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TRANSPORT OF ORGANIC ANIONS AND CATIONS IN ISOLATED RENAL PLASMA MEMBRANES

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INTRODUCTION

The transepithelial transport in proximal renal tubular cells has been well documented for both organic anions and cations, previously referred to as acids and bases. The latter nomenclature is incorrect as quaternary nitrogen-containing compounds are not chemical bases in the strictest sense. The sequence of movement of organic ions is transport across the basolateral membrane, accumulation in the cells, followed by efflux from the cell across the brush border into the tubular fluid (see 1–6).

The techniques for demonstrating these phenomena are numerous and have been outlined in reviews by Torretti & Weiner (7) and Rennick (8). They include such in vivo techniques as renal clearance, Sperber technique, stop-flow, retrograde intraureteral injection, and micropuncture. In vitro transport has been studied in renal slices and isolated renal tubules. Collectively these techniques have established that the two systems are separate and distinct and that both groups of compounds are actively secreted by "carrier"-mediated transport. Both anions and cations are capable of being transported "uphill" (movement against a concentration gradient). Each system is saturable, inhibitable by metabolic inhibitors, demonstrates competition between compounds within the same class, and demonstrates bidirectional transport.

While all of these findings have generated a great deal of information about the overall sequence of events, they have failed to yield specific information on the relative contributions of the brush border and basolateral membranes to the overall transepithelial phenomena. Therefore, this review emphasizes the contributions made as well as the questions raised

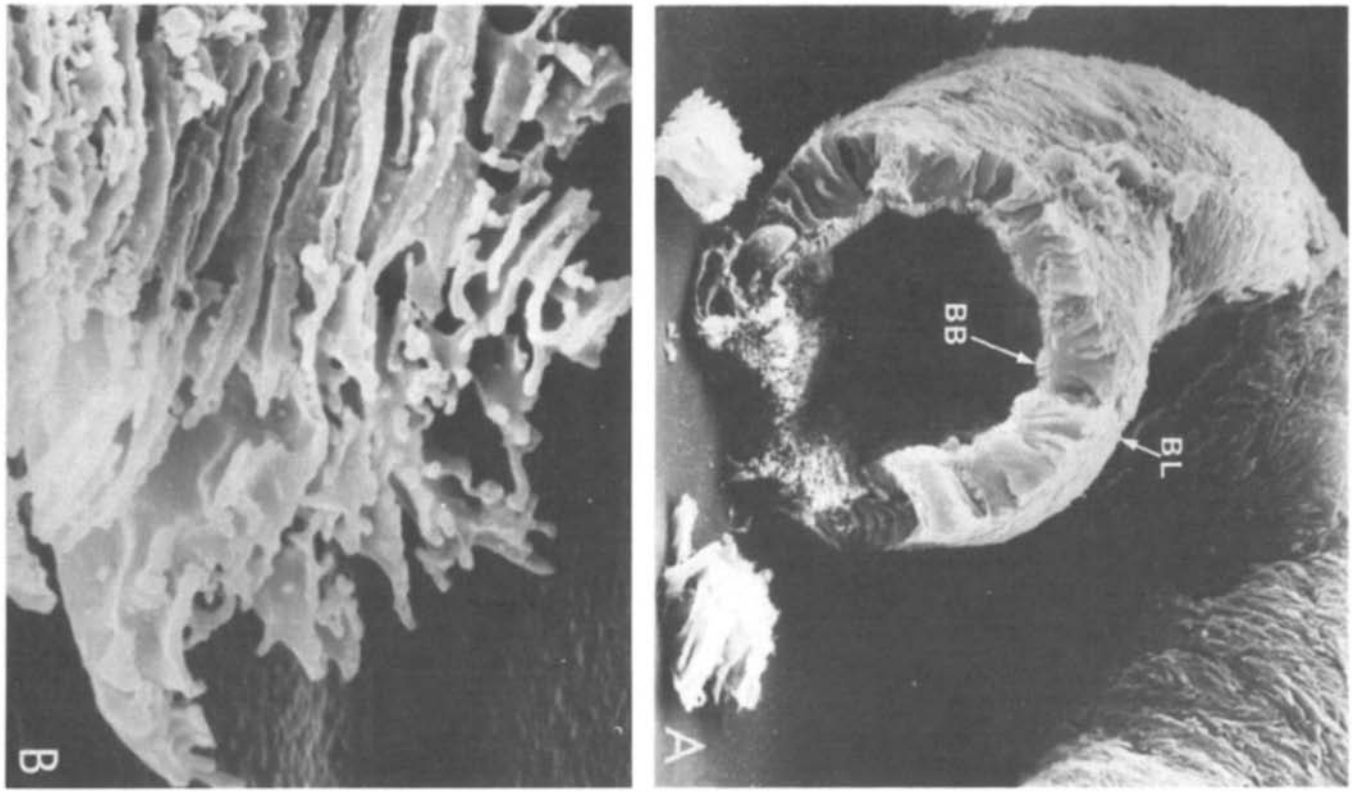
by studies on isolated renal plasma membrane vesicles in regard to our understanding of the renal transport of organic anions and cations.

