Characteristics of Glutathione Biosynthesis by Freshly Isolated Rat Kidney Cells*

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Suspensions of freshly isolated rat kidney cells were used to study glutathione biosynthesis and utilization for amino acid translocation. Incubation of cells isolated from diethylmaleate-perfused kidneys in the presence of glutamate, glycine, and cystine led to replenishment of cellular glutathione and to accumulation of glutathione disulfides in the medium. Cystine could be replaced by cysteine (<0.5 mM) but not by methionine plus serine indicating that the cystathionine pathway was insufficient to support optimal glutathione biosynthesis. Uptake of cystine was associated with enhanced intracellular concentration of acid-soluble thiols independently of whether glutathione biosynthesis was inhibited or not. Incubation of control cells in an amino acid-free medium resulted in a slow decrease in cellular glutathione level which was accentuated in the presence of the inhibitor of glutathione biosynthesis, methionine sulfoximine. Glutathione loss was markedly accelerated by addition of various amino acids or dipeptides to the medium (e.g. glycylglycine, methionine, cysteine, cystine and glutamate); this effect was abolished by serine + borate, a potent inhibitor of y-glutamyltransferase. The uptake of several amino acids by cells isolated from diethylmaleate-perfused kidneys was inhibited by serine + borate; the uptake of methionine, cysteine, and cystine was markedly affected whereas that of glycine was not. The inhibition by serine + borate of cellular uptake of cystine was correlated to a similar inhibitory effect on glutathione biosynthesis. The results indicate major differences in the requirements for glutathione biosynthesis by isolated kidney and liver cells and provide strong support for the functioning of the y-glutamyl cycle in renal cells.

The tripeptide glutathione is present at high concentrations in a variety of mammalian cells, notably hepatocytes, erythrocytes, and the epithelial cells in the lens of the eye and the proximal convoluted tubule of the nephron (cf. Ref. 1 for review). Whereas intracellular glutathione is present mainly in the reduced form, the reverse is true for the relatively low levels of glutathione that exist extracellularly in plasma, lymph, bile, and interstitial fluid, where glutathione disulfide predominates over GSH. Excretion of both GSH and GSSG from liver is probably a major source of plasma glutathione (2-4). Recent studies have shown that extracellular GSH is rapidly oxidized to GSSG by an enzyme located in the plasma membrane of kidney epithelial cells (5, 6). The degradation of extracellular GSSG, in turn, involves y-glutamyltransferase (EC 2.3.2.2) and cysteinylglycine dipeptidase (EC 3.4.13.6), two enzymes which are also located in the plasma membrane of certain cells, e.g. kidney and intestinal epithelial cells (1, 6, 7).

In addition to its general functions as a potent oxidation-reduction system and cysteine store intracellular glutathione plays an important role as a defense mechanism against toxic reactive metabolites of oxygen and various drugs (cf. Ref. 8). Thus GSH serves as a reductant in peroxide metabolism by glutathione peroxidase (EC 1.11.1.9), a cofactor in the degradation of aldheyde and a-ketoaldehyde metabolism by cytosolic formylaldehyde dehydrogenase (EC 1.2.1.1), and a nucleophile in the formation of various drug conjugates; the latter reaction may occur spontaneously or by way of glutathione transferases (2.5.1.18; cf. Ref. 9). The liver is the main site for the detoxifying functions of glutathione, and in some recent publications we have reported on glutathione turnover and utilization during drug metabolism by isolated rat hepatocytes (10-13). In the course of these studies it became evident that drug conjugates with glutathione formed in rat liver are further metabolized to the corresponding cysteine and N-acetyl-cysteine derivatives preferentially in the kidney and small intestine (cf. Ref. 1, 6, and 14); the first reaction in this sequence is catalyzed by y-glutamyltranspeptidase (15). In support of this hypothesis paracetamol (N-acetyl-p-aminophenol) was found to yield the glutathione conjugate, in addition to glucuronide and sulfate conjugates, when incubated with isolated rat hepatocytes, whereas exposure of isolated rat kidney cells to this drug also resulted in the formation of both the cysteine and N-acetyl-cysteine conjugates (16). Under our experimental conditions the metabolism of the exogenously added glutathione conjugate of paracetamol by isolated renal cells was not associated with any observable effect on intracellular glutathione level (6).

