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Studies of organic anion and cation transport in isolated segments of proximal tubules

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The transport of organic anions and cations has interested physiologists for many years despite the generally held view that these transport mechanisms are involved principally in the elimination of foreign substances and metabolites from the body. The clearance and excretion of para-aminohippurate (PAH) has been used an an index of renal plasma flow, and the maximal rate of PAH secretion has been assumed to reflect the mass of functioning renal tissue in patients [1]. Clinicians also utilize the fractional excretion of urate as a guide to volume sufficiency of the extracellular fluid compartment and quantify the excretion rate of this organic solute in the evaluation of gout and urolithiasis [2]. The renal excretion of organic cations has been of principal interest to renal pharmacologists, although there is increased awareness among clinicians that this system is important in the regulation of endogenous levels of organic bases [1].

Prior to the introduction of the in vitro microperfusion method in 1965, studies utilizing clearance, stop-flow and in situ micropuncture methods had generally localized the site of weak organic acid and base transport to the proximal tubules. In the initial report on tubule perfusion in vitro, Burg, Grantham, Abramow, and Orloff [3] confirmed that proximal straight tubules secreted PAH from the bath into the urine. Subsequently, the isolated tubule method has been used to study the tubular secretion or reabsorption of chlorphenol red, diodrast, urate, prostaglandin E_2, lactate, oxalate, cimetidine, creatinine, and procainamide. The in vitro transport of these substances has been examined principally in the rabbit, but snake, frog, flounder, and human tubules have been studied as well.

Organic anion secretion—PAH as a prototype. The study by Tune, Burg, and Patlak [4] set the tone for subsequent investigations of organic anion transport in isolated proximal tubules. Their study antedated the recent division of proximal tubules into three axial segments and the further differentiation among tubules originating in superficial and juxtamedullary glomeruli. Nonetheless, the crude division of nephrons into convoluted and straight proximal tubule segments gave a clear distinction between the transport capacities of these segments for the secretion of PAH and introduced the heterogeneity concept in relation to tubular function. With 2.5×10^{-5} M PAH in the bathing medium (commercial rabbit serum), the rate of PAH secretion of straight segments was found to be three to five times greater than in convoluted segments. Since the cellular concentration of PAH was higher than that in the bath or in the tubule fluid during the steady-state secretion of PAH from bath to lumen, the PAH transporter was localized to the basolateral plasma membrane (peritubular membrane) of the cells. The basolateral location of the transport was also supported by the finding that PAH was accumulated to high levels in the cells from the bathing medium in non-perfused tubules with collapsed lumens. Probenecid in the bathing medium blocked cellular accumulation and transtubule secretion of PAH, and PAH secretion was unaffected by changing the rate of tubular perfusion, providing support for the view that PAH was transported actively into the cells, with "downhill" movement into the urine. The simple model derived from these studies is reproduced in Figure 1.

On the basis of these initial studies, it was clear that proximal straight tubules possessed a powerful secretory transporter in the basolateral membrane capable of increasing the intracellular level of PAH several hundredfold higher than in the bathing medium. In a fortuitous series of studies performed by Grantham, Qualizza, and Irwin (5), Grantham et al [6], and Grantham (7), it was found that the PAH secretory process was strong enough to reverse the normal direction of fluid transport in proximal straight tubules. The demonstration of net fluid secretion was made in non-perfused tubules and in perfused tubules in which the distal end had been occluded. In a bath of rabbit serum, fluid secretion could be detected with PAH levels as low as 10 μM, the maximal rate of fluid secretion being observed with about 1 mM PAH in the medium. In the course of net fluid secretion the tubules elevated the lumen PAH concentration to about 40 mM at all levels of bath PAH concentration. Since the secreted fluid was isosmotic, this suggested that PAH was secreted into the cells and then into the lumen where the anion promoted the movement of sodium, potassium, chloride, and water into the urine, possibly through paracellular channels that were not permeable to PAH. The phenomenon of fluid secretion initiated by PAH is associated with hyperpolarization of the transtubular electrical potential, that is, the lumen becomes electrically more negative by a few millivolts [5]. This, together with the net accumulation of a relatively impermeant anion in the tubule fluid, suggests that fluid secretion is driven by anion transport, rather than the secretion of a cation. Recent studies also show that in the initial phase of fluid secretion driven by PAH transport, the volume of the tubule cells does not change appreciably [8]. This suggests that PAH may enter the cytoplasm in exchange for another diffusible intracellular

