Na+-dependent GSH transport in basal-lateral membrane vesicles from rat kidney exhibited saturation kinetics while Na+-independent flux increased linearly up to 10 mM GSH. Inhibitor studies showed that GSH transport was not dependent upon the catalytic activity of \( \gamma \)-glutamyltransferase. K\(^+\), choline and NH\(_2\) ions did not stimulate GSH transport, but Li\(^+\) partially substituted for Na\(^+\). Na+-dependent GSH transport was inhibited by other \( \gamma \)-glutamyl amino acids. The membrane also showed Na+-dependent transport of glutathione disulfide (GSSG) and \( \gamma \)-glutamylglutamate. These results show that specificity resides in the \( \gamma \)-glutamyl moiety and suggest that this system may be a general transport system for \( \gamma \)-glutamyl compounds. Results from four types of experiments showed that Na+-dependent GSH transport was electronegic. Transport was stimulated by negative and inhibited by positive valinomycin-induced K\(^+\) diffusion potentials; the transport rate was influenced by the anion component of the Na\(^+\) salt in the order NaSCN > NaCl > Na\(_2\)SO\(_4\); analysis of the Na\(^+\) concentration dependence indicated coupling of at least 2 Na\(^+\)/GSH; comparison of GSH-dependent Na\(^+\) transport and Na+-dependent GSH transport gave a Na\(^+\)/GSH stoichiometry of 2:1. Thus, energy is coupled to the transport of GSH in the form of the cellular Na\(^+\) gradient and the membrane potential. This system, if it functions in vivo, can act as a mechanism for extraction of GSH from the renal circulation.

The primary organ for clearance of circulating glutathione is the kidney (1, 2), which has been estimated to account for 50-67% of net plasma glutathione turnover (3, 4). Several investigators have found that during a single pass through the kidney, 80% or more of the plasma glutathione is extracted, greatly exceeding the amount which could be accounted for by glomerular filtration (4-8). While the filtered glutathione is degraded stepwise by the action of the brush-border enzyme, \( \gamma \)-glutamyltransferase and cysteinylglycine dipeptidase (9-11), the fate of the nonfiltered fraction remains unclear. Three alternative fates are possible based upon current knowledge: degradation, oxidation, and uptake. While considerable information is available concerning the first two processes, very little is known about the transport of glutathione in kidney.

Transport of glutathione across the basal-lateral membrane was first indicated in studies with perfused kidney and isolated renal cells (8, 12). Previous work in our laboratory demonstrated that GSH\(^+\) is transported intact by a Na\(^+\)-dependent process in renal basal-lateral membrane vesicles (13). In the presence of a Na\(^+\) gradient, but not a K\(^+\) gradient, a pronounced overshoot was observed indicating that the Na\(^+\) gradient can provide a driving force for intravesicular accumulation of GSH.

In the present study, we have characterized the kinetics and specificity of the GSH transport system under conditions where both degradation and oxidation are inhibited. The results demonstrate that the transport system has a specificity for \( \gamma \)-glutamyl compounds and is an electronegative system with a sufficient driving force to allow uptake of GSH into kidney cells against the GSH concentration gradient.

**EXPERIMENTAL PROCEDURES**

**Materials**—Phenylmethylsulfonyl fluoride, GSH, GSSG, Potassium, probenecid, \( \gamma \)-glutamyl p-nitroanilide, \( \gamma \)-glutamylglutamate, valinomycin, and 1-fluoro-2,4-dinitrobenzene were purchased from Sigma. Ophthalmic acid was purchased from Bachem (Torrance, CA). AT-125 was a gift from Dr. Donald J. Reed, Oregon State University.

