# THE EFFECT OF DIETARY MOLYBDENUM UPON THE UTILIZATION OF NUTRIENTS BY THE RAT

by

Herman L. Johnson, B. A., M. S.

Thesis submitted to the Graduate Faculty of the
Virginia Polytechnic Institute
in candidacy for the degree of

DOCTOR OF PHILOSOPHY

in

Biochemistry and Nutrition

May 15, 1963

Blacksburg, Virginia

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# I. INTRODUCTION

The effect of molybdenum toxicity in animals has been reviewed recently (1, 2, 3). Generally, the symptoms of a chronic molybdenum toxicity resemble those of a copper deficiency and are; namely, diarrhea, anemia, emaciation, weakness, alopecia and decolorization of the hair. Considerable species differences, both in dietary levels of molybdenum necessary to produce a toxicity and in the syndrome, have been observed. Briefly, the ruminants appear most susceptible to molybdenosis; next are the monogastric animals; and aves appear most tolerant. Other dietary factors, such as protein, copper and sulfate levels, influence the animal's response to high levels of molybdenum. The practical problem of molybdenosis in cattle and sheep has been eliminated through the dietary supplementation of copper and/or sulfate; however, the mechanism of molybdenum toxicity remains as an intriguing problem of academic interest.

The major portion of this dissertation will be essentially the same as a manuscript to be submitted to the Journal of Nutrition. The dissertation will be extended to include data and discussion which were not considered pertinent for journal publication and will also include hypotheses regarding the mechanism of molybdenum toxicity which could not be included in the publication due to the lack of sufficient experimental evidence.

A marked diminution of nutrient utilization by the molybdenum-fed rat has been reported by Johnson and Miller (4) using pair-fed and restricted-fed rats as controls. Since there was a large biological variation in the susceptibility of indivudual rats to the same level of dietary molybdenum, no statistical significance could be obtained for the trend for better weight gain of rats fed the basal diets in amounts equal to that consumed by the molybdenum-fed rats. Including a group of rats fed the basal diet in restricted amounts so that their weight gain was parallel to that of rats consuming the basal diet plus 600 ppm molybdenum revealed that the difference in food consumption between the two groups of rats was highly significant.

Monty and Click (5) have demonstrated that the rat could detect which diet contained added molybdenum and, when given a choice, would consume the diet free of added molybdenum. These workers attributed the depressed weight gain of molybdenum-fed rats to diminished food consumption.

The effect of molybdenum upon the rat is influenced by several different factors and some of these are included here. Miller and Price (6) observed that the growth depression in rats fed diets containing 100 ppm added molybdenum decreased as the dietary casein was increased from 8 to 18%. Gray and Daniel (7) reported that 1.2% dietary D,L-methionine alleviated the growth depressing effects of 800 ppm molybdenum and also increased the efficiency of feed utilization. They reported the same effects from the addition of 300 ppm added

copper as $CuSO_4 \cdot 5H_2O$ to their molybdenum diets. In this laboratory (4), the addition of 50 ppm copper as $CuCl_2$ to the diets containing 600 ppm molybdenum did not improve growth, while 250 to 500 ppm added dietary sulfate, as equimolar $K_2SO_4$ and $Na_2SO_4$, reversed 40 to 50% of the molybdenum-induced growth depression. From these data, it appears that the effects which Gray and Daniel attributed to the added copper may have been due to the added sulfate (about 450 ppm). Brinkman (8) observed that the age and nutritional status of the rat had a pronounced influence upon molybdenosis. Weanling rats were very susceptible to molybdenosis, but with only one week's growth beyond weaning tolerance to the same dietary level of molybdenum had greatly increased. Large variations within the same strain of weanling rats toward the same dietary level of molybdenum were attributed to differences in the nutritional status of the rats when received; i.e. liver copper stores of one strain varied almost 10-fold between shipments.

The observations that molybdenum depressed nutrient utilization for weight gain and that increased dietary levels of casein or methionine alleviated the growth depression of molybdenum-fed rats indicated that molybdenum may influence nitrogen metabolism. The following experiments were designed to observe if nitrogen metabolism was altered in molybdenosis of the rat and, if so, what alterations had occurred.

## II. METHODS

Male weanling albino rats derived from the Sprague-Dawley strain[1] were individually housed in stainless-steel metabolism cages constructed so that the urine and feces were collected separately. The rats were randomly assigned to treatment lots. In each of Experiments One, Two, and Three, four treatments (Lots A through D, respectively) were used: four rats were fed the basal diet ad libitum; five rats were fed ad libitum the basal diet containing added molybdenum (600 ppm in Experiment One and 400 ppm in Experiments Two and Three); each of five rats was paired to a rat in Lot B and was fed the same amount of the basal diet daily as its mate consumed during the previous 24 hours; and four rats were fed the basal diet restricted in amounts so that their average weight gain paralleled the average gain of the rats in Lot B. In Experiment Four, two treatments of nine rats each were used: the rats in Lot B were fed the basal diet plus 400 ppm molybdenum ad libitum and the rats in Lot C were fed the basal diet in amounts, daily, equal to the average consumed during the previous 24 hours by the rats in Lot B. Two treatments (nine rats per treatment) were used in Experiment Five. Lot B was fed as in Experiment Four and each rat in Lot D received the basal diets in amounts, daily, equal to 75% of the average consumed by the rats in Lot B.

