Uptake and Biliary Excretion of Cu^{64} in Rabbits in Relation to Blood Ceruloplasmin.* (30639)

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It has been postulated that one of the important biological functions of the alpha-2 globulin, ceruloplasmin, is the regulation of the biliary excretion of inorganic copper(1). However, there is little experimental evidence to support this theory(2,3). With the availability of an experimental technique for modifying the blood ceruloplasmin levels(4), it became feasible to reexamine the problem as to possible correlations between serum ceruloplasmin, liver uptake, and biliary excretion of Cu^{64} in the rabbit.

Three groups containing 9 to 12 male albino rabbits (12-13 weeks old) were employed. One group was maintained for 10-15 days on a commercial dry ration containing molybdate-sulfate, a diet known to reduce ceruloplasmin levels in the rabbit and greatly elevate total blood copper(4,5). A second group, which served as controls, was maintained on the commercial ration without added salts. A third group was maintained on a ration mixed with inorganic copper, 0.005% copper as Cu_2SO_4 for 4 weeks in order to elevate serum ceruloplasmin levels (5,6). Ceruloplasmin was measured as p-phenylenediamine oxidase activity in each rabbit serum 24 hours before administration of the isotope(7). Animals were given an intravenous injection of 75 microcuries of high activity Cu^{64} acetate (6 μg copper) *via* the marginal ear vein and sacrificed 2, 25, and 50 hours later. Levels of Cu^{64} activity were measured in 0.1-0.5 ml of bile immediately withdrawn from the gall bladder. Suitable samples of liver and cross sections of cortex and medulla of the kidney were hydrolyzed with sulfuric, perchloric and nitric acids(8). Each sample was returned to a standard volume and Cu^{64} activity determined. The values obtained were corrected for radioactive decay and expressed as percent of the dose administered (Table I). Radioactive copper was determined in a well-type, thallium-activated sodium iodide crystal scintillation counter.

In those rabbits maintained on a high molybdate-sulfate diet there resulted a marked diminution of serum ceruloplasmin levels, uptake of Cu^{64} by liver, and excretion of copper at 2, 25, and 50 hours (Table I). Cu^{64} uptake by liver in this group was reduced to 35.7% of the control level 2 hours after injection. Along with a 20% reduction in serum ceruloplasmin, the biliary excretion of Cu^{64} was virtually zero. This sharp suppression in liver uptake and biliary copper excretion in 2, 25, and 50 hours occurred despite an elevation in the non-ceruloplasmin copper pool(4,5). On the other hand, a significant increase in the concentration of Cu^{64} in the kidney was noted in this group as compared to the other 2 groups.

Inorganic copper loading in the third group was accompanied by higher ceruloplasmin levels and Cu^{64} uptake by the liver, in keeping with observations previously reported for rats and mice(9,10). While the uptake of Cu^{64} by liver in the third group of rabbits at the end of the first 2 hours was found to be 1.4 times greater than the control, it was 4 times that in the animals on the high molybdate-sulfate diet. Following the same pattern, bile Cu^{64} level during the first 2 hours in copper-loaded rabbits was found to be 3 times that of the controls, in agreement with previous reports(9,10), and 352 times that of the group on the high molybdate-sulfate diet. Cu^{64} activity in the kidney of copper-loaded rabbits at 2, 25 and 50 hours was somewhat lower than that found in the control group.

A preliminary study was undertaken of Cu^{64} uptake by the brains of animals main-

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TABLE I. Liver Uptake, Biliary Excretion, and Kidney Concentration of Cu^{64} in Rabbits with Altered Ceruloplasmin Levels.*

Group	Serum ceruloplasmin (mg/100 ml)	Bile† % of injected dose/ml 2 hr	25 hr	50 hr	Liver† % of injected dose/g 2 hr	25 hr	50 hr	Kidney† % of injected dose/g 2 hr	25 hr	50 hr
Control‡	11.7 ± 2.5	11.3 ± 5.7	14.8 ± 5.2	11.1 ± 2.4	39.3 ± 7.9	20.7 ± 5.0	14.5 ± 4.2	7.3 ± .7	12.2 ± 4.1	10.4 ± 1.8
High§ Mo-SO_4	2.4 ± 1.1	.1 ± .03	1.3 ± .6	.5 ± .02	14.0 ± 3.0	7.4 ± .5	9.5 ± 4.3	12.5 ± 1.9	15.0 ± 3.3	18.6 ± 4.6
High‖ Cu	23.3 ± 4.4	35.2 ± 3.7	20.5 ± 7.3	14.2 ± 7.2	55.9 ± 12.1	25.7 ± 3.1	19.3 ± 2.2	6.2 ± .7	6.3 ± 2.2	6.6 ± 1.3

