THE FINE STRUCTURE OF CONNECTIVE TISSUE FIBRILS

by

A. W. PRATT AND RALPH W. G. WYCKOFF

Laboratory of Physical Biology, Experimental Biology and Medicine Institute, National Institutes of Health, Bethesda, Maryland (U.S.A.)

The evident regularities in their fine structure have made collagen fibers, from the first, objects of special interest to electron microscopy. Thus it was early[1] seen that the fibrils of tendon are regularly segmented cylinders (Fig. 1) which, though of very different diameters, have a distance of 600-650 Å between segments. Most studies to date have dealt with the value of this large spacing and the factors that influence it. They have shown that its exact value depends more on the way the fibril has been prepared than on the animal from which it has been taken. There exists, however, another group of questions which deals with the fine structure of the fibrils and their segmentations, and with the way they are bound together in different forms of connective tissue. The electron micrographs of the present paper contain information bearing on these latter problems.

No other white connective tissue seems as uniform and as simply organized as tendon. As seen in Achilles or tail tendon, for example, it consists essentially of bundles of parallel collagen fibrils so arranged that their segments often, though not always, have the excellent alignment of Fig. 2. These bundles are enclosed in a sheath which must be torn away to free the fibrils for examination, and they are embedded in a mucoid material that usually clings to them in amounts sufficient to mask the macromolecular detail about the individual fibrils. Connective tissues from other parts of the animal body contain similar collagen fibrils which sometimes can be obtained freer from this contaminating material and thereby show more clearly the nature of their segmentation. Examination of these has revealed the presence of a certain number of fibrils having a more or less unbroken sequence of cross-striations that seems different from that of Fig. 1 and 2. The electron micrographs that follow show new details of the striae and bring out structural relationships that exist between the two apparently dissimilar forms of collagen.

Several types of preparation have been used in connection with this work. To make one type, excised pieces of connective tissue, either fresh or fixed in formalin or osmic acid, have been teased apart in a small volume of water or shredded in a WARING blendor. Drops of the resulting suspensions have been placed on pieces of formvar-covered 200 mesh screen and, when dry, washed by dipping in several changes of distilled water. After subsequent shadowing with palladium, desired areas have been punched from the screening for electron microscopy. Another type of preparation has been obtained from fragments or thin frozen sections cleaned of other than connective tissue by extraction with salts or by prolonged digestion on the surface of a trypsin

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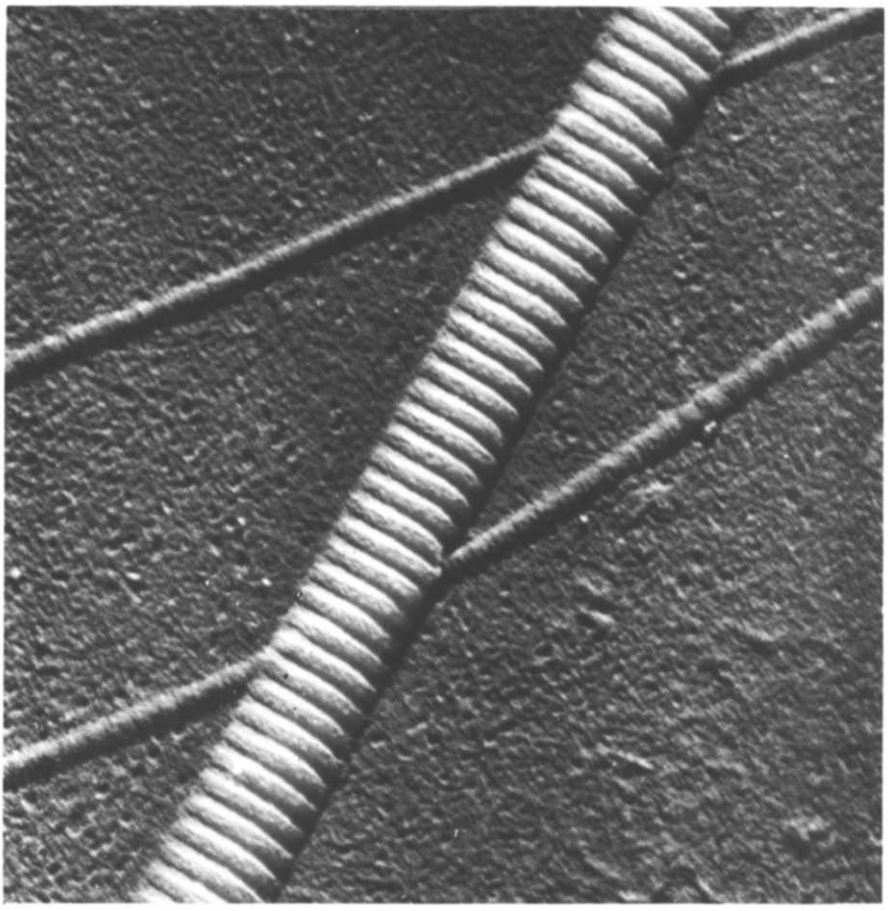