While isolated renal membrane vesicles have only recently been widely used as an in vitro model system for studying renal transport, the technique for isolating plasma membranes has been available for a number of years. In the late 1960s Fitzpatrick (9) isolated brush border (luminal) and basolateral (antiluminal) plasma membranes from isotonic homogenates of rat kidney by differential centrifugation. Heidrick et al (10) took advantage of the difference in polarity of the membranes of the renal proximal tubule and improved the purification of the two membranes using preparative free-flow electrophoresis. However, this technique has the disadvantage of producing a low yield of basolateral membranes. Others have improved the yield by modifying the differential centrifugation procedure (11–14) or by using a combination of differential centrifugation and free-flow electrophoresis (15). Despite the differences in isolation procedures, the data thus far indicate that the transport properties of the various isolated membrane preparations are comparable (16).

Electron micrographs of brush border microvilli have been shown in numerous publications; however, photographs of the basolateral membrane are quite rare. For this reason, we have included Figure 1 to help the reader visualize the possible source of basolateral membrane vesicles. Panel *A* is a scanning electron micrograph of a dog S_2 segment of the proximal convoluted tubule. Panel *B* is a magnification of the basolateral membrane of one cell. Note the inverted T-shaped basal and microvillous basal processes. The processes break off during homogenization and probably form the basolateral membrane vesicles to which we refer in this review.

Since there have been several recent reviews on various aspects of transport using membrane vesicles (17–21), only a brief description of the advantages and disadvantages of using membrane vesicles need be given. The advantages are: (*a*) vesicles are free of metabolic reactions; (*b*) the composition of the intra- and extravesicular spaces can be manipulated, allowing analysis of the driving forces for transport; (*c*) the optimal conditions for intracellular, extracellular, and membrane-related factors can be determined; and (*d*) the transport properties of the two membranes can be studied separately. The disadvantages are: (*a*) the preparations take time and some degradation may occur; (*b*) the membranes must be somewhat

Figure 1 (*A*) is a scanning electron micrograph of a dog S_2 segment of the proximal convoluted tubule (BB-brush border and BL-basolateral). Magnification is X 1400. (*B*) is a higher magnification (X 12,770) of the basolateral membrane from one cell. The photograph is of the lateral edge of the basal surface. The isolated cell was prepared by tryptic digestion of glutaraldehyde fixed tissue.



heterogeneous since they are prepared from a heterogenous population of cells (although proximal tubular cells predominate in renal cortex); (*c*) demonstration of a transport system in isolated membranes is evidence for its existence in the intact structure, but absence of a system in isolated membranes does not preclude its existence in vivo. In addition, the existence of a transport mechanism in the isolated membranes does not necessarily mean that the mechanism plays a prominent physiological role. Fortunately, there is a large body of information on the renal transport of organic anions and cations against which the observations made in isolated membrane vesicles can be evaluated. Another factor to be considered is the possibility that endogenous factors or regulators may be removed during the preparation of membranes. While this appears to be a limitation, the role of these putative factors can best be analyzed by adding components back to isolated membranes.

GENERAL METHODS

For the reader's convenience, a brief discussion of the experimental strategies used and the nomenclature encountered when reading papers concerning membrane vesicle studies is included.

An essential objective is to demonstrate that the preparations used have an intravesicular space into and out of which substrate molecules are moved. A demonstration of an osmotically active space usually satisfies this requirement. The data obtained from these studies can be used to estimate the extent of substrate binding to the membrane (22).

The next objective is to demonstrate specifically mediated transport, which presumably consists of the following steps: binding of the substrate at one face of the membrane, translocation across the membrane, and debinding at the opposite face. It is assumed that the association-dissociation reactions are faster than the translocation event. The criteria for demonstrating mediated transport are: (*a*) saturation; (*b*) specificity; and (*c*) countertransport. The structures responsible for mediating transport are termed "transporters" and presumably are integral membrane proteins.

Saturation is attainable if a substrate molecule forms a complex with the transporter, and then, as the substrate concentration is increased, the initial rate of transport is increased until a limiting value is approached.

The second criterion, specificity, implies some structural requirements for interaction between the substrate molecules and the transporter. The experiments are usually conducted by following the uptake of radioactively labeled substrates in the presence or absence of unlabeled competitors. Ordinarily, these experiments are conducted with both the labeled substrate

and a large excess of competitor on the same side of the membrane (*cis*-inhibition).

