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# RAT KIDNEY FUNCTION RELATED TO TISSUE GLUTATHIONE LEVELS

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**Abstract**—Rat renal function was evaluated during acute depletion of glutathione (GSH) produced by different doses of diethyl-maleate (DEM). Significant alterations in renal function were observed when the GSH level diminished. The replenishment of GSH and the restoration of renal function were also investigated at various times after the injection. Similar time courses were observed of both the GSH level and renal functions, but the former was shortest. This suggests that the restoration to normal of GSH renal content was necessary in order to regain appropriate kidney function. Furthermore, the fact that impairment of sodium excretion occurred simultaneously with GSH depletion may be considered as evidence of the first event in GSH protective action. It may be hypothetized that the thick ascending limb is the principal renal target for this deficiency.

Glutathione (GSH) is a tripeptide present at high concentration in mammalian cells essentially in its reduced form. This molecule is the most abundant cellular peptide and a major nonprotein thiol of most cells.

GSH plays an important role in several biological phenomena, such as detoxification of electrophilic metabolites of xenobiotics [1] and protection against oxidative damage to membrane proteins and lipids resulting from the formation of hydrogen peroxide, superoxides and other free radicals [2]. As an example, a decreased GSH pool has been associated with decreased cell viability and increased sensitivity of cells to the effects of irradiation [3].

In this connection, GSH may have a special role in maintaining renal function and structure [4], and GSH depletion caused by acetaminophen and phenacetin overdosage has been invoked in the pathogenesis of chronic analgesic nephropathy [5]. Such studies, however, do not establish whether the functional defects observed in association with low levels of GSH in the kidney are specific consequences of GSH depletion.

In the present investigation the rat renal content of GSH was modified using diethyl-maleate (DEM), a potent GSH depletor with no intrinsic toxic effects, at least on liver [6, 7] and kidney cells [8]. The effects of different renal GSH levels on renal function were evaluated by administering different doses of DEM. The time courses of restoration of both the renal GSH pool and renal function were also analyzed.

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## MATERIALS AND METHODS

*Animals and treatment.* Male Wistar rats weighing 300–350 g were used. They were housed two per cage and maintained on a standard diet and water *ad lib.* until the experiment. Room temperature was kept at 21–24° with a 12-hr cycle of light and dark. All experiments were concluded between 1:00 and 2:00 p.m. in order to minimize the influence of circadian variations.

Four experimental groups were studied: (i) Rats (N = 10) injected with a single dose of DEM (4.0 mmoles/kg body wt, i.p.) which was reported to be effective in depleting liver GSH levels [9]; (ii) Animals that received decreasing single doses of DEM as follows: 2.0 mmoles/kg body wt, i.p. (N = 4), 0.75 mmole/kg body wt, i.p. (N = 10), and 0.25 mmole/kg body wt, i.p. (N = 7). After the injection, the animals of groups (i) and (ii) were kept in cages and used 1 hr later for renal clearance studies; (iii) Animals injected with the single maximal dose of DEM used in this study (4.0 mmoles/kg body wt, i.p.) that were prepared for clearance studies 1 hr (N = 4), 6 hr (N = 4), 24 hr (N = 6), 48 hr (N = 5), and 72 hr (N = 6) post-injection; (iv) Control rats (N = 8) that received sunflower oil alone, the solvent of DEM used in all the other experimental groups. These rats were also used for renal clearance studies 1 hr after the injection.

At the end of the experiments, the kidneys were removed promptly, and the GSH content was assayed in whole renal tissue homogenates. In a group of rats, GSH levels were measured in both cortical and medullary sections.

*Experimental procedures.* The animals were anesthetized with sodium pentobarbital (50 mg/kg body wt, i.p.). The femoral vein and femoral artery were

cannulated (P.E.-50, Intramedic, U.S.A.), and a bladder catheter (3-mm i.d.) was inserted through a suprapubic incision. Animals were maintained in restraining cages throughout the experiment to facilitate collection of urine.

