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MOLYBDENUM: An Essential Trace Element in Human Nutrition

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INTRODUCTION

The essentiality of molybdenum for plant growth and the functional role played by the metal in several bacterial, plant, and animal enzymes have long been recognized. However, unequivocal evidence for the importance of the element in human health has been difficult to adduce owing to the absence of any documented instance of nutritional deficiency of the metal, or of any pathological condition attributable to a dysfunctional Mo-containing enzyme. Attempts at producing molybdenum deficiency in laboratory animals, as determined by the tissue levels of xanthine oxidase activity, have been

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thwarted by the fact that it is nearly impossible to eliminate low levels of the metal from synthetic diets. The problem has been further compounded by the apparent low daily requirement for the metal.

The severity of the pathology associated with simple sulfite oxidase deficiency, first described in 1967 (1), and the subsequent characterization of the enzyme as a molybdenum-containing protein in 1969 (2), established the essentiality of Mo for normal human development. The marked neurological lesions observed since then in a number of patients exhibiting combined deficiency of all molybdenum-containing enzymes (3) reinforce the importance of the metal in human health. The more recent studies (4) on the nature of the Mo cofactor, a prosthetic group presumed to be common to all molybdoenzymes save nitrogenase, have introduced a new perspective on the assimilation and utilization of the metal. It seems quite likely that in the absence of these recent developments in the biochemistry of the Mo-containing enzymes the symptoms associated with the above-mentioned genetic diseases might not have been correlated with the biochemical functioning of molybdenum. This sobering reflection raises important issues such as the adequacy of existing information on the nutritional requirements for the element and the possibility that nutritional deficiency of molybdenum, especially in infants and children, might go undiagnosed. The goal of this review is to evaluate several different facets of the present state of knowledge regarding the nutritional aspects of Mo and to provide a basis for future directions in research dealing with complete characterization of its biological functions, as a means of recognizing possible isolated or epidemiological instances of nutritional deficiency of the metal.

BIOCHEMISTRY OF MOLYBDENUM

The biological functions of Mo in animals can be traced to its role as a prosthetic group in three enzymes: xanthine oxidase/dehydrogenase, aldehyde oxidase, and sulfite oxidase, all of which catalyze oxidation-reduction reactions and contain, in addition to Mo, other prosthetic groups. Some properties of the three molybdoenzymes are summarized in Table 1. The extreme tightness of association of the metal to the proteins has so far precluded resolution and reconstitution of the metal, or replacement with other metals in vitro, in any Mo-containing enzyme. A vast amount of incisive information about the chemical state of the protein-bound metal has been obtained through the use of specialized techniques such as electron paramagnetic resonance and extended X-ray absorption fine structure spectroscopy. Several review articles (5, 6) and an extensive book (7) have been devoted to molybdenum chemistry and biochemistry. Following are brief descriptions of the three animal molybdoenzymes.

Xanthine Oxidase/Dehydrogenase

The nutritional studies of Richert & Westerfeld in 1953, demonstrating that supplementation of a synthetic diet with molybdenum was essential for the development and maintenance of tissue xanthine oxidase in weanling rats (8), set the stage for the characterization of that enzyme as a molybdenum-containing protein. Xanthine-oxidizing enzymes from several sources have been shown to be dimers of 300,000 molecular weight and to contain one flavin adenine dinucleotide (FAD), two Fe_2/S_2 clusters, and one molybdenum center per subunit. The enzyme has been purified from a multitude of sources such as animal tissues, plants, fungi, and bacteria (7). In animals the liver is the predominant site of xanthine oxidase activity, with lesser amounts in lung, kidney, and intestinal mucosa (Table 2). Watts et al (9) and Brunschede & Krooth (10) reported that cultured human cells from several different lines are devoid of xanthine oxidase activity. In contrast, cultured Chinese hamster, rat, and mouse cells contained variable amounts of activity (10).

Bovine milk xanthine oxidase is the prototype of a group of enzymes known as molybdenum hydroxylases that catalyze the oxidative hydroxylation of a number of purines, pteridines, pyrimidines, and other heterocyclic nitrogenous aromatic molecules (11–13). Substrate specificity of this group of enzymes is extremely broad for both oxidizing and reducing substrates (7, 11, 12). The molybdenum center of xanthine oxidase is directly involved in the oxidative hydroxylation of the substrates (7). Electron paramagnetic resonance studies have demonstrated that transfer of electrons from the substrate leads to the reduction of Mo(VI) to the Mo(IV) state (5, 7), and that the electrons are then transported to the FAD before eventual transfer to an oxidizing substrate molecule. Oxidizing substrates include NAD (nicotinamide adenine dinucleotide), oxygen, and ferredoxin as physiological acceptors, and methylene blue, cytochrome *c*, nitroblue tetrazolium, and dichlorophenolindophenol as artificial electron acceptors (7).

Table 1 Molybdenum enzymes in animal tissues

Enzyme	Organism	Prosthetic groups	Subunits	Molecular weight
Aldehyde oxidase	Mouse, pig, rabbit, *Drosophila*	Mo, FAD, 2 Fe_2S_2	2	270,000
Sulfite oxidase	Rat liver,	Mo, heme	2	120,000
	human liver,	Mo, heme	2	122,000
	chicken liver,	Mo, heme	2	110,000
	Drosophila	Mo, heme	2	—
Xanthine oxidase/ dehydrogenase	Bovine milk	Mo, FAD, 2 Fe_2S_2	2	300,000
	Chicken liver	Mo, FAD, 2 Fe_2S_2		300,000
	Rat liver	Mo, FAD, 2 Fe_2S_2		
	Turkey liver	Mo, FAD, 2 Fe_2S_2		280,000

Table 2 Distribution of molybdenum enzymes and molybdenum cofactor in different tissues

Tissue	Xanthine oxidase activity (m units/g)				Sulfite oxidase[a]	Cofactor activity[a,b]
	Cat	Dog	Sheep	Cow		
	(m units/g)					
Liver	17	19	12	29	191	133
Kidney	4.8	1.8	0.004	1.6	75	13.2
Lung	6.0	24	0.032	48	4.7	4.9
Adrenal	0.06	0.71	0.015	—	5.4	<1.0
Heart	0.001	0.3	0.007	0.22	10.8	5.6
Thymus	—	—	—	—	0.8	2.2
Brain	0	0.07	0.005	—	0.8	1.9
Muscle	0	0.2	0.022	0.33	0.8	<1.0
Blood	0.3[c]	23[c]	0[c]	62[c]	ND[c]	ND[c]
Intestine	—	19	0.058	12	—	—
Adipose	0.06	1.2	0.004	0.20	0.8	<1.0

[a]In rats (units/g).
[b]Units sulfite oxidase reconstituted.
[c]Milli international units/l.
[d]ND–not detected.

