Transport of GSH was studied in isolated rat kidney cortical brush-border membrane vesicles in which \( \gamma \)-glutamyltransferase had been inactivated by a specific affinity labeling reagent, \( L-(\alpha S,\beta S)-\alpha \text{-amino-3-chloro-4,5-dihydro-5-isoxazoloocteic acid} \) (AT-125). Transport of intact 2-\( ^3 \)H-glycine-labeled GSH occurred into an osmotically active intravesicular space of AT-125-treated membranes. The initial rate of transport followed saturation kinetics with respect to GSH concentrations; an apparent \( K_a \) of 0.21 mM and \( V_{\text{max}} \) of 0.23 nmol/mg protein \( \times 20 \) were calculated at 25 °C with a 0.1 M NaCl gradient (vesicle inside < vesicle outside). Sodium chloride in the transport medium could be replaced with KCl without affecting transport activity. The rate of GSH uptake was enhanced by replacing KCl in the transport medium with KSCN, providing a less permeant anion, and was reduced by replacing KCl with KSCN, providing a more permeant anion. The rate of GSH transport markedly decreased in the absence of a K\(^+\) gradient across the vesicular membranes and was enhanced by a valinomycin-induced K\(^+\) diffusion potential (vesicle-inside-positive). These results indicate that GSH transport is dependent on membrane potential and involves the transfer of negative charge. The rate of GSH transport was inhibited by \( S \)-benzyl glutathione but not by glycine, glutamic acid, and \( \gamma \)-glutamyl-p-nitroanilide.

When incubated with [2-\( ^3 \)H]glycine-labeled GSH, intact untreated vesicles also accumulated radioactivity; the rate of uptake was significantly higher in a Na\(^+\) gradient than in a K\(^+\) gradient. Sodium-dependent transport, but not sodium-independent uptake, was almost completely inhibited by a high concentration of unlabeled glycine. At equilibrium, most of the radioactivity accumulated in the intravesicular space was accounted for by free glycine. These results suggest that GSH which is secreted into the tubular lumen by a specific translocase in the luminal membranes or filtered by the glomerulus may be degraded in \( \text{situ} \) by membranous \( \gamma \)-glutamyltransferase and peptidase activities which hydrolyze peptide bonds of cysteinylglycine and its derivatives. The resulting free amino acids can be reabsorbed into tubule cells by sodium-dependent transport systems in renal cortical brush-border membranes.

There is a high (up to 10 mM) concentration of GSH in many cells. Since enzymes that degrade GSH and its derivatives are localized on the outer surface of the plasma membranes in many tissues (1-5), translocation of these tripeptides out of cells is important in their metabolism (6, 7). In the liver, GSH is transported from hepatocytes into both bile and plasma (8, 9). Recent studies in separated rat liver sinusoidal and canalicular plasma membrane vesicles (10-12) provided direct evidence for carrier-mediated transport systems for GSH and its derivatives in each plasma membrane preparation. Other cell types, such as erythrocytes, also possess transport systems for glutathione derivatives (13, 14). GSH and its derivatives in plasma are extracted mainly by the kidney (15, 16). Renal extraction of plasma GSH occurs by glomerular filtration and a nonfiltrating peritubular mechanism; 20-30% of GSH in renal arterial plasma is eliminated by the former and 50-60% by the latter mechanism during a single pass through the kidney (17, 18).

\( \gamma \)-Glutamyltransferase is the only known enzyme which hydrolyzes the \( \gamma \)-glutamyl linkage of GSH and its derivatives and is located on the outer surface of renal brush-border (1-4) and basolateral plasma membranes (19, 20). Thus, GSH in the lumenal and peritubular spaces can be degraded in \( \text{situ} \) by renal \( \gamma \)-glutamyltransferase. Since the turnover rate of GSH in the kidney is very high (21), large amounts of GSH may be translocated out of renal cells and subsequently undergo hydrolysis of peptide bonds. Recent studies in \( \text{vivo} \) (22-24) suggest that GSH in the kidney may be transported from tubule cells into the lumenal space; the mechanism of this transport is not known.

The present studies report transport of intact GSH by renal brush-border membrane vesicles in which \( \gamma \)-glutamyltransferase was extensively inactivated by a specific affinity-labeling agent, AT-125' (25, 26). Kinetic analysis provided direct evidence for membrane potential-dependent GSH secretory transport by renal cortical brush-border membrane.

