Alterations in Hepatic Pyrimidine and Nucleic Acid Metabolism in Magnesium-Deficient Rats[1] (36542)

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In the liver ammonia is mainly utilized for the synthesis of carbamyl phosphate. This compound serves as a substrate for two enzymes, ornithine transcarbamylase (OTC) and aspartate transcarbamylase (ATC). The former belongs to the catabolic pathway leading to the synthesis of urea, while the latter forms part of the anabolic reactions which end in the formation of the pyrimidine bases for ribonucleic (RNA) and deoxyribonucleic acids (DNA).

Our earlier studies in Mg^{2+}-deficient rats demonstrated a reduced hepatic OTC activity but no significant change in carbamyl phosphate synthetase (CPS) (1), Fig. 1. We then postulated that those findings were suggestive of an increased incorporation of the carbamyl group of carbamyl phosphate into the pyrimidine precursors.

To test this hypothesis we have studied the activity of ATC and determined the levels of RNA and DNA in the liver of magnesium-deficient rats. We also studied the incorporation of ^{14}C-ureidosuccinic acid into RNA and DNA and determined the activity of liver ribonucleases.

Materials and Methods. Albino female rats (Holtzman) of initial weight 150–200 g were divided into control and deficient groups. Magnesium-deficient and control diets (pellets) were purchased from General Biochemicals, Chagrin Falls, OH. The control diet differed from the deficient only in the magnesium content. The consumption of food by the animals was estimated by adding 100 g of diet to each cage every other day and subtracting the residual amount at the end of each 2-day period.

Animals were maintained on experimental diets for 6 months. In order to reduce mortality the deficient animals received control diet for 2 consecutive days every 6 weeks. At the time of sacrifice, they had remained on the magnesium-deficient diet for 2 consecutive months. At the termination of the experimental period, 2.5 μCi of DL-ureido-^{14}C succinic acid (3.26 mCi/mmole, New England Nuclear, Boston, MA) were injected intraperitoneally. One hour later the animals were sacrificed by exsanguination and the livers were removed, weighed, and prepared for histological examination and biochemical assays.

Using a Potter–Elvenhjem glass homogenizer and a motor-driven pestle, three separate fractions from each liver were homogenized in ice-cold deionized water, 0.25 *M* and 1.44 *M* sucrose. The last two homogenates were used for the ATC and ribonuclease determinations, the first one for the remaining biochemical and isotopic studies, as described below.

RNA was determined by the Fleck and Munro procedure (2), and DNA according to Ceriotti (3). Aspartate transcarbamylase activity was assayed as suggested by Bresnick and Mosse' (4) and the ureidosuccinic acid produced was determined colorimetrically by the method of Koritz and Cohen (5) as modified by Gerhart and Pardee (6). Alkaline and acid ribonucleases were assayed according to Shortman (7).

Plasma magnesium determinations were performed by a standard analytical technique utilizing atomic absorption spectrophotometry. Liver protein content was determined by the biuret method using bovine albumin as standard (8). Tests for significance (*t* test

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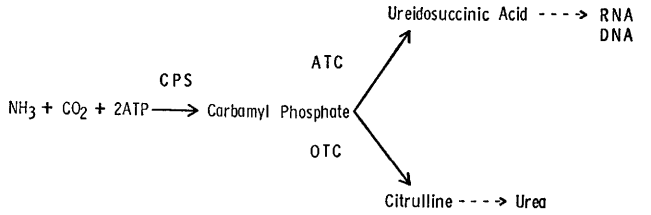

FIG. 1. Schematic representation of the pathways leading to the formation and utilization of carbamyl phosphate. CPS, carbamyl phosphate synthetase; ATC, aspartate transcarbamylase; OTC, ornithine transcarbamylase.

for nonpaired variates) were calculated with the aid of a digital computer.

Results and Discussion. Throughout the study the animals consumed nearly equal amounts of food with a daily mean per rat of 15.3 g for the deficient group and 15.6 g for the controls.

Typical manifestations of magnesium depletion developed as previously reported (9). Irritability and ear hyperemia promptly diminished after refeeding of the control diet.

