The Effect of Molybdenum on Ascorbic Acid Metabolism in Rats

By N. SASMAL, N. C. KAR, DIPTI MUKHERJEE AND G. C. CHATTERJEE
*Departments of Applied Chemistry and Biochemistry, University Colleges of Science
and Technology, Calcutta 9, India*

(Received 22 September 1967)

1. The effect of dietary molybdenum on the growth rate and also on ascorbic acid metabolism in rats was studied. An excess of dietary molybdenum resulted in growth retardation and loss of weight. Tolerance to molybdenum was affected by the nature of the molybdenum salt administered. 2. Molybdenum ingestion altered certain aspects of ascorbic acid metabolism in rats. The conversion of D-glucuronolactone into L-ascorbic acid *in vitro* and the oxidative breakdown of L-ascorbic acid by liver enzymes decreased with high molybdenum intakes. The activity of liver uronolactonase was slightly inhibited. The activities of L-gulonate dehydrogenase and L-gulonate decarboxylase were not affected appreciably. 3. Molybdenum supplementation of the control diet resulted in an increase in ascorbic acid content of spleen and adrenal gland, and in a marked decrease in the urinary excretion of ascorbic acid and glucuronic acid. The implications of these findings are discussed.

The precise functional role of ascorbic acid in cellular metabolism is not clearly understood, although this vitamin has been found to be involved in the metabolism of various substances. The deprivation of ascorbic acid leads to the development of several metabolic derangements that can be cured by the administration of the vitamin. The interrelationship between minerals and ascorbic acid metabolism has lately been the topic of interest, and a few preliminary reports have appeared, mainly on the effect of metal ions on the tissue reserve of the vitamin in both rats and rabbits (Chatterjee, 1967). The effect of molybdenum on ascorbic acid metabolism has been investigated by Russian workers, who have noted increased ascorbic acid content in liver, muscles and blood of rats and rabbits after the administration of sodium molybdate or ammonium molybdate orally or by subcutaneous injection (Malevannaya, 1963). Under conditions of scurvy in guinea pigs, the molybdenum content of the liver is slightly decreased, but it is increased in the bones, with a significant increase in the kidney (Smolinska, 1962).

From these interesting leads it was thought worth while to study the various aspects of ascorbic acid metabolism in rats and their interrelationships with molybdenum. This paper deals with studies on the liver and the kidney enzymes related to the glucuronic acid pathway of ascorbic acid meta-

bolism (Ashwell, Kanfer, Smiley & Burns, 1961) in an attempt to correlate the interactions between molybdenum and ascorbic acid metabolism.

METHODS

Details of the methods for the determination of total ascorbic acid in tissues and the preparation of rat liver and kidney homogenates, of the incubation media for studying the biosynthesis of L-ascorbic acid, of the test system to study the catabolism of L-ascorbic acid, of the incubation system to study the synthesis of L-xylulose, and of the methods for the determination of uronolactonase activity, protein and glucuronic acid were all given in the preceding paper (Mukherjee, Kar, Sasmal & Chatterjee, 1968).

Determination of molybdenum in urine. The molybdenum content of the urine was determined by the thiocyanate-stannous chloride method (Sandell, 1959).

Animal experiments. 1. Feeding of various amounts of molybdenum as sodium molybdate ($\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$) to rats. Male albino rats weighing 60–80 g., which had been maintained on stock diet, were divided into several groups and given the experimental diets *ad libitum*. The basal diet contained 18% casein, 72.6% corn starch, 5% groundnut oil, 4% Steenbock salt mixture no. 40 (Steenbock & Nelson, 1923), 0.3% DL-methionine and 0.1% DL-tryptophan. The experimental diets contained in addition 0–5000 p.p.m. of molybdenum as $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, reagent grade. Each rat was given an oral supplement of water-soluble vitamins daily. Six to ten animals were used in each group. The animals were weighed twice a week. Rats were killed 2 or 4 weeks after being given the experimental diets.