**EXPERIMENTAL PROCEDURES**

**MATERIALS**—Valinomycin, carboxyl chloride \( p \)-trifluoromethoxyphenyl hydrazone, \( \gamma \)-glutamyl-p-nitroanilide, and glutathione reductase were purchased from Sigma. \( L \)-Cystinylbisglycine was obtained from Bachem Co. Thin-layer chromatographic aluminum plates (Kieselgel 60F\( _2 \)) were purchased from Merck. [2-\( ^3 \)H]Glycine-labeled GSH and 2-\( ^3 \)H-labeled glycine were purchased from New England Nuclear. \( S \)-Benzy glutathione was synthesized as described previously (10). AT-125 was a gift from Dr. R. Davis, National Institutes of Health. Other reagents used were of analytical grade.

**Preparation of Renal Brush-border Vesicles**—Brush-border membrane vesicles were prepared from the renal cortex of male Wistar rats by a calcium-precipitation method as described previously (27). The vesicle samples were enriched at least 15-fold in alkaline phosphatase activity.

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DIRECT EVIDENCE FOR THE ROLE OF THE MEMBRANE POTENTIAL IN GLUTATHIONE TRANSPORT BY RENAL BRUSH-BORDER MEMBRANES

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phatase and \( \gamma \)-glutamyltransferase as compared with the crude homogenate. Vesicles were stored at \(-70^\circ C\) in 20 mM Hepes-Tris buffer, pH 7.4, and contained 0.1 M mannitol; storage of vesicles at this temperature did not alter the transport properties for D-glucose, glycine, and alanine for 3 months.

**Enzyme Activity**—\( \gamma \)-Glutamyltransferase and alkaline phosphatase activities in vesicles were determined as described (6). One unit of enzyme activity was defined as the amount of the enzyme required for formation of 1 \( \mu \)mol of product/min at 37°C.

**Affinity Labeling of \( \gamma \)-Glutamyltransferase**—\( \gamma \)-Glutamyltransferase in the brush-border vesicles was inactivated by AT-125 as described previously (10, 12). The reaction mixture contained, in a final volume of 0.5 ml, 20 mM Hepes-Tris buffer, pH 7.4, 0.1 M mannitol, 20 mg of vesicles, and 1 mM AT-125. The reaction was started by adding AT-125. At indicated times after incubation at 25°C, aliquots of 2–20 \( \mu \)l were withdrawn and remaining transferase activity was determined in the presence or absence of glycylglycine. After 30 min of incubation, the mixture was added to 1 ml of an ice-cold buffer solution which did not contain AT-125 and centrifuged for 30 min at 15,000 rpm in an Eppendorf centrifuge to eliminate excess AT-125. The precipitated vesicles were resuspended in the ice-cold buffer solution and kept on ice until used.

**Transport**—Since commercially available \(^3\)H-labeled GSH and glycine were dissolved in 0.05 N acetic acid and 0.01 N HCl, respectively, the labeled compounds were used for experiments after lyophilization of the labeled solutions to avoid contamination of these acids into transport medium. The lyophilized radioactive GSH and glycine were dissolved in appropriate concentrations of unlabeled GSH and glycine, respectively. The radioactive ligands thus prepared were subjected to silica gel thin-layer chromatography in butan-1-ol/acetic acid/water (25:4:10) under nitrogen. The ninhydrin-positive spot responsible for GSH (\( R_F = 0.05 \)) was scraped off and determined for radioactivity; more than 95% of radioactivity applied on the chromatographic plate was recovered from this spot. Under the identical conditions, \(^3\)H-labeled glycine also gave a single ninhydrin-positive spot (\( R_F = 0.12 \)); more than 96% of radioactivity applied on the plate was recovered from this spot. Transport of radioactive GSH and glycine was performed by a rapid filtration method as described previously (10). Unless otherwise stated, the transport medium contained, in a final volume of 0.15 ml, 20 mM Hepes-Tris buffer, pH 7.4, varying concentrations of the radioactive ligand, and 0.1 M NaCl or KCl. Transport was started by adding membrane vesicles. At the indicated times after incubation at 25°C, aliquots of 20 \( \mu \)l were withdrawn, diluted into 1 ml of ice-cold transport medium which contained 0.1 M NaCl, immediately filtered through Millipore filter (HA, 0.45 \( \mu \)m), and washed with 3 ml of the ice-cold medium. Vesicle-associated radioactivity on the filters was determined in a liquid scintillation counter using ASC-II (Amersham Corp.) as scintillator. All values were corrected for radioactivity found on the filters in the absence of membrane vesicles. There were small variations in transport activity with different membrane preparations; however, all experiments were repeated at least three times with similar results (deviation, 28%). It has been known that washing of membrane vesicles with a hypotonic solution eliminates most of intravesicularly transported free-form ligands (28). Thus, after equilibrium uptake, vesicle-associated radioactivity on the filters was eluted with 0.5 ml of water and concentrated by lyophilization to a small volume; no detectable radioactivity was found to remain on the filters by this washing procedure. Chemical analysis of the eluted radioactive was performed by glutathione reductase method using unlabeled GSH (20–3000 ng/ml of assay mixture) as the standard (29). To the concentrated solution was added 1 ml of reaction mixture containing 0.1 mg potassium phosphate buffer, pH 7.1, 1 mM EDTA, 0.2 mg of 5,5′-dithiobis(2-nitrobenzoic acid), 75 \( \mu \)g of NaHCO\(_3\), and 80 \( \mu \)g of NADPH. The reaction was started by adding 0.25 unit of glutathione reductase, and the change of absorbancy at 412 nm was recorded. The enzymic analysis revealed that more than 90% of radioactivity associated with AT-125-treated vesicles was recovered from intact glutathione. In contrast, no detectable glutathione was found to associate with the intact vesicles as determined by the reductase method. Thin-layer chromatographic analysis revealed that more than 93% of radioactivity eluted from vesicles was co-chromatographed with an authentic glycine (\( R_F = 0.45 \)) in propan-1-ol/water/acetic acid (10:5:1). Under identical conditions, \( R_F \) values for authentic GSH and cysteinylibiglycine were 0.34 and 0.09, respectively.