The concentration of glutathione in proximal tubular cells of the kidney is in the millimolar range, decreasing distally in the nephron (17). A rapid turnover of kidney glutathione has been demonstrated and taken to indicate a highly dynamic role of glutathione in this tissue (18). In a series of elegant investigations Meister and collaborators have obtained evidence for a linkage between the turnover of renal glutathione and the transport of certain amino acids and peptides across the tubular cell membrane (4, 18-20). According to their theory a major function of renal glutathione is to act as a donor of its y-glutamyl group to extracellular acceptor amino acids or peptides, thus forming y-glutamyl amino acids which are subsequently translocated into the cell. The transfer of the y-glutamyl moiety is mediated by y-glutamyltransferase which is located in the brush border of proximal convoluted tubular cells and exhibits a much higher activity in kidney than in any other mammalian tissue (18).

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Glutathione Synthesis by Renal Cells

In view of these findings it appeared important to pursue our investigation of the metabolism of exogenous glutathione and glutathione conjugates by isolated rat kidney cells. Thus, the present study has been focused on the biosynthesis and utilization of intracellular glutathione under various experimental conditions. The results obtained indicate marked differences in requirement for precursor amino acids during glutathione biosynthesis by isolated kidney cells, as compared to hepatocytes, and support the previous assumption of a direct function of kidney glutathione in the translocation of certain amino acids through the cell membrane.

**MATERIALS AND METHODS**

Chemicals—Collagenase (Grade II), GSH, and GSSG were obtained from Boehringer/Mannheim GmbH, Mannheim, Germany. Radiolabeled amino acids, \(^{35}\)S-methionine (865 Ci/mmol, radiochemical purity 95%), \(^{14}\)C-glutamate (325 Ci/mmol, radiochemical purity 96%), and \(^{14}\)C-glycine (600 Ci/mmol, radiochemical purity 94%) were obtained from the Radiochemical Center, Amersham, Bucks, England. Radio-labeled cysteine was obtained by leaving \(^{35}\)S-cysteine to auto-oxidize under room temperature for 2 h. All other chemicals were of analytical grade and obtained from local commercial sources. The L form of the various amino acids was used in all experiments.

**Cell Preparation**—Isolated kidney cells were prepared from male Sprague-Dawley rats (weighing 180 to 230 g, fed ad libitum) as described by Jones et al. (16). To obtain cells partly depleted of GSH the kidneys were perfused for 6 min with a calcium-free Hank’s solution containing 4 mM diethylmaleate, diluted in dimethyl sulfoxide 1:100, before adding collagenase to the perfusate and proceeding with the cell isolation procedure as described. Cells were counted in a Bürker chamber in the presence of 0.2% trypan blue; routinely ~90% of the freshly isolated cells excluded the dye.

The cytosolic fraction of a 20% (w/v) rat kidney homogenate in 50 mM sodium phosphate buffer, pH 7.4, containing 5 mM EDTA, was obtained by centrifugation at 105,000 g for 1 h. The isolated cytosolic fraction was dialyzed against 0.15 M KCl overnight. The kidneys were perfused for 6 min with a calcium-free Hank’s solution containing 4 mM diethylmaleate, diluted in dimethyl sulfoxide 1:100, before adding collagenase to the perfusate and proceeding with the cell isolation procedure as described. Cells were counted in a Bürker chamber in the presence of 0.2% trypan blue; routinely ~90% of the freshly isolated cells excluded the dye.

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**Experimental Procedure**—Cell incubations were performed in Krebs-Henseleit buffer, pH 7.4, containing 25 mM Hepes at 37°C under an atmosphere of 95% O₂, 5% CO₂ in rotating round-bottom flasks (21). In experiments where \(\gamma\)-glutamyltransferase was to be inhibited, L-serine and boric acid were added to the incubate, each at 20 mM concentration (22). To inhibit glutathione biosynthesis, methionine sulfoximine (a potent inhibitor of \(\gamma\)-glutamylcysteine synthetase (23)) was added to the incubate at a concentration of 5 mM.

