

Aggravation of Polycystic Kidney Disease in Han:SPRD Rats by Buthionine Sulfoximine

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Abstract. The administration of ammonium chloride or of sodium or potassium bicarbonate has marked effects on the development of polycystic kidney disease (PKD) in Han:SPRD rats. Because of the possibility that these effects are mediated by changes in redox metabolism, the aim of this study was to determine whether depletion of glutathione, the most abundant and important cellular thiol and scavenger of reactive oxygen species, would affect the development of PKD in this animal model. *+/+* and *cy/+* Han:SPRD rats were treated with: (1) L-buthionine(*S,R*)-sulfoximine (BSO), a specific inhibitor of γ -glutamylcysteine synthetase, the rate-limiting enzyme for the synthesis of glutathione; (2) glutathione monoethyl ester (GME), a compound that is known to increase the intracellular levels of glutathione; or (3) BSO and GME. Treatment with these drugs was started at 3 wk of age, and the animals were killed at 6 or 8 wk of age. Renal levels of oxidized glutathione were significantly higher in *cy/+* than in *+/+* rats, whereas no significant differences in reduced glutathione were detected. The administration of BSO caused a marked reduction in the

levels of glutathione. The administration of GME caused a significant increase in the levels of glutathione at 2 h, but not at 12 h, after the administration. The increase in the renal levels of glutathione 2 h after the administration of GME was less in the rats treated with BSO than in the rats not receiving this drug, indicating that in part the increase in glutathione level was due to *de novo* synthesis. BSO-induced glutathione depletion was accompanied by a marked aggravation of the renal cystic disease, as reflected by kidney weights, histological scores, and plasma urea concentrations. However, the administration of GME did not lessen the cystic disease and did not reverse the effects of BSO. The transient effect of GME administration and the simultaneous increases in the levels of cysteine and oxidized glutathione, in addition to reduced glutathione, may explain the lack of protection by GME. These data support the notion that changes in redox metabolism may affect the development of PKD. (*J Am Soc Nephrol* 8: 1283–1291, 1997)

The Han:SPRD rat with autosomal dominant polycystic kidney disease (PKD) is a useful model for the study of human autosomal dominant PKD (1–2). In this model, the cystic disease begins in and affects predominantly the proximal tubules. Administration of ammonium chloride or of sodium or potassium bicarbonate has marked enhancing or lessening effects on the development of PKD in Han:SPRD rats, suggesting that renal ammoniogenesis *per se* or a metabolic process linked to renal ammoniogenesis is important in the pathogenesis of PKD in this animal model (3,4). The extracellular pH has tissue-specific effects on the tyrosine phosphorylation of specific proteins, the mRNA levels of *c-fos*, *c-jun*, and early growth response-1, and the mRNA levels and activity of the Na/H antiporter in proximal tubular epithelial cells (5–7). Because reactive oxygen intermediates in a variety of cells also affect protein tyrosine kinase activation (8–11) and the expression of immediate response genes (12,13) and Na/H antiporter

(14), it is possible that the effects of acidification and alkalization on the development of PKD in Han:SPRD rats are mediated by changes in the redox metabolism of the proximal tubular epithelial cells. In addition, the activities of glutathione peroxidase and glutathione reductase in proximal tubules are inhibited by acid pH (15,16). To determine whether alterations in redox metabolism may affect the development of PKD in Han:SPRD rats, these animals were treated with: (1) L-buthionine(*S,R*)-sulfoximine (BSO), a specific inhibitor of γ -glutamylcysteine synthetase, the rate-limiting enzyme for the synthesis of glutathione (17); (2) glutathione monoethyl ester (GME), a compound that is known to increase the intracellular levels of glutathione in a variety of tissues (18,19); or (3) BSO and GME.

Materials and Methods

Experimental Animals

A colony of Han:SPRD rats, obtained from the polycystic kidney program at the University of Kansas Medical Center, has been maintained in our animal care facility since 1991. The animals used were the offspring of heterozygous rats. The rats with homozygous disease (*cy/cy*) were recognized at 1 wk of age by marked renal enlargement, died of uremia at 3 to 4 wk of age (1), and were not used in the study. The remaining homozygous normal (*+/+*) and heterozygous diseased (*cy/+*) animals were divided into different groups. Because the se-

Received October 7, 1996. Accepted December 12, 1996.

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1046-6673/0808-1283\$03.00/0

Journal of the American Society of Nephrology

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verity of the cystic disease differed in male and female *cy/+* rats (1), the data for male and female animals were analyzed separately. Impairment of renal function is noticeable by 8 wk of age in male *cy/+* rats, whereas female *cy/+* rats have a milder disease.