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[1] Dublin Laboratory Animals, Box 846, Dublin, Virginia

Semi-purified diets were used, and the basal is described in Table 1. The composition of the mineral mix and the vitamin mix are

TABLE 1

Composition of the basal diet
(per kilogram diet)

| | |
|---|---|
| Vitamin-free casein[1] | 100 g. |
| Sucrose | 815 g. |
| Fat (Wesson Oil) | 50 g. |
| Mineral Mix | 25 g. |
| Vitamin Mix | 10 g. |
| Choline Chloride (20% aqueous solution) | 5 ml. |
| Copper solution (1.6 ppm Cu as $CuSO_4 \cdot 5H_2O$) | 5 ml. |
| Molybdenum solution[2] (aqueous $Na_2MoO_4$) | 10 ml. |

[1] Nutritional Biochemicals Corporation, Cleveland 28, Ohio.

[2] Where appropriate

TABLE 2

Composition of mineral mix
(grams per kilogram diet)

| | |
|---|---|
| $Ca_3(PO_4)_2$ | 12.5 |
| NaCl | 6.5 |
| KCl | 5.5 |
| MgO | 0.7 |
| $Fe_2O_3$ | 0.15 |
| $MnO_2$ | 0.10 |
| KI | 0.03 |
| $ZnCO_3$ | 0.02 |

given in Tables 2 and 3, respectively. The fat soluble vitamins were administered weekly per os as two drops of a solution in oil. Tap water was provided ad libitum. Urine from each rat was collected in 5 ml of

1 N HCl in 48-hour composites. The feces were collected simultaneously. Both urine and feces were stored at -20° C. until the end of each 5-day collection period and then were analyzed for nitrogen content. All collections reported here were made between the 20th and 40th days of feeding. In Experiments 2 and 5, consecutive collection periods were used

TABLE 3

Composition of vitamin mix
(milligrams per kilogram diet)

| | |
|---|---|
| Sucrose | 7,945 |
| L-cystine | 2,000 |
| Calcium pantothenate | 20 |
| Niacin | 10 |
| Inositol | 10 |
| Thiamine-HCl | 5 |
| Menadione | 4 |
| Riboflavin | 3 |
| Pyridoxine-HCl | 3 |
| Folic acid | 0.2 |
| Biotin | 0.01 |
| Vitamin $B_{12}$ | 0.001 |

Dietary, fecal and total urinary nitrogen were determined by the Kjeldahl procedure. Free ammonia and urea, after urease treatment, were estimated by Nesslerization (9); creatine and creatinine by the Jaffe reaction (10). Free alpha-amino nitrogen was estimated by the naphthoquinone sulfonic acid method of Frame, et al. (11) after ammonia removal. After adjusting the urine pH to 10-12 with 10% NaOH, ammonia was removed by vacuum desiccation (concentrated sulfuric acid as the desiccant), decreasing the sample volume by approximately 50%. Values obtained by this method were comparable to those obtained with the

ninhydrin method of Khachadurian et al. (12). An automatic amino acid analyzer (13) was used for the quantitative determination of the individual amino acids of pooled urine samples (one sample from five mo-fed rats and one from five paired-fed rats).

Whole blood and plasma alpha-amino nitrogen were determined by the method of Frame, et al. (11).

The sixth experiment will be discussed in the results.

Descending paper chromatography was used to separate the individual amino acids and molybdenum in fresh urine samples of molybdenum-fed rats. Whatman Number one paper and the following three solvents were used: (a) isopropanol: water (80:20), (b) isopropanol:water:acetic acid (80:19:1), and (c) isopropanol:water:ammonium hydroxide (80:19:1). The amino acids were detected by spraying the dried paper with 0.5% ninhydrin in ethanol and the molybdate by spraying with 10% stannous chloride in 10% hydrochloric acid followed immediately by spraying with 10% ammonium thiocyanate.

Histopathological studies of livers and kidneys were conducted using standard procedures, by Dr. Osborne, Animal Pathology Department, V.P.I.

Urea synthesis in liver tissue of the rats were observed <u>in vitro</u> as described later.

Standard methods for statistical analyses were used.

## III. RESULTS

Molybdenosis in the rat was accompanied by a reduced food consumption and a depressed rate of weight gain. This is demonstrated in Table 4, which is a summary of the average final weights, weight gains and food and nitrogen consumption of the rats used in five experiments. The rats consuming the basal diet plus 600 ppm molybdenum (Exp. 1, Lot B) did not gain any weight. In order to demonstrate the differences in nutrient utilization, some weight gain in the molybdenum-fed rat appeared desirable. This was accomplished in the following experiments by decreasing the level of molybdenum, added to the diet, to 400 ppm. A consistent trend for better weight gain in the pair-fed rats (Lot C) in comparison to the molybdenum-fed rats (Lot B) was observed; however, this trend was not statistically significant because the standard deviations were so large. The restricted-fed rats (Lot D) consumed significantly less feed than the molybdenum-fed rats while their rates of weight gain were parallel to those of the molybdenum-fed rats. The increased weight gain of the pair-fed rats (Lot C) and the decreased food consumption of the restricted-fed rats (Lot D) in comparison to the molybdenum-fed rats (Lot B) demonstrated that nutrient utilization by the molybdenum-fed rats was decreased.

