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Tensile Properties of Tendon in Copper-Deficient Swine[1] (39554)

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Reduced tensile strength and elastic moduli of aorta (1) and aortic elastin (2) in copper-deficient swine have been related to the decrease of intermolecular crosslinks in elastin (3) due to a deficiency of a copper-containing enzyme, lysyl oxidase (4). Collagen is also a substrate for this enzyme and similar cross-linkages are accredited with the stabilization of collagen fibrils (5). The relative extent to which collagen as opposed to elastin cross-linkages contribute to the tensile properties of copper-deficient tissues has been difficult to determine (6). The present study was undertaken to compare the effect of copper deficiency upon the tensile properties of a purely collagenous tendon with its known effect upon the complex collagenous and elastic tissue of aorta.

Materials and methods. Seven crossbred pigs from two litters were reared from 2 days of age on a basal diet of evaporated cow's milk diluted with sulfide water, supplemented with iron (7). From age 2 weeks, three of the pigs were supplemented with 0.5 mg of copper/kg body weight per day as aqueous copper sulfate to serve as controls. The volume of packed red blood cells (VPRC) and serum copper levels (by atomic absorption spectrometry) were monitored weekly after age 1 month. All the pigs were sacrificed by exsanguination under pentobarbital anesthesia at ages 61–68 days when the VPRC and serum copper levels of the experimental group indicated severe deficiency (Table I). Segments of descending thoracic aorta and tails were stored at −70° until tested. Samples of tendon were fixed for electronmicroscopy in 3% glutaraldehyde in phosphate buffer, postfixed in 2% OsO_4, dehydrated, stained with phosphotungstic acid in absolute ethanol, and embedded in Durcupan ACM[2] for sectioning. Sections stained with uranyl acetate and lead citrate were examined with a Siemens Elmiskop IA.

Aortic rings, 2 mm wide, were stretched on the device described previously (1) in phosphate-buffered saline at pH 7.4 with simultaneous recording of load measured by a Statham transducer and extension at a constant rate of 5 mm/min. Tendons were stripped of their sheaths, gripped in the jaws of alligator clamps between filter papers, and stretched on the same apparatus. Calculations of elastic moduli and ultimate tensile strength were made from measurements of cross-sectional diameters and unstrained length with the ocular micrometer of a stereomicroscope. Ultimate tensile strength was defined as the force (grams) at the breaking point divided by cross-sectional area (square centimeters) at rest. Extensibility was defined as increment of length (millimeters) at the yield point divided by the initial extended length at zero force. Elastic modulus was defined by the formula $(\Delta F/A)/(\Delta L/L)$, in grams per square centimeter per 100% elongation, utilizing load (F), cross-sectional area (A), and initial length (L). Elastic recoil was determined by repeated elongation under 50-g increments of load up to 400 g, returning to the initial length between successive elongations. Stress-relaxation behavior was determined at successive 50-g increments of load by time tracings until relaxation was complete.

Results. Parameters of copper deficiency in the experimental group are given in Table I. The serum copper levels were about 5% of the controls. The aortic tensile strength and elastic modulus were less than 30% of the controls, indicating severe weakening of the aortic walls.

Tail tendons of the same deficient pigs failed to show any significant change in mechanical properties. Tensile strength, elastic modulus, and strain at breaking were the

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[2] Registered trademark of Fluka AG Chemische Fabrik, 9470 Buchs SG, Switzerland.

same as those of the controls at the 5% level of confidence (Table II). Elastic recoil (Fig. 1) was unchanged below the yield point. Decay of stress, due to relaxation at constant elongation, amounted to about 50% of the maximal stress in the linear phase of the stress–strain curve (Fig. 2) and was indistinguishable from the control.

The ultramicroscopic structure of the deficient tail tendons, in keeping with their mechanical properties, did not differ appreciably from the controls. The tendon proper contained no elastin (Fig. 3), although the sheaths, which were removed before stretching, contained small amounts.

Discussion. The syndrome of copper deficiency is characterized by spontaneous rupture of arteries (7, 8). Defects of vascular elastic membranes precede the ruptures (9). Loss of mechanical strength of whole aorta in copper-deficient swine (1) is accompanied by a commensurate loss of strength of the isolated aortic elastin (2). Repair of aortic elastin and restoration of mechanical strength follow administration of copper to deficient swine (10). The change in mechanical properties of elastin is correlated with a decrease in lysine-derived intermolecular cross-links (3) and their aldehyde intermediates (11). This has been attributed to deficiency of a copper-containing lysyl oxidase which oxidizes the ϵ-amino group of peptidyl lysine to the corresponding aldehyde (4).

