SECTION III

BASIC SCIENCES AND PATHOLOGY

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Trace Elements in Tendon Collagen

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Our interest in the structure and properties of bone, collagen, and bone mineral has led us to investigate the importance of the trace element content of these materials.[1-3,5] The regular occurrence of copper, for example, suggests a more than trivial relationship between trace amounts of this metal and the structure of bone.[1,3] In order to obtain further information on the association of certain trace elements with collagenous structures, we felt a study of the inorganic constituents of bovine tendon collagen was appropriate. Furthermore, some idea of the consistency of the concentration of these elements and the tenacity with which they are incorporated would help establish their relationship to the tendon collagen matrix, and thereby, to other analogous structures. To our knowledge, no general analysis of this nature has been reported for tendon.

METHODS

This investigation involved 2 groups of bovine Achilles tendons. The first group consisted of tendon collagen from 2 lots, obtained commercially,¶ which had been prepared according to the method used by Einbinder and Schubert.[4] This process removes salt-soluble proteins and mucopolysaccharides, yielding tendon collagen with less than 0.1 per cent hexosamine.

The second group consisted of tendons obtained in Syracuse, N. Y., and prepared in our laboratory. Five individual bovine Achilles tendons were removed from 5 freshly slaughtered animals and stripped of all loose connective tissue. The fresh weight of a typical tendon sample was 20 g. The tendon was cut into small pieces and dried for 24 hours at 100°C to a constant dry weight, found to be about 45 per cent of the initial wet weight. No attempt was made to wash, extract, or otherwise reduce the integrity of the specimens in this group.

All samples were ashed in a muffle furnace by raising the temperature in 50° increments over a 2-day period until 450°C was reached. Ashing was completed by keeping the samples at 450°C for an additional 24 hours. The ash

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¶ Schwartz Bioresearch.

per cent of dry weight averaged 0.4 per cent for the commercially-extracted tendons and 1.5 per cent for the locally-obtained tendons.

To obtain as much information as possible on a great many elements with high sensitivity, d-c arc emission spectroscopy was used for analysis. A Jarrell-Ash 1.5 m Wadsworth stigmatic spectrograph was employed in conjunction with a 220-volt, 9-ampere, free-current d-c arc source. Sample ash was diluted 1:9 and 1:99 with a 1:1 mixture of spectrographic grade Li_2CO_3 and graphite. The mixed samples and similarly prepared multi-element standards were excited in preformed graphite anode electrodes and the radiation was focused on a 10 μ slit on the spectrograph. Second-order spectra were recorded on Eastman S.A.-1 film and intensities measured on a comparator–microphotometer (Jarrell-Ash) in the range 2,300 A–4,300 A. The data were reduced and concentrations estimated using working curves of log transmission versus log concentration.[3]

RESULTS

The following elements were detected in one or more tendon samples: aluminum, calcium, copper, iron, lead, magnesium, manganese, nickel, phosphorus, potassium, silicon, silver, sodium, strontium, titanium, and zinc. Of these, sodium, potassium, calcium, phosphorus, and magnesium occured in larger than trace amounts, all having significantly higher concentrations (three-fold) in the dry, untreated material than in the dry extracted tendon. The average concentrations of the detected elements in ppm of dry sample are given in Figure 1 where the results for untreated and extracted tendon are shown and the estimated limits of detection are indicated. A contamination during the extraction procedure is suggested by the distinctly higher concentrations of many trace elements in the samples of extracted tendon.

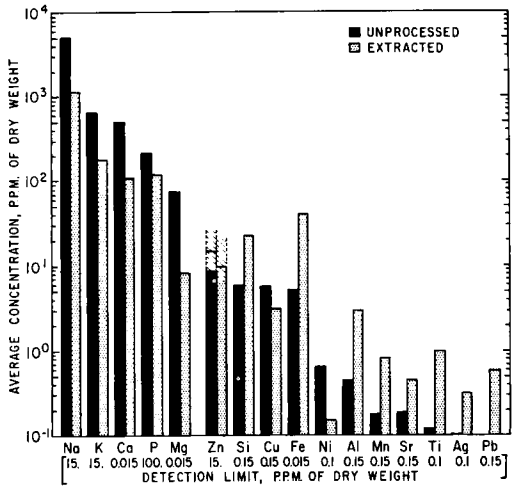

FIG. 1. Average concentrations of the elements detected in untreated tendon and commercially extracted tendon collagen in ppm of dry material on a log scale. The abundant elements are grouped separately from the trace constituents and the estimated limits of detection are indicated below the elements. The compositional differences between the extracted and untreated tendon are obvious as is the absence of lead in the untreated samples.

When compared on the same basis, the concentrations of sodium, potassium, calcium, and magnesium agree, within experimental error, with those reported by Urist, *et al.*[10] for fresh rat tail and rabbit Achilles tendon. The values for phosphorus by present methods, however, are approximately one-tenth as large as those of Urist, *et al.* with positive chemical methods.

