Copper: Relation to Penicillamine-Induced Defect in Collagen

Abstract. *When rats were depleted of copper by administration of a copper-deficient diet, no abnormalities developed in dermal collagen. In contrast, marked changes were produced by penicillamine although the degree of copper-deficiency induced by the drug was less. Large additions of copper to the diet failed to prevent the penicillamine-induced collagen defect when the drug was given parenterally. The effect of penicillamine on soft-tissue collagen appears to be unrelated to its copper-chelating properties.*

Administration of penicillamine to weanling rats results in increase in the extractable collagen fraction in the skin (*1*); similar changes in dermal collagen have been demonstrated in human patients given this drug (*2*). Because penicillamine is both a sulfhydryl compound and a copper chelator, the depletion of copper by the drug was considered a possible mechanism for its effect on collagen. Animals raised on copper-deficient diets develop abnormalities in connective tissue, especially in bone and aortic elastin, that bear some similarities to experimental lathyrism (*3*). Since copper is believed to be necessary for formation of the aldehyde groups that are needed for collagen cross-linking (*4*), relative deficiency in copper could cause the drug-induced increase in soluble collagen.

In the first of two experiments for study of the role of copper in the composition of dermal collagen, deficiency in copper was induced in young rats by dietary means, and the changes in dermal collagen were compared to those produced by penicillamine. In the second, large supplements of copper were added to the regular diet, and penicillamine was injected concurrently; the effect on the collagen was studied.

Male rats of the Holtzman strain (weighing 100 to 120 g) were divided into four groups: group 1, receiving standard Purina rat chow, served as controls; group 2 received a special formula diet derived from milk, containing copper at 0.8 part per million (ppm) (5); group 3 received a similar diet with a copper content of 16 ppm. In all other respects the vitamin and mineral composition of these diets was the same as standard Purina rat chow. Animals receiving the special diets were placed in individual aluminum cages and given deionized water in copper-free plastic dispensers. Animals in group 4 received Purina rat chow with penicillamine added at 0.25 percent.

After 8 weeks the rats were decapitated and bled into heparinized copper-free tubes. Plasma was analyzed for total copper with a Perkin-Elmer model-303 atomic-absorption spectrophotometer. After yielding 100-mg samples of skin for collagen analysis, the carcasses (with the remaining skin) were completely charred in 200 ml of concentrated sulfuric acid. A mixture of 1 part concentrated perchloric acid and 6 parts 90-percent nitric acid was added slowly until the solution cleared. This solution was evaporated to dryness, and the residue was washed five times with distilled water. The supernatants were pooled and brought to a volume of 100 ml with distilled water. The copper content of this solution was measured against standards containing copper at 5, 10, 15, and 20 ppm in the atomic-absorption spectrophotometer.

Plasma and total body copper (Table 1) were significantly reduced by the copper-deficient diet (group 2) relative to the same diet supplemented with copper and to the standard Purina chow. Group 4 animals were similarly depleted in plasma copper, but total body copper was not reduced as much; this finding could be explained by the decrease in plasma copper. The mean values for body weight were only moderately lower in groups 2 and 3; group 2 animals showed no symptoms.

Tensile strength of skin fragments was measured with a Chatillon strain gauge; the force in grams after a 1-cm stretch of a fragment of dorsal skin (5 mm wide and 35 mm long) was determined on three samples, and the results were averaged. For determination of total collagen, a piece of dorsal skin weighing 30 to 40 mg was cleaned, minced, and hydrolyzed in 6*N* HCl for 3 hours in the autoclave, and analyzed for hydroxyproline content (*6*). Another sample of skin was extracted successively with 0.15*M* NaCl, 0.5*M* NaCl, and 0.5*M* citrate, *p*H 3.6, at 4°C. The extracts were dialyzed, pooled, hydrolyzed with acid, and analyzed for hydroxyproline for determination of the soluble collagen fraction. The results appear in Table 1.

Rats made deficient in copper by dietary means had normal values for tensile strength of skin and for soluble collagen. Total collagen values ranged from 38 to 48 μg of hydroxyproline per milligram of wet skin without difference between groups. Only the animals from group 4 showed reduction in tensile strength and increase in the soluble collagen fraction.

Male Holtzman rats weighing 100 to 120 g were divided into four groups and fed standard Purina rat chow. Group 1 served as controls; groups 2 and 3 received daily supplements of 1 and 25 mg of copper, respectively; groups 2, 3, and 4 received daily 25 mg of penicillamine intraperitoneally.

Table 1. Mean values obtained for plasma and total body copper, tensile strength, and soluble collagen for the groups of animals in the first series of experiments. See text.

Rats		Copper in		Tensile strength (g/cm)	Hydroxyproline (wet skin, μg/mg)
Group	Number	Plasma (μg/100 ml)	Body (μg/g)		
1	3	167.0 (155–185)	1.40 (1.13–1.63)	14.0 (13–17)	3.6 (2.3–3.7)
2	6	49.6 (30–70)	0.52 (0.24–0.69)	17.0 (14–19)	4.1 (3.1–4.4)
3	6	144.0 (125–175)	1.56 (1.05–1.75)	16.0 (13–20)	3.7 (3.2–4.0)
4	4	42.0 (34–50)	1.07 (1.0–1.12)	7.5 (7–8)	8.8 (6.6–10.0)

Table 2. Mean values of tensile strength and total and soluble collagen for the four groups of animals in the second series of experiments. See text.

