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Molybdenum Toxicity in the Rat.* (22358)

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The symptoms of molybdenum toxicity have been thoroughly described in several species(1), yet little is known of the underlying defects responsible for these symptoms, except that the resultant anemia can be remedied by copper sulfate supplementation. Weil-Malherbe and Green(2) and Lutwak and Sacks(3) have noted that compounds such as adenosine triphosphate (ATP) and acetyl phosphate are rapidly hydrolyzed in the presence of molybdenum salts. Since molybdate ions *in vitro* catalyze the acid hydrolysis of "high-energy" phosphate esters, the possibility existed that molybdenum might have a similar effect *in vivo*. If this were so, a decrease in the supply of "high-energy" phosphate compounds might then be fundamental to some of the symptoms of molybdenum toxicity. The acetylation of p-aminobenzoic acid (p-ABA) and the conjugation of glycine with benzoic acid have been used extensively in tests for liver function. Both reactions involve ATP, coenzyme A, magnesium ions, and the appropriate enzymes. Lipmann(4) has shown that ATP is involved in the acetylation of sulfonamides by pigeon liver preparations, and Chantrenne(5) has established a requirement for ATP in the enzymatic formation of hippuric acid. If molybdate ions *in vivo* should catalyze the hydrolysis of organic phosphate esters, then the supply of "high-energy" phosphate compounds might be reduced, and presumably, the reduction would be reflected in the ability of the animal to acetylate p-aminobenzoic acid or to conjugate benzoic acid.

The experiments reported here tested this hypothesis. Studies were also conducted on the effect of molybdenum toxicity on the alkaline phosphatase activity of various tissues.

Methods. The basal diet had the following

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percentage composition: casein, 20.0; glucose, 69.0; corn oil, 6.6; salt mixture, U.S.P. XIV, 4.0; choline chloride, 0.1; 2500 I.U. vit. A, 250 I.U. vit. D, 15 mg alpha-tocopherol in corn oil, 0.3. Other vitamins were provided in the following quantities, mg/100 g diet: thiamine hydrochloride, 0.5; riboflavin, 0.8; niacin, 4.0; pyridoxine hydrochloride, 0.5; calcium pantothenate, 4.0; biotin, 0.04; folic acid, 0.2; menadione, 0.5; vit. B_{12}, 0.003. The basal diet was supplemented with 4.0 mg copper/100 g in order to provide sufficient copper to prevent anemia. Albino rats of the Wistar strain were obtained from commercial sources and housed in individual cages. In the first experiment sodium molybdate was added to provide a dietary level of 0.08% molybdenum. The average initial weight of the 8 pairs of rats studied was 94 g. For each pair, the food intake of the control rat fed the basal diet was limited to the intake of the rat fed the molybdenum-supplemented diet. After one week on the experimental diets, the rats were placed in metabolism cages and injected intraperitoneally with a 2.5 mg dose of p-aminobenzoic acid. Urine was collected for 24 hours after injection. This procedure was carried out once a week for 4 successive weeks with each pair of rats. The animals were sacrificed at the end of 6 weeks. In the second experiment, the dietary levels of molybdenum were 0.08%, 0.12% and 0.14%. The average initial weight of the rats was 79 g. All diets were fed *ad libitum*. After 2, 3, and 4 weeks, rats from each group were injected with a 2.5 mg dose of p-aminobenzoic acid as described in Exp. 1. Total and free urinary p-aminobenzoic acid were measured by the method of Bratton and Marshall(6). Urinary hippuric acid was measured following injection of benzoic acid by the method of Alexander *et al.*(7). Alkaline phosphatase activity was determined by the method of Bessey *et al.*(8), in which the hydrolysis of p-nitrophenylphosphate to p-nitrophenol is measured. Activities are expressed as micromoles of phosphate released in 30 minutes per mg protein. Tissues were homogenized in appropriate amounts of ice water in a Ten Broeck homogenizer. The protein content of the homogenates was estimated by the method of Lowry *et al.*(9).

TABLE I. Effects of Dietary Molybdenum in Pair-Fed Rats.

Group	Body wt (g)	Urinary acetyl-p-aminobenzoic acid (% of total p-ABA excreted)	Liver alkaline phosphatase (%)
Control	191	49.0 (28–72)	100
+Mo 800 p.p.m.	188	53.6 (37–80)	177

Eight pairs of rats used. Urinary p-ABA values represent means of 4 replicates at 1 wk intervals or 32 assays. Range of values is given in parentheses. Liver alkaline phosphatase values are means of 8 assays.

Results. Growth and food intake. In the first experiment (Table I), the addition of molybdenum to the diets at a level of 0.08% caused a negligible depression in growth in contrast to previous results from this laboratory when younger animals from a different commercial source were used(10). Since a dietary level of 0.08% molybdenum failed to produce a severe toxicity in the first experiment, 3 levels of molybdenum were used in Experiment 2 and, in addition, younger animals were used. In this experiment, molybdenum, at all 3 levels, definitely retarded growth. The average weights at 5 weeks were 171, 121, and 87 g, respectively, for the rats fed the 0.08%, 0.12%, and 0.14% levels whereas the average weight of the control group was 218 g (Table II). Many of the rats developed an intermittent diarrhea, but there was no mortality. Prior to start of the experiment, all the rats gained normally on the basal diet. However, within 2 days after the start of the experimental period, the presence of molybdenum in the diet depressed food intake, especially at the two higher levels, and the weight gains reflected the reduced food intake. This was not due to decreased "palatability" of the diet since 5 rats given sodium molybdate by stomach tube, also showed poor food intake.

