Copper, Molybendum and Zinc Interrelationships in Rats and Swine.* (20687)

R. KULWICH,† SAM L. HANSARD, C. L. COMAR, AND G. K. DAVIS.

From the University of Tennessee-Atomic Energy Commission Agricultural Research Program, Oak Ridge, and the Florida Agricultural Experiment Station.

It is generally recognized that naturally occurring molybdenum in pasture plants of certain areas produces a severe disease of cattle and sheep which is apparently cured or prevented by supplemental copper. The literature on molybdenum in relation to the health of farm animals has been reviewed to 1944 by Russell(1) and the influence of molybdenum on the copper metabolism of animals has been reviewed to 1952 by Marston(2). Most of this experimental work has been done with rats, sheep and cattle. While chronic molybdenum toxicity symptoms are similar to those of copper deficiency, there does not seem to be a simple stoichiometric relationship between the two elements, and the existence of other variables is indicated. The role of molybdenum and copper in various flavin enzyme systems(3-5) may need to be considered in any explanation of toxic effects and antagonisms. Of particular interest are the findings that under some conditions molybdenum causes a depletion in copper reserves, whereas under others, there occurs a tissue accumulation of copper coincidental with symptoms of copper deficiency(2). There has been some indication of a relationship between zinc and copper in that some of the effects of high dietary zinc in rats were overcome by copper supplementation(6,7). However, this relationship has not been well established. This study shows the effects of high levels of molybdenum, zinc and copper on the tissue storage of these elements and also upon the distribution of radioactive copper. Rats and swine were used, the latter because there is little or no information in regard to copper-molybdenum relationships in this species.

Methods. Weanling rats from a highly inbred Wistar strain and 10-week-old Hampshire pigs were each lotted into 6 groups and maintained on a basal ration to which copper, molybdenum, zinc or combinations were added at levels as indicated in Tables I and II. Molybdenum was supplied as sodium molybdate, and copper and zinc as the sulfate. The swine basal ration consisted of 61.3% ground yellow corn, 30% soybean oil meal (solvent process), 7% alfalfa meal, 1% sodium chloride and 0.7% calcium carbonate. It was found to contain by analysis 8.9, 2.1 and 39.2 p.p.m. of copper, molybdenum and zinc respectively. One group of the rats received a ration identical to that fed the swine, and the second group received a synthetic ration previously described(8) to which the copper, molybdenum and zinc supplements were added. The growth response and tissue analyses of the rats for the two groups were similar, and for brevity only results on the natural ration will be discussed. A third short term study with old and young rats involved administration of a molybdenum solution by stomach tube for 8 consecutive days to determine the effect of such supplementation upon tissue uptake of radiocopper intramuscularly administered and these results are presented in the discussion.

Swine were maintained for 27 weeks and rats for 14 weeks on the experimental rations. They were then dosed orally with radiocopper and placed in metabolism units for 24 hours previous to sacrifice for tissue distribution studies and tissue analyses(9-11). Each rat received 320 micrograms of labeled copper with an initial activity of about 100 microcuries and the swine received 75-100 mg of copper as the chloride containing an initial

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† Oak Ridge Institute of Nuclear Studies Graduate Fellow. Present address, Bureau of Animal Industry, U. S. Department of Agriculture, Beltsville, Md.

TABLE I. Effects of Dietary Levels upon Accumulation of Copper, Molybdenum and Zinc in Rat Tissues and upon Uptake of Copper 64.*