Rats			Tensile strength (g/cm)	Hydroxyproline (wet skin, μg/mg)	
Group	Number	Final weight (g)		Total	Soluble
1	3	187(160–190)	6.3(5.2–8.0)	31.0(30–33)	3.7(3.0–4.0)
2	4	185(177–206)	2.4(1.5–4.2)	29.5(26–31)	9.7(8.2–11.8)
3	4	125(115–129)	3.1(2.1–3.8)	29.0(25–35)	9.2(7.3–11.7)
4	3	185(166–209)	3.2(3.0–4.0)	30.2(24–34)	10.5(8.3–13.5)

After 2 weeks the rats were killed and collagen was analyzed (see Table 2).

All animals receiving penicillamine (groups 2 to 4) developed decrease in tensile strength of skin and increase in the soluble collagen fraction. Oral supplementation of copper in low concentration (group 2), and in a concentration sufficiently high to be toxic and retard growth (group 3), failed to prevent development of the drug-induced collagen defect.

In the first series of experiments, the penicillamine-induced abnormalities in soft-tissue collagen occurred with only a moderate decrease in total body copper, while a far greater degree of copper depletion, produced by the copper-deficient diet, failed to result in similar changes. This finding suggested that the mechanism of action of penicillamine on collagen was not due to copper-depletion by chelation.

In the second experiment the rats received orally large supplements of copper, the penicillamine being administered parenterally to avoid formation of a penicillamine-copper chelate in the gastrointestinal tract, which would have rendered the drug unavailable. The results show that, even with massive doses of copper, administration of penicillamine produced the expected alterations in dermal collagen. The penicillamine-induced collagen defect is believed to be independent of copper, probably being related to the particular sulfhydryl reactivity of the molecule—perhaps by formation of a stable thiazolidine compound with aldehyde groups that would interfere with the cross-linkage of the collagen fibrils (7).

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Hypertrophic, Hypoactive Smooth Endoplasmic Reticulum: A Sensitive Indicator of Hepatotoxicity Exemplified by Dieldrin

Abstract. *Rats with hypertrophic smooth endoplasmic reticulum (ER) and increased activities of the drug-handling enzymes induced by dieldrin were stressed with larger doses of the pesticide. The activity of the drug-handling enzymes was thus reduced, but liver weight, smooth ER, and P-450 hemoprotein remained elevated. While no changes were apparent by light microscopy, the hypertrophic, hypoactive smooth ER was recognized as tight clusters of tubular membranes associated with abnormalities of the mitochondrial membrane. Similar but not identical morphologic changes were noted in human liver diseases associated with hepatic insufficiency. Hypertrophic, hypoactive smooth ER may indicate transition from adaptation of injury, and can be used as a sensitive parameter of toxicity.*

Ingestion of any of a wide variety of substances [including drugs (*1*), alcohol (*2*), and food contaminants such as pesticides (*3*)] effects enlargement of the liver and hypertrophy of hepatocellular smooth endoplasmic reticulum (ER). The ER is the site of drug-handling enzymes such as aniline hydroxylase and *p*-nitroreductase. These enzymes are part of a nicotinamide-adenine dinucleotide phosphate–dependent electron-transfer chain, the terminal component of which is the P-450 hemoprotein (*4*).

Usually the hypertrophic smooth ER is associated with increased activity of the drug-handling enzymes (*5*), and may thus reflect adaptation. However, it is also found in the presence of recognizable hepatic injury, such as experimental biliary obstruction (*6*), after administration of a carcinogen (*7*), and in diseases of the human liver in which detoxification is known to be impaired. Thus the following questions are raised: (i) Can hypertrophic smooth ER be hypofunctioning? (ii) Does hypertrophic, hypofunctioning smooth ER reflect toxicity, and does it precede changes detectable by light microscopy? (iii) Are there associated alterations of other organelles?

Rats in which tolerance to dieldrin had been induced were stressed with larger doses of this pesticide, and ultrastructural changes were compared with the activity of the drug-handling enzymes and the level of P-450 hemopro-

Table 1. Effects of administration of dieldrin on liver weight, on contents of microsomal protein and P-450 hemoprotein, on the activities of microsomal drug-handling enzymes, on mitochondrial proline oxidation, and on oxidative phosphorylation. Rats received dieldrin at 0.2 mg/100 g daily for 28 days, and at 0.5 mg/100 g daily during the following 24 days. Phases: NSS, new steady state; Decom., decompensation.

Item	Days on dieldrin			
	0 (Control)	14 (NSS)	28 (NSS)	52 (Decom.)
Liver weight (g/100 g)	3.35 ± 0.18§	4.03 ± 0.23	3.83 ± 0.025	4.00 ± 0.30
Microsomal protein (mg)*	71.0 ± .8‖	99.0 ± 12.0	96.0 ± 14.0	102.0 ± 11.7
Aniline hydroxylase (mμmoles)†	10.8 ± 1.3§	20.2 ± 4.2	17.5 ± 4.1‖	12.4 ± 1.8
p-Nitroreductase (mμmoles)†	105.0 ± 17.0§	196.0 ± 37.0	186.0 ± 17.0§	99.0 ± 9.0
P-450 hemoprotein (mμmoles)‡	0.55	1.01		1.10
Aniline hydroxylase: P-450	19.6	20.0		11.3
Proline oxidase (μmoles)*	2.53 ± 0.21	2.46 ± 0.16	2.49 ± 0.24§	1.76 ± 0.33
Oxidative phosphorylation	2.25	2.11	1.99	1.18

* Mean ± standard deviation (S.D.) relative to total liver per 100 g of body weight (ten determinations). † Enzyme activity per milligram of microsomal protein, ± S.D. (ten determinations). ‡ Average of four determinations. § Difference between the mean and the following mean is statistically significant ($p > .001$). ‖ Difference between the mean and the following mean is statistically significant ($p > .01$).