All of the transporters described thus far exhibit the phenomenon of countertransport, i. e. a *trans* concentration of the transported substrate or presumed competitor will affect the translocation of the labeled substrate. The *trans* compartment is defined as that toward which the radioactive species is moving. The presence of nonradioactive substrate or competitor in the *trans* compartment may accelerate or inhibit the movement of the radioactive material in the orthograde direction. The magnitude and the direction of the effect of the *trans* material is determined by a complex of factors including its concentration, its affinity for the transporter, its rate of translocation, and the volume of the *trans* compartment relative to the *cis* compartment.

Transport against a concentration gradient is termed "concentrative" or "uphill" transport. Energy for uphill transport can be supplied directly by a chemical reaction (e. g. ATP hydrolysis), or through coupling to the flux of some other solute down its electrochemical gradient in the same (cotransport or symport) or in the opposite direction (exchange or antiport) of substrate movement. These processes are termed "secondarily active". The scope of secondarily active transport has been reviewed by West (23). Aronson (24) has delineated experimental approaches and strategies available for identifying secondarily active transport in epithelial tissue.

A standard experimental procedure used when studying coupled transport is to employ a large gradient of the co-ion (or "driver" ion) in the presence of a much smaller gradient (i. e. low concentration) of the substrate under study ("driven ion"). The movement of the two ions is coupled. Therefore, the labeled substrate will be driven into the intravesicular space until the driver ion cencentrations come to equilibrium. Since the labeled substrate will be transported until the much greater gradient of the driver ion is dissipated, it will be transiently accumulated producing an "overshoot" of its equilibrium value, and then will back-flux until equilibrium is achieved.

A feature of some coupled transport systems is that there is a net change in the charge distribution across the membrane as a result of the transport process. Reactions of this type are termed electrogenic. Electroneutral mechanisms on the other hand are ones in which there is no change in the net charge distribution as a result of transport.

The assay most commonly employed is some variation of a rapid filtration technique where the membrane vesicles are caught in a filter and the amount of substrate transported calculated by standard liquid scintillation counting techniques.

For a comprehensive review of membrane transport, the reader is referred to Heinz (25, 26).

ORGANIC CATIONS

The general characteristics of the renal organic cation transport system have been recently reviewed by Rennick (8). Therefore, only a summary of these characteristics will be given. In vivo transport studies with the organic cations are limited because these compounds often lead to circulatory disturbances. While a diverse group of organic cations are transported by the proximal renal tubule cells, all of these compounds are either primary, secondary, tertiary, or quaternary amines. This includes endogenous agents such as catecholamines and choline. Most studies have been confined to two compounds, tetraethylammonium (TEA) and N^1 methylnicotinamide (NMN). Studies of organic cation transport in isolated vesicles have been confined to NMN and choline.

Specifically mediated transport of NMN has been shown in both brush border and basolateral membrane vesicles prepared from dog kidney (27). The two systems differ from one another in the following ways: 1. The transporter in the brush border had the higher affinity while the V_{max} values in the two membranes were similar (27). 2. The activation energy for transport was lower in the basolateral membrane (28). 3. The countertransport patterns were different (28). The data obtained from these countertransport studies were interpreted as indicating different molecular mechanisms. It was suggested that the transporter in the brush border had properties consistent with alternating binding sites (29–32), while the transporter in the basolateral membrane had properties more consistent with its substrate binding sites being simultaneously exposed to both sides of the membrane (29–32). 4. The countertransport behavior elicited in the brush border membrane by a series of organic cations that were known to be secreted led to the conclusion that although all were transported, they were translocated across the membrane at different rates (28).

The energetics of secretion are of primary importance. It was known that an organic cation can be accumulated in the urine against its electrochemical gradient (33). The question that was addressed was, at which face of the renal cell does active transport occur? The authors hypothesized that since energy would be required to concentrate a cation from the negative interior of the cell to the less negative urine, the transport across the brush border membrane would be the active step in transepithelial transport. A possible energy source for secretion was suggested by the results showing that a proton gradient could drive the "uphill" accumulation of NMN (34). Since a large standing gradient of protons does not exist in the proximal tubule, other gradients were explored. A $Na^+:H^+$ acidifying mechanism had been demonstrated in brush border vesicles (35, 36). Under appropriate conditions, it was shown that a Na^+ gradient would stimulate NMN transport

(34). The interpretation of these experiments was that the Na^+ gradient did not drive NMN transport directly, but rather exchanged for a H^+, creating a proton gradient that provided the energy for NMN transport. This suggested that two functionally linked antiport systems drive organic cation secretion.

ORGANIC ANIONS

p-Aminohippurate

The organic anion transport system(s) has been the subject of extensive investigation. Most commonly, p-aminohippurate (PAH) has been used as the prototype anion with inhibition by probenecid taken as qualitative evidence for the existence of specifically mediated transport.

