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Research Article

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Cellular Accumulation of L-Cystine in Rat Kidney Cortex In Vivo

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ABSTRACT Cellular accumulation of L-cystine in rat kidney cortex in vivo has been studied using L-[^{35}S] cystine. The L-[^{35}S]cystine radioactivity in plasma decreases to less than 10% of the initially calculated value by 15 min. Four ^{35}S-containing intracellular products of L-cystine metabolism were identified including cystine, cysteine, reduced glutathione, and an as yet unidentified compound. The latter is probably taurine, cysteinesulphinate, or cysteic acid. Cellular accumulation of these products was found to be more rapid in vivo than in vitro. Cellular accumulation of the products of L-cystine metabolism was found to be essentially unchanged in the presence of ureter ligation. Unlabeled L-lysine administered simultaneously with L-[^{35}S]cystine, in both the presence and absence or ureter ligation, enhanced the cellular accumulation of intracellular metabolic products of L-[^{35}S]cystine. Simultaneous ^{35}S cellular accumulation and L-cystine clearance studies were performed both in the presence and absence of L-lysine. L-Lysine enhanced cellular accumulation of ^{35}S-products despite an accompanying increase in L-cystine clearance. The results are interpreted as evidence for a dissociation between cellular accumulation and transepithelial transport. This evidence for independent luminal transport and peritubular cellular accumulation could explain the apparent paradox in the disease cystinuria where there appears to be a luminal transport defect for L-cystine, but no defect for cellular accumulation of L-cystine metabolic products in vitro.

INTRODUCTION

Two recent reviews have summarized current knowledge of human cystinuria, an hereditary disorder characterized by defective renal tubule and gastrointestinal mucosa amino acid transport (1, 2). L-Cystine and the dibasic amino acids L-lysine, L-arginine, and L-ornithine appear in large quantities in the urine of human cystinuric subjects despite normal plasma levels (3, 4). In addition, reports of renal clearance studies (5, 6) demonstrating mutual interference among the dibasic amino acids and cystine led to the proposal that there is a common reabsorptive system in kidney tubules for cystine and the dibasic amino acids (7). Dent and Rose (7) postulated that this reabsorptive system is defective or absent in cystinuric patients. Subsequent studies in vitro have raised some doubt that cystine participates in the same transport process as the dibasic amino acids. Cellular accumulation of lysine and arginine in kidney tissue slices from cystinuric patients in vitro is defective, but cellular accumulation of cystine is normal (8). Studies on rat and human renal cortex slices in vitro have shown that the dibasic amino acids do share a common transport system, but no inhibition of cystine accumulation by lysine or lysine accumulation by cystine was found (8, 9). Lysine participates in exchange diffusion with other dibasic amino acids but not with cystine (10). Furthermore, biochemical (11, 12) and ontogenetic differences (13) have clearly established that the transport systems for lysine and cystine in rat kidney cortex in vitro are not the same.

The role of cysteine, the principal form of cystine found intracellularly, is unknown. Kinetic (12) and ontogenetic (14) studies in rat kidney cortex in vitro provide strong evidence that systems for cellular accumulation of cystine and cysteine are different. No interaction between cysteine and the dibasic amino acids for entry into tubule cells has been found (15). It has been demonstrated, however, that the dibasic amino acids inhibit the efflux of intracellular cysteine from rat kidney slices (15).

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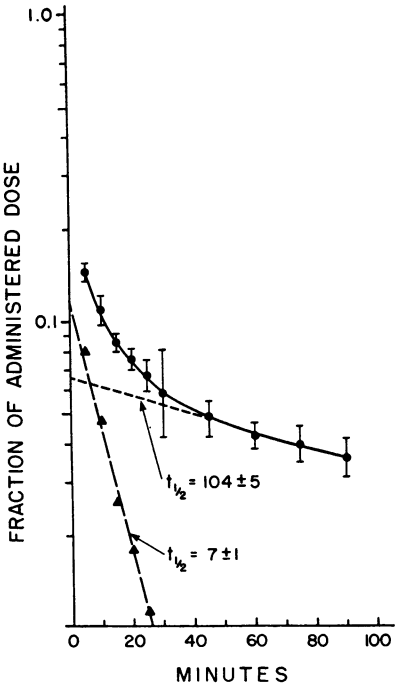

FIGURE 1 The rate of disappearance of plasma radioactivity after injection of 28.8 μCi of L-[^{35}S]cystine. The log of the fraction of the administered dose in plasma and extracellular fluid is plotted vs. time after injection. Each point is the average ±SE of six determinations.