Previous observations that increased dietary levels of casein alleviated molybdenosis in the rat indicated that dietary molybdenum may influence nitrogen metabolism. A summarization of the nitrogen balance

TABLE 4

Weight gain and feed consumption as regulated by dietary molybdenum, paired-feeding and restricted-feeding in rats

| Dietary Treatment | Days on Exp. | Weights | | Consumption | |
|---|---|---|---|---|---|
| | | Final | Gain | Feed | Nitrogen |
| | | g ± S.D.[1] | g ± S.D. | g ± S.D. | mg ± S.D. |
| Experiment 1[2] | 7 | | | | |
| Basal diet, ad libitum | | 166.8 ± 16.2 | 20.8 ± 3.0 | 64.4 ± 5.7 | 914.3 ± 80.4 |
| Basal + 600 ppm Mo, ad libitum | | 41.2 ± 5.7 | 0.2 ± 0.4 | 19.1 ± 2.4 | 267.0 ± 33.8 |
| Basal diet, pair-fed | | 59.2 ± 7.3 | 4.2 ± 1.8 | 18.6 ± 2.5 | 264.7 ± 36.0 |
| Basal diet, restricted-fed | | 49.5 ± 1.9 | 4.5 ± 0.6 | 15.0 | 213.0 |
| Experiment 2-a[3;4] | 10 | | | | |
| Basal diet, ad libitum | | 111.0 ± 12.7 | 29.8 ± 6.7 | 97.2 ± 13.2 | 1434.8 ± 194.2 |
| Basal + 400 ppm Mo, ad libitum | | 68.2 ± 6.2 | 10.0 ± 3.5 | 55.7 ± 9.6 | 821.9 ± 142.3 |
| Basal diet, pair-fed | | 75.6 ± 4.8 | 16.2 ± 2.2 | 54.9 ± 9.9 | 810.5 ± 146.8 |
| Basal diet, restricted-fed | | 64.2 ± 1.7 | 14.5 ± 1.0 | 45.0 | 664.2 |
| Experiment 2-b | 5 | | | | |
| Basal diet, ad libitum | | 123.8 ± 14.4 | 12.8 ± 3.9 | 47.5 ± 12.3 | 701.5 ± 180.2 |
| Basal + 400 ppm Mo, ad libitum | | 72.2 ± 9.9 | 4.0 ± 4.2 | 29.6 ± 5.0 | 436.7 ± 73.1 |
| Basal diet, pair-fed | | 84.8 ± 10.3 | 9.2 ± 3.4 | 29.8 ± 4.5 | 450.0 ± 67.1 |
| Basal diet, restricted-fed | | 68.8 ± 1.5 | 4.5 ± 0.6 | 22.5 | 332.1 |
| Experiment 2-c | 5 | | | | |
| Basal diet, ad libitum | | 141.2 ± 13.6 | 17.5 ± 1.9 | 57.0 ± 5.8 | 841.6 ± 85.7 |
| Basal + 400 ppm Mo, ad libitum | | 76.6 ± 10.9 | 4.4 ± 2.1 | 28.2 ± 3.7 | 416.2 ± 51.4 |
| Basal diet, pair-fed | | 90.8 ± 11.4 | 6.0 ± 1.9 | 29.0 ± 3.8 | 427.6 ± 55.8 |
| Basal diet, restricted-fed | | 71.8 ± 1.5 | 3.0 ± 0.0 | 22.5 | 332.1 |

(TABLE 4 - continued)

| Dietary Treatment | Days on Exp. | Weights | | Consumption | |
|---|---|---|---|---|---|
| | | Final | Gain | Feed | Nitrogen |
| | | g ± S.D. | g ± S.D. | g ± S.D. | mg ± S.D. |
| Experiment 3[5] | 5 | | | | |
| Basal diet, ad libitum | | 137.5 ± 10.8 | 18.8 ± 5.3 | 57.3 ± 5.4 | 786.9 ± 74.4 |
| Basal + 400 ppm Mo, ad libitum | | 70.2 ± 12.0 | 3.6 ± 1.7 | 25.8 ± 2.7 | 354.7 ± 36.9 |
| Basal diet, pair-fed | | 86.4 ± 8.7 | 5.2 ± 1.3 | 26.0 ± 3.1 | 356.3 ± 43.0 |
| Basal diet, restricted-fed | | 72.5 ± 4.4 | 5.0 ± 0.8 | 21.0 | 288.2 |
| Experiment 4[3] | 5 | | | | |
| Basal + 400 ppm Mo, ad libitum | | 57.2 ± 14.6 | 7.1 ± 3.8 | 27.8 ± 9.4 | 398.5 ± 134.4 |
| Basal diet, pair-fed | | 61.2 ± 6.4 | 9.8 ± 2.2 | 26.2 ± 3.6 | 375.4 ± 50.3 |
| Experiment 5-a[3,4] | 5 | | | | |
| Basal + 400 ppm Mo, ad libitum | | 65.3 ± 8.5 | 4.4 ± 3.1 | 29.5 ± 7.2 | 434.8 ± 105.0 |
| Basal diet, restricted-fed | | 65.7 ± 2.2 | 5.2 ± 0.6 | 22.6 | 328.7 |
| Experiment 5-b | 5 | | | | |
| Basal + 400 ppm Mo, ad libitum | | 69.1 ± 9.8 | 3.8 ± 2.4 | 28.3 ± 5.9 | 415.7 ± 87.0 |
| Basal diet, restricted-fed | | 70.3 ± 2.7 | 4.7 ± 0.9 | 21.6 | 317.8 |
| Experiment 5-c | 5 | | | | |
| Basal + 400 ppm Mo, ad libitum | | 74.6 ± 11.0 | 5.0 ± 2.4 | 27.9 ± 4.0 | 411.6 ± 58.6 |
| Basal diet, restricted-fed | | 76.1 ± 2.6 | 5.8 ± 1.0 | 20.7 | 305.6 |
| Experiment 5-d | 5 | | | | |
| Basal + 400 ppm Mo, ad libitum | | 77.7 ± 12.1 | 3.1 ± 2.3 | 28.9 ± 5.2 | 425.6 ± 76.0 |
| Basal diet, restricted-fed | | 79.3 ± 2.6 | 3.2 ± 0.8 | 21.8 | 321.0 |

[1] Average ± standard deviation.

