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Effect of Dietary Molybdenum on Esophageal Carcinogenesis in Rats Induced by *N*-Methyl-*N*-benzylnitrosamine

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ABSTRACT

The influence of dietary molybdenum on esophageal carcinogenesis induced by *N*-methyl-*N*-benzylnitrosamine (2.5 mg per kg of body weight once a week for 20 wk s.c.) was studied in male F344 rats. The tumor incidence and tumor development in the esophagus were significantly lower in the rats in the high-molybdenum (2 ppm) diet group than in the rats in the low-molybdenum (0.032 ppm) diet group; *i.e.*, 44.4% (0.6 ± 0.8) and 73.2% (2.2 ± 2.0), respectively. The molybdenum levels in the esophagus-forestomach, liver, and serum were significantly higher in the high-molybdenum diet group than in the low-molybdenum diet group. Xanthine oxidase activity in the esophagus and forestomach in the high-molybdenum diet group was significantly higher than that in the low-molybdenum diet group, whereas liver and serum xanthine oxidase activities were not significantly different between these two groups. These results suggest that xanthine oxidase in the esophagus plays a significant role in the inhibitory effect of molybdenum on esophageal carcinogenesis.

INTRODUCTION

In 1966, Burrell *et al.* (1) reported that the Bantu of the Transkei district in the Republic of South Africa had a high incidence of esophageal cancer. They attributed the cause to the consumption of food locally grown in soil low in molybdenum. In 1980, Yang (2) showed that the molybdenum contents of serum, hair, and urine samples from a high-incidence area of esophageal cancer in China was significantly lower than that of samples from a low-incidence area. He also noted that an inverse correlation was found between the esophageal cancer mortality rate and the contents of molybdenum, magnesium, and zinc in hair samples. Luo *et al.* (3) reported that the addition of molybdenum to drinking water significantly inhibited *N*-nitrososarcosine ethyl ester-induced esophageal and forestomach carcinogenesis in SD rats. They also revealed that the addition of 200 ppm of tungsten, which is antagonistic to molybdenum (4), significantly countered the inhibitory effect of a low level of molybdenum naturally occurring in the diet.

The first indication of the biological role of molybdenum came in 1953 when two groups of workers independently discovered that the flavoprotein enzyme, XOD,[2] is a molybdenum-containing metalloenzyme that is dependent on the presence of this metal for its activity (5, 6). XOD, which oxidizes hypoxanthine and xanthine to uric acid, is the last enzyme in the pathway of the degradation of purine derivatives from nucleic acids and is assumed to be a rate-limiting step in purine catabolism (7).

In this study, we examined the effect of dietary molybdenum on esophageal carcinogenesis induced in rats by MBN (8) with special reference to the molybdenum levels and XOD activities in the upper gastrointestinal tract, liver, and serum.

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[2] The abbreviations used are: XOD, xanthine oxidase; MBN, *N*-methyl-*N*-benzylnitrosamine.

MATERIALS AND METHODS

Chemicals. MBN was purchased from Iwai Chemical Co. (Tokyo, Japan). The purity was checked by high-pressure liquid chromatography and found to be greater than 99%. Sodium molybdate, nitric acid, perchloric acid, hydrochloric acid, and olive oil (all certified to be of special reagent grade) were purchased from Nakarai Chemical Co. (Kyoto, Japan). Trizma base and NAD^+ were purchased from Sigma Chemical Co. (St. Louis, MO).

Animals. Weanling male F344 rats (21 days old) were purchased from Kitayama Laboratories, Inc. (Kyoto, Japan). The animals were housed in suspended stainless steel cages (4 rats/cage) in a controlled environment with the temperature and humidity maintained at 22 ± 1°C and 55 ± 5%, respectively, and a 12-h light, 12-h dark cycle. The animals were handled in accordance with the guiding principles in the care and use of animals approved by the American Physiological Society.

Diets. The basal semipurified diets (Table 1) were prepared by the Oriental Yeast Co. (Tokyo, Japan) and stored at 4°C for no longer than 1 mo before use. The basal molybdenum level in this diet was 0.032 ppm determined by a flameless atomic absorption spectrophotometer (Shimadzu Model AA-670G; Kyoto, Japan) (9) and did not contain any detectable tungsten by a colorimetric method (10). This basal diet and one supplemented with 2.0 ppm of molybdenum as sodium molybdate served as low- and high-molybdenum diets, respectively.