PAH is concentrated in the urine against its electrochemical gradient. Dissecting the transepithelial transport into its component parts, the transport across the basolateral membrane appears to be the active step since PAH is accumulated intracellularly in an energy-dependent fashion (37–40). In the mammalian kidney, the movement of PAH from cell to urine was found to be a passive process (41); but it was left unresolved whether it was simple (42–45) or facilitated diffusion (46, 47).

This ambiguity was not a problem with nonmammalian species. In vivo experiments in *Necturus* kidney showed mediated transport across both faces of the renal cell (48). Similarly there is evidence for an analogous situation in flounder kidney (49, 50). Confirmatory evidence came from studies using basolateral and brush border membrane vesicles prepared from the flounder kidney (51, 52). In the study by Eveloff et al (51), probenecid inhibitable uptake was demonstrated in both membranes, although the brush border was less sensitive, suggesting differences in affinity between the two membranes for organic anions. Consistent with this idea was the finding that the transporter in the brush border was more sensitive to inhibition by the anion inhibitor, 4-acetamido-4'-isothiocyano-2-2'-disulfonic stilbene (SITS), than was the basolateral transporter. Extensive characterization of the transport mechanism was not described with the exception of showing that PAH transport in brush border membrane vesicles was independent of a sodium gradient but sensitive to potential difference (P.D.).

The authors argued that the P.D. responsiveness is important for the following reason: in the flounder kidney, the urine concentration of organic anions is an order of magnitude greater than the cellular concentration (50, 53), necessitating an additional driving force for transport from cell to urine, a force provided by the electrical potential.

Returning to those studies with mammalian kidneys, the first reports

presented evidence that PAH uptake in brush border vesicles was a non-mediated process (22, 54). In the study with rat kidney vesicles (22), the authors were unable to demonstrate probenecid inhibitable uptake, whereas in the study using rabbit brush border vesicles 1 mM probenecid decreased PAH uptake of 10 μM by 50%, a value the authors considered too low to be meaningful. The conclusion from these two studies was that PAH transport across the brush border membrane occurred by a simple diffusion-ion trapping mechanism.

Quite a different conclusion was reached by other workers who examined PAH transport in brush border vesicles. Kinsella et al (27), using dog kidney, demonstrated specifically mediated transport. They showed that the transporter in the brush border had a lower affinity but a higher capacity for PAH transport than did the basolateral membrane. Confirmation of mediated transport of PAH in the brush border came from Blomstedt & Aronson (55), who also used dog kidney. Their work extended our knowledge by showing that transport could occur by an electroneutral $PAH^-:OH^-$ exchange mechanism. More recently, specifically mediated transport has also been shown to occur in brush border membrane vesicles prepared from rat kidney (56). The evidence thus far suggests that in all species studied, PAH transport in the brush border membrane is less sensitive to probenecid inhibition than is the basolateral membrane. This may account for the discrepancy with earlier reports in that in the first studies relatively low concentrations of probenecid were used.

As stated, presumably the active step for organic anion secretions occurs at the basolateral membrane. The underlying mechanism was unknown. Burg & Orloff (39) showed in isolated tubules that inhibition of Na^+,K^+ ATPase diminished the uptake of PAH. Since this enzyme regulates the cellular levels of Na^+ and K^+, these results indicated the requirement of a functional Na^+ pump as well as proper electrolyte gradients for organic anion uptake, a conclusion which has been amply documented (57–59). In a well conceived experiment, Podevin et al (60) demonstrated Na^+ gradient (out $>$ in)-stimulated uptake of PAH in Na^+- and K^+-depleted rabbit kidney slices. Others have not been able to repeat this experiment (61). The complexity of the system was demonstrated by showing a Na^+-dependent and a Na^+-independent component in the uptake of PAH by kidney slices (62).

Attempts to resolve these complexities have been undertaken using isolated vesicles. The pioneering work with regard to understanding the energetics of PAH coupled transport was carried out by Berner & Kinne (22). They demonstrated specifically mediated transport of PAH which was stimulated by a Na^+ gradient (out $>$ in). However, they were unable to achieve uphill transport. Their conclusion was that a Na^+ gradient was not

the driving force for the intracellular accumulation of PAH. Similar findings were reported by Kinsella et al (27).

Recently, the role of Na^+ in PAH transport has been more clearly defined. Using rat kidney basolateral membrane vesicles, Kasher et al (63) showed a direct interaction of Na^+ with the organic anion transporter by demonstrating that in the presence of a Na^+ gradient the K_m for PAH decreased from 250 μM to 90 μM with a concomitant increase in V_{max} from 95 to 300 p moles/mg·min. In addition, the inhibition produced by probenecid increased from 20 to 90 percent in the presence of a Na^+ gradient. While these results indicated a direct effect of Na^+ on PAH transport, a Na^+ gradient alone did not drive the accumulation of PAH, a result similar to those of previously cited studies. The studies were extended to examine the effect of PAH on its own countertransport. A 5-fold gradient of PAH (in > out) stimulated the influx of [^{3}H] PAH, but did not yield a transient accumulation of label (i. e. an overshoot). When the two gradients were combined (Na^+ out > in and PAH in > out) the uphill transport of PAH was observed. The interpretation of these data was that PAH transport across the basolateral membrane occurs by a Na^+ gradient-stimulated anion exchange mechanism. In these studies the only anion tested was PAH itself. What other anions might function in this mechanism and whether or not their identification would be consistent with the known cellular gradients remains to be examined.