**Assays**—For assay of GSH and GSSG, samples removed from the incubation flask were immediately deproteinized by treatment with either trichloroacetic acid (6.5%) or phosphoric acid (5%) followed by centrifugation. GSH was estimated by the colorimetric method of Saville (24), which measures total acid-soluble thiols, or the fluorometric method of Hissin and Hilf (25). Glutathione disulfides were estimated fluorometrically.

Amino acid uptake was determined by incubation of isolated kidney cells with radiolabeled amino acid, separation of the cells from the medium by rapid Millipore (100 mesh) filtration, and counting cellular radioactivity in a Beckman LS 100 liquid scintillation counter.

**RESULTS**

Suspensions of freshly isolated kidney cells obtained by organ perfusion in the presence of collagenase were used as experimental model in this study, and some characteristics of such preparations are given in Table I. Trypan blue and NADH penetration frequencies (21) were used routinely as viability tests, and perfuision of the kidneys with diethylmaleate was employed to lower the concentration of glutathione in the freshly isolated cells (ref. Ref. 26). The selection of the latter procedure was based on a systematic investigation of various means to affect kidney glutathione level including starvation, injection of diethylmaleate into the animals prior to cell isolation, and preincubation of the isolated cells with diethylmaleate. As shown in Table I 1h preperfusion with diethylmaleate decreased the GSH level of the isolated cells by two-thirds without other apparent effects on the viability of the cell suspension. Both control cells and cells isolated from diethylmaleate-perfused kidneys could be incubated in an amino acid-free medium for up to 2 h with little decrease in viability (trypan blue exclusion frequency decreased from 85 to 90% to 75 to 80%); incubation for 2 h in a medium supplemented with amino acids resulted in no loss of cell viability.

When cells isolated from control kidneys were incubated in an amino acid-free medium, they lost about 7% of their GSH content during the first hour (Fig. 1). When methionine sulfoximine was also present in the medium, the decrease in GSH level was about 20%, indicating continuous GSH resynthesis from endogenous precursors during cell incubation in the presence of diethylmaleate.

**TABLE I**

Characteristics of isolated kidney cell preparations from control and diethylmaleate-perfused rat kidneys

<table>
<thead>
<tr>
<th></th>
<th><strong>Control cells</strong>*</th>
<th><strong>Cells from diethylmaleate-perfused kidneys</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Yield of cells per preparation using kidneys from two rats</td>
<td>32.5 ± 6.4 × 10⁶ cells (22)</td>
<td>33.8 ± 5.0 × 10⁶ cells (12)</td>
</tr>
<tr>
<td>Packed volume per 10⁶ cells</td>
<td>14.3 ± 1.2 μl (16)</td>
<td>14.0 ± 1.6 μl (4)</td>
</tr>
<tr>
<td>Trypan blue exclusion frequency</td>
<td>86.1 ± 1.4% (18)</td>
<td>90.2 ± 3.5% (12)</td>
</tr>
<tr>
<td>NADH penetration frequency</td>
<td>15.4 ± 1.4% (12)</td>
<td>18.2 ± 1.8% (3)</td>
</tr>
<tr>
<td>GSH content</td>
<td>28.9 ± 2.7 nmol/10⁶ cells (16)</td>
<td>9.5 ± 2.2 nmol/10⁶ cells (10)</td>
</tr>
</tbody>
</table>

* Data from Ref. 16.