Acetylation of p-aminobenzoic acid. The addition of 0.08% molybdenum to the diet in the first experiment did not alter the ability of the pair-fed rats to acetylate a 2.5 mg dose of p-aminobenzoic acid (Table I). Likewise,

TABLE II. Influence of Various Levels of Dietary Molybdenum on Rat Growth, Tissue Phosphatase, and Acetylation of p-ABA.

Groups	Body wt (g)	Tissue alkaline phosphatase Liver	Heart	Kidney	Urinary acetyl p-ABA (% total p-ABA excreted)
		μM phosphate/30′/mg protein			
Control	218	.041 ± .004	.224 ± .021	3.709 ± .102	67 (47–87)
+Mo 800 p.p.m.	171	.085 ± .008	.178 ± .013	3.671 ± .192	53 (46–60)
+Mo 1200 p.p.m.	121	.263 ± .025	.203 ± .027	2.395 ± .364	60 (44–83)
+Mo 1400 p.p.m.	87	.220 ± .016	.218 ± .006	2.477 ± .166	52 (42–68)

Urinary p-ABA values were obtained on 6 rats/group; values in parentheses give range of values.

higher levels of molybdenum had little effect on the acetylation (Table II). Under these conditions, then, the supply of "high-energy" phosphate esters, and presumably the amount of acetyl coenzyme A available for the acetylation of p-aminobenzoic acid, was not affected by the presence of molybdenum *in vivo*.

Conjugation of benzoic acid. Following the injection of benzoic acid, the hippuric acid was isolated from urine in a one part butanol, 5 parts chloroform mixture, hydrolyzed and the glycine determined as formaldehyde with chromotropic acid. A considerable variation in response was observed to the intraperitoneal injection of 24 mg of benzoic acid. Both control and molybdenum-toxic animals were inconsistent in the excretion of hippuric acid, however, there did not appear to be any tendency for the toxic animals to excrete less of the conjugate. In one experiment of 10 control and 10 toxic rats the mean excretion was 2.2 and 2.8 mg glycine of hippuric acid, respectively, in 24 hours following administration. This supports the observations on p-ABA acetylation that molybdenum does not interfere to any extent in the occurrence of these endothermic reactions. It seems reasonable that molybdenum does not have an effect because of the higher pH of the tissue systems or due to a conjugation of the mineral with proteins of the tissues.

Tissue alkaline phosphatase. While the gross effects of molybdenum were rather slight in the first experiment, in every case, liver alkaline phosphatase activity was greater in the molybdenum-fed animal than in the pair-fed control (Table I). Although the significance of the increase in enzyme activity is unknown, the activity of this enzyme does seem to be a sensitive indicator of dietary molybdenum, and may be of potential use in the detection of molybdenum toxicity.

In the second experiment (Table II) all 3 levels of molybdenum significantly increased the activity of the liver enzyme ($P<0.01$), and the activity was significantly greater in the rats fed the 0.12% and the 0.14% levels than in the rats fed the 0.08% level ($P<0.01$). Increasing the level of molybdenum from 0.12% to 0.14% produced no additional increase in liver alkaline phosphatase activity. Heart alkaline phosphatase was unaffected.

Upon autopsy, the kidneys of the rats fed the 0.12% and the 0.14% levels of molybdenum were noticeably paler than those of the control rats or the rats fed 0.08% molybdenum, although the kidneys of the latter animals frequently were paler than those of the controls. These differences appeared consistently although the gross structure of the kidney seemed normal. This alteration, together with the possible role of alkaline phosphatase in the reabsorption of glucose in the kidney tubule(12), suggested that a study of kidney alkaline phosphatase might be valuable. The data in Table II show the effect of molybdenum on kidney alkaline phosphatase. In the rats which had been fed the diets for 4-7 weeks, the 0.12% and 0.14% levels of molybdenum significantly ($P<0.02$) depressed the activity of the kidney enzyme.

Some studies were made of the influence of dietary molybdenum on intestinal alkaline phosphatase activity. It was observed that after one week on the experimental regimen 5 rats receiving 1200 p.p.m. molybdenum had significantly less ($P<0.01$) intestinal phosphatase activity than 5 controls. Control and toxic values were 16.58 ± 1.64 and 4.46 ± 0.73 μM phosphate released/30'/mg protein respectively. Because of this rapid and pronounced effect, the influence of sodium molybdate on the intestinal phosphatase assay system was tried. Molybdate concentrations up to 10^{-4} M had no influence on a semi-purified preparation of intestinal phosphatase. At 10^{-3} M molybdate, a 20% inhibition of activity was observed. This is in agreement with experiments on liver preparations(10) and suggests that the decrease in activity observed in toxicity is probably a reflection of altered synthesis of the enzyme rather than a direct influence of the mineral on the enzyme assay system.

Summary. 1. High levels of dietary molybdenum did not alter ability of rat to acetylate p-aminobenzoic acid or to conjugate benzoic acid with glycine. 2. A depression of food intake and growth was evident within 24 hours after addition of 0.12% molybdenum to the diet. 3. Liver alkaline phosphatase activity was significantly increased in molybdenum-toxic rats whereas the activities of the kidney and intestinal alkaline phosphatases were significantly reduced.

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Structure and Function of Mitochondria in Irreversible Hemorrhagic Shock, I.*† (22359)

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Irreversible hemorrhagic shock is seen in humans and experimental animals who have suffered a prolonged period of hemorrhagic hypotension and are refractory to blood volume replacement therapy. Battlefield experience with massive blood transfusions in World War II and the Korean conflict(1) strengthened the belief of some workers that an irreversible intracellular metabolic change was taking place. Several studies involving tissue analyses have appeared during the last decade and have been ably summarized by Engel(2). In general, high levels of glucose lactate, pyruvate, amino acids and inorganic

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