Distribution of Copper and Molybdenum in Liver Centrifugal Fractions.*† (26721)

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The toxicity of molybdenum has been recently reviewed(1,2). Feeding excess molybdenum to rats resulted in elevated liver levels of molybdenum and copper(3). Addition of inorganic sulfate to the diet reduced these elevated liver molybdenum and copper levels. The distribution of molybdenum in liver and kidney centrifugal fractions of chicks fed a tracer dose of Mo^{99} has been determined by Teekell and Lyke(4). Mills§ has determined the distribution of copper and molybdenum in liver centrifugal fractions. In other studies the distribution of copper as influenced by hypophysectomy and growth hormone was ascertained(5).

Despite the work done on molybdenum toxicity, there is little information concerning the mechanism of toxicity or the effect of molybdenum on copper metabolism. It has been suggested that molybdenum may form a complex with copper in the tissues which renders the copper unavailable(6). Molybdenum may also directly interfere in enzyme processes(7,8,9). Although in many instances molybdenum toxicity closely resembles a copper deficiency that can be overcome by dietary copper additions, attempts to demonstrate a copper deficiency at the enzyme level have often been unsuccessful(9, 10). However, the activity of calf heart cytochrome oxidase(11) and sheep skin tyrosine oxidase(7) may be reduced with molybdenum feeding.

The results reported here were obtained from studies to determine the influence of dietary molybdenum and sulfate upon distribution of copper and molybdenum in particular fractions isolated from the livers of rats and perhaps furnish information about the mechanism of molybdenum toxicity.

Methods. The rats used in these studies (weanling Sprague-Dawley strain males) were individually housed in stainless steel wire floored cages and were provided food and water *ad libitum*. The basal diet was essentially the same as described elsewhere (3) and contained in per cent: sucrose 81.5, vitamin-free casein (N. B. Co.) 10.0, Wesson oil 5.0, mineral mixture 2.5, and vitamin mixture 1.0. Copper at the level of 1.6 ppm was added as the sulfate to bring total copper, as analyzed, to approximately 2.5 ppm. Four levels of molybdenum and 3 levels of sulfate were added to the diets of the 6 treatment groups as indicated in Table I. After feeding the experimental diets for 6 weeks, the rats were sacrificed by decapitation and the livers removed. Approximately half of each liver was dried, then analyzed for copper and molybdenum. The other portion of the fresh liver was immediately placed on ice and homogenized with 9 volumes of cold 0.25 M sucrose for 2.5 minutes. The homogenate was centrifuged at 700 × g for 10 minutes, the supernatant removed, the nuclei and debris precipitate resuspended in approximately 5 ml of ice cold 0.25 M sucrose and recentrifuged at 700 × g for 10 minutes. The 2 supernatants were combined and centrifuged at 5,000 × g for 10 minutes. As with the nuclei and debris fraction, the sedimented mitochondria were resuspended in sucrose and recentrifuged at 5,000 × g for 10 minutes. The supernatants were combined and designated as the supernatant fraction in the case where no further fractionation was done. In a second study a microsomal fraction was prepared by centrifuging the supernatant, from which the mitochondria had been isolated, at 75,000 × g for one hour. The microsomal pellet was washed but not resuspended. A standard wet ashing method, using nitric and perchloric acids, was used to

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§ Personal communication, Dr. C. F. Mills, Rowett Institute, Scotland.

TABLE I. Distribution of Cu and Mo in 3 Liver Centrifugal Fractions (% of Total, 5 Rats/Treatment).

Diet additions							
ppm Mo*	0	200	300	400	500	500	500
ppm SO_4†	0	0	0	0	400	750	1500
Wt gain (g)‡	174 ±26	97 ±33	65 ±23	38 ±11	38 ±13	72 ±19	124 ±29
			Cu				
Nuclei and debris	14 ± 3.7	25 ± 4.5	23 ± 7.2	28 ± 7.4	30 ± 4.7	34 ± 8.5	24 ± 6.4
Mitochondria	20 ± 4.7	28 ± 3.4	27 ± 2.4	28 ± 1.9	29 ± 6.2	28 ± 8.5	27 ± 4.5
Supernatant	66 ± 5.4	46 ± 4.6	50 ± 2.5	44 ± 8.3	42 ± 9.2	38 ± 3.7	48 ± 8.3
Total (μg)§	31 ± 3.8	36 ± 6.1	27 ± 3.5	27 ± 5.3	25 ± 8.1	33 ± 2.2	34 ± 9.5
Conc. (ppm)‖	10 ± 2.8	15 ± 4.4	18 ± 3.0	29 ±11	20 ± 8.0	19 ± 4.0	14 ± 3.6
			Mo				
Nuclei and debris	—	10 ± 2.9	8 ± 3.3	13 ± 4.3	13 ± 3.3	17 ± 3.1	10 ± 3.8
Mitochondria	—	15 ± 1.0	14 ± 4.1	16 ± 1.9	15 ± 3.5	17 ± 4.0	16 ± 5.2
Supernatant	—	76 ± 3.4	78 ± 6.7	72 ± 5.8	72 ± 6.4	66 ± 3.5	74 ± 7.5
Total (μg)§	2 ± .4	40 ±13	45 ±13	31 ± 8.6	42 ±17	36 ±10	43 ±11
Conc. (ppm)‖	.7± .2	20 ± 5.5	33 ±10	35 ±14	35 ±13	21 ± 3.2	18 ± 6.6