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**Membrane Preparation**—Male white rats (Sprague-Dawley derived, barrier-reared outbred albino, King Animal Laboratories, Oregon, WI, 200-300 g) were anesthetized with diethyl ether and sacrificed by cutting through the diaphragm. The kidneys were immediately removed and placed in ice-cold Na\(^+\)-free 10 mM Tris-Hepes buffer, pH 7.6, containing 250 mM sucrose and 0.1 mM phenylmethylsulfonyl fluoride to inhibit proteolysis. The brush-border and basal-lateral regions of the plasma membrane were prepared by the Percoll density-gradient centrifugation method of Scala et al. (14) with the modification of the buffer as described above. Membrane fractions were identified by the use of marker enzymes as previously described (15). \( \gamma \)-Glutamyltransferase (EC 2.3.2.2) was assayed by the method of Orlovski and Meister (16) with \( \gamma \)-glutamyl-p-nitroanilide as substrate and glycylglycine as \( \gamma \)-glutamyl acceptor. Brush-border contamination of the basal-lateral fraction was estimated to be less than 5% by comparison of the distribution of marker enzymes (15). For use in transport studies, fractions from the Percoll gradient corresponding to the basal-lateral region of the plasma membrane were pooled and concentrated as previously described (13). This step allowed removal of Percoll. Protein was determined by the method of Bradford (17) with bovine serum albumin as standard.

**Transport Measurements**—Membrane vesicles were preincubated with 0.25 mM AT-125 to inhibit GSH catabolism (18) unless otherwise indicated.

\(^1\)The abbreviations used are: GSH and GSSG, reduced and oxidized glutathione, respectively; AT-125, 1-(\( \alpha \),5,5-\( \alpha \))-\( \alpha \)-amino-3-chloro-4,5-dihydro-5-isoxazolacetic acid; HPLC, high-pressure liquid chromatography; Tris, 2-amino-2-(hydroxymethyl)-1,3-propanediol; Hepes, 4-(\( \alpha \)-hydroxyethyl)-1-piperazineethanesulfonic acid; when the term "glutathione" is used in the text, the oxidation state is not being specified.

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indicated. All solutions were made anaerobic to prevent GSH oxidation by bubbling with prepurified nitrogen or ultrapure argon (Union Carbide Corporation, Linde Division, Somerset, NJ). GSH uptake was measured at room temperature by filtration on 0.45-μm nitrocellulose filters as previously described (13) or by the HPLC method of Reed et al. (19) with modifications (20). Separation of GSH and its metabolites was achieved on a 10-μ Ultrasil-NH2 column (4.6 mm x 25 cm; Beckman Instruments, Norcross, GA). The purity of the radiolabeled GSH was routinely assessed by derivatizing it with iodoacetic acid and 2-fluoro-2,4-dinitrobenzene followed by analysis by HPLC. Greater than 92% of the 3H counts eluted in a single peak which coincided with that of the derivative of authentic GSH.

In experiments in which the effect of membrane potential on GSH transport was studied, vesicles were pre-equilibrated with valinomycin (20 μg/ml) and subsequently exposed to different intravesicular/extravesicular ratios of K+. The intravesicular solution contained 10 mM Tris-Hepes, pH 7.6, 50 mM KCl, 100 mM sucrose, and 100 mM Tris-Cl; pH was readjusted to 7.6 with Tris base. In some experiments, Tris-Cl was substituted for KCl or NaCl. The pH of these solutions was readjusted to pH 7.6 with Tris base, resulting in an osmotic gradient when Tris concentrations were not equivalent on the two sides of the membrane. Because of the high initial osmolarity, the maximal magnitude of this gradient was less than 5% and was not corrected for in calculations of transport rates. Vesicles were diluted 10-fold with the appropriate incubation solution (detailed in figure legends), and uptake was measured as above. For some experiments, the membrane potential was "clamped" at 0 mV by addition of valinomycin to membrane vesicles with equimolar concentrations of K+ in the intra- and extravesicular solutions. This procedure has been described to effectively short circuit the membrane potential (21). In the absence of K+, valinomycin had no effect on transport. Valinomycin was added in ethanol; the final concentration of ethanol was 0.2%. Control measurements showed that this concentration of ethanol had no effect on GSH transport.

Labeled GSSG was formed by incubating 2 μCi of [glutamic-2-3H]GSH (0.3 ml with unlabeled GSSG (final concentration, 0.5 mM) for 30 min. Its formation was verified by HPLC (19, 20). Uptake of GSSG was measured by filtration as above.

Na+ uptake was measured by incubating membrane vesicles under anaerobic conditions with 10 mM Tris-Hepes, pH 7.6, 0.2 μCi NaCl/0.3 ml incubation mixture, 10 mM unlabeled NaCl, and 240 mM sucrose. Aliquots were filtered and radioactivity was determined in a Beckman γ counter.

