SOME OBSERVATIONS ON DERMAL COLLAGEN FIBRILS IN ULTRA-THIN SECTIONS*

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The ultra-structure of collagen fibrils has been known for a long time (1, 2, 3). The number of typical cross bands in one period depends on the methods of preparation employed. Wolpers (2, 3) originally described only two cross bands, but more advanced methods now permit us to detect up to 14 (4, 5, 6, 7) and to correlate them with the structural arrangement of macromolecules (6, 8, 9). Thus the ultrastructure of the single collagen fibril down to the elementary microfibrils (7, 10, 11, 12) has been well defined by means of the electron microscope, whereas the interfibrillary relationships of the collagen fiber have received far less attention and are as a result less well-known.

The reason is largely that in the past such investigations were only seldom done on ultra-thin tissue sections. It is technically difficult to demonstrate the pattern of the detailed intraperiod-fine-structure of the fibrils (13). Usually only a few interbands are visible within the cross bands of the fibrils, or only one or two fibrils with highly subdivided cross striation pattern were demonstrable in a collagen fiber (13). These examinations were mainly carried out on animal tendons; so far we know nothing of investigations on the behavior of normal human skin collagen in ultrathin sections.

MATERIAL AND METHODS

From the thigh of a 27 year old male person some healthy skin was excised under local anesthesia. The biopsy material was fixed in cooled, buffered, isotonic osmium-tetroxide solution (1%) at pH 7.2. The fixation time was 4 hours. After dehydration in acetone, the specimens were embedded in Vestopal W and sectioned in the routine manner using LKB-ultrotome. The ultra-thin sections were stained with PTA (0.5%) solution and studied by means of Siemens (Elmiskop I)-electron microscope in the Laboratory of Electron-Microscopy (Chief: W. Vogell, Ph.D.). For the purpose of an exact estimation of the periodicity in part of our sections, the grids and sections, after staining, were covered with Latex balls of an identical diameter (of 0.333 μm).

RESULTS

We were able to make certain interesting observations on collagen in ultra-thin tissue sections of normal human dermis.

The axial spacing of cutaneous collagen fibrils in our material was found to be 570 Å. In each period up to 8 intraperiod bands could be distinguished after PTA staining (Fig. 1). The diameter of fibrils averaged 900 Å in 100 fibrils measured. This varied between 1100 Å and 600 Å.

1. The Antiparallel Arrangement of Fibrils in the Collagen Fiber: An exact analysis of our material showed that within one collagen fiber two types of fibrils are clearly recognizable by the orientation of their cross band pattern. The characteristic of these two types of fibrils is the antiparallel orientation of their interperiod bands. They may be referred to as Right-type and Left-type (R- and L-type), whereas their diameter, periodic spacing and also the arrangement of the sequence of their cross bands do not differ. As shown in Fig. 1, this kind of inversed sequential arrangement may be observed in several adjacent fibrils within a collagen fiber and we can distinguish alternating R- and L-types. According to the results so far obtained, the quantitative proportion of the two fibril types is approximately 1:1. It is interesting to note that the more definite a-bands (for nomenclature cf. Schmitt and Gross (14)) are frequently aligned in transversal direction of the whole fiber.

2. The Branching of Fibrils. So far we have observed this phenomenon only once during our investigations. Although we are aware of the fact that some authors (15) would regard this as an artefact, the phenomenon was so striking that we like to describe it here. As shown in Fig. 2, the fibril marked x, appears to be branching in two parts (y and z). Whether these two branches continue into the adjacent fibrils cannot be stated with absolute certainty.

3. The Visualization of Elementary Micro-
fibrils in One Collagen Fibril. Under normal conditions the visualization within one collagen fibril of elementary microfibrils is a rare occurrence. However, it need by no means be a pathological symptom: Fig. 3 shows normal healthy skin.

DISCUSSION

Our results demonstrate that even ultra-thin sections are eminently suitable for detailed studies on the ultrastructure of dermal collagen fibrils and their interfibrillar relations.

In our material (i.e. in normal dermis) the periodic spacing of collagen fibrils was 570 Å; Fisher and Rodnan (16) found 540 Å. Even under pathological conditions no significant deviations from these values were observed; the results of examinations in the case of scleroderma (540 Å) by Fisher and Rodnan (16) and observations made by us on dermatosclerosis (590 Å) would appear to be sufficient proof. Although these repeating axial periods deviate noticeably from the usual spacing of 640 Å (1, 3), they cannot be interpreted as evidence of pathological changes in collagen fibrils.

The diameter of collagen fibrils does vary under physiological conditions from 800 to 1400 Å (17 and others) and depends on the age of the subject (18, 19). Our average values of 900 Å are quite in agreement with these findings and other evidence obtained by physical fragmentation of tissue examined.