Experiment 1. A total of 102 rats were randomized to one of two diets, a low-molybdenum diet or a high-molybdenum diet. After a 4-wk period of adaptation, 45 rats in each group received 20 weekly s.c. injections of MBN dissolved in 0.2 ml of olive oil at a dose level of 2.5 mg/kg of body weight (8). Six controls from each group were given 20 weekly s.c. injections of 0.2 ml of olive oil alone. The rats were allowed to freely drink demineralized distilled water, which had no detectable molybdenum or tungsten. The water was provided in molybdenum-free stainless steel, butyl gum, and polycarbonate water bottles (Clea Japan, Inc., Tokyo, Japan). The stainless steel of the cages was certified to contain no detectable molybdenum or tungsten. The cages and water bottles were washed with 0.1 N HCl solution and rinsed with molybdenum-free deionized distilled water before use.

The rats were examined and weighed each week. They were killed when moribund, and autopsies were performed. Moribund rats exhibited cachexia and dyspnea, judged to be due to large obstructive lesions of the esophagus and the resultant pressure on the trachea. Rats that survived for more than 17 wk from the start of the experiment were included in the effective number of rats, because the first death was caused by esophageal tumors at 17 wk, and no death occurred by then with an exception of 4 rats in the low-molybdenum group, which died of pneumonia. The remaining rats including controls were killed at 28 wk from the start of the experiment.

The esophagus and forestomach were totally excised with the tongue, pharynx, and larynx and fixed in 10% buffered formalin. Other organs were examined carefully and fixed if any lesion was found. Esophageal tumors with diameters of more than 1 mm were excised and stained with hematoxylin-eosin for histopathological examination. The lesions of any other organs were also stained.

Lesions greater than 1 mm in diameter were counted and divided into six histological classes, *i.e.*, hyperplastic, mildly dysplastic, moderately dysplastic, severely dysplastic, carcinoma in situ, and infiltrative carcinoma (11, 12). The lesions which were classified as moderately or severely dysplastic were regarded as precancerous lesions.

Survival curves were analyzed by the generalized Wilcoxon test. The incidence of carcinoma was analyzed by the χ^2 test. Body weight and

Table 1 *Composition of basal diet*

Component	%
Glucose	61.0
Milk casein	18.0
Corn oil	8.0
Cellulose powder	5.0
Mineral mix[a]	6.0
Vitamin mix[b]	2.0
	100.0

[a] Mineral mix: $CaHPO_4 \cdot 2H_2O$, 14.56%; KH_2PO_4, 25.72%; NaH_2PO_4, 9.35%; NaCl, 4.66%; calcium lactate, 35.09%; iron citrate, 3.18%; $MgSO_4$, 7.17%; $ZnCO_3$, 0.11%; $MnSO_4 \cdot 4$ to $6\ H_2O$, 0.12%; $CuSO_4 \cdot 5H_2O$, 0.03%; KI, 0.01%.

[b] Vitamin mix (per 100 g): vitamin A acetate, 50,000 IU; vitamin D_3, 10,000 IU; vitamin E acetate, 500 mg; vitamin K_3, 520 mg; vitamin B_1 chloride, 120 mg; vitamin B_2, 400 mg; vitamin B_6 chloride, 80 mg; vitamin B_{12}, 0.05 mg; vitamin C, 3000 mg; D-biotin, 2 mg; folic acid, 20 mg; calcium-DL-pantothenate, 500 mg; paraamino benzoic acid, 500 mg; niacin, 600 mg; inositol, 600 mg; choline chloride, 20,000 mg.

number of tumor developments per rat were analyzed with the Student *t* test.

Experiment 2. A total of 120 rats were randomly divided into 2 groups and fed the low- or high-molybdenum diet in the same manner as in Experiment 1. After a 4-wk period of adaptation, each group was divided into two groups, one receiving a MBN s.c. injection and the other not. Eight rats in each group were killed at 0, 4, 11, 18, and 25 wk. The entire esophagus-forestomach, liver, and blood were removed from each rat and used for molybdenum content and XOD activity analysis.

Molybdenum Analysis. The molybdenum concentration was determined as follows. After wet digestion with nitric and perchloric acids, the digestate was extracted with hydrochloric acid (1:5) (13). Twenty μl of the extraction were applied to a flameless atomic absorption spectrophotometer and the absorption was measured at 313.3 nm (9).

XOD Activity Determination. XOD (NAD^+-dependent and O_2-dependent) activities were assayed by a modified method of Rowe and Wyngaarden (14, 15). The esophagus-forestomach and liver were removed, and homogenization was carried out in 0.1 M Tris-HCl buffer at pH 8.1 (1 g of tissue plus 5 ml of buffer) in a Potter-Elvehjem homogenizer with a Teflon pestle. The homogenate was sonically treated with a Branson Sonifier Model 200 (Branson Sonic Power Co., Danbury, CT) and centrifuged at 600 × *g* for 20 min at −4°C, and the supernatant fluid was centrifuged at 105,000 × *g* for 60 min at 4°C. Blood samples were centrifuged at 300 × *g* for 10 min. The supernatant fluid and serum were dialyzed against the same buffer at 4°C overnight and assayed for XOD activity and protein concentration. The reaction mixture was added to 0.01 ml of 0.5 mM NAD^+ solution, and the rate of change in absorbance at 292 nm was recorded by a Beckman Model DU-40 spectrophotometer (Fullerton, CA) at 37°C. Enzyme activity was expressed as units (the formation of 1 μmol of uric acid per min) per g of protein. Protein concentration was measured by the method of Lowry *et al.* (16). Statistics were analyzed by the Student *t* test.