Zero time measurements were made by first collecting the vesicles on the filter and then adding the solution containing the appropriate amount of labeled and unlabeled substrates. The filter was then washed and radioactivity measured as above. The filtration time was less than 0.5 s, and uptake should be minimal so that this step allowed measurement of nonspecific binding to the filter and membranes. Counts retained on the filter were approximately 5% of those measured after 1 min of incubation for GSH and GSSG uptake and approximately 10% of those measured after 1 min of incubation for Na+ uptake and were subtracted from all determinations. After the initial wash of filtered samples, no additional loss of counts was observed by further washing of the filter, indicating that negligible loss of intravesicular label occurred in this procedure and that those counts which were removed were due to nonspecific binding. Statistical significance was assessed using the Student’s t test, with differences at the 0.05 level considered significant.

**RESULTS**

Quantitative measurement of GSH transport in basolateral membrane vesicles isolated from rat kidney required inhibition of both GSH oxidation by thiol oxidase (7, 15, 22, 23) and GSH catabolism by converting brush-border γ-glutamyltransferase. Oxidation of GSH was effectively prevented by using deoxygenated solutions (7). Catabolism of GSH was inhibited by pretreatment of vesicles with AT-125 (18).

The effect of AT-125 on γ-glutamyltransferase activity present in the basolateral fraction was compared to its effect on GSH uptake (Fig. 1). AT-125-dependent inhibition of γ-glutamyltransferase was half-maximal at 13 μM and essentially complete at 0.25 mM. These results are contrasted with the effect at AT-125 on GSH uptake, where 1 mM was required to produce detectable inhibition. Thus, the optimal concentration to inhibit γ-glutamyltransferase but not affect GSH transport was in the range of 0.25–1.0 mM, and 0.25 mM AT-125 was used subsequently. The comparison of sensitivities of γ-glutamyltransferase activity and GSH uptake demonstrated that uptake of GSH in the basolateral membrane does not require the presence γ-glutamyltransferase activity.

To test whether or not the observed GSH transport was due to the presence of contaminating brush-border membranes, the ability of purified brush-border membrane vesicles (pretreated with AT-125) to transport GSH was compared to that of basolateral membrane vesicles in the presence and absence of 100 mM NaSCN. The initial rate of Na+-dependent GSH uptake in brush-border membranes (0.71 nmol/min/mg of protein) was less than 10% of the rate in basolateral membranes. While basolateral membrane vesicles exhibited a transient accumulation of GSH above equilibrium in the presence of NaSCN (an overshoot), as previously demonstrated (13), no overshoot was observed in brush-border membrane vesicles (data not shown). Therefore, the small amount of brush-border contamination was not responsible for the measured Na+-dependent uptake of radiolabeled GSH in the basolateral membrane preparation.

Since our previous work showed that Na+ ions stimulated uptake of GSH (13), we studied the cosubstrate specificity of GSH transport in more detail (Table I). In experiment A, the membrane potential was clamped at 0 mV to prevent simple

**FIG. 1. Effect of AT-125 on GSH metabolism and transport.** Basal-lateral membrane vesicles were pretreated with the indicated concentrations of AT-125. γ-Glutamyltransferase activity (O) present in the basolateral fraction was assayed as described under "Experimental Procedures." Results are the mean of 4 preparations, each assayed in duplicate. 100% activity was 1.02 ± 0.23 pmol of p-nitroanilide released/min/mg of protein. Uptake of 1 mM GSH (○) in basal-lateral membrane vesicles was measured after 1 min of incubation by filtration and determination of radioactivity as described under "Experimental Procedures." The intravesicular solution contained 10 mM Tris-Hepes, pH 7.6, and 250 mM sucrose. Extravesicular solution was the same as the intravesicular solution for Na+-free incubations and contained 10 mM Tris-Hepes, pH 7.6, 100 mM NaSCN, and 50 mM sucrose for Na+-containing incubations. Results are the mean of 3 preparations and are shown corrected for Na+-independent GSH uptake. 100% activity was 6.45 ± 0.03 nmol of GSH/min/mg of protein.
charge effects due to the presence of the added cations. A gradient of choline or NH₄ ions did not enhance transport, while a gradient of Na⁺ ions produced a 3.4-fold stimulation of the stimulation produced by NaCl was observed with LiCl, suggesting that Li⁺ ions can replace Na⁺ to a limited extent. A philic anion, SCN⁻, was added with Na⁺ (4.9-fold). The stimulation of GSH uptake was observed in the presence of KCl or KSCN, which argues against simple electrical coupling as an explanation for the effect of Na⁺ on GSH.