2. Feeding of various amounts of molybdenum as ammonium molybdate $[(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}]$ to rats. The experimental diets in these cases contained 0–7000 p.p.m. of molybdenum as ammonium molybdate. Other conditions were the same as described above.

Fig. 1. Effect of various amounts of dietary molybdenum on the growth of rats. Conditions are given in the Methods section or the preceding paper (Mukherjee *et al.* 1968). Δ , Basal diet; $\circ$, basal diet+500 p.p.m. of Mo as sodium molybdate; $\bullet$, basal diet+500 p.p.m. of Mo as ammonium molybdate; $\square$, basal diet+5000 p.p.m. of Mo as sodium molybdate; $\blacksquare$, basal diet+7000 p.p.m. of Mo as ammonium molybdate; $\blacktriangle$, basal diet+1000 p.p.m. of Mo as sodium molybdate.

RESULTS

Growth studies. The data on the effect of various concentrations of dietary molybdenum on the growth of rats are presented in Fig. 1. Molybdenum given as ammonium molybdate had a growth-depressing effect even at 500 p.p.m. in the diet. Molybdenum when given as the sodium salt at dosages of 500 p.p.m. and 1000 p.p.m. was without any adverse effect on growth. But at the higher concentrations in the diet molybdenum was found to be toxic to rats irrespective of the nature of the salt used.

Tissue ascorbic acid concentrations. The data on the storage of the vitamin in liver, kidney, brain, spleen and adrenal gland are presented in Table 1. The ascorbic acid contents in liver, kidney and brain decreased, with a corresponding increase in contents in spleen and adrenal gland in rats given a high dose of molybdenum. Molybdenum-induced changes of tissue reserves of the vitamin did not depend on the nature of the salt administered.

Synthesis of L-ascorbic acid from D-glucuronolactone and L-gulonolactone by rat liver homogenates. The formation of L-ascorbic acid from D-glucuronolactone and L-gulonolactone by liver homogenates of rats fed on a diet supplemented with molybdenum has been studied and the results are presented in Table 2. From the results in Table 2 it can be calculated that there was a significant decrease in the synthesis of L-ascorbic acid from D-glucuronolactone by the liver tissues of rats fed on diets containing molybdenum either as sodium molybdate (1000 p.p.m. and 5000 p.p.m.) or as ammonium molybdate (1750 p.p.m.). The *P* values were <0.001 , <0.01 and <0.001 respectively. The synthesis of L-ascorbic acid from L-gulonolactone

Table 1. Influence of excess dietary molybdenum on tissue ascorbic acid content in rats

Each result is expressed as the mean $\pm$ S.D. of six experiments, each on a different animal.

Experimental period (days)	Dietary supplement	Concn. of ascorbic acid in tissue (mg./100g.)				
		Liver	Kidney	Brain	Spleen	Adrenal gland
29	None	47.8 $\pm$ 2.86	27.5 $\pm$ 4.43	57.5 $\pm$ 4.33	60.4 $\pm$ 6.52	256 $\pm$ 28.3
30	1000 p.p.m. of Mo as sodium molybdate	47.8 $\pm$ 4.03	35.7 $\pm$ 3.07	55.8 $\pm$ 3.64	57.4 $\pm$ 5.22†	436 $\pm$ 30.7*
15	5000 p.p.m. of Mo as sodium molybdate	40.3 $\pm$ 4.23	25.7 $\pm$ 4.82	53.0 $\pm$ 4.18	64.4 $\pm$ 6.12†	343 $\pm$ 21.5*
19	7000 p.p.m. of Mo as ammonium molybdate	40.5 $\pm$ 5.50	24.5 $\pm$ 3.27	52.4 $\pm$ 3.66	69.7 $\pm$ 4.95†	335 $\pm$ 26.8*

* Mean values significantly different from the controls (*P* values <0.001).

† Not significant.