RESULTS

Body Weight and Survival Curves: Experiment 1. There was no significant difference in body weight between the low-molybdenum and high-molybdenum diet groups (Fig. 1) or in the survival rate (Fig. 2). The mean body weight of both these groups receiving s.c. MBN injections reached a plateau at 15 wk after starting the experiment. Then, it decreased gradually to about 60% of the level of the control groups. Four rats in the low-molybdenum diet group died within 13 wk. Autopsies revealed no specific change other than pneumonia.

Tumor Incidence. The first death was caused by esophageal tumors 17 wk following the start of the experiment. A complete autopsy revealed that all the rats that died after 17 wk had developed multiple sessile or pedunculated lesions of the esophagus and localized thickening of the esophageal wall (Fig. 3). These rats developed cachexia or aspiration pneumonia caused

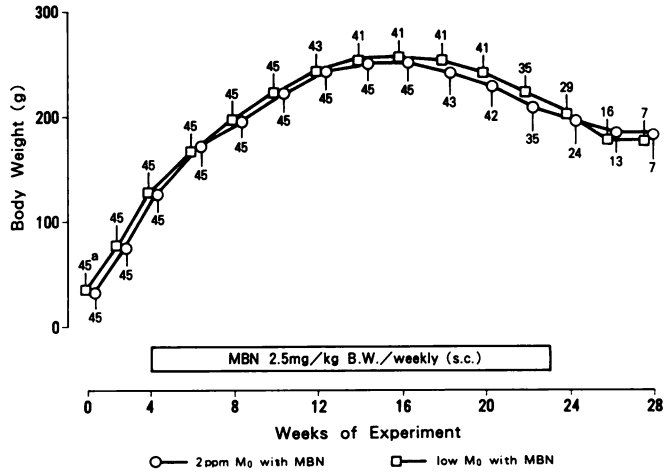

Fig. 1. Body weight of rats fed a high-molybdenum diet and treated with MBN (O) and those fed a low-molybdenum diet with MBN (□). *a*, number of rats surviving at each time point. *Mo*, molybdenum; *B.W.*, body weight.

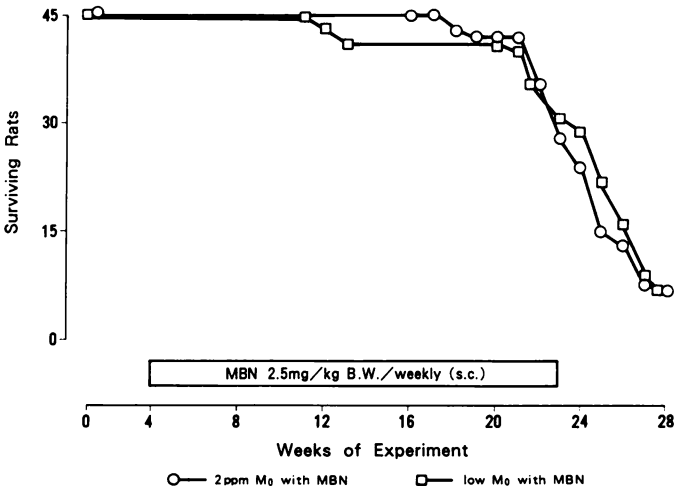

Fig. 2. Number of surviving rats fed a high-molybdenum diet and treated with MBN (O) and that of rats fed a low-molybdenum diet with MBN (□). *Mo*, molybdenum; *B.W.*, body weight.

by the obstruction by these lesions. The rats of the low-molybdenum diet group that died before 13 wk had no tumors. The development of esophageal carcinoma was significantly lower in the high-molybdenum diet group ($P < 0.01$) as shown in Table 2. Few lesions were found in the tongue, pharynx, larynx, or forestomach. There was no statistical difference in the tongue, pharynx, and larynx carcinoma between the low- and high-molybdenum diet groups. There was no carcinoma development in the forestomach. The induced carcinoma of all these organs was squamous cell carcinoma. The rats fed the high-molybdenum diet had significantly fewer tumors ($P < 0.001$), precancerous lesions ($P < 0.01$), and carcinoma ($P < 0.001$) than the rats fed the low-molybdenum diet (Table 3). No primary lesions of a neoplastic or preneoplastic nature were found in any other organs, and there were no metastases or invasions of adjacent tissues found.