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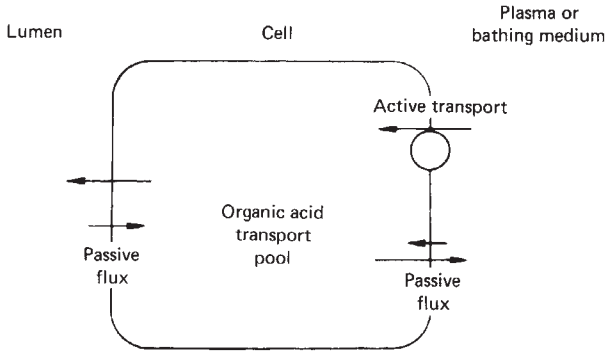

Fig. 1. *Tune-Burg-Patlak model of PAH transport in proximal tubule.* PAH is driven into the cells by a transporter in the basolateral membrane. The PAH diffuses preferentially into the lumen because the permeability of the luminal membrane to the organic anion is about 16 times greater than that of the basolateral membrane. (Reprinted with permission from [4]).

anion. Chloride and bicarbonate are the most logical candidates for the intracellular anions exchanged for PAH. A model of fluid secretion driven by PAH transport is illustrated in Figure 2.

Fluid secretion is observed in non-perfused proximal straight tubules in response to several organic anions including hippurate, benzoate, cephalothin, carbenicillin, and salicylurate. Organic cations (tetraethylammonium, quinine, and N-methylnicotinamide) do not cause fluid secretion [9]. Net fluid secretion has been demonstrated in a single human proximal straight tubule [10]. Of further interest is the fact that serum obtained from uremic rabbits and uremic patients also causes fluid secretion in proximal straight tubules [6, 9]. Some uremic patients have exceedingly high levels of a secretagogue that is removed by hemodialysis. Hippurate accounts for about one third of the secretory activity in human uremic serum [9]. The remainder is comprised of a myriad of organic anions, all transported by the PAH mechanism.

Net fluid secretion driven by organic anion transport has minimal influence on net tubule fluid absorption in normal or uremic subjects [11] despite an early supposition by the author [5, 6]. Recent studies show that in intact nephrons with relatively normal tubule fluid perfusion rates, the concentration of PAH does not increase to levels in tubule fluid high enough to counteract the net absorption of sodium chloride and sodium bicarbonate in the proximal tubule [11, 12]. In obstructed nephrons or during extreme reductions in GFR, the levels of PAH (and other endogenous secretagogues) may increase to high enough levels in tubule fluid to decrease the fractional reabsorption of tubule fluid or even cause net secretion. This probably accounts for the widely dilated proximal tubules commonly observed in fixed specimens obtained from patients with so-called "end-stage" renal diseases in which the glomeruli are damaged severely and single nephron glomerular filtration rate markedly reduced. It remains to be determined whether or not the newly recognized acquired-type of multicystic disease in dialysis patients is derived as a consequence of net fluid secretion in obstructed nephrons. Thus, the phenomenon of net fluid secretion driven by organic anion transport in slowly perfused proximal tubules illustrates a latent mechanism that may be important in certain pathologic states.

