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Developmental and Diamide-Induced Differences in Rat Renal Cortical Glutathione Levels[1] (40728)

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Many conditions which adversely affect metabolic functions in adult rat kidney have now been shown to have little or no effect upon these functions in newborn rat kidney (1–3). Presently there are several reports in the literature concerning the response of intracellular reduced glutathione (GSH) in adult rat kidney cortex slices to a variety of incubation conditions (4–7). However, no information is available concerning the GSH levels of newborn kidney and the response to these conditions. We have, therefore, studied GSH levels in newborn Sprague–Dawley rat kidney slices under standard incubation conditions and in the presence of diamide [diazene dicarboxylic acid bis(*N*,*N*-dimethylamide)]. A comparison of these data with those obtained from adult rat kidney cortex comprises the basis for this report.

Materials and methods. Adult male (150–200 g) and pregnant Sprague–Dawley female rats 14 days postconception were purchased from Charles River Breeding Laboratories (Wilmington, Mass.). Newborn pups were used within 72 hr after birth without regard to sex. The animals were stunned and decapitated; the kidneys were rapidly removed and placed in ice-cold Krebs Ringer bicarbonate buffer, pH 7.4 (KRB). Kidney cortex slices were made as previously described (8). Incubation flasks used in these experiments contained either three adult slices weighing 60–100 mg or 10 newborn cortical slices averaging 40–50 mg. The technique used for incubation of the slices has been described previously (8, 9). Reduced glutathione was determined colorimetrically according to the Ellman method (10) as modified by Tietze (11). Results are expressed in milligrams of GSH per gram wet weight. Statistical analysis was performed using Student's *t* test.

Results and discussion. Incubations of up to 90 min were carried out, using both newborn and adult kidney cortex slices in parallel experiments. The data shown in Table 1 demonstrate the striking ability of the newborn slice to maintain constant GSH levels throughout the incubation, while adult GSH levels fell approximately 50% under identical conditions. These observations parallel those in a recent report on free amino acid pools in renal cortex (12). These authors observed that intracellular free amino acid pools in newborn cortex, incubated under conditions identical to those used in the present experiments, remained constant or increased over 120 min. This was not the case, however, in adult renal cortical slice, in which total free amino acid pools dropped to approximately 50% of control levels within 120 min. Among the individual amino acids found to increase in the newborn with incubation were glutamic acid and glycine, both of which are biosynthetic precursors of glutathione. It is possible that the slow efflux known to be characteristic of newborn renal cortex (8, 9) is related to the maintenance of high free amino acid pools and therefore GSH levels in the newborn.

The effect of diamide, a compound known to oxidize reduced glutathione, was tested in both newborn and adult preparations. Varying concentrations of diamide were added to the incubation flasks and the incubations carried out for 30 min. The results of these experiments are shown in Fig.

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TABLE 1. EFFECT OF INCUBATION CONDITIONS ON INTRACELLULAR GLUTATHIONE[a]

	Time of incubation (min)					
	0	5	15	30	60	90
Newborn	0.604 ± 0.037 (8)	0.613 ± 0.021 (6)	0.576 ± 0.036 (6)	0.627 ± 0.021 (8)	0.579 ± 0.027 (6)	0.569 ± 0.038 (7)
Adult	0.652 ± 0.037 (8)	0.635 ± 0.041 (6)	0.586 ± 0.035 (6)	0.480 ± 0.026 (8)	0.367 ± 0.041 (5)	0.336 ± 0.021 (6)
P	<0.2	<0.6	<0.8	<0.001	<0.001	<0.001

[a] Slices were incubated under standard conditions, as described in text. Intracellular glutathione was measured by the method of Tietze (11), and results are expressed in mg/g tissue. Slices measured at "0" time were assayed immediately without incubation. Numbers in parentheses are the numbers of separate determinations, and the values given represent the means ± standard errors. Probabilities were calculated using Student's *t* test.

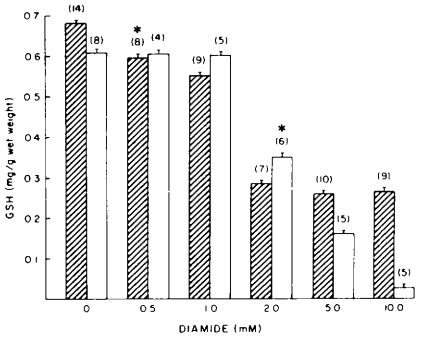

FIG. 1. Effect of diamide on renal cortical GSH levels. Slices from newborn (open bars) and adult (shaded bars) Sprague–Dawley rats were incubated in the presence of various concentrations of diamide for 30 min. GSH levels were measured by the method of Tietze (11). GSH is expressed as mg/g wet wt against the diamide concentration in mmole per liter (m*M*). Numbers in parentheses indicate the number of separate determinations. Asterisks (*) indicate the lowest concentrations of diamide at which GSH levels have been reduced significantly below those of the controls.