It is beyond the scope of this review to cover PAH transport in all species. However, an interesting paper by Holliday & Miller (64) showed in the urinary bladder of an invertebrate (rockcrab) that the uphill step occurred at the brush border membrane with passive exit at the basolateral membrane. The concentrative transport was a Na^+ gradient-dependent process.

Endogenous Anions

Our emphasis has been on PAH transport because it is considered to represent renal transport of anionic drugs. However, transport systems for endogenous anions do exist. The contribution made by studies on isolated membrane vesicles in understanding their transport is outlined below.

NICOTINIC ACID The pleiotrophic nature of the renal organic anion transport systems was demonstrated by Boumendil-Podevin & Podevin (65), who studied nicotinic acid uptake by brush border membrane vesicles prepared from rabbit kidney. They showed nicotinic acid was taken up by a Na^+-dependent, electroneutral process. These authors attempted a structure-activity relationship and found the system to be specific for monocarboxylic acids (based upon inhibitor studies). Benzoic and pyrazinoic acid were effective inhibitors, probenecid produced only slight inhibition, and

PAH was ineffective. The data with pyrazinoic acid is of interest because of its relationship to uric acid transport (for review see 66–68).

URIC ACID Uric acid transport has been extensively reviewed (66–72). Briefly, the current data suggest that in some species PAH and urate are transported by a common mechanism at both faces of the renal cell; in others they share common transport at only one face; and in a third group they are separate and distinct at both surfaces. The use of membrane vesicles has helped clarify the issue.

The initial work in isolated vesicles was carried out by Abramson et al (73) who showed urate transport in both basolateral and brush border membranes of rats. Unfortunately, the results were somewhat compromised by the presence of uricase in their preparations. Recently, this problem has been reexamined and it was found that the extent of urate metabolism was influenced by the amount of copper in the membrane preparation (74). Interestingly, removal of Cu^{++} seemed to affect both the metabolism and the transport of urate. Urate uptake was found to be greater in the basolateral membrane than in the brush border. While a Na^+ gradient stimulated transport in both membranes, the mechanism did not appear to be a Na^+:urate symport.

Other studies dealt with the brush border membrane exclusively. Two groups used rabbit (54, 75); Blomstedt & Aronson (55) examined dog kidney. The latter authors demonstrated that a pH gradient (inside alkaline) could drive the uphill transport of either urate or PAH, which indicated a proton symport or a hydroxyl antiport mechanism. Based upon mutual inhibition, their conclusion was that urate and PAH share a common transport system in the brush border membrane. Boumendil-Podevin et al (75) also showed pH-stimulated transport of urate. There appears to be general agreement that urate transport in the brush border membrane is independent of a Na^+ gradient, but rather responds to a pH gradient. Kahn & Weinman (76), using brush border membranes from rat kidney, proposed that other anions, such as bicarbonate, could replace hydroxyl in the exchange mechanism. The concept was extended by Aronson and coworkers (77, 78) who proposed that the coupling of two transport systems can drive urate reabsorption. That is, lactate is reabsorbed by a Na^+ gradient-stimulated mechanism, resulting in intracellular accumulation of lactate (or hydroxyl produced by metabolism), which in turn drives the urate exchange mechanism, leading to urate reabsorption.

The interrelationship between urate and PAH was extended by showing that either would stimulate the countertransport of the other in dog kidney brush border membrane vesicles (79). This finding is consistent with the hypothesis that PAH and urate share a common transport mechanism in

that membrane. On the other hand, neither urate nor PAH affected one another in basolateral membrane vesicles, which suggests independent transport. In the dog kidney urate shows net reabsorption and PAH net secretion. Since they share a common mechanism in the brush border, it appears as if net movement is controlled by the events at the basolateral membrane.

LACTATE Lactate uptake has been demonstrated in both brush border and basolateral membrane vesicles prepared from rat kidney (80, 81). Differences do exist between the two. Lactate transport in the brush border was a Na^+-dependent, electroneutral process, whereas transport in the basolateral was independent of Na^+. Lactate transport in the brush border was sensitive to D-lactate, 2-thiolactate, and L-3-phenyllactate, but the transport in the basolateral membrane was insensitive to these inhibitions. Basolateral transport was inhibited by 3-thiolactate, 2 hydroxybutyrate, and phloretin. Phloretin did show slight inhibition in the brush border (80).