**Affinity Labeling of Vesicle-bound Transferase**—\( \gamma \)-Glutamyltransferase, aminopeptidase M, and dipeptidase which hydrolyzes cysteinylbiglycine are localized on the outer surface of renal brush-border membranes (1–4). These ectoenzymes catalyze degradation of GSH and its derivatives into constituent amino acids; the transferase catalyzes the initial step of degradation. To determine whether renal brush-border membranes possess a translocase which is specific for intact GSH, it is essential to prepare vesicles which virtually lack \( \gamma \)-glutamyltransferase activity (10, 12, 30). Fig. 1 shows the effect of AT-125, an affinity labeling reagent for glutamine amidotransferases (25), on vesicle-bound transferase activity; AT-125 effectively inactivated transferase activity in a time-dependent manner. As with hepatic canalicular membrane vesicles (10), inactivation followed pseudo-first-order kinetics, and the rate constant of 0.692/min for inactivation was obtained with 1 mM AT-125 at 25°C. After 30 min of incubation, the residual transferase activity was less than 0.001% of that found in intact untreated vesicles.

**Transport of Intact GSH into an Osmotically Active Intravesicular Space**—\(^3\)H-labeled GSH was taken up by AT-125-treated vesicles and vesicle-associated radioactivity increased with incubation time (Fig. 2). Since the brush-border membranes virtually lacked \( \gamma \)-glutamyltransferase activity, intact GSH fully accounted for vesicle-associated radioactivity. After 30 min incubation, uptake reached equilibrium; about 1.2 nmol of GSH was associated with 1 mg of vesicles with 1 mM \(^3\)H-labeled GSH. The apparent rate of uptake was not affected by replacing NaCl in the transport medium by the same concentration of KCI.

GSH can form mixed disulfides with many thiol compounds including cysteinyl moieties of membrane proteins (31). To test whether vesicle-associated GSH reflects transmembrane movement or binding to membranous components, the effect of medium osmolarity on the equilibrium uptake was observed at different GSH concentrations (Fig. 3). After 30 min incubation, increasing the osmotic gradient across the membranes at raffinose concentration of 200–500 mM decreased GSH uptake by 50–54%. Although it is difficult to measure binding accurately, values obtained from extrapolation to infinitely high osmolarity suggest that the binding of GSH is less than 9%; most membrane-associated GSH represented transmembrane glucose transport activity.
Membrane Potential-dependent Secretory Transport of Glutathione

Fig. 2. Transport of intact GSH by AT-125-treated vesicles. Inactivation of vesicle-associated transferase was performed as described in Fig. 1. AT-125-treated vesicles retained less than 0.001% of the residual transferase activity of intact untreated membranes. Transport medium contained, in a final volume of 0.15 ml, 20 mM Hepes-Tris buffer, pH 7.4, 1 mM of [2-3H]glycine-labeled GSH, about 0.1 mg of membrane samples in protein, and 0.1 mM NaCl (open circles) or KCl (closed circles). Transport was started by adding membrane samples at 25°C. Other conditions were as described in the text.

