# CRYSTALLINE THREE-DIMENSIONAL PACKING IS A GENERAL CHARACTERISTIC OF TYPE I COLLAGEN FIBRILS

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## 1. Introduction

The X-ray diffraction pattern of moistened and stretched rat tail tendon has been known for several years [1,2] and demonstrates a crystalline packing of the collagen molecules within the fibrils. However, controversies have arisen about the meaning of this pattern leading to the proposal of several models (e.g. [3–6]). Furthermore it was proposed that this pattern was restricted to rat tail tendon and that it was not a general feature of collagen fibrils [7]. It has been found that the tail tendons of several mammals (such as mouse, grey squirrel, water vole or cat) do produce patterns similar to those for rat tail [8]. But these findings were restricted to tail material and trials on other tissues, e.g. turkey leg tendon [9] or bones [10], did not show any lattice.

We report here that the characteristic diffraction pattern given by rat-tail tendon is also produced by three other types of tissues (non-tail tissues) composed of Type I collagen: chicken- and turkey-leg tendon, bovine achilles tendon.

## 2. Material and methods

Freshly dissected material was kept in a freezer until use. The thinnest possible fibres were then chosen from the bird leg or dissected from the whole achilles tendon to avoid too high X-ray absorption. In order to enhance the 38 Å reflections [2], which are used as a criterion of the presence of the lattice, experiments were made on fixed fibres (2 h in 4% formaldehyde). Rat, chicken, turkey and bovine fibres were subsequently stained 2 h in 1% phosphotungstic acid pH 4.7 [11,12]. During the run, fibres were kept humid in a closed cell and stretched by about 10% to eliminate the crimp. X-Ray diffraction was performed on an Elliott GX 20 rotating-anode X-ray tube with a specimen–film distance of 17.5 cm.

## 3. Results

Although the diffraction pattern of chicken-leg tendon (fig.1) has the general characteristics of the pattern given by rat tail tendon (fig.2), it does not have the same crystallinity: in particular the reflections at about 13 Å spacing, corresponding to the interference between the neighbouring molecules [6]

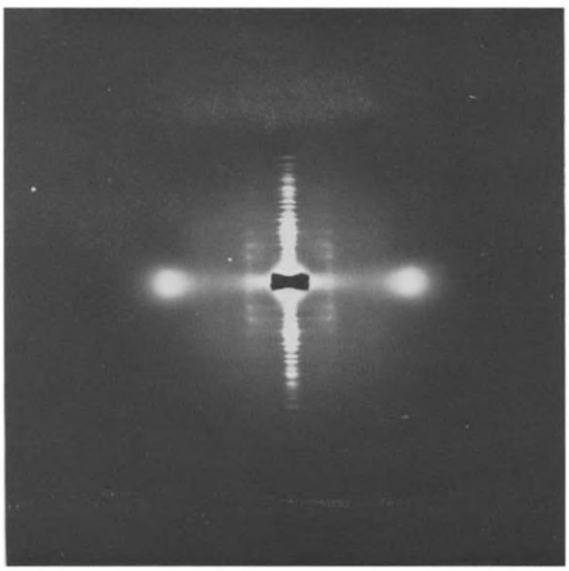

Fig.1. X-ray diffraction pattern of fixed and stained chicken leg tendon.

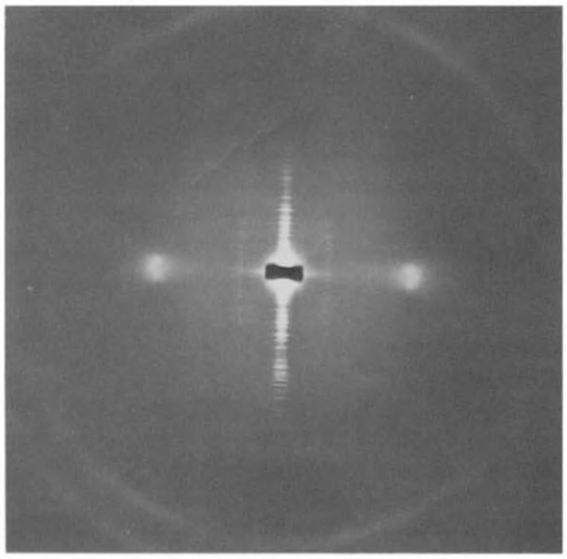

Fig.2. X-ray diffraction pattern of fixed and stained rat tail tendon.