Fig. 1. Fibril of tendon freshly teased from the tail of the rat and examined without cleaning. Chromium shadowing. Magnification = 55 000 ×.

solution. These bits of collagen have commonly been picked up on glass slides, washed by being floated onto the surface of several changes of water and finally dried on a glass surface. They have then either been shadowed with palladium, covered with a thin supporting layer of collodion and stripped from the glass or have first been coated with collodion, stripped and then shadowed. Connective tissue in the neighborhood of muscle has been rendered cleaner by treatment with KCl according to the methods of SZENT-GYÖRGYI[2]. Yellow connective tissue has been digested with trypsin to leave behind a fibrous residue for examination. Connective tissue from a variety of sources has been studied but the present photographs are limited to Achilles and tail tendon and collagen from the heart of dog and rat.

Previously published photographs in common with such electron micrographs as those of Fig. 1 and 2 do not show clearly the structure responsible for the segmentation

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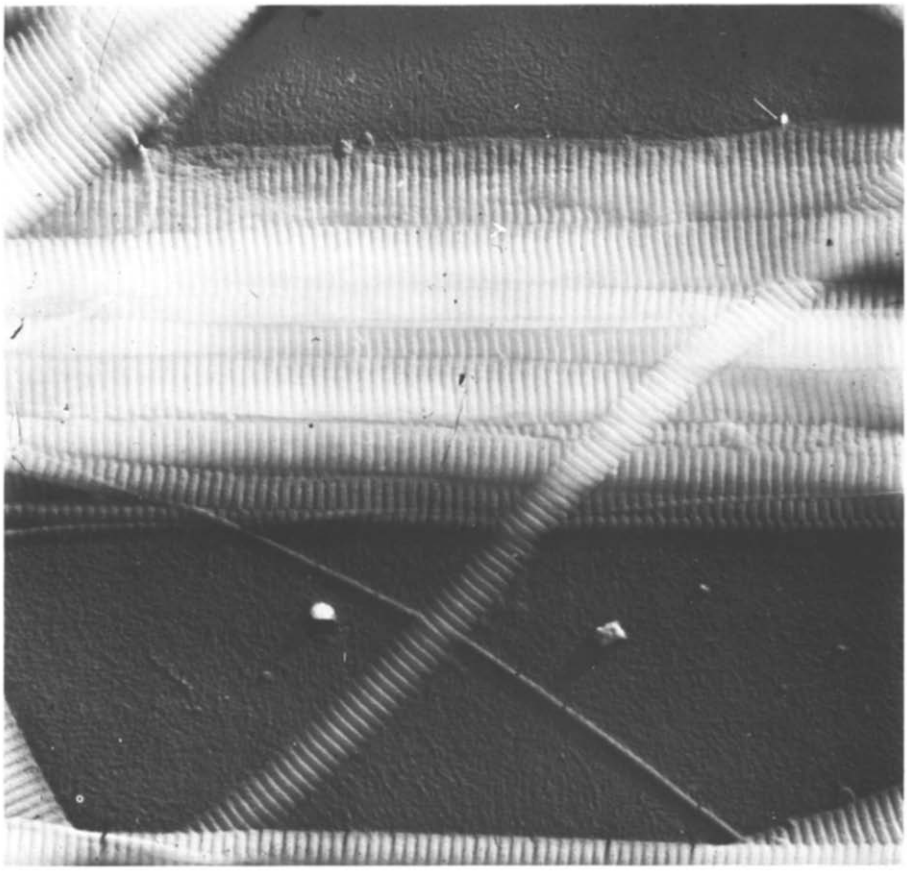