The effect of membrane potential under controlled conditions was examined by systematically varying the calculated Nernst K⁺-diffusion potential from −60 mV to +18 mV using valinomycin in the presence of intra- to extravesicular K⁺ gradients of from 10:1 to 1:2 (Fig. 2). Na⁺-independent GSH transport was not significantly affected by membrane potential (1.92 ± 0.06 nmol/min/mg of protein at −60 mV versus 1.87 ± 0.03 nmol/min/mg of protein at +18 mV; n = 6). In contrast, the rate of Na⁺-dependent GSH transport decreased as the membranes were depolarized, indicating that Na⁺-GSH cotransport is electrogenic and involves the net movement of positive charge with each turnover. Relative to 0 mV, a potential of −80 mV produced 50% stimulation, and a potential of +18 mV produced 20% inhibition.

The kinetics of GSH uptake were studied in the presence and absence of 100 mM NaCl with membrane potential clamped at 0 mV (Fig. 3A). In the absence of Na⁺, the initial rate of GSH transport increased linearly with GSH concentration up to 10 mM. In the presence of Na⁺, a nonlinear relationship was observed between the GSH concentration and the initial uptake rate. Subtraction of uptake rates in the absence of Na⁺ from those in the presence of Na⁺ produced a curve indicating a saturable transport mechanism, which appeared to follow simple Michaelis-Menten kinetics. An Eadie-Hofstee plot (Fig. 3B) of the Na⁺-dependent transport of GSH indicated a single transport system with an apparent Kₘ for GSH of 3.0 mM and a Vₘₐₓ of 19.5 nmol/min/mg of protein.

The effect of extravesicular Na⁺ concentration on the transport of GSH was studied under voltage-clamped conditions (Fig. 4A). Increasing the NaCl concentration produced stimulation of GSH uptake (shown corrected for Na⁺-independent uptake). This stimulation was not hyperbolic, as judged by the nonlinearity of the Eadie-Hofstee plot of these data (not shown), suggesting the involvement of multiple Na⁺ ions in the transport of each molecule of GSH. Therefore, the kinetic characteristics of the interaction of Na⁺ with the GSH trans-

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**TABLE I**

**Cosubstrate specificity of GSH transport**

| Cosubstrate | Initial rate | Control |
|-------------|-------------|---------|
|             | nmol/min/mg protein | %       |
| A. None     | 2.23 ± 0.09  | 100     |
| 100 mM NaCl | 7.57 ± 0.44  | 339     |
| 100 mM choline Cl | 2.36 ± 0.15 | 106     |
| 100 mM NH₄Cl | 2.23 ± 0.11  | 100     |
| 100 mM LiCl | 4.82 ± 0.23  | 215     |
| B. None     | 2.07 ± 0.10  | 100     |
| 100 mM NaCl | 7.17 ± 0.25  | 346     |
| 100 mM NaSCN| 10.10 ± 0.31 | 488     |
| 50 mM Na₂SO₄| 5.60 ± 0.38  | 271     |
| 100 mM KCl | 2.18 ± 0.13  | 105     |
| 100 mM KSCN| 2.21 ± 0.09  | 107     |

