Activity of Lactate and Glucose-6-Phosphate Dehydrogenases in Glomeruli and Proximal Tubules of Magnesium-Deficient Rats* (32970)

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Rats fed diets deficient in magnesium promptly develop hypomagnesemia and hypercalcemia. Focal deposition of calcium occurs about the cortico–medullary zones of the kidneys. Mitochondrial swelling is first evident in the distal segment of the proximal convoluted tubules and is accompanied by an increase of diaphorase and dehydrogenase activities (1). We undertook to provide quantitative data of enzymatic changes in the glomeruli and proximal tubules in the kidneys of magnesium-deficient rats. It was found that after 9 days on a magnesium-deficient diet, the activity of glucose-6-phosphate dehydrogenase diminished in the *pars recta* of the proximal tubules of the experimental rats as compared to the pair-fed controls.

Materials and Methods. Two enzymes, involved in the glycolytic pathway (lactate dehydrogenase) and the pentose phosphate shunt (glucose-6-phosphate dehydrogenase) were investigated. Fourteen female hooded rats (85 gm) were assigned to two groups and fed respectively magnesium-deficient and magnesium-supplemented diets similar to those used by MacIntyre and Davidsson (2). Rats were pair-fed; one member of the pair receiving the magnesium-deficient diet and one the magnesium-supplemented diet. The control animal on the magnesium-supplemented diet was offered the amount by weight of food eaten the previous day by his opposite number on the magnesium-deficient diet. Three rats from the experimental and control groups respectively were killed on the third day after the institution of the semi-synthetic diets. The remaining four pairs were killed after 9 days of feeding. The animals were stunned by a blow to the head and the kidneys were removed at once. One kidney was frozen immediately in liquid nitrogen while the other was fixed in formalin. We used the technique of microdissection of Lowry (3) as adapted by Kissane (4) to obtain glomeruli and proximal tubules from the frozen dried sample. Necrotic or calcified areas in the cortex were avoided. Hydronephrotic tubules were not included. Glomeruli and proximal tubules were separately incubated in specific buffer substrate solutions for a time designed so that the amount of change in substrate was proportional to the amount of tissue added (4,5). The oxidized or reduced pyridine nucleotides produced, an index of the enzyme activity, were measured by fluorometry (6). Serum magnesium and serum calcium were determined for all animals on blood taken at the time of killing. The significance between the mean values for each set of determinations was assessed by Student's *t* test.

Results. The serum magnesium of the experimental animals had decreased by the third day (0.99 meq/liter vs 1.52 meq/liter for controls) and was significantly lower at the conclusion of the experiment, at the end of 9 days (0.89 meq/liter vs 2.30 meq/liter) at which time hypercalcemia was also evident (6.86 meq/liter vs 5.84 meq/liter). Foci of calcification could be seen in histological sections of kidneys of magnesium-deficient rats fed on the diet for 9 days.

There was no difference in the activity of glucose-6-phosphate dehydrogenase or lactate dehydrogenase in the glomeruli of the experimental and control animals. There was an increase in activity of glucose-6-phosphate dehydrogenase but not in lactate dehydrogenase, in the proximal portion of the proximal tubule (*pars convoluta*) in the experimental animals. This difference was statistically significant at 3 days but not at 9 days (Table I).

There was no difference in the activity of lactate dehydrogenase in the distal portion of

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TABLE I. Enzymatic Activity of Glomeruli and Proximal Tubules.[a]

Kidney part	Enzyme: / Days on diet:	Lactate dehydrogenase 3	Lactate dehydrogenase 9	Glucose-6-phosphate dehydrogenase 3	Glucose-6-phosphate dehydrogenase 9
Glomeruli	Magnesium deficient	21.17 ± 1.30	31.76 ± 0.70	0.93 ± 0.10	1.06 ± 0.03
	Magnesium supplemented	25.43 ± 1.40	29.03 ± 2.0	0.94 ± 0.10	1.05 ± 0.05
Proximal tubules (*pars convoluta*)	Magnesium deficient	66.25 ± 2.42	69.5 ± 2.6	0.83 ± 0.04[b]	0.75 ± 0.09
	Magnesium supplemented	62.51 ± 2.19	77.5 ± 4.1	0.67 ± 0.05[b]	0.65 ± 0.07
Proximal tubules (*pars recta*)	Magnesium deficient	30.08 ± 1.30	33.66 ± 2.0	0.97 ± 0.10	0.71 ± 0.07[c]
	Magnesium supplemented	29.37 ± 1.90	33.91 ± 1.1	1.03 ± 0.10	1.37 ± 0.08[c]

[a] Activity expressed as μM of substrate converted per mg of dry weight of tissue per hour. Values represent means ± SEM.

[b] Difference between means of control and experimental group significant at $p \leq 0.05$.

[c] Difference between means of control and experimental group significant at $p \leq 0.01$.

the proximal tubule (*pars recta*) between the two groups of rats. But activity in this region of glucose-6-phosphate dehydrogenase while it showed only a slight decrease at 3 days was significantly diminished at 9 days in the experimental animals as compared to controls (Table I).

Discussion. This study fails to show the increase in lactate dehydrogenase postulated on the basis of histochemical (tinctorial) studies (1). Shank *et al.* (7) found a similar variation from tinctorial values when they studied the distribution of lactate dehydrogenase in the hepatic lobule by a quantitative histochemical method.

It is difficult at this stage to explain adequately the reason for the decrease in activity of glucose-6-phosphate dehydrogenase in the *pars recta* of the proximal tubules of magnesium-deficient rats. It is known that mitochondrial changes (swelling and decreased osmotic resistance) are an early although nonspecific manifestation of magnesium deficiency (1). One might speculate that decreased activity of glucose-6-phosphate dehydrogenase is a reflection of a greater segregation of intermediary substrates into a subcellular compartment, in this case the mitochondria, thereby making them less available to the pentose–phosphate shunt pathway.

Summary. Two groups of rats were fed respectively a magnesium-deficient and a magnesium-supplemented diet. No alteration in activity of lactate dehydrogenase in glomeruli and proximal tubules was evident in the magnesium-deficient animals after either 3 or 9 days on the diet. Activity of glucose-6-phosphate dehydrogenase increased significantly in the proximal portion of the proximal tubules after 3 days while it decreased in the distal portion of the proximal tubules after 9 days in the magnesium-deficient rats as compared to the controls. The change in activity of glucose-6-phosphate dehydrogenase in the distal portion of proximal tubules may be secondary to a redistribution of intermediary substrates into subcellular compartments.

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