Fig. 2. Several fibrils from Achilles tendon. Failure to tease them apart has left their segments in parallel array. Some structure is visible on the upper thin edge of the topmost fibril. Chromium shadowing. Magnification = 25 000 ×.

of collagen fibrils. In Fig. 1 the segments appear only as raised or thickened regions devoid of well defined fine detail, but examination of cleaner fibrils such as those of Fig. 3 and 4 shows that a pair of filaments or threads traverses each segment. They are evident along the fibril that runs horizontally along the upper part of Fig. 3 and stand out equally well on the vertical fibril. They are well marked on the broad fibers of Fig. 4 and can be found in many parts of Fig. 5 and 6. The investigation of these and numerous other electron micrographs thus indicates that the segments consist of such pairs of cross-threads usually, as in Fig. 1 and 2, embedded in a structureless matrix. This matrix seems especially voluminous and hard to remove from the fibers of tendon; but even here the underlying threads can sometimes be seen, particularly on the thin edges of large fibers. When viewed in unshadowed preparations, both with and without phospho-

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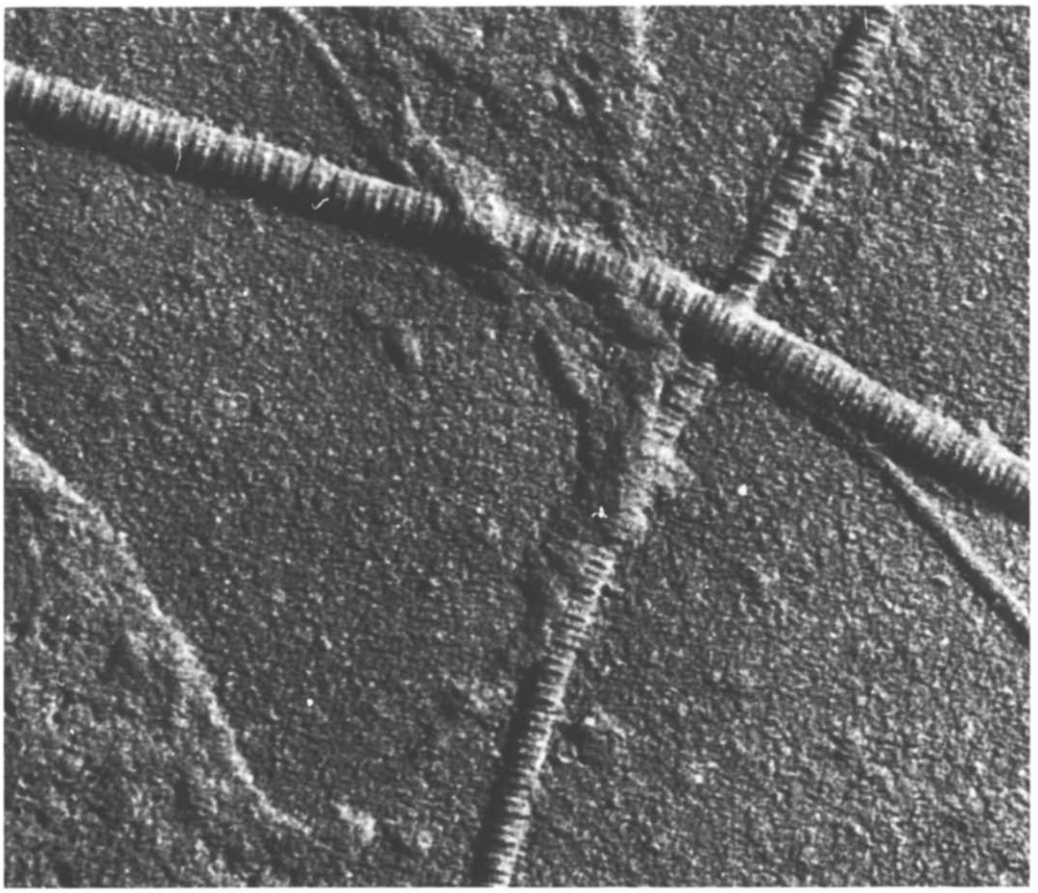

