free and conventional mice may reflect no more than the normal variability of response in such small groups of animals. Nevertheless, two facts from the present study suggest that the higher levels of spleen and liver interferon among germfree mice may be significant: (a) the tissue interferon levels were consistently higher among germfree mice whether tested at 2 hours or at 6 hours and whether tested in spleens or in livers, and (b) repetition of the test gave the same results: higher interferon levels in the spleen extracts from germfree mice.

The enhanced interferon response among the germfree mice may be due to higher storage levels of preformed interferon or of interferon precursors(14) because of their comparatively "pristine" condition. If so, the germfree animal may represent the optimum tool for demonstrating the existence of such entities.

Summary. A study was made to determine the relative capacity of germfree mice to respond to virus stimulation with interferon production *in vivo*. Germfree CFW mice responded to intravenously inoculated Newcastle disease virus with a pattern of serum interferon production similar to that of conventional mice. Moreover, the baseline inhibitory activity of sera from unstimulated control germfree and conventional mice were the same. However, there was some indication that the spleen and liver interferon responses of germfree mice may be higher than those of conventional mice. It is suggested that such higher responses in germfree mice might be due to higher storage levels of preformed interferon or of interferon precursors because of their comparatively "pristine" condition.

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Magnesium Deficiency and Urea Cycle Enzymes in Rat Liver.* (32414)

G. LIZARRALDE, V. E. MAZZOCCO,† AND E. B. FLINK
Department of Medicine, West Virginia University Medical Center, Morgantown

Induction of magnesium deficiency in the rat has been observed to produce several metabolic abnormalities. Recently we have reported a generalized aminoaciduria in the presence of an increase in the activity of sodium-potassium activated kidney adenosine triphosphatase, and the suggestion was made that the alteration in the urinary amino acid pattern was the result of renal tubular dysfunction rather than an "overflow of amino-aciduria"(1). Since protein metabolism was altered in the deficient animals, it seemed appropriate to study the influence of magnesium deficiency on the urea cycle enzymes. These enzymes have been shown to undergo adaptative changes whenever dietary protein

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† Trainee of USPHS (5-T1-AM-5381-04).

intake is quantitatively changed(2,3). The activity of the enzymes has been altered by adrenalectomy(4), growth hormone administration(5), or treatment with ethionine(6), carbon tetrachloride(7), or azo dyes(8). In all of these experimental designs, protein metabolism was affected either directly by stimulating catabolic or anabolic processes or indirectly by producing hormone imbalances with their concomitant alterations in general metabolic processes.

Materials and methods. Albino female rats (Holtzman) of initial weight of 150-200 g were divided into control and deficient groups. The magnesium deficient diet (General Biochemicals, Chagrin Falls, Ohio) was homogenized in water and each rat was given 7.5 g of the diet twice daily by gavage feeding; one milliequivalent of magnesium acetate was added to each feeding in the control rats. The diet was analyzed for magnesium content by methods previously reported(1) and with results as follows: control diet 2.0165 mEq/15 g; deficient diet 0.0165 mEq/15 g. At the end of 30 days, the animals were sacrificed by exsanguination, and the livers were quickly removed, weighed and homogenized in the ratio of 1 g of liver to 9 ml of 0.1% cetyltrimethyl ammonium bromide using a Potter-Elvehjem glass homogenizer and a motor driven pestle. The homogenates were then centrifuged at 4000 $\times$ g for 15 minutes at 5°C, and the clear supernate assayed for carbamyl phosphate synthetase, ornithine transcarbamylase, arginosuccinate synthetase, argininosuccinase and arginase by the method of Brown and Cohen(9). The results of the enzyme assays are expressed as micromoles of product formed per hour per g of liver.

Plasma magnesium determinations were done by the method of MacIntyre(10), and liver protein content was determined on the clear supernatant from the homogenates by the biuret method using bovine albumin as a standard(11).