Thus, though there is no apparent discrepancy between the clearance data and studies of dibasic amino acid accumulation in vitro, there is difficulty in trying to correlate renal clearance data for cystine with the studies of cellular accumulation of cystine in vitro. The intent of the present study has been to resolve the apparent inconsistencies between the renal clearance of cystine and the transport observations in vitro. In this study transepithelial transport is defined as the reabsorption of a filtered substance by the kidney tubular epithelium and is measured using clearance techniques. On the other hand, cellular accumulation is defined as the accumulation of isotope within the cell and is expressed as a distribution ratio, the ratio of intracellular to extracellular radioactivity. Cellular accumulation of ^{35}S has been examined in vivo after L-[^{35}S]cystine injection with regard to the rate of its disappearance from plasma, the rate of cellular accumulation in rat kidney cortex cells and the nature of the intracellular ^{35}S-containing metabolites. The interaction of cystine with lysine for cellular accumulation in kidney cells has been examined and correlated with the renal clearance of cystine. The influence of ureteral ligation on the cellular accumulation of cystine has also been determined. The results form the basis of this report.

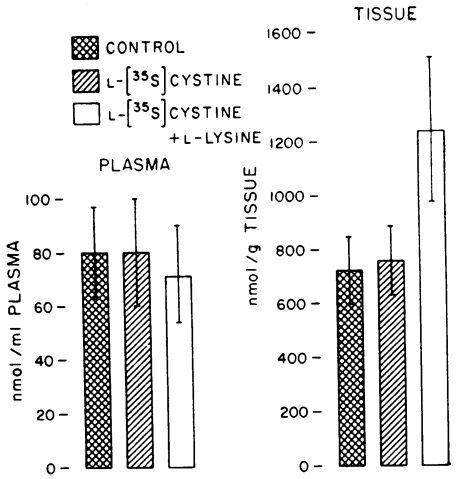

FIGURE 2 The effect of an injection of L-cystine or L-cystine plus L-lysine on total cyst(e)ine levels in rat plasma and kidney tissue. Total cyst(e)ine was determined using the spectrophotometric method of Gaitonde (22). Each value is the average ±SE determined from four rats.

METHODS

Animals. Adult male Sprague-Dawley rats weighing 150–250 g were maintained on a Purina Rat Chow diet (Ralston Purina Co., St. Louis, Mo.) and water ad lib.

Methods: cellular accumulation of L-[^{35}S]cystine. A solution of 1.6 mM L-[^{35}S]cystine with 3.26 μCi/ml was prepared by adding 1 ml of 1.6 mM L-[^{35}S]cystine in dilute NaOH to 6 ml of 1.6 mM L-cystine in isotonic saline. Solutions were made fresh each week since it had previously been found that cystine is oxidized to cysteic acid with time, but that less than 1% oxidation occurs within the 1st wk

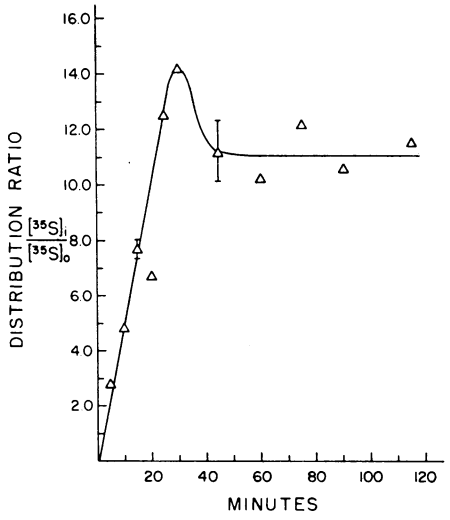

FIGURE 3 The DR (counts/minute per milliliter of intracellular fluid divided by counts/minute per milliliter of plasma) plotted as a function of time after injection of 1.14 μCi of L-[^{35}S]cystine. Points with error bars represent average ±SE of 36 slices from three rats. Other points represent average of 12 slices from individual rats.

TABLE I

Variation of Cellular Accumulation of ^{35}S after Injection with L-[^{35}S]*cystine by Serial Slices of Kidney Cortex and the Effect of Ureter Ligation*

Experiment*	Slice‡	Distribution ratios§ Control kidney	Ligated kidney	*t* test‖
1. L-[^{35}S] cystine injected	2	10.7±0.8	8.9±0.8	NS ($P > 0.05$)
	3	9.6±0.6	8.7±0.7	NS
	4	9.6±0.5	8.3±0.5	NS
2. L-[^{35}S] cystine+ L-lysine injected	2	12.9±0.4	11.5±0.4	NS
	3	22.1±2.0	14.6±1.2	$P < 0.01$
	4	26.8±1.6	17.8±1.1	$P < 0.0025$

* In experiment 1: 1.14 μCi L-[^{35}S] cystine was injected intravenously. In experiment 2: 450 μmol of L-lysine were given with the radioactive cystine as described in Methods.