Molybdenum Levels. In the esophagus-forestomach, the molybdenum levels in the rats fed the high-molybdenum diet were significantly ($P < 0.001$ to 0.01) higher than those of the rats fed the low-molybdenum diet (Fig. 4). In both of these groups, the presence or absence of MBN had no significant influence on the molybdenum concentration.

Serum molybdenum levels in the rats fed the high-molybdenum diet were significantly ($P < 0.001$) higher than those of

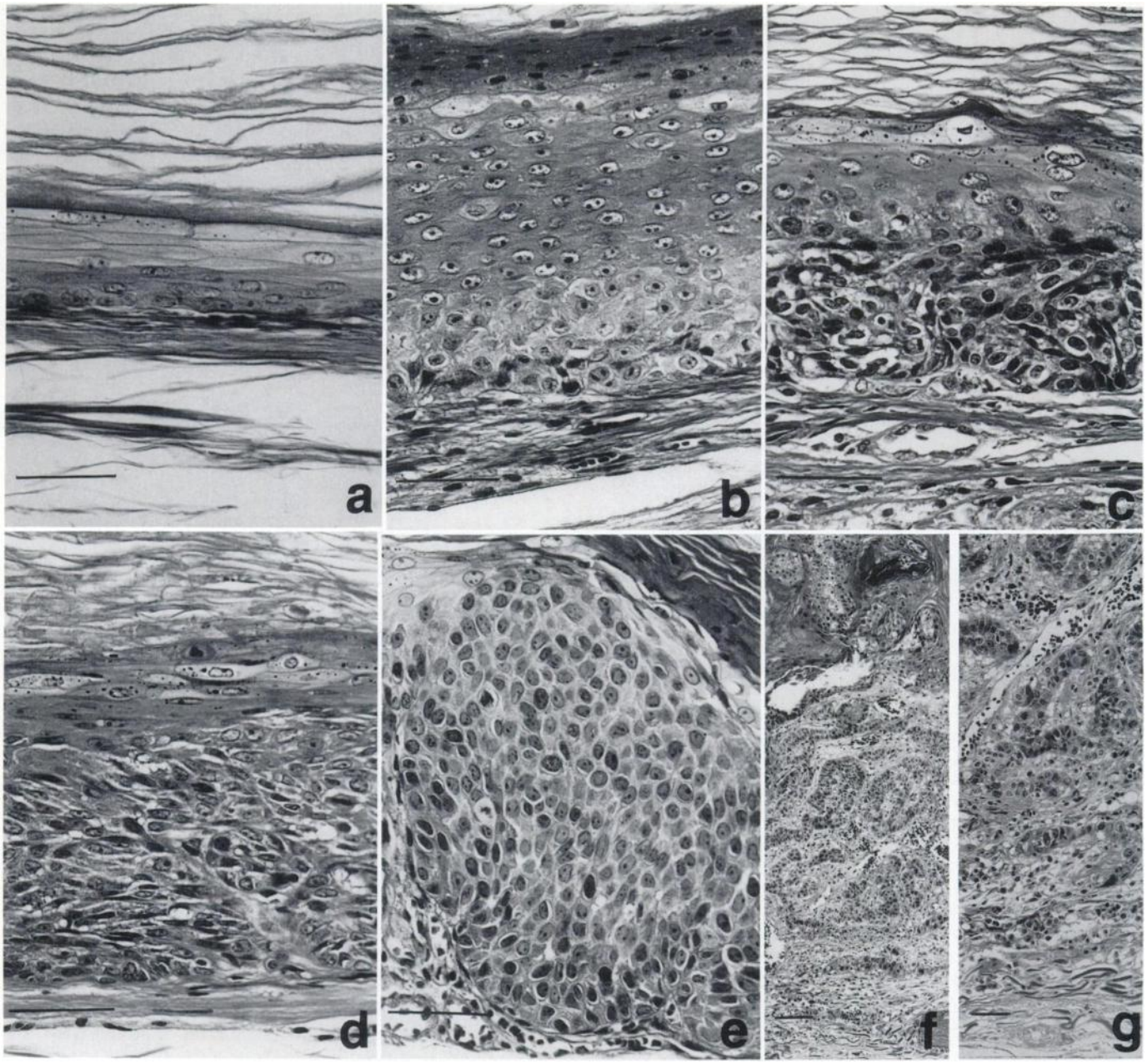

Fig. 3. Histopathological changes of rat esophagus. *a*, normal epithelium of esophagus consisting of a basal layer of prismatic cells, a layer of 2 to 5 rows of spinous cells with elongated nuclei, and a thin granular layer. H & E, × 100. *Bar* = 50 μm. *b*, mildly dysplastic lesion. It is characterized by thickening of the epithelium due to the increase in number and size of the cells. The large basal or spinous cells with large, light, round, or oval nuclei take up one-third of the thickened epithelium. Mitotic figures are frequent. H & E, × 100. *Bar* = 50 μm. *c*, moderately dysplastic lesion. The large basal or spinous cells take up one-half of the thickness of the epithelium. H & E, × 100. *Bar* = 50 μm. *d*, severely dysplastic lesion. Nearly all of the epithelium is composed of basal or squamous cells. Only a few layers of spinous and squamous cells remain on the surface. The stratified structure is disorganized. H & E, × 100. *Bar* = 50 μm. *e*, carcinoma in situ (squamous cell type). It is characterized by complete disorganization of the stratified structure, atypism of the spinous cells, and polymorphism. H & E, × 100. *Bar* = 50 μm. *f*, infiltrative carcinoma. Malignant cells penetrate the submucosa and infiltrate the muscular layer. H & E, × 25. *Bar* = 100 μm. *g*, higher power view of Fig. 3*f*. H & E, × 50. *Bar* = 50 μm.

the rats fed the low-molybdenum diet. In both of these groups, the presence or absence of MBN treatment had no significant influence on the molybdenum concentration.