A solution containing inulin (0.96 g/100 ml), *p*-aminohippuric acid (PAH) (0.3 g/100 ml) and D-mannitol (5.0 g/100 ml) was infused through the venous catheter employing a constant infusion pump (Unita Braun Melsungen, F.R.G.) at a rate of 4.5 ml/hr. After equilibration for 30 min, urine was collected during two 30-min periods. Blood from the femoral artery was obtained at the midpoint of each clearance period. Arterial blood pressure was estimated throughout the experiments with a manometer inserted in the femoral artery.

The glomerular filtration rate (GFR) was calculated from the clearance of inulin. Renal plasma flow was estimated by the clearance of PAH (CL PAH). The fractional excretion of water (FE % $H_2O$), sodium (FE % $Na^+$) and potassium (FE % $K^+$) were also calculated by conventional formulae for each animal.

*Analytical methods.* PAH concentrations in serum and urine were determined by Brun's method as modified by Waugh and Beall [10]. Inulin concentrations in the same samples were determined by the procedure of Roe *et al.* [11]. Sodium and potassium were measured by flame photometry and the volume of urine by gravimetry. Osmolality was determined in a vapor pressure osmometer (Wescor 5100 C, U.S.A.). Determination of renal GSH (nonprotein sulfydryls) was carried out in homogenates prepared in cold 5% trichloroacetic acid in 0.01 M HCl and measured as described by Ellman [12].

*Statistical analysis.* Statistical analyses were performed using an unpaired *t*-test, and multiple comparisons were made by analysis of variance and Scheffe' test; P values less than or equal to 0.05 were considered significant. Values are expressed as mean ± standard error.

*Chemicals.* All chemicals were of the highest grade commercially available. Inulin, PAH, D-mannitol, 5,5'-dithiobis-nitrobenzoic acid and DEM were purchased from the Sigma Chemical Co. (U.S.A.).

## RESULTS

*Effect of a single maximal dose of DEM on renal function and the GSH pool 1 hr after the injection.* The results are shown in Table 1. Blood pressure did not change during the experiments, and there were no differences between groups. When the GSH content was assayed separately in each zone of the renal tissue, the values were 3.31 ± 0.33 μmoles/g in control cortical tissue (N = 5) and 2.09 ± 0.10 μmoles/g in control medullary tissue (N = 5) versus 1.44 ± 0.22 μmoles/g (N = 5) and 0.70 ± 0.16 μmole/g (N = 5), respectively, in kidneys from DEM-treated rats.

*Relationship between GSH renal content and renal function impairment.* As presented in Table 2, different degrees of GSH depletion were obtained following the injection of different doses of DEM. The measured parameters of renal function are presented in Fig. 1. It can be seen (Fig. 1A) that the GFR and the CL PAH decreased in parallel with the impairment of GSH renal content.

Both the electrolyte and water excretion were also impaired, as indicated by their increase with an increase in the administered dose of DEM (Fig. 1B). The ratio of the osmolality of urine to plasma (Uosm/Posm) was also impaired with the reduction of renal GSH levels (Fig. 1C).

*Time course variations of renal GSH pool and function after a single dose of DEM (4 mmoles/kg body wt, i.p.).* As shown in Fig. 2, a marked and rapid depletion of GSH was observed after the injection of DEM. GSH levels 1 hr after treatment reached 24.5% of control values. Six hours after DEM injection, however, recovery was initiated, and renal GSH levels reached approximately 85% of control, and at 72 hr they were almost normal. Figure 2 shows the sharp depression in GFR and in CL PAH 1 hr after the injection of DEM (panel A) and the simultaneous increase in sodium and water excretion (panel B). At 48 hr after DEM injection, the parameters of renal function were no different from controls.

## DISCUSSION

There is considerable information regarding GSH metabolism in the kidney. Although the plasma concentration of GSH is only about 5 μM, its turnover is rapid, and more than 80% of plasma GSH is removed by the kidney in a single pass [13, 14]. Intracellular GSH is not distributed homogeneously in kidney tissue; the highest levels are found in the glomeruli and in the proximal convoluted tubules, but considerable amounts of GSH are normally present throughout the renal structure, including the cells of the thick ascending limb [15].