Purified xanthine oxidases from several sources have been characterized as O_2-reducing enzymes, and hence called oxidases. It is now evident that most of the xanthine-oxidizing enzymes, especially those of animals, exist in vivo as dehydrogenases, using NAD rather than O_2 as the physiological electron acceptor (14, 15). The studies of Stirpe & Della Corte (14) and Waud & Rajagopalan (15, 16) demonstrated that freshly purified mammalian enzymes such as from bovine milk (16) and rat liver (14, 15) reduce NAD, but spontaneously lose this ability when stored in the absence of thiol compounds such as mercaptoethanol or dithiothreitol. A variety of treatments—including proteolysis, heating, storage at −20°C, bubbling with oxygen, mixing with organic solvents, and use of sulfhydryl-modifying reagents—convert the dehydrogenase to the oxidase form (14). The dehydrogenase-to-oxidase conversion results in the enhanced production of superoxide radical concomitant with substrate oxidation (17).

The evolutionary advantage of the dehydrogenase-to-oxidase switch in animal xanthine dehydrogenase is not clear. Since lysosome-mediated proteolysis might be one mechanism of formation of the oxidase, the conversion might be triggered by any event leading to cellular damage. The oxidase form of the enzyme, being capable of generating superoxide radicals, could thus be a means of protecting the tissue by superoxide-mediated damage to the cellulolytic agent. Apropos of this, there are several reports of induction of xanthine dehydrogenase (oxidase) by interferon or interferon-inducing agents

(18, 19). It was also reported that the increase in activity was principally of the oxidase form (18) and that the oxidase activity was fully convertible to the dehydrogenase form by treatment with dithiothreitol. It was not clear whether the oxidase form was the primary product of induction or was produced from the dehydrogenase form by a secondary effect of interferon.

Ischemia-induced injury to tissues such as heart, brain, intestine, and kidney had long been attributed to the ischemic state itself. Recently, however, numerous studies have provided persuasive evidence that tissue damage occurs during postischemic injury, and that oxygen-derived radicals might be responsible for tissue necrosis. Many of these studies have implicated the dehydrogenase-to-oxidase conversion of xanthine dehydrogenase, presumed to occur during the anaerobic state, as the source of augmented production of superoxide radicals, which in turn generate a cascade of events culminating in tissue damage. An excellent discussion of the hypothesis and supporting data has been given by McCord (20).

The amount of the enzyme activity in vivo is controlled by a variety of mechanisms, including synthesis of the apoprotein, conversion of inactive forms to active enzyme and product inhibition. In mammals, hepatic xanthine oxidase activity has been found to be related to the level of dietary protein (21), riboflavin, molybdenum, and iron (22) and inversely related to the vitamin E status of the animal (23). Transcriptional control of enzyme synthesis has been shown to be involved in both types of variation in activity (23, 24). In uricotelic organisms such as the chicken, xanthine dehydrogenase activity in the liver and pancreas also depends upon protein intake (25, 26). Itoh et al (27) found that adaptation of chickens to a low-protein diet decreased not only the amount of liver xanthine dehydrogenase cross-reacting material but also the apparent molecular specific activity. This alteration was attributed to a chemical modification other than changes in the molybdenum or flavin content of the enzyme molecules. The mechanism of control was suggested to be the addition or deletion of a cyanolyzable sulfur group, which is a ligand of the Mo atom at the active site of the enzyme, and which is essential for the activity of xanthine dehydrogenase and xanthine oxidase (28).

Genetic control of enzyme synthesis has been proposed in malignant cells. Prajda & Weber (29) found increased levels of 5-phosphoribosyl-1-pyrophosphate (PRPP) amidotransferase activity with decreased xanthine oxidase activity in a number of transplantable rat hepatomas, which could not be ascribed to differences in the growth rate of the tumors (29). The authors concluded that neoplastic transformation of rat liver cells had altered the purine metabolic pathways toward elevated purine concentrations by increasing purine synthesis (increased amidotransferase) and decreasing catabolism (decreased xanthine oxidase).

Aldehyde Oxidase

The molybdoprotein aldehyde oxidase is an enzyme distinct from the group of other aldehyde-metabolizing enzymes, the aldehyde dehydrogenases. The enzyme seems to be restricted to animal cells. Rabbit liver aldehyde oxidase is present in the cytosol (30), whereas the pig liver enzyme is located in the mitochondria (31). The highest activity is present in the liver, but information on the tissue distribution of the enzyme is not available. Aldehyde oxidase is very similar to xanthine oxidase/dehydrogenase in size, cofactor composition, and substrate specificity (Table 1) but does not use NAD or NADP as electron acceptors, and presumably functions as a true oxidase using oxygen as its physiologic electron acceptor (7, 12). Aldehyde oxidase activity has been demonstrated to be under genetic and hormonal control in the mouse (32–34); it is not known whether nutritional control is exerted as seen with xanthine dehydrogenase.

The exact biological function of xanthine dehydrogenase and aldehyde oxidase remains a matter for speculation. It is clear that xanthine dehydrogenase plays a role in the terminal metabolism of purines, the pathway of which is shown in Figure 1. But the importance of this function has yet to be unequivocally established. It has also been suggested that intestinal xan-

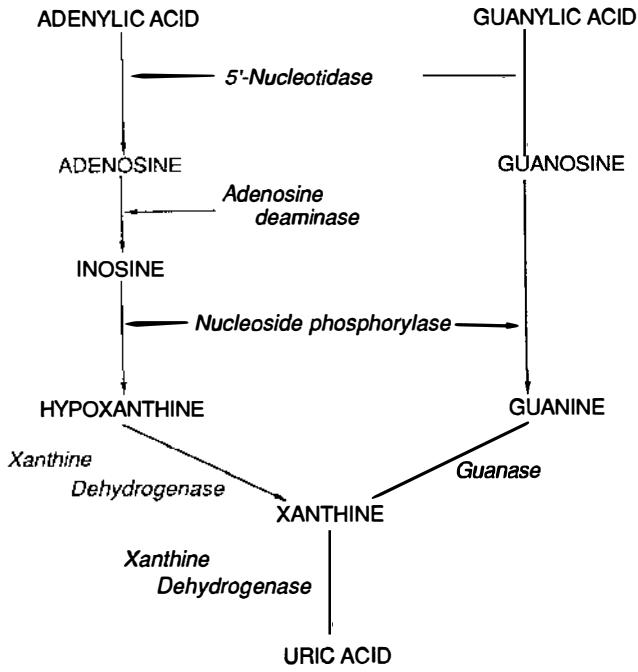

Figure 1 The pathways of purine nucleotide metabolism in man.

thine oxidase may play a role in the absorption of iron (35, 36). However, it is unlikely that xanthine oxidase is essential for intestinal iron uptake. In xanthinuria, a human genetic deficiency of xanthine oxidase, the affected individuals appear to be mostly asymptomatic, with only an occasional tendency toward myopathy and/or uropathy secondary to deposition of xanthine crystals in muscles and the renal calyces (37). Anemia attributable to defective iron uptake has never been observed. Additionally, animals rendered functionally deficient in xanthine dehydrogenase (by treatment with allopurinol or with dietary tungsten to deplete the tissue molybdenum) grow and reproduce normally, which demonstrates the lack of lasting effects from loss of enzyme activity despite changes in their excretion of oxidized purines (38).