Serial slices were made with a Stadie-Riggs microtome from the poles of each kidney.

‡ Slice 2 contains only cortex while levels 3 and 4 have increasing amounts of medulla.

§ The DR is the ratio of counts/minute per milliliter intracellular fluid to counts/minute per milliliter plasma. Each value is the average ±SD for five slices from five rats.

‖ Significance of DR difference for a given tissue level between the control and ligated kidneys.

(14). An intravenous injection of 0.35 ml of this solution was made into the tail vein of each rat. At the specified times the experiments were terminated by stunning and decapitating the rats. Blood was collected in heparinized beakers and the kidneys removed. The kidneys were cut in half and sectioned serially from outer pole to outer medulla with a Stadie-Riggs microtome. Four slices were obtained from each kidney half. Slices from three kidney halves were grouped at their respective levels. The intracellular and extracellular radioactivity were assessed as described previously (16) for studies in vitro as modified for studies in vivo by Ausiello, Segal, and Thier (17). The separate groups of kidney slices were boiled in 2 ml of water to extract the tissue amino acid pool. Plasma was separated by centrifugation. Portions of the tissue extract and blood plasma were assayed for ^{35}S by liquid scintillation counting. The distribution ratio (DR),[1] the ratio of counts/minute per milliliter of intracellular fluid to counts/minute per milliliter of plasma (medium in in vitro studies) was calculated according to the technique of Rosenberg, Blair, and Segal (16). The DR refers to the ratio of ^{35}S and therefore is a measure of the accumulation of cystine and its metabolites. Separate studies were also conducted using injections of 0.75 ml saline containing 0.56 μmol and 1.14 μCi of L-[^{35}S]cystine and 450 μmol of unlabeled L-lysine.

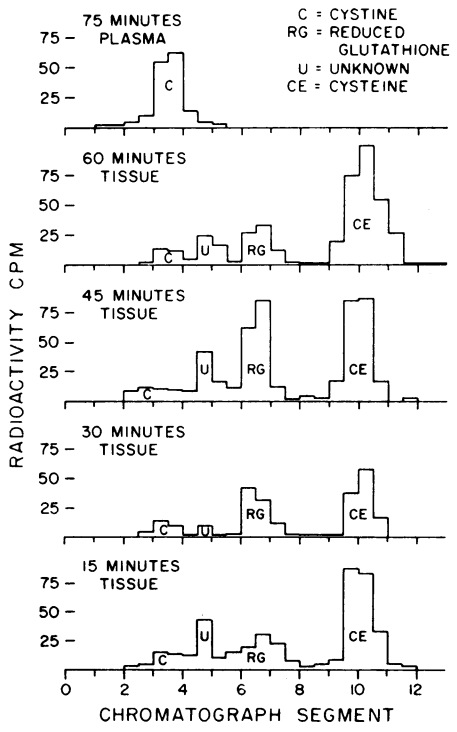

FIGURE 4 Representative TLC at various times after injection of L-[^{35}S]cystine.

Ureteral ligation. In the ureteral ligation experiments small abdominal incisions were made under ether or Inactin [5-ethyl-5-1′-methyl-propyl)-2-thiobarbituric acid] anesthesia and the left ureters tied just below the renal pelvis. The incisions were closed with clips and the animals revived. After 1 h the animals were injected as described above. 45 min later the animals were stunned, decapitated, and plasma and renal cortex assayed for radioactivity. Similar studies were also performed with L-[^{35}S]cystine injected 10 min after ureteral ligation and tissue taken 10 min later.

Plasma disappearance. Rats were anesthetized with Inactin (100 mg/kg), placed on a heated operating table, and

[1] *Abbreviations used in this paper:* DR, distribution ratio; NEM, *N*-ethylmaleimide.

a tracheostomy was performed. In these studies a solution of 1.6 mM L-[^{35}S]cystine containing 82.2 μCi/ml was used. An injection of 0.35 ml of this solution was given intravenously. At various times thereafter 100-μl samples of blood from the tail vein were collected in hematocrit tubes, centrifuged, and 20-μl portions of the plasma counted in a liquid scintillation counter.