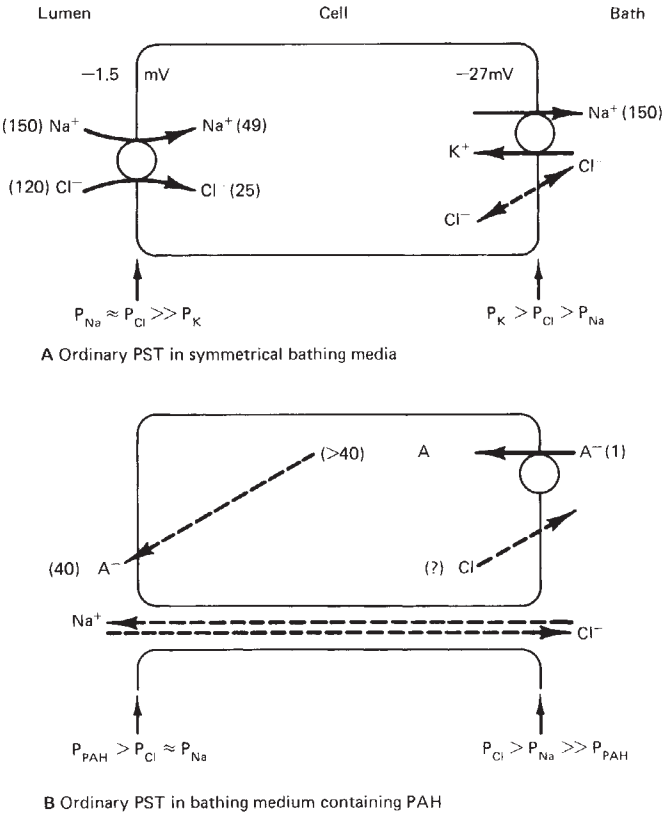

Fig. 2. *Model of fluid secretion driven by PAH transport in S_2 proximal tubule.* **A** *An "ordinary" cell, the principal elements being the sodium-potassium exchange pump (Na+K, ATPase), gradients for Na and Cl directed into the cells across the basolateral and luminal membranes, a sodium-chloride cotransport entry mechanism in the luminal membrane, and assymetric membrane permeabilities to K, Cl, and Na.* The transmembrane PD (cell to bath) is −27 mV, and the transtubule PD (lumen to bath) is −1.5 mV. **B** *A much simplified scheme of how PAH (A−) causes fluid and solutes to move from bath to lumen.* The hippurate anion is pumped into the cell to a concentration greater than 40 mM forcing the loss of anion (Cl^- and possibly HCO_3^-) from the cell. The hippurate anion diffuses into the lumen (when the lumen is collapsed in non-perfused tubules, the hippurate diffuses into the thin layer of fluid that separates adjacent membranes) causing increased lumen negativity, which in turn causes sodium to enter and chloride to leave the lumen via the paracellular pathway. Since the reflection coefficient for A^- in the lumen is higher than that for chloride and bicarbonate in the external medium, fluid moves in bulk from the bath into the lumen. A concentration of PAH in the lumen of about 40 mM is evidently needed to "balance" and slightly exceed the action of the sodium pump to promote fluid reabsorption. This model predicts that if the hippurate transporter is not dependent on the inward sodium gradient across the basolateral membrane, then inhibition of the sodium pump should accelerate the rate of fluid secretion for a fixed level of PAH secretion. (Reprinted with permission from [8]).

Cellular basis for PAH anion secretion. Woodhall et al [13] further defined the tubular sites of PAH secretion in the rabbit. Each proximal tubule is made up of at least three axially contiguous segments that transport PAH at different rates and have characteristic ultrastructural features. These segments have been designated S_1, S_2, and S_3. The initial S_1 segment, beginning with the glomerulus, secretes PAH at a relatively low rate, as does the terminal portion of the proximal straight tubule, S_3, or pars recta. By contrast that segment in between,