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**FIG. 2.** Effect of membrane potential on GSH uptake. The initial rate of 1 mM GSH uptake was measured by incubation for 1 min as described under "Experimental Procedures." The calculated Nernst K⁺-diffusion potential (Eₚ) was varied using valinomycin (20 μg/ml) in the presence of various intra- to extravesicular KCl gradients. Under all conditions, solutions contained 100 mM sucrose and 10 mM Tris-Hepes, pH 7.6. The intravesicular solution contained 50 mM KCl and 100 mM Tris-Cl (adjusted to pH 7.6 with Tris base). For Na⁺-free incubations, the extravesicular solutions contained KCl and Tris-Cl, respectively, given in mM: 5, 145; 15, 135; 50, 100; 70, 80; 100, 50. For solutions containing 50 mM NaCl, the extravesicular solutions also contained KCl and Tris-Cl, respectively, given in mM: 5, 95; 15, 85; 50, 50; 70, 30; 100, 0. Rates are for Na⁺-dependent uptake calculated relative to 0 mV and are the mean ± S.E. of 7 experiments and are plotted versus either extravesicular K⁺ concentration (log scale) or Eₚ.
Fig. 3. Effect of GSH concentration on rate of GSH uptake. A, the initial rate of GSH uptake was measured after 1 min of incubation in the presence (○) or absence (●) of 100 mM NaCl as described under “Experimental Procedures.” Membrane potential was clamped at 0 mV by using valinomycin (20 µg/ml) and equimolar concentrations of KCl on both sides of the membrane. Under all conditions, solutions contained 50 mM KCl, 100 mM sucrose, and 10 mM Tris-Hepes, pH 7.6. The intravesicular solution contained 100 mM Tris-Cl (adjusted to pH 7.6 with Tris base). For Na+-free incubations, the extravesicular solution contained 100 mM Tris-Cl (adjusted to pH 7.6 with Tris base). For Na+-containing incubations, the extravesicular solution contained 100 mM NaCl. The curve for Na+-dependent GSH uptake (---) was obtained by subtracting uptake in the absence of Na⁺ from that in the presence of Na⁺. Results are the mean ± S.E. of 3 preparations, each performed in duplicate. Where error bars are not shown, the error was smaller than the graphical representation of the data point. Inset, Rates of GSH uptake ± 100 mM NaCl at GSH concentrations of 5-100 µM. B, Eadie-Hofstee plot of Na⁺-dependent GSH uptake.

Fig. 4. Effect of Na⁺ concentration on rate of GSH uptake. A, the initial rate of 1 mM GSH uptake was measured as described under “Experimental Procedures.” Membrane potential was clamped at 0 mV by using valinomycin and equimolar concentrations of KCl on both sides of the membrane. Under all conditions, solutions contained 50 mM KCl, 100 mM sucrose, and 10 mM Tris-Hepes, pH 7.6. The intravesicular solution contained 100 mM Tris-Cl (adjusted to pH 7.6 with Tris base). The extravesicular solutions contained NaCl and Tris-Cl, respectively, given in mM: 0, 100; 5, 95; 10, 90; 20, 80; 40, 60; 60, 40; 80, 20; 100, 0. Rates shown have been corrected for GSH uptake in the absence of Na⁺ (1.91 ± 0.06 nmol of GSH/min/mg of protein; n = 5) and are the mean ± S.E. of 5 determinations, each performed in duplicate. B, linear transformation of the equation of Garay and Garrahan (25, 26) with n values of 1, 3, and 3.

port system were analyzed by the equation of Garay and Garrahan (25) for multiple substrate/activator reactions, using a linear transformation derived by Wright et al. (26). The equation relates GSH transport to [Na⁺] and the number of Na⁺-binding sites on the carrier.

\[
\frac{[\text{Na}^+]^{1/n}}{V_0^{1/n}} = \frac{[\text{Na}^+]^{1/n}}{V_{\text{max}}^{1/n}} + \frac{[\text{Na}^+]^{1/n}}{V_{\text{max}}^{1/n}}
\]
Renal GSH Transport

Fig. 5. Measurement of GSH-dependent Na\(^+\) uptake. A, uptake of 10 mM NaCl was measured in the presence (■) and absence (□) of 1 mM GSH as described under "Experimental Procedures." Intravascular solution contained 250 mM sucrose and 10 mM Tris-Hepes, pH 7.6. Extravascular solutions contained 10 mM Tris-Hepes, pH 7.6, and either 250 mM sucrose for Na\(^+\)-free incubations or 10 mM NaCl and 240 mM sucrose for Na\(^+\)-containing incubations. Measurements of uptake in the presence and absence of GSH were paired and are expressed as the mean ± S.E. of 3 preparations each performed in duplicate. Where error bars are not shown, the error was smaller than the graphical representation of the data point. B, time course of GSH-dependent Na\(^+\) uptake, obtained by subtracting uptake in the absence of GSH from that in the presence of GSH for paired experiments.