Sulfite Oxidase

Sulfite oxidase, a protein located in the mitochondrial intermembrane space (39), catalyzes the terminal step in the metabolism of sulfur-containing amino acids. As shown in Table 1, purified sulfite oxidase from various sources ranges in molecular weight from 110,000 to 122,000 and consists of two identical subunits, each containing one molybdenum center and one cytochrome-b_5-type heme. Sulfite oxidase has a very narrow substrate specificity, and does not oxidize compounds other than sulfite (SO_3^{2-}) to any extent. Cytochrome *c* is the physiological electron acceptor for the enzyme, with oxygen and ferricyanide serving as alternative oxidizing substrates. Oxidation of sulfite to sulfate occurs at the molybdenum center, the electrons are transferred singly to the heme domain, and ultimately transferred to cytochrome *c* on the intermembrane space side of the inner mitochondrial membrane. Thus, sulfite oxidase is more appropriately named sulfite: ferricytochrome *c* oxidoreductase. The enzyme has been found in all animals surveyed, but only rarely in plants, bacteria, fungi, or yeast (39).

The biological role of sulfite oxidase is unquestionably associated with the reaction of the oxidation of sulfite to sulfate. From the levels of tissue sulfite oxidase it is apparent that the capacity of the mammalian enzyme to oxidize sulfite is vastly in excess of the load imposed by the sum of the endogenous and exogenous sources of the compound. Thus, it has been estimated that the rat has the capacity to oxidize 750 mmol of sulfite per kilogram of body weight per day (40). In accord with this, Gunnison et al (41) estimated a half-life of less than one minute for intravenously administered sulfite in the rat. Additionally, Gibson & Strong (42) found that prolonged dietary administration of bisulfite to rats, even at doses 100–500 times the estimated daily human intake, did not saturate their capacity to oxidize the compound. From measurements of sulfite oxidase activity in autopsy samples of human liver it has been estimated (43) that the total sulfite-oxidizing capacity of adult liver is

4000–8000 mmol/day, compared to the average endogenous production of 25 mmol/day and the maximum exogenous intake of 2.5 mmol/day (44). These data lead to the reasonable conclusion that under normal circumstances there is little likelihood of toxicity in humans from sulfite at the levels it is consumed as food additives or the levels at which it can be formed from respired ambient SO_2.

Human genetic deficiency of sulfite oxidase was first described in an infant in 1967, manifest in a syndrome of mild to moderate seizures, severe mental retardation, and dislocated ocular lenses, and fatality shortly after hospitalization (1). The biochemical abnormalities in this and a subsequent case included elevated urinary excretion of sulfite, thiosulfate, and S-sulfocysteine, which are abnormal metabolic products of sulfur amino acid degradation (45, 46), and virtually no inorganic sulfate. The relevant pathway of sulfur amino acid metabolism is shown in Figure 2. Examination of autopsy samples of tissues obtained from the patient demonstrated a deficiency of sulfite oxidase in liver, brain, and kidney. Brain sulfatide concentration was found to be normal, but the weight of the child's brain was 50% less than expected (47). Histologic examination of the brain tissue revealed a diffuse deficiency of myelin (48). Percy et al (47) concluded that the total sulfatide content was reduced, but could not decide whether the causative factor for the pathological findings was sulfate deficiency or metabolite toxicity from sulfite.

The fact that the reaction catalyzed by sulfite oxidase is the terminal step in the excretory metabolism of sulfur amino acids sets a limit on the possible origins of the neurological damage. These are (*a*) toxicity from the accumulated levels of sulfite, (*b*) toxicity from a byproduct such as S-sulfocysteine, or (*c*) absence of sulfate, the product of the reaction.

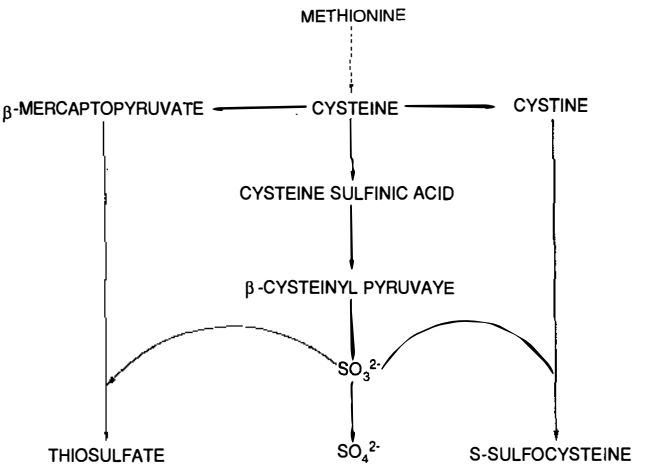

Figure 2 The formation of S-sulfocysteine and thiosulfate from cysteine.

There are numerous in vitro studies dealing with the reactivity of sulfite with various biological molecules. The data from such studies have been discussed from the perspective of potential mammalian toxicity in excellent reviews by Hayatsu (49), Shapiro (50), and Gunnison (51) and in a report by the Ad Hoc Review Panel of the FDA in 1985 (52). Some of the reported actions of sulfite are listed in Table 3. However, it is now well established that sulfite is not present at detectable levels in body fluids of normal humans or control experimental animals (45, 46, 51). From these observations, it has been hypothesized that sulfite oxidase prevents the accumulation of the toxic metabolite sulfite produced during endogenous metabolism of methionine and cysteine. The sulfate derived from the oxidation is utilized in synthetic reactions producing sulfolipids, mucopolysaccharides, and sulfated glycoproteins (39). The presence of the enzyme in developing tissues may be essential for providing sulfate in specific tissues. This might be especially true for pre- and postnatal nervous tissue if the pathologic changes seen in sulfite oxidase deficiency are attributable to sulfate deficiency.

The presence of Mo in sulfite oxidase has made it possible to generate an

Table 3 In vitro and in vivo effects of sulfite

Type of reaction	Examples
Addition	a. Formation of hydroxysulfonate compounds with aldehydes and latones
	b. Reaction with c=c bonds with pyridine nucleotides, flavin nucleotides, flavin nucleotides, uracil, cytosine
Sulfitolysis	a. Cleavage of thiamine
	b. Lysis of disulfide bonds
	c. Formation of S-sulfocysteine, thiosulfate
Free radical reactions	a. Reaction with superoxide by chain mechanism
	b. Oxidation of cellular constituents
Effect on enzymes	a. Alkylation of several flavoproteins
	b. Potent inhibition of sulfatases
Reaction with nucleic acids	a. Modification of DNA with loss of template activity
	b. Inactivation of bacteriophages
	c. Cross-linking of DNA and RNA with proteins
Effects on cells in culture	a. Inhibition of DNA synthesis
	b. Inhibition of growth of fibroblasts
	c. Cytotoxicity to lymphocytes
Effects on Mo-fed rats	a. No significant effects
Effects on Mo-deficient rats	a. No apparent effect on health
	b. Increased urinary levels of sulfite, S-sulfocysteine, and thiosulfate
	c. Higher incidence of adenosarcoma?

experimental model for deficiency of the enzyme in rats by feeding high levels of tungstate as a competitive inhibitor of molybdenum uptake. Exposing rats to more than 100 ppm of tungsten in a synthetic low-Mo diet caused a time-dependent decrease in tissue levels of sulfite oxidase, with a half-life of 4.7 days (38). Adult rats with less than 1% residual liver sulfite oxidase activity grew normally, showed no signs of neurological damage, and remained healthy indefinitely, which indicates an absence of any stressful condition under normal conditions of laboratory maintenance. When the effects of acute exposure to sulfite were examined, it was found that the LD_{50} for intraperitoneally administered bisulfite was 3-fold higher for control rats than for tungsten-treated rats (40). These findings clearly demonstrated the ability of tissue sulfite oxidase to effect rapid detoxification of large amounts of sulfite.