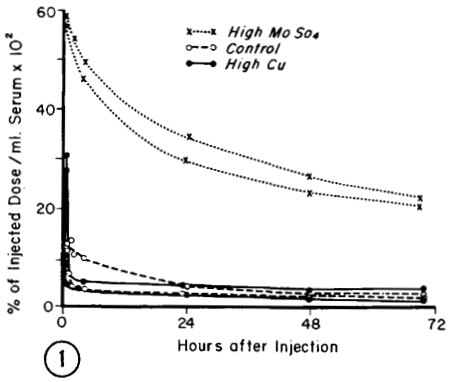
* Means ± standard deviation.
† % activity found in bile (1 ml) or tissue (1 g) of injected dose $\times 10^2$.
‡ Commercial dry ration containing a daily intake of 1-2 mg copper.
§ Rabbits maintained for 10-14 days on a commercial diet sprayed with an aqueous solution of 0.08% molybdenum as Na_2MoO_4 and 1.36% sulfate as Na_2SO_4.
‖ Rabbits maintained on commercial diet mixed with .005% copper as Cu_2SO_4 for 4 weeks.

tained on a high molybdate diet. As much as a 15% increase over control level was observed in the midbrain 25-50 hours after administration of the Cu^{64}. Further studies on Cu^{64} uptake into various regions of the brain are in progress.

In order further to investigate Cu^{64} activity levels in both serum and globulin fractions, of which ceruloplasmin is a component (11), a second experiment was undertaken. Two animals from each group were given an intravenous injection of 70 μc of Cu^{64} acetate and the activity in 2 ml of serum was determined at various intervals during a 72-hour period. An equal and similarly-treated number of animals was given the same dose. Two ml of serum were taken after 10 minutes, 2, 3, 5, 24 and 48 hours and Cu^{64} bound globulin was measured after precipitation twice with 45% saturated ammonium sulfate, pH 7.0.

The initial levels of activity in serum were higher in the rabbits fed the high molybdate-sulfate diet, followed by a gradual decrease during 72 hours (Fig. 1). The control animals showed a lower initial level followed by a pronounced fall between 2 and 5 hours after injection [presumably due to liver uptake (10,14)]; then the level of activity remained the same or slightly elevated in the controls and copper-loaded animals.

Cu^{64} incorporation into the globulin fraction in animals from all groups decreased slightly during the first few hours and then increased gradually for the next 24 hours.



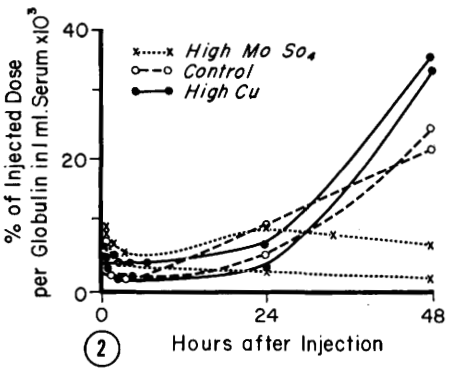


FIG. 1. Cu^{64} activity in serum plotted against time following intravenous administration of 70 microcuries Cu^{64} acetate in rabbits fed control, high Mo-SO_4, and high Cu diets.

FIG. 2. Cu^{64} activity in globulin fractions plotted against time following intravenous administration of 70 microcuries Cu^{64} in rabbits fed control, high Mo-SO_4 diets and high Cu diets. 45% saturated $(NH_4)_2SO_4$, pH 7.0, was used for globulin precipitation. Ordinate expresses % of total serum Cu^{64} present in the globulin fraction.

After 24 hours the rate of incorporation continued unchanged in the control group, increased markedly in the group maintained on a high Cu diet, and decreased slightly in the group maintained on the molybdate diet. Observations previously reported by other workers show the same pattern of incorporation of copper into the globulin fraction of rats and humans(13,14). The lowest activity at 48 hours was found in serum of rabbits on the high molybdate-sulfate diet (Fig. 2).

The pH of bile from the gall bladder was found to be between 5.2 and 8.1 with a mean of 6.2. Comparable values of 5.6 and 8.6 with a mean of 6.0 for humans and 6.4 and 6.7 for rabbits have been reported(15). There was no significant difference between the pH of bile from the 3 different groups of animals in the present study. The increased nonceruloplasmin copper levels previously reported(4,5), together with impaired Cu^{64} uptake by the liver and incorporation into serum globulin observed in the present study of rabbits fed a high molybdate-sulfate diet, are strikingly reminiscent of metabolic aberrations reported for Wilson's Disease(12,14, 16-18). Of particular significance is the fact that the Cu uptake by the brains of animals on a high molybdate diet is elevated at a time when the animals exhibit tremors. This finding lends further credence to the argument that the elevated Cu levels in the basal ganglia may be responsible for the neurological symptoms of Wilson's Disease.

This work substantiates previous suggestions(14,19) proposing an enzymic mechanism operative in the liver, which incorporates inorganic copper into the ceruloplasmin molecule, preparatory to biliary excretion. Inhibition of this enzyme system, which presumably occurred in the rabbits fed a high molybdate-sulfate diet, resulted in decreased liver copper uptake, an inhibition of ceruloplasmin biosynthesis, and a defect in biliary excretion of the metal. However, this assumption does not exclude the possibility that molybdenum may either have interfered with Cu incorporation or could have replaced bound Cu in the active site and thus altered ceruloplasmin activity. Further studies are necessary.

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