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MOLYBDENUM – A TOXICOLOGICAL APPRAISAL

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MOLYBDENUM - A TOXICOLOGICAL APPRAISAL

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CHAPTER 1 INTRODUCTION

In 1972, a preliminary review on molybdenum (by Karl-Henrik Robèrt, M.D. and Pamela Boston, B.A.) was submitted to the U.S. Environmental Protection Agency according to a contract between that agency and the Department of Environmental Hygiene of the Karolinska Institute. Based on this report, it was decided to make a more extensive and detailed review. The present document is a result of that decision and has been carried out under contract 68-02-1210 between the same two organizations. The project officer for both the preliminary and the final report has been Robert J.M. Horton, M.D. of the U.S. Environmental Protection Agency.

The focus of "Molybdenum - A Toxicological Appraisal" is upon an evaluation of the metabolism and toxic effects of molybdenum which can be of relevance for human beings. Some data have been gathered and assessed in connection with analytical methods, production, uses and occurrence.

The document has been prepared by a group on molybdenum. The names of the members are listed on the title page. Individual chapters were drafted by Magnus Piscator, M.D. (chapters 2-3) and Karl-Henrik Robèrt, M.D. (chapters 4-7).

In addition, Velimir Vouk,[x)] Ph.D. of the World Health Organization kindly contributed by drafting all sections dealing with the U.S.S.R. literature available only in the original language and also gave valuable criticism on the work as a whole. A collaboration with the WHO has further been under way through the participation

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of Dr. Piscator in two meetings on molybdenum organized by that body.

At the same time that this document was submitted to the U.S. Environmental Protection Agency, a conference on molybdenum in the environment was held in Denver, Colorado. Papers presented during that conference have not been possible to take into consideration in the present report. For further details the reader may get in touch with The Molybdenum Project[x)].

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CHAPTER 2 ANALYTICAL METHODS

Molybdenum is a transition element in group 6B of the periodic table. It can occur in 6 valence states: 0, +2, +3, +4, +5 and +6, making its chemistry very complicated and hence creating difficulties in analysis. More than 50 inorganic forms are known (Handbook of Chemistry and Physics, 1974/1975). With its great complexing power, molybdenum easily forms chelates, a property which has been used for analytical purposes. Colorimetric methods have been used for decades for determination of molybdenum, but during later years neutron activation, atomic absorption and X-ray fluorescence have also come into the picture. For more detailed discussions on the determination of molybdenum reference is made to recent reviews by Meglen and Glaze, 1973, 1974.

2.1 COLORIMETRIC METHODS

More than 100 years ago it was discovered that reduced molybdenum in an acid solution formed a colored complex with thiocyanate, which could be extracted into an organic solvent. This formed the principle for the most common method for determination of molybdenum - the thiocyanate colorimetric method. Numerous articles have been published on this method and many modifications have been proposed (e.g. Dick and Bingley, 1947, 1951, Karlsson, 1961, Fishman and Mallory, 1968, Kim and Zeitlin, 1968, Duval, 1971, Khosla and Rao, 1971, Meglen and Glaze, 1973, Savariar, Arunachalam and Hariharan, 1974, Yatirajam and Ram, 1974). The basic procedure has been to take up the sample in diluted hydro-chloric acid after wet or dry ashing. Thiocyanate, usually the potassium salt, and tin (II) chloride are added together with an iron salt. Hexavalent molybdenum is reduced to pentavalent molybdenum, but reduction to other valence states may occur simultaneously to some degree. The colored complex formed is extracted into an organic solvent, which may be ethylether, isoamylalcohol, butylacetate, isopropyl ether, methyl isobutyl ketone or N-benzylaniline

in chloroform or a mixture of chloroform and isoamyl alcohol. The color is usually read in a spectrophotometer at a wavelength varying from 465 to 500 nm depending on the method used.

The thiocyanate method is very complicated, as indicated by the many different modifications proposed. One important point seems to be that tin chloride causes reduction not only to pentavalent molybdenum, but also to other valence states. This has been claimed to be surmountable by using copper and thiourea as reducing agents, and extracting with N-benzylaniline (Khosla and Rao, 1971) or by using hydrazine as a reducing agent and tribenzylamine in chloroform as an extracting agent (Yatirajam and Ram, 1974). Another factor is that small amounts of iron must be present (Dick and Bingley, 1947). Many compounds, especially tungsten, may interfere with the determination of molybdenum by the thiocyanate method. Interference from other compounds seems to depend to a large extent on the procedure chosen. No consistent pattern has appeared, but titanium, platinum, silver, silica, chromates and nitrates have been shown to interfere in some procedures.

Some media, e.g. water, have generally low molybdenum content which may make pre-concentration necessary. Ion exchange (Fishman and Mallory, 1968) or precipitation with oxine and tannic acid (Kim and Zeitlin, 1968) have been used. The thiocyanate method seems to allow the determination of about 0.01-0.1 ug molybdenum/g sample. Precision has been reported to be about 10% in vegetables with a molybdenum content of 0.1-0.2 ug/g and 1-2% at concentrations above 1 ug/g. In one study (Fishman and Mallory, 1968) the thiocyanate method was compared with three other methods. As shown in Table 2:1 it was proven to be accurate when determining molybdenum in water. In plant material (clover) the thiocyanate method showed a mean of 0.96 ug/g (3 determinations) and neutron activation 0.94 ug/g (9 determi-

nations) (Van Zanten, Decat and Leliaert, 1962).

Another colorimetric method is the dithiol method, according to which hexavalent molybdenum is combined with toluene-3,4-dithiol to form a colored compound, which can then be extracted into an organic solvent (Stanton and Hardwick, 1967, 1968, Ssekaalo, 1971, Cardenas and Mortenson, 1974, Quin and Brooks, 1975). As seen in Table 2:1 this method gives similar results as the thiocyanate method for determination of molybdenum in water.

The dithiol method has had many applications and has been subjected to many modifications. For more details and references see e.g. Quin and Brooks, 1975.

Another method which involves complexing of hexavalent molybdenum uses 2-amino - 4-chlorobenzenethiol hydrochloride, also forming a colored complex, which can be extracted with chloroform (Ssekaalo and Johnson, 1969). This method was tested on barley and milk, the latter material requiring a separation of molybdenum from phosphate prior to final analysis.

2.2 EMISSION SPECTROGRAPHY

Spectrographic methods have been used to determine molybdenum in plants and water, and in animal and human tissues (e.g. Yip, Shaw and Nace, 1961, Meltzer et al., 1962, D´ Alonzo and Pell, 1963, Tipton et al., 1963, Niedermeier and Griggs, 1971). Molybdenum has been precipitated with e.g. 8-hydroxyquinoline (Heggen and Strock, 1953, Fishman and Mallory, 1968) as a pre-concentration step. In Table 2:1 such a method is seen to give similar results as other methods for determination of molybdenum in water. By concentrating and extracting from large samples concentrations of a few ng/g can be measured. Concentration of molybdenum in the sample has also been accomplished by complexing with tert-carbate (N-pyrrolidinodithiocarbamic acid-sodium salt) and extraction

into chloroform (Voth, 1963).

2.3 NEUTRON ACTIVATION

Neutron activation analysis has been employed in several investigations (Bowen, 1959, Van Zanten, Decat and Leliaert, 1962, Samsahl and Brune, 1965, Livingston and Smith, 1967, Kjellin, 1968, Lunde, 1968, Pillay and Thomas, 1971, Morgan and Holmes, 1972, Plantin, 1973). After irradiation molybdenum is separated by a complexing agent or ion-exchange. Both ^{99}Mo and ^{101}Mo may be used. The detection limit is reported to be 0.1 ug for ^{101}Mo (Van Zanten, Decat and Leliaert, 1962) and 0.2 ng for ^{99}Mo (Morgan and Holmes, 1972). Good agreement was found when the ^{101}Mo method was compared to a colorimetric method (see section 2.1).

2.4 ATOMIC ABSORPTION

Molybdenum is not well suited for analysis by atomic absorption in flame (Johnson, West and Dagnall, 1973, Meglen and Glaze, 1973). A claimed sensitivity of around 0.01 ug/ml (Roussos and Morrow, 1968) has not been supported by other authors. The sensitivity is generally relatively low. Molybdenum is not easily atomized, forming refractory oxides in the flame.

After extraction into organic solvents concentrations of 1-3 ug/g in fertilizers could be determined (Hoover and Duren, 1967, Koirtyohann and Hamilton, 1971). In fresh water molybdenum has been determined at concentrations of from 230 to 3200 ug/l, in which range the agreement with other methods is good (see Table 2:1). In this case the analysis was carried out on a dithiol-MIBK extract (Fishman and Mallory, 1968).

Flameless methods have been recommended, but data are not available as to how they compare to other methods. A sensitivity of 0.035 ug/g has been reported (Johnson, West and Dagnall, 1973). Muzzarelli and Rocchetti, 1973, determined

molybdenum in seawater concentration by ion-exchange with a graphite atomizer after separation and from their data it can be calculated that about 0.03 ug/ml can be detected in the concentrated solution.

2.5 QUALITY CONTROL AND INTRA-LABORATORY COMPARISONS

A considerable number of methods have been used to determine molybdenum in different media. In many studies, the method proposed has only been tested on artificial solutions or on a very limited number of samples, and generally not been compared with other methods. Comparisons among different methods inside the laboratory have been carried out, as in the study by Fishman and Mallory, 1968 (see Table 2:1). In other studies a few standard samples have been tested. Very few data on inter-laboratory comparisons have emerged.

Meglen and Glaze, 1974, performed intra-laboratory checks in which they compared their own colorimetric (thiocyanate) method with an atomic absorption method, and they found a good agreement for water samples with a very high content of molybdenum, i.e. in the mg/l range. In an inter-laboratory check, they compared their own results on the thiocyanate method for water samples with two other laboratories, one using a colorimetric method and the other both colorimetric and atomic absorption methods. All water samples contained between 250 and 450 ug/l, and there was a good agreement between laboratories.

The U.S. Geological Survey distributed 6 mineral or soil samples to 85 laboratories in the U.S. for the determination of molybdenum and 14 other elements. The values submitted on the molybdenum samples varied widely, e.g. in one mineral sample from 1 to 500 mg/kg (Allcott and Lakin, 1974).

No data on the accuracy of methods used for the determination of molybenum in animal tissues and body fluids are available.

TABLE 2:1 MOLYBDENUM IN FRESH WATER (ug/l) DETERMINED BY FOUR DIFFERENT METHODS. (Modified from Fishman and Mallory, 1968).

Sample	Thiocyanate	Spectrographic	Dithiol	Atomic absorption
1	920	900	960	970
2	6	2	6	
3	3000	2800	3200	3200
4	400	300	390	
5	8	7	7	
6	290	270	320	
7	330	280	330	
8	3800	3500	3900	
9	260	220	250	230
10	19	14	21	
11	1800	1600	1900	
12	260	230	300	280
13	550	500	550	530
14	2	3	1	

CHAPTER 3 PRODUCTION, USES AND OCCURRENCE

A vast literature has accumulated on the aspects of molybdenum treated in this chapter. A complete review of the environmental behavior of molybdenum has not been possible to give here. The distribution and transport of molybdenum in environmental compartments present a very complex pattern, intimately connected with the basic properties of the earth's surface.

For details on production technology, supply-demand relationships, consumption patterns and the like, see reviews by Morning, 1969, and Sheridan, 1970.

Recently an extensive project on transport, distribution and effects of molybdenum has been undertaken in Colorado, where the largest molybdenum deposit in the world is situated and mined. Two comprehensive progress reports (Transport and the Biological Effects of Molybdenum in the Environment, Progress Report, January 1, 1973, and Progress Report, January 1, 1974, University of Colorado, Boulder, Colorado and Colorado State University, Fort Collins, Colorado) have already emerged from the University of Colorado and Colorado State University. Readers interested in the flow of molybdenum in the environment are referred to that project for more extensive information[x)].

Some data on the occurrence of molybdenum in soil, plants and food will also be found in Chapter 6 in connection with effects on livestock and human beings.

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x) The leader of this project is Dr. Willard R. Chappell; His address: The Molybdenum Project, Duane F-1033, University of Colorado, Boulder, Colorado 80302.

3.1 PRODUCTION

Molybdenum does not occur in the native state, but is obtained from minerals, such as molybdenite (MoS_2), wulfenite ($PbMo_4$), ferrimolybdate ($FeMoO_3 \times H_2O$) and jordisite (amorphous MoS_2). Many other minerals contain molybdenum, but these four are the ones used for commercial exploitation.

Molybdenite is the greatest source of molybdenum, the largest deposit being the one in Climax, Colorado. 75% of the world's known reserves of molybdenum are in the western parts of North and South America. Large deposits are also known to exist in the U.S.S.R., Canada and Chile. In the U.S. the main part of the produced molybdenum comes as a primary product from the processing of molybdenite, but a substantial fraction is obtained in connection with processing of copper, tungsten or uranium ores. Molybdenum is also obtained by recycling of scrap materials. In Table 3:1 the total known production of molybdenum, excluding U.S.S.R. and some other countries in the eastern hemisphere, is shown.

3.2 USES

In Table 3:2 the consumption of different forms of molybdenum in the U.S.A. during a one-year period is depicted.

Molybdenum's ability to harden steel has made it a metal of growing importance in this century. About 85% of the produced molybdenum is used in the manufacturing of alloys, such as alloy steels, tool and high speed steels, stainless steel, alloy cast iron and alloys with nonferrous metals. Molybdenum may be used in steel alone or in combination with other alloy materials, such as chromium, manganese, nickel and tungsten. Especially the weapon and aircraft industries have found great use for molybdenum alloys. Molybdenum has a low coefficient

of expansion and high tensile strength, making it very suitable for the latter industry.

Other products which may contain molybdenum are spark plugs, certain parts of X-ray tubes and electrodes in electrically heated glass furnaces. In the chemical industry molybdenum compounds are used as catalysts and as chemical reagents, e.g. for the determination of phosphorus. There are also many pigments that contain molybdenum compounds. Molybdenum is an essential element in plant and animal nutrition, and molybdenum compounds are added to soils or waters for increasing plant and fish production respectively.

3.3 OCCURRENCE

3.3.1 In ambient air and fuel

In the U.S. molybdenum concentrations in ambient air have been reported to be 10-30 ng/m^3 in urban areas and 0.1-3.2 ng/m^3 in non-urban areas (Air Quality Data, 1966). The concentrations of molybdenum in air are thus low. It has been suggested that its high boiling point (melting point 2617°C, boiling point 4612°C) allows molybdenum to remain to a large extent in the ash after combustion of e.g. coal.

Analyses of fly ash from 18 power stations in the U.K. showed molybdenum concentrations ranging from 10-40 ug per gram, with one sample showing 180 ug/g (Smith, 1958). Molybdenum concentrations in coal from different parts of the U.S. varied from 0.28-15 ug/g (Kaakinen and Jorden, 1974). Andersson and Grennfelt (1973) reported that molybdenum concentrations in light oils were below 0.1 ug/g and in heavier oils up to 0.52 ug/g. It was estimated that the total emission from burning of oils in Sweden was about 2.5 tons/year. Lindau and Sundberg, 1974, cal-

culated that the total yearly emission of molybdenum in air in Sweden amounted to about 70 tons, 45 tons coming from the iron-alloying industries.

Deposition of molybdenum from air emissions has been measured in Sweden by determining molybdenum in moss. The normal molybdenum concentration in moss (Hypnum cypressiforme) was found to be around 1 ug/g. The maximum value in moss in Stockholm of 7.6 ug/g was found near a waste disposal plant, where the molybdenum came from incinerated material. In a Swedish city with large metal processing industries, levels of up to 400 ug/g were found in moss. Near a large steel works a maximum level of 560 ug/g was found (Lindau and Sundberg, 1974).

3.3.2 In work environment

The only data on industrial levels available to us have been those reported from the U.S.S.R. by Mogilevskaya, 1963, and Golyakova, 1971, each author reporting from two different plants. For effects associated with these levels, see Chapter 6.

In 40 samples taken above a crucible during a smelting process involving MoO_3 (analysis by thiocyanate method, sampling time not stated), Mogilevskaya found an average value of 1.39 mg/m^3 when the molybdenum content of the alloy was 4% and an average of 5.4 mg/m^3 when it was 17%. The area above the crucible was distinguished from the breathing zone of the workers, where the mean concentration was 0.22 mg/m^3 and sometimes reached 0.4-0.5 mg/m^3. In another factory, this one producing molybdenum wire, she stated that the air concentration of MoO_3 fluctuated between 6.4 and 19 mg/m^3.

Golyakova, 1971, reported some values found during the processing of the final products in the hydrometallurgy of tungsten and molybdenum salts and oxides. In air

samples from two plants (sampling time not stated) she recorded a range of 0.5-200 and 0.2-30 mg Mo/m^3, respectively, in the main working places.

3.3.3 In water and marine organisms

Harvey, 1945, summarized earlier data which indicated levels of molybdenum in sea water below 1 ug/l. More recent data indicate that the concentration is around 10 ug/l (Black and Mitchell, 1952, Sugawara et al., 1962, Head and Burton, 1970, Sournia and Citeau, 1972, Muzzarelli and Rocchetti, 1973). Fresh water generally contains less than 10 ug/l. Durfor and Becker, 1964, in a study of the water supplies of the 100 largest cities in U.S., found that the median concentration was 1.4 ug/l. Only in one supply was a high value of 68 ug/l found. Runnells et al., 1974, found average concentrations from 1.2-4.1 ug/l in 4 rivers of the American West, whereas in the Climax area in Colorado where mining is extensive, molybdenum concentrations were as high as several mg/l of water. In the same study molybdenum concentrations in the sediments varied in the 4 rivers between 17 and 57 mg/kg dry weight, whereas in the Climax area an average of 530 mg/kg dry weight was found, the highest value being 1760 mg/kg. These data agree well with earlier reported data by Turekian and Scott, 1967, who found that molybdenum concentrations in sediments of rivers in the eastern parts of the U.S. ranged from 5-35 mg/kg.

In 170 California lakes concentrations of molybdenum from < 0.3 to 100 ug/l (median value 0.4) were found (Bradford, Bair and Hunsaker, 1968). Soluble molybdenum concentrations in one California lake have been shown to have large seasonal variations, from < 1 to 15 ug/l during one year (Dumont, 1972).

Runnells et al., 1974, also reported no difference between molybdenum concentrations in unfiltered and fil-

tered water indicating that molybdenum was not bound to particles in the water phase.

Boström and Wester, 1967, determined molybdenum in water in three Swedish cities. Samples were taken from the raw water, the water works, a water reservoir and from the tap. As seen in Table 3:3 treatment and distribution do not cause any changes in molybdenum levels. These results indicate a low removal of molybdenum in water treatment plants, support to which is given by Zemansky and Jorden, 1973, who found that only between 11-15% of the molybdenum was removed during treatment.

Molybdenum in tap water was determined in 17 Swiss cities. In 16 the concentrations were around 1 ug/l or less, whereas in one city a concentration of 29 ug/l was found (Wenger and Högl, 1968). In the Tomsk region in U.S.S.R. concentrations between 0.11 and 0.15 ug/l were found in summer, whereas in winter between 0.03 and 0.06 ug/l were found (Osmolovskaja, 1967). High concentrations of molybdenum in tap water were found in Denver, Colorado. The source is the Dillon reservoir, which drains the mining area in Climax. In the reservoir molybdenum concentrations vary between 200 and 400 ug/l. Values above 50 ug/l are considered to be due to mining activities (Chappell, 1974). High molybdenum concentrations (25,000 ug/l) in ground water have been found in mining areas in Colorado (Chappell, 1974).

The fate of molybdenum released into water from molybdenum mines was discussed by Asmangulyan, 1965. Molybdenum sulfide, which is the compound released, is only slightly soluble in water, but is oxidized to more soluble molybdates and gradually converted to a molybdenum sulfate complex.

In the marine environment molybdenum has been determined in algae. Black and Mitchell, 1952, found that molybdenum

concentrations in algae were relatively low, the concentrations being between 30 and 240 ug/kg wet weight, 100-1320 ug/kg dry weight. Lunde, 1970, determined molybdenum in seaweed from the Norwegian coast and recorded concentrations from 300 to 5,800 ug/kg. It is not stated in the article whether the values are on dry or wet weight basis. Young and Langille, 1958, found concentrations of molybdenum from 230 to more than 1,000 ug/kg on a dry weight basis in algae from the Atlantic coast of Canada.

3.3.4 In soils

Ever since molybdenum was shown to be essential to plants and excess molybdenum in soil was shown to cause high concentrations in herbage, in turn causing the so-called "teart" disease in cattle (Ferguson, Lewis and Watson, 1943), many reports from different parts of the world have been devoted to the occurrence and properties of molybdenum in soil.

In this section no attempt is made to discuss different types of soil with regard to geochemical properties. By going to the original references, the reader in many cases may find such details.

A general question has been that of molybdenum availability to plants, since this depends not primarily upon the total molybdenum content of the soil, but upon such factors as whether the soil is acid or alkaline, organic matter and levels of phosphate and sulfate. Whereas most other metals are more available in acid soils, molybdenum is more available in neutral or alkaline soils.

Data from some earlier studies in different areas of the world have been compiled by Iyer and Satyanaryan, 1958, and Reddy, 1964, the concentrations reported varying from 0.1-10 mg/kg of total molybdenum. In mining areas and near molybdenum-emitting industries considerably higher values have been reported (Chappell, 1974).