Amino acid clearances. Clearance studies were conducted using a modification of a technique described by Buckalew, Ramirez, and Goldberg (18). Rats were weighed and anesthetized with Inactin (100 mg/kg). A tracheotomy was performed. The bladder and both jugular veins were catheterized. The temperature of each rat was maintained between 37 and 38°C. A 10% mannitol solution in 0.9% NaCl was given at a rate of 0.1 ml/min until a total of 1% of the rat's weight had been administered. The mannitol solution was then slowed to 0.05 ml/min throughout the duration of the experiment. [*methoxy*-^{3}H]Inulin with 50 μCi/ml was administered as a primer at 1.2 μCi/100 g body weight and a sustaining solution of 5.30 μCi/ml was infused at 0.03 ml/min. When a uniform rate of urine flow was obtained two control urine samples were collected and two midpoint blood samples were obtained from the tail vein. In half the rats an intravenous dose of 0.35 ml of 1.6 mM L-[^{35}S]cystine (8.0 μCi) was injected. In the other half in addition to the L-[^{35}S]cystine another 0.4 ml of unlabeled L-lysine (450 μmol) was injected. Three 15-min urine collections were obtained with midpoint blood samples. Urine flow greater than 0.05 ml/min was replaced with 0.45 N saline. After the last urine collection the animals were sacrificed and the kidney slices and plasma prepared and assayed for radioactivity as described above. Portions (20 μl) of urine and plasma were pipetted into 10.0 ml of Scintisol and 1.0 ml of H_2O. The ^{35}S and ^{3}H content was determined in a liquid scintillation counter. Corrections were made for background, quenching, and reciprocal interference of ^{3}H and ^{35}S channels. Several representative urine samples from each clearance period were assayed for amino acid content in a Beckman 120 C Amino Acid Analyzer (Beckman Instruments, Inc., Fullerton, Calif.).

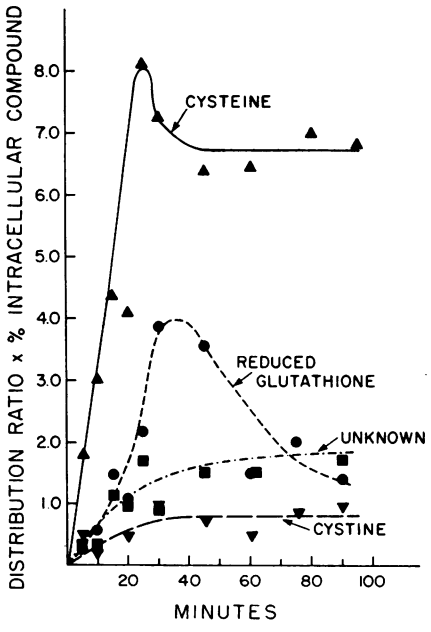

FIGURE 5 The ratio of intracellular radioactivity for a given metabolite of cystine to extracellular radioactivity due to L-[^{35}S]cystine for various times after injection of 1.14 μCi L-[^{35}S]cystine.

TABLE II

Clearance of L-*Cystine and Cellular Accumulation of* ^{35}S

Time period	Fractional clearance ($C_{cystine}/C_{inulin}$)	Distribution ratio	Urine lysine‡
min	±*SE*	±*SD*	*μmol/ml*
Group 1			
0 to 15	4.02±0.52	7.7±0.3*	§
15 to 30	4.60±1.51		§
30 to 45	5.17±1.12	11.8±1.5	§
		11.2±1.3*	
Group 2			
0 to 15	24±0.7	11.5±2.3*	6.15
15 to 30	5.2±1.3	21.0±2.1*	0.0465
30 to 45	5.0±1.1	19.5±2.2	0.0028
		25.6±1.6*	

For the clearance studies each group consists of three rats. Group 1 represents control rats where labeled cystine was injected at 0 time. Rats in group 2 were injected with L-lysine in addition as described in Methods.

* These values are intended for comparison and were determined on a comparable group of rats not in the clearance studies. Each value is the average ±SD for 48 tissue slices from four rats. The other values are obtained from kidneys removed at the end of the clearance studies.

‡ Representative urine lysine concentrations are given for a single rat in each time period.

§ Unmeasurably low, less than 0.001 μmol/ml.