[2] Experiment started after 35 days of feeding.

[3] Experiment started after 20 days of feeding.

[4] a, b, c, and d refer to sequential feeding periods of the duration shown

[5] Experiment started after 30 days of feeding

data for a twenty-day trial (Exp. 5) is presented in Table 5. The difference in fecal nitrogen excretion (although statistically significant) is minor and would not contribute significantly to the decreased nutrient utilization. The molybdenum-fed rats retained only 70% as much of the ingested nitrogen as did the rats fed the basal diet. This difference in retention was accounted for by the increased urinary nitrogen excretion of molybdenum-fed rats (62% more than for the rats fed the basal diet). The increased urinary nitrogen excretion was attributed to the increased excretion of urea plus ammonia (41% over that for controls) and free alpha-amino nitrogen (540% over that for controls). The determination of creatine, creatinine and urinary protein revealed that the excretion of these components was not affected by molybdenum feeding; therefore, they were expressed as part of the remaining urinary nitrogen. Although the control rats were permitted to consume only 75% as much feed and nitrogen as was consumed by the molybdenum-fed rats, apparent nitrogen retention in milligrams was slightly more than that of the molybdenum-fed rats.

Body size affects the amount of urinary nitrogen excretion. In order to minimize this variable, urinary nitrogen components were calculated for the 100-gram rat for all experiments and all treatments (Figure 1). The total urinary nitrogen excretion by the molybdenum-fed rats was 69 to 143% greater than for the rats fed the basal diet. Per 100 grams of body weight, the molybdenum-fed rats consistently excreted more urea plus ammonia than the rats in any of the lots fed the basal diet.

TABLE 5

Effect of dietary molybdenum on nitrogen balance and urinary nitrogen components in rats

(9 rats per treatment, experimental period of 20 days)

| | Restricted-fed Basal Diet | Ad libitum Basal + 400 ppm Mo |
|---|---|---|
| | (% of consumed) | (% of consumed) |
| Fecal Nitrogen | 5.0 ± 1.2 | 6.9 ± 1.2[3] |
| Total Urinary Nitrogen | 29.5 ± 2.6 | 47.9 ± 8.1[2] |
| α-amino nitrogen | 1.3 ± 0.1 | 8.5 ± 5.3[2] |
| urea plus ammonia nitrogen | 20.1 ± 2.3 | 28.3 ± 4.0[2] |
| remaining urinary nitrogen | 8.1 ± 1.0 | 11.1 ± 3.3[4] |
| Nitrogen Retained (by difference) | 65.5 ± 3.4 | 45.2 ± 8.4[2] |
| Consumed (mg Nitrogen) | 1273 | 1689 ± 270 |
| Retained (mg Nitrogen) | 834 ± 42 | 774 ± 239 |

[1] Average ± standard deviation

[2] Significantly different ($P < .001$)

[3] Significantly different ($P < .01$)

[4] Significantly different ($P < .05$)