Collagen is also a substrate for lysyl oxidase and analogous lysine-derived intermolecular cross-links occur in collagen (12). Decrease in cross-linkage of collagen in copper deficiency is indicated by an increase in salt-soluble collagen in pig aorta (3) and chick Achilles tendon (13) and in acid-soluble collagen of chick bone (14). Alteration of the tensile properties of aortic collagen would be expected in copper deficiency but it has not been demonstrated because the physical structure of aortic collagen renders it unsuitable for stress-strain measurements (6). The structure of tail tendon fulfills the requirements which recommends its use for the purpose.

In view of the foregoing considerations, the failure of severe copper deficiency to affect the tensile properties of tail tendon is unexpected and warrants explanation. It suggests the possibility that tendon collagen cross-linkage may not be affected by copper deficiency, at least not to the extent that aortic elastin cross-linkage is affected. Previously reported work on chick bone and tendon makes this appear unlikely. Experiments have been undertaken to explore that possibility.

Meanwhile the alternative must be considered, namely, that the cross-links affected by copper deficiency may not be the limiting factor in the tensile properties of tendon that were tested, as they apparently are in elastin. While it is generally believed that these cross-links are responsible for the mechanical stabilization of collagen fibers, direct proof of this has not been found (15).

An important difference between the structure of elastic membranes and collagenous fibers may be a critical consideration in this problem. Elastin is a polymeric continuum and breakage of elastin laminae neces-

TABLE I. PARAMETERS OF COPPER DEFICIENCY.

Group	No. pigs	No. samples	Serum copper (μg/100 ml)	Aortic tensile strength (kg/cm^2)	Aortic elastic modulus (kg/cm^2/100% elongation)
Control	3	12	104.0 ± 4.0	11.30 ± 1.12	81.90 ± 15.37
Copper deficient	4	16	5.3 ± 0.7[a]	3.36 ± 0.58[a]	21.85 ± 5.73[a]

[a] $P < 0.001$.

TABLE II. TENSILE PROPERTIES OF PIG TAIL TENDON.

Group	No. pigs	No. tendons	Tensile strength (kg/cm^2)	Elastic modulus (kg/cm^2/100% elongation)	Strain at breaking point (% elongation)
Control	3	18	352 ± 57	1321 ± 133	36.0 ± 2.0
Copper deficient	4	27	327 ± 83[a]	1338 ± 82[a]	36.8 ± 2.0[a]

[a] $P > 0.05$.

sarily involves rupture of covalent bonds. Collagen consists of discontinuous subunits (fibrils) which may separate from one another by slippage, rather than by breakage (16). The role of weak bonds may be predominant in determining mechanical behavior of the highly ordered collagen molecules within the fibrils (17). The elastic recoil and stress-relaxation behavior indicate that these bonds are not affected by copper deficiency.

Summary. Young pigs were rendered copper deficient as demonstrated by serum copper levels and aortic tensile properties. Tail tendons of the same pigs had normal ultimate tensile strength, elastic modulus, elastic recoil, and stress-relaxation compared to controls. These results suggest that lysine-derived cross-links of insoluble collagen of tendon may not be decreased in copper deficiency, as are the analogous elastin cross-links, or that such cross-linkages may not be the limiting factor in determining tensile strength of collagen, as they appear to be in elastin.

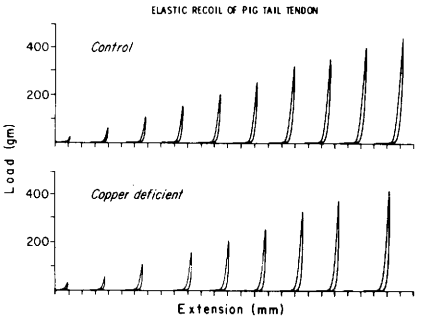

FIG. 1. Elastic recoil of pig tail tendon repeatedly extended at rate of 5 mm/min by increasing loads.

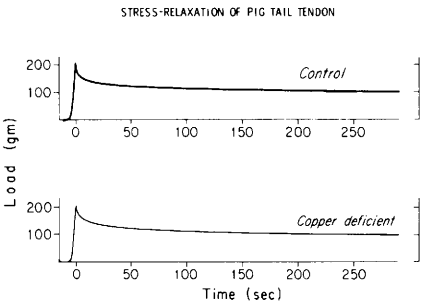

FIG. 2. Stress-relaxation of pig tail tendon maintained at constant extension.

FIG. 3. Electronmicrograph of control pig tail tendon. Small elastic fibers (arrow) are confined to the sheath (S). Collagenous fibrils of the tendon (T), fixed under extension by 50-g load, appear to have a low-pitched helical arrangement.

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