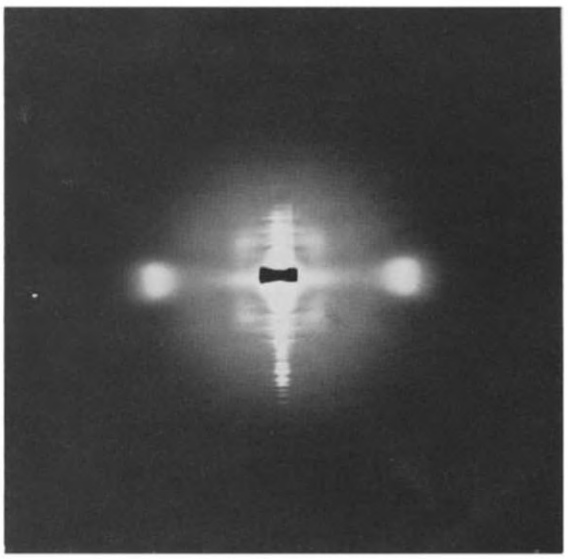

Fig.4. X-ray diffraction pattern of fixed and stained bovine Achilles tendon.

are not as well defined. A slight increase of the spacing of the '38 Å' row line (42.6 Å instead of 38.5 Å) might correspond to a general swelling of the molecular packing. The situation is the same in uncalcified turkey-leg tendon (fig.3) where the row line is at 39.9 Å. From calcified turkey-leg tendon, however, no sharp reflections could be observed in the equatorial plane thus confirming the findings of others [9,10]. Bovine achilles tendon (fig.4) also possesses a regular 3-D structure as demonstrated by reflections at 43 Å and 40 Å respectively.

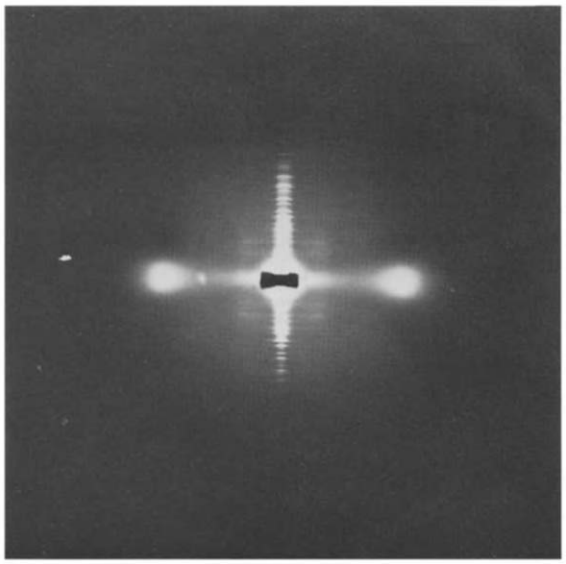

Fig.3. X-ray diffraction pattern of fixed and stained uncalcified turkey leg tendon.

## 4. Discussion

These findings demonstrate that the 3-D structure of collagen fibrils is not only a feature of tail tendons but can be found in other Type I collagen fibrils. The reason why the crystalline state has been observed up to now mainly in rat tail tendon and not in other tissues is due to the particular purity and orientation of the material. After staining, however, the enhanced reflections can be visualized in other tissues.

These results are not surprising if it is recalled that:

(i) the 3-D assembly of proteins depends primarily on their amino acid sequence;

(ii) the sequence of a given type of collagen (Type I, II, III or IV) varies little from one species to another [13,14].

Hence we may expect that, unless influenced by other molecules, collagen will assemble in the same way in different tissues. A crystalline arrangement,

such as demonstrated by the '38 Å' row-line, implies regular intermolecular interactions. This, in turn, permits the formation of regular intermolecular covalent crosslinks. What the above results show is that in some cases the geometrical regularity can be lost and yet the regular topology of crosslinks still preserved. Certain treatment such as fixing and staining can enhance the geometrical regularity so that it produces row-lines. However it is hazardous to conclude that the failure to observe sharp reflections in the equatorial region of the X-ray diffraction pattern implies lack of regular intermolecular topology.

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