Fig. 3. Uptake of GSH into an osmotically active intravesicular space. Incubation medium contained, in a final volume of 0.15 ml, 0.1 mg of AT-125-treated vesicles, 0.1 mM KCl, 20 mM Hepes-Tris buffer, pH 7.4, varying concentrations of raffinose (200-500 mM), and 1 mM (closed circles) or 6 mM (open circles) radioactive GSH. After 30 min of incubation at 25°C, vesicle-associated radioactivity was determined as in Fig. 2 with the stop solution which contained 0.3 mM NaCl. The inverse values for raffinose concentration in the transport medium are plotted on the abscissa. Amount of vesicle-associated radioactivity in the absence of raffinose was 1.2 and 7.2 nmol/mg x 30 min at 1 and 6 mM GSH concentration, respectively. Each point represents the mean value of triplicate determinations.

brane movement of the ligand rather than binding to the membranes. The osmotically active intravesicular space of the membrane samples was calculated to be 1.2 µl/mg membrane protein.

Since AT-125 alkylates various membrane proteins (32, 33), possible changes in transport or membrane permeability of treated vesicles for various ligands may have occurred. To test this possibility, the transport of various ligands was compared with the intact and AT-125-treated vesicles. In the presence of a sodium gradient (vesicle inside < vesicle outside), an overshooting uptake was observed in the intact vesicles (Fig. 4A). With a potassium gradient, the rate of uptake was low, and overshoot was not observed. At equilibrium, vesicle-associated radioactivity was identical in the two transport medium; 0.2 nmol of glycine was taken up by 1 mg of the vesicles in protein. AT-125-treated vesicles retained Na⁺-dependent transport activity for 3H-labeled glycine (Fig. 4B). Osmotic dependency of equilibrium uptake also revealed no significant binding of glycine to the membrane surface (data not shown). Thus, AT-125-treated membranes samples had 1.13 µl of intravesicular volume/mg of membranes in protein, which compares favorably with vesicle-associated GSH at equilibrium uptake. AT-125-treated vesicles also retained Na⁺-dependent overshoot for D-glucose uptake; equilibrium uptake gave a value of 1.2 µl of the intravesicular volume/mg of membranes in protein under identical conditions as in Fig. 2. These results indicate that AT-125 specifically labeled the vesicle-associated transferase without having a significant effect on active transport systems for various ligands and that vesicle-associated GSH, glycine, and D-glucose reflect transmembrane transport into the intravesicular compartment of AT-125-treated membranes.

Dose-dependent Transport of GSH—Fig. 5 shows the dose dependence of the initial rate of GSH transport by AT-125-treated brush-border vesicles. The rate of uptake plotted as a function of GSH concentration seemed to be best described as the sum of saturable and nonsaturable diffusion components. Double-reciprocal plots of the saturable uptake revealed a carrier-mediated transport for GSH with an apparent Kₐ of 0.21 mM and Vₐ of 0.23 nmol/mg protein x 30 s.

Effect of Membrane Potential on GSH Uptake—The potential difference across biomembranes represents an important driving force for electrogenic solute transport. Since GSH is negatively charged under physiological pH, the anionic nature of GSH may contribute to its transmembrane movement. To test this possibility, the effect of an artificially imposed membrane potential difference on transport activity was studied using anion replacement (Fig. 6). In the presence of a potassium gradient (K⁺ inside < K⁺ outside), replacement of Cl⁻ by a more permeant anion, SCN⁻, decreased the rate of GSH uptake. When a less permeant SO₄⁻ was used instead of Cl⁻, the rate of uptake increased, indicating that transport of GSH occurred as an anion without simultaneous symport of a cation or antiport of anion. Further evidence was obtained from experiments involving a valinomycin-induced K⁺ diffusion potential (Fig. 7). Treatment of vesicles with valinomycin increased the rate of GSH transport in the presence of KCl. Furthermore, preloading of the vesicles with the transport medium markedly decreased the rate of GSH uptake. These
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FIG. 5. Dose-dependent transport of GSH. Incubation medium contained, in a final volume of 0.15 ml, 20 mM Hepes-Tris buffer, pH 7.4, 0.1 M KCl, and varying concentrations of radioactive GSH. Transport was started by adding membrane vesicles. After 20 s of incubation at 25 °C, vesicle-associated radioactivity was determined as in Fig. 2. Closed circles, vesicle-associated GSH; ----, unsaturable diffusion component obtained from uptake at high GSH concentrations; open circles, saturable uptake which was obtained by subtracting diffusion component from the total uptake activity. A, double-reciprocal plots of saturable uptake in A.

