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ROLE OF DIETARY MAGNESIUM IN THE METABOLISM OF DRUGS BY NADPH-DEPENDENT RAT LIVER MICROSOMAL ENZYMES

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Abstract—Metabolism *in vitro* and *in vivo* of aniline and aminopyrine metabolism *in vitro* were significantly reduced in magnesium-deficient rats. Magnesium depletion did not alter the rate of metabolism *in vitro* of *p*-nitrobenzoic acid or pentobarbital, nor the duration of action of pentobarbital. Microsomal protein and RNA levels were maintained during magnesium depletion, but a significant decrease in the cytochrome P-450 content of the microsomes was noted in magnesium-deficient rats at approximately the same time as the lower rate of drug metabolism was noted. Hepatic enzyme activities and P-450 levels were restored to normal by feeding magnesium-deficient rats a magnesium-containing diet for 8 days. The results indicate that the impairment of hepatic drug metabolism in magnesium deficiency results from a decrease in the specific activity of the microsomal enzymes or from an alteration in the cytochrome P-450 levels.

THE NEED for information concerning the effects of the total environment on the ability of man to tolerate foreign compounds (i.e. drugs, pesticides, antioxidants) becomes more important as the use of these compounds increases. Kato *et al.*[1] have shown the effect of dietary protein levels and of starvation[2] on the metabolism of drugs. The effect of ascorbic acid-deficient diets on drug metabolism has also been reported.[3] Varying the level of thiamine in the diet has recently been shown to alter markedly the rate of hepatic drug metabolism,[4] but very little information has been reported on the effects of mineral imbalance or deficiency on drug metabolism. Of the minerals known to be essential in the diet, calcium[5] and zinc[6] deficiencies have been shown to alter the rate of hepatic metabolism of drugs.

With the development of suitable analytical techniques, the occurrence of hypomagnesemia in humans, due in part to prolonged use of diuretics, alcoholism, pregnancy, etc., has been shown to be more prevalent than earlier reports would have indicated.[7,8] It therefore seemed important to obtain information on the effect of a low daily intake of magnesium on the rate of hepatic drug metabolism. This report summarizes the effect of magnesium depletion on the rate of drug metabolism *in vitro* and *in vivo* by rat liver. Preliminary results on the role of dietary magnesium in the microsomal electron transport system will be presented.

MATERIALS AND METHODS

Male rats of the Wistar strain, weighing approximately 125 g, were divided into three comparable groups as described previously for zinc deficiency studies.[6] The control diet contained in per cent: vitamin-free casein, 25; corn starch, 20; dextrose,

30; corn oil, 14; minerals,[9] 4; vitamins,[6] 1; and cellulose, 6. To induce magnesium deficiency (Mg-deficient), rats were fed a similar diet except that magnesium was omitted from the mineral mixture. As determined by atomic-absorption spectrophotometry,* the magnesium content of the diets was 5 mg/100 g (deficient) and 78 mg/100 g (control). All animals received glass-distilled water *ad lib.*

Enzyme preparation. Microsomes, and crude enzyme preparations (17,500 *g* supernatants) for drug metabolism assays *in vitro*, were prepared as previously described.[6]

Enzyme assays. Assay conditions for the determination of the metabolism *in vitro* of pentobarbital, *p*-nitrobenzoic acid, aniline and aminopyrine and glucose 6-phosphate dehydrogenase activity were those previously described.[6] Drug metabolism *in vitro* was reported as millimicromoles metabolized per milligram of crude protein per hour. Isocitrate dehydrogenase activity was measured as outlined by Plaut.[10]

Microsomal NADPH-dehydrogenase was assayed as described by Gillette *et al.*[11] and microsomal NADPH-cytochrome c reductase activity was determined by the method of Williams and Kamin.[12]

Chemical analyses. Protein, microsomal RNA and cytochrome P-450 were determined as outlined previously.[6] The levels of P-450 were expressed as millimicromoles per milligram of microsomal protein.

Drug metabolism in vivo. As an indication of the metabolism *in vivo* of pentobarbital, sleeping times were determined as outlined previously.[6]

The metabolism *in vivo* of aniline was determined by monitoring 24-hr urine samples, collected in 1 N HCl, for free and total *p*-aminophenol. Aniline (60 mg/kg) was administered i.p. as the hydrochloride dissolved in isotonic potassium phosphate at pH 5·8. The total amount of *p*-aminophenol excreted was assayed after hydrolysis of an aliquot of urine in 1 N HCl at 100° for 1 hr. The color reaction for *p*-aminophenol was that described by Schenkman *et al.*[13]

RESULTS

The rate of metabolism *in vitro* and *in vivo* of aniline during magnesium depletion was the first indication that magnesium deficiency altered the rate of hepatic drug metabolism. The results of these studies are shown in Table 1. A lowered rate of aniline metabolism was noted, both *in vitro* and *in vivo*, a few days after the disappearance of the peripheral vasodilation which is the earliest gross manifestation of magnesium deficiency in rats.[7] The return to control values after 8 days of magnesium repletion indicated that the alteration in the rate of aniline metabolism was a direct result of the low level of magnesium in the diet.