Liver molybdenum levels of the high-molybdenum diet group were significantly ($P < 0.001$ to 0.05) higher than those of the low-molybdenum diet group. In the low-molybdenum diet group, there was a significant ($P < 0.05$) difference between the rats treated with MBN and those not treated with MBN at 11 wk.

XOD Activities. At 4, 11, and 18 wk, the XOD activities in the esophagus-forestomach of the rats fed the high-molybdenum diet were significantly ($P < 0.001$ to 0.05) higher than those of the rats fed the low-molybdenum diet (Fig. 5). There was a significant ($P < 0.01$) difference at 25 wk between these two groups of rats not treated with MBN. However, in the rats treated with MBN, there was no significant difference between these two groups. In MBN-treated rats of both groups, the XOD activities increased lineally after 4 wk and were always slightly higher than those of the rats not treated with MBN. However, there was no significant difference between these two groups.

Serum XOD activities showed no significant difference be-

Table 2 *Effect of dietary molybdenum on the incidence of carcinoma of the esophagus*

	Low molybdenum	2 ppm of molybdenum	*P* value
Effective no. of rats	41	45	NS[a]
No. of carcinoma-bearing rats	30 (73.2)[b]	20 (44.4)	<0.01

[a] NS, not significant.
[b] Numbers in parentheses, percentage of the number of carcinoma-bearing *versus* effective rats.

Table 3 *Incidence and histopathology of esophageal tumors in rats fed low- and 2-ppm molybdenum diets*

	Low molybdenum	2 ppm of molybdenum	*P* value
Effective no. of rats	41	45	NS[a]
Total no. of tumors	670	462	
No. of tumors/rat	16.3	10.3	<0.001
No. of precancerous lesions	262	179	
No. of precancerous lesions/rat	6.4	3.9	<0.01
No. of carcinomas	91	27	
No. of carcinomas/rat	2.2	0.6	<0.001

[a] NS, not significant.

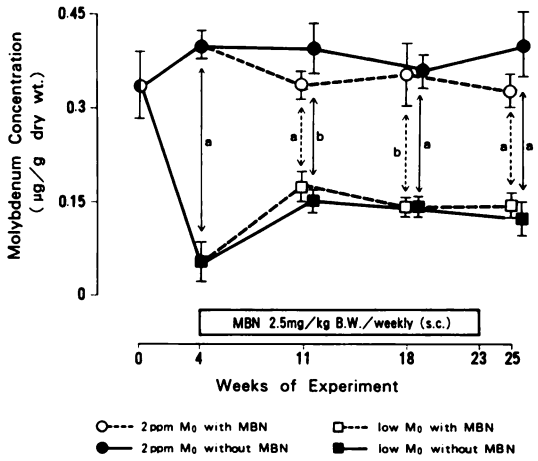

Fig. 4. Molybdenum (*Mo*) levels in the esophagus-forestomach of rats fed a high-molybdenum diet and treated with MBN (○) without MBN (●), fed a low-molybdenum diet with MBN (□), and without MBN (■). MBN treatment was started during the fifth experimental wk and continued for 19 wk. The statistical difference between the high- and low-molybdenum diet groups was $P < 0.001$ (*a*) and $P < 0.01$ (*b*). *B.W.*, body weight; points and bars, mean ± SD.