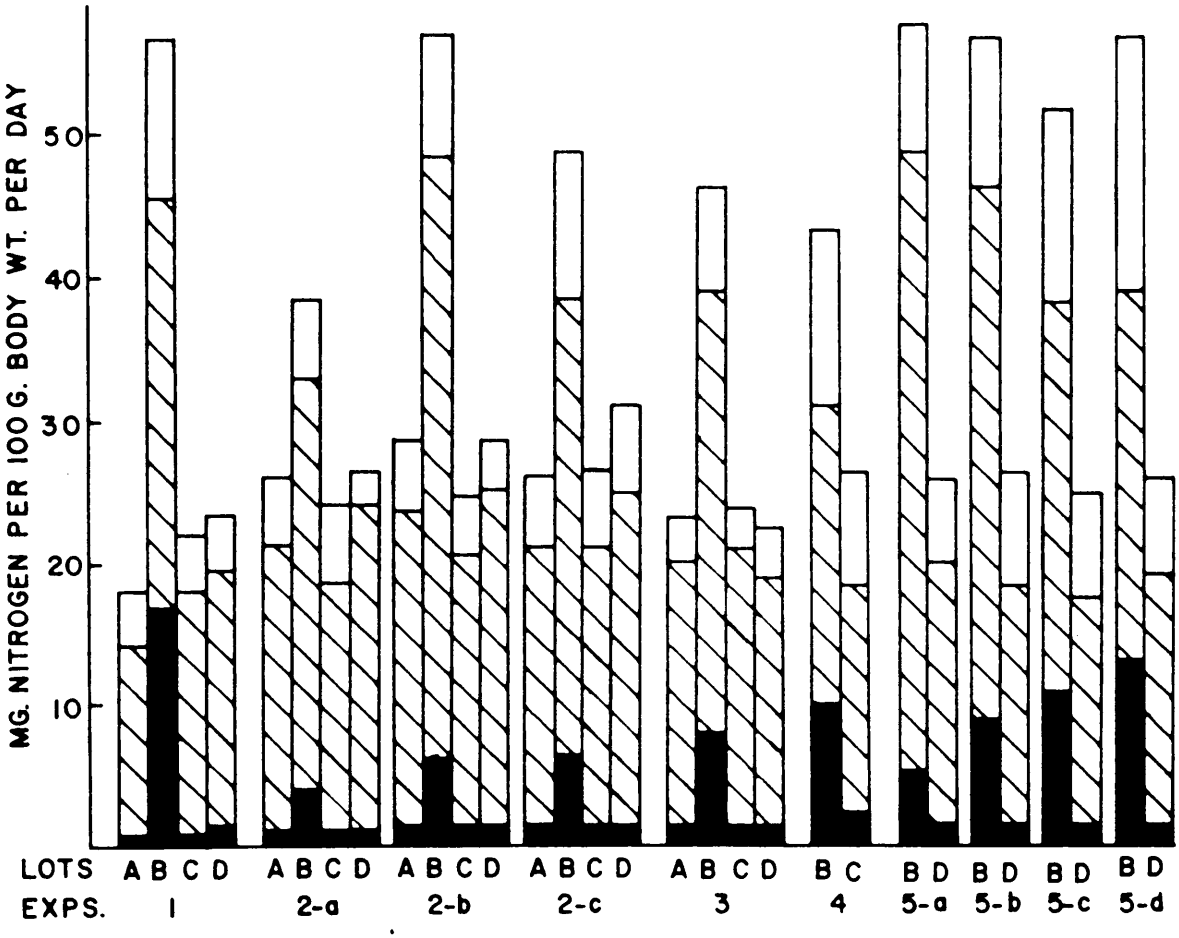


Figure 1. Urinary nitrogen excretion. Full bar is total urinary nitrogen, black area is alpha-amino nitrogen and cross-marked area is urea plus ammonia nitrogen.

However, rats fed the basal diet, ad libitum, excreted more urea plus ammonia per rat per day since they were two to four times as large as the molybdenum-fed rats. The largest comparative difference observed was the excretion of alpha-amino nitrogen, with the molybdenum-fed rats excreting four to 17 times as much as the rats fed the basal diet. An increased amino aciduria with a higher level of dietary molybdenum is demonstrated by comparing Lot B, Exp. 1 (fed the basal diet plus 600 ppm molybdenum) to Lot B in any of the other experiments (fed the basal plus 400 ppm molybdenum). Increasing amino aciduria with an increasing length of feeding period was observed and is shown in Experiments Two and Five where the letters "a" through "c" and "a" through "d" represent consecutive collection periods. As the amino aciduria increased, urea plus ammonia excretion decreased. The amino aciduria could not be detected during the first 14 days of feeding molybdenum and the collections represented here were made between the 20th and 40th days. Total urinary nitrogen, urea plus ammonia nitrogen and alpha-amino nitrogen of the rats fed the basal diet were not significantly affected by the method of feeding, whether ad libitum or restricted (Experiments 1, 2, and 3).

The amino aciduria accompanying molybdenosis was significant and could provide an indication of the mechanism of the toxicity; so, it was investigated further. The free, individual amino acid levels in pooled urine samples from rats in Lots B and C of Experiment Three are

presented (Table 6). It should be emphasized that these data are not conclusive. They were obtained from pooled samples which were desiccated at pH 10 to remove excess ammonia and then were stored for six weeks at pH 2.2 and -20° C., so that some of the labile amino acids may have been partially or totally destroyed or lost. Citrulline, a urea cycle intermediate, and the aromatic amino acids, including histidine, were found only in the urine of the molybdenum-fed rats. The only non-dispensable amino acid excreted in large amounts by the molybdenum-fed rat was threonine. The other hydroxy-amino acid, serine, was also excreted in large amounts.

To determine whether or not the increased excretion of alpha-amino nitrogen was a reflection of the blood levels, alpha-amino nitrogen was determined on blood obtained by heart puncture. The rats had been fed the diets for 23 days (Experiment 4) and then fasted for 24 hours prior to having the blood drawn. The whole blood alpha-amino nitrogen level was significantly lower (11.42 $\pm$ 1.17 vs. 13.09 $\pm$ 0.95 mg per 100 ml blood) while the plasma level was significantly higher (10.16 $\pm$ 0.59 vs. 8.08 $\pm$ 0.89 mg per 100 ml plasma) in the molybdenum-fed rat compared to the pair-fed control. Significance was determined by the t-test ($P < .01$) with 10 rats per treatment.

Threonine, valine and tryptophan have been involved in amino acid imbalances and were the most limiting amino acids in this diet. The possibility that the urinary excretion of these compounds may have induced an amino acid imbalance was investigated. A summarization of

