Acta Dermatovener (Stockholm) 51: 27–34, 1971

HYALURONATE (?) MICROFIBRILS IN HUMAN DERMIS

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Abstract. Microfibrils unrelated to collagen and elastic fibrils were demonstrated in connective tissue. They were located between collagen and elastic fibrils of human dermis and showed two different patterns, both with smooth profiles. One was thread-like with thicknesses varying from 20 Å to less than 400 Å in width. The thicker thread-like microfibrils ramified into thinner branches, and anastomosing thin threads were seen forming large meshes. The other type showed a lattice-like pattern located between collagen fibrils. These microfibrils are presumed to represent hyaluronic acid or a hyaluronate-protein complex.

Microfibrils have been found between collagen and elastic fibres of aorta, skin, ligamentum nuchae and synovium in man and experimental animals (7, 26, 10, 11, 13, 19, 22). Their elastic nature had not been clarified until recent electron microscopic studies of elastic fibres in bovine ligamentum nuchae (17, 26) and in human skin (16) were published. These studies revealed a fibrillar substructure of elastic fibres called "microfibrils of elastic fiber" (7, 26) or "elastic fibrils" (16). Further electron microscopic studies of normal and diseased human skin revealed a different type of microfibril, the ultrastructure of which is described below.

MATERIAL AND METHODS

Biopsied skin pieces of 3 normal human individuals were immediately fixed in an ice-cold 6% glutaraldehyde solution with 10% sucrose, buffered at pH 7.4 by a veronal acetate buffer. The specimens remained in this fixative for 1 hour. Some biopsies were fixed in the same fixative containing alcian blue at a concentration of 1%. The alcian blue changed the pH of the fixative to 6.2. All specimens were post-fixed in a 1% osmic acid solution in the same buffer with 10% sucrose at 4°C for 1 hour. After dehydration, the specimens were embedded in Epon 812. Ultrathin sections were stained by uranyl acetate plus lead citrate or separately by lead citrate or uranyl acetate. Hyaluronidase was applied for 6 hours at 37°C to fresh samples and to glutaraldehyde-fixed tissue sections. The enzyme solution contained 3 mg (about 1100 i.u.) of testicular hyaluronidase (Sigma) per ml 0.1 mol phosphate buffer, pH 7.1. For control of the hyaluronidase effect the plain solvent was used. Both hyaluronidase-influenced and control ultrathin sections were stained by lead citrate. A Siemens electron microscope (Elmiskop IA) was operated at 80 kV with double condensors, a 200 nm condensor aperture, and a 30 nm objective aperture.

OBSERVATIONS

Two different types of microfibril were seen, one as branched threads, the other as a lattice-like pattern (Fig. 1). The former was found among collagen fibril bundles and elastic fibres of various thicknesses and lengths. The thickest was 300 to 400 Å wide and ramified to thinner branches (Fig. 3). The thinnest was less than 20 Å wide. They showed smooth profiles, and the anastomosing thin branches tended to form large meshes (Figs. 2 and 3). Occasionally, thicker threads showed indistinct segments at intervals of about 400 Å (Fig. 3) or dense fine granules along their surfaces (Fig. 5). No increased contrast of the microfibrils was seen after alcian blue-glutaraldehyde fixation. If stained with lead citrate, however, the microfibrils showed good contrast.

Lattice-like patterns were seen crossing the spaces between collagen fibrils, occasionally continuing into the first-mentioned threads (Figs. 1 and 4). The sizes and numbers of the microfibrils varied from one specimen to another. Uranyl acetate stained the fibrils faintly to a contrast simulating the surrounding collagen fibrils (Fig. 6), while lead citrate yielded a stronger contrast than collagen (Fig. 5).