Identification of intracellular ^{35}S-containing compounds and cyst(e)ine quantification. Kidney slices were washed in saline, dried, weighed, and then homogenized in 1.0 ml of 2 mM *N*-ethylmaleimide (NEM) in 0.1 M phosphate buffer (pH 7.4) to form cysteine and reduced glutathione adducts as described previously (19). 1-ml plasma samples were added directly to the NEM solution. The homogenate was deproteinized immediately by adding 1.0 ml of 10% trichloroacetic acid (TCA) and then centrifuged. Unreacted NEM and TCA in the supernate were extracted three times with a volume of ether equivalent to two times the supernatant volume. The aqueous phase was acidified with one drop of 2 N HCL and a 20–40 μl sample chromatographed on Eastman Chromagram cellulose sheets (Eastman Kodak Co., Rochester, N. Y.) employing *n*-butanol/pyridine acetic acid/H_2O in the ration 3:2:0.6:1.5 for development according to method of States and Segal (20). The appropriate cysteine-NEM, glutathione-NEM, and cystine standards were prepared by the method of Smyth, Nagamatsu, and Fruton (21). The chromatograph sheet from each experiment was then cut into half-centimeter segments and each segment was assayed for ^{35}S activity using standard liquid scintillation techniques. The segments corresponding to cystine, cystine-NEM, and glutathione were identified by comparison to the location of simultaneously run standards stained with ninhydrin.

The concentrations of cyst(e)ine (cystine plus cysteine) in rat plasma and homogenized kidney were determined spectrophotometrically after reduction by dithiotreitol

(DTT) using the method of Gaitonde (22) as modified for increased sensitivity by States and Segal.[2] Samples of plasma and kidney were obtained from rats which had received injections of saline, L-[^{35}S]cystine or L-[^{35}S]cystine and 450 μmol of L-lysine. The sample was deproteinized by addition of an equal volume of 5% TCA, centrifuged, and the supernate transferred to a 10 ml Coleman tube (Coleman Instruments, Maywood, Ill.). One drop of phenolphthalein and 0.5 ml 0.5 M Tris buffer (pH 8.0) were added. Approximately 0.03 ml 2 N NaOH was added to insure a pH between 8.0 and 9.0, and 0.01 ml of 100 mM DTT prepared in Tris buffer (pH 8.0) was added to reduce cystine to cysteine. After 30 min incubation at room temperature, 1 ml of glacial acetic acid followed by 1 ml of Gaitonde's ninhydrin reagent 2 were added to each tube. The remainder of the procedure was the same as described by Gaitonde. A blank and several cysteine standards were prepared under the same conditions. The samples were read at 560 nm.

L-[^{35}S]Cystine was purchased from Schwarz/Mann, Orangeburg, N. Y., and was found to be 98–100% pure by high voltage electrophoresis (19) and thin-layer chromatography (TLC) (20).

RESULTS

Plasma cystine levels and rate of cellular accumulation of cystine and its metabolites. The rate of disappearance of radioactivity from plasma at various times after injection of [^{35}S]cystine into six rats is shown in Fig. 1. The rate of decrease of radioactivity in plasma can be plotted as the sum of two exponential curves. The initial more rapidly decreasing curve was found to have $t_{\frac{1}{2}}$ of 7±1 min. The second exponential component was found to have a $t_{\frac{1}{2}}$ of 104±5 min. Fig. 2 shows the results for spectrophotometric determination of total cyst(e)ine in plasma 45 min after injection of [^{35}S]cystine or [^{35}S]cystine plus lysine. It can be seen that the plasma levels of cyst(e)ine in the control animals were comparable with those in the animals injected with [^{35}S]cystine. The presence of lysine did not affect the plasma cyst(e)ine level.

The time curve for cellular accumulation of ^{35}S after administration of [^{35}S]cystine by rat kidney in vivo is shown in Fig. 3. A peak DR of 14.2 is reached at 30 min, decreases slightly to 11.3 at 45 min, and remains relatively constant thereafter. In Table I it can be seen that in the control group the DR for ^{35}S does not change significantly with depth of slice. Fig. 2 shows the results for spectrophotometric determination of total cyst(e)ine in kidney tissue 45 min after injection of [^{35}S]cystine. By comparison of Table I and Fig. 2 it can be seen that the radioactive label reflects real changes in cyst(e)ine concentrations in rat kidney and the DR calculated from radioactivity is therefore a valid measure of cyst(e)ine cellular accumulation.

Intracellular ^{35}S-containing metabolites of cystine. In this study both the plasma and kidney tissue from each rat were assayed for ^{35}S-labeled compounds using the TLC technique. Data from several representative thin-layer chromatographs are shown in Fig. 4. Four discrete peaks of ^{35}S-radioactivity were found on the chromatographs of the kidney tissue homogenates. Three of these were identified as cystine, reduced glutathione, and cysteine. The fourth peak has not yet been identified. The only ^{35}S-labeled compound found in plasma was cystine. There were no other discrete radioactive peaks detected in the plasma even at 90 min after the intravenous injection of L-[^{35}S]cystine.