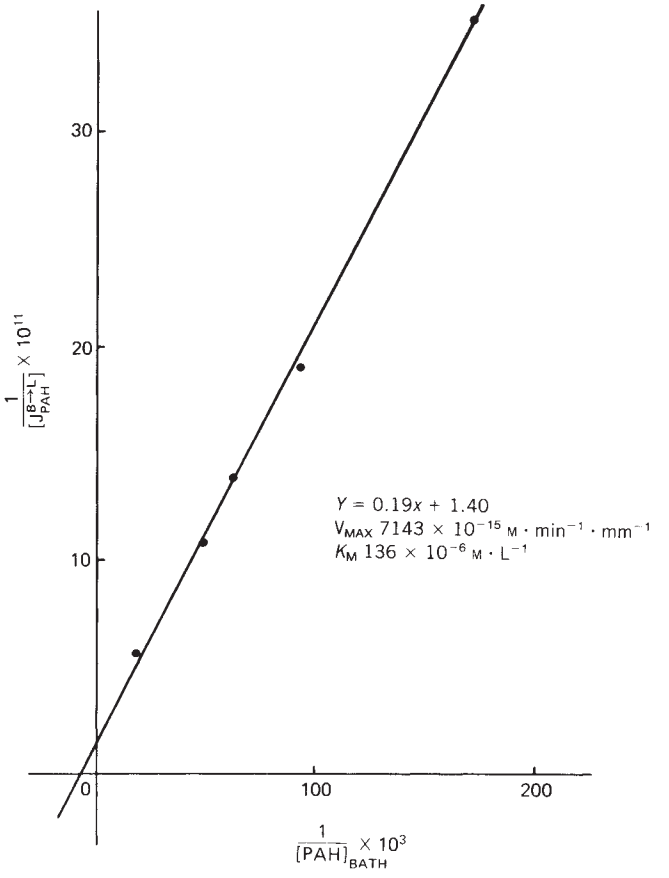

Fig. 3. *Lineweaver-Burk plot of the relation between bath PAH concentration and the net transtubule secretion of PAH in an S_2 segment of proximal tubule.*

S_2, which is convoluted at the beginning and straight at the end, transports PAH at rates some three- to fivefold greater than S_1 and S_3. Grantham and Irish [14] showed that the axial transition from S_2 to S_3 is probably gradual, rather than abrupt. In this regard, it should be noted that net fluid secretion driven by PAH transport is observed only in S_2 segments.

The initial designation of tubule heterogeneity for PAH transport was defined on the basis of a single concentration of PAH in the bathing medium (24 μM). Shimomura, Chonko, and Grantham [15] were concerned that this concentration of bath PAH might not give maximal rates of PAH secretion in the different tubule segments, so a study was performed to define the kinetic basis for PAH secretion in S_1, S_2, and S_3 segments. The relation between bath and tubule PAH secretion was determined in each of the segments. The passive movement of PAH from bath to lumen was determined for each segment using several strategies. The relation between bath and net PAH secretion was hyperbolic in all three segments and yielded linear plots when analyzed by the Lineweaver-Burk method (Fig. 3). The maximal rate of PAH secretion, V_{max}, was greater in S_2 than in S_1 or S_3 confirming the observation by Woodhall et al [13]. Also, the apparent affinity for PAH transport, K_m, was not different among the segments indicating that the heterogeneity in secretion rates may be due to a variable density of PAH transporters of common affinity in the S_1, S_2, and S_3 segments of the proximal tubule.