Experimental Groups

The studies were initiated at 3 wk of age. The aim of study 1 was to determine whether the administration of BSO enhances the development of renal cystic disease. Male and female *cy/+* and *+/+* Han:SPRD rats were divided into a control group drinking water and an experimental group drinking 20 mM BSO. The aim of study 2 was to determine whether the administration of GME protected against the development of renal cystic disease. Male *cy/+* and *+/+* Han:SPRD rats were divided into an experimental group treated with 250 mM GME hemisulfate at a dose of 2.5 mmol/kg body wt given intraperitoneally every 12 h, and two control groups, one treated with Na_2SO_4 adjusted at the concentrations measured in the GME solutions (100 to 125 mM) and another receiving sham injections only. The aim of study 3 was to determine whether the administration of GME could reverse the effects produced by the administration of BSO. Female *cy/+* and *+/+* Han:SPRD rats were divided into four groups: one control group receiving sham injections only, one group treated with BSO, one group treated with BSO and Na_2SO_4 , and one group receiving BSO and GME hemisulfate. In this study, the BSO was given in the drinking water (20 mM) as well as intraperitoneally (1 mmol/kg body wt every 12 h). The administration of GME and Na_2SO_4 was as in study 2. BSO and Na_2SO_4 were obtained from Sigma Chemical Co. (St. Louis, MO). GME hemisulfate was prepared in our laboratory. All rats were fed a standard rodent diet containing 23% protein (Purina Mills, Inc., Richmond, IN).

Experimental Protocol

At 6 wk (study 1) or 8 wk (studies 2 and 3) of age, the rats were placed in metabolic cages and kept fasting from 8 p.m. to 8 a.m. for collection of urine under mineral oil. Approximately half of the rats treated with GME received this drug intraperitoneally at 8:00 a.m. and were studied 2 h later. The remaining rats treated with GME did not receive the morning dose and were studied 12 to 14 h after the last dose. The rats were weighed and anesthetized with pentobarbital 50 mg/kg body wt intraperitoneally.

Heparinized blood samples were obtained by cardiac puncture, and the abdomen was opened. The left kidney was rapidly removed and cut through the hilus into two equal portions; one or both halves were immediately frozen in liquid nitrogen and kept at -70°C until used for the determination of glutathione. In 16 rats of study 1, the inner medulla and cortex were dissected from one-half of the kidney, immediately frozen in liquid nitrogen, and kept at -70°C until used for the determination of tissue concentrations of ammonia (20). The right kidneys were removed, cut into 3-mm slices, placed in pre-weighed containers with 10% formaldehyde, weighed, fixed overnight at 4°C , and embedded in paraffin for histological studies.

Preparation of GME

GME was prepared as described by Anderson and Meister (21). Twenty-five grams of glutathione were added to a solution of 7 ml of H_2SO_4 into 250 ml of anhydrous ethanol. The reaction mixture was stirred and maintained at 30 to 40°C . Progress of the reaction was followed by HPLC. A typical reaction was found to be 96% complete after 6 h. Seventy-five percent of the product was identified as GME by mass spectroscopy (Figure 1). The reaction produced three additional products, one of which was identified by mass spectroscopy as

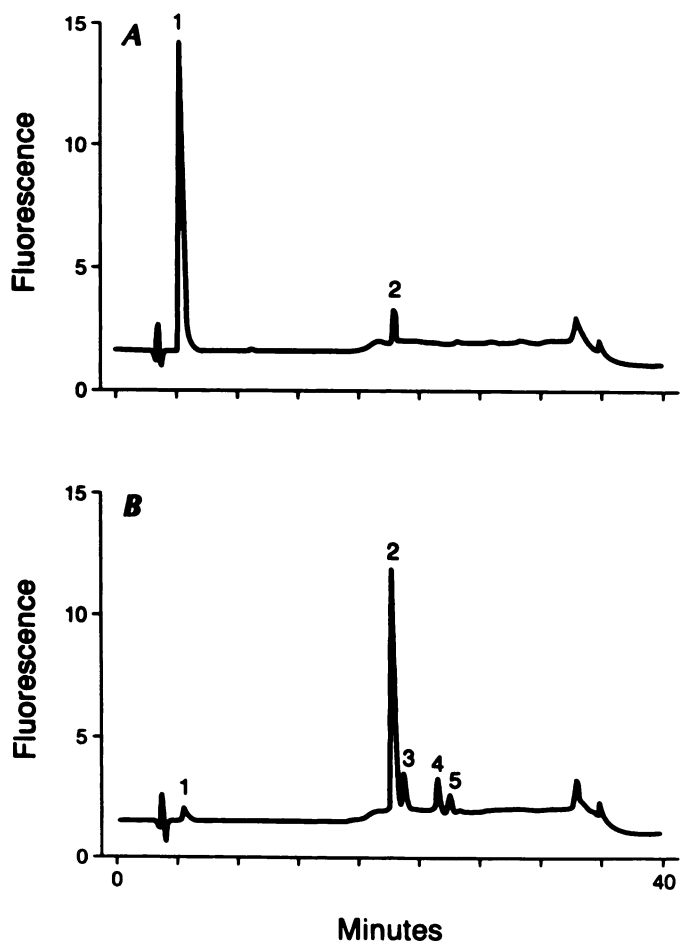


Figure 1. HPLC elution profile of the reaction mixture used for the synthesis of glutathione monoethyl ester (GME). (A) 5 min. (B) 6 h. Peaks 1, 2, and 4 correspond to glutathione, GME, and glutathione diethyl ester. Peaks 3 and 5 were not identified.