TABLE 6

Urinary amino acids

(Experiment 3, urine collected after 28 days of feeding)

| | Pair-fed Basal Diet | Basal + 400 ppm Mo | Ratios of Mole %[1] |
|---|---|---|---|
| | µm/day/100g rat | µm/day/100g rat | Mo-fed/control |
| histidine | --- | 8.54 | --- |
| citrulline | --- | 7.98 | --- |
| tyrosine | --- | 4.28 | --- |
| phenylalanine | --- | 3.42 | --- |
| hydroxyproline | .09 | 6.70 | 3.40 |
| asparagine | .42 | 28.21 | 3.07 |
| serine | .46 | 30.76 | 3.06 |
| threonine | .38 | 23.94 | 2.89 |
| valine | .04 | 2.00 | 2.26 |
| alanine | 1.30 | 39.88 | 1.40 |
| glycine | 2.04 | 59.54 | 1.34 |
| isoleucine | .04 | 1.14 | 1.30 |
| glutamic acid | 1.20 | 26.78 | 1.02 |
| leucine | .10 | 2.00 | .92 |
| lysine | .28 | 4.56 | .75 |
| proline | 2.32 | 33.34 | .66 |
| ethanolamine | .32 | 3.85 | .55 |
| methionine | .10 | .86 | .39 |
| taurine | 4.40 | 35.90 | .37 |
| beta-alanine | .37 | 1.85 | .23 |
| aspartic acid | .38 | 1.70 | .23 |
| 1-methyl histidine | .23 | 1.00 | .21 |
| hydroxylysine | .02 | .09 | .20 |
| ornithine | .14 | .28 | .10 |
| sarcosine | .42 | .40 | .04 |
| TOTAL | 15.05 | 329.00 | |

[1] Ratios of mole % =

$$100 \times \frac{\mu M \text{ of individual amino acid/day/Mo-fed rat}}{\text{total } \mu M \text{ of amino acids/day/Mo-fed rat}}$$
$$\overline{100 \times \frac{\mu M \text{ of same amino acid/day/control rat}}{\text{total } \mu M \text{ of amino acids/day/control rat}}}$$

the dietary supplementation and effect upon weight gains are presented in Table 7. None of the supplementations significantly affected the weight gains of either the basal diet or basal diet plus 400 ppm molybdenum-fed rats.

TABLE 7

The effect of amino acid supplementation on growth of weanling rats receiving the basal diet with and without molybdenum
( 5 rats per treatment, 6-week feeding period)

| Dietary Supplementation | | | Weight Gains | |
|---|---|---|---|---|
| 0.2% L-threonine | 0.2% L-tryptophan | 0.2% L-valine | Basal Diet | Basal + 400 ppm Mo |
| | | | g | g |
| | | | 168.5 ± 12.8[1] | 37.8 ± 10.3 |
| + | | | 180.0 ± 20.3 | 42.8 ± 12.2 |
| | + | | 180.8 ± 21.2 | 36.2 ± 8.9 |
| | | + | 163.2 ± 17.6 | 30.6 ± 12.3 |
| + | + | | 189.6 ± 15.6 | 29.4 ± 14.4 |
| + | | + | 186.6 ± 16.2 | 51.4 ± 5.5 |
| | + | + | 183.4 ± 29.6 | 35.2 ± 11.4 |
| + | + | + | 178.2 ± 16.0 | 50.0 ± 23.6 |

[1] Average ± standard deviation

## IV. DISCUSSION

Molybdenosis in the rat was accompanied by depressed weight gain and decreased nutrient consumption and utilization. These observations are consistent with previous reports (4, 5, 7). The decreased utilization of nutrients by the molybdenum-fed rats was demonstrated by the trend toward better weight gain of the pair-fed rats and the decreased consumption, with parallel gain, of the restricted-fed rats receiving the basal diet (-molybdenum). The restricted-fed lot of rats was included when it was observed (4) that the pair-fed rats were not adequate controls because the molybdenum-fed rats had a depressed utilization of ingested nutrients for weight gain. This depression in nutrient utilization may be attributable to either an increased need for energy or a decreased efficiency of utilization of ingested nutrients by the molybdenum-fed rats. Since the molybdenum-fed rat consumed less diet than rats fed the basal diet, an increased nutrient requirement did not appear consistent and the efficiency of nitrogen utilization was investigated.

Only a minor impairment in digestion and absorption of dietary protein was found, concommitantly with molybdenosis, as indicated by the small increase in fecal nitrogen. The increased level of total urinary nitrogen accompanying molybdenum feeding indicated a significant alteration in nitrogen metabolism. This may account for the observation that increased levels of dietary casein alleviated the molybdenosis

syndrome (6). However, this increased excretion of urinary nitrogen could have resulted from an increased utilization of dietary protein for energy rather than a defect in nitrogen metabolism. The increased excretion of urea plus ammonia by the molybdenum-fed rat indicates that the deamination of amino acids was increased and this could be attributed to an increased utilization of amino acids to satisfy caloric requirements. An increased utilization of dietary protein for energy would contribute to the decreased ingestion of diet since food consumption is regulated, extensively, in animals by caloric requirements (14). However, this would not explain the increased rate of gain of the pair-fed rats or the decreased food consumption of the restricted-fed rats receiving the basal diet unless the molybdenum-fed rat had an increased requirement for energy.

The increased deamination of amino acids could have resulted from an increased catabolism of body protein or decreased anabolism of dietary protein. Creatine and creatinine excretion by the molybdenum-fed rat was not affected; therefore, increased catabolism of tissue protein appears improbable because these compounds are used to estimate muscle protein catabolism. However, these urinary components are not affected by the turn-over of some of the more transient body proteins, such as those of the liver and blood; consequently, more definitive experiments (i.e. using labeled amino acids) are needed to determine the source of the urinary nitrogen. The increased excretion of free alpha-amino nitrogen by the molybdenum-fed rat represents a loss of metabolizable energy and was a contributing factor to the depressed nutrient utilization.

Nitrogen retention paralleled weight gain so that the percent of body protein would probably be similar in the two treatments.