Gunnison et al (41, 53) carried out detailed studies of tungsten-treated rats as a model for metabolic characterization of sulfite oxidase deficiency. They observed a clear inverse relationship between hepatic sulfite oxidase activity and tissue or urine concentrations of sulfite and of the two sulfite derivatives S-sulfocysteine and thiosulfate. However, despite prolonged exposure to these endogenously generated metabolites, the animals remained outwardly indistinguishable from control rats, which suggests that depletion of sulfite oxidase activity may not lead to physiological damage in adult animals. If this were so, it is possible that adult onset of sulfite oxidase deficiency in humans might be a benign condition in the absence of other contributory factors.

The Molybdenum Cofactor

Despite extensive knowledge concerning the other prosthetic groups of molybdenum enzymes (i.e FAD, heme, nonheme iron sulfur centers), little was known about the chemical nature of the molybdenum centers until recently. One of the most exciting new areas in the biochemistry of molybdenum is the identification and characterization of molybdopterin, the organic moiety of the molybdenum cofactor responsible for the anchoring of the molybdenum atom at its active site.

The genetic studies of Patemen et al (54) in 1964 on a group of pleiotropic mutants of *Aspergillus nidulans* led to the proposal that there existed a common cofactor for the molybdoenzymes nitrate reductase and xanthine dehydrogenase. Since then a variety of studies have established that in all molybdenum-containing enzymes, except nitrogenase, Mo exists as part of a complex called the "molybdenum cofactor" (55). Molybdenum cofactor was functionally characterized by its ability to reconstitute the apoprotein of nitrate reductase in crude extracts of cells of the *nit-1* mutant of *Neurospora crassa* (56). Nason and coworkers (57) showed that the cofactor could be released from purified molybdoenzymes by a variety of procedures, including

heating, acidification, and treatment with denaturing agents. However, the Mo cofactor released by such procedures was extremely unstable and was rapidly inactivated by unknown degradative pathways, especially on exposure to air. As a consequence, it has not been possible, even to date, to purify and directly characterize the structure the active cofactor.

An important clue to the chemical nature of the cofactor was the finding in our laboratory that an identical 6-substituted pterin could be isolated from sulfite oxidase, xanthine dehydrogenase, and other molybdoenzymes after treatment with oxidizing agents (58). In an effort to obtain at least partial structural information on the unstable cofactor, efforts were directed at characterizing stable inactive oxidized derivatives of the cofactor. This approach was highly successful and resulted in complete characterization of two fluorescent derivatives, Form A (Figure 3*A*) and Form B (Figure 3*B*), which in themselves define many of the features of the active species. The details of the chemical characterization of Form A and Form B have been published (59). Despite the apparent striking difference between the two compounds, it can be seen that the fused thiophene ring of Form B could have arisen from a linear C-6 side chain, by the reaction of a sulfhydryl group attached to a side chain carbon at the C-7 position of the pterin ring. The structure of Form B also gave direct evidence for the presence of sulfur in the molybdenum cofactor. The generation of Form A and Form B from molybdoenzymes under defined conditions has been used as a probe for the presence or absence of molybdenum cofactor in various systems such as purified enzymes and whole cells (60). All molybdoenzymes and all normal cells tested yielded the two derivatives, whereas nonmolybdoproteins or cells known to be pleiotropically deficient in molybdoenzymes failed to do so.

Further studies revealed the existence of a third, naturally occurring inactive derivative of the molybdenum cofactor that appears to be formed in

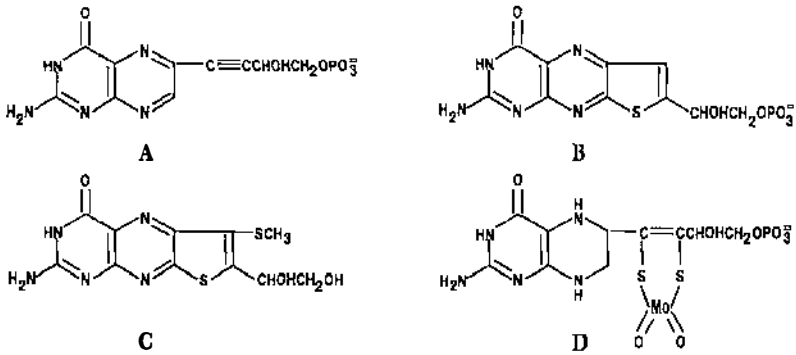

Figure 3 The structures of molybdenum cofactor and its derivatives.

vivo. The compound urothione (Figure 3*C*) with the same thienopterin ring system found in Form B was isolated from human urine in 1940 (61) and structurally characterized in 1969 (62). The function and metabolic origin of urothione remained obscure until recently, when a connection was established with a newly identified human genetic disorder in which the patients appeared to be deficient in all molybdoenzyme functions.

MOLYBDENUM COFACTOR DEFICIENCY In the 11 years since the first report of a patient exhibiting combined deficiencies of xanthine oxidase and sulfite oxidase (3), more than 20 cases of molybdenum cofactor deficiency have been identified. The autosomal recessive condition has been detected in populations of the US, central and southern Europe, northern Africa, Turkey, and Asia, and thus is not restricted to any ethnic group. The deficiency disease is fatal, with about half of the patients failing to survive beyond early infancy. The surviving patients are severely ill, and in some cases, cared for in institutions. Pathological studies on two molybdenum cofactor deficiency patients (63) and a sulfite-oxidase-deficient patient (45) showed similar cerebral atrophy characterized by brain shrinkage and severe loss of neurons. The severity of brain lesions was unlike those generated by ischemia or anoxia or those seen in cystic encephalomalacia of infancy. It is apparent that the damage to the central nervous system in the cofactor-deficient patients is the direct result of sulfite oxidase deficiency.

The clinical abnormalities in the cofactor deficiency state are explicable as being due to the absence of xanthine oxidase and sulfite oxidase. Urinary levels of urate are very low, with a corresponding increase in xanthine and hypoxanthine. The elevation in systemic sulfite concentrations results in the formation and excretion of abnormally high levels of S-sulfocysteine and thiosulfate. As would be expected, the concentration of inorganic sulfate in the urine is greatly diminished.