Basolateral PAH transport. Several studies of isolated proximal tubules give strong evidence that the principal PAH transporter is located in the basolateral plasma membrane. The uptake of cellular PAH in non-perfused tubules or in tubules with lumens filled with oil suggests a basolateral location for the transporter [3, 14, 16–18]. Dantzler [16] showed that, during the steady state movement of PAH from lumen to bath in snake proximal tubules, the cellular concentration of PAH was lower than in the perfusion medium. This experiment excludes intracellular binding as a possible cause for the high cellular levels of PAH when the anion is placed exclusively in the bath, and the pK_a of PAH is so low that nonionic diffusion cannot explain the cellular accumulation of PAH. We have recently measured the basolateral transmembrane electrical potential of non-perfused S_2 segments and found a mean value of −27 mV [19, 20]. In perfused tubules the potential is even more negative. Therefore, the PAH anion enters the cells against steep electrical and chemical gradients implicating a primary or secondary active transport mechanism. The chemical potential gradient for sodium entry into the cells across the basolateral membrane seems too low to account for the fact that PAH can be accumulated in the cells to levels nearly 200 times greater than in the bathing medium [8]. Although these considerations weigh against a secondary active mechanism for PAH secretion tied to the sodium gradient, it is well known that elimination of the transmembrane sodium gradient with ouabain [5, 13], replacement of medium sodium with choline [21] or elimination of medium potassium [22] markedly inhibit PAH transport. Dantzler and Bentley [22] have argued that the effects of low sodium medium causes secondary effects, such as increasing the basolateral permeability to PAH. Furthermore, in their studies complete removal of medium sodium reduced, but did not eliminate, PAH movement from the bath into the cells. Consequently, the studies of isolated proximal tubules appear to favor the existence of a PAH transporter fueled by an energy source other than the transmembrane sodium gradient. Whether this mechanism uses ATP directly in the manner of a primary solute "pump," or whether other as yet untested gradients (H^+, OH^-) provide the motive force remains to be determined.

Lumen membrane transport. The lumenal membrane of rabbit, snake, and frog proximal tubules is much more permeable to PAH than the basolateral membrane [4, 16, 17]. Tune, Burg, and Patlak [4] proposed that the lumen membrane was more permeable to PAH than the basolateral membrane and that the flux of the anion into the urine proceeded by simple passive diffusion. This suggestion was offered, however, before it was appreciated that transporters may be driven by ionic gradients. Today, a simple passive diffusion of PAH into the urine seems less attractive when one considers the fact that the lipid layer of membranes is a firm barrier to the diffusive movement of charged solutes. Moreover, in at least one species there is evidence to suggest that an organic anion may move from the cell into the lumen against a steep chemical gradient [23]. Dantzler and Bentley [24, 25] have also provided recent data suggesting that PAH may move from the cytoplasm into the lumen via a carrier in the lumen membrane. The inclusion of probenecid or a sulfonic stilbene (SITS) in the tubule perfusate strongly inhibited PAH secretion from bath to lumen. These

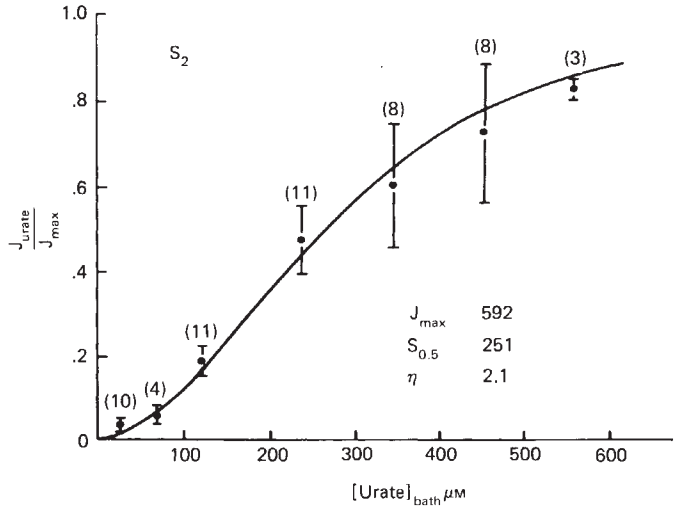

Fig. 4. *Relation between bath urate concentration and the net secretion rate for urate in S_2 segments.* J_{max} is the maximal secretory rate, $S_{0.5}$ is the concentration of urate to cause a secretion rate equal to one-half J_{max}, and η is the number of urate binding sites adduced from a Hill plot. The sigmoid curve is based on the J_{max}, $S_{0.5}$ and η values listed in the figure. See [32] for details. (Reprinted with permission from [32]).

experiments suggest, but do not prove, that PAH may enter the lumen from the cytoplasm via a specific transporter. There is no evidence in isolated tubule studies to indicate that PAH can accumulate in the tubule fluid to levels higher than in the cytoplasm, a finding that would give even clearer evidence for a primary or secondary anion transporter in the lumen membrane.