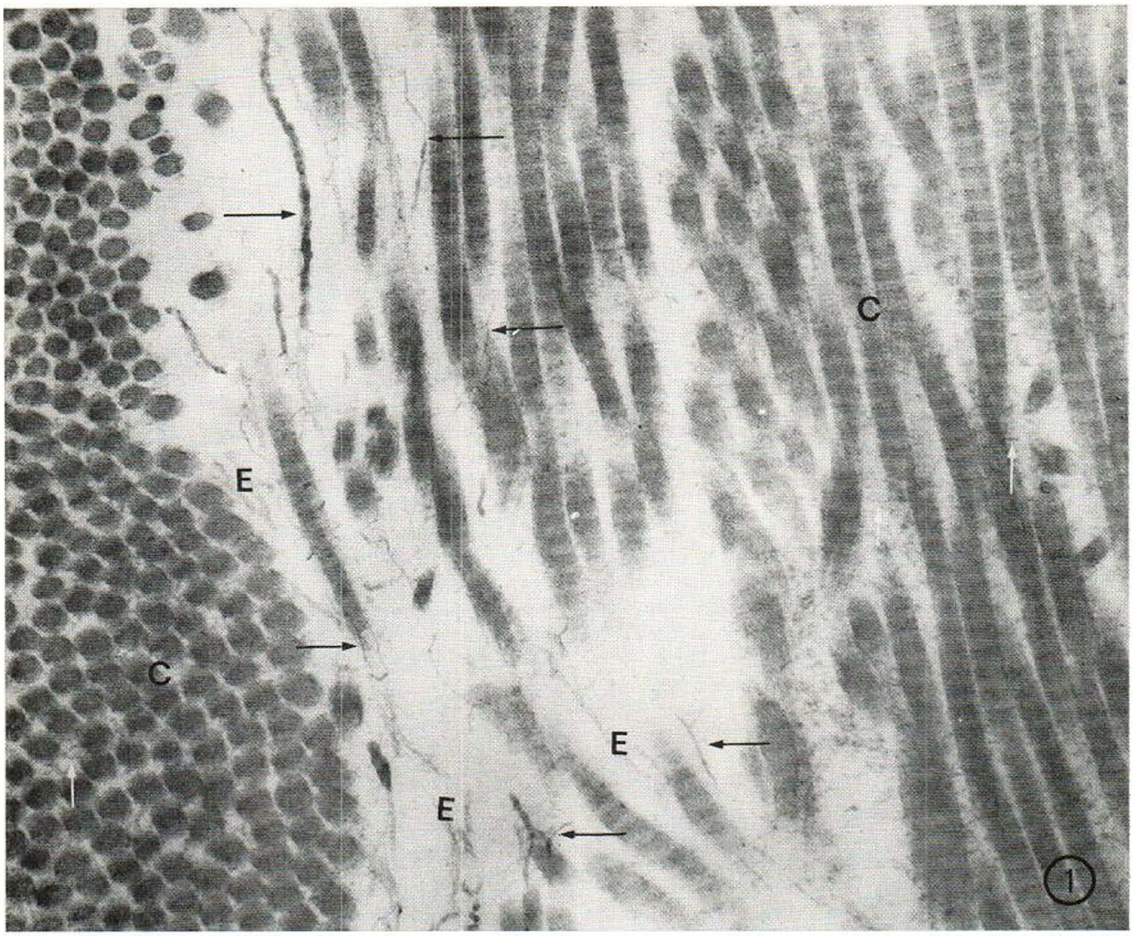

Fig. 1. Middle portion of normal human dermis. Two types of microfibrils are seen among collagen (*C*) and elastic (*E*) fibrils. Threads (*black arrows*) show various thicknessess, branches and anastomoses. Lattice-like microfibrils are located between collagen fibrils (*white arrows*). Uranyl acetate plus lead citrate stain. × 49 000.

After the influence of testicular hyaluronidase, the thick thread-like microfibrils showed no definite fibrillar shape and there was a considerable reduction of contrast, in comparison to the contrast of the surrounding collagen fibrils. Thinner microfibrils could not be demonstrated at all (Figs. 7, 8, 9 and 10). The control sections showed figures similar to those of non-treated specimens. The lattice-like and the thread-like microfibrils underwent identical changes (Figs. 9 and 10). Glutaraldehyde did not interfere with the effect of hyaluronidase (Figs. 7, 8, 9 and 10).

DISCUSSION

In a recent paper, the elastic fibril was described as a 90 Å wide thread showing a somewhat granular profile and occasional beads at 90 Å intervals after uranyl acetate plus lead citrate stain. A tendency to twisting was also noticed, while no branching was reported (16). These electron microscopic characteristics of the elastic fibrils differ definitely from those of the microfibrils described above. Hitherto, the ultrastructure of mucopolysaccharides has posed an unsolved problem for the electron microscopist. However, lead citrate is known to stain polysaccharide-rich tissue components, i.e. epithelial mucin (5), glycogen (5) and mast cell granules (15). Recently, possible ultrastructural patterns of mucopolysaccharides in connective tissue have been described by some authors as fine filaments and dense spots in cartilage (33), aorta (6, 31), synovium (6) and stroma