In the mammalian kidney GSH turnover is

Table 1. Effect of a single maximal dose of DEM (4.0 mmoles/kg body wt i.p.) on renal GSH levels and renal function 1 hr after the injection

| Rats | GFR (ml/min/100 g) | CL PAH (ml/min/100 g) | Uosm/Posm | FE % $Na^+$ | FE % $K^+$ | FE % $H_2O$ | $[GSH]_r$ (μmoles/g) |
|---|---|---|---|---|---|---|---|
| Control (N = 8) | 0.70 ± 0.02 | 4.47 ± 0.54 | 2.82 ± 0.11 | 0.71 ± 0.10 | 22 ± 8 | 2.57 ± 0.32 | 2.01 ± 0.05 |
| DEM-treated (N – 10) | 0.40 ± 0.03 | 1.60 ± 0.21 | 1.86 ± 0.11 | 1.91 ± 0.24 | 77 ± 6 | 4.64 ± 0.32 | 0.49 ± 0.02 |

Data are mean values ± S.E.M. All the differences between groups are statistically significant. ($P \leq 0.05$).

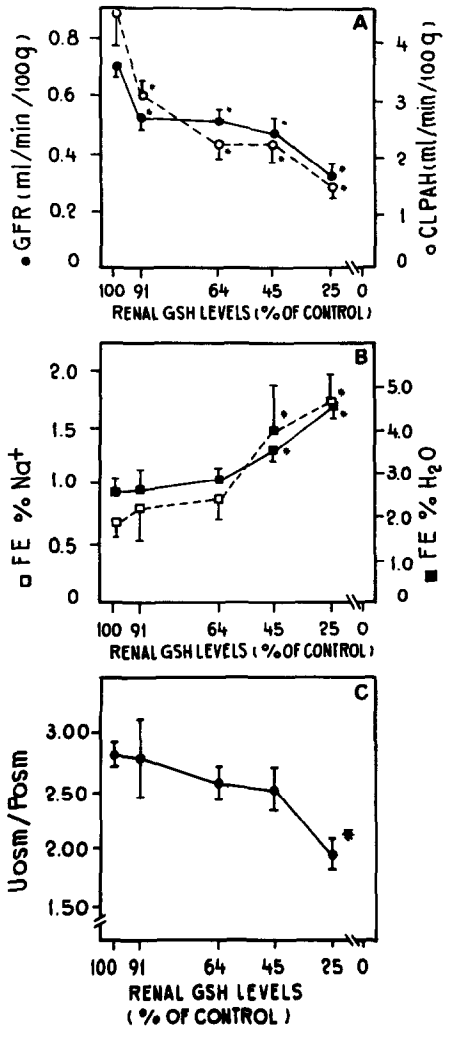


Fig. 1. Effect of renal glutathione levels on rat renal functions. All the parameters are plotted against GSH kidney content expressed as a percentage of control levels. Results are given as the mean ± S.E.M. Key: (*) $P < 0.05$, compared with control; FE % $Na^+$, sodium fractional excretion; and FE % $H_2O$, water fractional excretion.

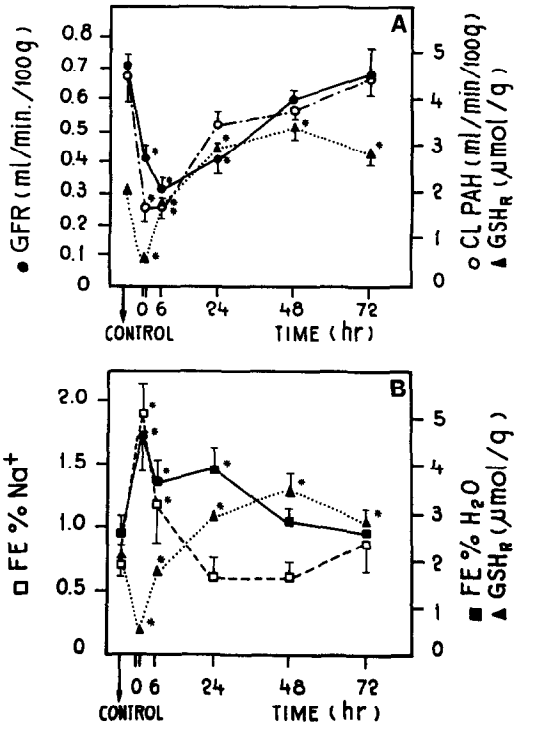


Fig. 2. Time course changes of renal GSH concentration ($GSH_r$) and rat renal functions after a single dose of DEM. Values are plotted against time course after i.p. administration of a single dose of DEM (4.00 mmoles/kg body wt). Results are given as the mean ± S.E.M. Key: (*) $P < 0.05$, compared with control.