‡ Significant (*P* <0.02).

Table 2. *Effect of added dietary molybdenum on the synthesis of L-ascorbic acid by rat liver homogenates*

Experimental details are given in the Methods section or the preceding paper (Mukherjee *et al.* 1968). Each result is expressed as the mean $\pm$ s.d. of four experiments, each on a different animal.

Dietary supplement	L-Ascorbic acid synthesized (μ moles/g. of protein)	
	From D-glucuronolactone	From L-gulonolactone
None	14.0 $\pm$ 1.28	10.2 $\pm$ 2.63
1000 p.p.m. of Mo as sodium molybdate	7.7 $\pm$ 0.12*	9.7 $\pm$ 3.14†
5000 p.p.m. of Mo as sodium molybdate	9.2 $\pm$ 1.71**	9.5 $\pm$ 1.29†
1750 p.p.m. of Mo as ammonium molybdate	6.7 $\pm$ 1.71*	9.0 $\pm$ 3.16†

* and ** Mean values significantly different from the controls (*P* values < 0.001* and < 0.01**).
† Not significantly different from the controls.

Table 3. *Effect of dietary molybdenum on the catabolism of L-ascorbic acid by rat liver dehydroascorbatase*

The conditions are given in the Methods section or the preceding paper (Mukherjee *et al.* 1968). Each result is expressed as the mean $\pm$ s.d. of six experiments, each on a different animal.

Dietary supplement	Dioxogulonic acid formed (μ mole/mg. of protein)
None	0.27 $\pm$ 0.035
1000 p.p.m. of Mo as sodium molybdate	0.34 $\pm$ 0.095*
5000 p.p.m. of Mo as sodium molybdate	0.16 $\pm$ 0.032†
7000 p.p.m. of Mo as ammonium molybdate	0.16 $\pm$ 0.026†

* Not significant.
† Significant (*P* values < 0.001).

Table 4. *Effect of dietary molybdenum on the activity of rat liver uronolactonase*

The conditions are given in the Methods section or the preceding paper (Mukherjee *et al.* 1968). Each result is expressed as the mean $\pm$ s.d. of four experiments, each on a different animal.

Dietary supplement	D-Glucuronolactone hydrolysed (μ mole/mg. of protein)
None	0.53 $\pm$ 0.025
7000 p.p.m. of Mo as ammonium molybdate	0.40 $\pm$ 0.039*

* Significant (*P* < 0.01).

in vitro by the tissues from molybdenum-fed animals was not affected appreciably.

Formation of 2,3-dioxogulonic acid from dehydro-ascorbic acid by rat liver homogenates. The data on the effect of molybdenum on the formation of

Table 5. *Effect of dietary molybdenum on the synthesis of L-xylulose from sodium L-gulonate by rat kidney homogenates*

The conditions are given in the Methods section or the preceding paper (Mukherjee *et al.* 1968). Each result is expressed as the mean $\pm$ s.d. of five experiments, each on a different animal.

Dietary supplement	L-Xylulose formed (μ mole/mg. of protein)
None	0.56 $\pm$ 0.027
1000 p.p.m. of Mo as sodium molybdate	0.56 $\pm$ 0.073
5000 p.p.m. of Mo as sodium molybdate	0.54 $\pm$ 0.052*
7000 p.p.m. of Mo as ammonium molybdate	0.52 $\pm$ 0.051*

* Not significant.

2,3-dioxogulonic acid from dehydroascorbic acid by liver dehydroascorbatase are presented in Table 3. It can be seen that high intakes of molybdenum (5000 p.p.m. and 7000 p.p.m.) markedly inhibited the activity of dehydroascorbatase. The *P* values were both < 0.001.

Conversion of D-glucuronolactone into D-glucuronic acid by rat liver uronolactonase. Dietary molybdenum at a concentration of 7000 p.p.m. was inhibitory to the conversion of D-glucuronolactone into its free acid by the action of rat liver uronolactonase. The results are presented in Table 4.