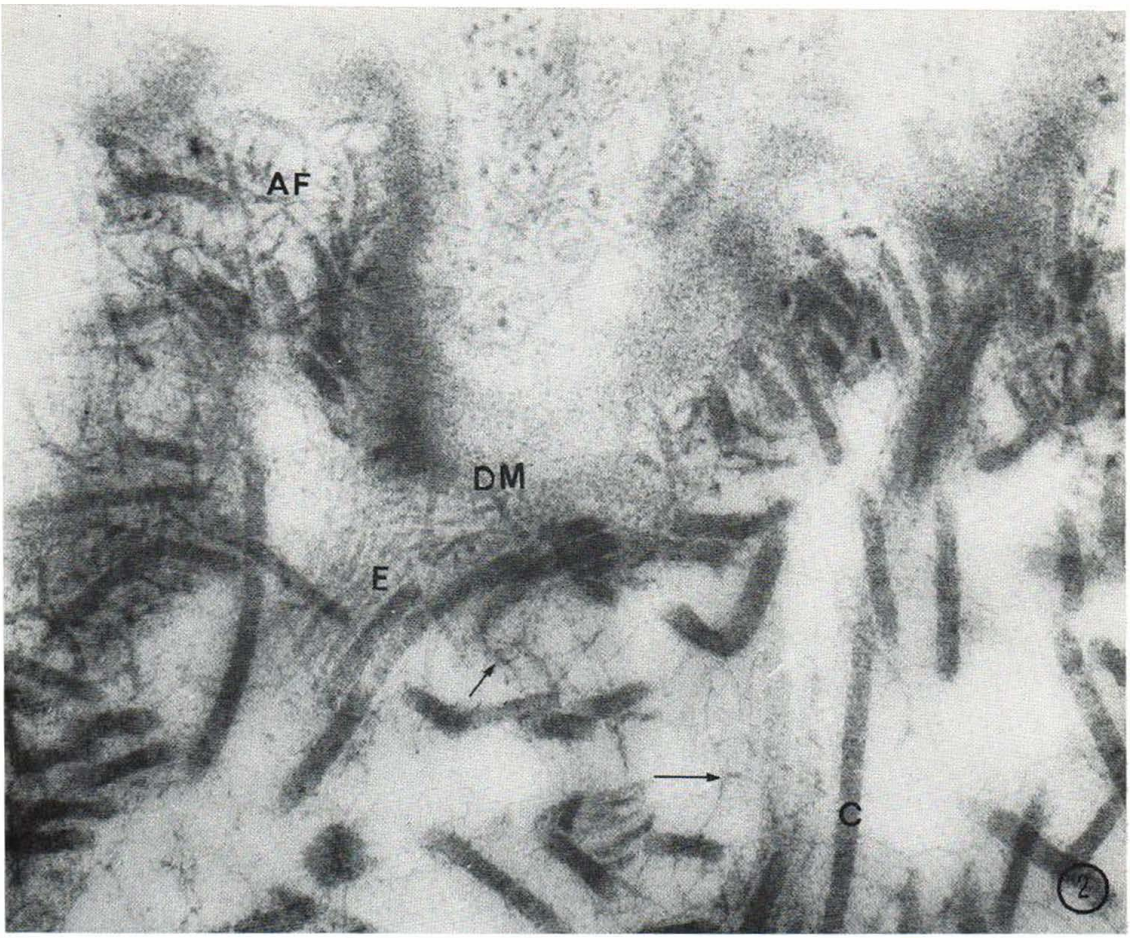

Fig. 2. Dermis close to the dermal membrane (*DM*). Microfibrils form large meshes by anastomosis (*arrows*). Elastic fibrils (*E*); collagen fibrils (*C*); anchoring fibrils (*AF*). Uranyl acetate plus lead citrate stain. × 98 000.

of basal cell carcinoma (17). The molecule of potassium hyaluronate was observed by Jensen & Carlsen (12) as a filament which was several thousand Å long and less than 30 Å wide. A heparin-protein complex from ox liver capsule has been demonstrated as a beaded filament composed of particles of about 35 Å in diameter (29), and chondroitin sulfate from bovine nasal cartilage as a chain of particles of a length varying from 100 Å to 1500 Å. Each of these particles had a diameter of about 30 Å (28).