where \( V \) is the rate of transport of GSH at a given Na\(^+\) concentration, \( V_{\text{max}} \) is the maximal rate of transport, and \( K_s \) is a constant describing the affinity of Na\(^+\) for \( n \) equivalent and noninteracting binding sites. If the number of Na\(^+\) binding sites/carrier is \( n \), a plot of \([\text{Na}^+] / V_{\text{max}}\) versus \([\text{Na}^+]\) will yield a straight line with a slope of \( 1/V_{\text{max}} \). Fig. 4B shows a series of such plots for the data in Fig. 4A, assuming \( n \) values of 1, 2, and 3. Inspection of these plots indicates that a value of \( n = 2 \) provides the best fit, although the \( n = 3 \) plot gave a reasonably good fit at all but the two lowest Na\(^+\) concentrations. From the slope of the \( n = 2 \) plot, the \( V_{\text{max}} \) for Na\(^+\)-dependent GSH transport as a function of Na\(^+\) concentration was 7.7 nmol/min/mg of protein at 1 mM GSH. These results suggest that at least 2 Na\(^+\) ions are coupled to the transport of each GSH molecule.

Fukuhara and Turner (27) have suggested that the Garay and Garrahan model (28) has a potential flaw because it does not consider that the transport carriers may be oriented with their Na\(^+\)-binding sites on either side of the membrane. Thus, when Na\(^+\) is added to one side of the membrane, some sites may not be accessible for binding. The model used by Fukuhara and Turner to determine the number, \( n \), of Na\(^+\)-binding sites involves plotting \( V/[\text{Na}^+] \) versus \( V \); the plot is linear with the appropriate value of \( n \). Analysis of the data in Fig. 4A by this model (not shown) did not yield a straight line for integral values of \( n \). A value of \( n = 1.7 \) gave the best fit, indicating the movement of multiple Na\(^+\) ions.

A direct measure of the stoichiometry of Na\(^+\)-GSH cotransport was obtained by comparing the Na\(^+\)-dependent GSH transport rate to the Na\(^+\)-dependent Na\(^+\) transport rate. 1 mM GSH stimulated uptake of NaCl (present at 10 mM) by approximately 15% (Fig. 5A). Although this difference is small relative to total Na\(^+\) uptake, the measurements were paired and the error for the GSH-dependent Na\(^+\) uptake was small. The GSH-dependent Na\(^+\) uptake was linear for approximately 2 min and gave an initial uptake rate of 2.3 ± 0.1 nmol of Na\(^+\)/min/mg of protein (\( n = 3 \)) (Fig. 5E). The initial rate of Na\(^+\)-dependent GSH uptake measured under the same conditions and with the same membrane preparations was 1.1 ± 0.1 nmol of GSH/min/mg of protein (\( n = 5 \)). The ratio of these two transport rates gives a Na\(^+\)/GSH transport stoichiometry of 2.1/1.0, in agreement with the results presented in Fig. 4.

Probenecid, a competitive inhibitor of the renal secretion of a variety of acids (28), inhibits extraction of glutathione by perfused kidney. Therefore, we examined the effect of probenecid on GSH transport in renal basal-lateral membrane vesicles (Fig. 6). GSH uptake in the absence of Na\(^+\) was not affected by probenecid. However, Na\(^+\)-dependent GSH transport was markedly inhibited, with 50% inhibition occurring at 0.1 mM probenecid. Although the molecular basis for the probenecid effect is not clear, it suggests that the acidic nature of the GSH molecule plays a role in the transport process.

Competition experiments between GSH and its constituent amino acids and other γ-glutamyl compounds were performed to determine the specificity of the GSH transport system (Table II). 1 mM GSH was incubated with a 5-fold excess of each of the compounds tested under voltage-clamped conditions in the presence and absence of 100 mM NaCl. None of the test compounds inhibited Na\(^+\)-independent GSH transport. Glycine, cysteine, and glutamate caused significant but small amounts of inhibition of the Na\(^+\)-dependent flux, while cysteinylglycine did not cause significant inhibition. The effect of the γ-glutamyl compounds was more striking with γ-glutamylglutamate causing 59% inhibition. Ophthalmic acid, a GSH analog found in lens tissue which possesses α-aminoisobutyrate substituted for cysteine, produced 70% inhibition.

