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Copper Loading in the Turkey* (33083)

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Decreased serum ceruloplasmin accompanies hepatolenticular degeneration (Wilson's disease). This deficiency may play a role in the pathogenesis by allowing excess deposition of tissue copper (1). It was thought that an animal lacking ceruloplasmin might respond to copper loading by developing the symptoms of Wilson's disease. The broad-breasted white turkey gave promise of serving as an experimental model (2); it has no measurable ceruloplasmin oxidase activity and has the lowest reported plasma copper levels (3).

Materials and Methods. Thirty-nine turkeys of the broad-breasted white strain were fed starting ration for 6 weeks and then maintained an oats, barley, and water *ad libitum*. Copper sulfate pentahydrate, dissolved in distilled water to a Cu concentration of 1 mg/ml, was injected subcutaneously daily beginning at age 91 days. Group I received 0.5 mg of Cu for 7 days; group 2, 0.5 mg for 90 days; group 3, 0.5 mg for 84 days, then 5.0 mg for 17 days. Samples of blood, liver, brain, kidney, and muscle of experimental and control birds were taken at the time of sacrifice. The blood was heparinized and centrifuged; plasma ceruloplasmin oxidase activity was determined by the method of Houchin (4). Values are reported as units of optical density (Coleman junior spectrophotometer) because a conversion factor to mg of ceruloplasmin is not available for the turkey. Fresh tissue samples were weighed to the nearest mg. Tissue and plasma Cu were determined by the wet ash method (5). Significance of data was determined by the student *t* test.

Results. No symptoms suggestive of hepatolenticular degeneration were observed. Experimental birds of groups 1 and 2 appeared as healthy as controls. About 3 days prior to autopsy, copper-injected group 3 birds were observed to have diarrhea and appeared pale with ruffled feathers. This presumably was

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TABLE I. Ceruloplasmin Values and Tissue Copper in Control and Copper-Loaded Turkeys.[a]

Group	(N)	Total Cu injected (mg)	Ceruloplasmin values (optical density)	Cu (μg/100 ml of plasma)	Cu (μg/gm of tissue)			
					Liver	Kidney	Muscle	Brain
Group 1 (7 days)								
Control	(3)		0	9.4 ± 3.0	3.2 ± 0.3	3.6 ± 0.1	0.7 ± 0.3	2.3 ± 0.6
Cu-injected	(8)	3.5	.029 ± .014[b]	35.8 ± 8.6[b]	41.6 ± 15.3[b]	4.4 ± 2.0	0.9 ± 0.2	2.4 ± 0.5
Group 2 (90 days)								
Control	(7)				3.8 ± 0.6	4.1 ± 0.4		2.1 ± 0.4
Cu-injected	(10)	45.0			90.1 ± 80.0[b]	3.9 ± 1.1		3.2 ± 0.8
Group 3 (101 days)								
Control	(3)		0	8.0 ± 7.0	3.1 ± 0.8	3.2 ± 0.6		1.8 ± 0.3
Cu-injected	(8)	127.0	.017 ± .013[b]	42.1 ±12.6[b]	80.0 ± 71.0[b]	4.7 ± 0.3[b]		3.0 ± 1.2

[a] Results given as mean ± SD. (N) = no. of birds.
[b] $p < .01$.

evidence of copper intoxication resulting from copper loading.

Plasma ceruloplasmin activity and plasma copper increased the first week of copper injections and were maintained at these levels, irrespective of further injections (Table I).

Liver copper concentrations were highly variable in injected animals but increased from the control mean of 3 to 43 μg/gm after 3.5 mg of Cu. Mean values of only twice this were reached after 127 mg of Cu (Table I). Copper increases in other organs were small and in most cases insignificant.

Discussion. The control liver copper values reported here agree well with those of Underwood (6), who gives 13.5 μg/gm of dry weight liver. Using a wet/dry ratio of 4/1, our figure would be approximately 14 μg/gm. We have not found values for other organs of the turkey with which to compare our data.

In species which normally have ceruloplasmin, the ratio with copper atoms is well established. Such ceruloplasmin has a molecular weight of 151,000–160,000 and contains 0.34% copper, or 8 atoms per molecule of ceruloplasmin (3). However, ceruloplasmin induced by stress in the chick (which, like the turkey, normally lacks ceruloplasmin) has the same molecular weight (158,000) but contains only 0.20% copper, or 5 atoms per molecule (7). Our attempts to measure free plasma copper (8) were unsuccessful, nor did we ascertain what type of ceruloplasmin was induced by copper loading in the turkey.

The increase in plasma and tissue copper shown by the turkey is similar to the reaction of other species to copper injection. The copper-loaded mammal (9), however, may achieve liver copper concentrations 5 times those recorded for the turkey, and also shows increased levels of copper in kidney and heart but not brain. Normally in the mammal, essentially all the plasma copper is bound to ceruloplasmin, but with heavy copper injections, it is possible to increase free plasma copper (8). From the present study, it appears that in the turkey the liver removes copper from the blood and excretes it, maintaining the plasma copper at a level

which is not raised by prolonged and massive copper injection. Wilson's disease is characterized by decreased ceruloplasmin, increased nonceruloplasmin copper, and accumulation of copper in the brain and other tissues. The course of the disease can be altered by administration of penicillamine, which reacts with free plasma copper to form a compound readily excreted by the kidneys. Although it is possible experimentally to increase copper levels in some tissues, Wilson's disease remains the only known instance of increased copper in the brain.

Summary. Chronic copper intoxication evidenced by diarrhea and failure to thrive was induced in the broad-breasted white turkey by heavy copper loading. None of the overt signs of Wilson's disease were noted. The present study suggests that metabolic and excretory pathways for copper in the turkey, and possibly other fowl, are different from those of the mammal.

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Errata

Vol. **127**, No. 2 (1968), in the article, "Enrichment of Antibody Plaque-Forming Cells of Spleen by Sedimentation at Unit Gravity," by Michael G. Mage, Warren H. Evans, and Elbert A. Peterson, pp. 478–481:

Page 479, column 2, lines 9–13 should read:

". . . The gradient was formed by connecting three vessels in series. The first vessel (connected to the inlet of the sedimentation chamber) contained 800 ml of 0.30% sucrose in Hanks' solution. . ."

Vol. **127**, No. 4 (1968), in the article, "Hydroxyurea Derivatives: 1-Hydroxybiuret and 3-Hydroxybiuret," by Glen R. Gale, Alayne B. Smith, and John B. Hynes, pp. 1191–1196:

Page 1192, legend of Figure 2, the last line should read:

"Abscissa: log ($M \times 10^5$).