In regard to the cofactor itself, autopsy samples of tissues are devoid of sulfite oxidase and xanthine oxidase activities, as well as reconstitutively active molybdenum cofactor activity (4). Sulfite oxidase deficiency can also be demonstrated in cultured fibroblasts (46). In addition, tested liver tissue samples lack molybdopterin, as evidenced by the failure of the samples to generate Form A under specified conditions. It is reasonable to conclude that the pathological sequelae of molybdenum cofactor deficiency are in a large measure due to sulfite oxidase deficiency.

Because of the obvious similarities between urothione and Form B, we considered the possibility that urothione is metabolically related to the molybdenum cofactor. Urine samples from normal individuals were found to have urothione, whereas urine from molybdenum-cofactor-deficient patients were devoid of the compound (4), a fact confirming the proposed metabolic link.

On the basis of the structural elements noted in the three oxidized derivatives, we proposed structure D (Figure 3) for the active molybdenum cofactor, a complex of Mo and the organic component termed molybdopterin. The significant structural features of molybdopterin are (*a*) the presence of a reduced pterin ring and (*b*) a 4-carbon side chain containing an enedithiol and a terminal phosphate ester. We have carried out a variety of experiments to test the proposed structure, and the results are in full agreement with the suggested structure for molybdopterin (64). Since the role of molybdopterin as an essential trace element revolve entirely on its interaction with molybdopterin, information on the current state of knowledge on some of the essential aspects of the molybdenum cofactor is summarized below.

MOLYBDOPTERIN IS A UNIVERSAL COFACTOR A number of molybdo- and nonmolybdoenzymes have been examined for the presence of molybdopterin, and the results are summarized in Table 4. All molybdoenzymes examined so far contain molybdopterin or a pterin of a similar nature. Formate dehydrogenase from *Clostridium thermoaceticum* is unusual in that it appears to contain tungsten, rather than molybdenum, as the functional metal. Nevertheless, the purified enzyme yields fluorescent species indistinguishable from Form A and Form B. All of the nonmolybdoenzymes tested so far are devoid of molybdopterin.

THE MO COFACTOR IS A COMPLEX OF MO AND MOLYBDOPTERIN In order to demonstrate that molybdopterin is capable of complexing Mo, the effects of varying the conditions used for release of Mo cofactor from sulfite oxidase

Table 4 Assay for molybdopterin in various molybdo- and nonmolybdoenzymes

Enzyme	Source	Mo	Molybdopterin
Xanthine oxidase	Bovine milk	+	+
Xanthine oxidase	Bovine milk (Sigma)	+	+
Xanthine dehydrogenase	Chicken liver	+	+
Aldehyde oxidase	Rabbit liver	+	+
Nitrate reductase	*Chlorella vulgaris*	+	+
Nitrate reductase	*Cucurbita maxima*	+	+
Sulfite oxidase	*Thiobacillus novellus*	+	+
Demolybdosulfite oxidase	Tungsten-fed rats	−	+
Purine hydroxylase	*Aspergillus nidulans*	+	+
Carbon monoxide dehydrogenase	*Pseudomonas carboxydovorans*	+	+
Formate dehydrogenase	*Methanobacterium formicicum*	+	+
Formate dehydrogenase	*Clostridium thermoaceticum* (tungsten)	−	+

were studied (65). Exposing sulfite oxidase to 6-M guanidine-HCl for 2 to 5 min led to dissociation of Mo cofactor. Subsequent gel filtration on Sephadex G-25 yielded protein-free cofactor. In the reconstitution assay using the apoprotein of *N. crassa nit-1* nitrate reductase as the acceptor, the reconstitutive ability of cofactor released at pH 7.0 was independent of the presence of Mo in the reconstitution mixture, whereas cofactor released at pH 8.0 was totally dependent on added Mo. In the latter case, very high Mo concentrations were needed, in contrast to the levels of Mo expected in cofactor released at pH 7.0. Under all conditions, the presence of molybdopterin in the cofactor-containing fractions from Sephadex G-25 was established by oxidative conversion to the highly fluorescent derivative, Form A. The reconstituting activity of the cofactor released at pH 7.0 was abolished by sulfhydryl-reactive reagents such as Hg^{2+} and arsenite; thus, in the active, intact cofactor the Mo is complexed to the -SH groups of molybdopterin. Studies with xanthine dehydrogenase yielded similar results.

ABSENCE OF MOLYBDOPTERIN IN THE *nit-1* MUTANT OF *NEUROSPORA CRASSA* The oxidized pterins Form A and Form B can be readily generated from mycelia of wild-type *Neurospora crassa* (66). In contrast, cells of the pleiotropic mutant *nit-1* are virtually devoid of material giving rise to these compounds. Similar mutants in *E. coli* also fail to yield these pterins (67). Nor can these derivatives be obtained from tissues of the Mo-cofactor-deficient patients. These findings categorically show that molybdopterin is the molecule that is lacking in these mutant organisms.

MOLYBDOPTERIN IS TRANSFERRED TO *nit-1* NITRATE REDUCTASE DURING RECONSTITUTION A procedure for preparing highly active molybdopterin from denatured sulfite oxidase has been described (66). The preparation, containing ascorbate as a stabilizing agent, was free of Mo, which shows that molybdopterin can remain active even in the absence of Mo. On the basis of its phosphate content and a quantitation of nitrate reductase units reconstituted, the preparation was judged to be greater than 50% active. The phosphate group is essential for the biological function of molybdopterin, since treatment of the isolated material with alkaline phosphatase led to total loss of reconstitutive ability. The molybdopterin preparation was free of any peptides. Using the isolated sample of molybdopterin, it was demonstrated that, after reconstitution of nitrate reductase in *nit-1* extracts and subsequent chromatography on Sephadex G-200, fractions displaying nitrate reductase activity were highly enriched in molybdopterin. These data further corroborated the conclusion that molybdopterin is the sole organic constituent of Mo cofactor and that transfer of a peptide is not required for the reconstitution of *nit-1* nitrate reductase.

NUTRITIONAL ASPECTS OF MOLYBDENUM

Molybdenum in Tissues

Under normal dietary conditions, the molybdenum content of tissues is quite low [0.1–1.0 μg/g wet weight in humans and chickens (68), with lower amounts in rats (69)]. In humans, liver, kidney, adrenal gland, and bone contain the most molybdenum in both quantity and concentration, with lesser but still significant quantities in lung, spleen, and muscle (68). Retention of the metal appears to be low; the major part of the administered metal is excreted within a few hours (70).

Tissue levels of Mo can be affected by ingestion of foodstuffs grown on soil of high molybdenum content or experimentally supplemented diets. Raising the molybdenum content of rat diets from 1 to 30 ppm altered the hepatic molybdenum from 1 to 11–12 ppm, while the molybdenum content of bone increased from 0.2 to 9–12 ppm (71). Other experiments with various species have demonstrated similar responses, depending upon the dietary conditions. Elevations of molybdenum content of soft tissues by factors of 100 or more are not uncommon if the dietary content of molybdenum, copper, and sulfate are appropriately altered (72).

Dietary inorganic sulfate (300–3000 ppm) decreases the tissue molybdenum content in rats (73, 74), sheep (72), and cattle (72), presumably by competing for absorption in the intestine. More detailed information concerning the uptake of molybdenum is reviewed by Underwood (72).