* Mo added to diet as $Na_2MoO_4 \cdot 2H_2O$.
† SO_4 added to diet as an equimolar mixture K_2SO_4 : Na_2SO_4.
‡ 6-wk gain in body wt.
§ Calculated from total liver wt and analysis of an aliquot.
‖ Dry wt basis.

digest the samples. Copper was determined by the carbamate method of the A.O.A.C. (12) and molybdenum by the thiocyanate method of Evans *et al.*(13).

Results. The feeding of up to 400 ppm of molybdenum severely limited rat growth and increased the liver levels of copper and molybdenum (Table I). Additions of inorganic sulfate improved the growth of molybdenum-fed rats and reversed the tendency toward increased concentration of liver copper and molybdenum. Distributions of copper and molybdenum in the centrifugal fractions are also given in Table I. An average of 66% of the total copper was present in the supernatant of the livers of the rats fed the control diet (–Mo) while in the molybdenum-fed rats, supernatant copper was approximately 45% of the total. This difference was statistically significant. The percentage of the total copper in the nuclei and debris and mitochondria fractions prepared from the livers of rats fed molybdenum was increased over that found in the same fractions prepared from livers of rats fed the control diet. When molybdenum was fed, it was found predominately in the supernatant. There was no significant effect of increasing dietary molybdenum above the lowest level added upon distribution of molybdenum or copper. It appeared that dietary sulfate had no significant effect on distribution of copper and molybdenum in the particulate fractions of liver.

To determine the amount of copper and molybdenum associated with the microsomes as a result of molybdenum feeding a second experiment was initiated. Six rats were fed the basal diet and 6 were fed basal plus 400 ppm molybdenum for 6 weeks. From the livers of rats in this trial, a microsomal fraction was prepared in addition to the other particulate fractions. The distribution results were qualitatively similar to those reported in Table I. In addition, the copper associated with the microsomal fraction represented about 10% of the total and did not appear to be altered when molybdenum was included in the diet. Likewise, the amount of molybdenum in the microsomal fraction was approximately 10% of the total.

Discussion. The largest amount of copper and molybdenum on an absolute and molar basis was not found in the same liver fractions, thus it appears unlikely that molybdenum causes copper concentration to rise by simply forming a copper-molybdenum complex. The increase in copper concentration in the particulate fractions may constitute a compensatory mechanism, namely, an at-

tempt to overcome a copper deficiency induced by a molybdenum-block of a copper dependent metabolic reaction. Perhaps the failure to demonstrate a copper deficiency at the enzyme level is due to this extreme compensation.

The copper appeared to be associated with the particulate fractions of liver containing a high concentration of protein. The converse appears to be true for molybdenum. This suggests that the copper may be bound to protein as it is in blood.

Summary. In the liver of control rats (–Mo) the copper appeared to be concentrated (> 55%) in the supernatant fraction (nuclei and debris < 15%; mitochondria 20%; microsomes 10%), while in the liver from molybdenum-fed rats more copper was found in the nuclei and debris (27%) and mitochondria (27%) fractions and less in the supernatant (<37%). The percentage of total copper in the microsomes appeared to be unaltered by dietary molybdenum. The molybdenum was usually found to be concentrated (>60%) in the supernatant (nuclei and debris < 15%; mitochondria 15%; microsomes 10%). The feeding of sulfate had little or no effect on distribution of copper or molybdenum.

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Normal and Experimental Involution of Rat Mammary Gland.* (26722)

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Previous studies in this laboratory have employed desoxyribonucleic acid (DNA) as a quantitative index of mammary gland proliferation during various experimental conditions(1,2,3,4) and during normal pregnancy and lactation in the rat and mouse(4,5,6,7). It was thought desirable to investigate reversion of DNA during normal involution of the rat mammary gland and to determine the effect of the ovarian hormones estrogen and progesterone upon this physiological process. These data will serve as a basic reference for further experiments on prevention of mammary gland involution.

Materials and methods. Data were obtained from primiparous Sprague-Dawley-Rolfsmeyer rats. Young were separated from dam on day 1 of lactation and mammary gland DNA was determined on days 5, 10, 14, and 20 after weaning. Experimental animals received subcutaneous injections of 1 μg E (estradiol benzoate) or 1 μg E + 3 mg P (progesterone) from day of weaning.

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