	Basal ration	Basal + 1000 p.p.m. Mo	Basal + 1000 p.p.m. Zn	Basal + 200 p.p.m. Cu	Basal + Mo and Cu	Basal + Zn and Cu
Wt gain, g/8 wk	115	38	105	119	40	107
Copper (p.p.m.)						
Liver	14	48	13	17	415	13
Kidney	20	152	21	21	131	24
Molybdenum (p.p.m.)						
Liver	2.1	33	2.6	1.6	36	2.2
Kidney	1.0	146	1.7	<1	57	<1
Femur	<1	19	1.7	<1	19	1.8
Zinc (p.p.m.)						
Liver	65	101	100	77	127	71
Kidney	97	99	96	100	102	91
Femur	149	120	177	144	129	229
Copper 64†						
Blood	29	186	10	5	36	7
Liver	49	164	61	79	164	94
Kidney	257	397	298	233	136	273
Spleen	19	66	12	49	38	9
Muscle	2.1	16.5	1.2	1.8	4.0	1.7
Skin	6.0	22.9	8.2	5.4	13.7	3.6
Red bone marrow	395	650	86	125	158	151

* Values represent averages of individual tissues from 3-5 animals except for kidneys which were pooled. Femurs calculated on fresh wt basis, all others on dry wt.

† Reported as % of ingested dose $\times 10^3$/g of fresh tissue corrected to body wt of 275 g.

activity of 25 to 30 millicuries. Samples for radiocopper assay were prepared by wet-ashing in concentrated nitric acid and diluting to a volume of 10 ml(12). Samples with sufficient radioactivity were transferred to a 60 mm Petri dish and counted directly with a thin mica end-window Geiger counter. With samples containing low levels of Cu 64, a one milliliter aliquot was pipetted into a tared stainless steel planchet and evaporated to dryness under an infra-red lamp. The dried samples were then counted in a restricted atmosphere proportional counter. Self-absorption corrections were applied as determined from an empirical self-absorption curve. Samples which were to be analyzed for total copper, molybdenum or zinc in addition to radioactivity measurements, were treated after radioassay, according to the method of Lindner and Harley(13) to yield a completely oxidized sample suitable for the chemical determinations. The carbamate method(14) was used for copper determinations, using isoamyl alcohol as the solvent. The thiocyanate-stannous chloride method, essentially as described by Perrin(15) was used for molybdenum determinations. Zinc was determined by the dithizone method(16).

Results with rats. To conserve space only average values are given. While there was considerable variation between individuals, the differences observed were so large that there is little question as to significance. As noted in Table I, the addition of 200 p.p.m. of copper or 1000 p.p.m. of zinc had no apparent effect upon growth rate of the rats whereas the addition of 1000 p.p.m. of molybdenum resulted in a reduced growth rate. The supplement of 200 p.p.m. of copper did not alter the effects on growth of the high molybdenum intake. When the rats were drenched daily with molybdenum the growth was also retarded and the effect was greater in weanlings than in 24-week-old animals.

Analyses of livers, kidneys, and femurs of the rats indicated that supplementary zinc or copper resulted in little increase in the concentration of copper, zinc or molybdenum in tissues after 14 weeks on the ration. However, rats receiving supplementary molybdenum either with or without copper showed appreciably higher copper concentrations in the liver and kidney, ranging from a 3-fold to a 30-fold increase. High dietary molybdenum

TABLE II. Effects of Dietary Levels upon Accumulation of Copper, Molybdenum and Zinc in Swine Tissues and upon Uptake of Copper 64.*

	Basal ration	Basal + 1000 p.p.m. Mo	Basal + 1000 p.p.m. Zn	Basal + 200 p.p.m. Cu	Basal + Mo and Cu	Basal + Zn and Cu
Daily gain (lb)	1.1	.95	1.0	.98	.96	1.0
Feed/100 lb of gain (lb)	423	462	415	397	417	444
Copper (p.p.m.)						
Liver	29	149	27	60	239	32
Kidney	40	144	47	59	60	43
Spleen	6.6	25	3.1	4.4	6.9	4.8
Plasma	238	376	260	231	330	247
Molybdenum (p.p.m.)						
Liver	5.0	67	5.6	4.6	32	5.0
Kidney	4.4	75	3.7	3.2	25	4.9
Spleen	1.1	21	.9	.4	8.8	.5
Femur shaft	1.9	32	.2	1.5	22	1.1
Copper 64†						
Blood	14	19	10	10	10	10
Liver	66	137	48	42	40	46
Kidney	75	80	59	50	37	67
Spleen	4.9	7.7	3.1	2.3	2.9	2.9
Muscle	2.4	2.2	1.3	2.1	1.5	1.2
Skin	2.6	2.7	4.9	3.9	2.7	4.1
Red bone marrow	1.2	3.4	1.3	.4	2.9	1.3

* Values represent averages of individual tissues from 3 animals in each lot. Plasma calculated as $\mu g/100$ ml, all other analytical results as parts per million on a dry matter basis.