The renal handling of lactate is envisioned by these authors as consisting of active reabsorption of lactate from the urine by a Na^+ gradient-dependent mechanism. The movement across the basolateral membrane is dependent upon the relative cellular and plasma concentrations of lactate. Thus lactate would be recovered in the renal cell for metabolism or reabsorption back to the blood depending upon the metabolic state.

DI AND TRICARBOXY ACIDS The tricarboxylic acid (TCA) cycle intermediates are an important class of endogenous anions. Understanding of their renal handling has been advanced by studies with isolated plasma membrane vesicles. The emphasis has been on the events occurring at the brush border. Kippen et al (82), using brush border membrane vesicles prepared from rabbit kidney, demonstrated the Na^+ gradient-stimulated uptake of citrate and α-ketoglutarate. The kinetic parameters were: Km values for citrate and α-ketoglutarate were 0.18 mM and 1.0 mM respectively with the V_{max} for each being 17 nmoles/mg. Other TCA cycle intermediates inhibited the uptake of either citrate or α-ketoglutarate by greater than 90%, whereas monocarboxylic acids (e. g. pyruvate, lacate, PAH) were much less effective, with inhibition of approximately 20%.

The system was examined in greater depth by these same workers by determining the relative inhibitory constants of forty compounds (83). They confirmed their earlier work by showing that the system is relatively insensitive to monocarboxylic acids, but highly specific for 4 carbon dicarboxylic acids in the *trans* configuration (e. g. fumaric acid had a 50-fold lower affinity than maleic acid). The structure-activity relationships indicated that

either the α or the β carbon could be substituted, but if both were substituted, the congener displayed lower affinity.

The question of whether or not the inhibitors were translocated was resolved by establishing the response of a fluorescent dye (84). The Na^+-stimulated electrogenetic transport of citrate and succinate produce a characteristic change in fluorescence. They found that those effective inhibitors produced the same fluorescent changes, but the weak inhibitors (i. e. the monocarboxylic acids) caused little or no fluorescent change. They concluded that the potent inhibitors were translocated by this system but the weak ones were not.

The stoichiometry of Na^+ to succinate was studied (85). Succinate transport in rabbit brush border membrane vesicles was shown to be dependent upon the absolute magnitude of the Na^+ gradient and not upon the ratio of concentration of Na^+ outside to concentration of Na^+ inside. Graphical analysis yielded a sigmoidal relationship for the effect of the NaCl gradient on succinate uptake. Two different models were used to explain the Na^+ effect. The conclusion of the analysis was that at least 2 Na^+ must be transported per substrate molecule. Since the data indicated an electrogenetic mechanism, 2 Na^+ per $succinate^{2-}$ would not lead to a net change in charge distribution. They proposed, therefore, 3 Na^+ per substrate molecule. Ordinarily citrate is a trivalent anion at physiological pH, and 3 Na^+ per $citrate^{3-}$ would not be consistent with an electrogenic process. The issue was resolved by proposing that it is either the mono- or divalent species of citrate that is transported, rather than the trivalent form (86). The conclusion was reached by following the pH dependence of transport: lowering the pH from 7.5 to 5.5 increased the uptake of citrate 10-fold but had little effect (slight inhibitory) on succinate uptake.

A stoichiometry greater than one would have important physiological implications in that it would give thermodynamic advantage for uphill transport and could explain the efficient renal reabsorbtion of these important metabolites.

AMINO ACIDS

The renal handling of amino acids has been extensively reviewed (87–91). The use of kidney membrane vesicles to study amino acid transport has also been reviewed (92–94). Therefore, it is not our intent to present an exhaustive review of amino acid transport, but rather to present some salient features that may aid in our understanding of renal function.

It is generally agreed that the proximal tubule efficiently reabsorbs most of the amino acids presented to its luminal border by glomerular filtration. Reabsorption is a Na^+ gradient-dependent process although, as will be

discussed, some subtleties do exist. Transport at the basolateral membrane can occur by Na^+-dependent as well as Na^+-independent mechanisms. Conflicting reports concerning the transport of dipeptides in the brush border have appeared. Are they taken up as dipeptides (95) or must they first be hydrolyzed to their constituent amino acids (96)? These conflicting reports may reflect species differences. For a review of dipeptide transport see Carone & Peterson (97).

Sodium gradient-dependent, stereospecific uptake by the brush border has been demonstrated for alanine (98–100), proline (98, 100–102), glycine (101), phenylalanine (103), and glutamic acid (104–108). The situation for the basic amino acids was less clear in that a Na^+ gradient stimulated L-arginine uptake but did not produce concentrative transport (105, 109). However, uphill transport could be demonstrated when a transmembrane potential difference (inside negative) was imposed on top of the Na^+ gradient (109, 110). Tuarine (111) and cystine are transported by at least two different systems, one of which is shared by the basic amino acids and glutamine (112). Glutamine uptake has been shown to possess a Na^+ gradient-dependent as well as a Na^+ gradient-independent pathway (113–114).