**FIG. 1.** GSH content in isolated kidney cells and the effect of incubation in the absence or presence of various amino acids. Cells (1 × 10⁶/ml) were incubated in Krebs-Henseleit buffer, pH 7.4 (containing 25 mM Hepes) in rotating round-bottom flasks. Amino acids known to be \(\gamma\)-glutamyl acceptors in the transpeptidation reaction were added to individual flasks along with the inhibitor of \(\gamma\)-glutamylcysteine synthetase, methionine sulfoximine. O, without \(\gamma\)-glutamyl acceptor, without methionine sulfoximine; O, without \(\gamma\)-glutamyl acceptor, plus methionine sulfoximine, 5 mM; V, plus methionine, 2 mM; plus methionine sulfoximine, 5 mM; plus cystine, 0.2 mM, plus methionine sulfoximine, 5 mM; plus glycine, 2 mM; plus methionine sulfoximine, 5 mM; plus serine-borate, 20 mM. One experiment typical of three.
absence of the inhibitor. Addition to the incubate of amino acids or dipeptides, known as potent acceptors of the \(\gamma\)-glutamyl moiety of glutathione in the \(\gamma\)-glutamyl transpeptidation reaction (27), produced a marked decrease in cellular GSH level, in analogy with previous findings by Griffith et al. in vitro experiments (20). Glycylglycine had the most pronounced effect also in our system (Fig. 1). Inhibition of \(\gamma\)-glutamyltransferase by serine-borate abolished the acceptor-mediated decrease in cellular glutathione concentration completely (shown for methionine in Fig. 1). Inhibition was observed only in the presence of the combination of serine and borate, since neither serine nor borate had any inhibitory effect when added separately at 20 mM concentrations. GSH loss, observed in the presence of methionine sulfoximine and absence of extracellular \(\gamma\)-glutamyl acceptor, or with serine-borate added, probably reflected the amount of GSH lost by secretion and as a result of leakage and cell death.

Cells isolated from kidneys perfused with diethylmaleate contained only 30% of the their normal glutathione level. During incubation in amino acid-free medium, they were able to resynthesize glutathione at a net rate of about 5 nmol/10^6 cells/h. Addition of various amino acids to the incubation medium affected the rate of GSH resynthesis as shown in Table II. In contrast to what has been observed previously with hepatocytes (12, 28), methionine was unable to support reaccumulation of GSH in the partly depleted cells. On the contrary, this amino acid seemed to diminish the net rate of GSH replenishment, which was in accordance with its effect on glutathione concentration in control cells (cf. Fig. 1). In contrast to methionine, addition of cystine to the incubate caused an increase in intracellular glutathione, despite the GSH-lowering effect of cystine seen in control cells when GSH resynthesis was blocked by methionine sulfoximine. As further shown in Table II, the presence in the medium of a combination of glycine, glutamate, and cysteine was required for optimal GSH reaccumulation by cells isolated from diethylmaleate-perfused kidneys (occurring at a rate of \(\sim 20\) nmol/10^6 cells/h). Cystine could be replaced by cysteine at low concentrations, whereas higher concentrations of cysteine decreased cellular GSH reaccumulation rate. Analysis of the cellular GSH reaccumulation rate as a function of time suggested that the effect of exogenous cysteine was not mediated by cysteine formed by auto-oxidation (see under "Discussion").

Addition of methionine or glycylglycine to the incubate counteracted the replenishment of cellular glutathione obtained with glycine, glutamate, and cysteine, again suggesting utilization of intracellular GSH as a \(\gamma\)-glutamyl donor to extracellular acceptor amino acids or dipeptides.

Rates of uptake of amino acids were studied with both control cells and cells isolated from diethylmaleate-perfused kidneys; no apparent differences in the rates of translocation of the amino acids used were noted between the two models. As shown in Fig. 2 the rate of cysteine uptake by cells isolated from diethylmaleate-perfused kidneys was fastest (\(\sim 40\) nmol/10^6 cells/30 min) and that of cystine slowest (\(\sim 12\) nmol/10^6 cells/30 min). Inhibition of \(\gamma\)-glutamyltransferase by serine-borate lowered the rates of uptake of methionine, glutamate, cysteine, and cystine (the latter was inhibited by 50%), whereas glycine uptake was unaffected by the inhibitor (Fig. 2). Neither serine alone, nor borate alone, affected amino acid uptake to any observable extent.

Since cysteine uptake by the cells isolated from diethylmaleate-perfused kidneys occurred more slowly than that of the

Table II

Resynthesis of glutathione by cells isolated from kidneys perfused with 4 \(\mu\)M diethylmaleate to lower intracellular glutathione concentration