† Values reported as % of ingested dose $\times 10^5$/g fresh wt corrected to a body wt of 200 lb. The No. of animals per lot were as follows: 2, basal; 1, basal + Mo; 3, basal + Zn; 2, basal + Cu; 3, basal + Mo and Cu; 3, basal + Zn and Cu.

was also found to increase the molybdenum content of the liver, kidney and femur.

The high molybdenum rats on the purified rations, the individual values for which are omitted, showed a 3-fold increase in skin copper concentration at the basal copper level and an even greater, though less uniform increase at the high copper level. Skin samples were taken from the back without removing the hair. It is of interest that Hundley and Ing(17) reported a 4-fold increase in copper concentration of skin samples of pantothenic acid-deficient black rats which showed a graying of the hair coat. These authors suggested that gray hair production in pantothenic acid deficiency may involve interference with copper utilization. Singer and Davis(18) reported graying of hair coat in piebald rats on a copper deficient ration which was corrected either by additional dietary copper or calcium pantothenate. These findings support the viewpoint that the high copper concentrations in skins of rats showing graying of the hair coat, after being fed rations deficient in pantothenic acid but not in copper, may reflect the presence of physiologically unavailable copper. In the light of these findings it is plausible to assume that the high copper concentrations found in the skin and other tissues of rats maintained on high levels of dietary molybdenum may also represent copper that is physiologically unavailable.

Results obtained from the tissue distribution of radiocopper (Table I) provide further evidence of increased copper retention by animals fed supplementary molybdenum. At the basal copper level, uptake of copper 64 by blood, liver, kidney, spleen, muscle, skin and red bone marrow was increased in those rats receiving high levels of molybdenum, although there was considerable variability within groups. At 200 p.p.m. dietary copper, the high molybdenum groups showed increased radiocopper uptake by all tissues examined except kidney and spleen. The groups receiving the high level of dietary zinc at the basal copper level showed evidence of lowered blood and red bone marrow copper 64 accumulation, but this did not occur at the high level of copper intake.

Drenching with molybdenum for 8 consecu-

tive days was likewise shown to increase the uptake of Cu 64 administered intramuscularly to 24-week-old rats. The blood showed a 3-fold increase; spleen, skin and liver showed smaller increases but the kidney value was lowered. A somewhat similar pattern was noted with weanling rats. Copper 64 uptake by the blood in the molybdenum groups was increased over 3 times that found in the control group. The skin and liver showed a slight increase in Cu 64 uptake for the molybdenum-drenched group, while kidney and spleen values were lower than those for controls.

Results with pigs. Growth rate and feed consumption data for the various lots of swine (Table II) indicated that the addition of 200 p.p.m. of copper or 1000 p.p.m. of molybdenum or zinc to a natural ration did not result in any marked growth retardation nor did it influence the feed utilization efficiency. Pigs fed 1000 p.p.m. of zinc in this experiment showed no ill effects although they received a lower level of zinc, on a dry matter basis, than did those in the experiments reported by Grimmett *et al.*(19), in which 1000 p.p.m. of zinc as the lactate was added to whole milk with pigs becoming lame and unthrifty within two months. Apparently zinc accumulation in the present experiment had not reached the toxic level.

Histological examination of sections taken from the cerebrum, cerebellum, cervical cord, thoracic cord and lumbar cord of pigs from all lots showed no degenerative changes and were believed to represent normal tissues. Leg joints and muscles taken from the gluteal region showed no pathological change.