The following elements were sought but not found present in sufficient quantities to be detected: As, Au, B, Ba, Be, Bi, Cd, Co, Cr, Dy, Ga, Hf, Hg, Ho, In, Ir, La, Mo, Os, Pd, Pt, Sb, Sn, Te, U, V, W. Their detection limits in ppm of dry weight were approximately an order of magnitude below those stated for the same elements in a previous report.[3]

Figure 2 illustrates the per cent standard deviations in the measured concentrations of each detected element in unprocessed tendon samples; these figures comprise both sample and experimental variations. Noteworthy is the remarkable consistency in the values obtained for iron and copper in the 5 ppm range. Of the elements investigated, copper showed the most consistency among all samples, including extracted as well as unprocessed tendon on a ppm of dry weight basis. The mean values for copper were 3.2 ppm

for dry extracted tendon and 5.7 ppm for the dry unprocessed tendon. Copper, therefore, is present in natural tendon, as well as in extracted tendon, and for the most part, is not removed during the extraction process.

Due to interferences, the zinc concentrations obtained in these experiments are only estimates; this element was present in all samples, however, in the range of 5 to 50 ppm of dry weight. Lead was notably absent from untreated samples, but present (0.6 ppm of dry weight) in extracted tendon collagen, probably as a processing contaminant.

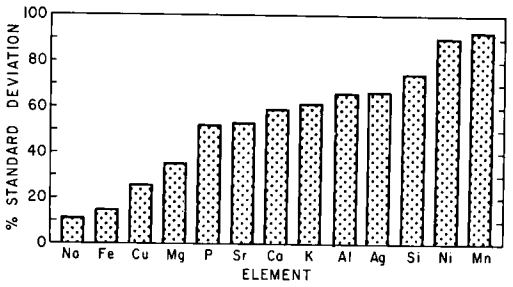

FIG. 2. The per cent standard deviations in the measured concentrations of elements found in the various untreated tendon samples examined. The values include both sample and experimental variations. Noteworthy are the relatively low values for iron and copper. Insufficient data were available to estimate the deviations for zinc, lead, and titanium.

DISCUSSION

The presence of large amounts of sodium, potassium, calcium, phosphorus, and magnesium in all tendon samples examined cannot be ignored. Considerable reductions in their concentrations appear to take place upon the extraction of soluble material from the whole tendon. The implication, therefore, is that these elements may be primarily associated with either the soluble protein or the mucopolysaccharides, or both. Even so, it should be pointed out that while tendon is not thought to be a calcified tissue, the ratio of dry weight concentrations of calcium to phosphorous was found here to be about 2.1 in the unprocessed tendon. Its similarity to the Ca/P ratio of hydroxyapatite (2.15) is evident. In addition, the sodium content of the unprocessed tendon is of the same magnitude as in bone,[6] but the ratio of magnesium to calcium is 10 times that of bone. Further investigation of the role of this group of elements in tendon is clearly necessary.

A second group of elements (Fe, Si, Al, Ti, Mn, Sr, Pb, Ag) was found in much higher concentrations in processed tendon collagen than in untreated tendon. Contamination with low-grade reagents during processing was probably the cause. Of this group, titanium and silver were detected in only one untreated sample and may be supposed to be relatively unimportant to tendon collagen structure in the concentration ranges accessible to us in this experiment.

Lead was the only member of this group of elements undetected in all untreated tendon samples. Lead is a common contaminant in contemporary human bone (50 ppm),[3,7-9] and is thought to exchange for calcium in that tissue.[6] Its absence (< 0.2 ppm) in untreated tendon, unless due to a lack of exposure *in vivo,* seems to confirm that lead is associated only with mineralized tissue. This leaves open the interesting possibility, however, that there may be structural differences between mineralizable and nonmineralizable collagenous matrices reflected in their relative ability to fix lead.

Copper and zinc comprise a third group of elements found in both types of tendon examined in approximately the same amount. Copper concentrations ($3 \rightarrow 5$ ppm) were extremely constant in all samples and had values consistent with those of bone ($\sim$ 3 ppm),[3,9] and human tendon (< 9 ppm).[5] This striking result supports the hypothesis of the importance of copper in collagenous structures and may indicate that copper atoms in some form are involved in the intramolecular, intermolecular or interfibrillar organization of collagen *in vivo.*

Zinc was present in all samples in signifi-

cant concentrations, and thus may rank with copper in importance, as in bone.[3] Further discussion of zinc must await a more refined analysis of this element.

Attention is similarly drawn to iron. Despite concentrations an order of magnitude higher in processed tendon, iron was found in untreated samples in 5 ppm of dry weight, with the smallest standard deviation of all the trace elements detected (Fig. 2). This concentration is approximately that found for this element in isolated human cortical bone.[3] The absolute value and constancy of the iron concentrations in untreated bovine tendon and human bone suggest that iron, if not a necessary component in these collagenous systems, may be associated with them in a non-trivial fashion. Furthermore, we have not failed to observe the similarities between the results for iron and copper in this experiment. Unless the similarities are coincidental, it may be that these ions have conplementary roles in collagen structure.

SUMMARY

A wealth of trace elements in tendon, usually ignored, is revealed by present investigations. Some important differences and similarities between mineralized and non-mineralized collagenous tissue believed to be unobserved previously are noted for further research work.

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