The major part of tissue molybdenum is presumably associated with molybdoenzymes. The extreme instability of molybdopterin, referred to above, makes it very unlikely that tissues would contain significant levels of free molybdenum cofactor, the complex between Mo and molybdopterin. However, there is strong evidence that storage forms of the cofactor exist in all systems, as observed in bacteria (75–78) and rats (77). Johnson et al (77) examined the distribution of molybdenum in the livers of rats fed a control diet containing 0.02–0.03 ppm molybdenum. As seen in Table 5, sulfite oxidase and xanthine oxidase accounted for 60% of the molybdenum, while the molybdenum associated with the molybdenum cofactor contained the remainder. Also of interest is the reported absence of detectable amounts of Mo in the liver tissue of molybdenum-cofactor-deficient patients. It thus appears that in normal dietary status all tissue molybdenum is quantitatively associated with molybdopterin, but it is possible that under conditions of more variable exposure of molybdenum, other forms of molybdenum, including free molybdate ion, might be extant. The unusually high content of Mo in bone tissue remains unexplained, but could have relevance to the reported anticariogenic role of the metal (70).

Table 5 Distribution of molybdenum in rat liver

	Animal 1	Animal 2	Animal 3
Liver weight (g)	9.0	8.7	12.5
Activity[a] (units/g)			
Sulfite oxidase	231	212	196
Xanthine oxidase	3.01	3.44	3.24
Molybdenum cofactor	182	189	157
Molybdenum contribution (μg/g)			
Sulfite oxidase	0.255	0.234	0.216
Xanthine oxidase	0.077	0.088	0.083
Molybdenum cofactor	0.201	0.209	0.173
Molybdenum expected (μg/g)	0.53	0.53	0.47
Molybdenum detected (μg/g)	0.52	0.58	0.48

[a]Activity of Mo cofactor is expressed as units of sulfite activity reconstituted in samples of purified demolybdoenzyme.

[b]Molybdenum contribution of the individual components was calculated assuming a specific activity of 1500 units/mg and 1.656 μg of Mo per mg for sulfite oxidase, and 2.5 units/mg and 0.640 μg of Mo for xanthine oxidase. Molybdenum contribution of the cofactor was calculated as for sulfite oxidase.

Molybdenum in Blood

There is wide variation in the estimated blood levels of Mo in humans, but most of the data indicate a normal value of only a few ng/ml of whole blood (70). Allaway et al (79) described average levels of 5 ng/ml (whole blood) or less for 75% of normal subjects in 19 areas of the US, but did find two areas where 70% or more of the population had levels exceeding 5 ng/ml. It has been reported that people in selected areas with high concentrations of soil Mo in USSR or Africa may manifest blood levels as high as 170 ng/ml (170 ppb) (70).

PATHOLOGIC STATES OF MOLYBDENUM INTAKE

Dietary Sources of Molybdenum

Several lists of Mo content of foods have been published (70, 80, 81). Some of the published values are presented in Table 6. The richest sources of the element include legumes, cereal grains (and hence bread and baked products), leafy vegetables, milk, beans, liver, and kidney. Fruits, stem and root vegetables, and muscle meats are among the poorest. Tsongas et al (81) concluded that milk, beans, cereals, pastas, breads, bakery items, and vegetable mixtures are the major sources of Mo in the average diet. The same authors

calculated a daily intake of 120–240 μg Mo/day, depending on age, sex, and income. This estimate is lower than the value 210–460 μg/day calculated earlier by Tipton et al (80).

Daily Requirements

Prior to the discovery of the importance of sulfite oxidase for normal human development, there was no compelling evidence to show that Mo was an essential element for human health. Nutritional studies on the dietary requirement for the metal were sporadic, limited in scope, and quite inconclusive. A few molybdenum balance studies have been reported, but have not yielded consensus data. The state of affairs in this area was summarized by Underwood: "Molybdenum is apparently of so little practical significance in human nutrition, either in health or disease, that few people have been stimulated to undertake studies with this element" (72). There were several deterrents to nutritional studies, including the difficulty of detecting small levels of the metal, the apparent low dietary requirement for the metal, lack of biochemical indices for quantifying the molybdenum status of individuals, and the inability to produce dietary molybdenum deficiency in animals or humans. Apparently the intestinal absorption of the element is extremely efficient even at very low dietary levels. Though a Recommended Daily Allowance has not been established, a range of 0.15–0.5 mg/day has been estimated as adequate for adults (82).

Molybdenum Deficiency

True molybdenum deficiency has not been achieved in experimental animals. Early efforts to produce molybdenum deficiency using "low-molybdenum" diets caused attenuation of rat liver xanthine oxidase to 10% of normal without affecting the health of the animals or changing the excretion of uric acid or allantoin (83, 84). Similar experiments with chickens receiving low-molybdenum diets (0.03 mg/kg) failed to show consistent changes in growth or development. It was reported that certain pathologic conditions involving the appearance of altered feather maturation (clubbed down) and femoral epiphyseal changes (scabby hip syndrome) in chicks could be reversed by molybdenum supplementation (85–87). However, a later report suggested that a deficiency of dietary components besides molybdenum may be required to produce the symptoms described (88). In light of the existence of the molybdenum cofactor, which may be an essential dietary requirement for some animals, the syndromes need further study.

At least two studies have presented evidence of the importance of Mo in early developmental stages. In experiments with goats, Anke et al (89) found that feeding of diets low in Mo to dams led to a decrease in conception rate and poor fetal survival rate. Payne (86) reported that a condition leading to

Table 6 Mo content of various foods (μg/g wet weight)

	Tsongas et al (81)	Schroeder et al (68)
Vegetables		
Onions	0.02	0.00
Corn	0.08	—
Squash	0.05	0.02–0.16
Pumpkin	0.05	—
Spinach	0.07	0.26
Lettuce	0.03	0.02–0.11
Potatoes	0.07	0.03
Tomatoes	0.05	0.00
Cabbage	0.06	0.02
Green beans	0.15	0.66
Wax beans	0.80	0.43
Lima beans	0.80	0.31
Lima bean seeds	8.70	4.80
Black-eyed peas	1.20	—
Green peas	1.30	3.5
Mushrooms	—	0.00
Carrots	—	0.08
Cauliflower	—	0.00
Fruit		
Banana	0.08	0.03
Plum	0.01	0.06
Pineapple	0.09	—
Oranges	0.01	—
Orange juice	—	0.79
Eggs		
Various sizes	0.09	0.49
Butter	<0.01	0.00–0.19
Fish		
Halibut	0.02	0.04
Shrimp	0.01	0.03
Mussels (freeze-dried)	—	0.56
Lobster (frozen)	—	0.23
Poultry		
Chicken (white)	0.05	0.00
Chicken (dark)	0.05	—
Beef		
Steak	0.04	0.00
Kidney	—	21.4
Liver	—	1.97

Table 6 (*Continued*)

	Tsongas et al (81)	Schroeder et al (68)
Pork		
Sausage	0.02	—
Chops	0.04	3.68
Milk		
Skim 2%	0.05	—
Skim, powder	—	0.29
Whole	0.05	0.20
Ice cream	0.05	0.00
Cheese		
Cottage	0.09	—
American	—	0.05
Grains and cereals		
Rice	0.29	—
Wheat	—	0.64–5.87
Rye	—	0.61–1.47
Oats	—	1.37
Bread		
All types	0.21	—
Sugars		
All kinds	—	0.00–0.16
Oils and Fats		
All kinds	0.00	0.00

high embryonic mortality and abnormal growth and development of chicks could be abolished by including low levels of Mo in the diets. The high concentration of Cu in the commercial diets used in these studies was presumably the cause of the syndrome.