The ratio of intracellular radioactivity for a given metabolite of cystine to extracellular radioactivity due to [^{35}S]cystine was determined as the product of the DR (from Fig. 3) and the fractional contribution for that metabolite (obtained from thin-layer data). This quantitative analysis of the intracellular ^{35}S-labeled metabolites of cystine is plotted in Fig. 5. Most of the intracellular ^{35}S-radioactivity is in cysteine. However, there is also substantial incorporation of the ^{35}S label into reduced glutathione. This intracellular analysis in vivo differs from previous studies in vitro in two respects. The first is that low concentrations of [^{35}S]cystine have been found in vivo, even after correction for the amount in extracellular fluid by the method described by Segal and Crawhall (23); none was found intracellularly in vitro. Secondly, there is an additional compound. not yet identified (but its position on the chromatograph would suggest that it is cysteic acid, cysteinesulphinic acid, or taurine) which has not been described from studies in vitro.

Effect of ureter ligation on cellular accumulation of ^{35}S after the injection of [^{35}S]cystine. Table I shows the effect of ureter ligation on the accumulation of ^{35}S. There is a small difference in the DR at each tissue slice level between the kidney with the ligated ureter and the control kidney when [^{35}S]cystine is injected alone. This difference is not significant ($P > 0.05$). The same results were obtained whether a 10 or 60 min waiting period was allowed between ligation and injection. Thus, ureter ligation and marked inhibition of glomerular filtration rate does not result in a marked reduction in the DR.

Effect of lysine on cellular accumulation of ^{35}S after the injection of [^{35}S]cystine. Table II shows the effect of lysine (in concentrations known to be inhibitory for arginine and ornithine, but not cystine cellular accumulation in vitro) on the DR (labeled with asterisks) of ^{35}S at 15, 30, and 45 min. The intracellular distribution of the ^{35}S radioactivity among the various sulfur-containing compounds of cystine metabolism was not changed significantly by the presence of high concentrations of lysine. Fig. 2 shows the results of the spectrophometric determination of total cyst(e)ine in rat kidney tissue after injection of [^{35}S]cystine or [^{35}S]cys-

[2] States, B., and S. Segal. *Clin. Chim. Acta.* In press.

tine plus lysine. Comparison of Table I and Fig. 2 allows several points to be made. First, the presence of high lysine concentrations results in an increase in the amount of radioactivity intracellularly. Spectrophotometric determinations confirm that lysine causes an increase in total cyst(e)ine, which chromatography demonstrates to be primarily cysteine. Thus, the radioactive label reflects real changes in cyst(e)ine concentrations. Secondly, the large standard error for tissue "total cysteine" in the presence of high concentrations of lysine is unexplained but is probably related to the increase in DR with depth of slice (discussed in the next section).

Effect of ureter ligation and high concentrations of lysine on cellular accumulation of ^{35}S after the injection of L-[^{35}S]*cystine.* Table I shows the effect of ureter ligation in the presence of high concentrations of lysine on the cellular accumulation of ^{35}S. The presence of high concentrations of plasma lysine resulted in several differences between the ligated and nonligated side. First, although there was no significant difference in the DR for ^{35}S between the two sides for tissue slice 2, there was a large and significant difference in the DR between the two sides for tissue slices 3 and 4 ($P < 0.01$ and 0.0025, respectively). Second, unlike the results obtained when only [^{35}S]cystine was injected, the cellular accumulation of ^{35}S in the presence of lysine increased significantly with increasing amounts of medulla. At each level, both on the ligated and nonligated side, the DR were greater than those obtained when cystine alone was injected. However, the increase was not nearly as large on the ligated side. It can be concluded that enhancement of cellular accumulation of ^{35}S by simultaneous administration of large concentrations of lysine can be observed both in the presence and absence of ureter ligation and can be observed to increase with the increasing amounts of medulla.