The highly subdivided units of collagen fibrils of the normal human skin after staining with PTA could also be demonstrated on ultra-thin sections.
Fig. 3. Collagen fibrils (ultra-thin skin section). Visualization of elementary microfibrils within a normal collagen fibril. 10,000 X.

of the normal dermis. We found up to 8 cross bands per period, and were able to definitely identify the following bands (adopting the nomenclature of Schmitt and Gross (14)): a, b₁, b₂, c (often occurring as a doublet), d, e (likewise often occurring as a doublet). Until now only a few authors have demonstrated the highly subdivided cross banding of fibrils on other types of material of animal origin (10, 13, 20). The arrangement of the highly subdivided periods is entirely in agreement with previous observations made on single isolated fibrils (4, 6, 10, 14).

The most important observation seems to be that in normal human dermis the collagen fibrils within a fiber show an antiparallel arrangement, i.e. an inverse sequence of the cross bands in adjacent fibrils. This behavior has not previously been observed in human skin collagen. On the basis of their investigations on animal tissues Nemetschek (4, 10) and Karrer (21) pointed to the possible antiparallel orientation of adjacent collagen fibrils. Gieseking (13) not only observed on ultra-thin sections of rat's tail tendon an antiparallel alignment of adjacent fibrils but also found that both types of fibrils (L-type and R-type) occur in a ratio of approximately 1:1. These findings are particularly noteworthy as, according to Harkness (15), the antiparallel alignments of fibrils is not usual. The significance of the antiparallel orientation of adjacent fibrils is at present unknown (21). According to Gieseking (13), this arrangement prevents adjacent fibrils from fusing in spite of their dense parallel packing and the presence of a large amount of available reactive groups on their surfaces. However, two similar types of fibrils may be adjacent without fusion taking place, and this would appear to contradict this suggestion. We would suggest that the antiparallel orientation of the cross banding in adjoining fibrils produces, through the Beyersdorfer effect* a state of internal tension within the bundle of fibrils and may play an important role in the cohesion of the collagen fiber (22, 23).

In this connection, the observations of Schmitt et al. (1) are of interest. They have shown that individual collagen fibrils are highly extensible and can be stretched to 10 times (to 6000 Å) their original length. The whole collagen fiber, however, cannot be extended to such a degree and they interpret these findings as being due to cohesive forces between fibrils or because of the presence of some binding material. These results can be explained by the antiparallel alignment of fibrils in a collagen fiber as a consequence of the Beyersdorfer effect, i.e. by the tendency of identical cross bands to attract each other within one period of adjacent fibrils. These remarks would mean that cohesion of identical structural elements was important not only during collagen formation (10) but also for the cohesive forces existing between fibrils in the mature collagen fiber.

We may conclude from present investigations that the antiparallel orientation of periodicity in adjacent fibrils is a general structural principle in the formation of the collagen fiber which, as could be shown by us, also applies to the collagen of the human skin.

A branching and anastomosing of collagen fibrils is generally rejected (24, 25, 26). However, Harkness (15) believes that in highly collagenous tissues of primary mechanical function such as tendon and sclera collagen fibrils “do join to form an almost continuous structure”. Our observation (Fig. 2) shows that also in human dermis a branching and perhaps an anastomosing of collagen fibrils may occur.

In normal human dermis the visualization of...

* I.e. mutual attraction of identical structural elements.
elementary microfibrils within fibrils is only seldom observed. These closely resemble those microfibrils artificially produced by acetic acid (10). The structures, we described, were not produced by artificial means and this would seem to confirm the current conception of the structure of collagen fibers (7, 10). It ought to be pointed out that such a rare occurrence as the appearance of elementary microfibrils within a collagen fibril must not always be considered to be of pathological significance.

SUMMARY

Collagen fibrils and interfibrillary relations within collagen fibers were investigated on ultrathin sections of normal human skin. The following observations were made:

1. Collagen fibrils usually showed repeating axial periods of 570 Å. By means of PTA-staining it was possible to demonstrate the intraperiod fine structure up to 8 intraperiod bands. The average diameter of fibrils was 900 Å.

2. Adjacent collagen fibrils within a bundle often showed inverse sequence of cross bands (antiparallel arrangements). Therefore there exists in the human skin two types of fibrils, L-type and R-type which are characterized by inverse orientation of their intraperiod cross bands. It may be assumed that this kind of antiparallel orientation of adjacent fibrils constitutes an important feature in the formation of the collagen fiber. The possible role of the Beyersdorfer effect was discussed in relation to the cohesion of the fibrils within the collagen fiber.

3. Branching of collagen fibrils and visualization of one fibril into elementary microfibrils is also occasionally observed in normal dermis. It follows that the appearance of these microfibrils should not necessarily be assumed to be pathological.

REFERENCES