glutathione diethyl ester (8.5%). Glutathione diethyl ester is also known to increase the cellular levels of glutathione (22). The other two products were not identified. After 6.5 h, the reaction mixture was concentrated to approximately 150 ml and precipitated with 1000 ml of diethyl ether at 4°C for 16 h. The ether was decanted and the product was dried under N_2 for 4 h. The partially dried product was then placed on the lyophilizer until preparation for use in the animals. GME solutions were neutralized to pH 7.0 before injection into the rats.

Laboratory Methods

Glutathione was measured by the assay described by Griffith (23). Because of the high levels in the kidney of gamma glutamyl transpeptidase, which cleaves glutathione, the tissues were powdered under liquid nitrogen and transferred without thawing into a homogenizer containing 1% picric acid. The treatment with acid inactivates the gamma glutamyl transpeptidase. We have found that the levels of glutathione in tissue treated this way are not different from those found in tissue rapidly homogenized without freezing. After homogenization and centrifugation, total glutathione was determined in the supernatant with an enzymatic recycling assay based on glutathione reductase. 2-Vinylpyridine was used for derivatization of reduced glutathione (GSH) and determination of oxidized glutathione (GSSG).

GSH values were calculated by subtracting GSSG from total glutathione. Urinary ammonia was measured by the Berthelot reaction (24). To determine the cortical and inner medullary concentrations of ammonia, the tissues were homogenized in ice-cold 0.9% saline with chilled glass homogenizers (20). After addition of cold 10% trichloroacetic acid and centrifugation, the supernatants were neutralized with 10 mM Na₂HPO₄ in 9N NaOH, and the ammonia concentrations were measured using Kodak Ektachem clinical chemistry slides (NH₃/AMON) and a Kodak Ektachem 700 analyzer (25). Protein was measured in the tissue homogenates using a bicinchoninic acid protein acid reagent. Plasma urea, creatinine, bicarbonate, sodium, potassium, and chloride were measured using a Hitachi 977 analyzer (26).

Morphologic Analysis

Four micrometer transverse tissue sections, including cortex, medulla, and papilla, were stained with hematoxylin and eosin. These sections were graded without knowledge of group assignment according to the extent of cystic changes (0, absence of cysts; 1, 2, 3, and 4 cysts in <20%, 20 to 40%, 40 to 60%, and >60% of renal cortex, respectively) and the extent and severity of the interstitial infiltration by inflammatory cells (0, absence of infiltrates; 1, focal, mild; 2, focal, moderate; 3, diffuse, mild; 4, diffuse, moderate or severe) (3).

Statistical Analysis

The results are presented as means \pm SD. ANOVA using the Bonferroni test for simultaneous two-group comparisons was used to detect differences between groups. Differences were considered significant if $P < 0.05$.

Results

Renal Levels of Glutathione

The renal levels of GSH and GSSG in the control +/+ and cy/+ Han:SPRD rats and effects of the various treatments on

these levels are summarized in Table 1. The renal levels of GSH were similar in the untreated control cy/+ and +/+ rats. The renal levels of GSSG were significantly higher in untreated control cy/+ when compared with +/+ rats by a two-way ANOVA, but not when male and female rats were analyzed independently. The administration of sodium sulfate had no effect on the levels of glutathione. The administration of BSO caused a marked reduction in the levels of GSH and a less consistent reduction in the levels of GSSG. The intraperitoneal injection of GME to rats not pretreated with BSO increased the renal levels of GSH and GSSG at 2 h, but not at 12 h, after administration. The renal levels of glutathione 2 h after the administration of GME to rats treated with BSO were not different from those observed in the control animals, but at 12 h they had returned to the levels seen in the rats treated with BSO only. The increase in the renal levels of glutathione 2 h after the administration of GME was less in the rats treated with BSO than in those not receiving this drug.