extremely rapid with a half-life of only 30 min [14]. This rapid turnover implies not only GSH breakdown but also considerable and continuous GSH synthesis within the kidney. Therefore, GSH turnover could be considered as a special function of the kidney related to protection against foreign compounds.

In this connection, the present experiments were carried out to study the effects of GSH depletion on renal function, and the ability of the kidney to restore GSH levels and the related renal functions. DEM was used to modify renal GSH levels; it has been described as a potent GSH depletor [1]. This compound, due to the fact that it conjugates only with GSH, has a short duration effect, since GSH synthesis increases rapidly after acute depletion [16]. On the other hand, it has also been described as lacking other toxic effects, at least in liver [6, 7] and kidney [8].

When renal GSH concentration diminished, the renal parameters measured suffered deleterious modification, whereas blood pressure remained unchanged, eliminating general effects on the circulation. The fall in GSH tissue levels was

Table 2. GSH levels in renal tissue 1 hr after the injection of various doses of DEM

| DEM (mmoles/kg body wt i.p.) | $[GSH]_r$ (μmoles/g tissue) | N | % of Control value |
|---|---|---|---|
| None | 2.01 ± 0.05 | 8 | 100 |
| 0.25 | 1.83 ± 0.09 | 7 | 91 ± 4 |
| 0.75 | 1.28 ± 0.03 | 10 | 64 ± 1 |
| 2.00 | 0.91 ± 0.06 | 4 | 45 ± 3 |
| 4.00 | 0.50 ± 0.02 | 10 | 25 ± 1 |

Data are mean values ± S.E.M.; N = number of experiments.

accompanied by parallel reductions of GFR and CL PAH. If we were to assume that CL PAH can be used to estimate renal plasma flow, it would suggest that the filtration fraction remained constant in all experimental groups. These data together favour an increased resistance of the afferent arterioles. When the results on tubular functions were analyzed, an increased FE % $Na^+$ and FE % $H_2O$ also were seen, and the ratio Uosm/Posm diminished simultaneously with the reduction in renal GSH level.

After a single dose of DEM, the patterns of GSH and the renal function parameters changing over time were strongly related. It was noted, however, that restoration of renal functions with time was not as rapid as the repletion of renal GSH levels. This might indicate that restoration of the renal GSH pool is necessary prior to restoration of renal function. It was also noticed that the peak of sodium excretion impairment was most coincident with the GSH minimum level obtained after injection of DEM. This suggests that a primary defect of the sodium excretion mechanism was induced by GSH depletion.

All these data together reinforce the idea that the thick ascending-limb cells may have a special sensitivity to the effects of GSH depletion, as described by others [4, 17]. Thus, defective distal sodium reabsorption by this segment would be responsible for an augmented delivered load of sodium and water to the distal portions, causing increased potassium secretion. Simultaneously, a diminution in the medullar interstitial osmolality could be expected as shown by the alteration in Uosm/Posm ratio. Moreover, defective distal sodium reabsorption might conceivably cause reduced GFR and CL PAH by negative tubuloglomerular feedback [18].

Nevertheless, a direct effect of GSH depletion in each zone of the nephron could not be discarded. In this sense, the GSH levels measured in cortical and medullary slices reveal a marked diminution, probably higher in the medulla, but significant in both zones.

The results of the present study suggest a close relationship between renal GSH levels and renal function and, also, a marked ability of renal tissue to replenish its GSH content after a maneuver that decreases GSH concentration without altering its metabolic pathways, such as the presence of DEM.

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