In retrospect it seems possible that molybdenum deficiency in adult humans or laboratory animals is a benign condition and therefore asymptomatic. The aforementioned nutritional studies with rats, goats, and chicks and the severity of the pathology associated with molybdenum cofactor deficiency in humans suggest that sulfite oxidase plays a major essential role only during the in utero stage and the postnatal developmental period. Arguing against this possibility is the report of induced Mo deficiency in an individual under total parenteral nutrition (TPN) (90). The patient, a 24-year-old male with

Crohn's disease, had been on TPN for a prolonged period but developed gradual symptoms of intolerance to the TPN solution used as a source of nutrients. A metabolic study on urinary metabolites showed high levels of sulfite and thiosulfate and low sulfate, symptomatic of sulfite oxidase deficiency, and high levels of hypoxanthine and xanthine and low uric acid, as would be seen in xanthine oxidase deficiency. Supplementation of the TPN solution with 300 μg/day of Mo led to a dramatic decrease in the urinary content of the abnormal metabolites and the capacity to tolerate the TPN treatment. Despite the subsequent death of the patient due to massive gastrointestinal hemorrhage, these important findings provide a convincing argument for supplementation of TPN regimes with Mo. Since it is difficult to isolate the possible consequences of Mo deficiency from the major illness of this patient, it is not possible to determine whether some of the observed pathology was attributable to Mo deficiency.

At present, the best way to produce molybdenum deficiency (as judged by extremely low tissue levels of xanthine dehydrogenase, sulfite oxidase, and molybdenum content) is through dietary administration of tungstate as an antagonist of molybdenum uptake. Tungsten is placed in the periodic table in the group immediately beneath Mo but has a similar atomic size and number of valence states. It has been used extensively in many biological systems to demonstrate the presence (or requirement) of molybdenum in enzymes that exist in low amounts or are unstable to conditions needed for purification. Molybdenum deficiency induced by tungsten treatment could be used in studies in which biochemical functions attributed to any molybdoenzyme could be verified.

One of the findings made by Gunnison et al (53) was an increased incidence of mammary adenocarcinoma in sulfite-oxidase-deficient (tungsten-treated) rats. Though the observed incidence was low (4/149) and not statistically significant, it was argued that the rarity of spontaneous tumors of this type among rats could indicate that the observed carcinomas may be treatment related. These studies of Gunnison et al may be of significance in relation to certain reports of the relationship between Mo deficiency and esophageal cancer, first reported in the Bantu of the Transkei in 1966 (91) and subsequently in the Hunan Province in China (92). In the latter case, analysis of a large number of samples of cereals and drinking water showed an inverse relationship between the mortality rate from esophageal cancer and the dietary content of a number of minerals, including Mo. Levels of Mo in the serum of inhabitants of the high-risk area were lower than those seen in low-risk areas. In animal experiments designed to confirm the relationship between Mo deficiency and cancer, Yang and coworkers reported that Mo supplementation significantly inhibited the esophageal and forestomach cancer induced in male rats by N-nitrososarcosine ethyl ester and the mammary carcinoma induced in

female rats by N-nitroso-N-methylurea (93–95). The usefulness of tungsten-treated rats as a model of Mo deficiency in corroborating the suggested link between deficiency of the metal and the incidence of cancer is obvious.

Molybdenum Toxicity

The effect of excessive molybdenum intake on animals appears to depend upon a number of variables, including the age and species of the animal, the previous dietary history of the animal, the relative amounts of molybdenum, copper, and sulfur in the diet, and the levels of other dietary constituents, not all of which have been identified (72, 85, 96). The symptoms vary among species, but may include failure to thrive (i.e. poor weight gain, poor growth, loss of weight or condition), anemia, dermatologic changes, anorexia and diarrhea, and other unique species-specific symptoms (96). Ruminants such as cows and sheep show extreme sensitivity to dietary molybdenum levels as low as 2–30 ppm, while horses and pigs can show tolerance to levels that are much higher (1000 ppm). In many ways, the symptoms resemble those of copper deficiency, and treatment with supplemental copper usually reverses them. However, symptoms may be produced where the dietary copper is "normal" but the molybdenum content is considerably higher than "normal."

It has been suggested that the underlying biochemical events leading to molybdenum toxicosis take place within the gastrointestinal tract, where the interactions among dietary copper, molybdenum, and sulfur are maximal. Huisingh et al (97) proposed that copper becomes unavailable when it interacts with molybdate to form the biologically inactive cupric molybdate. The formation of insoluble copper sulfide complexes from intestinal or ruminal bacterial evolution of sulfide is another possible way of removing copper. An alternative hypothesis proposed by Dick et al (98) stated that toxicity is the consequence of the gut flora reducing sulfate or other sulfur compounds to sulfide, followed by the sulfide reacting with molybdate to form thiomolybdates. Thiomolybdates are more toxic than molybdenum oxides when administered to animals that lack rumen (85). The protective action of copper against Mo toxicity in ruminants was explained by its forming a complex with thiomolybdate to render the molybdenum complex nutritionally ineffective. When the diet is low in Cu, intestinal absorption of free thiomolybdate would occur, followed by a reaction of the absorbed thiomolybdate with intracellular copper and other metals or proteins to produce the toxic effects. This theory would explain the differences in susceptibility between ruminants and nonruminants subjected to the same dose of molybdenum in the diet. The lack of a rumen and the associated flora would prevent toxicity in rats (except at high concentrations of molybdenum), while the presence of sulfide-producing organisms in the cow rumen would render the animal extremely susceptible to thiomolybdate production. Variations in copper intake would affect the level

of thiomolybdates absorbed and determine the extent of observed toxicity. More complete discussions of animal molybdenum toxicosis are presented in the reviews by Underwood (72), Pitt (96), and Mills & Bremner (85).