Concentrations of available molybdenum between 0.2 and 0.7 mg/kg have been regarded as "normal", whereas concentrations below or above are regarded as "deficient" or "excessive" (Pines, 1963). One common method for the determination of available molybdenum is extraction with ammonium oxalate at pH 3.3 according to Grigg, 1953. Extraction with oxalate of pH 3.0 (Gupta and McKay, 1966) with ammonium acetate, disodium EDTA or water (Williams and Thornton, 1973) has also been used.

Pines, 1963, found that in soils in Israel with pH from 6.7 to 8 total molybdenum varied from 1.6-8.4 mg/kg, whereas available molybdenum varied from 0.2-4.4 mg/kg. Gupta and McKay, 1966, found in soils from eastern Canada, that available molybdenum varied from less than 0.01-0.1 mg/kg. The pH of these soils varied from 4.5-7.8 and the available molybdenum was significantly correlated to the pH of the soil.

In Ireland, Brogan, Fleming and Byrne, 1973, determined molybdenum in a large number of samples. The available molybdenum varied from 0.05-6.5 mg/kg. In this study the highest levels of available molybdenum were found in acid soils. In India Iyer and Satyanarian, 1958, found available molybdenum ranging from 0.01-0.22 mg/kg, with the lowest values found in soils with low pH.

Reddy, 1964, analyzed both total and available molybdenum in a large number of soils in a region in India. The former varied from 0.5-4.1 mg/kg and the latter from 0.12-2.26 mg/kg. On an average the total molybdenum concentration was 26 times the available concentration. Up to pH 7.9 there was an increase in available molybdenum with increasing pH.

Grigg, 1960, 1961, has presented exhaustive data on total and available molybdenum in soils of different types on the northern and southern islands of New Zealand. Total

molybdenum on the northern island varied between 0.8 and 4.6 mg/kg and available molybdenum from 0.03-1.11 mg/kg. On the southern island available molybdenum concentrations ranged from 0.05-0.45 mg/kg.

Molybdenum has been determined in sewage sludge by Berrow and Webber, 1972. They found that molybdenum concentrations varied between 2 and 30 mg/kg dry weight, with 35 of 42 samples below 10 mg/kg. Andersson and Nilsson, 1972, found that adding sewage sludge containing 0.68 mg/kg dry weight of molybdenum to soil with a total molybdenum content of 0.53 mg/kg dry weight caused an increase with about 50% in the molybdenum concentrations in the plants. Molybdenum in fertilizers was determined by Hoover and Duren, 1967, who found levels from 2.6-5.9 mg/kg in four samples. In five samples Koirtyohann and Hamilton, 1971, found from < 2 to 5.2 mg/kg.

3.3.5 In food

A number of reports on the molybdenum content of various food items have appeared, showing variations both among and within different classes of foodstuffs. An extensive review on molybdenum concentrations in foodstuffs has been given by Schlettwein-Gsell and Mommsen-Straub, 1973, who compiled data from 26 original papers.

In Table 3:4 data from several reports have been assembled on molybdenum in some common foodstuffs. The highest amount of molybdenum has been found in the parts of the vegetables above ground, whereas root parts generally have considerably lower contents. Especially legumes and cauliflower can have very high concentrations of molybdenum. Meat and other animal products generally have low concentrations of molybdenum. The molybdenum content of milk has attracted interest since it may reflect variations in molybdenum content of animal feed.

Data on molybdenum in food will also be found in Table 6:3 in connection with a description of human effects in a molybdenum-rich area.

Archibald, 1951, reported normal cow milk to average 73 ug Mo/kg, with a range of 18-120. Feeding the same cows 500 mg Mo as ammonium molybdate daily raised the concentration in milk to 371 ug/kg. Molybdenum in the milk of cows is bound to the enzyme xanthine oxidase, meaning that the activity of the enzyme in such milk is proportional to the content of molybdenum (Hart, Owen and Proudfoot, 1967).

Whether the differences between plant products depend entirely on uptake of molybdenum from soil or somewhat on the contribution of analytical differences is hard to evaluate. The good agreement between different analyses of milk gives some assurance that the different methods give similar results.

3.3.6 In tobacco

In tobacco Voss and Nicol, 1960, found molybdenum concentrations of 0.3-1.76 ug/g, mean 0.87. Reddy, 1964, found 0.36 ug/g in Indian tobacco.

3.3.7 Daily intake

Schroeder, 1970, estimated that in the U.S. the average diet contained 335 ug molybdenum, range 210-460. In the U.S.S.R. estimates of intake in children were 156-161 ug/day (Vorobjeva and Osmolovskaya, 1970) and in adults 329-376 ug/day (Gabovich, 1964). According to Smolyar, 1972, diets of both children and adults in the U.S.S.R. contain between 200 and 500 ug of molybdenum. Hamilton and Minski, 1972/1973, determined molybdenum in total diet samples collected from different regions of the U.K. They found an average of 128 ug/day (S.D.± 34). Tipton and Stewart, 1970, followed three subjects for long periods in a balance study. The daily intakes were on an average

110, 210 and 460 ug. Wester, 1971, determined the daily intake of molybdenum in two hospital patients by neutron activation analysis of duplicate samples of the hospital diet. Average amounts in 6 periods of 5 days each varied from 250 to 1,000 ug. Wester, 1974a,studied also 4 healthy subjects in a metabolic study. The daily intake of molybdenum varied between 115 and 245 ug (average for 10 days).

The above mentioned data show quite a good mutual agreement. From the discussions in the section on molybdenum in foodstuffs, it is obvious that if a diet is mainly based on leafy vegetables and legumes, the intake may be considerably higher. This is illustrated by the metabolic study by Robinson et al., 1973, in which the diet was based on meatloaf, and included no other vegetables, resulting in a low daily intake, less than 100 ug molybdenum. The intake from water and air will be small compared to the intake from food, except in some areas where mining activities may cause increases in molybdenum concentrations in drinking water. It is not known if any molybdenum is released from cigarettes during smoking.

3.3.8 Conclusions

The natural variations of molybdenum concentrations in the environment are large, depending on geological factors. Concentrations in water and soil may vary with a factor of more than 10 causing both deficient and excessive concentrations for plants and ruminants in some parts of the world. In areas where molybdenum ore is processed, concentrations in soil and water may increase considerably. In soil the available molybdenum is of greater importance than the total amount of molybdenum for plant nutrition. The availability is dependent on pH and other factors in the soil, being greater in alkaline soils. Molybdenum concentrations in air are generally low. The variations in foodstuffs, especially plants, are very greatly dependent both on species and soil characteristics. In general,

high concentrations are found in leafy vegetables and legumes, whereas edible roots have a lower content. Animal products are generally low in molybdenum. Daily intake of molybdenum can be estimated to be between 100 and 500 ug. In areas where molybdenum ore is mined considerable contamination may occur, which can cause high concentrations in drinking water and thus daily intakes of more than 1,000 ug.

TABLE 3:1 WORLD PRODUCTION OF MOLYBDENUM, EXCLUDING U.S.S.R. AND SOME OTHER COUNTRIES IN THE EASTERN HEMISPHERE, 1956-1969. (From Morning, 1969).

Year	Total (in thousand pounds)[x]
1956-60	63,300
1961	74,000
1962	59,300
1963	75,000
1964	78,000
1965	98,531
1966	124,988
1967	126,273
1968	125,735
1969	142,802

[x]1,000 pounds = 0.45 tons

TABLE 3:2 CONSUMPTION OF MOLYBDENUM (in thousand pounds contained molybdenum) BY END USES IN THE UNITED STATES IN 1969. (From Morning, 1969).

End Uses	Molybdic Oxides	Ferro-molybdenum[x]	Ammonium and Sodium Molybdenum	Other Mo Materials[xx]	Totals
Steel:					
Carbon	3,068	468		W	3,536
Stainless and heatresisting	4,259	1,883		117	6,259
Alloys	18,768	2,309		28	21,105
Tools	2,045	1,204		237	3,486
Cast irons	1,050	3,140		84	4,274
Superalloys	338	597		1,581	2,516
Alloys for cutting and wear-resistant materials	W	W		3	3
Alloys for welding; for hard-facing rods and materials		383		29	412
Magnetic alloys		W		W	W
Other alloys	W	87		109	196
Mill products made from metal powder	412	W		1,899	1,311
Chemical and Ceramic Uses:					
Pigments	731		371	W	1,102
Catalysts	1,514		W		1,514
Other	35	W	W	785	820
Misc.	2,128	1,065	418	478	4,089
Totals (indep. roundings)	34,349	11,135	789	5,348	51,622

x) Incl. calcium molybdate

xx) Incl. purified Mo disulfide, molybdenite concentrate, molybdenite concentrate added directly to steel, Mo metal powder, Mo metal pellets

W = witheld, confidential

TABLE 3:3 MOLYBDENUM CONCENTRATIONS IN WATER (ug/l) IN THREE SWEDISH CITIES. (From Boström and Wester, 1967).

	Raw water	Water works	Water reservoir	Tap water
Stockholm	4	3	4	3
Gothenburg	2	2	1	3
Malmö	9	9	6	9

TABLE 3:4 MOLYBDENUM CONCENTRATIONS (ug/kg wet weight) IN DIFFERENT FOODSTUFFS.

Average concentration	Reference	Country
Milk		
73	Archibald, 1951	U.S.A.
29	Kirchgessner, 1957	Germany
36	Voth, 1963	U.S.A.
5[x)]-51[xx)]	Kiermeier and Capellari, 1958	Germany
25	Stanton and Hardwick, 1968	U.K.
200	Schroeder, Balassa and Tipton, 1970	U.S.A.
17	Lupea and Vranceanu, 1972	Roumania
38-48[xxx)]	Ward, 1974	Colorado, U.S.A.
Potatoes		
780	Reddy, 1964	India
100[Ψ)]	Le Riche, 1968	U.K.
20-180[ΨΨ)]	Warren, Delavault and Fletcher, 1971	Canada "Industrial areas"
160	Warren, Delavault and Fletcher, 1971	Canada "Normal area"
30	Schroeder, Balassa and Tipton, 1970	U.S.A.
100	Lupea and Vranceanu, 1972	Roumania "Area 1"
55	Lupea and Vranceanu, 1972	Roumania "Area 2"
< 20[ΨΨΨ)]	MacLean and Langille, 1973	Canada "Light farming"
80[ΨΨΨ)]	MacLean and Langille, 1973	Canada "Intensive farming"
Cabbage		
460	Reddy, 1964	India
20	Schroeder, Balassa and Tipton, 1970	U.S.A.
340[ΨΨΨ)]	MacLean and Langille, 1973	Canada "Light farming"
570[ΨΨΨ)]	MacLean and Langille, 1973	Canada "Intensive farming"

x) From cattle grazing on pastures with acid soils.
xx) From cattle grazing on pastures with alkaline soils.
xxx) Averages for 5 sampling periods during one year.
Ψ) Calculated from dry weight values in the article, assuming a ratio of 1:5 of dry weight to wet weight.
ΨΨ) Calculated from ash values in the article.
ΨΨΨ) Air-dry basis.

TABLE 3:4 CONTINUED

Average concentration	Reference	Country
Cauliflower		
1,920	Reddy, 1974	India
260-2,200	Bovay and Rod, 1964	Switzerland
Legumes		
1,000 (peas)	Schall and Schall, 1962	Germany
750-2040	Reddy, 1964	India
6,030 (peas)	Los, Piatnizkaja and Samsonova, 1966	U.S.S.R.
1,099 (peas)	Lupea, Vranceanu and Waltraut, 1969	Roumania
310-4,800	Schroeder, Balassa and Tipton, 1970	U.S.A.
180 (peas)	MacLean and Langille, 1973	Canada "Intensive farming"
Wheat		
230-400	Schall and Schall, 1962	Germany
490	Reddy, 1964	India
300	Kirchgessner and Friesecke, 1969	Germany
190-780	Basargin and Peregudova, 1969	U.S.S.R.
199	Lupea, Vranceanu and Waltraut, 1969	Roumania
640-5,870	Schroeder, Balassa and Tipton, 1970	U.S.A.
179-222	Lupea and Vranceanu, 1972	Roumania

CHAPTER 4 METABOLISM

4.1 UPTAKE AND ABSORPTION

Data presented in the immediately following sections do not cover interactions of molybdenum with other compounds such as sulfur and copper. Such studies are treated separately in Chapter 7. For general aspects of accumulation of toxic metals, their absorption, excretion and biological half-times, the reader is referred to reports by the Task Group on Metal Accumulation, 1973, and the Task Group on Metal Toxicity, in press.

For understanding the metabolism and toxicity of molybdenum, the solubility of the different compounds must be taken into consideration. With regard to solubility in biological fluids there are only data available from Mogilevskaja, 1963, who stated that the solubilities of molybdenum metal and molybdenum trioxide were 3.8 and 125 mg/100 ml serum and 4.6 and 50 mg/100 ml gastric juice respectively. The solubility was higher in alkaline media (Na_2CO_3) than in acid media (0.3% HCl). Ammonium paramolybdate was easily dissolved in water, serum and gastric juice. Calcium molybdate is practically insoluble in water, whereas sodium molybdate has a high solubility (Handbook of Chemistry and Physics, 1974/1975).

4.1.1 Absorption following inhalation

4.1.1.1 In animals

Fairhall et al., 1945, exposed groups of guinea pigs to the dusts (particle size not given) of molybdenite (average concentration 285.9 mg Mo/m^3), calcium molybdate (158.9 mg Mo/m^3), molybdenum trioxide (204.7 mg Mo/m^3) and a fume of molybdenum trioxide (53.0 mg Mo/m^3), one hour daily, five times per week, for five weeks. From the resultant distribution (see Table 4:1) in organs, it is obvious that hexavalent compounds (calcium molybdate, molybdenum trioxide)

are absorbed, even though no quantitative evaluations can be made. Tissue concentrations, with the exception of the lungs, after exposure to molybdenite were not higher than among controls. The trioxide fume gave unexpectedly lower concentrations in the tissues than did the trioxide dust.

Confining themselves to distribution among different organs, the authors did not discuss how large the total body retention was, nor what proportion of the molybdenum retention was actually gastrointestinally absorbed following mucociliary lung clearance.

4.1.1.2 In human beings

No data are available.

4.1.1.3 Conclusions

One study on animals shows that different molybdenum compounds are absorbed, but a quantitative evaluation cannot be made from the data on hand. No human data on absorption after inhalation are available.

4.1.2 Gastrointestinal absorption

4.1.2.1 In animals

Fairhall et al., 1945, gave 50 mg of Mo as MoO_3 by oral administration with a syringe to three pairs of guinea pigs weighing between 350 and 450 grams. The distribution among organs at intervals of 4 hours, 16 hours, and 48 hours after administration is given in Table 4:2. The cumulative amount found in the feces up to 48 hours was 5.8 mg, the remaining portion in the gastrointestinal tract being 1.29. If all of this is counted as a not-absorbed amount, the total absorption would be around 85%. Fairhall et al. further gave evidence of a rapid absorption of molybdenum when they measured the blood values of molybdenum after the administration of 100

mg of MoO_3 to two rabbits weighing 1,880 and 1,620 g by means of a stomach tube. The blood values increased from 2 ug/g blood before exposure to more than 10 times the initial value 400 min. after administration (15 and 20 ug/g blood respectively). Considerable doubt must be expressed as to the validity of the blood values. One reason for this is the large variation seen in Table 4:2; another reason is the very large difference seen between Fairhall et al.'s "normal" values in animals and "normal" values in humans (section 4.3.2). The latter are 100-1,000 times lower than the values given by Fairhall et al., 1945.

In an experiment by Neilands, Strong and Elvehjem, 1948, 13.34 mg of Mo given as radioactive MoO_3 (hexavalent) was introduced into the back of the throat of 12 rats that had been starved for one day before the experiment. The study is not suitable for an exact assessment but shows that at least 10-20% of the molybdenum had been absorbed as early as 2.5 hours after administration. At that time the liver contained 28 ug and the kidneys 60 ug of molybdenum. In the stomach there was 1,537 ug and in the intestines 215 ug. The authors did not state whether these figures meant tissue alone or tissue plus contents. The carcass had a concentration of approximately 825 ug. After 2 days at least 35% of the given dose must have been absorbed since during day two, 4,692 ug was excreted with the urine and molybdenum was still present in internal organs.

Van Campen and Mitchell, 1965, studied absorption of $(NH_4)_2{}^{99}MoO_4$ from ligated segments of the female rat gastrointestinal tract. The abdominal cavity was opened, the segment ligated, and molybdenum thereafter injected into it. The isotope was readily absorbed from the stomach as well as from the intestines at all levels down to the colon (absorp-

tion from the last was not examined). The uptake was counted as the total uptake by the blood, heart, kidneys and liver in percentage of given dose two hours after administration (2.7 uMol Mo). The means for uptake from the different segments fell in the following order: the duodenum (9.46%) > ileum (8.28%) > midsection (6.75%)> stomach (3.6%). Each figure is the mean of 5 animals (5 animals in each "segment group").

Anke et al., 1971, studied the absorption of molybdenum in four lactating goats following oral ingestion. The animals were fed a diet to which was added 1.5 mg Mo as MoO_3 per day and animal for two weeks. Subsequently, 3 mCi of $^{99}MoO_3$ was injected via a stomach tube and levels of ^{99}Mo in the feces, urine and different organs were determined for a period of 96 hours. It was calculated that on an average at least 34.7% of the given amount of ^{99}Mo was absorbed from the gastrointestinal tract (based on molybdenum found in tissues, urine and milk). The total percentage found in the feces, 51%, was counted as the not-absorbed amount.

Miller et al., 1972, compared absorption in calves and pigs after doses of ^{99}Mo. Pigs were given the isotope in feed, whereas calves were given the isotope in milk by nipple pails (abomasal dose) or by gelatin capsules (rumen dose). It was found that if the rumen was by-passed, absorption in calves was similar to that in pigs whereas rumen doses were absorbed to a lesser extent. It was postulated that molybdenum became less available by some process in the rumen.

4.1.2.2 In human beings

Tipton, Stewart and Dickson, 1969, analyzed 22 elements in the complete daily diets (self-chosen), and in the total daily urinary and fecal excreta of three men for a

period of 50 weeks. Analysis was made by arc emission spectrography. As can be seen from Table 4:3, all three subjects had a positive balance of molybdenum. Subjects A and B had more than 50% of the total urinary-fecal contents in their urine. Considering the dietary/fecal ratio of these two subjects it seems likely that the absorption was well over 50%. In subject C the absorption was probably less than 50%.

A three-day balance study was conducted on 11 healthy schoolchildren by Alexander, Clayton and Delves, 1974. The analytical method employed was atomic absorption. They concluded that 77% of the ingested molybdenum was absorbed.

In an 18-day balance study on 4 women, Robinson et al., 1973, found that between 38 and 72% of the daily intake was in feces, indicating that from 28 to 62% had been absorbed. Molybdenum was determined by a dithiol method.

Boström and Wester, 1968, studied one healthy man and two patients with osteomalacia for two 5-day balance periods. From their data it can be seen that from 30 to 76% of the molybdenum might have been absorbed by the patients. The values for the healthy man were 38 and 76% in the two periods. Molybdenum was determined by neutron activation. Wester, 1974a, studied 4 healthy men during two 5-day periods, the one period involving low and the other high calcium intake. From his data it can be estimated that absorption was 52% (44-62) and 47% (29-65) during the two periods.

4.1.2.3 Conclusions

Hexavalent molybdenum is readily absorbed from the gastro-intestinal tract, 40%-85%, in animals. Naturally occurring molybdenum in human food is absorbed to a high degree, probably 25-75%. The data on humans are based on a very limited number of observations.

4.2 EXCRETION

4.2.1 In animals

4.2.1.1 Urine and alimentary tract

Fairhall et al., 1945, gave guinea pigs molybdenum as MoO_3 per os in doses of about 20 mg daily for ten days. After two days, the daily total urinary and fecal contents were equalizing the administered doses. The urine contained about three times the amount that appeared in the feces. The excretion of the absorbed molybdenum seemed almost complete already three days after the ten-day exposure period.

Neilands, Strong and Elvehjem, 1948, in their experiment on rats, referred to in section 4.1.2.1, found after single oral introduction of 13.34 mg of Mo (given as radioactive MoO_3) 4,692 ug Mo in the urine during the second day after exposure, compared to only 697 ug in feces.

In the study by Anke et al., 1971, on lactating goats (section 4.1.2.1), the cumulative urinary excretion of ^{99}Mo during 4 days following a single peroral dose of $^{99}MoO_3$ was 25.4% out of the ingested amount while 51% was in the stools.

Robinson et al., 1964, reported on the excretion of intravenously administered ^{99}Mo as sodium molybdate in water to two groups of six cows each. The cows in one of the

groups had been on a deficient diet and had symptoms of reduced growth, low milk production and low serum copper levels. The excretion of ^{99}Mo in urine followed an exponential pattern during the observation period (80 hours) with a half-time of 19.9 ± 1.4 (S.E.) hours in the normal cows and 18.7 ± 1.2 in the nutritionally deficient cows. The biological half-time for the body as a whole cannot be evaluated as excretion via feces and milk was only followed for a short period.

In two experiments, Amon, Scheler and Peters, 1967, studied the excretion of Mo in rats following two s.c. doses of 2 mg of $^{99}MoO_3$ given on day 1 and 4 of the experiments. Within 14 days 43.5% and 79.4% respectively had been excreted in the urine, and 2.6% and 9.7% in the feces.