1, demonstrating the relative resistance of immature renal cortical tissue to oxidation of intracellular GSH by low concentrations of diamide, up to 1.0 m*M*. Thereafter, further increase in diamide concentration resulted in a sharp decrease in GSH. On the other hand, the response of adult cortical tissue to diamide exposure is somewhat different. At diamide concentrations up to 1.0 m*M* GSH levels in mature tissue appeared to fall linearly in proportion to increases in inhibitor. There was a marked decrease between 1.0 and 2.0 m*M* diamide, with little change thereafter, up to 10 m*M* diamide. Thus, the two types of tissue appear to respond to increasing diamide concentrations in opposite fashions. This may be due to the unique ability of the newborn cortical slice to maintain high levels of glutathione biosynthetic precursors, or it may reflect a difference in membrane permeability to diamide in newborn tissue.

The effect of 2 m*M* diamide with time on intracellular GSH was examined in newborn and adult slices. Figure 2 demonstrates the comparative sensitivity of mature and immature cortical slices to 2 m*M* diamide. GSH levels were reduced in the newborn at a far more rapid rate than the adult, reaching 52% of the control level

within the first 15 min of incubation, compared to the adult which required 20–30 min to reach approximately 50% of its control level. Thus, the greater sensitivity of the newborn tissue to diamide at concentrations greater than 1.0 m*M* is confirmed by the data shown in Fig. 2. Although the levels reached by 30 min in both tissues approximate each other, as shown for 2.0 m*M* diamide in Fig. 1, the rate at which GSH levels fall in the newborn is much faster than the adult. The finding that adult tissue GSH is maximally reduced by diamide concentrations in the range from 2.0–5.0 m*M* is in agreement with the observations of Reynolds *et al.* (13).

Our data may be interpreted to indicate a slower entry rate of diamide into the adult renal tubular cell, with a slower but more prolonged decrease in GSH levels. On the other hand, it is also possible that the newborn renal tubule has a slower rate of GSH turnover than the adult, resulting in a more rapid equilibration at a lower level. A third possibility is a difference in metabolic disposition of diamide. Although the present observations will not permit discrimination between these possibilities, they are sufficient to suggest that studies of GSH turnover in renal cortical tissue from rats of different maturational stages are necessary.

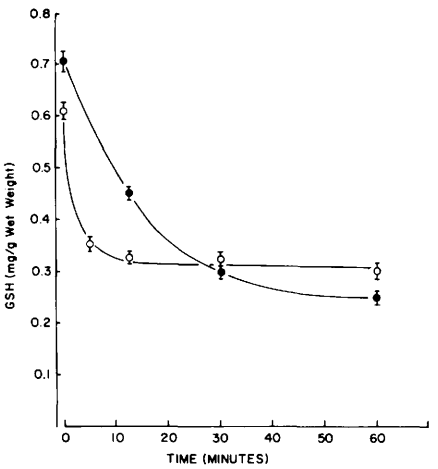

FIG. 2. Time course of the effect of 2 m*M* diamide on renal cortical GSH levels. Slices from newborn (○) and adult (●) animals were incubated in parallel experiments for the times shown, at which point GSH levels were determined. Each point shown represents the average ± standard error of at least three experiments.

The recent observation that 2.0 m*M* diamide decreases the initial rate of glycine uptake in adult rat brush border membrane vesicles (13) is immediately relevant to the present data. Such an observation suggests that diamide acts upon the structural conformation of the carrier or the whole membrane, or both—probably at the site of thiol groups located within the protein moieties. We have observed distinct differences in the rates of decrease in GSH levels of adult and newborn tissue induced by diamide. It is possible that this is related to interactions between the membrane thiol groups and resultant conformational changes of the membrane which, in turn, determine the rate of diamide penetration into the cell. If this is the case, our observations suggest distinct differences, as well, in the structures of newborn and adult renal tubular cell membranes. Diamide may well constitute a useful probe for further investigation of these differences.

Summary. The effect of prolonged incubations (up to 90 min) on reduced glutathione (GSH) levels has been studied and compared in renal cortical slices from newborn and adult Sprague–Dawley rats. While newborn levels of GSH remained constant during incubation, adult levels continued to fall, reaching about 50% of control levels by 90 min. Significant decrease in GSH of adult slices could be achieved by 30 min of incubation using 0.5 m*M* diamide, a known glutathione oxidant. On the other hand, 2.0 m*M* diamide, was required in newborn slices to achieve a significant reduction in GSH levels. Using 2.0 m*M* diamide in both newborn and adult slices, the time course of the effect of diamide on GSH was examined. Newborn GSH levels dropped to 52% of controls within 15 min, while adult slices were able to maintain levels between 40 and 50% of controls until about 30 min. These differences are attributed to developmental changes in membrane–diamide interactions, making diamide a potentially useful probe for investigation of developmental alterations in membrane structure.

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