Molybdenum toxicity in humans has rarely been reported, but reports of exposure to high Mo are extant. In 1961, Kovalskii et al (99) described a gout-like syndrome with arthralgia and hyperuricemia in the population of a province in Armenia. In two villages that were epidemiologically investigated, 71 of 362 adults were noted to have symptoms that typically included recurrent joint pain in knees, interphalangeal joints of the hands, and metatarsal-phalangeal joints of the feet associated with erythema, edema, and joint deformity. Other symptoms and signs of hepatomegaly, gastrointestinal changes, and kidney disease were also described, but specific details were not given. Increases of serum uric acid were noted both in the symptomatic (mean $\pm$ SE = 81 $\pm$ 0.4 mg/100 ml) and asymptomatic (5.3 $\pm$ 0.4 mg/100 ml) individuals living in the villages as compared to the lower levels for individuals living outside the area (3.8 $\pm$ 0.5 mg/100 ml). Symptomatic individuals also demonstrated hyperuricosuria and elevation in blood Mo content (symptomatic 310 $\pm$ 20 ppb, asymptomatic 170 $\pm$ 10 ppb, control 60 $\pm$ 10 ppb). Blood copper levels were slightly though significantly lower. The dietary intakes were 10–15 mg of molybdenum and 5–10 mg of copper per day in the study population, and 1–2 mg Mo and 10–15 mg copper for individuals outside the area. No evidence of uric acid crystal deposition in joints was presented nor was the possibility of significant liver disease as the cause of elevations in uric acid ruled out. The pathogenesis of the disorder remains to be explained.

The question of possible toxicity in workers exposed to molybdenum in the dust of a molybdenite roasting plant was addressed by Walravens et al (100). Evaluation of clinical and biochemical parameters revealed the lack of specific physical complaints, but did show elevations in serum ceruloplasmin (50.5 $\pm$ 1.4 mg/100 ml for workers, 30.5 $\pm$ 1.3 mg/100 ml for controls), nonsignificant elevations in uric acid, and significant elevations in blood and urine Mo without elevations in urinary copper excretion. Exposure was calculated to be 10.2 mg/day of molybdenum as dust. The high worker turnover rendered epidemiologic studies difficult and made the description of possible harmful effects impossible to assess. The elevations in ceruloplasmin suggest that the workers may be reacting to the excessive molybdenum, but much more research is needed to study the long-term effects of exposure to high levels of molybdenum in dust.

NEW PERSPECTIVES

The accumulation of nutritional, pathological, and biochemical information concerning molybdenum metabolism has stimulated interest in a number of

areas for future investigation. In view of the invariant association of molybdenum with its cofactor in known molybdenum enzymes and storage proteins, future studies of absorption and transport of molybdenum in tissues and body fluids need to investigate the possible role of the cofactor or similar molecules in the assimilation of the metal. Similarly, the site(s) and mechanism of synthesis of the cofactor provide an exciting area for study. A synthetic process similar to the pathway for heme synthesis may be envisaged, as well as the possibility for control of protein synthesis or posttranslational modification of molybdenum enzymes.

From the pathology viewpoint, a number of problems may be studied at the biochemical level. The presence of significant quantities of molybdenum beyond the amount contained by the three known molybdoenzymes and cofactor in human liver or tissues of molybdotoxic animals raises several questions: How do animals store molybdenum? What are the biochemical effects of excessive molybdenum and thiomolybdate intake in humans and animals? Are there other molybdoenzymes whose activities might be lacking in patients with molybdenum cofactor deficiency? With the advant of techniques for long-term total parenteral nutrition for debilitated patients with chronic bowel disease, malignancy, or other disease processes, deficiencies of trace elements and other organic nutrients are increasingly possible. Investigations into the Mo requirements of such patients may be necessary in order to avoid a nutritional deficiency of the metal. Now that the essentiality of the metal for normal human health and development is established, these and other studies on various aspects of molybdenum metabolism take on added significance.

It is now abundantly clear that molybdenum is an essential trace element for humans, at least during the early developmental period. The primary biochemical function in which this essentiality is expressed is the oxidation of sulfite to sulfate. The genetic absence of the enzyme leads to severe neurological impairment and, in most cases, death. The molecular basis for the neuropathology is either toxicity from the sulfite accumulating in the body or the failure to generate sulfate in situ in the cells of the central nervous system. The brain abnormality seen in sulfite oxidase deficiency suggests that failure to form adequate amounts of sulfolipids and sulfoproteins, resulting from decreased availability of sulfate, could well be the primary cause of the lesions. The presence of some sulfolipids in affected brain tissue could be due to the ability of inorganic sulfate to supply part of the pool normally needed for the sulfation reactions.

It is not clear whether sulfite oxidase deficiency could occur in adulthood, and if so, whether the condition would be deleterious. Certainly adult rats maintained on a tungsten-containing diet appear to thrive well and are indistinguishable from normal rats. This finding suggests that tungsten-treated adult rats may not be the appropriate model for human molybdenum cofactor

deficiency. A better procedure would be to maintain pregnant rats on tungsten. Under these circumstances, the fetuses would develop under conditions similar to human sulfite oxidase deficiency. The progeny could then be examined for survival, molybdenum enzyme activities, presence of abnormal sulfur-containing metabolites, neurological damage, etc. If pathology is observed, the possibility of reversal by treatment with Mo could be tested.

So far all of the patients exhibiting combined deficiencies of molybdoenzyme activities appear to be deficient in molybdopterin rather than molybdenum itself. The symptoms of Mo deficiency would be indistinguishable from those of cofactor deficiency; but the Mo deficiency state should be correctable by administering the element by an appropriate route. It is possible that because of specific circumstances fetal Mo deficiency could occur, even if only to a partial degree. Children born under such circumstances might have sufficient brain damage to suffer from mental retardation.

The above scenario is but one of many that could result in isolated instances of Mo deficiency in children. There is greater need now than ever before for nutritional studies on human molybdenum requirement and assimilation. The biochemistry of the function of Mo is now well understood and should serve as the basis for designing the protocols for these studies. The carefully performed studies of Gunnison et al (41, 53) in experimental rats should serve as the model for human nutritional studies. Monitoring urinary levels of thiosulfate and S-sulfocysteine, the abnormal metabolites arising from sulfite accumulation, and the ratio of hyperxanthine plus xanthine versus uric acid should provide the means of assessing molybdenum status and obviate the need for the more cumbersome balance studies.

The reported existence of low-molybdenum areas in China and the suggested relationship of Mo deficiency to increased incidence of cancer both represent new developments in the study of molybdenum. Assessment of the Mo status of the population of the affected area using the procedures mentioned above could provide valuable information on methods to be used in epidemiological studies. If the increased incidence of cancer in the Mo-deficient population is statistically significant, these findings might lend credence to the suggestion that even low levels of intracellular sulfite are mutagenic. These observations clearly need further investigation.

It is not known whether humans can synthesize molybdopterin de novo or from a dietary precursor. There are two other pterins of importance in animal metabolism. Folic acid, which serves as a carrier of single carbon units in bacteria, plants, and animals, is a dietarily essential vitamin for animals. Biopterin, the other major pterin, is a cosubstrate for the hydroxylation of phenylalanine, tyrosine, and tryptophan in animals. These reactions do not occur in plants, and animals must perforce synthesize biopterin de novo. Like folic acid, molybdopterin is present in plants, the ultimate food source for

animals, and could conceivably be obtained in the diet by animals. However, the extreme lability of molybdopterin might lead to its destruction at a stage before intestinal absorption. Of course it is possible that the dietary factor would not be molybdopterin itself, but a precursor molecule, possibly a pterin. In any event, it might be a fruitful endeavor to measure molybdopterin content of various foodstuffs, either by conversion to Form A and Form B or by using the activity assay, and correlate it with the Mo content of the samples.

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