4.2.1.2 Milk

In Anke's experiment referred to in 4.1.2.1 (Anke et al., 1971), a cumulative amount of 2.4% of ingested molybdenum was found in the milk of lactating goats four days after administration.

4.2.1.3 Bile

Caujolle and Roche, 1935, studied the biliary excretion of molybdenum in two dogs with bile fistulas following s.c. injections of molybdenum salts. One dog weighing 25 kg received 5 g of sodium molybdate and the other weighing 15 kg 0.2 g of ammonium molybdate. Six hours after the injection, the first dog had excreted 33.5 mg of molybdenum in the bile and the second dog 2.2 mg. For comparison the corresponding urinary amount was 91.9 mg and 16 mg respectively.

Fairhall et al., 1945, at autopsy of their experimental guinea pigs, also found a considerable amount of molybdenum in bile (see sections 4.3.1.1 and 4.3.1.2).

4.2.1.4 Hair

The total amount of molybdenum retained in the body after 96 hours in the experiment by Anke et al., 1971, was 6.9%. Out of the retained amount 0.2% appeared in the hair. The authors pointed at a steady rise of molybdenum concentration in hair as long as 96 hours after the administration. The activity in hair at this point was 6 times higher than the activity 24 hours after administration.

4.2.2 In human beings

Excretion in man was studied by Rosoff and Spencer, 1964. Carrier-free ^{99}Mo in ammonium hydroxide was injected intravenously (50-100 uCi) and the subsequent urinary and fecal excretion was determined. The cumulative urinary ^{99}Mo excretion in 10 days was 24% in one subject and 29% in another, while the corresponding fecal excretion was 6.8% and less than 1%, respectively (Figure 4:1). The retention of the metal seems higher in man than in animals from this study. What part the considerably smaller doses could have played is not known.

The total excretion of molybdenum under "normal" steady state conditions is not known. Data from Tipton, Stewart and Dickson, 1969, tend to show that 25-50% of the ingested amount is excreted via urine.

In Figure 4:2, data have been compiled on urinary excretion of molybdenum in relation to dietary intake in individuals studied by various investigators. It is obvious that a relationship exists.

Data on daily urinary excretion of molybdenum in "normal" human subjects have also been given by Meltzer et al., 1962 (spectrography) and Wester, 1973, 1974b. On the average, the excretion in the groups varied between 49 and 81 ug per day. In New Guinea Adkins, Barmes and Schamschula, 1974, found daily averages ranging from 4 to 20 ug of molybdenum (method not stated) in 10 groups of "normal" subjects.

4.2.3 Conclusions

Studies referred to have dealt with hexavalent molybdenum compounds. Limited animal data indicate a rapid excretion. A more or less complete excretion of molybdenum took place during the first two weeks after a single s.c. exposure.

In one study on humans the retention 10 days after an intravenous injection of molybdenum was higher, only 30-40% of the dose being excreted.

The main channel of excretion is through the kidneys. Excretion through bile is likely to be the main channel of fecal excretion. There are indications of a small excretion through milk, sweat and hair.

4.3 TISSUE DISTRIBUTION

4.3.1 In animals

4.3.1.1 Single exposure

4.3.1.1.1 Oral route

In Fairhall et al.'s study (1945) three pairs of guinea pigs were given 50 mg of molybdenum as molybdenum trioxide by oral administration with a syringe, and were killed at 4 hours, 16 hours and 48 hours afterwards. Molybdenum was analyzed with the thiocyanate method. The molybdenum concentration in kidneys decreased from 46 ug/g wet weight after 4 hours to 7 ug/g after 48 hours. In the liver there was a decrease from 20 to 3 ug/g and in the spleen from 26

to 18 ug/g. The concentration in the bile was between 20-30 ug/g during the whole study (Table 4:2).

A similar study on 12 rats divided into 6 groups of two rats each was made by Neilands, Strong and Elvehjem, 1948. The rats were given 13.34 mg of molybdenum trioxide labelled with ^{99}Mo in the back of the throat via a syringe. The rats in three of the groups were also given immediately thereafter 0.5 ml of a solution of copper sulfate corresponding to 5 mg of copper. Two rats from both the non-copper and the copper groups were killed after 2 hours, 26 hours and 51 hours.

The molybdenum concentrations in different organs and biological material are given in Table 4:4. The distribution was rather similar to the one found by Fairhall et al., 1945. It is further seen that bones contained appreciable amounts of molybdenum. The excretion from the organs was rapid in these experiments.

4.3.1.1.2 Intravenous route

Durbin, Scott and Hamilton, 1957, administered 3 uCi of ^{99}Mo as Na_2MoO_4 intravenously to three rats. After 4 hours 1/3 was excreted via the urine, 1/3 was in the liver, and the rest was distributed among the gastrointestinal tract, blood, soft tissues and skeleton. Liver, kidney and pancreas contained the highest concentrations of ^{99}Mo.

4.3.1.2 Repeated exposure

4.3.1.2.1 Oral route

Fairhall et al., 1945, performed a long-term experiment on rats fed different molybdenum compounds which varied as to concentration (10-500 mg molybdenum per rat and day) and as to period of time (10-500 days). In rats given molybdenite there was no mortality and concentrations

of molybdenum in liver, kidney and bone at the end of the experiment were low (2-6 ug/g wet weight) and the same regardless of whether the rats were given 10 or 500 mg molybdenum per day during a 44-day period. Control animals had 3-6 ug molybdenum per gram tissue, the studies thus indicating that there could only be a very low absorption of molybdenite.

Molybdenum trioxide, calcium molybdate and ammonium molybdate were absorbed and the mortality was between 25-100%. In some cases the data were based on surviving animals while in other cases deceased (time of death not given). This makes it impossible to evaluate accumulation tendencies and dose-related differences. The thiocyanate method was used for analysis.

In four lactating cows, Huber, Price and Engel, 1971, found concentrations of molybdenum in kidney and spleen to be several times higher than those in the liver, 42.3 and 95.0 versus 10.4 ug/g dry weight following a six month's intake of a basal ration of 53 mg Mo/kg diet given as sodium molybdate. Blood, brain and muscles contained considerably less metal. Bones were not analyzed. Four other animals were fed 173 mg Mo/kg diet. In the spleen there was no increase in molybdenum compared to the 53 mg/kg group, while in blood, kidneys and liver the concentration increased with a factor of 2-3.

In the study by Anke et al., 1971, four goats were each fed 1.5 mg molybdenum as molybdenum trioxide daily for two weeks. Hereafter 3 mCi of $^{99}MoO_3$ were administered through a stomach tube. 96 hours after this the animals were sacrificed and their tissues analyzed for ^{99}Mo. The results are presented in Tables 4:5 and 4:6. 6.9% of the administered dose was found in the body, excluding the gastrointestinal tract, and the figures in the tables are

given in percent of this amount. The bones contained the highest amount, 27.4%, followed by the liver, 19.9%, and the kidneys, only 3.4%. The concentration (radioactivity per gram dry weight) was highest in the kidneys, being followed by the liver.

4.3.1.2.2 Inhalation

Groups of guinea pigs were exposed to the fumes and dusts of different molybdenum compounds one hour daily, five times per week for five weeks (Fairhall et al., 1945). In Table 4:1 the different tissue concentrations in animals dying during the test, sacrificed at the end of the test, and sacrificed two weeks after the test, are given. As expected, molybdenite after inhalation also gave very low concentrations in the tissues, apart from the lungs. For more details see 4.1.1.1.

At the end of the exposure the concentrations of molybdenum were higher in animals exposed to molybdenum trioxide dust than in those exposed to calcium molybdate or molybdenum trioxide fume. The low concentration after exposure to molybdenum trioxide fume is remarkable as is the fact that exposure to fumes gave the lowest mortality. The possibility of methodological errors must be borne in mind but the authors offered some explanation in differences in solubility of the two compounds.

The drop in the molybdenum content of most tissues two weeks after the end of exposure indicates low retention of the metal. No estimations or calculations on the total retained amount of molybdenum are given.

4.3.1.3 Distribution within organs

In a study by Brinkman, Miller and Engel, 1961, groups of rats were fed between 200 and 500 mg Mo/kg diet for six

weeks. Hereafter the livers of the animals were removed, homogenized and centrifuged. Molybdenum was usually found to be concentrated in the supernatant (>60%) while nuclei and debris contained <15%, mitochondria 15% and microsomes 10%.

4.3.2 In human beings, "normal exposure"

It has not been possible to find any studies dealing with distribution in man following experimental administration or industrial exposure to molybdenum.

Butt et al., 1964, spectrographically determined molybdenum concentrations in blood in three groups of individuals in the Los Angeles area, totally 266 subjects in two different hospitals (two patient groups and one personnel group) and found values between non-detectable to 34 ng per ml. Mertz et al., 1968, also using spectrography, found an average concentration of 6 ng/ml serum, but described a wide scatter (0.1-30 ng/ml) among individuals (69 subjects in Germany).

By neutron activation analysis Morgan and Holmes, 1972, found average concentrations in whole blood of 0.8 and 1.2 ng/ml in 8 normal men and 5 normal women respectively in the U.K. Brune et al., 1966, found 3.3 ng/ml in whole blood and Kjellin, 1968, detected <1 ng/ml in cerebrospinal fluid and 15 ng/ml in brain tissue. Wester, 1973, found an average concentration in serum of 5.6 ng/ml in 8 healthy subjects. The three latter studies involved subjects in Sweden.

Allaway et al., 1968, studied the influence of the geographical area on the blood molybdenum content. The study encompassed 229 subjects from 19 different areas in the United States. 75% of the samples examined by the thiocyanate method showed a concentration of molybdenum of

less than 5 ng/ml of whole blood. Still, they found two collection sites where more than 70% contained more than this amount, and in one of these areas the maximum detected value was 410 ng/ml.

Polonskaja, 1968, determined molybdenum in the blood of 47 pregnant women and 12 non-pregnant controls in the U.S.S.R. The controls had 166 ng/ml of blood; in the pregnant women, the blood level of molybdenum first decreased and then increased again. From the 12th to the 19th week of pregnancy, the concentration was 129 ng/ml; from the 28th to the 38th week: 152 ng/ml; from the 39th to the 40th week: 234 ng/ml. After parturition the concentration remained high for 5-6 days (213 ng/ml). The reason that these blood values were high compared to the other reports is not known.

Tipton and Cook, 1963, reported levels of 27 different trace elements in 29 spectrographically analyzed tissues of 150 adult subjects from the United States. As for molybdenum, this metal was observed in every sample of liver (147/147), in almost every sample of kidney (140/144), and adrenals (10/13), and only occasionally in other tissues. The median values were, respectively, 1 ug/g, 0.3 ug/g and 0.7 ug/g wet weight.

Plantin, 1973, determined molybdenum in kidney cortex, liver and heart by neutron activation in 8 autopsy cases in Sweden, 51-63 years of age. The average concentrations in the respective organs were 0.2, 0.88 and 0.022 ug/g wet weight. Samsahl, Brune and Wester, 1965, also in Sweden, found 0.41 and 0.50 ug/g wet weight in two livers by neutron activation.

Schroeder, Balassa and Tipton, 1970, gave the changes with age in the main concentrations of hepatic and renal

molybdenum by decade of life in the United States and in other countries as seen in Figure 4:3. The method of analyses was emission spectroscopy. Here the concentrations are given in ug/g ash. According to Tipton and Cook, 1963, the ash percents of wet weight for liver and kidney are 1.3% and 1.1% respectively. There were no significant differences between the United States and the non-U.S. subjects. Molybdenum was relatively low in the newborn, rising to a peak (about 1.1 ug/g in livers and 0.4 ug/g in kidneys) in the second and third decade of life, and declining thereafter, particularly in the liver. In this material total body content of the United States subjects was less than 9 mg.

Pribluda, 1964, reported the molybdenum concentrations in liver and kidney of 80 subjects aged 17 to 77 years in the U.S.S.R. The subjects died accidentally and had no signs of organic disease. The thiocyanate method was used for determining molybdenum. The results were evaluated statistically and are shown in Table 4:7. The concentration of molybdenum in the human liver was about 2-3 times higher than in the kidneys.

In another report from the U.S.S.R., Gurskaja, 1966, determined molybdenum by the thiocyanate method in the ash of the amnion of 60 fetuses and the (villous) chorion of 90 fetuses. The arithmetic mean concentration of molybdenum in the amnion was 3.5 ± 0.27 (S.E.) ug/g ash; in the (villous) chorion 0.6 ± 0.02 ug/g ash. The ash content of the tissue was 0.47 and 1.05% respectively. Fetuses from women in the age group 21-25 had more molybdenum in the amnion (4 ug/g ash) than from those in the 31-35 year group (2.3 ug/g ash). The difference was statistically significant ($P < 0.02$). There was no age-related difference with regard to the molybdenum concentration in the (villous) chorion.

4.3.3 Conclusions

There is a fairly good uniformity in results between the different animal studies dealing with the tissue distribution of molybdenum. Thus, it seems clear that the metal is concentrated to a higher degree in kidneys, liver, and bone tissue, the highest values invariably being in the kidneys. On the other hand, the retention of the metal is low, the major part of administered doses in animal experiments being excreted within hours or days, meaning a biological half-time of an approximately similar value.

Human studies have only been concerned with "normal exposure", that is, no studies dealing with distribution following experimental or industrial exposure have been published. Estimations of blood levels in different individuals show a wide scatter, but most data support a normal value of only a few ng/ml whole blood. The geographical area may be of considerable importance. There are indications of an increasing molybdenum level in the human liver and probably kidneys to the second and third decades of life, and a slight decline thereafter. The liver values are higher than the kidney values, the liver reaching on the average a concentration of 0.5-1 ug/g wet weight and the kidney about 0.25 ug/g wet weight. There are no studies implying an accumulation of the metal throughout life.

TABLE 4:1. DISTRIBUTION OF MOLYBDENUM (ug Mo/g wet weight) IN GUINEA PIGS FOLLOWING INHALATION OF VARIOUS MOLYBDENUM COMPOUNDS (Modified from Fairhall et al., 1945).

Compound	Total number exposure	Average concentration (mg. Mo/m^3	Percent mortality	Animals died during test					Animals killed at end of test					Animals killed two weeks after test				
				Liver	Kidney	Lung	Spleen	Bone	Liver	Kidney	Lung	Spleen	Bone	Liver	Kidney	Lung	Spleen	Bone
Molybdenum sulfide	24	286	4.2						2	1	387	1	2					
Molybdenum trioxide dust	24	205	51.0	12	21	17	16	52[1]	10	27	20	17	24	2	3	5	7	11
Calcium molybdate	26	159	20.8	4	6	126	8	20	3	11	179	6	21	3	6	132	4	12
Molybdenum trioxide fume	25	191	8.3		5	69		13	2	4	8	6	6	1	2	3	8	5
Molybdenum trioxide fume	25	53	0						2	4	3	4	6	2	1	2	4	5
Controls									1	1	1	5	3					

[1]Includes weak animals killed after 10 exposures.

TABLE 4:2 DISTRIBUTION[x)] OF MOLYBDENUM IN TISSUES OF GUINEA PIGS AT DIFFERENT INTERVALS FOLLOWING ORAL ADMINISTRATION OF 50 mg OF MOLYBDENUM AS MOLYBDENUM TRIOXIDE. (Modified from Fairhall et al., 1945).

	Interval after dosage		
	4 hours	16 hours	48 hours
Kidneys	46	20	7
Spleen	26	12	18
Blood[xx)]	26	5	50
Bile	28	20	30
Liver	20	8	3
Lungs	31	10	9
Muscle		2	1
Feces (total Mo)	10	990	5,800
Urine (total Mo)	2,200	11,100	13,200
Gastrointestinal tract (total Mo)	19,900	5,080	1,290

x) Values for tissues given as ug Mo/g wet weight and for feces, urine and gastrointestinal tract as total cumulative molybdenum in ug.

xx) Special comments, see text.

TABLE 4:3 MOLYBDENUM IN DIET, URINE AND FECES (ug/day, mean ± S.E.) IN THREE HUMAN SUBJECTS. (Modified from Tipton, Stewart and Dickson, 1969).

Subject	Diet	Feces	Urine
A	210 ± 20	88 ± 17	110 ± 10
B	460 ± 80	99 ± 15	130 ± 10
C	110 ± 10	71 ± 3	26 ± 6

TABLE 4:4 DISTRIBUTION[x)] OF RADIOACTIVE MOLYBDENUM IN TISSUES OF RATS GIVEN A SINGLE ORAL DOSE OF MoO_3 LABELLED WITH ^{99}Mo ALONE AND WITH COPPER. (Modified from Neilands, Strong and Elvehjem, 1948).

Group No.	1	2	3	4	5	6
Average weight, g	132.6	150.3	142.9	155.8	96.2	71.4
Time interval between dose and death, hours	2.1	26.1	51.0	2.5	26.4	51.4
Mo dose, mg	13.34	13.34	13.34	13.34	13.34	13.34
Cu dose, mg				5.00	5.00	5.00
Molybdenum in tissues (ug/g wet weight)						
Blood	28.8	2.3	1.8	13.2	3.0	2.3
Stomach	176.4	5.8	2.9	9.3	19.3	4.6
Kidneys	18.5	7.7	7.1	14.3	9.7	2.8
Liver	9.1	1.2	0.8	5.4	1.7	1.6
Intestine	13.9	25.4	6.2	9.0	25.5	14.4
Bone	10.9	4.8	3.0	10.3	6.4	3.9
Heart	9.0	1.5	1.0	10.0	1.9	1.6
Lungs	15.8	1.6	1.2	12.4	3.4	2.2
Carcass	8.5	3.2	0.6	8.0	3.9	1.5
Feces			290.9			597.4

x) Each column represents the average from two rats.

TABLE 4:5 RELATIVE PERCENTAGE DISTRIBUTION (MEAN AND S.D.) OF RETAINED ^{99}Mo IN TISSUES OF FOUR GOATS 96 HOURS AFTER ORAL ADMINISTRATION. (From Anke et al., 1971).

	Skeleton	Liver	"Rest"	Head	Muscle	Blood	Kidneys	Hair	Ovaries
Mean	27.4	19.9	14.4	14.3	11.6	8.7	3.4	0.2	0.1
S.D.	9.2	4.7	7.2	13.6	12.7	9.3	1.5	0.18	0.004

TABLE 4:6 RELATIVE CONCENTRATION[x)] (MEAN AND S.D.) OF RETAINED ^{99}Mo IN TISSUES OF FOUR GOATS 96 HOURS AFTER ORAL ADMINISTRATION. (From Anke et al., 1971).

	Kidney	Liver	Ovaries	Blood	Head	Skeleton	"Rest"	Hair	Muscle
Mean	100	60	28	18	12	12	7	3	3
S.D.	16	22	10	2	10	6	2	4	2

[x)] Given as radioactivity/g dry weight. Highest activity = 100.

TABLE 4:7 MOLYBDENUM IN LIVER AND KIDNEYS (ug/g wet weight) OF "NORMAL" HUMANS OF DIFFERENT AGES. (Modified from Pribluda, 1964).

	Liver				Kidneys			
Age, years	17-25	26-44	45-59	60-77	17-25	26-44	45-69	60-77
Number	23	27	20	10	23	27	20	10
Arithmetic mean	0.64	0.61	0.54	0.57	0.22	0.24	0.24	0.24
Standard deviation	0.21	0.18	0.12	0.15	0.06	0.06	0.09	0.06

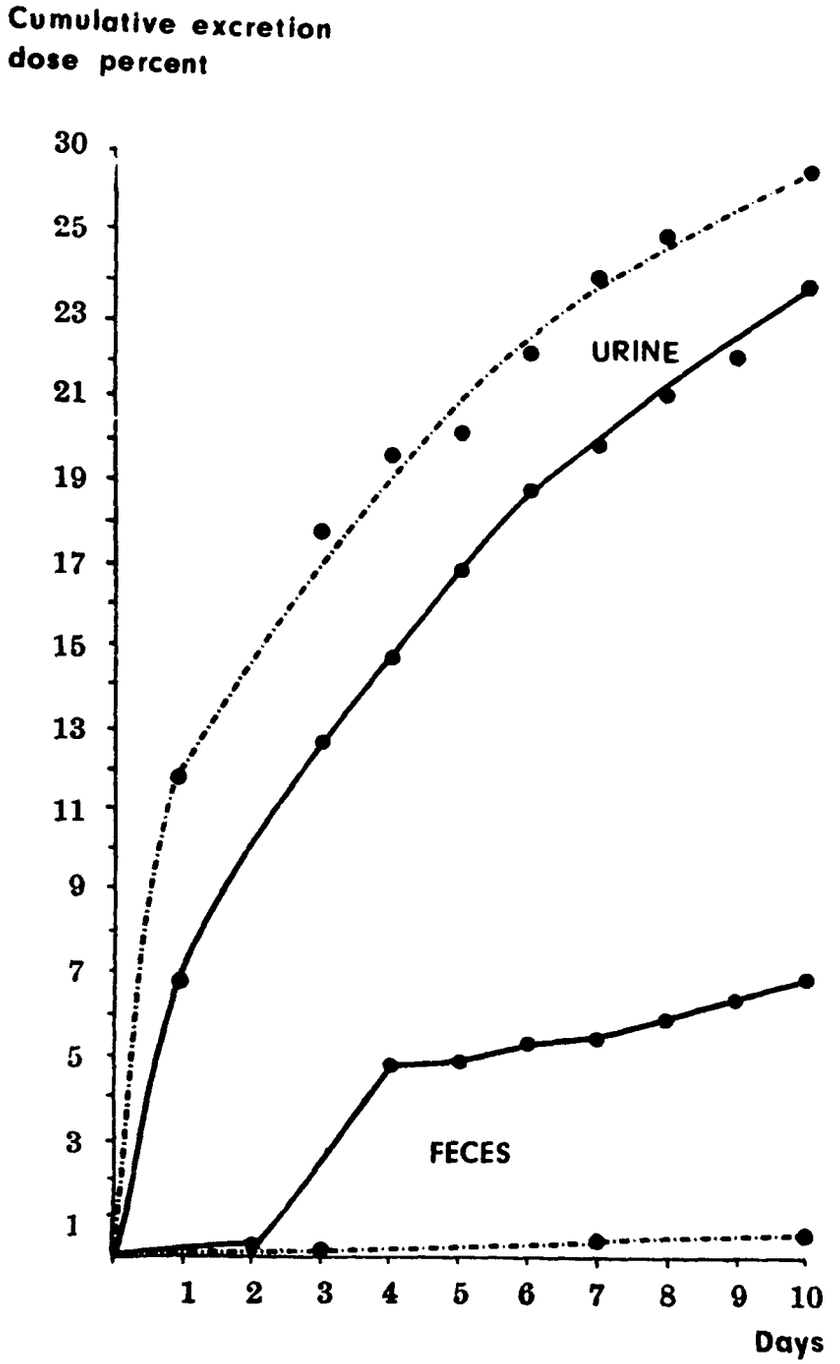

FIGURE 4:1 Cumulative excretion of ^{99}Mo in man after an intravenous injection of carrier-free ^{99}Mo in ammonium hydroxide. ———— Subject 1; -----Subject 2. (From Rosoff and Spencer, 1964).