The amino aciduria would not account for the total depression in nutrient utilization for weight gain because the growth depression was observed immediately after feeding molybdenum and the amino aciduria could not be detected until after about 20 days of feeding. Gross kidney damage resulting from molybdenum feeding and contributing to the amino aciduria appeared unlikely since no proteinuria was observed. However, histopathological studies of the kidneys showed that some fatty infiltration had occurred. The fat was deposited in fine droplets on or near the basement membranes of the proximal convoluted tubules and a slight degree of regeneration had occurred in the epithelium of the tubules. Since the kidney damage was not extensive, the possibility that it could account for the observed amino aciduria does not appear probable because the renal threshold for most of the amino acids is high and the kidney has a large reserve function, i.e. normal kidney functions are maintained in animals and humans with only one kidney.

It is known that ingested molybdenum is excreted primarily via the urine. The possibility that the animal detoxifies molybdenum by the formation of an amino acid-molybdenum complex prior to excretion was investigated with paper chromatography. Preliminary studies indicated that this was not probable because the molybdenum did not move as a distinct spot and did not correspond to any ninhydrin-positive area. It is possible that a relatively unstable complex was formed and had

dissociated either before or during the chromatographic separation so that it was not detected. Also, the molybdenum could have bound the amino acid in such a manner that the amino acid would not give a positive ninhydrin reaction.

An inhibition, by molybdenum, of the resorptive mechanism for amino acids in the kidney is possible. These mechanisms are specific for groups of amino acids (15) and if one or more of them were inhibited, the group or groups of amino acids would be excreted and this selective excretion could result in an "induced" amino acid imbalance. Other heavy metals, such as cadmium, lead, uranium, and mercury, have caused amino acidurias (16) and in most cases, serine and threonine were found in increased concentrations in the urine. This could indicate that the resorptive mechanism for these two amino acids was more susceptible to inhibition by heavy metals. Amino acid imbalances are sometimes accompanied by amino acidurias (17, 18, 19) and are more easily induced with low protein diets. Nine percent casein diets have been used to induce amino acid imbalances which could be alleviated by threonine supplementation (20, 21). Threonine was the most limiting amino acid in the diet used in these studies since it was supplemented with cystine (22). The last experiment described herein was designed to determine if this loss of threonine caused the growth depression through an imbalance. Since the dietary supplementation with these three amino acids had no significant effect upon weight gain during the six-week feeding period, it was concluded that the elevated excretion of these amino acids did not result in an amino acid imbalance within the body.

Shimke (23, 24) reported that the activity of the liver urea cycle enzymes is correlated with the amount of urea produced and with the dietary protein level. Feeding high levels of protein in the diet or fasting increases the enzymatic activities while feeding low levels of protein or protein-free diets decreases the activity. Using the method of Ratner and Pappas (25), a preliminary study of the urea synthesis as affected by the duration of feeding molybdenum was conducted. The data indicated that the in vitro activity in liver tissue of rats fed the basal diet decreased rapidly and plateaued within two weeks while the liver activity of the rats receiving molybdenum decreased gradually and plateaued at about the same level after three weeks. The rapid decrease in liver urea cycle enzymes of the rats fed the basal diet would be expected because the level of dietary protein was low and it would be conserved for protein synthesis. The delayed decline of activity in the molybdenum-fed rat livers will be discussed later.

The observations reported here are compatable with the hypothesis that dietary molybdenum inhibits some facet of protein synthesis. This hypothesis could explain these observations. The increased urinary nitrogen excretion would occur if the dietary protein was not being anabolized to body protein. Immediately after feeding molybdenum, the absorbed protein moieties would be utilized for energy; thereby, increasing urea plus ammonia excretion and decreasing food consumption. Food consumption would be further diminished since there would be less demand for energy for protein anabolism. The urea cycle activity of the

molybdenum-fed rats would remain high since there were large amounts of amino acids which needed deamination. However, assuming that protein synthesis was inhibited, the protein moieties of new enzyme molecules would not be synthesized in sufficient amounts and the activity of the liver urea cycle would gradually decrease. The excess amino acids would then be excreted in increasing amounts resulting in the observed increasing amino aciduria concommitantly with the decreased excretion of urea plus ammonia. The observed citrullinuria is consistent with proposed insufficient urea cycle hypothesis. Also, histopathological studies of the livers revealed a uniform lesion in all livers from the molybdenum-fed rats consisting of (a.) marked fatty infiltration and fatty metamorphosis of the parenchymal cells diffusely distributed throughout the liver lobules and (b.) numerous mitotic figures indicating extensive regeneration. A severe impairment of the liver functions was probable. Lower whole blood levels of alpha-amino nitrogen in the molybdenum-fed rat, after fasting, compared to that of rats consuming the basal diet could be attributed to less free amino acids present in the red blood cells while the higher plasma levels could indicate the diminished catabolism and anabolism of the available amino acids. Decreasing growth depression accompanying molybdenosis with increasing dietary casein levels is also compatable with this hypothesis. Increasing dietary casein would increase the concentrations of the reactants for protein synthesis which would then be increased by mass

action. Furthermore, *in vitro* studies of the effect of minerals upon the incorporation of amino acids by rat liver preparations (26) have demonstrated an inhibition by molybdenum (added *in vitro*) at the physiological levels found in the livers of molybdenum-fed rats.

The observations which have been noted and discussed are consistent with the proposed hypothesis that molybdenum inhibits some facet of protein synthesis *in vivo*. The hypothesis warrants further investigation and with the rapid improvements in protein synthesis experimentation, definitive experiments should be possible.