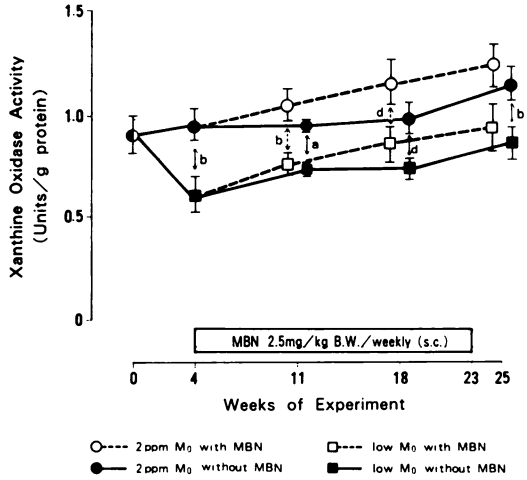

Fig. 5. Xanthine oxidase activities in the esophagus-forestomach of rats fed a high-molybdenum diet with MBN (○) and without MBN (●) and fed a low-molybdenum diet with MBN (□) and without MBN (■). MBN treatment was started during the fifth experimental wk and continued for 19 wk. The statistical difference between the high- and low-molybdenum diet groups was $P < 0.001$ (*a*), $P < 0.01$ (*b*) and $P < 0.05$ (*d*). *Mo*, molybdenum; *B.W.*, body weight; points and bars, mean ± SD.

tween the high-molybdenum diet group and the low-molybdenum diet group with an exception at 4 wk ($P < 0.001$). At 25 wk, there was a significant ($P < 0.05$) difference between the rats treated with MBN and those not treated with MBN in the high-molybdenum diet group.

Liver XOD activities also showed no significant difference between the high-molybdenum diet group and the low-molybdenum diet group with an exception at 4 wk ($P < 0.01$). In both of these groups, the presence or absence of MBN had no significant influence on the liver XOD activities.

DISCUSSION

The observations that esophageal cancer patients have lower tissue concentrations of molybdenum than do matched controls (2) support the hypothesis that diminished molybdenum reserves may sensitize the esophagus to environmental carcinogens such as nitrosamines.

N-Nitroso compounds show a high degree of organ-specific carcinogenicity (17). Stinson *et al.* (8) found that weekly doses of 2.5 mg of MBN/kg of body weight (s.c.) to F344 rats produced pedunculated papillary carcinomas exclusively in the esophagus and, in a few cases, carcinoma of the tongue, pharynx, and larynx. Iizuka *et al.* (18) revealed that MBN had a specific affinity for the esophagus in a study using autoradiography. When *N*-nitroso [*methyl*-^{14}C]benzylnitrosamine was injected i.v., methylation of purine bases in DNA was most extensive in the esophagus (19). The enzymic removal of O^6-methylguanine from esophageal DNA might be relatively slow. On the other hand, Mehta *et al.* (20) indicated that microsomal metabolic activation appeared to be necessary but not a sufficient stimulus for the carcinogenic effects of MBN.

Molybdenum is a constituent element of XOD (5, 6). This enzyme catalyzes the reduction of O_2, cytochrome *c*, and various quinones and dyes by aldehyde and purines (7), and it generates superoxide anion radicals (7, 21). XOD is thought to catalyze the action of various carcinogens (22) and also to catalyze mutagens into DNA adducts that induce mutation of microorganisms (23, 24).

In this study, both molybdenum levels and XOD activities in the esophagus-forestomach tissue in the rats fed the high-molybdenum diet were significantly higher than those of the rats fed the low-molybdenum diet. Furthermore, the XOD activity in MBN-treated rats increased gradually, but that of nontreated rats was maintained at almost the same values throughout the experiment. Westerfeld and Richert (25) showed that liver XOD activity was mainly affected by the protein concentration in the diet. Since the casein concentration in the basal diet used for this experiment was uniform, these two groups showed the same liver XOD activities.

XOD encompasses a wide spectrum of substrate specificity for oxidizable substrates (7, 26) and can interact with compounds containing aromatic nitro groups (27). The primary role of this enzyme might be in detoxification because of its wide specificity (26, 28). The reduction of organic nitro compounds to hydroxyamino derivatives by XOD may prevent the formation of potentially dangerous nitroso compounds. Although purine *N*-oxides are known to be oncogenic (29), XOD can reduce purine *N*-oxides to free bases (30). Fried *et al.* (31) suggested that XOD serves *in vivo* to generate the H_2O_2 required for detoxication reactions using H_2O_2 as the oxidizing agent.

Lu and Lin (32) revealed that dietary molybdenum (1 mg/rat/day) inhibited DNA methylation of hepatic cells by [^{14}C]diethylnitrosamine. This suggests that molybdenum directly detoxicates carcinogens independently of XOD. These studies and our data suggest that *in vivo* metabolism of MBN in the esophagus-forestomach is more important than that in the liver, and XOD which exists in the cytosol fraction (33) plays a role in catalyzing MBN to noncarcinogenic metabolites.