Other hepatic drug-metabolizing enzyme systems studied were not found to be as sensitive to magnesium depletion as was aniline hydroxylation. A marked decrease in the rate of *N*-demethylation of aminopyrine *in vitro* was noted only after 14 days on the low magnesium diet (Table 2). The rate of aminopyrine metabolism *in vitro* returned to control values when magnesium-deficient rats were given the diet containing magnesium for 8 days.

No alteration in the rate of oxidation *in vitro* of pentobarbital was noted after 22 days on the low magnesium diet. No alteration in the sleeping time, after pentobarbital administration, was found during magnesium depletion, reflecting the equal rate of pentobarbital metabolism *in vitro* in magnesium-deficient and control rats.

* As outlined in: *Official Methods of Analysis*, tenth edn., p. 23. Assoc. Offic. Agr. Chemists (1965).

TABLE 1. METABOLISM *IN VITRO* AND *IN VIVO* OF ANILINE DURING MAGNESIUM DEPLETION*

Diet	Days on test	*In vitro*	*In vivo*	
		p-Aminophenol (mμmoles/mg protein/hr)	*p*-Aminophenol excretion (mg/24 hr)	
			Free	Total
Ad lib. control		2·64 ± 0·15		
Isocaloric control	11	2·45 ± 0·19	2·01 ± 0·23	6·46 ± 0·26
Mg-deficient		1·60 ± 0·11†	1·22 ± 0·15†	4·65 ± 0·28†
Ad lib. control		3·66 ± 0·29	1·47 ± 0·10	6·76 ± 0·30
Isocaloric control	22	3·88 ± 0·30	1·39 ± 0·21	6·92 ± 0·37
Mg-deficient		2·32 ± 0·21†	0·80 ± 0·07†	5·22 ± 0·23†
Isocaloric control		3·88 ± 0·10	1·70 ± 0·18	6·73 ± 0·22
Mg-deficient + Mg	24 + 8	3·73 ± 0·15	1·54 ± 0·11	6·49 ± 0·29

* Results are expressed as the mean value obtained with at least 4 rats ± S.E.M. Drug metabolism *in vitro* in rat liver 17,500 *g* supernatants was reported as mμmoles *p*-aminophenol formed/mg crude protein/hr.
† Significantly different from control values, P = 0·01.
‡ Significantly different from control values, P = 0·05.

TABLE 2. METABOLISM *IN VITRO* OF AMINOPYRINE DURING MAGNESIUM DEPLETION*

Diet	Days on test	4-Aminoantipyrine (mμmoles/mg protein/hr)
Isocaloric control		6·52 ± 0·56
Mg-deficient	14	5·23 ± 0·12†
Ad lib. control		7·56 ± 0·95
Isocaloric control	22	7·02 ± 0·81
Mg-deficient		4·11 ± 0·48‡
Isocaloric control		7·64 ± 0·56
Mg-deficient + Mg	24 + 8	7·13 ± 0·38

* Results are expressed as the mean value obtained with at least 4 rats ± S.E.M. Drug metabolism *in vitro* in rat liver 17,500 *g* supernatants was reported as mμmoles 4-aminoantipyrine formed/mg crude protein/hr.
† Significantly different from control values, P = 0·05.
‡ Significantly different from control values, P = 0·01.

TABLE 3. REDUCTION OF NADP *IN VITRO* BY RAT LIVER HOMOGENATES DURING MAGNESIUM DEPLETION*

Diet	Days on test	Dehydrogenase	
		Glucose 6-phosphate	Isocitrate
Ad lib. control		35 ± 2·9	157·6 ± 6·4
Isocaloric control	14	22·6 ± 3·9	152·0 ± 2·9
Mg-deficient		30·6 ± 1·6	148·2 ± 3·1
Isocaloric control		41·5 ± 4·5	190·2 ± 7·6
Mg-deficient	28	32·3 ± 1·6†	174·0 ± 4·4

* Results are expressed as the mean value obtained with at least 4 rats ± S.E.M. The values represent mμmoles NADPH/mg protein/min.
† Significantly different from control values, P = 0·07.

No alteration in *p*-nitroreductase activity was found even when rats were fed the magnesium-deficient diet for 28 days.

It is apparent from the data given in Table 3 that the decreased metabolism of aniline and aminopyrine noted in magnesium-depleted rats cannot be explained by a lowered rate of NADPH production. No alteration in glucose 6-phosphate dehydrogenase or isocitrate dehydrogenase activities was noted prior to 28 days on the low magnesium diet. Even at 28 days, the glucose 6-phosphate dehydrogenase activity in deficient rats was not markedly reduced from that in control animals.