Study 1

The administration of BSO was well tolerated. The differences in body weight between control and experimental animals at 6 wk of age were not statistically significant. Both male gender and the administration of BSO had a significant, independent aggravating effect on renal cystic disease as reflected by relative kidney weights, histological scores, and plasma concentrations of urea (Table 2, Figure 2). The concentration of plasma bicarbonate was significantly lower in cy/+ rats receiving BSO. No differences were detected in the urinary excretion (Table 2) or in the cortical (22.8 ± 12.4 versus 23.4 ± 9.5 nmol/mg of protein, $n = 10$) or inner medullary

Table 1. Renal tissue levels of GSH and GSSG glutathione^a

Group	GSH (nmol/mg of protein)				GSSG (nmol/mg of protein)			
	Male		Female		Male		Female	
	+/+	Cy/+	+/+	Cy/+	+/+	Cy/+	+/+	Cy/+
Control	19.0 \pm 4.1 ($n = 7$) ^b	20.6 \pm 8.9 ($n = 9$)	17.8 \pm 7.8 ($n = 7$)	26.3 \pm 10.6 ($n = 9$)	0.24 \pm 0.09 ($n = 7$)	0.33 \pm 0.09 ($n = 9$)	0.35 \pm 0.06 ($n = 7$)	0.54 \pm 0.18 ($n = 9$)
BSO	4.6 \pm 1.5 ^c ($n = 3$)	5.2 \pm 1.1 ^c ($n = 5$)	5.5 \pm 2.1 ^c ($n = 4$)	7.4 \pm 3.6 ^c ($n = 7$)	0.11 \pm 0.04 ^c ($n = 3$)	0.29 \pm 0.09 ($n = 5$)	0.17 \pm 0.10 ($n = 4$)	0.29 \pm 0.14 ^c ($n = 7$)
Na ₂ SO ₄	16.0 \pm 4.2 ($n = 4$)	30.2 \pm 16.8 ($n = 6$)			0.22 \pm 0.11 ($n = 4$)	0.52 \pm 0.15 ($n = 6$)		
GME (2 h)	51.3 \pm 12.7 ^c ($n = 3$) ^b	45.3 \pm 9.7 ^c ($n = 3$) ^b			2.52 \pm 2.27 ^c ($n = 3$)	1.24 \pm 0.67 ^c ($n = 3$)		
GME (12 h)	26.0 \pm 7.0 ($n = 4$)	17.0 \pm 2.6 ($n = 3$)			0.27 \pm 0.08 ($n = 4$)	0.42 \pm 0.06 ($n = 3$)		
BSO + Na ₂ SO ₄			6.3 \pm 2.4 ^c ($n = 6$)	11.8 \pm 1.3 ^c ($n = 5$)			0.12 \pm 0.08 ^c ($n = 6$)	0.19 \pm 0.11 ^c ($n = 5$)
BSO + GME (2 h)			12.0 \pm 1.6 ($n = 5$)	15.8 \pm 4.3 ($n = 4$)			0.75 \pm 0.34 ^c ($n = 5$)	0.38 \pm 0.16 ($n = 4$)
BSO + GME (12 h)			4.3 \pm 3.4 ^c ($n = 4$)	6.0 ($n = 1$)			0.17 \pm 0.08 ^c ($n = 4$)	0.15 ($n = 1$)

^a The results represent means \pm SD. GSH, reduced glutathione; GSSG, oxidized glutathione; BSO, buthionine sulfoximine; GME, glutathione monoethyl ester.

^b Includes data from five acute experiments (single sham or GME injections 2 h before being euthanized).

^c $P < 0.05$ versus control.

Table 2. Effect of BSO on renal morphology and function in *cy/+* Han:SPRD rats^a

Characteristic	Male		Female		BSO Effect by Two-Way ANOVA (P Value)
	Control (n = 5)	BSO (n = 5)	Control (n = 4)	BSO (n = 4)	
Body weight (g)	172 ± 27	145 ± 21	122 ± 22	112 ± 27	NS
Kidney weight (percentage of body weight)	1.73 ± 0.34	3.23 ± 1.01	1.45 ± 0.22	2.34 ± 0.67	<0.001
Cystic dilation (0 to 4)	1.20 ± 0.27	2.60 ± 0.89	1.13 ± 0.14	1.56 ± 0.38	<0.002
Interstitial inflammation (0 to 4)	0.60 ± 0.22	1.95 ± 0.48	0.56 ± 0.31	1.19 ± 0.55	<0.001
Plasma urea (mg/dl)	31.2 ± 5.2	53.2 ± 14.6	28.3 ± 1.5	34.7 ± 11.7	<0.005
Plasma creatinine (mg/dl)	0.28 ± 0.04	0.32 ± 0.04	0.28 ± 0.05	0.28 ± 0.05	NS
Plasma bicarbonate (mEq/L)	23.8 ± 2.9	19.6 ± 2.9	22.3 ± 1.7	20.0 ± 1.6	<0.002
Urine ammonia (μmol/h per 100 g body wt)	11.4 ± 3.8	10.1 ± 1.5	11.6 ± 2.9	10.1 ± 3.3	NS

^a The results represent mean ± SD. Abbreviations as in Table 1.