Transport of other (non-PAH) anions in isolated tubules

Uric acid. The renal handling of uric acid is complex. At physiologic pH uric acid is ionized predominately as urate in the plasma and in the fluid of proximal tubules. The transport of this substance in many respects parallels the organic anion transport mechanisms typified above for PAH. But there are important differences. Whereas PAH transport occurs almost exclusively by secretion in the proximal tubules of most species, urate is reabsorbed and secreted by the proximal tubule to a variable degree among species. Snake and rabbit proximal tubules have been studied most extensively by the isolated tubule method. Renal handling in these animals is principally by glomerular filtration and tubular secretion, whereas in man and other primates, urate is filtered, extensively reabsorbed and extensively secreted. Despite the obvious limitation as a model for man, snake and rabbit tubules have provided important information about the secretory component of urate handling.

Tubular secretion of urate occurs exclusively in the proximal tubules, and is heterogeneous [26, 27]. Urate accumulates in the cells from the bath and appears to move down a concentration gradient into the urine in both snakes and rabbit proximal tubules [18, 28, 29]. In contrast to PAH, however, the net secretion rate is highly dependent on the urinary flow rate [26, 28]. This seems to be due to the fact that the permeability of the tubules to urate is much higher than to PAH; that is, urate is a gradient as opposed to a capacity-limited transport process. The high permeability to urate appears to be due to a higher basolateral membrane and paracellular permeability to urate than to PAH ([31] and Grantham, Tanner, and Chonko, unpublished observations). In other words, during steady-state transport from bath to lumen, there is probably extensive recycling of urate from bath to cell to bath and from cell to lumen to bath, thereby reducing the chance that a molecule of bath urate placed into the cell by the basolateral transporter will end up in the tubule fluid. By contrast, due to the relatively low permeability of the basolateral membrane and paracellular junctions, molecules of bath PAH have a greater chance of being deposited in the tubule fluid.

Shimomura et al [32] evaluated the bases for the heterogeneity of urate transport in rabbit proximal tubules. The conventional system of kinetic analysis could not be used for urate, however, since the relationship between bath urate concentration and urate secretion rate was sigmoidal, rather than hyperbolic (Fig. 4). As for PAH the $S_{0.5}$ values (the concentration of urate to cause ½ maximal J_{urate}) for S_1, S_2, and S_3 were similar, whereas the V_{max} values were oriented $S_2 \approx S_1 > S_3$. These data were interpreted to mean that heterogeneity of urate secretion depends on a variable density of urate transporters of common affinity along the proximal tubule. The pattern of heterogeneity differed from that for PAH in that the PAH V_{max} was $S_2 > S_1 = S_3$ [15]. In a preliminary report Weber, Kokko, and Jacobson [27] found that superficial proximal convoluted tubules secreted urate at a rate twice that of superficial S_2 segments. By contrast, juxtamedullary proximal convoluted tubules showed a small rate of net urate reabsorption. Thus, internephron as well as intranephron heterogeneity exists in rabbit proximal tubules.

Kinetics of PAH and urate transport. PAH and urate transport in intact proximal tubules has been analyzed from a kinetic perspective by several researchers. The analyses depend on the measurement of steady state fluxes from bath to urine in response to changes of bath anion concentration. Such studies provide "operational" values for affinities and maximal rates of transport but cannot be viewed as yielding true transport constants. PAH transport is best suited for the analysis since the anion is not bound extensively to plasma proteins, the pump to leak ratio is relatively high across the basolateral and paracellular pathways, the net secretion rate is not influenced appreciably by changes in tubule perfusion rate, and there is no carrier-mediated reabsorptive mechanism. Urate is more of a problem for such an analysis since it "leaks" across the basolateral membrane, the net flux is strongly dependent on tubule perfusion rate, and carrier mediated reabsorption exists in some species. Fortunately, in snakes and rabbits, the two species in which proximal tubules can be dissected easily, urate absorption is miniscule and not appreciably transported by facilitated means. The studies have been done at relatively high perfusion rates to diminish the effect of lumen to bath backflux of secreted urate and PAH.