FIG. 6. Effect of anion replacement on the uptake. Transport of GSH was measured at 25 °C in incubation medium containing, in a final volume of 0.15 ml, 20 mM Hepes-Tris buffer, pH 7.4, 1 mM radioactive GSH, and 0.1 M KC1 (open circles). Potassium chloride in the transport medium was replaced by the same concentration of KSCN (triangles) or 50 mM K2SO4 plus 50 mM mannitol (closed circles). Other conditions were as described in Fig. 2.

Transport profiles are characteristic of a membrane potential-dependent transport system.

Specificity of GSH Transport—Renal brush-border membranes possess various translocases which are responsible for transport of different ligands. Experiments in vivo with various γ-glutamyl compounds suggest that the kidney may also absorb intact γ-glutamyl peptides (34, 35). Since GSH is a γ-glutamyl peptide, its transport may be affected by other γ-glutamyl peptides or structurally related compounds. To test the structural specificity of the GSH transport system in vesicular membranes, GSH transport was measured in the presence or absence of various compounds. The rate of GSH transport was not affected by γ-glutamyl-p-nitroanilide, glycine, and glutamic acid (Fig. 8). In contrast, S-benzyl glutathione markedly reduced the rate of GSH uptake by the vesicles.

Transport by Untreated Renal Brush-border Vesicles—Fig. 9 shows uptake of radioactivity by untreated brush-border vesicles after incubation with [2-3H]glycine-labeled GSH. Vesicle-associated radioactivity increased with incubation time; uptake was significantly higher in a sodium than in a potassium gradient. These results are in contrast with GSH transport by AT-125-treated vesicles in which sodium dependency was not observed at any ligand concentration. Thin layer chromatographic analysis revealed that more than 90% of radioactivity which accumulated in the intravesicular space
possible participation of proton gradients in GSH transport, the port was determined by a "down-hill" electrochemical potential difference (vesicle-inside-positive). Since the lumenal side of renal tubules is more positively charged than the intracellular space under physiological conditions, the luminal side of renal brush-border membranes is more positively charged than the intracellular side. Because renal brush-border membranes have abundant \( \gamma \)-glutamyltransferase activity, the transport process for intact GSH was observed in vesicle preparations only if pH gradients were established across the membranes in the presence of a high concentration of unlabeled glycine. Sodium-dependent uptake of radioactivity by the vesicles was inhibited by 96% and Na\(^+\)-independent uptake of radioactivity was inhibited only slightly (8–20%).

**DISCUSSION**

The present study provides direct evidence for a GSH transport system in brush-border membranes of rat kidney cortical tubules. Because renal brush-border membranes have abundant \( \gamma \)-glutamyltransferase activity, the transport process for intact GSH was observed in vesicle preparations only if anion replacement and valinomycin-inhibited K\(^+\) diffusion potential suggest that GSH transport across the brush-border membranes depends on the membrane potential and involves transfer of negative charge. Under physiological conditions, GSH is negatively charged. The present studies were performed by measuring GSH uptake into vesicles which were oriented exclusively right-side-out (36). The rate of GSH uptake was enhanced by an artificially imposed membrane potential, and the direction of transport was determined by a "down-hill" electrochemical potential difference (vesicle-inside-positive). Since the luminal side of renal tubules is more positively charged than the intracellular space under physiological conditions (37), GSH could be secreted across the brush-border membranes. To test the possible participation of proton gradients in GSH transport, experiments were performed in which pH gradients were established across the membranes in the presence of an excess of 50 \( \mu \)M 

\[
\text{carboxyl chloride p-trifluoromethoxyphenyl hydrazone. A 10-fold proton gradient, either directed from outside to the inside or vice versa (pH 7.0–8.0), did not affect the transport process.}
\]