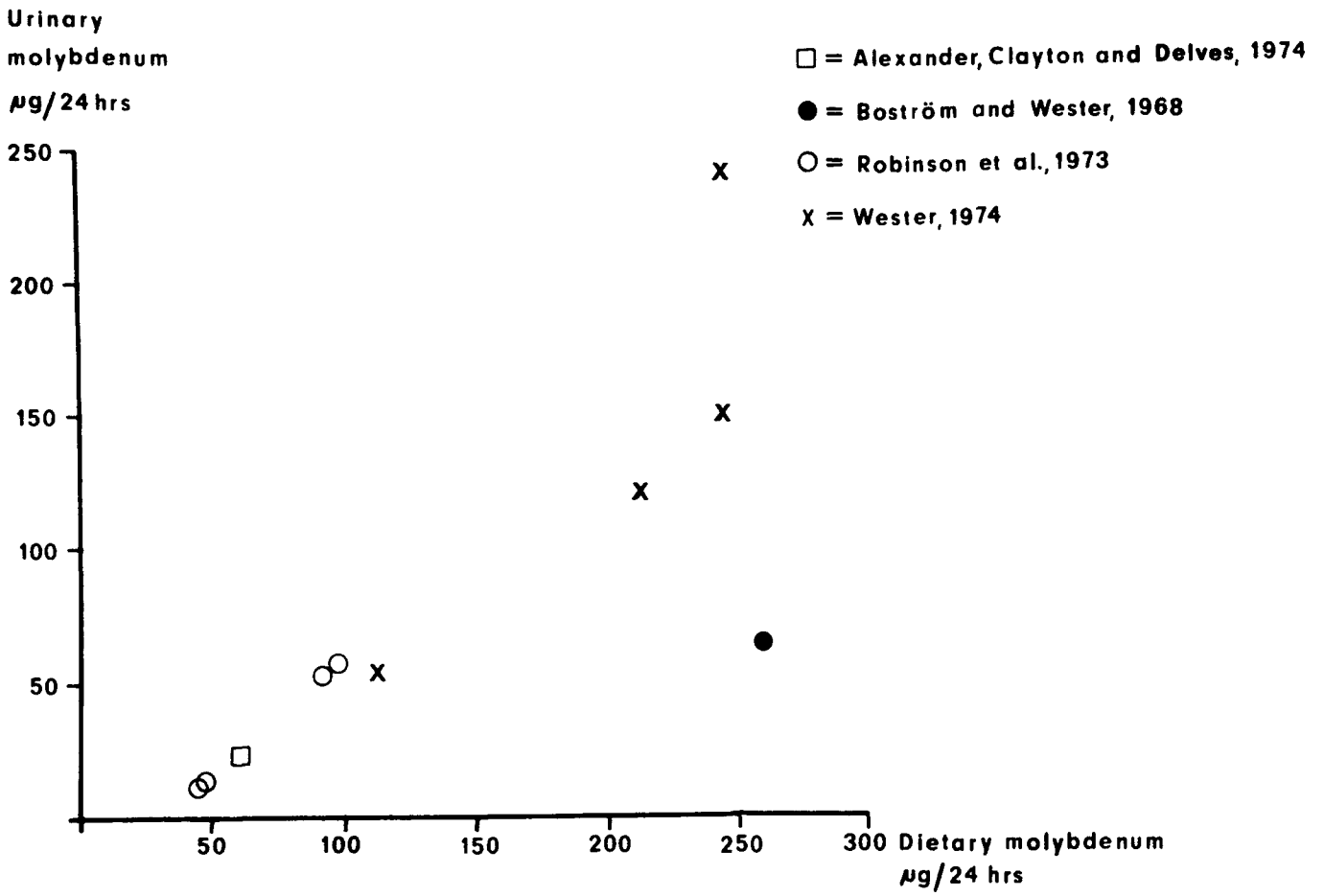

FIGURE 4:2 Relationship between daily intake and urinary excretion of molybdenum. All data are from balance studies on healthy individuals. The data from Boström and Wester, 1968, Robinson et al., 1973, and Wester, 1974a, represent individual values. The data from Alexander, Clayton and Delves, 1974, represent the mean values from eleven healthy schoolchildren. These values were originally given as ug/kg body weight and have been converted to 20 kg body weight.

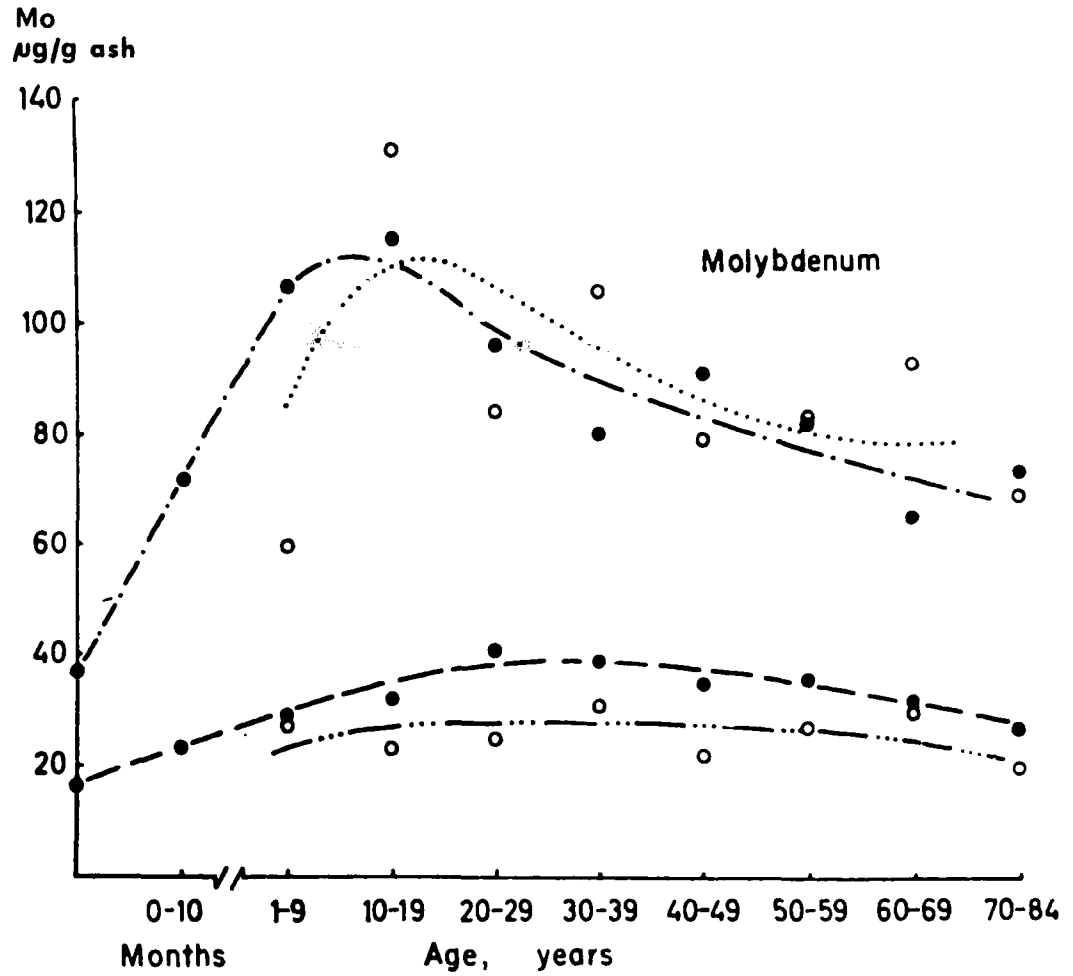

FIGURE 4:3 Mean concentrations of molybdenum, according to age, in livers (upper curves) and kidneys (lower curves) of United States and non-U.S. subjects, ug/g ash. There were 226 livers (solid dots, dash-dot line) and 217 kidneys (solid dots, dashed line) from U.S. subjects and 179 livers (open circles, dotted line) and 181 kidneys (open circles, dash and 3 dots) from non-U.S. subjects. Note the similarity of the curves. The first 3 points for U.S. values were based on 18, 33 and 3 samples respectively, the remainder on 10-45 samples in each decade. The first non-U.S. point was based on only 2 samples, the remainder on 7-37 samples in each decade. (From Schroeder, Balassa and Tipton, 1970).

CHAPTER 5 MOLYBDENUM AS AN ESSENTIAL ELEMENT IN ANIMAL NUTRITION

5.1 MOLYBDENUM DEFICIENCY AND WOLFRAMATE INHIBITION STUDIES

One way of studying the essentiality of an element is to exclude it from the diet of experimental animals. This can be technically difficult since complete absence of the element is often required before anything definite can be said about its role in metabolism. Tracer doses of some essential elements are sufficient to maintain a normal metabolism.

In an experiment by Higgins, Richert and Westerfeld, 1956, female weanling rats were raised and maintained on a purified diet poor in molybdenum, approximately 20 ug Mo/kg, for three generations. No difference arose in the development of these animals compared with a group that received an additional 200 ug molybdenum per kg given as $NaMoO_4$. The animals grew at a normal rate, reproduced, and appeared normal in all respects. The livers of the third generations of animals contained 1.89 ± 0.15 and 2.24 ± 0.19 ug/g dry weight of molybdenum respectively. In order to decrease the tissue levels of molybdenum further, six animals were given an inhibitory metal, wolframate, 4.5 mg W/kg diet in the form of Na_2WO_4 as a supplement to the molybdenum-low diet. This increased the urinary excretion of molybdenum from less than 0.07 ug/day to about 0.6 ug/day and decreased the liver molybdenum to 0.81 $\pm$ 0.18 ug/g dry weight. Still, no symptoms or effects on the animals could be found.

A similar experiment was carried out by the same authors on chicks (see Tables 5:1 and 5:2). Feeding a synthetic diet containing approximately 20 ug/kg of molybdenum to day-old chicks for five weeks (group 2 in Tables 5:1 and 5:2) failed to produce a molybdenum deficiency syndrome in

this species. At the simultaneous administration of tungsten, however, something that might have been molybdenum deficiency developed. 45 mg/kg of tungsten (as Na_2WO_4) given to 29 chicks (group 4) significantly reduced the growth of the animals compared to a control group given an "ordinary" chick diet. The mortality after five weeks was 24% compared to zero in the control group. The liver molybdenum was 0.22 ug/g dry weight compared to 3.56 ug/g in the control group (group 1) and other tissues of group 4 such as proventriculus, intestines, kidneys, brain and muscles contained from non-detectable to 0.08 ug/g dry weight. The adverse effects were largely overcome by adding more molybdenum to the diet. 22 chicks were given the same amount of tungsten (45 mg/kg) plus 2 mg/kg of molybdenum. These animals grew at a normal rate, showed normal values of molybdenum in their tissues, but still showed mortality, 9% after five weeks. To draw any definite conclusions from this experiment is impossible, since another element was given in high amounts.

The findings of Higgins, Richert and Westerfeld, 1956, are partly confirmed by Teekell and Watts, 1959. They studied the growth response of 1,790 chicks to the dietary supplementation of different levels of molybdenum. One group of chicks came from dams that received a regular diet, and another group from dams that received tungsten in their diet. The latter group showed a statistically significant positive growth response to molybdenum supplementation. The result is difficult to evaluate for several reasons, first because of the presence of another element, tungsten. Secondly, the tungsten supplement to dams did not, according to a table in this survey, decrease the tissue level of molybdenum in the chicks.

The tungsten in itself was transferred from the dams to the chicks. This would indicate that the "negative" growth response in chicks from tungsten-supplemented dams is a direct effect of the tungsten rather than an indirect effect of a decrease of molybdenum.

Tungsten was used by Cohen et al., 1973, and Johnson, Rajagopalan and Cohen, 1974, to produce molybdenum deficiency in rats. The activities of the enzymes sulfite oxidase and xanthine oxidase in liver were reduced to about 10 percent of the control activity when 100 mg tungsten per kg of diet was given, but no toxic signs appeared in the rats. Activity was rapidly restored by giving molybdenum (Cohen et al., 1973). Johnson, Rajagopalan and Cohen, 1974, could show that rats with decreased activities of sulfite oxidase were less resistant to bisulfite, the LD_{50} being considerably less in molybdenum-deficient rats. In such rats, survival times after inhalation of sulfur dioxide at concentrations of 925 and 2,350 ppm (2,313 and 5,815 mg/m^3) were much shorter than in controls while there was no difference when the exposure was 590 ppm (1,475 mg/m^3). It was concluded that at the higher exposures systemic effects of the bisulfite derived from the sulfur dioxide occurred, but that at the lower exposure the effect was a direct effect on the lungs. In that case, the sulfite oxidase activity in the lungs did not influence the outcome. Neither bisulfite nor sulfur dioxide induced sulfite oxidase activity. There was no difference in the 2-week mortality after 4 hours of exposure to sulfur dioxide at concentrations ranging from 224 to 1,319 ppm between controls and tungsten-treated animals.

Sheriha, Sirny and Tillman, 1962, fed a synthetic diet that contained approximately 10 ug/kg of molybdenum to

six lambs for 46 days. Three of those animals received an addition of 2 mg Mo/kg diet supplied as Na_2MoO_4, but this supplement did not result in higher weight gains compared to the other three animals. The authors concluded that under the experimental conditions of this trial, the molybdenum requirement of sheep was less than 0.01 mg Mo/kg diet.

5.2 THEORETICAL BACKGROUND TO THE QUESTION OF MOLYBDENUM ESSENTIALITY - ENZYME STUDIES

5.2.1 Xanthine oxidase

Metalloflavoproteins, playing a fundamental role in animal metabolism through the "electron transport chain", are a group of enzymes containing both metal and flavin firmly bound to protein. Molybdenum was found to be a constituent of the enzyme xanthine oxidase by Westerfeld and Richert, 1953, and DeRenzo et al., 1953. Earlier studies by Westerfeld and Richert had revealed evidence that the levels of liver and intestinal xanthine oxidase in rats were dependent on some unidentified dietary factor. It could be supplied by adding liver residue or soy flour to a purified diet and it was not destroyed by ashing. In 1953 this factor was isolated and identified as molybdenum. Xanthine oxidase from different animal tissues has later been purified and analyzed, and has been a subject of several studies (see Bray and Swann, 1972, for review of work on this enzyme and other molybdenum enzymes up to 1972).

In vitro studies have revealed a wide range of oxidations that are catalyzed by xanthine oxidase. Its main biological function is considered to be the oxidation of two purines, xanthine and hypoxanthine, to uric acid. In the experiment by Higgins, Richert and Westerfeld, 1956, commented on in 5.1, the molybdenum depletion

of rat and chick tissues caused by molybdenum-poor diets and tungsten supplement was accompanied by a decrease in xanthine oxidase activity in all tissues analyzed. Comparison between group 1 ("ordinary" chick diet) and group 4 (20 ug Mo/kg and 45 mg W/kg) revealed a significant, sharp decrease in xanthine oxidase activity and molybdenum content in the tissues of the latter group (see Tables 5:1 and 5:2). Further, this depletion was accompanied by changes in the uric acid excretion: about one half of the uric acid normally excreted by chicks was replaced by a mixture of xanthine and hypoxanthine. In a study by Leach et al., 1962, on chicks, the decrease of xanthine oxidase activity parallel to growth inhibition during tungsten-induced molybdenum deficiency was confirmed. Added dietary molybdenum reversed the tungsten effect on xanthine oxidase activity but growth inhibition was only partially reversed (mortality was not discussed). Hence the authors suggested that the growth inhibition was not caused by interference with the activity of this enzyme. It is difficult to compare this study with the study by Higgins, Richert and Westerfeld as the dietary level of molybdenum and the tungsten supplement were much higher (1.72 mg molybdenum per kg and 500-2,000 mg W per kg).

In vitro studies by Green and Mazur, 1957, demonstrated how xanthine oxidase, reduced by the oxidation of hypoxanthine or xanthine to uric acid, could release iron from hepatic ferritin. In 1958 the biological role of this mechanism had been confirmed in in vivo studies on guinea pigs, rabbits and dogs (Mazur et al., 1958). The administration of xanthine oxidase substrates (xanthine and hypoxanthine) to the animal led to an increase of plasma iron. Further, a reduction of blood pressure through hemorrhage led to a sharp increase in

plasma iron and uric acid in blood samples from the hepatic vein. According to the authors this is indicative of an increase in activity of this mechanism, mediated by the physiologic demand for iron at tissue hypoxia. The ferritin - xanthine oxidase system should be a part of the homeostatic mechanism for the regulation of iron in the circulation. For more details in this matter, see a review by Seelig, 1972.

5.2.2 Other molybdoflavoproteins

5.2.2.1 Sulfite oxidase

The occurrence of molybdenum in this enzyme was first established as a result of EPR work (electron paramagnetic resonance) by Cohen, Fridovich and Rajagopalan, 1971. This enzyme catalyzes the oxidation of sulfite to sulfate which is necessary in the mammalian metabolism of the sulfur amino acids and related sulfur-containing compounds. Irreverere et al., 1967, described a case of possible inherited disease: sulfite oxidase deficiency. The patient was born with neurological abnormalities and deteriorated to a decorticate state in nine months. The patient's urine contained abnormally increased amounts of S-sulfo L-cysteine, sulfite and thiosulfate, and markedly reduced amounts of inorganic sulfate. This pattern would be explained by the presence of a block in the conversion of sulfite to sulfate, a postulation that was confirmed by post mortem studies of the patient's tissues. The authors suggested that the pathologic changes could have been caused by the toxic effects of accumulation of sulfite or other metabolites (or deficiency of inorganic sulfate). Three of the patient's siblings had died in infancy with neurological disorders, leading the authors to suspect the disorder of being a hereditary metabolic defect, "sulfite oxidase deficiency".

5.2.2.2 Aldehyde oxidase

This enzyme is third of the mammalian molybdoenzymes and has many properties in common with xanthine oxidase (see review by Mahler and Green, 1954, Bray and Swann, 1972). It is known to catalyze the oxidation specifically of aldehydes and various nitrogen-containing aromatic heterocyclic compounds (Knox, 1946). In 1964 Rajagopalan and Handler showed that it is also capable of oxidizing hypoxanthine but not xanthine. Neither the need for this enzyme nor its correlation with molybdenum have been subjected to any studies so far.

5.3 MOLYBDENUM AND DENTAL CARIES

In 1953 Adler and Straub discovered that in certain communities in Hungary caries incidence was lower than would have been expected from the fluoride content of water in these areas. Later studies by Nagy and Polyik, 1955, showed that the drinking water contained unusually high concentrations of molybdenum, 100 ug/l, suggesting that this factor caused the reduction in caries. Up to the year 1967 three epidemiological studies of caries frequency in man had revealed evidence of a caries protective effect of molybdenum in the diet (review by Jenkins, 1967), in turn confirmed by several animal studies.

5.3.1 In animals

Adler, 1957, supplemented the drinking water of two generations of rats with 100 ug Mo/l given as ammonium molybdate and found a significantly reduced caries frequency in the second generation (4 out of 21 compared to 11 out of 22 in a control group). This result was confirmed by Adler and Porcsalmy, 1961. Van Reen, Ostrom and Berzinskas, 1967, found an approximately 10% reduction of caries frequency by the supplementation of 24 mg Mo/kg diet

Mo/l given as sodium molybdate or ammonium molybdate to rats, i.e., a much weaker effect of a much higher concentration of molybdenum. This experiment, however, was undertaken with only one generation of rats (21 days old, duration of experiments 5 weeks) and it is possible that the positive effect of the molybdenum is mainly active during the neonatal period and early development of the teeth.

In 1973, Bowen found no caries preventive effect of molybdenum when monkeys were given 2 mg sodium molybdate/l water in a 5-year experiment beginning at 11-17 months of age.