Protein, RNA and cytochrome P-450 levels in liver microsomes from magnesium-deficient and control animals are summarized in Table 4. Magnesium depletion did

TABLE 4. MICROSOMAL PROTEIN, RNA AND CYTOCHROME P-450 LEVELS IN MAGNESIUM-DEFICIENT RATS*

Diet	Days on test	Protein (mg/g wet liver)	RNA (mg/g wet liver)	Cyt. P-450 (mμmoles/mg protein)
Isocaloric control		27·8 ± 0·7	4·57 ± 0·38	0·85 ± 0·05
Mg-deficient	11	27·5 ± 1·7	5·09 ± 0·21	0·67 ± 0·07†
Ad lib. control		24·4 ± 1·4	4·32 ± 0·12	
Isocaloric control	22	22·8 ± 1·5	4·48 ± 0·28	0·94 ± 0·08
Mg-deficient		24·0 ± 1·7	4·85 ± 0·25	0·52 ± 0·07‡
Isocaloric control				1·01 ± 0·09
Mg-deficient	24 + 8			0·94 ± 0·08

* Results are expressed as the mean value obtained with four rats ± S.E.M.
† Significantly different from control values, $P = 0{\cdot}05$.
‡ Significantly different from control values, $P < 0{\cdot}01$.

TABLE 5. MICROSOMAL NADPH-DEHYDROGENASE AND NADPH-CYTOCHROME *c* REDUCTASE ACTIVITIES DURING MAGNESIUM DEPLETION*

Diet	Days on test	NADPH-dehydrogenase	NADPH-cyt. c reductase
Isocaloric control		13·98 ± 0·74	35·40 ± 3·34
Mg-deficient	14	12·20 ± 0·81	35·49 ± 1·98
Isocaloric control		12·87 ± 0·71	38·00 ± 0·88
Mg-deficient	22	10·42 ± 0·39†	30·00 ± 0·77†

* Results are expressed as the mean value obtained with four rats ± S.E.M. The values represent mμmoles/mg protein/min.
† Significantly different from control values, $P = 0{\cdot}05$.

not alter the amount of microsomal protein or RNA. Similar data were obtained from grossly deficient rats (i.e. 28–30 days on test), indicating little or no effect of magnesium depletion on total microsomal protein synthesis. After 11 days on the magnesium-deficient diet, the cytochrome P-450 content of microsomes from deficient animals was significantly lower than that of controls. This decrease in P-450 content was a direct result of magnesium depletion and not some other dietary stress, since repletion with magnesium increased the level of microsomal P-450 to that found in control animals.

The effect of magnesium depletion on the activity of two enzymes of the microsomal

electron transport system is shown in Table 5. The decrease in aniline and aminopyrine metabolism (Tables 1 and 2) noted at 11 and 14 days on the magnesium-deficient diet cannot be explained by a decreased activity in microsomal NADPH-dehydrogenase or cytochrome *c* reductase. As indicated in Table 5, only after 22 days on a magnesium-deficient diet was any significant decrease in these two enzyme activities noted.

DISCUSSION

Since hypocalcemia often co-exists with hypomagnesemia,[7] and calcium deficiency has been shown to lower the rate of hepatic drug metabolism,[5] it was considered possible that the lowered rate of drug metabolism found in rats given a magnesium-deficient diet might be a result of a low daily intake of calcium. However, lowered rates of aminopyrine, hexobarbital and *p*-nitrobenzoic acid metabolism were found only after 40 days on a calcium-deficient diet,[5] whereas only 11–14 days on the magnesium-deficient diet were required to lower the rate of aniline and aminopyrine metabolism. At no time in the present studies was an alteration in the rate of *p*-nitroreductase activity noted and all magnesium-deficient rats returned to normal activity after repletion with magnesium alone. It may be concluded, therefore, that simple magnesium deficiency alters the rate of aniline hydroxylation and aminopyrine *N*-demethylation.

Before one can conclude that magnesium deficiency exerts a specific effect on drug metabolism, it is necessary to eliminate the possibility that the results obtained in the present study could be explained by impaired utilization of the diet. If reduced food intake or utilization, with resultant partial starvation, were responsible for the observations noted herein, one would expect lower body weights as well as a decrease in the protein content of the liver microsomes of the magnesium-deficient animals. This was not observed during our studies (Table 4). When the body weights of isocaloric controls and magnesium-deficient animals were compared, no significant difference was noted prior to 15 days on test. It has been shown by Dixon *et al.*[14] that the effects of starvation on the oxidative pathways of drug metabolism are noted within 36–72 hr. In the present study, it was found necessary to maintain animals on the magnesium-deficient diet for 11–14 days before any significant decrease in drug metabolism was observed either *in vitro* or *in vivo*. The oxidative metabolism of pentobarbital was not altered at any time during our work. It seems highly probable, therefore, that magnesium deficiency causes a decrease in the specific activity of the microsomal enzymes responsible for ring hydroxylation and *N*-demethylation.

During the early stages of magnesium deficiency, no significant decrease in the microsomal electron transport system was noted other than a decreased P-450 content of microsomes. This could lead to a decrease in drug metabolism, but raises the question of why no decrease in pentobarbital oxidation or *p*-nitroreductase activity was noted. A recent report[15] has identified some properties of two forms of P-450 found in microsomal preparations. Magnesium deficiency may alter the binding of certain drugs to one or more of these forms of P-450, or possibly alter the rates of synthesis of these heme proteins. Work now in progress will attempt to delineate the mechanism(s) by which magnesium depletion alters the rate of only two of the drug metabolic pathways which were investigated.

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