Table 1 summarizes the apparent kinetic parameters adduced from studies of snake and rabbit tubules. The apparent K_m for PAH secretion in the snake is about one tenth of that in the rabbit proximal tubule. The plasma level of endogenous aryl anions is not known for snakes, but in rabbits the level is about 5 to 15 μM [9]. Thus, the rabbit secretory transporter has an apparent affinity some ten times higher than the normal level of

Table 1. Apparent PAH and urate transport constants for rabbit, snake, frog, and rat proximal tubules

	Segment	K_m μM	V_{max} 10^{-15} M/min/mm	Inhibited by Ouabain	Inhibited by Probenecid	K_m ($S_{0.5}$) μM	V_{max} 10^{-15} M/min/mm	Inhibited by Ouabain	Inhibited by Probenecid
		PAH				*Urate*			
In vitro									
Rabbit	S_1	139 [15]	1097 [15]	+	+	185 [32]	568 [32]	+	+
	S_2	195 [15]	7430 [15]	+	+	238 [34]	950 [34]	NT	+
						251 [32]	592 [32]	+	+
	S_3	113 [15]	1647 [15]	+	+	234 [32]	55 [32]	+	+
Snake	Distal-Proximal	10 [16]	325 [16]	NT	+	~150 [28]	~140 [28]	−	+
Frog	Proximal	15 [17]	659 [17]	+	NT				
In vivo									
Rat	Proximal Convoluted					410 [43]	4700 [43]	NT	+

Abbreviations: NT, not tested. References are in brackets.

aryl anions in the plasma. All segments of rabbit nephron have a greater capacity for PAH transport than the distal-proximal tubule of the snake. With this arrangement the rabbit is able to efficiently eliminate loads of hippurate-like material that may be ingested or produced as a consequence of metabolism. Anionic vasoactive substances, such as the prostaglandins, are avidly transported by this mechanism in the rabbit, and the mechanism may have a role to play in the primary intratubular delivery of prostaglandins to distal sites [33].

The plasma urate level in snakes is 400 to 500 μM [28] and in rabbits about 35 μM [26]. Thus, the apparent affinity for the urate transporter in snakes is lower than the plasma level by nearly threefold, suggesting that the secretory mechanism in snakes is saturated normally. If this is true, it seems unlikely that urate secretion rates would be modified importantly by changes in plasma urate level, and that other factors, for example, filtration, reabsorption, and urine flow rate might be more important than secretion in regulating plasma urate levels in these reptiles. By contrast, the plasma urate level in rabbits is about one seventh of the concentration required to produce secretion of urate at one half the maximal rate. Thus, proximal tubule urate secretion is probably controlled in an important way by the plasma urate level, and vice versa. It is well known that urinary urate excretion suppressible by probenecid is increased markedly by elevating the plasma urate level in rabbits (reviewed in [32]).

Urate secretion in the rabbit does not appear to follow simple Michaelis-Menten kinetics, in contrast to PAH. Shimomura et al [32] recently observed that in many S_2 and S_1 segments the relationship between the urate secretory flux and bath urate concentration was sigmoidal, rather than hyperbolic. Their studies suggested that urate may be secreted by an allosteric transporter with at least two binding sites for urate. Senekjian, Knight, and Weinman [34] did not find a sigmoidal pattern in their studies. Although the apparent affinities were similar in the two studies (Table 1), the V_{max} values were higher in Houston than in Kansas City by a factor of two. One explanation for this discrepancy is that Senekjian, Knight, and Weinman [34] studied S_2 segments in synthetic medium containing bovine serum albumin, whereas Shimomura et al [32] used rabbit serum or a synthetic salt solution containing rabbit serum proteins. Recent preliminary studies show that rabbit serum contains an inhibitor of urate transport that is capable of reducing the maximal accumulation of urate in the cells of S_2 tubules [35]. The inhibitor is not removed from serum by ultrafiltration or extensive dialysis suggesting that it is either a substance with a large molecular weight or is bound to plasma proteins. Of considerable importance is the finding that the inhibitor confers the sigmoidal appearance on the relationship between bath urate and tissue urate content. If rabbit serum contains an allosteric inhibitor, as postulated by Shimomura et al [32], it is possible that it may play an important role in the regulation of urate secretion in this species. It is interesting to note that in man urate secretion appears to be related to plasma urate in a sigmoidal pattern (reviewed in [32]).