The mechanism behind the caries preventive effect of molybdenum is a subject of controversy. Stookey and Muhler, 1959, 1964, and Stookey, Roberts and Muhler, 1962, have provided evidence of a synergistic effect of molybdenum and fluoride in rats and hamsters. Measurements of the fluoride retention in the carcass and skeleton of experimental animals showed higher values of fluoride in the molybdenum-exposed animals, indicating that molybdenum may act metabolically to increase the "availability" of the fluoride ion (a substance that has a documented anti-caries effect).

5.3.2 In human beings

Anderson, 1969, found evidence for the caries preventive effect in humans that had been postulated by early investigators (see Jenkins, 1967). 682 twelve-year-old children from a "molybdenum" area (or "teart" area; see Chapter 6) in Great Britain and from "control" areas were examined for the presence of dental caries. Further, drinking water, herbage, milk, urine and extracted teeth were analyzed for their molybdenum contents. Samples from the molybdenum area were found to hold slightly but sig-

nificantly higher levels of molybdenum compared to the control areas. Pooled urine samples from the molybdenum area contained 57 ug/l compared to 33 ug/l in the control area. The dental caries prevalence was said to be significantly lower in the molybdenum area than in the control area.

5.4 CONCLUSIONS

Data from enzymatic animal studies provide backing for the concept of molybdenum's essentiality. The metal is a constituent of three mammalian metalloflavoproteins, xanthine oxidase, aldehyde oxidase and sulfite oxidase. A report of congenital sulfite oxidase deficiency and its postulated correlation to a deadly syndrome is indicative of an absolute need for at least this enzyme (and consequently molybdenum). Animal experiments indicate that reduction in the activity of sulfite oxidase increases the susceptibility to bisulfite.

Trials to produce a "molybdenum deficiency syndrome" by feeding animals a diet low in molybdenum have failed in all instances so far. Assuming an absolute need for the metal, this is indicative of an extremely low requirement as it has evidently not been possible to reduce the dietary molybdenum level to a sufficient degree. Thus, it has been necessary to introduce an element with a known property of inhibiting molybdenum in the trials, tungsten. With this technique it has been possible to produce an assumed molybdenum deficiency syndrome in chicks consisting of reduced weight gain and even death. In rats, no toxic effects were noted, however.

There is evidence of a caries preventive effect of molybdenum both in animals and human beings.

TABLE 5:1 THE EFFECT ON GROWTH AND TISSUE XANTHINE DEHYDROGENASE ACTIVITIES OF CHICKS FED DIETS CONTAINING VARYING AMOUNTS OF TUNGSTEN (as Na_2WO_4) AND MOLYBDENUM (as Na_2MoO_4). (From Higgins, Richert and Westerfeld, 1956).

Diet group[1]	Additions to diet (mg/kg)		Body weight[2] (g)		Xanthine dehydrogenase activities (mm^3 O_2/20 min/flask[3])			
	W	Mo	At 3 wks	At 5 wks	Intestine	Liver	Kidney	Pancreas
1			218	343	8	32	11	6
2			217	311	8	32	12	4
3		0.2	203	339	5	27	11	5
4	45		169	311	1	4	1	1
5	45	2	196	339	7	34	11	4
6	45	20	203	332	7	35	17	5
7	45	60	186	322	6	29	14	7
8	94		149	274	1	3	2	1
9	94	60	177	324	5	29	12	5

1) Group 1 was fed Park and Pollard starter mash; group 2 was fed the synthetic diet to which no molybdenum was added, but which contained approximately 0.02 mg/kg Mo.

2) Starting weights averaged 40 to 44 g for all groups. Twenty-nine chicks per group for groups 4 and 8; 22 chicks per group for all others.

3) Xanthine dehydrogenase values were determined on 8 chicks from each group after 6 weeks on the diets, using 28.3 mg of liver or kidney and 84.9 mg of intestine or pancreas per flask.

TABLE 5:2 MOLYBDENUM CONTENT (ug Mo/g dry weight) OF CHICKEN TISSUES AFTER SIX WEEKS ON DIETS CONTAINING VARYING AMOUNTS OF TUNGSTEN AND MOLYBDENUM. (Same diet groups as in Table 5:1). (Modified from Higgins, Richert and Westerfeld, 1956).

Diet group	1	2	3	4	5	6	7	8	9
Proventriculus	0.21	0.15	0.18	0	0.19	0.83	1.51		6.79
Intestine and colon	0.51	0.24	0.35	0.05	0.76	1.11	1.82		>5.75
Kidney	4.44	3.09	3.48	0	1.65	6.85	>8.80		14.45
Brain			0.17	0	0.05	0.12	0.59		0.82
Skeletal muscle	0.14		0.10	0.08	0.10	0.44	1.07		0.77
Liver	3.56	2.52	3.35	0.22	2.73	3.55	4.43	0.20	4.11

CHAPTER 6 TOXICITY

6.1 "TEART" DISEASE - MOLYBDENUM POISONING IN RUMINANTS

Since the middle of the 19th century a bovine disease named "teart" has been known. The disease affects only ruminants of special pastures and is mainly characterized by diarrhea and concomitant emaciation. If left too long on teart land, the cattle may even die, but a very rapid recovery upon removal to non-teart pastures is typical. The illness is described as follows: diarrhea may start within 24 hours of putting the cattle in the pasture. The dung becomes extremely loose and watery. The animals lose condition rapidly, their coats becoming rough and lustreless and changing to a more greyish tone.

Sheep are affected to a certain degree, but horses and swine are resistant. The illness found its etiology in 1937 through spectrographic analyses of "teart" herbage from Somerset in England (Ferguson, Lewis and Watson, 1938), this herbage containing considerably higher amounts of molybdenum than herbage from non-teart fields close-by. The same authors presented a detailed study on the "teart" pastures of Somerset in 1943, and the data are reviewed by Ferguson, 1944.

The areas in question are in central Somerset, north Somerset, Gloucester and Warwick. The degree of teartness varies from field to field, from season to season and is proportional to the molybdenum content of herbage. From Table 6:1 it can be stated that a diffuse "borderline" should lie somewhere around 12 mg Mo/kg in dry matter. The molybdenum contents vary widely from 4 mg/kg to 59 mg/kg in different fields, at the same time of the year, and the seasonal variation at the same farm is also marked. Table 6:2 shows that "teart" is most pronounced

in autumn, after which the "teartness" decreases, the winter hay containing the lowest levels of molybdenum. Though containing not insignificant concentrations of molybdenum, winter hay rarely produces "teartness". This, according to the authors, stems from a lower proportion of water soluble molybdenum in hay.

The authors also tested the postulation that molybdenum is the etiologic factor by feeding molybdic salts to some animals, thus producing the same symptomatic picture (see 6.3).

In 1946 "teart" was also reported from Kern County in California by Britton and Goss. These authors described the same symptoms as those from Somerset, but in addition, a hypochromic microcytic anemia. The average morbidity in the cattle was about 80%. Analyses of alfalfa from the area in question revealed a molybdenum content of 10.3 mg/kg, i.e. only at Ferguson, Lewis and Watson's "borderline". Ferguson, Lewis and Watson compared the"teart" of Somerset with copper deficiency in cattle reported by Brouwer et al., 1938. The resemblance between the two illnesses made Ferguson, Lewis and Watson test copper as an antidote to "teart". This was quite successful, "teart" being completely cured by the addition of copper salts (1-2 g of copper sulfate/animal) to the diet. Thus, the etiology of the chronic molybdenum poisoning in cattle could be said to be more a matter of the balance in the dietary molybdenum-copper composition than a matter of the dietary molybdenum level per se. Accordingly, the relatively lower molybdenum content found in the Britton and Goss study might be explained by a low copper content in the herbage.

Hogan et al., 1971, reported on sheep from New Zealand. The animals were grazed from April to October on pastures

that contained about 5-7 mg/kg dry matter of copper, and 5-8 mg Mo per kg in the beginning of the trial period. During August the molybdenum content rose rapidly to approximately 20 mg/kg. (The authors supplemented the diet of certain groups with copper and selenium and compared these groups with "controls". Selenium plays a role in copper metabolism, but did not give any remarkable effects in this study). The animals demonstrated symptoms that were not described in cattle in the foregoing studies. Besides diarrhea and anemia, some animals were suffering from lameness. Out of 9 animals grazing on pastures with high levels of molybdenum, 4 were affected (this was noted in September) and were killed and autopsied. They showed lesions concerning the skeleton-muscle system: rib, humerus, and femur fractures, muscle insertions having been stripped off the bone together with periost, and hemorrhages of periost and muscle (see 6.3.3.4).

6.2 IN HUMAN BEINGS

6.2.1 Effects on lungs

Mogilevskaja, 1963, reported 3 cases of pneumoconiosis out of 19 workers exposed to metallic molybdenum and its oxide. A 44-year-old woman working in a molybdenum reducing shop, exposed for 5 years at concentrations in the range of 1-3 mg/m^3 and a 44-year-old man exposed for 4 years at concentrations fluctuating between 6-19 mg/m^3, showed early signs of pneumoconiosis on X-ray examination. The woman complained over difficulties in breathing, and general weakness, the man over dry cough. A 34-year-old man exposed for 7 years to concentrations between 6 and 19 mg/m^3 showed fully developed pneumoconiosis (multilayer shadows of a nodular nature and slight emphysema). This man complained over difficulties in breathing, pain in the chest and expectoration, particularly in the morning. The dust contained different

proportions of the metal and its oxide. 90% of the particles were below 5 u.

6.2.2 Hyperuricemia

Akopajan, 1964a, examined 73 workers in a copper-molybdenum plant and 10 control subjects. The highest levels of uric acid in blood were observed in miners who were the most exposed group. The increased blood uric acid was found in 34 out of 37 workers who complained of arthralgia. The results are difficult to evaluate because the author does not report the values of uric acid in blood; the exposure is not defined either. Avakajan, 1966a, also described hyperuricemia while studying 85 workers in a copper-molybdenum plant in Kadzaran. He further noted an increase in the concentrations of bilirubin, globulins and cholesterol in blood but no data were given in this study.

Kovalskii, Yarovaya and Shmavonyan, 1961,[x] and Yarovaya, 1964, reported on a high incidence of gout in an area of Armenia having 77 mg Mo/kg and 39 mg Cu/kg in the soil. Kovalskii, Yarovaya and Shmavonyan, 1961, on the basis of molybdenum levels in different food products, calculated the total molybdenum and copper intake for an adult man in this area compared to a man in a control area (10-15 mg molybdenum and 5-10 mg copper, versus 1-2 mg molybdenum and 10-15 mg copper respectively). The concentrations of molybdenum and copper in food products and in drinking water in the molybdenum-rich area were compared with those in the control area. In this region of the U.S.S.R., more than 50% of the diet is based on locally grown products. Selected values for molybdenum and copper concentrations in food are given in Table 6:3. As is seen throughout the table, the values from the molybdenum-rich area as regard molybdenum are considerably higher. It is not known however why the values in the control area are

[x] The treatment of this report is based upon our access to a complete translation made by Göran Pershagen, M.D. at the Department of Environmental Hygiene of the Karolinska Institute.

higher than usually seen in "normal" areas (see 3.3.7).

A medical survey of 400 subjects from two villages in the molybdenum-rich area of which 262 persons were 18 years or older revealed a prevalence of symptoms similar to gout in 31% from one of the villages and 18% from the other, these percentages pertaining to the adults only and making a total of 71 persons. The authors claimed that similar symptoms normally occurred in 1-4% of the population of the U.S.S.R. The symptoms were characterized as arthralgia in the knee-joints, hands and feet. Joint deformities were also reported. Symptoms from the gastro-intestinal tract, liver and kidney were said to be commonly observed. The description of the symptoms is not given in such a way as to allow a judgement as to whether they are actually identical with symptoms of gout. Concerning the symptoms from the gastrointestinal tract, liver and kidney, no details whatsoever are furnished.

A more detailed study was performed on 52 subjects in the molybdenum-rich province and on 5 subjects from a control area. In Table 6:4 the uric acid values in blood and urine from the 52 examined subjects, some with and some without symptoms of gout, are presented, along with the values of the 5 control subjects.

In the paper there are no data whatsoever regarding how the exposed and control subjects were chosen nor were any detailed data on age distribution given. Another point of difficulty is that only 5 controls were included, but it should be noted that their values agree with what is considered "normal" (Documenta Geigy, 1970). In spite of the difficulties in interpreting any bias in connection with the selection of the study groups, the data presented seem to show an increased uric acid level in blood and urine in the subjects living in the molybdenum-rich area.

Concerning concentrations of molybdenum in blood and urine, data from Table 6:5 reflect the excessive exposure to molybdenum inasmuch as the levels of molybdenum are considerably higher in the molybdenum-rich province than in the control area. The same reservations apply concerning the selection of subjects as were discussed in connection with Table 6:4.

6.2.3 Other effects

Eolajan, 1965, examined 500 workers in a molybdenum mine and plant and a control group of equal size (general population of the same area). The majority of workers were miners in copper-molybdenum mines (both underground and surface). According to the information of a previous study, dust levels exceeded, on the average, the MPC[x)] value 10-100 times (MPC for metallic Mo and insoluble Mo compounds is 6 mg/m^3 in the U.S.S.R.). (Note: the composition of dust is not given). A large percentage of workers had various unspecific symptoms and signs including general weakness, fatigue, headache, irritability, lack of appetite, epigastric pain, pains in joints and muscles, increasing loss of weight, red and moist skin, tremor of the hands, sweating and dizziness. On the basis of a neurological analysis of these symptoms and signs, the author concluded that a long-term exposure at the levels mentioned results in the impairment of the central nervous system.

6.3 IN ANIMAL EXPERIMENTS

6.3.1 Single exposure

6.3.1.1 Injection

Maresh, Lustok and Cohen, 1940, studied the acute toxicity of intraperitoneal injections of sodium molybdate. The authors gave 20 rats, weighing from 114 to 260 grams, from 45 to 350 mg Mo/kg body weight. Ten rats receiving 114 mg

x) MPC = Maximum Permissible Concentration

Mo/kg and below showed a few transitory symptoms and remained well. The 10 rats receiving from 117 mg Mo/kg and above died within a few hours.

Fairhall et al., 1945, studied the acute toxicity in guinea pigs following intraperitoneal injections of molybdenum compounds. They followed the animals 4 months after the injection and noted the mortality rate (see Table 6:6). In 12 animals receiving 800 mg ammonium molybdate per kg, mortality was 100% (probably within a few hours) whereas those receiving 80 mg/kg underwent no mortality at all. 75%, receiving 400 mg molybdenum trioxide/kg, died within 4 days, and the majority of those within 2 hours. The injection of calcium molybdate and molybdenite resulted in a much lower mortality.

In experiments on cats, Cilingarajan, 1965, found that a single i.v. injection of 25 mg/kg of sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$) produced a moderate increase in arterial blood pressure (measured in the carotid artery), and a stable prolonged increase in the tonus of the nictitating membrane. The pressor effect of noradrenaline was potentiated. Introduction of acetylcholine was followed by hypotension. A dose of 50 mg/kg reduced the pressor effect of noradrenaline significantly; the nictitating membrane contracted on stimulation of the preganglionic fibers of the cervical nerve.

6.3.1.2 Inhalation

Mogilevskaja, 1963, exposed 45 rats for one hour to the dusts of metallic molybdenum (25-30 g/m^3), molybdenum trioxide (12-15 g/m^3), molybdenum dioxide (10-12 g/m^3) and ammonium paramolybdate (3-5 g/m^3). The distribution and particle size are seen in Table 6:7. With the exception of some irritation of the upper respiratory passages, the

author could find no adverse effects upon the appearance of the animals, which were followed 4 weeks after administration.

In another experiment rats were exposed for 2 hours to an aerosol of molybdenum trioxide (concentrations of the dust being below 0.064 g/m^3). The animals showed no signs of poisoning, and increased in weight parallel to controls for 2 weeks after administration. Microscopical examination, however, revealed evidence of "dystrophic processes" in the heart, liver and kidneys, and "necrotic processes" and "signs of regeneration" in the liver.

In a third series of experiments, rats were given a single intratracheal dose of a 50-mg suspension of the dust of metallic molybdenum (17 rats) or molybdenum trioxide (8 rats). 85-90% of the particles had a diameter of less than 2 u. In the case of animals exposed to molybdenum dust a progressive interstitial process and a formation of dust granulomata were seen after two months. There were collagen fibers in the thickened intraalveolar septa as well as in the granulomata. The changes progressed and gave rise to peribronchial and perivascular fibrosis. At the sites of accumulation of dust, "whole fields of collagen fibers and emphysema" were found. Molybdenum trioxide also gave rise to interstitial fibrosis, but here the most marked changes were observed in the regional lymph nodes (proliferative processes and marked fibrosis),.

Dzukaev, 1970, studied the effects of the intratracheal administration of powdered (metallic) molybdenum on rabbits. 32 animals were given 1.5-2.0 ml of molybdenum powder suspended in a physiological solution (70-80 mg/kg body weight; particle size: 36% <2 um; 47% <5 um; 17% >5 um). Two animals died from pneumonia within 10 days. The remaining

30 animals were killed after 3, 6 and 9 months respectively. The author stated, "In 9 months, histological examinations showed slight diffuse pneumoconiosis with interstitial pneumonia." The author does not give a more detailed account of what number of animals was used in each group or what the prevalence of pathological findings was.

6.3.3 Repeated exposure

6.3.3.1 Oral administration, general aspects

6.3.3.1.1 Experiments with common laboratory animals

Fairhall et al., 1945, studied the toxicity of molybdenite, molybdenum trioxide, calcium molybdate and ammonium molybdate on white rats by feeding the animals from 10-500 mg/animal/day for a varying number of days up to 232 days. No signs of poisoning appeared in any of the groups ingesting molybdenite while all groups receiving hexavalent molybdenum, were affected: loss of appetite, loss of weight, "they became quiet and listless and their furs harsh and rough". Daily doses in excess of 100 mg were uniformly fatal. Moreover, the 10 mg groups showed a mortality varying from 25-50% during the test period (see Table 6:8). The approximate LD_{50} of daily repeated doses in mg of molybdenum per kg per day for molybdenum trioxide was given as 125, for calcium molybdate 100 and ammonium molybdate 333. These figures only serve as a comparative toxicity between the three compounds, as the "LD_{50}" in repeated exposure is a vague term.

Neilands, Strong and Elvehjem, 1948, placed 21-day-old rats in groups of 4 on a diet containing 500, 1,000 or 5,000 mg Mo/kg as sodium molybdate. All four rats receiving 5,000 mg Mo/kg died shortly after the first week. All groups showed growth retardation (see Figure 6:1) and emaciation, but the authors negated any other effects such as diarrhea (see 6.1) or changes in blood picture (see 6.3.3.3).

Growth retardation was also reported by Gray and Daniel, 1954, who fed 5 rats 800 mg Mo/kg in the diet given as sodium molybdate, this level being insufficient to cause any mortality within an experimental period of 6 weeks. Mean increase of weight for the five rats within this period was 94 g compared to 152 g for a control group.

Williams and Van Reen, 1956, fed groups of 6 rats 800 mg Mo/kg diet, 1,200 mg/kg and 1,400 mg/kg, respectively, given as sodium molybdate. After 5 weeks a control group had an average weight of 218 g, whereas the experimental groups, in order according to dose, weighed 171, 121 and 87 g. Many of the rats developed an intermittent diarrhea, but there was no mortality. The authors related the poor weight gain to reduced food intake, but excluded the possibility of palatability as a cause of the depressed intake as 5 rats given sodium molybdate by stomach tube also showed poor food intake.

Ostrom, Van Reen and Miller, 1961, fed rats 400 mg Mo/kg diet as sodium molybdate for 5 weeks. The authors confirmed the growth depression described by earlier investigators, but also found anemia and mandibular exostoses. These symptoms were highly correlated with the growth depression, i.e. all three symptoms tended to appear in those rats that were most affected. Thus, out of 20 rats, 10 rats showed the toxic signs, whereas 10 rats were relatively unaffected. The hemoglobin values (g/100 ml) of 7 rats with exostoses on an average were depressed from 13.8 to 8.4 while 8 rats without exostoses showed normal hemoglobin values, 13.2 on the average.

In order to find out whether the growth depression upon molybdenum intake is proportionally correlated to a reduced food intake or if some other metabolic process is causing the disorder, Arrington, Ammerman and Moore,

1965, pair-fed rats for 4 weeks. Each pair consisted of 1 rat receiving 500 or 1,000 mg Mo/kg diet given as sodium molybdate, and 1 control. The intake of the pair-control was limited to the reduced intake by the pair-mate. Still, the weight gains of the "molybdenum rats" were significantly lower than their pair-mates in both the 500 mg/kg and the 1,000 mg/kg groups. This effect was also studied by measuring the food intake of ad libitum fed rats for 6 weeks using the term "feed per unit gain". The corresponding figures for controls, 500 mg/kg and 1,000 mg/kg were 4.7, 5.1 and 8.6 respectively, the latter experimental group value being significantly different from controls. The matter of reduced feed efficiency is confirmed on rats by Gray and Daniel, 1954, already referred to in this chapter.

Greseva and Iliev, 1973, fed 4 groups of 36 white rats (body weight 100 $\pm$ 10 g) for 17 weeks with diets containing naturally occurring molybdenum bound in peas, or added molybdenum as MoO_3. The total dose received was 2,365 ug (controls, normal concentration of Mo in peas); 24,487 ug (peas + MoO_3); 11,550 ug (peas with naturally high content of Mo - grown on Mo-rich soil); and 24,497 ug (normal peas + MoO_3). The mortality increased from Group I to Group IV as follows: 14.2%, 13.4%, 34.6%, 57.2%.