## V. SUMMARY

Male weanling albino rats derived from the Sprague-Dawley strain were indivudually housed in stainless-steel metabolism cages. The rats were fed a semi-purified diet (with or without 400 or 600 ppm added molybdenum) which consisted of adequate minerals and water-soluble vitamins and the following: (in percent) vitamin-free casein, 10; sucrose, 81.5; and vegetable oil, 5. The fat-soluble vitamins were administered weekly per os. Tap water was provided ad libitum.

Urine and feces were collected separately. Nitrogen balance studies were conducted and the results of these studies are reported. Standard procedures for nitrogen determination were used.

Decreased food consumption and reduced weight gain, accompanying molybdenosis, were observed. Restricting food consumption (basal diet, no added molybdenum) for one group of rats to obtain weight gain parallel to that of the molybdenum-fed rat demonstrated that the molybdenum-fed rat utilized nutrients less efficiently for weight gain.

The determination of fecal nitrogen indicated that with toxic levels of dietary molybdenum, the decrease in digestion and absorption of dietary nitrogen was minor.

A significant alteration in nitrogen metabolism accompanying molybdenosis was detected when total urinary nitrogen was determined. As percent of nitrogen intake, the molybdenum-fed rat excreted 70% more urinary nitrogen, compared to the rats fed the basal diet. After

decreasing the variation in nitrogen excretion due to body size by expressing urinary nitrogen per unit body weight, the molybdenum-fed rat excreted 70 to 140 percent more than the control rats.

Within two days after initiating the experiment, the rats fed the molybdenum-containing diets had a decreased consumption and retention of nitrogen. For the first 14 days, the increased urinary nitrogen was attributable to elevated levels of urea and ammonia. After 20 days of feeding, an amino aciduria developed.

Elevated levels of urinary alpha-amino nitrogen were observed with a higher concentration of dietary molybdenum (600 vs. 400 ppm). The level of urinary alpha-amino nitrogen increased as the duration of feeding the molybdenum diets increased. Concommitantly with the increasing amino aciduria was a decreasing excretion of urea plus ammonia.

Individual amino acid levels in the urine were determined. Citrulline and the aromatic amino acids, including histidine, were detected only in the urine of the rats consuming the diets with added molybdenum. The only non-dispensable amino acid found in large concentrations in the molybdenum-fed rat's urine was threonine, which was accompanied by large amounts of the other hydroxy-amino acid, serine.

The diets were supplemented with all combinations of L-threonine, L-valine and L-tryptophan in one experiment. No significant effect upon weight of either the control or the molybdenum-fed rats attributable to the amino acid supplementation was observed.

Several hypotheses regarding the mechanism of molybdenum toxicity in the rat are discussed. The hypothesis that dietary molybdenum inhibits protein synthesis *in vivo* appears to be the most consistent with the experimental observations. Assuming that the inhibited protein synthesis hypothesis is true, the dietary protein would be metabolized for energy and would increase urea plus ammonia excretion. Since the protein moieties of the urea cycle enzymes were not being synthesized in sufficient amounts, amino acids were excreted via the urine. The citrullinuria is consistent with the hypothesis that the urea cycle is not functioning optimally.

## VI. ACKNOWLEDGEMENTS

The author wishes to take this opportunity to express appreciation to some of the people who assisted in the experimental work and in writing the thesis. To Dr. J. Clark Osborne for preparation and interpretation of the histopathological slides. To Dr. K. W. King and for assistance in chromatographing the amino acids and in calculating the results. To the members of the Biochemistry and Nutrition staff for assistance in preparing this manuscript. To for his assistance and inspiration. To Dr. Russell Miller whose stimulating discussion and vital nature was always a challenge to excel. To the graduate students in the department for stimulating discussion. To my wife for her patience during long hours in the laboratory. To the Nutrition Foundation, Inc., New York, for financial support in the research. To National Institutes of Health, Bethesda, Maryland, for the Traineeship grant and to Virginia Polytechnic Institute for the scholarship.

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# THE EFFECT OF DIETARY MOLYBDENUM UPON THE UTILIZATION OF NUTRIENTS BY THE RAT

Herman L. Johnson

## ABSTRACT

Male weanling albino rats derived from the Sprague-Dawley strain were individually housed in stainless-steel metabolism cages. The rats were fed a semi-purified diet (with or without 400 or 600 ppm added molybdenum) which consisted of adequate minerals and water-soluble vitamins and the following: (in percent) vitamin-free casein, 10; sucrose, 81.5; and vegetable oil, 5. The fat-soluble vitamins were administered weekly per os. Tap water was provided ad libitum.

Urine and feces were collected separately. Nitrogen balance studies were conducted and the results of these studies are reported. Standard procedures for nitrogen determinations were used.

Decreased food consumption and reduced weight gain, accompanying molybdenosis, were observed. Restricting food consumption (basal diet, no added molybdenum) for one group of rats to obtain weight gain parallel to that of the molybdenum-fed rat demonstrated that the molybdenum-fed rat utilized nutrients less efficiently for weight gain.

The determination of fecal nitrogen indicated that with toxic levels of dietary molybdenum, the decrease in digestion and absorption of dietary nitrogen was minor.

A significant alteration in nitrogen metabolism accompanying molybdenosis was detected when total urinary nitrogen was determined. As percent of nitrogen intake, the molybdenum-fed rat excreted 70% more urinary nitrogen, compared to the rats fed the basal diet. After decreasing the variation in nitrogen excretion due to body size by expressing urinary nitrogen per unit body weight, the molybdenum-fed rat excreted 70 to 140 percent more than the control rats.

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