<table>
<thead>
<tr>
<th>Amino acid in medium</th>
<th>Cellular GSH concentration</th>
<th>Number of experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 min</td>
<td>30 min</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>9.5 ± 2.4</td>
</tr>
<tr>
<td>Gly</td>
<td></td>
<td>9.2 ± 0.8</td>
</tr>
<tr>
<td>Glu</td>
<td></td>
<td>9.7 ± 0.7</td>
</tr>
<tr>
<td>Cys</td>
<td></td>
<td>8.9 ± 0.7</td>
</tr>
<tr>
<td>Cys (0.4 mM)</td>
<td></td>
<td>10.8</td>
</tr>
<tr>
<td>CysCys (0.2 mM)</td>
<td></td>
<td>10.0 ± 0.5</td>
</tr>
<tr>
<td>Met</td>
<td></td>
<td>8.3 ± 1.3</td>
</tr>
<tr>
<td>Met + Ser</td>
<td></td>
<td>8.6 ± 1.3</td>
</tr>
<tr>
<td>Gly + Glu</td>
<td></td>
<td>9.9 ± 0.6</td>
</tr>
<tr>
<td>Gly + Glu + Cys</td>
<td></td>
<td>8.9 ± 1.4</td>
</tr>
<tr>
<td>Gly + Glu + Cys (0.4 mM)</td>
<td></td>
<td>9.3</td>
</tr>
<tr>
<td>Gly + Glu + Met</td>
<td></td>
<td>8.9 ± 0.9</td>
</tr>
<tr>
<td>Gly + Glu + Met + Ser</td>
<td></td>
<td>8.7 ± 1.0</td>
</tr>
<tr>
<td>Gly + Glu + Cys</td>
<td></td>
<td>9.8 ± 1.5</td>
</tr>
<tr>
<td>Gly + Glu + Cys (0.2 mM)</td>
<td></td>
<td>9.6 ± 0.9</td>
</tr>
<tr>
<td>Gly + Glu + Met</td>
<td></td>
<td>9.3</td>
</tr>
<tr>
<td>Gly + Glu + Met + Ser</td>
<td></td>
<td>9.6</td>
</tr>
</tbody>
</table>

* Amino acid concentration was 1 mM unless otherwise stated.
* Incubation time.
* Mean ± range.

Fig. 2. Uptake of radiolabeled amino acids into isolated kidney cells incubated in the absence (○) or presence (●) of the \(\gamma\)-glutamyltransferase inhibitor, serine-borate (20 mM). In each experiment shown, one labeled precursor for GSH synthesis was present, the others were present unlabeled. The labeled amino acids were in each case: A, [\(^{[35}S\)]methionine; B, [\(^{14}C\)]glutamate; C, [\(^{14}C\)]cysteine; D, [\(^{[35}S\)]cysteine (●) or [\(^{[35}S\)]cystine (○). Amino acid concentrations in all cases were 1 mM, except for cysteine, which was present in 0.2 mM. Results are expressed as nanomoles of labeled amino acid taken up per 10^6 cells. Mean and range of four experiments.
other glutathione precursors (glutamate and glycine), it was of interest to compare the rates of cystine uptake and GSH reaccumulation stoichiometrically. As shown in Fig. 3 the rates were similar both in the absence and presence of serine-borate. Thus, it appears that cystine uptake was a rate-limiting factor for renal glutathione synthesis under our experimental conditions. Since the concentration of cystine in plasma is similar (normally 0.2 to 0.3 mM (29)), the rate of GSH accumulation observed with our model may reflect the synthetic capacity in vivo fairly well.

Incubation in a medium supplemented with the three precursor amino acids needed for optimal GSH reaccumulation by cells isolated from diethylmaleate-perfused kidneys led to a net increase in cellular GSH level over that observed with freshly isolated cells (i.e. from ~30 to ~36 nmol/10^6 cells). This increase was partly inhibited by serine-borate (but not by serine or borate alone), indicating the involvement of \( \gamma \)-glutamyltransferase in cystine (and glutamate) uptake by the cells. However, the level of glutathione retained inside the cells did not reflect the total rate of synthesis, since there was also a steady increase in glutathione in the incubation medium. This increase did not occur in the form of GSH, but rather as glutathione disulfides; i.e. GSSG or mixed disulfide (GS-Cys), or both. Since glutathione disulfides are substrates of \( \gamma \)-glutamyltransferase, and thus readily broken down by the cells when present in the medium, it was necessary to add serine-borate to the incubate to obtain a quantitative estimate of their rate of formation. On the other hand, this inhibitor affected the uptake of cystine and glutamate by the cells and thereby GSH synthesis (cf. Fig. 2). Thus, the data given in Fig. 4 must be regarded as minimum values for the formation of glutathione disulfides during incubation of kidney cells.