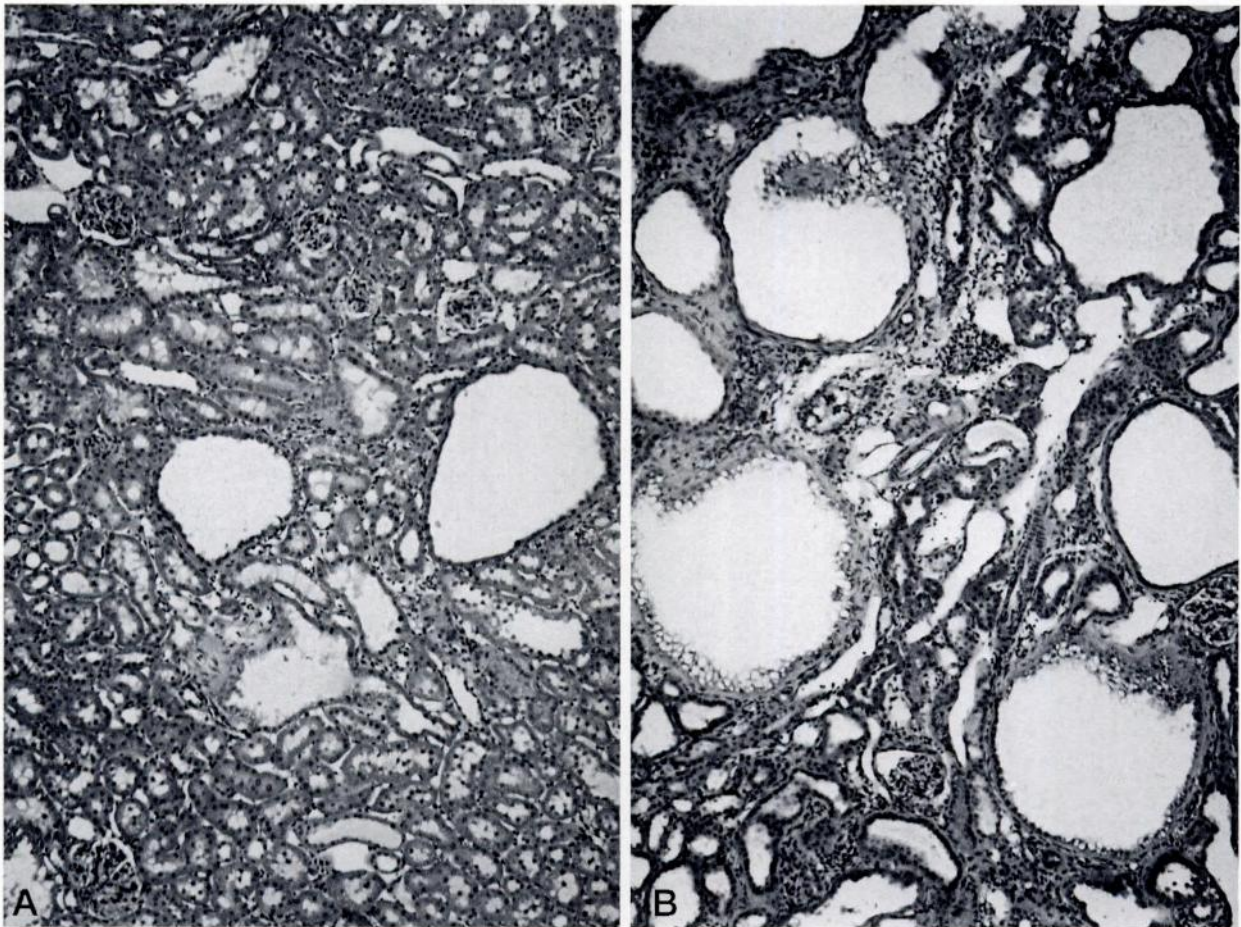


Figure 2. Four micrometer kidney sections from 6-wk-old male *cy/+* Han:SPRD rats from the control group (A) or from the group drinking 20 mM BSO (B). Hematoxylin and eosin, ×100.

(79.7 ± 61.5 versus 56.3 ± 25.4 nmol/mg of protein, *n* = 10) concentrations of ammonia between control *cy/+* rats and *cy/+* rats treated with BSO. In the *+/+* rats, the administration of BSO caused a slight but significant increase in the relative kidney weight (1.05 ± 0.06 versus 0.89 ± 0.04% of body weight, *n* = 12) without any effect on renal histology or biochemical parameters.

Study 2

The administration of GME for 5 wk was well tolerated and did not result in any obvious toxicity or growth retardation. In addition, administration of GME did not have a significant effect on the development of renal cystic disease as reflected by renal weight, histological scores, and concentrations of plasma urea and creatinine (Table 3).