Effect of lysine on cystine clearance. The clearance of cystine alone and cystine in the presence of large concentrations of lysine are shown in Table II. The results are expressed in terms of the fractional clearance ($C_{cystine}/C_{inulin}$). The glomerular filtration rate measured by the C inulin ranged from 2.2 to 2.6 ml/min throughout the experiments. The data indicate that the fractional clearance of cystine is increased about fivefold in the first collection period immediately after the administration of lysine, a result previously well documented (5, 6). The clearance of cystine then returns to near normal. The corresponding DR were obtained at the end of each clearance period. The DR labeled with asterisks were not obtained from the rats used in the clearance study but were obtained from separate comparable experiments. Despite the increased clearance of cystine in the presence of lysinuria, the DR at the end of the first clearance period remained significantly elevated above the control value. In fact, the data from the next two clearance periods indicate that the DR continues to increase or is maintained even after cystine clearance returns to control levels. This emphasizes the dissociation of luminal transport from cellular accumulation under these conditions. Cystinuria, on the other hand, appears to correlate with the magnitude of the urine lysine concentration.

DISCUSSION

Crawhall, Purkiss, and Young (24) while studying cystine metabolism in the dog described the disappearance of plasma cystine radioactivity in terms of a two component system. They concluded that after a single injection of [^{35}S]cystine the initial rapidly decreasing curve represented equilibration of the tracer with the intracellular space while the second less steeply decreasing component represented cystine turnover. This appears to be the case in our experiments since the distribution ratio approaches its maximum in roughly the same time (30 min) that is required for the more rapidly decreasing component of plasma radioactivity to undergo five half-lives ($t_{\frac{1}{2}} = 7 \pm 1$). After this period of time the distribution ratio remains relatively constant while the plasma cystine radioactivity decreases at a slower rate due to cystine turnover.

The studies reported here indicate that the accumulation of cystine and its metabolites in rat kidney cells is more rapid in vivo than in vitro. The maximum distribution ratio is 14.2 at 30 min after injection. This compares with studies in vitro where a maximum distribution ratio of five is reached only after 60 min incubation at comparable extracellular cystine concentrations.

Crawhall and Segal (19) used the formation of NEM adducts to trap intracellular sulfhydryl metabolites of cystine and high voltage electrophoresis for the separation of cystine and cysteine-NEM adducts in adult rat renal cortex. They found that the intracellular form after cystine transport was cysteine (100%). This was later re-examined in both 5-day old and adult rat kidney tissue using a TLC technique (14). Cysteine was again found to be the primary intracellular cystine metabolite (51–68%) but a substantial amount of ^{35}S incorporation into reduced glutathione (12–30%) was also noted in both groups using this method. In the present studies approximately 60% of intracellular radioactivity was cysteine, up to 30% reduced glutathione and the rest small amounts of intracellular cystine and an additional compound. The latter has not been identified as yet but its position on the chromatograph suggests that it is taurine, cysteic acid, or cysteinesulphinic acid. Segal and Smith (14) reported no intracellular cystine in

vitro unless high concentrations (500 μM) of cystine were used in the incubation media. At these concentrations intracellular reduction or conversion of cystine was found to be slightly impaired. At the concentrations (< 100 μM) of cystine used in this study reduction is not a limiting process.

The simultaneous administration of lysine with cystine resulted in an increase in the distribution ratio for ^{35}S to approximately two times its control value. This effect is similar to that obtained by Ausiello, Segal, and Thier (17) who obtained a severalfold increase in the distribution ratio for lysine when large amounts of arginine were administered simultaneously. The increased DR for ^{35}S obtained here could be partially due to artifact. One might argue that because of the larger urine cystine concentrations there could be increased trapping of labeled cystine in the tubular lumen. This trapped radioactivity would be measured as part of the intracellular radioactivity and spuriously elevate the DR. There is, however, a strong argument against this phenomenon having a significant effect on the DR. Tissue was analyzed 45 min after injection of lysine and cystine. Clearance studies indicate that at 45 min cystine clearance is no longer elevated above control levels. Furthermore, the DR for ^{35}S actually determined in the clearance studies remained elevated when lysine was administered simultaneously even though cystine clearance was normal at the time the kidney was removed for analysis. Thus, the contribution of trapped labeled cystine to the DR should be no greater 45 min postinjection in the rat injected with cystine plus lysine than in the control injected with cystine alone. Exchange diffusion is another plausible explanation for the increase in DR for cystine in the presence of large amounts of lysine. However, exchange diffusion between cystine and lysine does not occur in vitro (10). A third possibility is that the increase in cellular accumulation of ^{35}S is due to inhibition of cysteine efflux from the intracellular space. Cystine and lysine do not compete for the same transport system in vitro (15). Schwartzman, Blair, and Segal (15, 25) observed, however, that lysine inhibits cysteine efflux from kidney tissue cells in vitro. The fact that cysteine is the primary intracellular metabolite adds importance to the efflux interaction between cysteine and the dibasic amino acids.

