ings of LeRoy *et al*(4). The content of prolylhydroxyproline in serum is 0.21 μg per ml as such, or 0.12 μg per ml as hydroxyproline (e). This represents 1.1% of the total hydroxyproline of serum (b + c), and 4.0% of the total dialyzable hydroxyproline (b), whereas it is 17% of the bound dialyzable hydroxyproline (b—a). Only the latter value is significant at present since free hydroxyproline comprises only a small fraction of the total excreted in urine and since we know nothing about the hydroxyproline of serum that is present in the form of protein. Prolylhydroxyproline therefore is 17% of the bound dialyzable hydroxyproline of serum, whereas it is 50% of the total hydroxyproline of urine(5).

Summary. Estimates of the different forms of hydroxyproline in blood serum were made by methods which now account for all of the hydroxyproline. In micrograms per milliliter of serum they are: total, 10.1; as protein, 7.1; dialyzable, 3.0; free, 2.3; peptide, 0.7; prolylhydroxyproline, 0.12. The latter represents only 1% of the total hydroxyproline of serum whereas it is about 50% of the total in urine.

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Ceruloplasmin: *in vitro* Inhibition in the Rabbit.* (30256)

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Despite numerous investigations [see (1) for review] little information is available on the role of blood ceruloplasmin in body function in general or its relationship to copper distribution and metabolism. It has been known since the discovery of Wilson's disease that the central nervous system is particularly sensitive to a disturbance in copper metabolism, although the significance of low ceruloplasmin to the condition is unknown. With the important finding that a copper deficiency state could be produced in animals maintained on a diet containing high molybdate and sulfate, an experimental technique became available for studying the relationship of copper to neurological function. Ewes maintained on a similar diet gave birth to lambs with swayback disease, a demyelinating condition presumably similar to Wilson's disease(2). It was the objective of the present study to determine the effects of a high molybdate and sulfate diet on blood ceruloplasmin in an effort to help elucidate the role of this copper protein.

Materials and methods. Twenty-four white albino rabbits, 14-15 weeks old, were maintained on an intake of 0.05% molybdenum, as sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$) and 1.8% sulfate, as sodium sulfate (Na_2SO_4) mixed with a Purina rabbit dry ration containing 2 mg % copper. Deionized distilled drinking water was provided, *ad libitum*.

The weight of the animals, the level of total copper in blood, serum oxidase activity, hemoglobin levels, and alkaline phosphatase levels were examined weekly. Tissue copper determinations were performed after 10 weeks on one-third of the animals, while the rest of the determinations were made after death.

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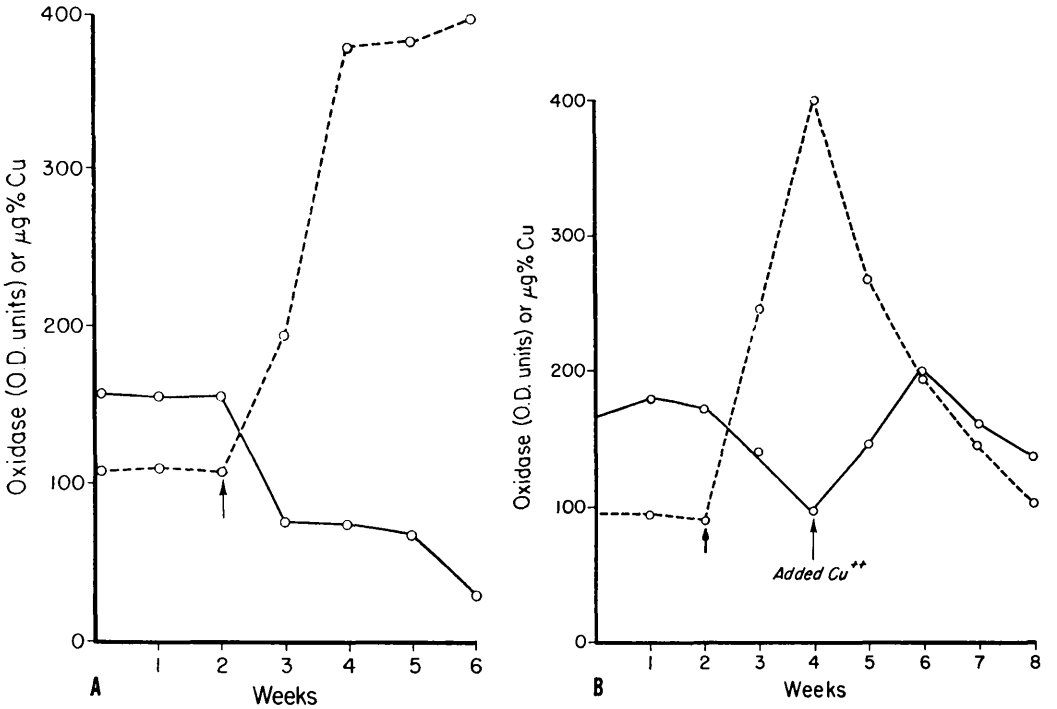

FIG. 1. A) Change in serum oxidase activity (solid line) and total blood copper (dotted line) of rabbits maintained on high molybdate and high sulfate diet (arrow indicates time when molybdate diet was begun). The standard error of the points in the curves is less than 8%. Each point is based on 4 animals. B) Effect of addition of copper (as Cu_2SO_4) on levels of serum oxidase (solid line) and total blood copper (dotted line) of rabbits maintained on described diet. Molybdate diet was begun at 2 weeks (first arrow) and copper added to the diet at 4 weeks (second arrow). The standard error of the points in the curves is less than 8%. Each point is based on 4 animals.