Study 3

Administration of BSO to cy/+ Han:SPRD rats for 5 wk resulted in growth retardation. Administration of BSO without or with Na₂SO₄ to cy/+ rats resulted in a marked aggravation of renal cystic disease as reflected by kidney weights, histological scores, and concentrations of plasma urea (Table 4, Figure 3). These effects were not prevented by the simultaneous administration of GME. Although the rats treated with BSO tended to have lower concentrations of plasma bicarbonate, the differences were not statistically significant. The urinary excretion of ammonia was similar in the four groups. The administration of BSO to +/+ rats resulted in growth retardation similar to that observed in cy/+ rats and a slight but significant increase in the relative kidney weight (1.02 ± 0.02 versus $0.76 \pm 0.03\%$ of body weight, $n = 6$) without detectable effects on renal histology or biochemical parameters.

Discussion

The results of this study demonstrate that administration of BSO enhances the development of PKD in cy/+ Han:SPRD rats. BSO is a specific inhibitor of γ -glutamylcysteine synthetase, an enzyme that catalyzes the synthesis of γ -glutamylcysteine from cysteine and glutamate (17). Glutathione is

formed by a combination of γ -glutamylcysteine and glycine by the action of glutathione synthetase. BSO does not undergo metabolism *in vivo*, is excreted unchanged into the urine, is well tolerated in chronic animal experiments, and is not known to have side effects other than those related to the depletion of cellular glutathione. In our study, the administration of BSO resulted in a marked reduction in the renal levels of glutathione. Therefore, it seems likely that aggravation of renal cystic disease induced by BSO is due to the cellular depletion of glutathione. Because glutathione is the most abundant cellular thiol and plays a major role in scavenging reactive oxygen species, these observations are consistent with the hypothesis that alterations in redox metabolism may alter the development of PKD in Han:SPRD rats.

As reported previously, the administration of GME significantly increased renal levels of glutathione. GME may increase the levels of glutathione by a number of mechanisms. It was initially thought that the effectiveness of GME in increasing the cellular levels of glutathione was because of the capacity of this compound to enter the cells more effectively and its conversion into glutathione intracellularly (18). However, more recent studies indicate that, to a large extent, GME acts as a slow release form of extracellular glutathione, which is

Table 3. Effect of GME on renal morphology and function in male cy/+ Han:SPRD rats^a

Characteristic	Control ($n = 4$)	Na ₂ SO ₄ ($n = 6$)	GME ($n = 4$)
Body weight (g)	248 ± 17	253 ± 15	258 ± 13
Kidney weight (percentage of body weight)	2.43 ± 0.32	2.80 ± 0.55	2.42 ± 0.26
Cystic dilation (0 to 4)	2.44 ± 0.55	2.63 ± 0.61	1.88 ± 0.25
Interstitial inflammation (0 to 4)	2.56 ± 0.31	2.50 ± 0.74	2.19 ± 0.38
Plasma urea (mg/dl)	58.0 ± 13.8	57.5 ± 15.0	43.8 ± 4.9
Plasma creatinine (mg/dl)	0.40 ± 0.00	0.40 ± 0.06	0.45 ± 0.10
Plasma bicarbonate (mEq/L)	25.2 ± 1.0	25.2 ± 1.0	25.0 ± 0.8
Urine ammonia (μmol/h per 10 g body wt)	7.9 ± 2.0	8.9 ± 0.7	13.0 ± 6.2

^a The results represent mean ± SD. Abbreviations as in Table 1.

Table 4. Effect of GME on the BSO-induced aggravation of PKD in female cy/+ Han:SPRD rats^a

Characteristic	Control ($n = 5$)	BSO ($n = 3$)	BSO + Na ₂ SO ₄ ($n = 5$)	BSO + GME ($n = 5$)
Body weight (g)	192 ± 10	150 ± 16 ^b	149 ± 10 ^b	161 ± 12 ^b
Kidney weight (percentage of body weight)	1.61 ± 0.04	3.40 ± 0.96 ^b	3.51 ± 0.35 ^b	3.45 ± 0.67 ^b
Cystic dilation (0 to 4)	1.33 ± 0.20	2.50 ± 0.66 ^b	2.90 ± 0.52 ^b	2.45 ± 0.54 ^b
Interstitial inflammation (0 to 4)	1.45 ± 0.40	2.83 ± 0.29 ^b	2.95 ± 0.41 ^b	3.00 ± 0.35 ^b
Plasma urea (mg/dl)	36.3 ± 11.4	74.7 ± 15.1 ^b	62.3 ± 5.1 ^b	67.6 ± 17.3 ^b
Plasma creatinine (mg/dl)	0.38 ± 0.05	0.43 ± 0.11	0.40 ± 0.00	0.56 ± 0.11
Plasma bicarbonate (mEq/L)	23.8 ± 2.6	20.0 ± 1.6	22.8 ± 1.5	21.0 ± 0.7
Urine ammonia (μmol/h per 100 g body wt)	14.3 ± 4.6	13.0 ± 5.2	15.6 ± 3.8	18.8 ± 3.5

^a The results represent mean ± SD. Abbreviations as in Table 1.