The low amount of glutathione disulfides recovered in the medium was similar for control and partly glutathione-depleted kidney cells incubated in the absence of amino acids indicating a low rate of synthesis and little secretion of glutathione by the cells under these conditions. In a medium supplemented with amino acids the rate of extracellular disulfide accumulation was much higher (Fig. 4). Especially with control cells secretion of glutathione to the medium was considerable. If we compensate for the serine-borate effect on GSH synthesis, we can get a rough quantitative estimate of the total rate of glutathione synthesis by the isolated kidney cells in terms of GS equivalents: control cells, 7 nmol (intracellular) + 15 nmol (extracellular) = 22 nmol/10^6 cells/h; partly depleted cells, 20 nmol (intracellular) + 9 nmol (extracellular) = 29 nmol/10^6 cells/h.

The ability of extracellular cystine and relative inefficiency of cysteine to support replenishment of the glutathione store in cells isolated from diethylmaleate-perfused kidneys was contrary to our previous findings with isolated hepatocytes (12, 28) and thus unexpected. Table II and Fig. 5 show that...
the effect of cysteine in this respect was in fact concentration dependent. At a concentration in the medium of 0.4 mM, cysteine was almost as efficient as cystine in supporting accumulation of intracellular glutathione. At a 1 mM concentration of cysteine, however, the increase in intracellular GSH was much lower. In contrast, the accumulation of glutathione disulfides in the medium was markedly enhanced in the presence of the high concentration of cysteine, suggesting that extracellular cysteine also at this concentration did in fact support glutathione biosynthesis by the isolated cells, but that this effect was masked by an increased secretion of glutathione (presumably as GSSG) to the medium.

Fig. 6 shows the effect on intracellular thiol and GSH concentrations of incubating cells isolated from diethylmaleate-perfused kidneys with cysteine or cystine in the absence or presence of the inhibitor of glutathione biosynthesis, methionine sulfoximine. The latter did not inhibit the accumulation of intracellular acid-soluble thiols with either cysteine or cystine present in the medium, indicating that the uptake of cysteine was associated with the conversion of the disulfide to thiol, independent of whether it was incorporated into glutathione or not. The rate of accumulation of intracellular thiol observed with cystine in the medium was somewhat lower (9 nmol/10^6 cells/h) than would be expected from the results of the assay of cysteine uptake by cells incubated under conditions of glutathione biosynthesis, suggesting that glutamate or glycine, or both, may stimulate cysteine uptake or, more probably, that intracellularly accumulated acid-soluble thiol (most probably cysteine) was subject to further metabolism also under conditions of inhibited GSH biosynthesis.

Thus it appears that cystine-supported glutathione biosynthesis by isolated kidney cells was preceded by reduction of cysteine, as indicated by enhanced intracellular thiol concentration also under conditions of inhibited glutathione synthesis. As suggested by the results presented in Fig. 7, this reaction may involve the cytosolic thioltransferase (glutathione disulfide oxidoreductase) as well as GSSG reductase (EC 1.6.4.2) (30). Thus, incubation of the dialyzed, postmicrosomal fraction of a rat kidney homogenate with cystine resulted in production of acid-soluble thiol, only when the incubate was supplemented with both GSH and reduced pyridine nucleotide; NADPH was much more efficient in this respect than NADH. The mixed disulfide of GSH and cystine, which may also form spontaneously, is a known substrate of thioltransferase which catalyzes its conversion to cysteine and GSSG; the subsequent reduction of the latter is mediated by GSSG reductase (30).

**DISCUSSION**

During recent years suspensions of freshly isolated cells have become an experimental tool frequently used in various metabolic investigations. Isolated hepatocytes have proven to be a valuable model system for studies of drug biotransformation including various conjugation reactions (31) and have also been used to investigate the biosynthesis of different cofactors utilized in these reactions, notably glutathione (12, 28). More recently an experimental model consisting of freshly isolated rat kidney cells has been developed and employed in similar studies (5, 6, 16). The viability of the isolated kidney cells is usually somewhat lower than that of hepatocytes but still sufficient for short-term (<5 h) incubation. As judged by light microscopy the suspensions of isolated rat kidney cells, as obtained by the technique used in this study, consist of two different cell populations, one of them probably being of proximal tubular origin and accounting for 70 to 80% of the total cell yield.