Because γ-glutamyl compounds inhibited GSH transport but cysteinylglycine did not, it appears that specificity resides in the γ-glutamyl moiety. To determine whether the inhibi-
Renal GSH Transport

In the present work, we have characterized GSH transport in vesicles from the basolateral region of the plasma membrane of rat kidney proximal tubule. This transport exhibited a dependence on Na" and was due to a single system. Enrichment of marker enzymes and the absence of a significant transport activity in purified brush-border membrane vesicles indicated that basolateral membranes were responsible for the observed transport of GSH.

Examination of the effect of AT-125 on γ-glutamyltransferase activity and on GSH transport showed that the two processes have a different sensitivity to the inhibitor thus indicating that they are unrelated. This difference in susceptibility to inhibition can be explained by the action of AT-125 as a nonspecific alkylating agent at high concentrations, in the presence of NaSCN and 75 nmol/min/mg of protein in the presence of 100 mM NaSCN. Thus, Na"-dependent uptake of γ-glutamylglutamate also occurs in these membranes and, therefore, the inhibition of GSH transport may be due to competition for the same carrier.

The presence of an active thiol oxidase on the renal basolateral membrane raises the question as to whether oxidized glutathione (GSSG) is also transported. A time course of GSSG uptake in the presence and absence of 100 mM NaSCN demonstrated that a Na" gradient can drive the transient accumulation of GSSG above equilibrium (Fig. 7). The initial rate of uptake of 0.5 mM GSSG was 5.6-fold greater in the presence of 100 mM NaSCN (2.11 nmol/min/mg of protein) than in the absence of Na" (0.38 nmol/min/mg of protein). A comparison of the rate of uptake of 0.5 mM GSSG with that of 1 mM GSH, a concentration equal in GSH equivalents, indicated that GSSG is transported at only 20% of the rate of GSH (cf. Table I and Ref. 13).

**DISCUSSION**

![Fig. 6. Inhibition of GSH uptake by probenecid. The initial rate of uptake of 1 mM GSH in the presence and absence of 100 mM NaSCN was measured by 1 min of incubation in the presence of the indicated concentrations of probenecid as described under “Experimental Procedures.” The intravesicular solution contained 250 mM sucrose and 10 mM Tris-Hepes, pH 7.6. The extravesicular solution was the same as the intravesicular solution for Na"-free incubations and contained 100 mM NaSCN, 50 mM sucrose, and 10 mM Tris-Hepes, pH 7.6, for Na"-containing incubations. Results are shown corrected for Na"-independent GSH uptake and are the mean ± S.E. of 3 preparations, each performed in duplicate.](Image)

**TABLE II**

| Addition         | Na"-dependent GSH transport | Inhibition |
|------------------|-------------------------------|------------|
|                  | nmol/min/mg protein           | %          |
| None             | 5.40 ± 0.34                   | 0          |
| Glycine          | 4.68 ± 0.31                   | 13.3*      |
| Cysteine         | 4.76 ± 0.29                   | 11.9*      |
| Glutamate        | 4.07 ± 0.26                   | 24.6*      |
| Cys-Gly          | 6.06 ± 0.26                   | 5.9        |
| γ-Glu-Glu        | 2.21 ± 0.14                   | 59.1*      |
| γ-Glu-α-AIB-Gly  | 1.62 ± 0.16                   | 70.0*      |

* Statistically significant difference from control (no addition) (p < 0.05).

α-AIB, α-alminoisobutyrate.

In the present work, we have characterized GSH transport in vesicles from the basolateral region of the plasma membrane of rat kidney proximal tubule. This transport exhibited a dependence on Na" and was due to a single system. Enrichment of marker enzymes and the absence of a significant transport activity in purified brush-border membrane vesicles indicated that basolateral membranes were responsible for the observed transport of GSH.