The copper content of the blood and tissue was estimated by a slight modification of the method of Eden and Green(3) with extreme care being taken to avoid contamination by heavy metal. Duplicate samples of blood were drawn from the marginal vein of the ear and transferred to test tubes containing sodium citrate as an anticoagulant. Serum oxidase activity was measured by a modification of the method of Abood *et al*(4) and the optical density converted to mg ceruloplasmin per cent. Hemoglobin, alkaline phosphatase, and uric acid were also determined(5,6).

Results. The addition to the diet of 0.05% molybdenum and 1.8% sulfate after 2 weeks caused a gradual increase in total blood copper to a level of 300-400% of the normal value (Fig. 1A). On the other hand, the oxidase activity correspondingly decreased until there was no demonstrable activity in serum at the end of a 4-week period.

When the molybdenum-containing diet was supplemented after 4 weeks with 0.002% copper as sulfate ($CuSO_4 \cdot 5H_2O$) the trend of the curves was reversed (Fig. 1B). A gradual decrease of total inorganic copper in blood and an increase in oxidase activity occurred along with the decrease in hemoglobin content. A striking similarity was observed in rate of disappearance of ceruloplasmin activity and hemoglobin concentration (Table I). A diminution in hemoglobin content is not unexpected in view of the finding that copper deficiency is associated with anemia(7). An increase in dietary copper concentration resulted in a 100% increase in ceruloplasmin with only a 30% increase in total blood copper.

There was no significant change in levels of alkaline phosphatase or uric acid. Copper determinations showed a variable increase in

TABLE I. Levels of Hemoglobin, Ceruloplasmin and Copper in Rabbits Maintained on 3 Different Diets.

Diet	Hemoglobin (mg/100 ml)	Serum ceruloplasmin (mg/100 ml)	Copper blood (μg/100 ml)
A (10)*	12.0 ± .88	11.1 ± 2.2	91.3 ± 8.4
B (10)*	4.3 ± 1.3	2.2 ± 1.3	415 ± 23
C (5)*	13.3 ± .81	24.5 ± 2.4	122 ± 16

* No. of animals.
Diet A is Purina dry ration.
Diet B is high sulfate-high molybdate diet for 4-5 weeks, as described in text.
Diet C is Diet A + 0.25% of copper sulfate. Animals were maintained on Diet C for 8 weeks.

some organs. The rabbits maintained on the molybdate-sulfate diet died within a period of 9-14 weeks, unless their diet was supplemented with copper. After the rabbits were kept on the molybdate diet they showed gradual signs of generalized weakness, tremors, ataxia, alopecia, and dermatitis. Supplementation of the diet with copper resulted in a gradual return of the animals to normal.

Discussion. The present results substantiate the work of Mills and Fell(2) showing that swayback symptoms are produced in lambs of ewes given molybdenum and sulfate. Although molybdenum toxicity in rats and rabbits was reported to be associated with symptoms like those of copper deficiency(8,9, 12), there were no determinations (in previous investigations) for ceruloplasmin or copper. One explanation presented for the "induced" copper deficiency produced by molybdenum is that molybdenum, which is excreted mainly in urine, in the presence of sulfate would over-exhaust the kidney and prevent the excretion of inorganic copper(10). It has been suggested that one function of ceruloplasmin may be to facilitate copper excretion through the bile(11). Any factor leading to a decreased ceruloplasmin would, on this basis, result in an accumulation of copper in blood and tissues. The preliminary data indicate that some relationship exists between the level of blood ceruloplasmin and the tendency for copper to accumulate in the brain as well as other tissues. Further details involving the use of radioactive copper will be reported later. It should be mentioned that molybdenum does not inhibit the oxidation of p-phenylenediamine when it is added to normal serum.

There appears to be increasing evidence that the changes in blood ceruloplasmin are a reflection of some basic underlying process associated with copper metabolism and not directly responsible for the pathological course(13). It is more likely, however, that neurological changes, such as demyelination, readily occur following an interference with copper absorption or utilization. It should be emphasized that the neurological symptoms appeared shortly after the fall in blood ceruloplasmin and long before the occurrence of generalized debilitation. A further analogy exists between the condition produced by the molybdate diet in rabbits and Wilson's disease; namely, in both situations the blood ceruloplasmin is decreased while the levels of copper in the body fluids and tissues are markedly elevated. The use of molybdenum may aid investigation of the mechanisms involved in formation of ceruloplasmin and, perhaps, clarify the role of this protein in copper metabolism.

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