Three groups of 8 guinea pigs in each were given 25, 100 and 200 mg molybdenum per animal per day of either molybdenum trioxide or calcium molybdate (Fairhall et al., 1945). The compounds were administered in a 10% gum arabic solution into the back of the animals' throats by means of a syringe. The oxide proved most toxic, 25 mg molybdenum per day leading to a 75% mortality, and both the 100 mg and 200 mg doses leading to 100% mortality. The corresponding numbers of days

on test were 99 days, 27 days and 6 days respectively. Calcium molybdate gave a 12.5% mortality at 25 mg molybdenum within 95 days and 25% at both 100 mg and 200 mg molybdenum per animal per day, also within 95 days. Toxicity symptoms were not described.

Arrington and Davis, 1953, fed sodium molybdate to 31 (22 weanlings and 9 adults) rabbits. Molybdenum was added to a commercial ration (containing 2.7 mg Mo/kg and 16.4 mg Mo/kg of copper) providing 140, 500, 1,000, 2,000 and 4,000 mg Mo/kg diet. Growth data were collected from weekly weights during 12 weeks of 22 weanling rabbits placed on the experiment at 6 weeks of age. Other symptoms as anemia and skeletal deformities were also studied, and the results are given in Table 6:9.

Rabbits fed 1,000 mg/kg diet or more developed a toxic syndrome, which was characterized by anorexia, loss of weight, alopecia, slight dermatosis, anemia and death. In some young rabbits an abnormality of the front legs developed. The syndrome that started to develop within 4 weeks for younger rabbits, and took a "somewhat longer time for mature animals", had a tendency to become worse until the animal died or was given copper therapy. Growth was retarded only in those rabbits which developed other symptoms of toxicity. At the two higher doses the animals lost weight instead of gaining. Hemoglobin values were depressed to between 3.2 g/100 ml and 8.0 g/100 ml compared to controls between 12.0 g/100 ml and 15.0 g/100 ml and the anemia also appeared together with the other symptoms.

McCarter, Riddell and Robinson, 1962, induced molybdenosis in 52 rabbits by feeding an oats-alfalfa diet, containing approximately 2,000 mg of sodium molybdate per kg diet. All animals were affected; by the 5th week

mortality was 65%. Epiphyseal line fractures appeared in some animals after only 12 days. Hemoglobin was decreased to approximately 65% and of the 34 animals that died, 33 had lost 4-41% of their body weight. These authors also noted diarrhea in some test animals.

Arrington, Ammerman and Moore, 1965, also studied feed efficiency in rabbits, and used the same method as already described for rats. The rabbits consumed less feed and gained less than controls, but in this species feed efficiency was not affected. 1,000 mg Mo/kg diet did not significantly reduce intake or gain in this study. Rats consume proportionally larger amounts of food than rabbits, and the authors suggested this fact to be at least a part of the explanation for the species differences. (Already 500 mg Mo/kg diet resulted in decreased feed efficiency in rats).

Probst, 1971, negated noxious effects in broilers at the supply of up to 6.95 mg Mo/kg feed given as ammonium molybdate. The author is actually recommending an addition of 5-5.5 mg molybdenum/kg feed to broilers as this level improves weight gain and feed efficiency in the animals.

Davies et al., 1960, tested from 100 to 8,000 mg Mo/kg given as sodium molybdate in the diet of chicks. Each group contained from 18 to 20 birds. A slightly decreased weight gain started to appear first at 500 mg/kg diet, but became more severe at higher doses and at 8,000 mg/kg, the 4 week weight was only 1:6 of that of the controls. Mortality was inconsistent at levels over 500 mg/kg (some of the groups had a slight mortality that was not correlated to the dose-level), but 6,000 and 8,000 mg/kg gave rise to a mortality of 33 and 61% respectively. Hemoglobin was slightly increased at levels up to 2,000 mg/kg, but still higher levels produced anemia.

Lepore and Miller, 1965, tested the egg production and embryonic viability in hens after the supplement of 0, 500, 1,000 or 2,000 mg Mo/kg diet added as sodium molybdate. Each group contained 6 animals. The animals laid 15% fewer eggs than controls when fed a diet containing 500 mg Mo/kg. The decreases at 1,000 and 2,000 mg Mo/kg were 50% and 80% respectively. The feeding of 500 mg Mo/kg diet resulted in an egg concentration of 16-20 mg Mo/kg, this concentration leading to the death of all embryos.

6.3.3.1.2 Experiments with cattle, trials to produce "artificial teartness"

As already commented upon in 6.1, Ferguson, Lewis and Watson, 1943, tested whether molybdenum could be a cause of "teart" by feeding molybdic salts to experimental animals. Serious diarrhea in three out of four cows arose in 5-10 days on non-teart pastures, by feeding the animals 1,710 mg of sodium molybdate daily. This dose contained 680 mg of molybdenum, according to the authors corresponding to 50 mg Mo/kg in the dry matter of a normal daily ration on pasture. These cows were brought up on "teart-land" which, according to the authors, makes animals weaker and more susceptible to molybdenum poisoning. Cattle on winter rations, and cattle brought up on sound land required higher doses (approximately 200 mg/kg dry matter) and some animals did not react even to these doses.

Britton and Goss, 1946, referred to in 6.1, gave 5,000 mg sodium molybdate daily to a heifer calf (6 months old) for 7 months. In 6 weeks the animal was thin, rough-coated and grey in color. The animal also became increasingly emaciated. At the end of the trial the weight was 355 pounds, compared to a control calf weighing 490 pounds. In spite of the demonstrated toxic signs, the

authors did not note any diarrhea or anemia. A month after the trial, the test calf was recovering rapidly.

Cook et al., 1966, treated 24 yearling steers with 0 mg (control), 1.5 mg (low level) and 3.0 mg (high level) of molybdenum per kg body weight daily. The weight of the animals was approximately 264 kg, and molybdenum was administered as sodium molybdate in gelatine capsules. The total daily amount of molybdenum would be, according to these figures, approximately 400 and 800 mg respectively, i.e. close to the amounts tested by Ferguson, Lewis and Watson, 1943. The experiment was planned for 150 days, but after 100 days three animals receiving the high level of molybdenum had died, making the authors reduce the daily intake to 1.5 mg per kg body weight for the remaining test animals for the final 50 days. Profuse diarrhea occurred within 2 weeks in most animals and was most pronounced in the high level animals. At 100 days the control animals had gained an average of 81 kg, whereas the low and high level groups lost 24 and 69 kg respectively. These authors also noted anemia in several animals.

Huber, Price and Engel, 1971, studied molybdenum toxicity in lactating dairy cows fed a basal ration containing 6 mg Cu/kg. Overt symptoms of molybdenum toxicity (diarrhea, "inanition" etc) were observed in three cows consuming a diet with 173-200 mg Mo/kg given as sodium molybdate. A diet containing 53-100 mg Mo/kg (trial period 6 months) failed to produce any symptoms in this study.

6.3.3.2 Repeated exposure through inhalation, common symptoms and effects on lungs

Groups of guinea pigs were exposed to the dusts (particle size not given) of molybdenite, calcium molybdate and molybdenum trioxide respectively one hour per day 5 times

per week (Fairhall et al., 1945, as referred to in 6.3.1 and 6.3.3.1). Twenty-five guinea pigs were exposed to molybdenite dust (average molybdenum content 286 mg/m^3). With the exception of one animal, dying after only 3 days, the appearance of these animals was normal (tissues levels were extremely low, see section 4.3). Fifty-one animals were exposed to an average of 205 mg Mo/m^3 as molybdenum trioxide. This exposure led to loss of appetite, loss of weight, diarrhea, muscular incoordination, loss of hair and a mortality rate of 50% during the experimental period. Upon histopathological examination the lungs showed a small to a moderate amount of alveolar and bronchial exsudate, but no other significant findings. The calcium molybdate dust was administered to 24 animals in an average concentration of 160 mg Mo/m^3 and led to a 20% mortality but "no other toxic signs" were evident. The same model of administration was used in another experment, exposing 12 guinea pigs to the fume of molybdenum trioxide 5 days/week for 5 weeks. Two levels of concentrations were used, 191 and 53 mg Mo/m^3. Only one animal given the high concentration died, and no further evidence of toxicity was apparent. As the fume generally is more toxic than the dust a more pronounced toxic effect had been anticipated.

Mogilevskaja, 1963, exposed rats to dusts of metallic molybdenum, 12-15 g/m^3, molybdenum dioxide 8-10 g/m^3, molybdenum trioxide 8-10 g/m^3 and ammonium paramolybdate 0.5-2.5 g/m^3 one hour daily for 30 days. With the exception of one death and slight growth depression the rats exposed to metallic molybdenum and molybdenum dioxide appeared normal during the exposure period. In lungs there was a considerable deposit of dust with thickening of the intra-alveolar septa, which contained connective tissue fibers. The toxic effects of molybdenum trioxide were, however, more pronounced. Weight gain in this group was only 8.6 g on the average, compared to 31 g in a control group. The

lungs showed macroscopical hemorrhages, marked perivascular edema, and large areas of hemorrhages into the alveolar spaces. Mortality rate was not mentioned. The exposure to ammonium paramolybdate showed the most marked toxic effects, a 100% mortality and more serious effects of the same type as described for molybdenum dioxide.

In another experiment by Mogilevskaja, 1963, rats were exposed to a condensation aerosol of molybdenum trioxide for 2 hours "on alternate days" for 2 months at a concentration of 3-10 mg/m^3. 90% of the particles were smaller than 1 u. During this entire period, no changes in the condition of the animals were noted. The lungs showed thickening of the alveolar walls, interstitial pneumonia and areas of collapse and emphysema.

Dzukaev, 1970, demonstrated a "slight diffuse pneumoconiosis" in rabbits 9 months after a single intratracheal administration of powdered molybdenum at 70-80 mg/kg. When 40 mg of molybdenum were administered a second time 6 months later, note was made of an intensified inflammatory process in the interstitial tissue resulting in a "vague pneumosclerosis and symptoms of focal pneumonia without the formation of cellular-fibros foci". The changes were not visible by radiography in vivo but could be demonstrated well by radiography on isolated lungs.

Lukasev, Siskova and Knys, 1971, exposed rabbits and rats (the number is not stated) for 3.5 months, 4 hours daily, to molybdenum trioxide dust by inhalation. The concentration changed cyclically from 210 to 10 mg/m^3 within 25 minutes. The animals were divided into 3 groups (the size of groups is not given): 1) controls; 2) exposed to molybdenum trioxide and 3) exposed to molybdenum trioxide and also given an oral dose of sodium sulfate solution (50 mg of sulfate per rabbit and 5 mg per rat). On autopsy, the animals exposed only to

molybdenum trioxide showed point hemorrhages in the lungs and in some cases polyemia. There were no changes in other organs. Microscopic examination revealed extensive incidence of hemorrhages, destruction of alveoli, emphysema, interstitial pneumonia and bronchitis. Rabbits which were treated with sodium sulfate showed interstitial pneumonia and bronchitis but no hemorrhages. In rats exposed only to molybdenum trioxide, there were no hemorrhages but interstitial pneumonia and bronchitis were well developed. Interstitial pneumonia and bronchitis were less well developed in rats treated with sulfate. The authors did not give any information on mortality or the prevalence of pathological changes. Collagen fibers were not found in either rabbits or rats. No changes were observed in the content of mucopolysacharides as compared with control animals.

6.3.3.3 Effects on blood

As is already described in 6.1, 6.3.3.1 and 6.3.3.2, anemia is a common constituent of the molybdenum toxicity syndrome in many species (cattle, sheep, rabbits, guinea pigs, rats, chicks).

Britton and Goss, 1946, referred to in 6.1 and 6.3.3.1, characterized the anemia appearing with "teart" disease in cattle as hypochromic and microcytic with a high lymphocyte and platelet count. Analysis of alfalfa from the area in question revealed a molybdenum content of 10 mg/kg.

Cunningham and Hogan, 1959, did not find hematological effects in sheep grazing on pastures providing 8 mg Mo/kg diet and 7 mg Cu/kg diet. Hogan et al., 1971, referred to in 6.1, in a study on sheep on pastures high in molybdenum (up to 20 mg/kg diet) reported, however, "significantly lower hemoglobin and hematocrit levels in those animals not receiving

copper as an antidote" but prevalence and actual values were not given.

Arrington and Davis, 1953, produced toxic symptoms in rabbits by feeding the animals sodium molybdate in doses from 1,000 mg Mo/kg diet and higher (see Table 6:10 which presents some "representative animals"). The first changes in hemoglobin and the number of red blood cells were observed at the same time as other evidence of toxicity appeared.

Anemia in rabbits has also been reported by McCarter, Riddell and Robinson, 1962. They fed the animals 2,000 or 2,340 mg sodium molybdate/kg feed for 5 weeks Hemoglobin and hematocrit was decreased to approximately 65 and 60% respectively. The anemia was characterized by the authors as a microcytic, hypochromic anemia, but detailed values are not given.

Molybdenosis was produced in 12 young rabbits by giving them 4,000 mg/kg as sodium molybdate in the diet for 4 weeks (Valli et al., 1969). Decreases in hemoglobin, hematocrit, but not in hemoglobin saturation were apparent after 11 days and were marked at the 18th day. The authors further described a marked erythroid hyperplasia of bone marrow (while the myeloid series was not so affected) developing in all test rabbits by the 11th day. In this experiment the prevalence of anemia was 100%, but neither hemoglobin nor hematocrit values were given.

Ostrom, Van Reen and Miller, 1961, referred to in 6.3.3.1, reported on anemia in 21-day-old rats fed 400 mg Mo/kg diet as sodium molybdate. Hemoglobin and hematocrit values were on an average significantly and markedly reduced in those rats that were also affected by exostoses (8.4 compared to 13.8 for controls, and 29.2 compared to 43.7 as mean values respectively). MCHC (mean corpuscular hemoglobin concentration in percent) was slightly de-

pressed, 29.5 compared to 31.5. White blood cells were not affected.

In an experiment by Gray and Daniel, 1954, hemoglobin values were only slightly depressed by the feeding of 800 mg Mo/kg diet as sodium molybdate to 21-day-old rats. The contemporaneous weight gain was less in the exposed group than in the control group. Thus, in 6 weeks the mean (5 animals) weight gain was 94 g and the mean hemoglobin value 14.28 g/100 ml while the corresponding values for a control group were 152 g and 14.49 g/100 ml. As the authors used the same molybdic salt and the same age of animals as in Ostrom, Van Reen and Miller's study, the difference in results is somewhat surprising. The difference was only affecting the blood picture as weight depression and mandibular exostoses were reported by both author groups. In Ostrom, Van Reen and Miller's study, albino rats of the NNRI-B strain were fed a "purified diet" while in the Gray and Daniel study rats of the Sprague-Dawley strain were fed a "basal diet" supplemented with copper. Either difference in strains or in dietary levels of copper "or other dietary constituents" should be responsible for this difference in result.

The existence of molybdenosis in rats without the contemporaneous existence of anemia is also described by Neilands, Strong and Elvehjem, 1948, in Sprague-Dawley rats on a purified ration which received 500, 1,000 or 5,000 mg Mo/kg diet as sodium molybdate. At all levels, depressed growth and emaciation were encountered but the blood picture was normal (hemoglobin, hematocrit, red blood cells and white blood cells). Sprague-Dawley rats also failed to develop anemia in an experiment by Johnson, Little and Bickley, 1969. The animals were fed up to 1,200 mg Mo/kg diet as sodium molybdate. The authors concluded that the hematological system of this rat strain is likely to be resistent to molybdenum intoxication.

The study by Davies et al., 1960, on chicks indicates a strong resistance of this species to high dietary levels of molybdenum. First at 4,000-6,000 mg Mo/kg diet were changes in blood pictures apparent.

6.3.3.4 Effects on bone and connective tissue

Osteolathyrism is a disease caused by ingestion of seeds from the sweet pea (Lathyrus odoratus). The disease can easily be experimentally reproduced by feeding the sweet pea or its toxic compound β-aminopropionitrile (BAPN), to animals. Its histological features are recaptured by Gardner, Dasler and Weinmann, 1958, and it is evident that the disease has many characteristics in common with the bone disorders in molybdenosis (disorders of epiphysis of long bones, periostal new bone formation at the sites of muscle attachments, loosening and detachment of tendonous insertions, tearing of the periostem etc.). It also, to some extent, resembles certain disorders in the bone tissue of man (osteogenesis imperfecta, osteopetrosis, Pagets disease, Perthes disease, Osgood-Schlatter disease).

Hogan et al., 1971, reported on connective tissue lesions in 4 out of 9 sheep on high molybdenum pastures (up to 20 mg/kg dry matter, corresponding copper values were 5-7 mg/kg ppm dry matter). One animal showed rib fractures and a transverse fracture of the humerus. Another one demonstrated "stripping" of 3 muscle insertions together with periost of the right humerus. A third demonstrated the same type of lesions on both humeri plus a fracture of the left femur. The fourth of the affected animals exhibited "stripping" at the same site as the secondly mentioned sheep.

Ostrom, Van Reen and Miller, 1961, demonstrated mandibular exostoses in 10 rats out of 20 fed 400 mg Mo/kg diet per day as sodium molybdate. At the start of the experiment,

the animals were 31 days of age, and the duration of the experiment was 5 weeks. The exostoses were situated at insertion sites of muscles and were according to the authors histologically resembling "osteolathyrism".

Lalich, Groupner and Jolin, 1965, fed rats 1,000, 1,500 and 2,000 mg of sodium molybdate per kg feed. The authors used young animals, 39-45 g, and checked the body weight every other day. If the rats stopped eating and lost weight, the diet was supplemented with 0.5 mg of copper sulfate per day for 1-3 days, but the experiment was continued for 6 weeks whereafter the animals were killed and autopsied. Twenty-seven of 33 rats developed deformities of the long bones, and femur, tibia and humerus were equally affected. The changes consisted of shortening of bones and increase of shaft diameter, enlargement of femoral condyles, and the tibial head, and gross deformities of knee joints. Several rats demonstrated palpable mandibular exostoses. At microscopical examination it was found that the greatest deformities occurred at the ends of the long bones, particularly the epiphyseal plates (widening of the plates and distortion of the chondroblastic alignment). In contrast to the alterations observed in the epiphyseal plates, only relatively minor changes were encountered in the trabeculae, and cortical bone. Some rats were given copper sulfate or copper carbonate from the start of the experiment, and none of these animals developed any bone disorders.

In the experiments on rabbits performed by Arrington and Davis, 1953, McCarter, Riddell and Robinson, 1962, and Valli et al., 1969, commented upon in Chapter 6.3.3.1 and 6.3.3.3, bone disorders appeared together with the other symptoms. In the first study "front leg abnormalities", i.e. deformities in joints and in one case twisting of humerus, developed in 7 weanling animals

out of 22 (1,000 mg Mo/kg diet and more were required to produce the symptoms). Five of 7 test animals that were X-rayed demonstrated epiphyseal line fractures and epiphyseal plate widening in the study by McCarter, Riddell and Robinson, 1962, and this finding was confirmed by Valli et al., 1969. In this study 6 out of 12 animals fed 4,000 mg Mo/kg diet as sodium molybdate for 4 weeks demonstrated intra-cartilaginous epiphyseal fractures in the humeri. The femoral epiphyseal plates were increased because of widening of the zone of chondrycytes. The latter authors compared the pathomorphology with "osteolathyrism", suggesting an interference with the same "metabolic pathway" in the two disorders.

6.3.3.5 Effects on liver

Fatty changes in the liver are a common result in histopathological studies on experimental molybdenosis. They have been reported by Fairhall et al., 1945 (25 mg and more per day of molybdenum dioxide orally to guinea pigs for 14 days); Asmangulyan, 1965 (50 mg/kg body weight per day, of ammonium molybdate orally to rabbits for 6 months); Rokicka, 1969 (120 mg molybdenum/kg/day as ammonium molybdate intraperitoneally to rats for 30 days); Mogilevskaja, 1963 (inhalation by rats of an aerosol of molybdenum trioxide in a concentration of 3-10 mg/m^3 on alternate days for 2 hours/day for 2 months); Valjcuk and Sromko, 1973 (5 mg/kg body weight/day as ammonium molybdate orally to rabbits for one year). Both Fairhall et al., 1945, and Mogilevskaja, 1963, also noted necrotic foci in the liver.

Grigorajan and Brutajan, 1968, reported significantly reduced cholesterol levels and increased bilirubin levels in sheep given large doses (>1.5 g) which generally produced molybdenosis.

Liver function was also studied by Avakajan, 1966b, on 6 dogs with 4 controls; 4 dogs were fed from 500 mg (20 mg/kg body weight) to 4,700 mg ammonium molybdate in their diets for 5.5 months; 2 dogs received the same doses of sodium molybdate for 3 months. The increase of bilirubin in blood serum of dogs exposed to ammonium molybdate was highly significant. The mean ± S.E. was for all dogs prior to the experiment 3 $\pm$ 0.28 ug/ml. At the end of the experiment, the exposed group showed values of 5.4 $\pm$ 0.29 ug/ml and the control group 3.1 $\pm$ 0.12 ug/ml. The same effect was seen on dogs exposed to sodium molybdate. At high doses (1,500 to 4,700 mg), serum cholesterol was significantly increased (220 mg per 100 ml as compared to 114 mg/100 ml in the controls), and esterified cholesterol reduced to 51%. Total serum proteins were somewhat increased and the albumin/globulin ratio significantly decreased (0.92 to 0.71).