Presumably the presence of high concentrations of lysine intracellularly reduces cysteine efflux from the cell resulting in an increase in the DR. The DR for ^{35}S does not vary with depth of tissue slice in rats injected only with labeled cystine. However, in rats injected with cystine plus lysine the DR for ^{35}S increases with depth of tissue slice. This effect is also noted in the presence of ureteral ligation. The reason for this effect is unknown.

Cellular accumulation has been thought to reflect transepithelial transport. There is evidence that the terminal straight segment of the proximal tubule is the site of maximal cellular accumulation while the proximal convoluted tubule is assumed to be the site of tubular reabsorption (26). In the present study evidence of a dissociation between cellular accumulation and transepithelial transport was obtained from ureteral ligation experiments. It is known that less than 6 min after ligation of a rat ureter there is a maximal rise in ureteral pressure (27). In order to demonstrate that the phenomenon of "reabsorption replacement" was minimal in these experiments two different waiting periods after ligation were used (10 and 60 min). There was ample time for glomerular filtration rate to be markedly inhibited. This reduces the contribution of tubular reabsorption of subsequently injected labeled cystine to a minimum. The distribution ratio for ^{35}S in this study has been shown, however, to be almost as well maintained on the ligated as the unligated side when only cystine is injected. Therefore, under these conditions, cellular accumulation primarily reflects the effect of peritubular processes. The work of Bergeron and Vadeboncoeur (28, 29) using micropuncture techniques would suggest that there is peritubular contribution to cellular accumulation.

The injection of labeled cystine with unlabeled lysine in the ligation experiments yielded several results. The distribution ratio for ^{35}S in both the ligated and nonligated kidneys was increased over that obtained in control ligated and nonligated kidney. However, unlike results obtained when only labeled cystine was injected, there was a statistically significant decrease in the DR on the ligated vs. the nonligated side. Furthermore, this difference between ligated and nonligated kidneys increased with increasing depth of slice. It might indicate that the contribution of tubular reabsorption to cellular accumulation increases with depth of slice. The increase in tissue water in a hydronephrotic kidney could account for some of the decrease in the DR on the ligated side.

In studies performed to correlate clearance data with the cellular accumulation the data indicate that high concentrations of urine lysine are associated with an increase in urine cystine concentrations. However, the urinary loss of cystine does not remain elevated throughout the duration of the clearance experiment. Despite the return of cystine clearance to control level the DR of ^{35}S continues to increase. This suggests that there is a dissociation of transepithelial transport and cellular accumulation under these conditions. The short duration of the increased urine cystine concentrations is due to rapidly decreasing levels of lysine in plasma.

Large amounts of lysine are lost in the urine. In addition, Waterlow and Stephen (30) have shown that lysine turnover in the rat is about 100 μmol/100 g per h for a 200 g rat. They found that after a single injection of L-[^{14}C]lysine the specific activity of free lysine fell to about 4% of the calculated initial activity by 15 min.

There is increasing evidence to suggest that amino acids are secreted by kidney tubules (31, 32). Tubular secretion of cystine could explain ratios of cystine clearances to inulin clearance > 1 observed in some cystinuric patients. Tissue studies in vitro (6) have failed to demonstrate a defect in cellular accumulation of cystine in cystinuric kidney. The present studies suggest that cellular accumulation in vivo occurs from both the peritubular and brush border (luminal) surfaces of the cell. The ureteral ligation experiments indicate that the peritubular side of the cell has the capacity to transport cystine. It is conceivable that in the tissue slice studies in vitro cellular accumulation occurs primarily from the peritubular side. Therefore, the conclusions to be drawn about luminal surface transport from kidney slice studies in vitro are in doubt. It is possible that cystine and lysine do compete for a common transport system on the luminal surface. When large amounts of lysine are infused increased amounts of cystine are excreted in the urine. This transport system could be defective in cystinuric patients. The defect, however, might not have been found in vitro because it was masked by normal cellular accumulation from the peritubular side. The peritubular side would presumably not have a common transport system and this would explain the finding in vitro of a lack of competition between L-lysine and L-cystine for cellular accumulation. Once cystine enters the cell and is converted in large part to cysteine, competition between cysteine and dibasic amino acid for efflux might occur. This interaction for efflux would explain the increase in ^{35}S intracellularly with the simultaneous injection of lysine and would explain why ^{35}S intracellularly might remain elevated even though plasma lysine concentrations are decreasing. This proposal for cystine transport is speculative but forms a basis for further investigation.

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