Analytical data for copper, molybdenum and radiocopper are also presented in Table II. Ingestion of 200 p.p.m. of supplementary copper did not cause any increase in spleen or plasma copper levels when compared to the basal group, although kidney and liver values were somewhat higher. Supplementary zinc did not appear to affect copper accumulation in these tissues studied. Dietary supplementation with molybdenum, on the other hand, appeared to markedly increase tissue copper accumulation.

At the basal copper level, pigs receiving supplementary molybdenum showed a 5-fold increase in liver and about a 4-fold increase in spleen and kidney copper concentration. Plasma copper levels were elevated about 58%. An increase in tissue copper concentration by molybdenum supplementation was also found at the high copper level; there was a 4-fold increase in liver copper concentration and a 43% increase in plasma copper concentration observed in the control group. However, when high dietary copper levels were fed, supplementary molybdenum did not appear to materially affect kidney or spleen copper concentration.

Ingestion of 1000 p.p.m. of molybdenum resulted in high molybdenum values for liver, kidney, femur and spleen. Molybdenum concentration in these tissues from groups receiving supplementary copper in addition to molybdenum, however, were considerably lower than corresponding values for groups receiving only supplementary molybdenum. This indicates that the excess copper intake lowered the accumulation of dietary molybdenum. Accumulation of molybdenum was apparently unaffected by the high zinc level.

Unfortunately, copper 64 uptake data were obtained for only one pig of the lot receiving supplementary molybdenum at the basal copper level. The data for this animal indicated an increased concentration in liver, spleen and red bone marrow of pigs on the basal ration plus high molybdenum. At the high copper level, however, the lot receiving supplementary molybdenum showed a definite increase in copper 64 uptake only in the red bone marrow. High dietary level of zinc did not appear to have any appreciable effect upon the uptake of labeled copper.

The increased tissue copper accumulation observed in swine fed high dietary molybdenum levels is in agreement with the rat data and the results of Marston with rats who reported that supplementation with 1 mg of molybdenum daily resulted in increased copper accumulation.

Summary and conclusions. Substantial increases in tissue copper concentrations and radiocopper accumulation resulted when a level of 1000 p.p.m. of molybdenum as sodium molybdate was fed to rats and swine on a

natural ration. This increase was especially pronounced in the kidneys and livers and is in accord with the hypothesis that excess copper, which is not physiologically effective, may be accumulated when a high level of molybdenum is fed. A dietary level of 1000 p.p.m. of zinc did not appear to have any appreciable effect upon copper metabolism. Growth depression in rats produced by 1000 p.p.m. of added molybdenum to a natural or synthetic ration was not counteracted by the addition of 200 p.p.m. dietary copper. Swine appeared to be more resistant to molybdenum toxicity than did rats. No degenerative changes were observed in the spinal cord, leg joints or muscle tissues of swine fed levels of 1000 p.p.m. of molybdenum or 1000 p.p.m. zinc for a 7-month experimental period. With the criteria of these experiments it appears that dietary zinc was not involved in copper-molybdenum imbalance.

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Fat Storage of an Estrogen in Women Following Orally Administered tri-*p*-anisylchloroethylene. (20688)

CHARLES R. THOMPSON AND HAROLD W. WERNER.
From the Research Laboratories, The Wm. S. Merrell Co., Cincinnati, Ohio

It was reported that tri-*p*-anisylchloroethylene (TACE*) administered orally to ovariectomized rats and monkeys produced estrogenic activity of long duration(1,2). This prolonged action following oral administration resulted from storage of estogenic material in the body fat and its slow release(1,2). Greenblatt and Brown(3) demonstrated estrogenic material in the body fat of human beings given TACE and none in that from patients receiving other synthetic and natural estrogens. Since fat storage of estrogen is of therapeutic significance, further studies extending those of Greenblatt and Brown are reported.

Methods. Fat biopsy specimens† obtained

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