Fig. 3. Two fibrils of Achilles tendon of dog clean enough to manifest the pair of threads that underlie each segment. The third thread can be seen in a few of the hollows between pairs. Palladium shadowing. Magnification = 64 000 ×.

tungstic acid staining, the thick segments show greater electron scattering. The cross-threads certainly contribute to this greater "opacity", but the present photographs do not show whether or not there is also a periodic alteration in composition of the fibrillar contents.

The narrow horizontal fibril that appears especially clearly at the left of Fig. 4 has threads traversing it in an unbroken sequence rather than in pairs. Fibrils at the very top and elsewhere in Fig. 5 show the same thing. Close study, particularly of Fig. 3, 4 and 5, demonstrates that this continuous cross-striation results when an additional thread or filament fills each hollow between pairs. A few such threads are still retained in hollows of fibrils of Fig. 3 and 4; they are to be found on most of the fibrils of Fig. 5 and 6. As a consequence of this partial retention of the third cross-thread and of its

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Fig. 4. A group of collagen fibrils from dog heart in which are apparent the pairs of threads composing each segment. The several smaller fibrils at the right seem to merge into two larger fibrils towards the left of the figure. Small fibrils running horizontally through the middle of the photograph and vertically at the left are continuously striated. The bottommost fibril shows transition between the two patterns of striation. Palladium shadowing. Magnification = 55 000 ×.

evident difference from the others, regions of paired-thread segmentation and of uninterrupted cross-striation often follow one another along the same fibril. Were it not for this irregular succession of cross-striation along a single fiber it would be tempting to interpret the filaments or threads as evidence that a collagen fiber is built of a succession of disks (WOLPERS[3]). Such disks could conceivably be regions of especially high protein density or minimum water content. Swelling experiments as well as many aspects of such electron micrographs as these do not, however, accord well with such interpretations.

The thread-like appearance of the cross-striations, on the other hand, suggests that they may play a role in binding adjacent fibrils into the bundles in which they naturally occur. Though adjacent fibrils are often well aligned, as in Fig. 2, their cross-striae usually do not extend over more than one fibril. Nevertheless, a region can occasionally be found where threads seem to span adjacent fibrils. Thus at the left center of Fig. 5

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Fig. 5. Collagen fibrils from heart muscle of the dog. Some are continuously striated; in others the paired threads are clearly brought out. Palladium shadowing. Magnification = 40 000 ×.

Fig. 6. Another group of connective tissue fibrils from heart muscle. A fabric-like arrangement is shown by the fibrils in the center of the photograph. Palladium shadowing. Magnification = 40 000 ×.

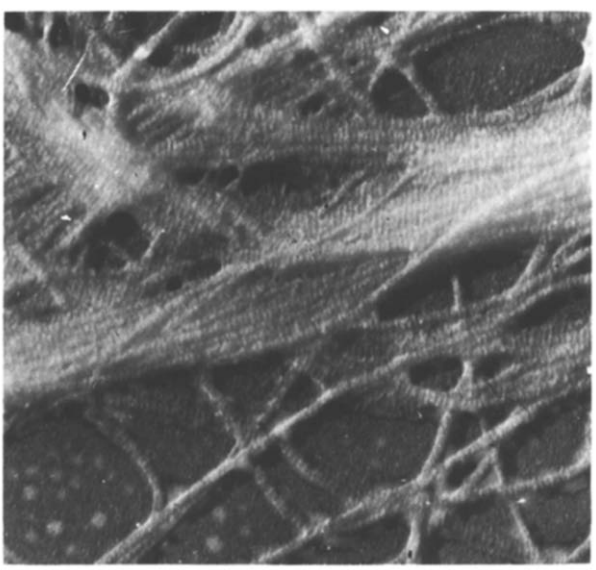

Fig. 7. Continuously cross-striated fibrils left behind after tryptic digestion of yellow connective tissue of the dog. Palladium shadowing. Magnification = 50 000 ×.