Reabsorption of the amino acids, therefore, entails active uptake from the urine by a Na^+ cotransport mechanism followed by passive diffusion from cell to plasma (91). A sodium-independent transport mechanism for proline has been demonstrated in basolateral membrane vesicles (102). On the other hand, it has been proposed that some amino acids may be accumulated in the cell from the peritubular space by an active process (91). Glutamate can be transiently accumulated in rat kidney basolateral membrane vesicles by a Na^+ gradient (out $>$ in)-dependent mechanism (108), consistent with uphill transport in that membrane. Interestingly a K^+ gradient (in $>$ out) will also drive uphill transport. An absolute requirement for Na^+ exists however, in that the K^+ gradient will only drive concentrative transport in the presence of a Na^+ gradient (out $>$ in) or if Na^+ is equilibrated across the membrane (i. e. $[Na^+]out = [Na^+]in$).

This mechanism for glutamate uptake appears to be exactly the same in the brush border membrane (104, 106, 107). Thus glutamate transport seems to be unique, in that the same mechanism is operative at both faces of the renal cell.

Amino acid uptake by the brush border can be either electroneutral or electrogenic (for review see 115). As far as glutamic acid transport is concerned, arguments have been presented supporting either viewpoint (104, 107). Resolution of this problem is essential for understanding the underlying mechanism. The arguments have been reviewed by Sacktor (94). For an electrogenic process, a mechanism consisting of an influx of 3 Na^+ and one $glutamate^{1-}$ per 1 K^+ efflux was proposed (104). From the

other viewpoint (107), two possible mechanisms were offered to explain electroneutral uptake of glutamic acid: (*a*) 2 $Na^+ \cdot 1$ glutamate^{1-} in/1 K^+ out; (*b*) 1 $Na^+ \cdot 1$ glutamate$^{1-} \cdot 1$ H^+ in/1 K^+ out (later modified to 1 $Na^+ \cdot 1$ glutamate^{1-} in per $K^+ \cdot 1$ OH^- out). This controversy points out some of the difficulties encountered when interpreting the data obtained from studies with vesicles. Burckhardt et al (104) found a sideness of the K^+ effect, i. e. K^+-stimulated only when it was present on the cytoplasmic side of the membrane. They also showed that glutamate uptake was potential-sensitive, when and only when K^+ was present intravesicularly, consistent with an electrogenic mechanism.

The debate seems to center around the maneuvers used to create the potential (inside negative). Burckhardt et al (104) created a H^+ diffusion potential with FCCP (carbonylcyanid-p-trifluormethoxy-phenylhydrazone) by changing the pH of the medium (i. e. dilution from pH 6.25 to pH 7.4). Sacktor argues that the results can be interpreted quite differently. Low pH is known to inhibit glutamate transport and in the diffusion potential created in the manner described by Burckhardt et al, it is possible that inhibition of transport would be relieved due to a higher intravesicular pH caused by H^+ exit. Sacktor postulates the interesting hypothesis that the OH^- gradient created (in $>$ out) might in fact be a driving force for glutamate influx. Obviously, we have not heard the end of this interesting and provocative debate.

A great deal more sorting out of the complexities of amino acid transport needs to be accomplished. Clearly, several amino acids are transported by more than one system (100, 101, 109, 112, 113).

SUMMARY

The use of membrane vesicles has helped clarify our understanding of the secretory and reabsorptive processes occurring in the proximal tubule. In our opinion, three important areas have been advanced.

First, those constituent steps of transepithelial transport that had been described as passive processes are now shown to be catalyzed by a transporter. This was found for PAH at the brush border and for proline, lactate, and urate at the basolateral membrane. The nature of their respective transport mechanisms is less clear. Do they all occur by some obligatory exchange mechanism? That is, for PAH secretion (Figure 2, reaction 1) must some anion (Cl^-?) be taken up for every molecule of PAH transported from the cell? Similarly, are cationic drugs taken up from the peritubular space (i. e. NMN, reaction 2, Figure 2) by an exchange mechanism? It has been proposed (27, 28) that NMN transport into the cell would be efficiently

driven by the potential difference, thereby negating the need for an exchange mechanism. However, this hypothesis has not been verified. Some neutral amino acids are transported across the basolateral membrane by a Na^+-independent process. Would a cation or anion exchange mechanism facilitate the exit of these zwitterions? While these questions have not been addressed, their resolutions are ones that can be accomplished using membrane vesicles. We think that the demonstration of specially mediated transport at both faces of the renal cell has important consequences with regard to site of action of some transport inhibitors and are potential sites of drug:drug interactions.