^b $P < 0.05$ versus controls.

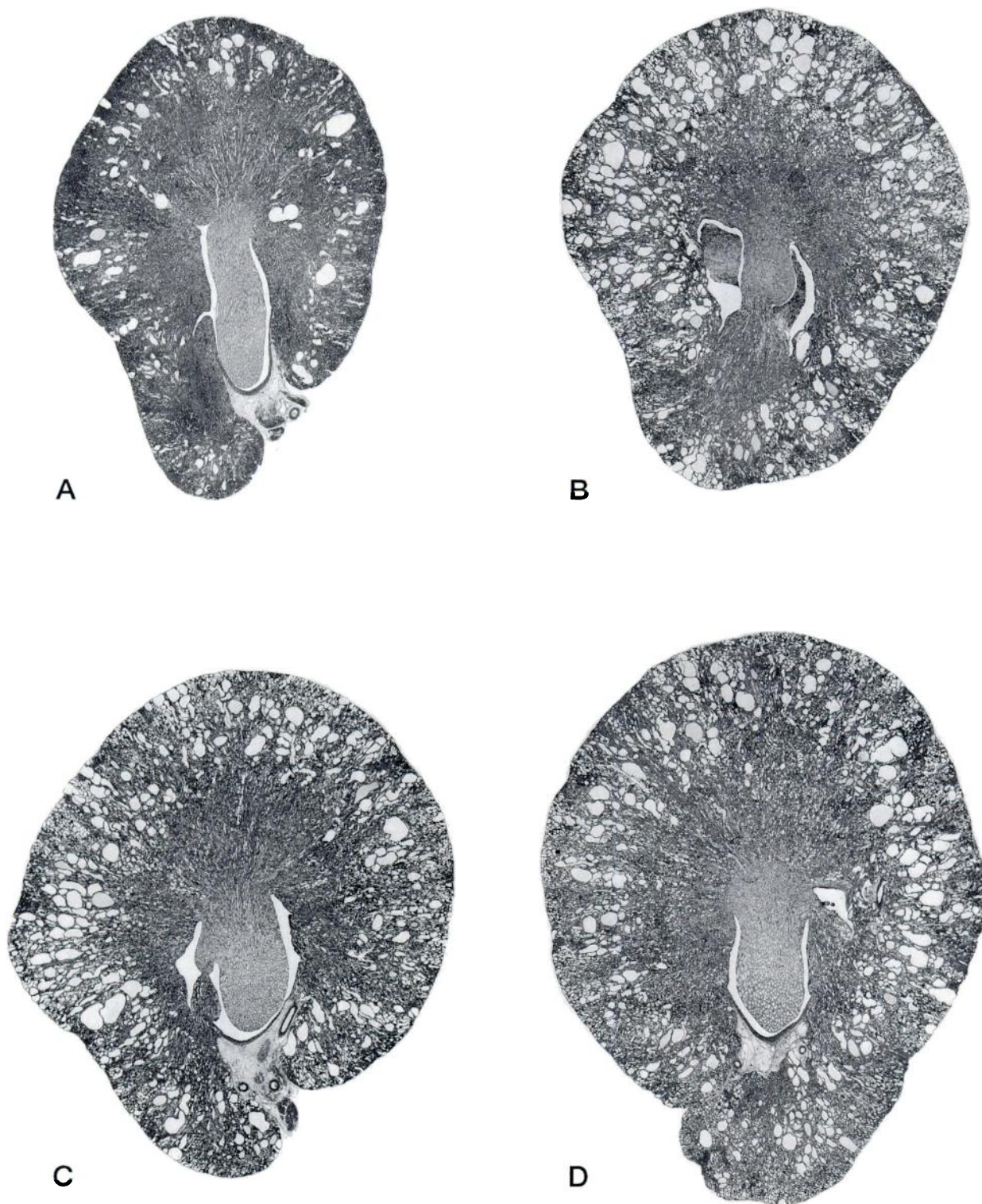


Figure 3. Representative transverse sections of kidneys from 8-wk-old female *cy/+* Han:SPRD rats from the control group (A) and from the groups treated with BSO (B), BSO and Na_2SO_4 (C), or BSO and GME hemisulfate (D). The administration of BSO increased the severity of the cystic disease, whereas the administration of Na_2SO_4 or GME hemisulfate had no additional effect.

then metabolized further by γ -glutamyl transpeptidase, thereby providing the precursor amino acids for *de novo* synthesis of glutathione (19). This may account for our ability to detect only modest increases in renal glutathione after the administration of GME to rats treated with BSO.