and along the horizontal fibrils crossing the middle of Fig. 6 a pair of fibrils may be spanned by every third cross-thread. Such a staggering is uncommon; usually, as in Fig. 2, the segments or their paired threads are aligned. Sometimes this alignment is so good that it is difficult to know whether one is seeing a single broad fiber or several well-aligned smaller ones. Thus in Fig. 4 it is uncertain whether or not the fibrils seen as separate at the top right have merged to form two broad fibers at the left of the field. It will be important to extend these studies of collagen to determine if the threads of its cross striations are indeed remnants of elaborate systems binding the fibrils together to establish the details of this fabric and the way its orderliness may be disturbed by disease.

All the present observations indicate that the continuously cross-striated fibrils are as much a component of healthy tissue as those in which only the familiar paired striations are apparent. Perhaps therefore they are not the same as the cross-striated "laminated" fibrils which WOLPERS[3] has considered as an embryonic or degenerate form of collagen. This will only become clear with the examination of diseased tissue.

Fibrils of small diameter occur in great numbers in other connective tissue than the tendons of tails and joints. Many of these are continuously cross-striated. Other cross-striated fibrils of small diameter are encountered in preparations of yellow connective tissue after digestion with trypsin (Fig. 7). Like the continuously cross-striated fibrils from white connective tissue they have a repetition distance along the fibril of ca 200 Å ($^1/_3$ of the basic 640 Å spacing of collagen), and they may constitute a collagenous framework to the elastic tissue.

SUMMARY

Electron micrographs of especially clean fibrils of collagen from dog and rat have shown their segments built up of pairs of threadlike striæ. Sometimes a third filament is seen in each hollow between pairs; then the fibril appears continuously cross-striated with a distance between striæ of ca $\frac{640 \text{ Å}}{3} = 210$ Å. Transitions between the two types of striation are often found in the same fiber. Perhaps these threads are the remains of a second transverse system of filaments which bind the separate collagen fibers into a fabric-like structure.

RÉSUMÉ

Les photographies prises au microscope électronique de fibres de collagène spécialement purifiées du chien et de rat montrent que les segments de ces fibres sont constitués par des paires de stries filiformes. Parfois un troisième filament est visible dans chacun des creux formés par deux paires de stries; la fibre apparaît alors comme striée transversalement de façon continue et la distance entre les stries est de $\frac{640 \text{ Å}}{3} = 210$ Å environ. On trouve parfois dans une même fibre des formes de transition entre les deux types de striure. Peut-être que ces stries sont les restes d'un second système transversal de filaments qui lient en texture les fibres de collagène isolées.

ZUSAMMENFASSUNG

Elektronen-Mikrogramme von besonders gereinigten Kollagenfibern vom Hund und der Ratte haben gezeigt, dass die Segmente dieser Fibern aus Paaren von fadenförmigen Streifen aufgebaut sind. Manchmal ist in jedem Zwischenraum zwischen den einzelnen Paaren ein dritter Faden sichtbar; dann erscheint die Fiber als durchlaufend quergestreift und zwar mit einer Entfernung zwischen den Streifen von ca $\frac{640 \text{ Å}}{3} = 210$ Å. Oft findet man in derselben Faser Übergangsformen zwischen den beiden Typen. Vielleicht sind diese Fäden die Überreste eines zweiten transversal verlaufenden Fasersystems das die einzelnen Kollagenfibern zu einer gewebeartigen Struktur verbindet.

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