Synthesis of L-xylulose from sodium L-gulonate by rat kidney homogenates. Table 5 shows that molybdenum supplementation of the basal diet did not appreciably alter the activities of L-gulonate dehydrogenase and L-gulonate decarboxylase, which catalyse the conversion of sodium L-gulonate into L-xylulose in the presence of NAD.

Urinary excretion of molybdenum, ascorbic acid and glucuronic acid. The data on the excretion of molybdenum, ascorbic acid and glucuronic acid in urine of rats given a high dose of molybdenum are

Table 6. *Effect of molybdenum ingestion on the urinary excretion of molybdenum, ascorbic acid and glucuronic acid in rats*

The conditions and methods are given in the Methods section or the preceding paper (Mukherjee *et al.* 1968). The results are expressed as the mean $\pm$ s.d. values and the numbers in parentheses denote the number of rats used in each experiment.

Dietary supplement	Amount excreted in the urine (mg./day)		
	Molybdenum	Ascorbic acid	Glucuronic acid
None	0.102 $\pm$ 0.044 (9)	0.437 $\pm$ 0.144 (8)	3.38 $\pm$ 0.85 (9)
1000 p.p.m. of Mo as sodium molybdate	0.182 $\pm$ 0.039 (7)	0.313 $\pm$ 0.014 (6)	1.35 $\pm$ 0.49 (7)
5000 p.p.m. of Mo as sodium molybdate	0.679 $\pm$ 0.334 (4)	0.081 $\pm$ 0.026 (6)	1.84 $\pm$ 0.49 (6)
7000 p.p.m. of Mo as ammonium molybdate	0.355 $\pm$ 0.182 (4)	0.129 $\pm$ 0.027 (4)	2.68 $\pm$ 0.30 (4)

Table 7. *Effect of addition of molybdenum in vitro on the synthesis and degradation of L-ascorbic acid by rat liver homogenates*

The conditions are given in the Methods section or the preceding paper (Mukherjee *et al.* 1968). Molybdenum was added to the reaction mixture in the form of sodium molybdate. Each result is expressed as the mean $\pm$ s.d. of four experiments, each on a different animal.

Molybdenum added (μ g.)	L-Ascorbic acid formed from D-glucuronolactone (μ moles/g. of protein)	Dioxogulonic acid formed from dehydroascorbic acid (μ mole/mg. of protein)
0	11.6 $\pm$ 0.63	0.22 $\pm$ 0.038
10	11.4 $\pm$ 1.58*	0.19 $\pm$ 0.044*
500	11.1 $\pm$ 1.78*	0.12 $\pm$ 0.048†

* Not significant.

† Significant ($P < 0.02$).

presented in Table 6. Inclusion of molybdenum in the diet increased the molybdenum content of urine and decreased the excretion of both ascorbic acid and glucuronic acid in the urine of rats.

Effect of addition of molybdenum in vitro on the synthesis and the degradation of L-ascorbic acid by rat liver enzymes. In an attempt to pinpoint the exact enzymic step affected by dietary molybdenum, the effect of addition of varying amounts of molybdenum as sodium molybdate *in vitro* on the synthesis and the degradation of L-ascorbic acid has been studied. The results are presented in Table 7. In contrast with the decreased ascorbic acid-synthesizing capacity of liver tissues in molybdenum-fed rats, the addition of molybdenum *in vitro* to the reaction mixture containing normal rat liver homogenate failed to elicit any significant alteration in ascorbic acid synthesis. But the liver dehydroascorbate which catalyses the conversion of dehydroascorbic acid into 2,3-dioxogulonic acid was found to be significantly inhibited by the addition of molybdenum (500 μ g.) *in vitro* to the reaction medium.