Freshly isolated renal cells contained a high concentration of glutathione which decreased slowly during incubation in an amino acid-deficient medium (cf. Fig. 1). This decrease was accelerated in the presence of methionine sulfoximine, an inhibitor of \( \gamma \)-glutamylcysteine synthetase (23), suggesting a continuous resynthesis of glutathione from endogenous precursors in the absence of the inhibitor. Under these conditions very little GSH or GSSG was seen to accumulate in the incubation medium. However, we cannot exclude that a certain amount of glutathione was continuously released from the cells, but subsequently metabolized extracellularly by \( \gamma \)-glutamyltransferase and dipeptidase, and thus escaped detection.

In the presence of the proper precursor amino acids cells isolated from diethylmaleate-perfused rat kidneys were capable of actively resynthesizing glutathione. This resulted in replenishment of the intracellular glutathione store and was also associated with an accumulation of glutathione disulfides in the medium; the latter was enhanced by increased cysteine concentration and was also more pronounced when incubation...
was performed with cells isolated from control kidneys (cf. Figs. 4 and 5). Since extracellular glutathione disulfides are rapidly broken down by γ-glutamyltransferase present in the plasma membrane, it was difficult to assay the total capacity for glutathione biosynthesis of the isolated kidney cells; 20 to 30 nmol/10^6 cells/h is a rough estimate of this capacity and similar to the rate of glutathione biosynthesis observed with isolated hepatocytes (12).

The finding that glutathione accumulation in isolated renal cells partly depleted of GSH requires the presence in the medium of a combination of glutamate, glycine, and cystine was unexpected (cf. Table II). This observation is at variance with our results with isolated hepatocytes which indicate that also in the absence of added glutamate and glycine, exogenous cysteine, or methionine, is capable of supporting cellular glutathione biosynthesis during short-term incubation (12, 28); cystine is not taken up, or taken up very slowly, by isolated liver cells. It thus appears that both the access to intracellular glutamate and glycine and the capability to produce cysteine for glutathione biosynthesis from methionine by way of the cystathionine pathway, are lesser in isolated kidney cells as compared to hepatocytes. Results suggesting that cysteine generation by the cystathionine pathway is insufficient as support of glutathione biosynthesis by kidney homogenate have been reported previously by Krebs et al. (32).

The observation that extracellular cysteine is efficiently utilized for replenishment of the glutathione pool by partly GSH-depleted renal cells was surprising, since exogenous cysteine does not support glutathione biosynthesis by hepatocytes (12). Apparently, uptake of cystine by kidney cells occurs much more readily and is associated with reduction of the disulfide to the thiol form, independently of whether it is subsequently incorporated into glutathione or not (cf. Fig. 6). Most probably the reduction of cystine involves a sequence of reactions catalyzed by cystosolic thioredoxinase (Reactions 1 and 2) and glutathione reductase (Reaction 3) and initiated by the formation of a mixed disulfide of cystine and intracellular glutathione (Reaction 1) (cf. Ref. 30); the latter reaction may also occur spontaneously.

\[
\text{CysCys + GSH} \leftrightarrow \text{Cys + GS-Cys} \quad (1)
\]

\[
\text{GS-Cys + GSH} \leftrightarrow \text{Cys + GSSG} \quad (2)
\]

\[
\text{GSSG + NADPH + H}^+ \rightarrow \text{2GSH + NADP}^+ \quad (3)
\]

Thus, the combined functions of cystosolic thioredoxinase and GSSG reductase seem to offer a potent mechanism for the reduction of cystine by renal cells. The possible existence of other pathways for reduction of cystine in kidney remains, however, to be investigated.