The results of the GME experiments may seem at first glance to contradict our interpretation of the effect of BSO administration on the development of PKD. GME did not have a protective effect when administered alone and did not reverse the effect of BSO when administered with this drug. Several

explanations may account for the failure of GME to influence the development of PKD. The administration of GME significantly increased the renal levels of glutathione at 2 h, but not at 12 h. γ -Glutamylcysteine synthetase is effectively feedback-inhibited by glutathione, and intermittent elevations of the cellular concentration of glutathione likely inhibit its *de novo* synthesis (17,18). Administration of glutathione or GME increases the cellular concentration of cysteine to a larger extent than that of glutathione (19,27,28). In the presence of BSO, which inhibits γ -glutamylcysteine synthetase, the increase of cysteine relative to that of glutathione may be even higher (19,27,28). Auto-oxidation of cysteine can result in the production of oxygen-free radicals (29–31) and can negate the possible beneficial effects of higher cellular levels of glutathione. Finally, administration of GME results in higher levels not only of GSH, but also of GSSG.

Studies in ischemic models of acute renal failure also suggest that the administration of large parenteral doses of glutathione or GME may not be the best way to assess the physiological role of locally produced glutathione in the renal defense against oxidative stress (32–35). Although the renal content of glutathione markedly decreases after temporary occlusion of the renal artery (32), the effects of parenteral administration of glutathione or GME have been inconsistent (32–37). In most studies, enhancement rather than reduction of the ischemic injury has been observed (33–35).

Previous studies have shown that oxidative stress induced by a diet deficient in vitamin E and selenium in 3-wk-old rats results in increased renal growth, interstitial inflammation, and renal ammoniogenesis without changes in plasma bicarbonate (38). It has been suggested that the accumulation of reactive oxygen species in the kidney stimulates renal ammoniogenic pathways and that the increased concentrations of ammonia result in enhanced renal growth and interstitial inflammation. Because we and others have shown that the administration of ammonium chloride markedly enhances the development of PKD in Han:SPRD rats (3–4), the possibility that BSO could stimulate renal ammoniogenesis must be considered. The results of our study, however, cannot be explained by this mechanism because the administration of BSO did not increase the urinary excretion or the renal concentrations of ammonia. Other mechanisms that may be directly related to alterations in redox metabolism must be involved. The higher levels of GSSG in the kidneys of cy/+ rats compared with +/+ rats in our study may also point to the participation of redox mechanisms in the pathogenesis of PKD in this animal model.

The hypothesis that redox metabolism is important in the pathogenesis of PKD is supported by recent observations in other models of renal cystic disease. Cystogens such as nordihydroguaiaretic acid and diphenylthiazole can cause oxidative stress by inhibiting catalase and epoxide hydrolase (39). Administration of diphenylthiazole causes enlargement of the endoplasmic reticulum and induces microsomal enzymes, which are an important source of reactive oxygen species. Another cystogen, diphenylamine, has pro-oxidant effects via a redox cycling mechanism (40). Lipopolysaccharide, a powerful inducer of oxygen-free radicals (41), triggers the development

of renal cystic disease in germ-free rats treated with nordihydroguaiaretic acid (42). Cyclosporine administration lowers intracellular glutathione in the renal cortex (43), increases the generation of oxygen-free radicals in renal and hepatic microsomes (43,44), and is associated with a greater prevalence and development of acquired renal cystic disease (45). Renal cystic disease in subtotally nephrectomized rats on a high-protein diet (46) is accompanied by an increased GSSG:GSH ratio (47). Gene expression of at least two antioxidant enzymes (plasma glutathione peroxidase and CuZnSOD) is markedly reduced in kidneys of cpk polycystic mice (48). Targeted disruption of the *Bcl-2* proto-oncogene, which has been shown to protect cells from apoptosis caused by oxidative damage or overexpression of *c-myc* (49), results in renal cystic disease (50,51). Finally, there is increasing evidence that oxygen-free radicals are important in the regulation of cell proliferation and apoptosis (52–54), both of which are prominent in human and experimental renal cystic diseases (55–57).

The mechanism by which alterations in redox metabolism may affect the development of PKD is not known. The controlled production of reactive oxygen species and the maintenance of the cellular redox state are implicated in the regulation of cell differentiation and proliferation (52–54) by a number of mechanisms (8–13,58–62). The results of the present study provide support to the evolving concept that alterations in redox metabolism may contribute to the pathogenesis of PKD.

Acknowledgment

This study was supported by National Institutes of Health Grant DK44863 (to Dr. Torres).

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