Examination of the effect of AT-125 on γ-glutamyltransferase activity and on GSH transport showed that the two processes have a different sensitivity to the inhibitor thus indicating that they are unrelated. This difference in susceptibility to inhibition can be explained by the action of AT-125 as a nonspecific alkylating agent at high concentrations,
whereas at low concentrations it is a specific glutamine antagonist (22).

Uptake of GSH across the renal basolateral membrane in vivo requires an input of energy because an approximately 200-fold concentration gradient for GSH exists between renal cells and plasma. For GSH transport coupled to Na⁺, thermodynamics requires that ΔGSH < zNaΔNNa, where ΔG is the electrochemical potential and z is the net charge (zNa = +1, zGSH = −1), ω and a are the extracellular (plasma) and intracellular activities (approximated by concentrations; GSH = 25 μM (30), GSH = 5 mM; Na⁺ = 140 mM, Na⁺ = 20 mM (31)), R is the gas constant, F is the Faraday constant, and T is the absolute temperature (T = 310 K), was used to calculate the equilibrium potentials of GSH and Na⁺ across the basolateral membrane (EGSH = +141.5 mV, ENa⁺ = +52.0 mV). With these values and a membrane potential across the basolateral membrane of −60 mV (31), ΔGSH = +201.5 mV and ΔNNa = +112.0 mV. Therefore, ΔG < 1.8 for Na⁺-GSH cotransport to be thermodynamically feasible.

Several lines of evidence in this study are in agreement with the thermodynamic calculations and indicate an electrogenic transport process. GSH transport was faster with NaSCN and lower with Na₂SO₄ relative to NaCl; depolarization of the basal-lateral membrane inhibited and hyperpolarization stimulated transport; analysis of the Na⁺ concentration dependence and comparison of GSH-dependent Na⁺ transport and Na⁺-dependent GSH transport rates indicated a Na⁺:GSH stoichiometry of 2:1.

The stoichiometry of the red blood cell (Na⁺ + K⁺)-stimulated ATPase is influenced by the cytoplasmic Na⁺ concentration (32). For technical reasons, a Na⁺ concentration of 10 mM was used in our measurement of the Na⁺:GSH transport stoichiometry so that a large enough increase in Na⁺ uptake due to GSH could be observed. Consequently, if a different concentration of Na⁺ was found, a different coupling ratio may have been observed. The results presented here do not exclude the possibility that under certain conditions the stoichiometry may be higher than 2:1 but clearly establish that it is not 1:1.

Recent in vivo experiments provide evidence for transport of γ-glutamyl compounds in the kidney (33-35). However, these studies did not provide evidence for GSH transport although we have previously shown that transport of GSH occurs in renal basolateral membrane vesicles. The current studies show that other γ-glutamyl compounds (GSSG and γ-glutamylglutamate) are also transported into basolateral membrane vesicles by Na⁺-dependent processes. Inhibition of GSH transport by γ-glutamyl amino acids and the lack of inhibition by cysteinylglycine showed that the most important structural feature for transport is the γ-glutamyl group. Therefore, the GSH transport system described here may function as a general transport system for γ-glutamyl compounds.

Intergen metabolism of glutathione has been the focus of several recent studies (1-8, 12, 30, 33). The kidney has been the center of this focus because it contains high activities of glutathione-metabolizing enzymes. In addition to the brush-border enzymes, γ-glutamyltransferase and cysteinylglycine dipeptidase, which degrade glutathione to its constituent amino acids (9-11), a thiol oxidase activity is present on the basolateral membrane (7, 15, 22, 23). Although GSH transport occurs under anaerobic conditions, this does not exclude the possibility that the oxidase is in some way functionally related to the transport system. Since GSSG transport across the basolateral membrane is slow relative to GSH, both in vesicles and in intact kidney (2, 30), the oxidase may regulate transport by oxidizing GSH to GSSG and decreasing renal extraction of plasma glutathione.

The characteristics of the transport process described in the present work are found in an in vitro system and may or may not apply in vivo. However, if this GSH transport system functions in vivo, it could explain the mechanism by which the kidney effectively removes most of the glutathione passing through the renal circulation. Future studies exploring the relationship between this transport system and the other renal enzymes acting on glutathione will provide a more complete understanding of the interaction of different intracellular and extracellular compartments in this metabolism.

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