The inability of extracellular cysteine at high concentration (1 mM) to support replenishment of the glutathione store of cells isolated from diethyldiamine-perfused kidneys appears to have a trivial explanation. Exogenous cysteine is readily auto-oxidized to yield cystine and hydrogen peroxide (33); H₂O₂ is metabolized by intracellular catalase and glutathione peroxidase. As a result of the latter reaction there is, at least with liver cells (13), enhanced secretion of GSSG which accumulates in the medium. In the present investigation we did observe increased concentrations of extracellular glutathione disulfides when kidney cells were incubated with increasing concentrations of cystine (cf. Fig. 5), suggesting that extracellular cysteine does in fact support glutathione biosynthesis by renal cells, but that this effect is masked by the utilization of GSH for metabolism of H₂O₂ resulting from auto-oxidation of exogenous cysteine. A similar mechanism has previously been proposed to explain GSH depletion in isolated hepatocytes caused by high concentrations of cysteine in the medium (32). Whether this mechanism was the only reason for the inefficiency of cysteine at high concentration to support replenishment of GSH in the partly depleted renal cells, or whether additional effects such as uncompensated utilization of intracellular GSH for cysteine uptake (see below) may have been contributory, is difficult to decide since the metabolism of secreted glutathione disulfides by γ-glutamyltransferase prevents exact calculation of the total capacity of the cells for glutathione biosynthesis. In any case the effect of relatively high concentrations of exogenous cysteine on renal glutathione biosynthesis is of limited physiological importance, since cysteine occurs in plasma predominantly in the oxidized form.

The results of the present investigation provide strong support for the function of glutathione in amino acid uptake in renal cells. However, the details in the uptake process, as proposed in the γ-glutamyl cycle (34), have not been firmly established. Under conditions of inhibited glutathione biosynthesis, the slow decrease in intracellular GSH level was markedly accelerated in the presence of various acceptor amino acids and dipeptides in the medium, and this effect was abolished by addition of the inhibitor of γ-glutamyltransferase, serine-borate (cf. Fig. 1). Thus, our experiments with isolated kidney cells confirm previous findings in vitro (20) and with kidney slices (35) in regard to the effect of exogenous γ-glutamyl acceptors on renal glutathione level. Also in our system glycylglycine was most efficient in this respect, leading to a 50% decrease in cellular GSH concentration after incubation of the kidney cells for 1 h.

The observed inhibition of amino acid uptake by isolated renal cells by serine-borate, which was correlated to a lowering effect of the same amino acids on intracellular GSH level under conditions of inhibited glutathione biosynthesis, indicates that γ-glutamyltransferase is involved in glutathione-linked amino acid translocation. The selective effect of serine-borate on this enzyme is supported by our finding that this agent caused no similar inhibition of amino acid uptake by isolated hepatocytes, which virtually lack γ-glutamyltransferase activity. The effect of serine-borate on the rate of amino acid uptake in kidney cells differed between the various amino acids; the translocation of methionine, cystine, and cysteine was more sensitive to serine-borate than that of glutamate, whereas there was no apparent inhibition of glycine uptake (cf. Fig. 3). In no instance did the inhibition by serine-borate exceed 50% of the basal rate of amino acid translocation. Since serine-borate is a very potent inhibitor of γ-glutamyltransferase, causing almost complete inhibition of this activity as tested with both physiological and artificial substrates (6, 36), it seems justified to propose that the effect of this inhibitor reflects the quantitative role of GSH-linked amino acid translocation in overall amino acid uptake by renal cells.

Whether intracellular glutathione concentration may become rate limiting for renal amino acid uptake under extreme conditions remains to be elucidated. In our experiments there was no observable difference between the rates of amino acid uptake by cells isolated from control (~30 nmol of GSH/10^6 cells) and diethyldiamine-perfused kidneys (~10 nmol of GSH/10^6 cells). However, in certain intoxications renal GSH level may decrease even further, and this may affect amino acid uptake. Conversely, the utilization of intracellular GSH for amino acid translocation may contribute to glutathione depletion under such extreme conditions. This effect is probably most pronounced in the case of amino acids which utilize intracellular GSH for translocation but cannot serve as renal glutathione precursors. In view of the findings of Griffith et al. (20) one may, for example, question the beneficial effect on the kidney of high doses of methionine administered to
support hepatic glutathione biosynthesis during severe paracetamol intoxication (37). However, further experimental work is needed to elucidate the physiological significance of this rather complex interaction. Also for this purpose suspensions of freshly isolated kidney cells may serve as a useful experimental tool.

REFERENCES

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