Second, vesicle studies have advanced hypotheses aimed at understanding the energetics of transport. Two basic operational modes have evolved. One is secondarily active processes such as the Na^+ gradient-dependent reabsorption across the brush border as exemplified by some amino acids; lactate; mono-, di-, and tricarboxylic acids. This mechanism is consistent with the standing gradient hypothesis proposed by Crane (116). A more complex phenomenon is the coupling of two secondarily

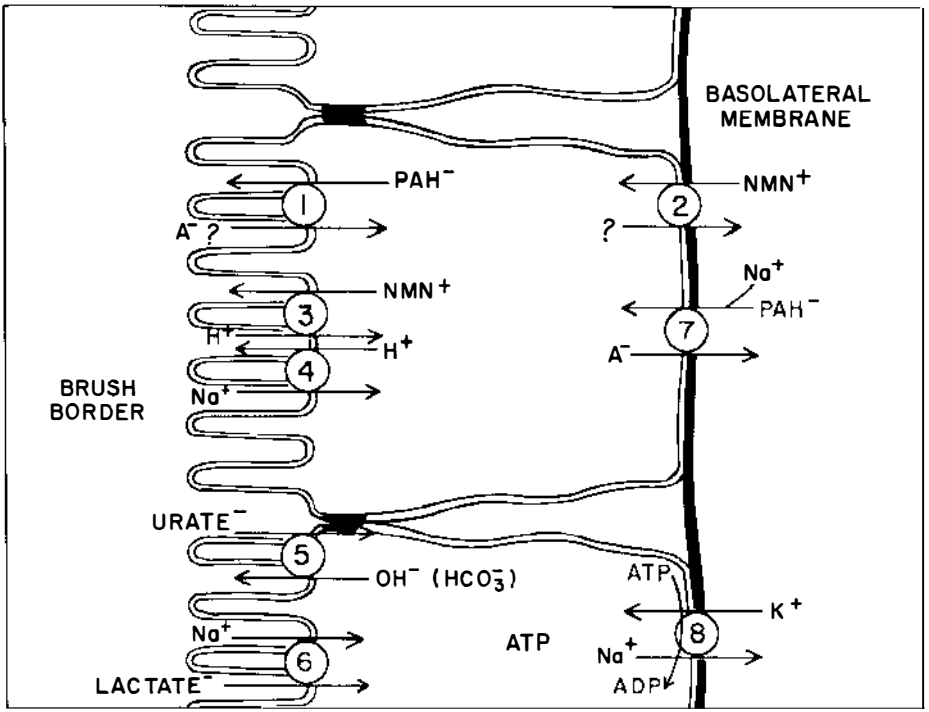


Figure 2 A schematic of a proximal tubular cell showing the location of various transporters discussed in the summary.

active mechanisms [it has been suggested (24) that these should be termed "tertiarily active"]. Examples of the latter are: the coupling of NMN secretion to urinary acidification (Figure 2, reactions 3 and 4, respectively; for the sake of simplicity, only one acidification mechanism is considered); the coupling of urate reabsorption by a hydroxyl exchange to the Na^+ gradient-dependent uptake and metabolism of lactate (Figure 2, reactions 5 and 6); and the Na^+ gradient-stimulated PAH:anion exchange in the basolateral membrane (Figure 2, reaction 7). The description of these coupled systems requires a modification of the standing gradient hypothesis. Ordinarily, the large standing Na^+ gradient is considered to be the sole energy source. The modification is that there is a shallow, regenerative gradient interposed between the sodium gradient and the transported ion. The sodium gradient in turn is created by the energy in ATP hydrolysis (Figure 2, reaction 8).

A third area advanced by vesicle studies is the elucidation of the underlying mechanisms of transport. While a great deal remains to be accomplished, some interesting findings have been reported. For example, those studies showing Na^+ stoichiometries greater than one have important mechanistic and thermodynamic implications. Kinsella & Aronson (117) have proposed an innovative approach for determining the stoichiometric ratios for exchange reactions.

Areas that need to be more fully developed and that are amenable to examination with vesicles include the effect of drugs on the transport of endogenous substrates, and the transport of amphoteric drugs. One study with dog kidney vesicles (118) has shown that the nephrotoxic cephalosporin antibiotic, cephaloridine, acts only as an anion in the basolateral membrane (Figure 2, reaction 7) and predominantly as a cation in the brush border membrane (Figure 2, reaction 3), thus raising the question about amphoteric drugs. Are they transported as anions or as cations?

A probable outcome of these studies will be a demonstration of the multiplicity of the renal transport systems, a phenomenon already described for the amino acids. A likely problem then is the origin of this redundancy. Is it due to cell heterogeneity or to closely related transporters with overlapping specifities? Resolution of this potential complication cannot be unequivocally accomplished by the technique at hand. Rather it must await methodologies which can achieve subfraction of the vesicle population.

Notwithstanding the current methodological limitation, there is a pressing need for more studies in the basolateral membrane to raise our understanding of the processes occurring in this membrane. For it will only be by describing events at both faces of the renal cell that we will begin to understand transepithelial transport.

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