DISCUSSION

The toxicity of excess of dietary molybdenum in rats has been demonstrated before by various

workers (Neilands, Strong & Elvehjem, 1948; Gray & Ellis, 1950; Jeter & Davis, 1954). The data presented in the present paper indicate that tolerance to molybdenum is affected by the chemical form in which the mineral is administered. Molybdenum ingested as ammonium molybdate is more toxic than sodium molybdate at lower intakes (500 p.p.m.). The reason for the greater toxicity of ammonium molybdate is not clear.

The data presented in the present paper indicate that molybdenosis results in diminished reserves of ascorbic acid in liver, kidney and brain, with a corresponding rise in spleen and adrenal gland. Some of these results seem to be at variance with those reported by Malevannaya (1963), who noted an increased ascorbic acid content in the liver of rats receiving excess of molybdenum. The discrepancy may be attributed to the difference in the mode of administration of molybdenum and the type of basal ration used.

High intake of molybdenum is found to exert a differential effect on the synthesis of L-ascorbic acid. The conversion of D-glucuronolactone into L-ascorbic acid by rat liver is significantly inhibited, whereas the synthesis of L-ascorbic acid from L-gulonolactone is not altered appreciably. Molybdenum also inhibits the oxidative breakdown of ascorbic acid. The synthesis of L-xylulose by rat

kidney enzymes, however, is not affected. The results indicate that molybdenum interferes with some aspects of ascorbic acid metabolism in rats.

The fact that addition of molybdenum *in vitro* to the reaction mixture containing normal rat liver homogenate results in an inhibition of the activity of dehydroascorbatase agrees quite well with the results obtained in experiments with molybdenum-fed rats *in vivo*. The result that the synthesis of L-ascorbic acid from D-glucuronolactone by normal rat liver enzymes is not inhibited by the addition of molybdenum to the incubation medium indicates its involvement in this process in some indirect manner, the exact nature of which is not known. From the results presented in the present paper it can be concluded that molybdenum does not augment the synthesis of L-ascorbic acid at all in the animal system, though an increase in the tissue reserves in spleen and adrenal gland has been observed. This contradicts the earlier suggestion put forward by Russian workers (Malevannaya, 1963), on the basis of increased tissue concentrations of ascorbic acid, that dietary molybdenum stimulates the synthesis of L-ascorbic acid.

The authors thank Dr S. C. Niyogy for his active interest in the work and Professor K. C. Bhattacharyya for his interest and the facilities offered. This research has been financed in part by Grant no. FG-In-172 A7-HN-4 made by the U.S. Department of Agriculture under Public Law 480.

REFERENCES

- Ashwell, G., Kanfer, J., Smiley, J. D. & Burns, J. J. (1961). *Ann. N.Y. Acad. Sci.* **92**, 105.
Chatterjee, G. C. (1967). In *The Vitamins*, 2nd ed., vol. 1, p. 407. Ed. by Sebrell, W. H. & Harris, R. H. New York: Academic Press Inc.
Gray, L. F. & Ellis, G. H. (1950). *J. Nutr.* **40**, 441.
Jeter, M. A. & Davis, G. K. (1954). *J. Nutr.* **54**, 215.
Malevannaya, E. M. (1963). *Vop. Pitan.* **22**, 63. Cited in *Chem. Abstr.* (1964), **61**, 9821c.
Mukherjee, D., Kar, N. C., Sasmal, N. & Chatterjee, G. C. (1968). *Biochem. J.*, **106**, 627.
Neilands, J. B., Strong, F. M. & Elvehjem, C. A. (1948). *J. biol. Chem.* **172**, 431.
Sandell, E. B. (1959). *Colorimetric Determination of Trace Elements*, vol. 3, p. 455. New York: Interscience Publishers Inc.
Smolinska, V. D. (1962). *Nauk. Zap. Med. Inst. I. Franka*, **5**, 19. Cited in *Chem. Abstr.* (1964), **60**, 4523a.
Steenbock, H. & Nelson, E. M. (1923). *J. biol. Chem.* **56**, 355.