Body and liver weight differed significantly between deficient and control animals (Table I). Although decreased rate of body growth is a known consequence of chronic Mg^{2+} deletion, no deficient biochemical or physiological reason for its occurrence is available. Likewise we are unable to explain the increased size and the flabby and friable consistency of the liver in the deficient animals.

The decreased plasma Mg^{2+} in the experimental group correlates well with the general manifestations of Mg^{2+} depletion observed in those animals.

The significant increase in hepatic ATC activity and in RNA and DNA content (Table II) supports the concept of an increased utilization of carbamyl phosphate as a substrate for pyrimidine synthesis. We believe that failure to find an increased incorporation of ^{14}C ureidosuccinic acid into the RNA nucleotides can be explained by an expanded nucleotide pool in the deficient rat, diluting the isotopic precursor. Therefore, when the radioactivity incorporated into RNA is expressed as a percentage of the total distintegrations per minute (DPM) found in the liver homogenate, no significant difference is seen. However, when the percentage of dpm incorporated into RNA is expressed in relation to 1 mg of RNA (RNA specific activity) a significant decrease in specific activity is seen in the deficient group.

Although the levels of alkaline ribonuclease were too low for accurate measurement, the activity of acid ribonuclease was significantly increased in the deficient group. Such an abnormality also has been reported in livers of Mg^{2+}-deficient guinea pigs (10).

Biochemical changes similar to those herein described have been reported in conditions in which there is an increased demand for protein synthesis, such as in the regenerating liver after partial hepatectomy (4, 11) and in

TABLE I. The Effects of Chronic Magnesium Deficiency on Body and Liver Weights, Liver Protein, and Plasma Magnesium of Rats.[a]

	Control group	Magnesium-deficient group	p value
No. of animals	8	7	
Final body weight (g)	320.5 ± 28.7	285 ± 17.25	$< .02$
Liver weight (g)	8.4 ± 0.36	9.2 ± 0.708	$< .05$
Total liver protein (mg)	935 ± 75.4	857 ± 74.1	NS
Plasma Mg^2 (mEq/liter)	1.64 ± 0.22	0.61 ± 0.17	$< .001$

[a] Values are means with SD.

TABLE II. The Effects of Chronic Magnesium Deficiency on Some Biochemical Parameters of Rat Liver.[a]

	Control group	Magnesium-deficient group	p value
Aspartate transcarbamylase (mmole/hr/100 g body weight)	157.2 ± 21.4	280 ± 41.8	< .001
RNA-P (mg/100 g body weight)	1.875 ± 0.228	2.565 ± 0.3	< .001
DNA (mg/100 g body weight)	7.073 ± 0.898	8.31 ± 1.37	< .05
dpm per RNA fraction (% of total liver dpm)	12.43 ± 1.65	12.11 ± 1.49	NS
dpm/mg RNA (% of total liver dpm)	6.68 ± 1.76	4.75 ± 0.655	< .01
Acid ribonuclease ($\Delta A_{260\ nm}$/30 min/100 g body weight)	16.27 ± 11	45.88 ± 27.61	< .05

[a] Values are means with SD.

some hepatomas (12). Despite a protein intake equal to that of the control group and biochemical alterations suggestive of increased protein synthesis, the Mg^{2+}-deficient rat demonstrates inability to generate protein, as manifested by diminished growth rate (9), low serum protein (13), and decreased synthesis of serum albumin (14).

Although our data suggest a compensatory biochemical response to a demand for a greater rate of protein synthesis, they do not explain why the latter does not occur. Because changes in the formation and stability of polysomes have been reported to occur in bacterial cultures maintained in a magnesium-deficient media (15) and smaller size polysomes have been described in the livers of magnesium-deficient guinea pigs (10), we feel that a similar biochemical abnormality may be an explanation for our findings. Further studies designed to evaluate the availability of nutrients to the liver cell, particularly amino acids, and to investigate the nature and functional characteristics of the excess RNA found in the deficient liver will contribute to the understanding of our investigation.

Summary. Chronic administration of magnesium-deficient diet to rats results in an increased hepatic aspartate transcarbamylase and acid ribonuclease activity. The liver content of RNA and DNA was also significantly increased. These findings suggest an adaptive biochemical response to activate protein synthesis.

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