelial resistance is approximately 1000 ohm-cm^2, a value which would suggest that the medullary collecting duct of the Golden hamster is a high resistance epithelium of the type which usually has a lanthanum impermeable occluding type of tight junction. However, the authors note that using their measured values for transepithelial resistance and potential difference, the calculated short circuit current, an estimate of net sodium transport, amounts to only 6.5 to 13% of the net sodium transport rate previously measured in rat medullary collecting duct, and suggest that this difference in the electrical properties of the collecting duct may represent species variation. In the absence of lanthanum tracer studies in the collecting duct of the Golden hamster, it is difficult to relate their electrophysiological findings to the present morphological data derived from the rat.

References

1. Tisher CC, Yarger WE: Lanthanum permeability of the tight junction (zonula occludens) in the renal tubule of the rat. *Kidney Int* 3:238–250, 1973
2. Martinez-Palomo A, Erlij D: The distribution of lanthanum in tight junctions of the kidney tubule. *Pflügers Arch* 343:267–272, 1973
3. Martinez-Palomo A, Erlij D, Bracho H: Localization of permeability barriers in the frog skin epithelium. *J Cell Biol* 50:277–287, 1971
4. Helman SI, Grantham JJ, Burg MB: Effect of vasopressin on electrical resistance of renal cortical collecting tubules. *Am J Physiol* 220:1825–1832, 1971
5. Cereijido M, Curran PF: Intracellular electrical potentials in frog skin. *J Gen Physiol* 48:543–557, 1965
6. Whittembury G, Rawlins FA: Evidence of a paracellular pathway for ion flow in the kidney proximal tubule: Electron microscopic demonstration of lanthanum precipitate in the tight junction. *Pflügers Arch* 330:302–309, 1971
7. Machen TE, Erlij D, Wooding FBP: Permeable junctional complexes: The movement of lanthanum across rabbit gall bladder and intestine. *J Cell Biol* 54:302–312, 1972
8. Hegel U, Frömter E, Wick T: Der elektrische Wandwiderstand des proximalen Konvolutes der Rattenniere. *Pflügers Arch* 292:274–290, 1967
9. Baldamus CA, Radke HW, Rumrich G, Sauer F, Ullrich KJ: Reflection coefficient and permeability of urea in the proximal convolution of the rat kidney. *J Membr Biol* 7: 377–390, 1972
10. Valtin H: Hereditary hypothalamic diabetes insipidus in rats (Brattleboro strain): A useful experimental model. *Am J Med* 42:814–827, 1967
11. Tisher CC, Cirksena WJ, Arstila AU, Trump BF: Subcellular localization of sodium in normal and injured proximal tubules. *Am J Pathol* 57:231–251, 1969
12. Revel JP, Karnovsky M: Hexagonal array of subunits in intercellular junctions of the mouse heart and liver. *J Cell Biol* 33:C7–C12, 1967
13. Tisher CC, Clapp JR: Intraluminal latex injection: An aid to the histological identification of renal tubules. *Kidney Int* 2:54–56, 1972
14. Luft JH: Improvements in epoxy resin embedding methods. *J Biophys Biochem Cytol* 9:409–414, 1961
15. Trump BF, Smuckler EA, Benditt EP: A method for staining epoxy sections for light microscopy. *J Ultrastruct Res* 5:343–348, 1961
16. Watson ML: Staining of tissue sections for electron microscopy with heavy metals. *J Biophys Biochem Cytol* 4:475–478, 1958
17. Reynolds ES: The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J Cell Biol* 17: 208–212, 1963
18. Führ J, Kaczmarck J, Krüttgen CD: Eine einfache colorimetrische method zur Inulinbestimmung für Nierenclearance-Untersuchungen bei Stoffwechselgesunden und Diabetikern. *Klin Wochenschr* 33:729–730, 1955
19. Frömter E: The route of passive ion movement through the epithelium of Necturus gallbladder. *J Membr Biol* 8:259–301, 1972
20. Boulpaep EL: Electrophysiological properties of the proximal tubule: Importance of cellular and intercellular pathways, in *Electrophysiology of Epithelial Cells* (Symposia Medica Höechst), edited by Giebisch G, Frömter E, New York, FK Schattauer Verlag, 1971, p. 91
21. Ussing HH: Anomalous transport of electrolytes and sucrose through the isolated frog skin induced by hypertonicity of the outside bathing solution. *Ann NY Acad Sci* 137:543–555, 1966
22. Ussing HH: Relationship between osmotic reactions and active sodium transport in frog skin. *Acta Physiol Scand* 63: 141–155, 1965
23. Erlij D, Martinez-Palomo A: Opening of tight junctions in frog skin by hypertonic urea solutions. *J Membr Biol* 9: 229–240, 1972
24. Mandel LJ, Curran PF: Response of the frog skin to steady-state voltage clamping: I. The shunt pathway. *J Gen Physiol* 59:503–518, 1972
25. Burg M, Helman S, Grantham J, Orloff J: Effect of vasopressin on the permeability of isolated rabbit cortical collecting tubules to urea, acetamide and thiourea, in *Urea and the Kidney*, edited by Schmidt-Nielsen B, Kerr DWS, Amsterdam, Excerpta Medica Foundation, 1970, p. 193
26. Schafer JA, Andreoli TE: The effect of antidiuretic hormone on solute flows in mammalian collecting tubules. *J Clin Invest* 51:1279–1286, 1972

27. SCHAFER JA, ANDREOLI TE: Cellular constraints to diffusion: The effect of antidiuretic hormone on water flows in isolated mammalian collecting tubule. *J Clin Invest* 51: 1264–1278, 1972
28. ROCHA AS, KOKKO JP: Permeability of the medullary nephron segments to urea and water: Effect of vasopressin. *Kidney Int* 6:146–156, 1974
29. GARDNER KD, JR, MAFFLY RH: An *in vivo* demonstration of increased collecting tubular permeability to urea in the presence of vasopressin. *J Clin Invest* 43:1968–1975, 1964
30. MORGAN T, BERLINER RW: Permeability of the loop of Henle, vasa recta, and collecting duct to water, urea, and sodium. *Am J Physiol* 215:108–115, 1968
31. JAENIKE JR: The influence of vasopressin on the permeability of the mamalian collecting duct to urea. *J Clin Invest* 40:144–151, 1961
32. BOWMAN FJ, FOULKES EC: Antidiuretic hormone and urea permeability of collecting ducts. *Am J. Physiol* 218:231–233, 1970