Specific histochemical visualization of mucopolysaccharides under the electron beam has been attempted by several authors. The techniques used were reduced silver solutions (silver methenamine, ammoniacal silver carbonate and silver proteinate) after periodic acid oxidation (3, 9, 14, 24, 32), colloidal iron (4, 30), thorotrast (1, 25), bismuth nitrate (28, 29) and phosphotungstic acid (27). Among these, thorotrast and colloidal iron stain acid mucopolysaccharides (1, 4, 25). Particles of colloidal iron were found to be deposited between collagen fibrils of the jejunal wall, and thorotrast in the ground substance of cartilage. These findings seemed to indicate acid mucopolysaccharides located in the interfibrillar spaces, but no morphologic figures of acid mucopolysaccharide were described. Alcian blue and ruthenium red, both histochemical dyes for acid mucopolysaccharide, have also been applied for electron microscopy (8, 20, 21, 23, 34). When the dyes were used for tissue slices in combination with glutaraldehyde fixation

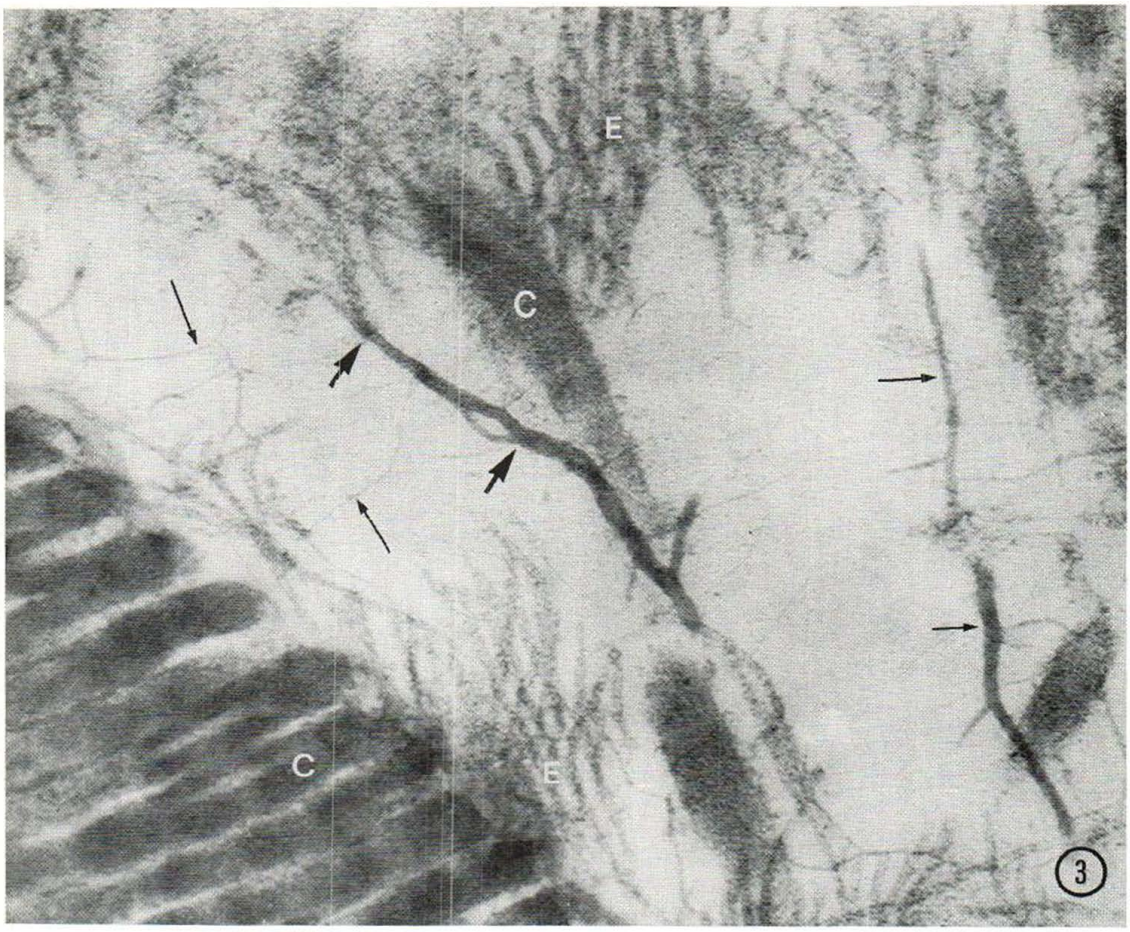

Fig. 3. Microfibrils of various thicknesses form large meshes (*arrows*) and show smoother profiles than elastic fibrils (*E*). Between thick arrows, a microfibril indicates indistinct segmentation at intervals of 400 Å. Collagen fibrils (*C*). Uranyl acetate plus lead citrate stain. × 98 000.

and osmification, the contrast increased on the cell surfaces (34). However, when applied to Epon-embedded ultrathin sections, sufficient contrast was only found in tissue components of very high mucopolysaccharide content, i.e. mast cell granules (18), while the cell surfaces did not show increased contrast (18).