It has been reported that there was no detectable XOD activity or marked decrease in activity in chemically induced carcinomas (34) or transplanted tumor as Novikoff hepatoma (35). Furthermore, Prajda *et al.* (36) noted that the key purine synthetic enzyme, glutamine 5′-phosphoribosyl 1-pyrophosphate amidotransferase, was increased, but the antagonizing catabolic enzyme, XOD, was decreased in hepatomas. Thus, they concluded that this enzymatic imbalance confers selective advantages to the cancer cells. The elevation of XOD activity may limit the salvage pathway by promoting conversion of hypoxanthine into uric acid. Haddow *et al.* (37) found that daily i.p. injection of XOD completely suppressed the growth of spontaneous mammary tumors in C3H and high-tumor C strain mice and increased the levels of XOD in the liver and tumors of the treated animals. They speculated that the active XOD molecules penetrated into the liver and tumors and altered growth rates of the tumors. Our results showed that the XOD level in the esophagus-forestomach of the high-molybdenum diet group with fewer tumors was higher than that of the low-molybdenum diet group with many tumors. Further studies are needed to clarify the mechanisms of the inhibitory effect of molybdenum.

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REFERENCES

1. Burrell, R. J. W., Roach, W. A., and Shadwell, A. Esophageal cancer in the Bantu of the Transkei associated with mineral deficiency in garden plants. J. Natl. Cancer Inst., *36:* 201–214, 1966.
2. Yang, C. S. Research on esophageal cancer in China: a review. Cancer Res., *40:* 2633–2644, 1980.
3. Luo, X. M., Wei, H. J., and Yang, S. P. Inhibitory effects of molybdenum on esophageal and forestomach carcinogenesis in rats. J. Natl. Cancer Inst., *71:* 75–80, 1983.
4. Higgins, E. S., Richert, D. A., and Westerfeld, W. W. Molybdenum deficiency and tungstate inhibition studies. J. Nutr., *59:* 539–559, 1956.
5. Richert, D. A., and Westerfeld, W. W. Isolation and identification of the xanthine oxidase factor as molybdenum. J. Biol. Chem., *203:* 915–923, 1953.
6. De Renzo, E. C., Kaleita, E., Heytler, P. G., Oleson, J. J., Hutchings, B. L., and Williams, J. H. Identification of the xanthine oxidase factor as molybdenum. Arch. Biochem. Biophys., *45:* 247–253, 1953.
7. Fried, R., and Fried, L. W. Xanthine oxidase (xanthine dehydrogenase). *In:* H. U. Bergmeyer (ed.), Methods of Enzymatic Analysis, Vol. 2, pp. 644–649. New York: Academic Press, Inc., 1974.
8. Stinson, S. F., Squire, R. A., and Sporn, M. B. Pathology of esophageal neoplasms and associated proliferative lesions induced in rats by *N*-methyl-*N*-benzylnitrosamine. J. Natl. Cancer Inst., *61:* 1471–1475, 1978.
9. Sohler, A., and Pfeiffer, C. C. Effect of molybdenum supplements on blood molybdenum. Trace Elements Med., *2:* 93–95, 1985.
10. Bickford, C. F., Jones, W. S., and Keene, J. S. Microdetermination of molybdenum and tungsten in organic materials. J. Am. Pharm. Assoc., *37:* 255–261, 1948.
11. Napalkov, N. P., and Pozhariski, K. M. Morphogenesis of experimental tumors of the esophagus. J. Natl. Cancer Inst., *42:* 922–940, 1969.
12. Pozhariski, K. M. Tumours of the oesophagus. *In:* V. S. Turusov (ed.), Pathology of Tumours in Laboratory Animals, Vol. 1, pp. 87–100. Lyon: International Agency for Research on Cancer, 1973.
13. Gorsuch, T. T. Individual elements: chromium, molybdenum, and tungsten. *In:* T. T. Gorsuch (ed.), The Destruction of Organic Matter, Chap. 8, pp. 114–116. Oxford: Pergamon Press, 1970.
14. Rowe, P. B., and Wyngaarden, J. B. The mechanism of dietary alterations in rat hepatic xanthine oxidase levels. J. Biol. Chem., *241:* 5571–5576, 1966.
15. Stirpe, F., and Della Corte, E. The regulation of rat liver xanthine oxidase; conversion *in vitro* of the enzyme activity from dehydrogenase (type D) to oxidase (type O). J. Biol. Chem., *244:* 3855–3863, 1969.
16. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. Protein measurement with the Folin phenol reagent. J. Biol. Chem., *193:* 265–275, 1951.
17. Druckrey, H., Preussmann, R., Ivankovic, S., and Schmähl, D. Organotrope carcinogene Wirkungen bei 65 verschiedenen *N*-Nitroso-Verbindungen an BD-Ratten. Z. Krebsforsch., *69:* 103–201, 1967.
18. Iizuka, T., Ichimura, S., and Kawachi, T. Autoradiography and organ distribution of *N*-methyl-*N*-nitrosobenzylamine in rats. Gann, *69:* 487–492, 1978.
19. Hodgson, R. M., Wiessler, M., and Kleihues, P. Preferential methylation of target organ DNA by the oesophageal carcinogen *N*-nitrosomethylbenzylamine. Carcinogenesis (Lond.), *1:* 861–866, 1980.
20. Mehta, R., Labuc, G. E., Urbanski, S. J., and Archer, M. C. Organ specificity in the microsomal activation and toxicity of *N*-nitrosomethylbenzylamine in various species. Cancer Res., *44:* 4017–4022, 1984.
21. McCord, J. M. Oxygen-derived free radicals in postischemic tissue injury. N. Engl. J. Med., *312:* 159–163, 1984.
22. Mori, M., Matsuhashi, T., Miyahara, T., Shibata, S., Izima, C., and Kozuka, H. Reduction of 2,4-dinitrotoluene by Wistar rat liver microsomal and cytosol fractions. Toxicol. Appl. Pharmacol., *76:* 105–112, 1984.
23. Howard, P. C., Heflich, R. H., Evans, F. E., and Beland, F. A. Formation of DNA adducts *in vitro* and in *Salmonella typhimurium* upon metabolic reduction of the environmental mutagen 1-nitropyrene. Cancer Res., *43:* 2052–2058, 1983.
24. Swaminathan, S., and Hatcher, J. F. Xanthine oxidase-mediated mutagenicity of the bladder carcinogen 4-nitrobiphenyl. Mutat. Res., *172:* 37–45, 1986.
25. Westerfeld, W. W., and Richert, D. A. Liver and intestinal xanthine oxidases in relation to diet. J. Biol. Chem., *192:* 35–48, 1951.
26. Krenitsky, T. A., Neil, S. M., Elion, G. B., and Hitchings, G. H. A comparison of the specificities of xanthine oxidase and aldehyde oxidase. Arch. Biochem. Biophys., *150:* 585–599, 1972.
27. Morita, M., Feller, D. R., and Gillette, J. R. Reduction of niridazole by rat liver xanthine oxidase. Biochem. Pharmacol., *20:* 217–226, 1971.
28. Rajagoparan, K. V. Xanthine oxidase and aldehyde oxidase. *In:* W. B. Jakoby (ed.), Enzymatic Basis of Detoxication, Vol. 1, pp. 295–309. New York: Academic Press, Inc., 1980.
29. Sugiura, K., and Brown, G. B. Purine *N*-oxides. XIX. On the oncogenic *N*-oxide derivatives of guanine and xanthine and a nononcogenic isomer of xanthine *N*-oxide. Cancer Res., *27:* 925–931, 1967.
30. Stöhrer, G., and Brown, G. B. Purine *N*-oxides. XXVIII. The reduction of purine *N*-oxides by xanthine oxidase. J. Biol. Chem., *244:* 2498–2502, 1969.
31. Fried, R., Fried, L. W., and Babin, D. R. Biological role of xanthine oxidase and tetrazolium reductase inhibitor. Eur. J. Biochem., *33:* 439–445, 1973.
32. Lu, S. H., and Lin, P. Recent research on the etiology of esophageal cancer in China. Z. Gastroenterol., *20:* 361–367, 1982.
33. Villera, G. G., Mitidieri, E., and Affonso, O. R. Intracellular distribution of xanthine oxidase in the rat liver. Nature (Lond.), *175:* 1087, 1955.
34. Reid, E., and Lewin, I. Adenosine deaminase, nucleoside phosphorylase, and xanthine oxidase in liver tumours. Br. J. Cancer, *11:* 494–498, 1957.
35. De Lamirande, G., Allard, C., and Cantero, A. Purine-metabolizing enzymes in normal rat liver Nandovikoff hepatoma. Cancer Res., *18:* 952–958, 1958.
36. Prajda, N., Morris, H. P., and Weber, G. Imbalance of purine metabolism in hepatomas of different growth rates as expressed in behavior of xanthine oxidase (EC 1.2.3.2). Cancer Res., *36:* 4639–4646, 1976.
37. Haddow, A., De Lamirande, G., Bergel, F., Bray, R. C., and Gilbert, D. A. Anti-tumour and biochemical effects of purified bovine xanthine oxidase in C3H and C mice. Nature (Lond.) *182:* 1144–1146, 1958.