Analysis of variance, standard error of the mean (S.E.M.), and paired Student's t test were performed according to Bailey(12).

Results and discussion. While the control animals gained weight and remained quite normal throughout the study, the deficient

TABLE I. Summary of Data Collected when Liver Enzymes of Urea Cycle from Magnesium Deficient Rats Were Assayed. Results are expressed as units and are given as means ± S.E.M. One unit is the amount of enzyme catalyzing the formation of one micromole of citrulline or urea/hour at 37° under the condition of the assay. P values greater than 0.1 are not given.

	Control group	Magnesium deficient group	p value
No. of animals	5	5	
Final body weight (g)	232.0 ± 5.7	210.0 ± 5.6	$<.05$
Liver weight (g)	6.4 ± .23	7.0 ± .71	
mg protein/g liver	86.25 ± 7.7	91.47 ± 6.5	
Plasma Mg^{++}	1.96 ± .09	.85 ± .11	$<.05$
Carbamyl phosphate synthetase			
Units/g of liver	328.3 ± 85.0	285.8 ± 46.0	
Units/total liver	1824.0 ± 130.0	2066.0 ± 210.0	
Ornithine transcarbamylase*			
Units/g of liver	9632.0 ± 822.0	7290.0 ± 422.0	$<.05$
Units/total liver	61426.0 ± 4550.0	49870.0 ± 2060.0	$<.05$
Argininosuccinate synthetase			
Units/g of liver	117.2 ± 21.0	97.92 ± 11.8	
Units/total liver	722.0 ± 123.0	780.0 ± 69.0	
Argininosuccinase			
Units/g of liver	441.9 ± 44.5	282.4 ± 65.0	$>.05$
Units/total liver	3532.0 ± 715.0	2234.0 ± 500.0	
Arginase			
Units/g of liver	13986.0 ± 2250.0	15200.0 ± 2460.0	
Units/total liver	84299.0 ± 14850.0	124760.0 ± 24800.0	

* 10 animals were used in each of both the control and the deficient group.

rats developed the known manifestations of magnesium deficiency(1). Final body weight was significantly less in the deficient animals; however, the liver weight and the liver protein content did not show any significant difference. Both tended to be slightly increased in the deficient group.

When the specific activity of the enzymes assayed was expressed either as micromoles of product formed per hour per gram of liver or as micromoles of product per hour per total liver, only ornithine transcarbamylase showed a statistically significant change (decreased) in the deficient animals. While the activity of carbamyl phosphate synthetase and argininosuccinase was decreased in the same group of rats, this difference was not statistically significant (Table I).

Decreased activity of ornithine transcarbamylase in rat liver has been previously reported in regenerating liver(13) in some hepatomas(14), and in rats fed the amino acid analog ethionine for a period of 2 weeks (6). These 3 conditions have been associated with increased synthesis of nucleic acids, and in the former two, an increase in the activity of aspartate transcarbamylase has also been described, suggesting that the decreased activity of ornithine transcarbamylase occurred in order to allow an increased incorporation of the carbamyl group into the pyrimidine precursors. In our experimental design and according to previously reported data(1), alterations related to protein metabolism in the magnesium deficient rat were mainly represented by a generalized aminoaciduria and an increase in total urinary nitrogen excretion.

The reason for the enzymatic changes observed in our magnesium deficient rats is unknown, but considerations which are being investigated include studies of the activity of the pathway leading to the synthesis of pyrimidines and the way in which magnesium deficiency may affect the enzyme kinetics.

Summary. A statistically significant decrease in ornithine transcarbamylase in livers of magnesium deficient rats is reported. Since such an isolated enzymatic deficiency has usually been described in conditions where increased synthesis of pyrimidines is required and in view of certain manifestations of protein deficiency present in the experimental animals, this enzymatic alteration suggests the possibility of an increased incorporation of carbamyl phosphate into pyrimidine precursors in an attempt to compensate for a deranged protein anabolism.

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