Basolateral and lumenal transport of urate. Since urate is elevated in the cells during the course of transtubule secretion in rabbits and snake proximal tubule, more than likely the principal secretory transporter is located in the basolateral membrane [18, 28, 29, 35]. In the rabbit the basolateral transporter binds a variety of organic anions, in decreasing affinity: probenecid > furosemide > sulfinpyrazone > para amminohippurate > pyrazinamide > ticrynafen > salicylate.

The basolateral urate transporter of the rabbit is exquisitely sensitive to ouabain [26, 36]. The snake proximal tubule urate transporter, however, is resistant to ouabain although urate uptake is slowed considerably when potassium is removed from the external medium. The relation between the sodium pump and the basolateral transport of urate is clouded further in the snake by the observation that removal of bath sodium had no effect on urate transport and elevation of medium potassium decreased urate secretion [37, 38]. Thus, the relation between the sodium pump (sodium gradient) and the basolateral transport of urate awaits further studies for clarification of the mechanism.

Organic cations. The kidneys of animals transport organic cations into the urine by efficient secretory mechanisms [39, 40]. Despite the fact that the rabbit aggressively transports tetraethylammonium (TEA), N^1-methylnicotinamide (NMN) and choline, few perfusers have studied these interesting substances. Recently, McKinney, Myers, and Speeg [41] published an initial report (the first of many I am sure) in which they

demonstrated that cimetidine, an important drug for the treatment of duodenal ulcer, is secreted by S_2 segments of rabbit proximal tubule. Cimetidine secretion was inhibited by both organic anion (probenecid) and organic base (quinine) inhibitors, although the cationic inhibitors were more potent than the anions. The authors suggest that cimetidine may be transported by organic cation and anion transporters. Because inhibition of cimetidine was seen only with high levels of anionic inhibitors, in the range that Shimomura et al [32] found to cause cell swelling in S_2 segments, it is important to "dissect" out in more detail whether or not organic cations can utilize the anion pathway and vice versa.

It appears that S_2 segments also secrete creatinine and procainamide via the organic cation pathway [42]. These studies are of considerable importance because of the fact that so many drugs are eliminated in this way, and their elimination is subject to adverse drug—drug interactions.

Organic cations show heterogeneity of transport, but, in contrast to organic anions, the pattern of secretion is $S_1 \geq S_2 > S_3$ [44]. The mechanism of the transcellular movement of organic cations has not been addressed in isolated perfused tubules, but studies are certainly within the reach of today's researchers.

Summary. The isolated tubule method has been a powerful tool for studying the mechanism of transport of organic anions and cations in renal tissue. The method has contributed to *(1)* our appreciation and understanding of intra- and internephron heterogeneity, *(2)* the awareness that renal tubules can secrete as well as reabsorb fluid in association with active ion transport, *(3)* an awareness that endogenous renal hormones (prostaglandins) and drugs (diuretics) may be secreted by the proximal tubule and delivered in the urine to pertinent luminal receptors down stream, and *(4)* a functional demonstration that transportable organic anions accumulate to high levels in patients with renal failure. On a cautionary note, recent studies of urate secretion indicate that transport mechanisms, to be equated with in vivo states, may have to be evaluated in media that closely simulate the normal extracellular fluids of the body.

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