6.3.3.6 Effects on kidney

Histopathological changes in kidney in experimental molybdenosis, mainly described as only "dystrophic changes" or "swelling" of cells are described by the following: Fairhall et al., 1945 (25-200 mg/animal per day of molybdenum trioxide orally to guinea pigs for 14 days); Mogilevskaja, 1963 (inhalation by rats of an aerosol of molybdenum trioxide in a concentration of 3-10 mg/m^3 2 hours/day on alternate days for 2 months); Asmangulyan, 1965 (50 mg/kg body weight/day of ammonium molybdate orally to rabbits for 6 months); Valjcuk and Sromko, 1973 (5 mg/kg body weight/day as ammonium molybdate orally to rabbits for one year); Lukasev, Siskova and Knys, 1971 (inhalation by rabbits and rats of molybdenum trioxide 4 hours/day for 3.5 months. Concentration of dust was changed cyclically between 210 and 10 mg/m^3). In the latter study the findings were described as "atrophy" of renal tubules. The number of animals and prevalence of pathomorphological changes were not given.

Grigorajan and Tatevosajan-Makarajan, 1970, exposed 4 groups of 10 rats to sodium molybdate by stomach tube (0.025 mg Mo/kg; 1 mg Mo/kg; 20 mg Mo/kg and 100 mg Mo/kg body weight). The first 3 groups received molybdenum each day for 150 days; the fourth group only 30 days as the large dose killed the animals rapidly. Animals were kept in metabolic cages to obtain 24 hours urine. The authors examined some physicochemical properties of urine, the presence of proteins, blood and sugar; and changes in weight and micromorphology of kidneys. Small doses of molybdenum (0.025 and 1 mg/kg) had no effect on renal function. High doses (20 and 100 mg/kg) impaired renal function.

6.3.3.7 Myocardial effects

Fairhall et al., 1945, fed up to 200 mg/day of molybdenum trioxide to guinea pigs for 14 days and did not find any significant histopathological changes in the heart. A similar opinion was given by Birjukova, 1971, injecting 70-100 ug/kg per day subcutaneously to 15 rats for 20 days. Valli et.al., 1969, and Lukasev, Siskova and Knys, 1971, on the other hand, did observe histopathological changes in the myocardium in connection with other signs of toxicity. Andreasajan, 1968, did electrocardiographic recordings on 6 dogs fed rising doses of molybdenum starting from 500 mg (20 mg/kg body weight) and reaching 4,700 mg daily for 3-5.5 months. Four dogs were given ammonium molybdate and two sodium molybdate. The author described a series of ECG abnormalities that were noted at the beginning of the first month.

6.3.3.8 Effects on the thyroid gland

Widjajakusuma, Basrur and Robinson, 1973, studied the effect on the thyroid gland of rabbits fed a diet containing 3,000 mg Mo/kg given as sodium molybdate. The animals were examined daily for gross signs of molybdenum toxicity, and

hemoglobin and body weight were checked weekly. When molybdenosis was evident, plasma thyroxin (T_4) concentrations were significantly decreased (mean for 14 molybdenum-fed rabbits being 2.31 compared to 4.40 for 19 controls).

6.4 CONCLUSIONS

There are few reports on molybdenum toxicity in man. In industry a few cases of pneumoconiosis and hyperuricemia attributed to exposure to molybdenum have been reported. Although the data have not been possible to analyze in strict detail due to lack of information on the epidemiological methodology in the report, one study has indicated that molybdenum might be a causal factor behind hyperuricemia and an increased prevalence of gout-like symptoms in a general population in two villages in Armenia, the molybdenum content in the soil in this area being very high. Concentrations in several vegetables were considerably higher in this area than in the control area (e.g. potatoes 11 mg/kg vrs 3.3 mg/kg and beans 82 mg/kg vrs 5.1 mg/kg).

A disease termed "teart" disease has been reported in ruminants grazing on pastures with high levels of molybdenum, the "risk zones" seeming to lie somewhere between 10 and 20 mg/kg dry matter in the herbage. This disease afflicts only cattle and sheep and is not seen in horses and swine. "Teart" is characterized mainly by diarrhea, anemia and emaciation that can be so pronounced as to lead to death. The animal typically recovers rapidly once it is removed to "non-teart" pastures low in molybdenum. The disease can also be cured by the addition of higher levels of copper to the diet.

Experimentally produced molybdenosis is reported in many species (cattle, sheep, rabbit, guinea pigs, rats, hens etc.) and is in repeated exposure experiments, mainly

characterized by reduced weight gain and emaciation, an anemia resembling iron deficiency anemia, and defects in the skeletal system and connective tissues resembling "osteolathyrism". The dietary levels used in long-term experiments for rats are generally over 400 mg Mo/kg diet, this level in one experiment being reported to produce molybdenosis in 50% of the exposed animals. A 75% mortality resulted among guinea pigs fed 25 mg molybdenum per day and animal, given as molybdenum trioxide while calcium molybdate brought a 25% mortality among the same species when they were fed 200 mg molybdenum per animal and day, indicating different toxicity levels for different compounds. The same study gave evidence that only the hexavalent molybdenum compounds (soluble) are toxic while molybdenite (molybdenum disulfide) is harmless.

The metal seems to be toxic to about the same "magnitude" in the different species, but its toxic manifestations seem to differ somewhat among different animals. Thus, skeletal deformities seem to be more a constituent in the molybdenum syndrome of rabbits and rats than in the other species. Diarrhea, perhaps the most typical manifestation of molybdenosis in cattle, is only rarely described in other species.

The mode of administration in the different experiments is generally through ingestion or administration via a stomach tube. A few reports on inhalation or intratracheal administration have been published. In some instances it has been possible to produce a pneumoconiosis-like picture, especially at intratracheal administrations of high levels of metallic molybdenum or molybdenum trioxide.

TABLE 6:1 AVERAGE MOLYBDENUM CONTENTS (mg/kg dry weight) OF HERBAGE SAMPLES[x] IN RELATION TO TEART DISEASE. (From Ferguson, Lewis and Watson, 1943).

Year	Farm	Teart	Mildly Teart	Non-Teart
1937	W. Hambridge	51(5)		4 (2)
	Go. Hambridge	14(2)		5 (1)
	Gr. West Camel		14(4)	
	Co. West Camel	25(3)		12 (2)
	D. West Camel		12(2)	
	K. Butleigh	20(6)		4 (2)
	Cd. Pilton	52(2)		4 (1)
	Wh. Kingsdon	40(1)		6 (1)
	M. Kingsdon	30(1)		
	P. Babcary	30(1)		3 (2)
	H. Pylle		24(1)	
	Lu. Kingsdon		13(1)	
1938	W. Hambridge	59(4)		4(12)
	Co. West Camel	49(2)		4 (4)
	K. Butleigh	18(2)		5(12)
	Wh. Kingsdon	27(1)		4 (3)
	Arithmetic mean, 1937	33	16	5
	Arithmetic mean, 1938	38		4

[x] The figures in parentheses refer to the number of samples analyzed.

TABLE 6:2 SEASONAL VARIATION IN MOLYBDENUM CONTENT (mg/kg dry weight) OF HERBAGE AT FARM W. (From Ferguson, Lewis and Watson, 1943).

	Apr	May	June	July	Aug	Sept	Oct	Dec
1937	32	34	26	48		80		29
1938		42	49		66		79	
1939 a	49	62		53	93	66		
1939 b	45	66		87	108	102		
1940	74		62		68			

TABLE 6:3 URIC ACID IN BLOOD AND URINE IN SELECTED PERSONS[x] WITH AND WITHOUT A GOUT-LIKE DISEASE IN A MOLYBDENUM AREA AND IN A CONTROL AREA. (Modified from Kovalskii, Yarovaya and Shmavonyan, 1961).

Area	Status of subjects	No. of subjects	Statistic	Blood mg/100 ml	No. of subjects	Urine mg/day
Molybdenum province	Ill	17	M_1	8.1	17	824
			s_1	1.8		285
			m_1	0.4		67
Molybdenum province	Healthy	35	M_2	5.3	34	649
			s_2	2.1		234
			m_2	0.4		40
			t (1-2)	5.1		2.2
Molybdenum province	Ill + Healthy	52	M_3	6.2	51	707
			s_3	2.3		247
			m_3	0.3		42
Control area	Controls	5	M_4	3.8	5	432
			s_4	1.0		89
			m_4	0.5		40
			t (2-4)	2.6		3.9
			t (3-4)	4.4		4.7

Note: M = arithmetic mean, m = standard error, s = standard deviation, t = t-test

x) Not all subjects with the gout-like disease are included in the table. It is not known on what basis the ones who are included were selected from the total group of 71 with clinically overt gout.

TABLE 6:4 CONCENTRATIONS OF MOLYBDENUM AND COPPER IN BLOOD AND URINE IN SELECTED[x)] PERSONS WITH AND WITHOUT A GOUT-LIKE DISEASE IN A MOLYBDENUM AREA AND IN A CONTROL AREA. (Modified from Kovalskii, Yarovaya and Shmavonyan, 1961).

			Blood			Urine		
Area	Status of subjects	Statistic	No. of subjects	Mo ug/ml	Cu ug/ml	No. of subjects	Mo ug/l	Cu ug/l
Molybdenum province	Ill	M	16	0.31	1.13	6	290	3,040
		s_1		0.08	0.25		20	140
		m_1		0.02	0.06		10	60
Molybdenum province	Healthy	M_2	31	0.17	1.38	11	280	1,840
		s_2		0.08	0.26		40	300
		m_2		0.01	0.05		10	90
		t(1-2)		6.4	3.1		0.7	12.0
Molybdenum province	Ill + healthy	M_3	47	0.22	1.29	17	280	2,260
		s_3		0.10	0.30		30	320
		m_3		0.01	0.04		10	80
Control area	Controls	M_4	5	0.06	1.83	4	160	1,200
		s_4		0.02	0.20		30	240
		m_4		0.01	0.09		20	120
		t(2-4)		7.1	4.5		5.5	4.6
		t(3-4)		11.4	5.5		5.5	7.6

Note: M = arithmetic mean, m = standard error, s = standard deviation, t = t-test

x) Not all subjects with the gout-like disease are included in the table. It is not known on what basis the ones who are included were selected from the total group of 71 with clinically overt gout.

TABLE 6:5 CONCENTRATIONS OF MOLYBDENUM AND COPPER IN SOME FOOD PRODUCTS IN A MOLYBDENUM-RICH AREA AND IN A NORMAL AREA. (Modified from Kovalskii, Yarovaya and Shmavonyan, 1961).

Food products x)	Molybdenum-rich area		Normal area	
	ug Mo/kg	ug Cu/kg	ug Mo/kg	ug Cu/kg
potatoes	11,000	3,200	3,300	7,000
cabbage	5,200	5,900	40	22,000
tomatoes	8,500	29,000	2,700	17,000
beans	82,000	17,000	5,100	14,000
bread, brown	280	740	410	5,900
bread, white	510	2,100	390	2,800
cow's milk	280	190	47	290
beef liver	340	2,100	98	11,000
beef	59	400	12	820
mutton	34	510	10	680
eggs	8	490	3	910
drinking water	10	270	5	110

x) vegetable products in dry weight
animal products in wet weight

TABLE 6:6 PERCENT MORTALITY FOLLOWING A SINGLE INTRAPERITONEAL INJECTION OF A MOLYBDENUM COMPOUND IN GUINEA PIGS. (Modified from Fairhall et al., 1945).

Molybdenum compound	Number of animals	Amount injected (mg/kg)	Mortality (%) 4 days	4 weeks	4 months
Molybdenite	12	800	17	17	25
Molybdenum trioxide	8	400	75	75	75
Ammonium molybdate	8	80	0	0	0
Ammonium molybdate	12	800	100		
Calcium molybdate	6	400	0	0	17

TABLE 6:7 PARTICLE SIZE DISTRIBUTION OF THE DUST OF METALLIC MOLYBDENUM AND OF MOLYBDENUM COMPOUNDS USED FOR INHALATION EXPERIMENTS ON RATS. (From Mogilevskaya, 1963).

Substance	Percentage of Particles of Different Sizes				
	less than 2.5 u	2.5-5 u	5-7.5 u	7.5-10 u	more than 10 u
Metallic molybdenum	55	35	6	3	1
Brown molybdenum dioxide	26	25	22	12	15
Molybdenum trioxide	24	25	30	14	7
Ammonium paramolybdate	33	31	18	8	10

TABLE 6:8 PERCENT MORTALITY AND DISTRIBUTION OF MOLYBDENUM IN TISSUES OF RATS AFTER PROLONGED INGESTION OF A MOLYBDENUM COMPOUND. (Modified from Fairhall et al., 1945).

Compound	No. of animals	Estimated daily consumption (mg Mo)	No. of days on test	Estimated total molybdenum intake (ug)	Percent mortality	Concentration of molybdenum (ug/g wet weight)		
						Liver	Kidney	Bone
Molybdenite	8	10	44	400	0	2	4	6
Molybdenite	8	100	44	4,400	0	2	6	5
Molybdenite	8	500	44	22,000	0	1	4	6
Molybdenum trioxide	8	10	120	900	50	12	11	23
Molybdenum trioxide	10	25	137	1,800	80	8	19	25
Molybdenum trioxide	10	50	137	1,700	90	36	33	40
Molybdenum trioxide	8	100	14	1,100	100	24	14	
Molybdenum trioxide	8	500	8	3,100	100	49	39	
Calcium molybdate	10	9	137	900	50	4	12	25
Calcium molybdate	10	21	128	1,300	100	5	21	28
Calcium molybdate	10	43	137	3,200	60	9	17	34
Calcium molybdate	10	86	57	3,600	100	8	17	79
Calcium molybdate	10	430	17	4,700	100	18	35	37
Ammonium molybdate	8	10	232	2,300	25	9	36	
Ammonium molybdate	8	100	13	1,000	100	16	16	41
Ammonium molybdate	8	500	9	3,200	100	16	8	
Control (average)	48	0	137	0	15	3	6	5

TABLE 6:9 SYMPTOMS IN RABBITS FED DIETS CONTAINING DIFFERENT CONCENTRATIONS OF MOLYBDENUM AS SODIUM MOLYBDATE. (Modified from Arrington and Davis, 1953).

Concentration of Molybdenum in diet (mg/kg)	No. of animals	Deaths	Average survival time	Body weight		Anemia	Alopecia and dermatosis	Front leg abnormality
				Initial, g	Final, g			
			Weanling rabbits					
4,000	2	2	30	967	803	2	0[1]	1
2,000	5	4	44	887	739	5	4	4
1,000	5	-[2]		716	1521	4	4	2
500	5	0		700	1948	0	0	0
140	5	0		671	1917	0	0	0
Control	5	0		738	1844	0	0	0
			Mature rabbits					
4,000	2	2	51			2	0	0
2,000	3	2	54			3	2	0
1,000	2	0				1	1	0
140	2	0				0	0	0
Control	4	0				0	0	0

1) Deaths occurred early and no alopecia observed.

2) Severe toxic symptoms developed in three rabbits and death impended; copper therapy initiated to prevent death.

TABLE 6:10 HEMOGLOBIN AND RED BLOOD CELL COUNTS IN RABBITS FED DIETS CONTAINING DIFFERENT CONCENTRATIONS OF MOLYBDENUM AS SODIUM MOLYBDATE.
(Modified from Arrington and Davis, 1953).

Concentration of Molybdenum in diet (mg/kg)	Week of experiment	Hemoglobin (g/100 ml blood)	Erythrocytes (million cells/mm^3)
Control	12	13.2	6.16
Control	17	15.0	6.42
Control	17	14.5	5.79
Control	17	12.0	5.08
1,000	13	3.5	1.55
1,000	7	6.3	3.39
1,000	5	6.3	3.16
1,000	3	8.0	3.46
2,000	4	5.5	1.89
2,000	17	7.3	3.50
2,000	13	3.2	1.20
2,000	9	4.5	1.53

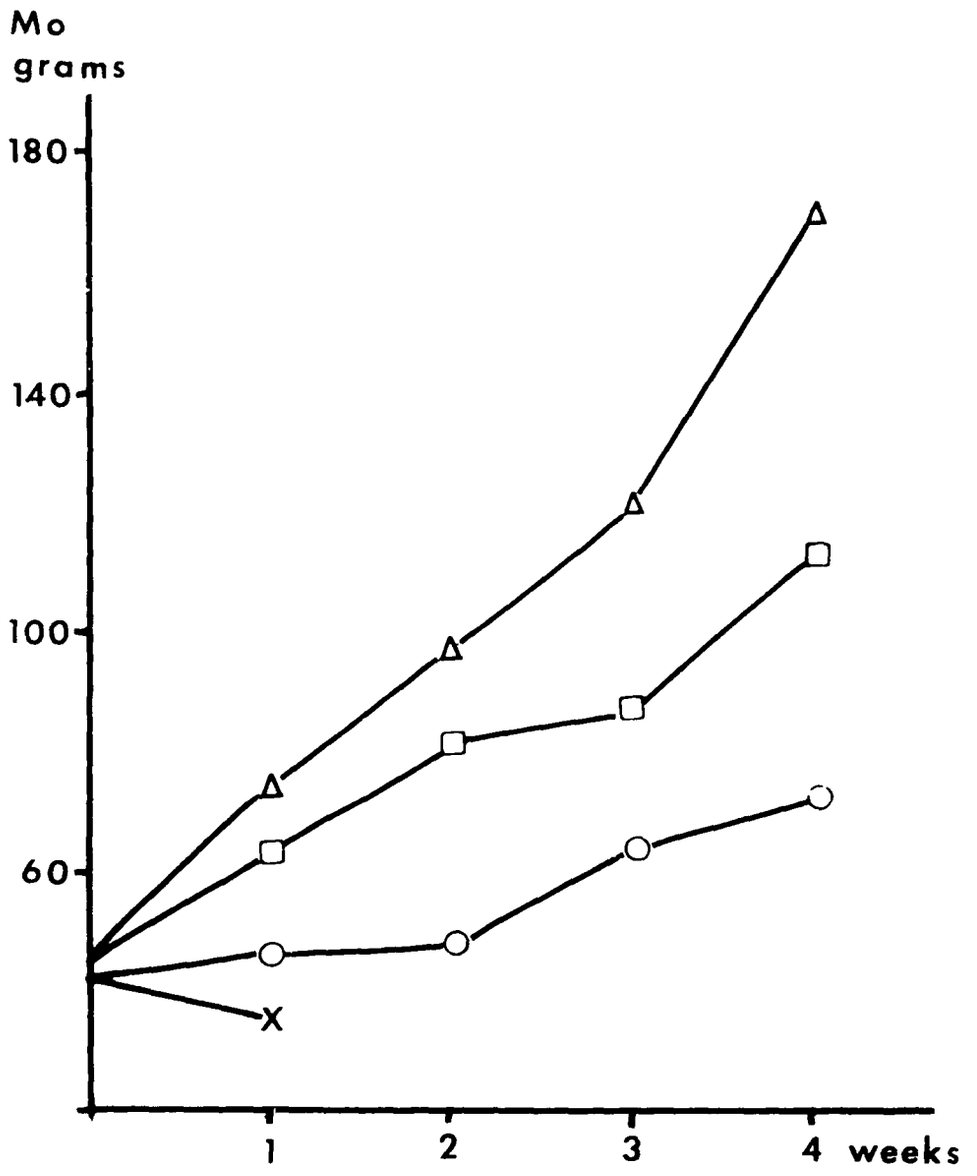

FIGURE 6:1 Growth retardation in rats fed a purified diet containing molybdenum ($Na_2MoO_4 \cdot 2H_2O$). Δ, diet without Mo(control); □, diet contained 500 mg Mo/kg; 0, diet contained 1,000 mg Mo/kg; X, diet contained 5,000 mg Mo/kg. (From Neilands, Strong and Elvehjem, 1948).

CHAPTER 7 INTERACTIONS OF MOLYBDENUM WITH COPPER, SULFATE, PURINES AND PROTEINS

7.1 INTERACTIONS WITH COPPER AND SULFATE

The toxicity of molybdenum in animals is dependent to a large extent upon the supply of other compounds. Most attention has been devoted to the influence of copper and sulfur compounds, as witnessed by the large number of reports on this subject. For penetration of certain aspects of this relationship, the reader is referred to Underwood, 1971. In most studies large amounts of molybdenum and other compounds have been given and the relevance of the findings for long-term exposure to excessive amounts of molybdenum in human beings is not known.

That the administration of copper sulfate could cure "teart" disease was shown by Ferguson, Lewis and Watson, 1943. They tried this compound as an antidote since Brouwer et al., 1938, had described a "teart-like" syndrome in copper-deficient cattle, which could be cured by copper sulfate.

Other copper compounds have also had a beneficial effect upon cattle diseases caused by excessive molybdenum, e.g. copper glycinate (Cunningham, 1957, Cook et al., 1966). Copper sulfate has been shown to be more effective than copper glycinate in curing molybdenotic heifers (Clawson et al., 1972).

Subsequent to the early experiments on cattle diseases, copper sulfate has been shown to counteract effects caused by excessive molybdenum in other species such as rats (Neilands, Strong and Elvehjem, 1948), rabbits (Arrington and Davis, 1953) and guinea pigs (Arthur, 1965).