In intact untreated vesicles, transport profiles of the radioactive ligand(s) were significantly different from those in AT-125-treated membrane samples. Uptake of radioactivity was higher in a sodium than in a potassium gradient (Fig. 9). In this context, Lash and Jones (38) reported a Na\(^+\)-dependent transport system for GSH in renal basolateral membranes which may translocate GSH from plasma into tubule cells. Thus, Na\(^+\)-dependent uptake of radioactivity by the intact brush-border membranes (Fig. 9) may also reflect transport of intact GSH into the vesicles. However, this possibility seemed unlikely. \( \gamma \)-Glutamyltransferase activity in the intact untreated vesicles was extremely high: about 0.8 \( \mu \)mol of GSH/min could be degraded by 1 mg of the vesicles at 25 °C and pH 7.4 in the absence of glycylglycine (38, 40). Renal brush-border vesicles also have abundant aminopeptidase M and dipeptidase activities which hydrolyze cysteinylglycine and its derivatives on the outer surface of the plasma membranes (1–4, 30). Thus, rapid degradation of [\( ^2\text{H}\)]glycine-labeled GSH into constituent amino acids would have occurred during incubation. For these reasons, it was not surprising that the transport profiles of radioactivity derived from [\( ^2\text{H}\)]glycine-labeled GSH were significantly different in intact and AT-125-treated vesicle preparations. To elucidate the quantitative aspect of transport and degradation of GSH by the intact vesicles, several kinetic parameters were compared. As reflected by the ratio of the respective \( V_{\text{max}}/K_m \) values (Table I), the enzymatic cleavage of GSH on the outer surface of the intact vesicles might be about 37,800 times more efficient than the uptake of GSH even when the transport system is optimized for uptake. Thus, sodium-dependent uptake of radioactivity by intact vesicles could reflect active transport of glycine which was formed extravesicularly by \( \gamma \)-glutamyltransferase and the peptidases. Consistent with this hypothesis is that sodium-dependent uptake of radioactivity was almost completely inhibited by a high concentration of unlabeled glycine. Thus, GSH which is filtered by the glomerulus or excreted by renal tubule cells into the luminal space can be rapidly degraded into component amino acids in situ; the resulting amino acids are efficiently reabsorbed by tubule cells (reabsorption) by the sodium-dependent amino acid transport systems in brush-border membranes. These observations support the proposal (22–24) that GSH secretion across the renal brush-border membranes, luminal hydrolysis, catabolite reabsorption, and intracellular resynthesis of GSH may operate in vivo.

GSH transport by AT-125-treated vesicles was markedly inhibited by S-benzyl glutathione. The presence of \( \gamma \)-glutamyl-p-nitroanilide, glycine, or glutamic acid did not affect the rate of GSH uptake. Buthionine sulfoximine, a specific inhibitor of \( \gamma \)-glutamylcysteine synthetase, and serine-borate complex, a transition state inhibitor of \( \gamma \)-glutamyltransferase, also failed to inhibit transport of GSH (data not shown). These observations suggested that the translocase may have high affinity for the tripeptide moiety of GSH and its derivatives. Recently, the presence of transport systems for glutathione S-conjugates was demonstrated in erythrocyte membranes (14) and hepatic canalicular plasma membranes (12). Since the kidney has high activity of glutathione S-transferases (41), renal tubule cells might also have secretory systems for S-substituted glutathiones. Transport of glutathione S-conjugates by brush-border membrane vesicles under the present experimental conditions is shown in Table I. The data show that the transport process is more efficient than the degradation process; in fact, the degradation process is at least 100 times less efficient than the transport process. The mechanism of GSH transport is shown in Table I. The transport process is inhibited by both \( \gamma \)-glutamyl-p-nitroanilide and glycine, while the degradation process is inhibited by both \( \gamma \)-glutamyl-p-nitroanilide and glutamic acid.

**TABLE I**

<table>
<thead>
<tr>
<th>Mechanism of GSH processing</th>
<th>( V_{\text{max}} ) (mol/20 ( \mu )l ( \times ) mg protein)</th>
<th>( K_m ) (M)</th>
<th>( V_{\text{max}}/K_m ) (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Transport</strong></td>
<td>( 0.23 \times 10^{-9} )</td>
<td>( 0.21 \times 10^{-9} )</td>
<td>( 1.19 \times 10^{-4} )</td>
</tr>
<tr>
<td><strong>Degradation by the ( \gamma )-transferase</strong></td>
<td>( 0.27 \times 10^{-4} )</td>
<td>( 6.0 \times 10^{-4} )</td>
<td>( 4.50 \times 10^{-2} )</td>
</tr>
</tbody>
</table>

*Value obtained from Ref. 30.
one S-conjugates across the renal brush-border membranes should be studied further.

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