In 1953 Dick showed that the dietary intake of sulfates other than copper sulfate was of importance for the met-

abolism and effects of molybdenum. Sheep ingesting 10 mg molybdenum per day excreted more molybdenum and had lower blood levels of molybdenum when they received hay with a high content of sulfates. Reduction in tissue levels of molybdenum was found in a later study (Dick, 1956). Dick, 1956, summarizing his studies on sheep, reported that when the intake of sulfate was low, daily doses of 0.3-100 mg for long periods had no effect on blood copper levels. In contrast, when intakes of sulfate were high, rapid rises in blood copper took place upon the administration of 60 or 90 mg of molybdenum. Even 15 mg of molybdenum had an effect on blood copper when more than 5 grams of sulfate were given. It was also shown that the rise in blood copper was wholly attributable to a rise in plasma copper. When sulfate was given in doses from 1,000 to 6,000 mg/day and molybdenum from 2.5 to 25 mg/day, it was found that decreases in levels of copper became greater with increasing sulfate intake. Thus 15 mg of molybdenum and 1,400 mg sulfate had the same effect as 5 mg molybdenum and 2,500 mg sulfate. These studies showed conclusively that effects of molybdenum on copper metabolism in sheep were intimately related to the intake of sulfates.

Throughout the subsequent years, the literature on the interrelationships of molybdenum, copper and sulfate has greatly expanded. Wynne and McClymont, 1955, reported that copper levels in liver of sheep on a low copper and molybdenum intake were not affected by the dose of 400 mg sulfate/kg diet, while 4,000 mg sulfate/kg caused lowered liver levels of copper and symptoms of copper deficiency. Similar results were obtained by Kline, Hays and Cromwell, 1971, when giving 2,200 mg sulfate/kg diet for 88 days to sheep receiving 11 mg of molybdenum and 15-33 mg of copper/kg diet. In pigs, however, the same authors did not find any changes in liver levels of copper even after prolonged exposure to high dietary levels of molybdenum (50-100 mg/kg) and sulfate (1,000-5,000 mg/kg).

Goodrich and Tillman, 1966, found that sheep given large doses of sulfate (4,000 mg/kg diet) for two months underwent a reduced weight gain when the molybdenum intake was low (2 mg/kg diet), but not when the molybdenum intake was higher (8 mg/kg diet). Marcilese et al., 1969, did not find that sulfate (4,000 mg/kg diet) had any impact upon copper metabolism in sheep on a low molybdenum intake, while 50 mg Mo/kg diet together with sulfate did produce changes in copper metabolism.

In cattle on a low intake of copper and sulfate for 300 days, liver concentrations of copper decreased when from 5 to 50 mg molybdenum/kg diet was given, but no toxic signs arose (Vanderveen and Keener, 1964). When sulfate (3,000 mg/kg diet) was given, 50 mg Mo/kg caused hair changes, which were reversed by copper. Higher molybdenum levels (100-200 mg/kg diet) together with sulfate resulted in signs of molybdenosis but liver levels of copper were not decreased. In calves, Cox et al., 1960, did not find any significant changes in liver concentrations of copper when molybdenum concentrations in the diet were 250 to 800 mg/kg.

In sheep sulfate increased the urinary excretion of molybdenum (Dick, 1953), whereas milk molybdenum levels fell when high doses of sulfate were given (Hogan and Hutchinson, 1965).

Miller, Price and Engel, 1956, gave rats molybdenum and sulfate for six weeks, the levels in food being 75-300 and 400-3,300 mg/kg respectively. Molybdenum alone caused increases in liver copper and reduced weight gains, but 800-2,200 mg/kg of sulfate alleviated both the weight reduction and the increase in liver copper at the lowest molybdenum exposures (75-100 mg/kg diet).

Johnson and Miller, 1961, found that the growth depression brought about in rats by 600 mg Mo/kg diet was partially ameliorated by 500 mg sulfate/kg diet, whereas copper had no effect.

In copper-deficient rats, Gray and Daniel, 1964, showed that small amounts of molybdenum in the diet (10 mg/kg) caused toxic symptoms, intensified when sulfate was also given. In copper-supplemented rats, larger doses of molybdenum (800 mg/kg) were needed to produce symptoms, in which cases sulfate prevented the effects of molybdenum. It was also found that methionine (1.2% in diet) prevented the copper accumulation and alleviated the symptoms caused by molybdenum, but to a lesser extent than sulfate.

Miller and Denton, 1959, showed that sulfate reduced molybdenum levels in liver of molybdenum-treated chicks. When copper was given as well, further reductions in molybdenum levels occurred. Copper supplementation did not improve growth in molybdenum-treated chicks. Molybdenum alone or together with sulfate slightly increased the liver levels of copper.

Davies et al., 1960, compared sodium sulfate to ammonium sulfate in chicks, finding that the latter compound was more toxic. Sodium sulfate promoted growth in molybdenum-treated chicks, but did not prevent accumulation of molybdenum. Methionine had no effect on the toxicity of molybdenum.

In guinea pigs, copper supplements prevented some of the drop in weight gain caused by molybdenum. Dietary levels of 200 mg Mo/kg diet caused a slight increase in liver levels of copper after 8 weeks, but higher molybdenum intake (1,000 and 2,000 mg/kg) caused reductions in liver copper.

Lukasev and Siskova, 1969, noted that ammonium paramolybdate administered orally to rabbits (5 mg Mo/kg body weight) for 5 days reduced the urinary excretion of free sulfates but increased the excretion of bound sulfates (calculated as the difference between total sulfates and free sulfates). When 50 mg/day of sulfate was given simultaneously with molybdenum, the urinary excretion of sulfates was normal. There was no effect of molybdenum on the urinary excretion of indican and thiocyanate. Lukasev and Siskova, 1971b, measured the distribution of molybdenum and copper in rabbits after inhalation exposure to molybdenum trioxide. Simultaneous exposure of rabbits to molybdenum (inhalation of MoO_3) and oral application of sodium sulfate (50 mg/day per animal) did not significantly affect the urinary excretion of molybdenum. The excretion of copper was increased following exposure to molybdenum; this effect was significantly reduced by sulfates ($P < 0.02$).

Several attempts have been made to explain the mechanisms behind the complex interrelationships of molybdenum, copper and sulfate.

To study the influence of molybdenum upon copper metabolism, Compère et al., 1965, injected ^{64}Cu as the chloride in molybdenum-treated animals and controls. Higher plasma levels of ^{64}Cu and a lowered uptake of ^{64}Cu in the liver were found in the molybdenum-treated animals compared to controls. In another group of molybdenum-treated rats, which were supplemented with copper chloride in the diet, the uptake of ^{64}Cu in the liver was higher than in controls. These results were interpreted as showing the blockage by molybdenum of copper metabolism in the liver.

Marcilese et al., 1969, gave molybdenum and sulfate in feed at concentrations of 50 and 4,000 mg/kg respectively for 120 days to a group of sheep. This group was compared

to two other groups, one a control group, and the other a group receiving 4,000 mg/kg diet of sulfate. The plasma clearance and distribution of ^{64}Cu were studied after injection of sheep's plasma incubated with the isotope. Whereas sulfate alone had no influence on either the metabolism of the radioisotope or stable copper, molybdenum together with sulfate caused considerable decreases in the uptake of ^{64}Cu in the liver and decreases in the contents of stable copper in the liver. The disappearance of ^{64}Cu from plasma was about twice as rapid in the control group as in the molybdenum-treated animals. It could also be shown that in control animals considerably more of the ^{64}Cu was in the ceruloplasmin fraction than in animals given molybdenum.

Molybdenum exposure has been shown to decrease the activity of the enzyme sulfide oxidase in the liver (Mills et al., 1958). Halverson, Phifer and Monty, 1960, found that excessive dietary cystine given rats on a high molybdate - low copper diet worsened symptoms caused by molybdenum. The toxic effects of cystine were prevented or reversed by copper. It was postulated that a decrease in sulfide oxidase activity could cause excessive amounts of sulfide in the tissues, which could interfere with copper metabolism.

Another factor could be the formation in vivo of a copper-molybdenum complex, as postulated by Dowdy and Matrone, 1968a, 1968b, and Dowdy, Kunz and Sauberlich, 1969. The copper in this complex would have low availability for the organism.

Regarding the role of sulfate, the data by Dick, 1953, 1956, suggested that sulfate mobilized stored molybdenum by interference with molybdenum transport across membranes.

7.2 INTERFERENCE WITH PURINE METABOLISM

Yarovaya, 1964, briefly reported changes of purine metabolism in humans under the conditions of high molybdenum concentrations in food products. It was established that "2-10 times higher than normal" content of molybdenum (and "relatively low copper concentrations") in daily food rations increased the concentration of molybdenum in blood approximately 4 times. This led to an increase of xanthine oxidase activity by 100%, which in turn was conjectured to result in the high uric acid content and high incidence of gout registered in these areas high in molybdenum. (Kovalskii, Yarovaya and Shmavonyan, 1961; see section 6.2).

Increased xanthine oxidase activity when molybdenum is given as a supplement in the food is confirmed by Gusev, 1969, The author studied effects of molybdenum on the purine metabolism in a chronic experiment on 50 rats. Rats were fed standard diet which included the essential elements. One group (10 animals) served as the control; the second group received the standard diet from which molybdenum and copper were excluded as far as possible. Three other groups of 10 animals received by stomach tube each day a solution of ammonium molybdate (5, 50 and 500 mg Mo/kg,[x] respectively). After six months the animals were killed and the following measurements made: xanthine oxidase in liver; uric acid in blood and urine; and ceruloplasmin in serum. The xanthine oxidase activity increased with increasing dose of molybdenum. The concentration of the uric acid in the control group was 0.95 mg/100 ml in blood and 0.71 mg/day in urine. The corresponding values in animals receiving 5, 50 and 500 ug Mo/day[x] were 1.26, 2.29 and 2.27 mg/100 ml in blood, and 0.88 mg, 1.04 mg and 0.87 mg/day in urine.

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[x] There is some discrepancy here due to the uncertainty as to how to interpret the U.S.S.R. units expressed as MKG.

Kovalsky and Vorotnitskaya, 1970, found two peaks of xanthine oxidase activity, and consequently of uric acid levels, in liver and kidney of rats at different "Cu/Mo-ratios" in the diet. One peak appeared at a Cu/Mo ratio of 1.6 (Cu/Mo = 80 ug/50 ug per day, copper given as copper sulfate and molybdenum as ammonium molybdate) and one at a Cu/Mo ratio of 16 (Cu/Mo = 320 ug/20 ug). In both instances xanthine oxidase activity was approximately doubled compared to controls (Cu/Mo = 4) but the ratio of 1.6 (relatively high molybdenum) produced a higher peak of uric acid than the ratio of 16 (relatively high copper). The two peaks made the authors suggest two different xanthine oxidase enzymes, one containing molybdenum and one containing copper. The reason for the higher uric acid response in the high molybdenum case is not clear but xanthine oxidase induction is suggested as the reason for the higher prevalence of gout in a molybdenum-endemic area (see section 6.2).

7.3 INTERFERENCE WITH PROTEIN METABOLISM, STUDIES ON ENZYME IMPAIRMENT

Mills et al., 1958, studied some enzyme systems in rats suffering from molybdenosis induced by feeding the animals 800 mg/kg diet of Mo and/or 0.29% sulfate, these elements being supplied as sodium molybdate and sodium sulfate, respectively. The molybdenum supplement resulted in a 36% depression of growth in a five week period and the inclusion of sulfate largely prevented this effect on growth. After this period the rats were sacrificed and their enzyme activities in liver and kidney were measured and compared to controls. It was found that the enzyme sulfide oxidase was depressed to about 56% of normal values in livers. Further, alkaline phosphatase was elevated in liver and depressed in kidney. The latter changes were prevented by the sulfate supplement. No effect was reported on the levels of activity of cysteine desulfhy-

drase and aryl sulfatase or on the rate of oxidation of cysteine sulfinic acid in tissue homogenates. Thus the authors failed to detect a dysfunction of sulfur metabolism which could account for the protective effect of sulfate against molybdenum toxicity. Further the significance of the determined effects of sulfide oxidase and alkaline phosphatase was discussed in terms of a possible impairment of protein metabolism. Indications of metabolic protein derangement were found in measurement of protein/DNA and protein/RNA ratios in liver and kidneys, but the possible linkage between the anticipated protein derangement and the changes in enzymatic impairment remains to be further explored.

Johnson and Miller, 1961, 1963, confirmed the concept of impairment by molybdenum of protein metabolism and alkaline phosphatase activity in rats. These authors found a depression of femur alkaline phosphatase activity and also reported an alleviation of the molybdenum-induced enzyme depression by added dietary sulfate. Through controlled feed intake studies it was shown that feed restrictions leading to a growth depression of the same magnitude as the molybdenum supplementation to "ad libitum" fed animals led to an equivalent depression of alkaline phosphatase activity. Thus, the authors concluded that the decrease in enzyme activity induced by molybdenum feeding was the result of growth depression and not of the ingestion of molybdenum per se. (Basal ration restriction to about 62% of the rations of "ad libitum" fed animals resulted in a growth depression equivalent to the one achieved by molybdenum supplement in the diet of 600 mg/kg given as sodium molybdate). Impairment of protein metabolism was established through an increased excretion in urine of nitrogen (47.9% of consumed nitrogen compared to 29.5% for controls) and amino acids by rats fed 400 mg/kg diet of molybdenum given as sodium molybdate.

A complicated derangement of the amino acid levels in blood of rabbits fed 25-1000 ug Mo as ammonium molybdate was reported by Val'chuk and and Koval'skaya, 1970.

Lukasev and Siskova, 1971a, found that an oral daily dose of 5 mg Mo/kg body weight (as ammonium molybdate) for seven months increased significantly the total amino acids (paper chromatography) in the liver and the kidneys. The comparison was made with a control group fed only the standard diet and another experimental group receiving the same dose of molybdenum and 50 mg/kg per day of sulfate. The addition of sulfate had no significant effect. When the molybdenum dose was reduced to 0.5 mg/kg daily and given for 4.5 months, the urinary excretion of amino acids first decreased and then increased again. There was no change in the amino acid content in serum and organs. The effect of sulfate (50 mg/kg) showed up only at the end of the experiment (increased urinary excretion of amino acids as compared to the control and molybdenum group). The distribution of individual amino acid content in the serum, liver and kidneys was changed in both groups receiving molybdenum (0.5 and 5 mg/kg). Sulfates had small influence.

Avakajan, 1966b, gave dogs (4 experimental and 2 control animals) ammonium molybdate daily in increasing oral doses from 500 mg (20 mg/kg body weight) to 4.7 g for 5.5 months, and measured cholinesterase activity in serum; thiol groups in serum, liver, spleen, kidney, lungs, heart and brain tissue; and molybdenum and copper in blood, liver, heart, spleen, brain, kidneys and lungs. Molybdenum in doses from 500 mg to 1.5 g in 1.5 months increased the activity of cholinesterase and the concentration of thiol groups in serum. Higher doses given for 4.5 months resulted in a decrease of both the cholinesterase activity and the con-

centration of thiol groups in serum and in organ homogenates.

In order to study the mechanism behind bone disorders of animals fed a high molydenum intake, Feaster and Davies, 1959, fed 2,000 mg/kg of molybdenum as sodium molybdate to rabbits and studied the resultant effect on the Ca-P metabolism of the animals. After having been fed the diet for five weeks (the animals showed the symptoms described in Chapter 6 including deformity of the joints, anemia and diarrhea) they received a single oral dose containing 3 uCi each of ^{45}Ca and ^{32}P and were killed at different intervals after 6 hours to 14 days. Neither soft tissue levels nor urinary or fecal excretion of total or radioactive Ca or P indicated any impairment in the absorption of these ions from the intestine. Further, there were no indications of any derangement of bone metabolism of Ca and P. The bones of the experimental animals did weigh significantly less than those of the controls, indicating a decrease of "organic matter" rather than "demineralization" as a causal factor behind the molybdenum-bone disorders observed in the animals.

7.4 SUMMARY

There is a complex relationship between molybdenum, copper, sulfate and probably also some other sulfur compounds. Species differences exist, e.g. sheep are more susceptible to imbalances between the elements than pigs. Copper generally has a beneficial effect on the symptoms caused by excessive molybdenum but the action of sulfur compounds, especially sulfate, is not so clearly understood, both positive and negative effects having been reported, depending upon the copper status. The association between molybdenum exposure, hyperuricemia and gout-like symptoms reported in Chapter 6 could find an explanation in terms of an impaired purine metabolism through an enhancement

of xanthine oxidase activity by molybdenum. Moreover, there are indications of an interference with other enzymes as sulfide oxidase and alkaline phosphatase.

CHAPTER 8 CONCLUSIONS AND DISCUSSION CONCERNING POSSIBLE HEALTH EFFECTS OF MOLYBDENUM ON HUMAN BEINGS

Molybdenum is considered to be an essential element. It is a constituent of some enzymes including xanthine oxidase which oxidizes xanthine or hypoxanthine to uric acid. Although there are no minimum requirement data for human beings, information from other mammals tends to show that the necessary amount of molybdenum is very small. Therefore, the concentration of molybdenum in a normal diet with all probability will be sufficient for maintaining physiological levels of the metal in humans.

Despite the fact that human beings are exposed to relatively high amounts of molybdenum (100-500 ug/day), primarily via food, there is no substantial age-related accumulation of molybdenum in the body. Concentrations in those organs in which molybdenum is mainly found, i.e. liver, kidney and bones, are relatively low, in the order of below 1 ug/g wet weight. Molybdenum occurring naturally in the diet is absorbed to a high degree from the gastrointestinal tract (probably 25-75%) but is eliminated rapidly, primarily via the urine. The biological half-time is not well established in humans but the major part of the absorbed molybdenum is eliminated within days or, at a maximum, a few weeks.

Animal data show that the metabolism of molybdenum is closely related to the metabolism of copper and sulfur compounds.

Most observations on the toxicity of molybdenum are based on data from animals. It is clearly shown that an excessive exposure to molybdenum via food can give

rise to a severe disease in ruminants which involves diarrhea, anemia and emaciation and may progress to death. The disease may be prevented or cured by the administration of copper compounds or removal of animals from the areas high in molybdenum. The disease is termed teart disease and has been possible to reproduce experimentally. It has also been possible to show toxic effects resembling teart disease in several of the common laboratory animals. In addition, several forms of bone deformities, including exostoses, have been induced. The doses required are excessive and much higher than those to which human beings eating a normal diet may be expected to be exposed, even in molybdenum-rich areas. In several studies 200-400 mg of soluble hexavalent molybdenum compounds per kg diet were required over prolonged periods.

Information into possible toxic effects in human beings is scarce. There are data from the U.S.S.R. literature pointing towards the possibility that exposure to some molybdenum dusts may give rise to pulmonary disorders in the form of pneumoconiosis. Similar effects have been reproduced in animals. No conclusions concerning the necessary exposures to give rise to this condition in humans can be drawn at this time.

There have also been reports on effects of molybdenum on purine metabolism. It has been shown in studies from the U.S.S.R. that people exposed to molybdenum (concentration not known) in industry have increased levels of uric acid in urine and in blood. Moreover, a report from the U.S.S.R. from 1961 has indicated that people living in an area of that country with high dietary intake of molybdenum (by the authors calculated to 10-15 mg/day as compared to 1-2 mg in the control area) show an increased prevalenceof joint-disorders claimed to be gout-like as well as an increase in blood levels of uric acid and an

increase in the urinary excretion of uric acid. The reason for the high daily intake value in the control area is not known. The results are very difficult to evaluate since no details are given concerning the study population and the selection of subjects; furthermore, the control group consisted of only five subjects. Nevertheless, the data fully motivate further studies, not only in the U.S.S.R. but also in regions of other countries where the exposure to molybdenum via diet is high, one reason being that from the theoretical standpoint, effects like those reported might well appear. Thus, molybdenum exposure could give rise to an increase in xanthine oxidase activity which in turn should give rise to an increase in uric acid formation. The reason that effects have not been reported from other parts of the world may be a lack of studies focusing on these effects. Target groups of special importance are 1) persons living in areas with a high molybdenum content in food, particularly in such areas in which the inhabitants are dependent upon locally produced food; 2) persons from non-contamined areas with consumption habits favoring a high molybdenum intake and an imbalance in relation to other dietary constituents of importance, e.g. copper.

Explanatory note: The spelling of the names of the U.S.S.R. authors does not follow any one exact system. Whenever reports from the U.S.S.R. have been published in an English-language journal or book, or quoted secondarily in such a work, the spelling has been taken just as it appeared in the English publication. In the reference list, such papers are treated just as any other report originally in English. No comment is made as to the fact that the authors are from the U.S.S.R. As regards individual translations or standard translations such as the one of the U.S.S.R. journal Gigiena i Sanitariya (Hygiene and Sanitation, published for the Environmental Protection Agency through the National Science Foundation, Washington, D.C., by the Israel Program for Scientific Translation, Jerusalem), the names of the authors are spelled according to the conventions used by the translator. In the reference list, the fact that the original publication was in the U.S.S.R. language will be noted as well as the availability of a translation. A third case refers to works read by Dr. Vouk and for which no translation is known to us. These names have been transliterated by Dr. Vouk according to the International System for the Translation of Cyrillic Characters. In the reference list the note "In Russian" will follow all such reports.

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16. ABSTRACT

This review presents and evaluates the available material on the metabolism and toxicology of molybdenum with emphasis on those aspects of relevance to human beings. Agricultural and environmental aspects of molybdenum are less completely covered. Special efforts have been made to include recent publications in Russian, and to coordinate this work with a similar effort under way in the Environmental Health Criteria Programme of the World Health Organization.

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