Using ruthenium red in the fixative, cartilage (23), mast cell granules (8) and ground substance of synovium (21) have been studied. In synovial membrane, amorphous and filamentous material similar to that of this report have been seen previously, but not interpreted (21). Furthermore, adsorption of the dyes and colloidal iron to cell surfaces and to interfibrillar spaces should be considered for interpretation of the above-mentioned papers (4, 20, 21, 23, 25, 34) in which these reagents were applied to tissue-slices. Therefore, no conclusive findings of acid mucopolysaccharide ultrastructure have been presented in the previous papers.

Some authors have recommended cetyl pyridinium chloride in the glutaraldehyde fixative for better preservation of mucopolysaccharides (4). Glutaraldehyde and osmic acid do not precipitate acid mucopolysaccharides, but low temperature (0° to 4°C) and a content of 10 % sucrose may prevent the extraction of mucopolysaccharides from the tissue until dehydration begins. Thus, the hyaluronidase-sensitive microfibrils seen in this study may represent hyaluronic acid or its protein complex.

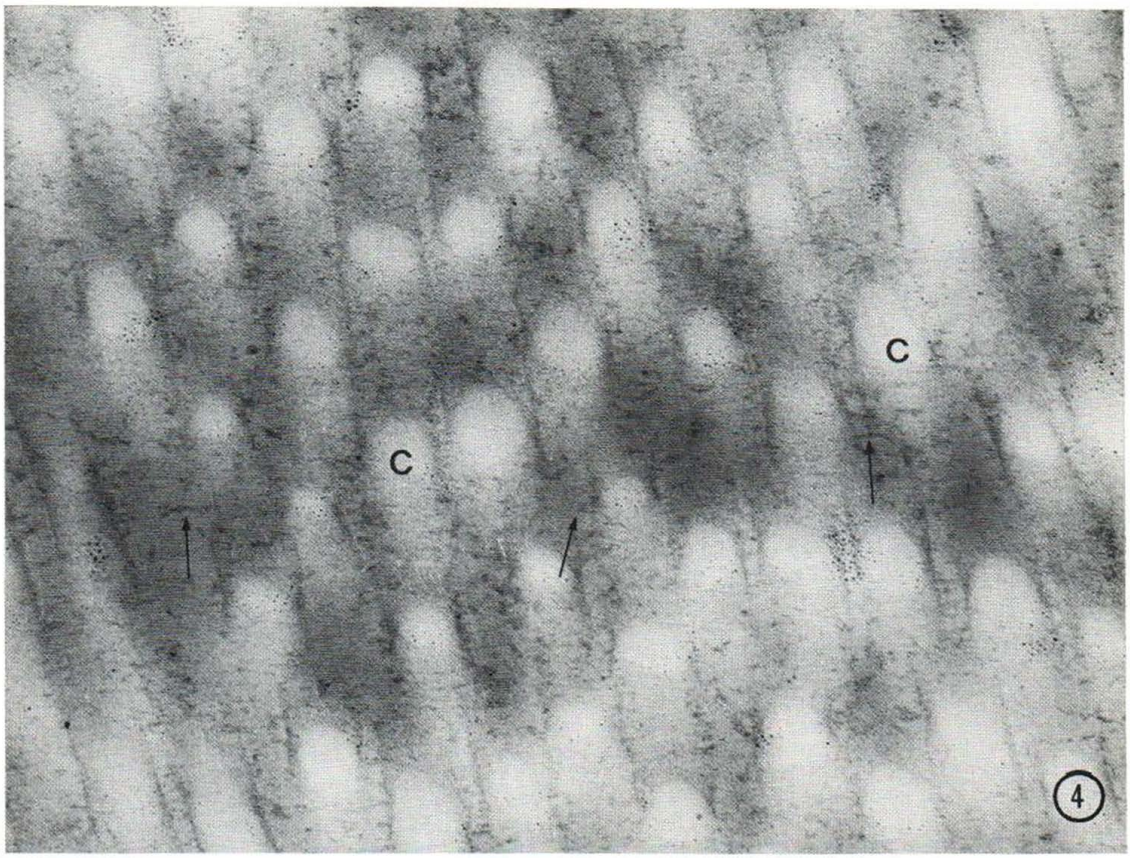

Fig. 4. Lattice-like microfibrils (*arrows*) between collagen fibrils (*C*). Lead citrate stain. × 98 000.

ACKNOWLEDGEMENT

The authors thank Mrs Nancy Hansen and Mr John Winther for their technical assistance.

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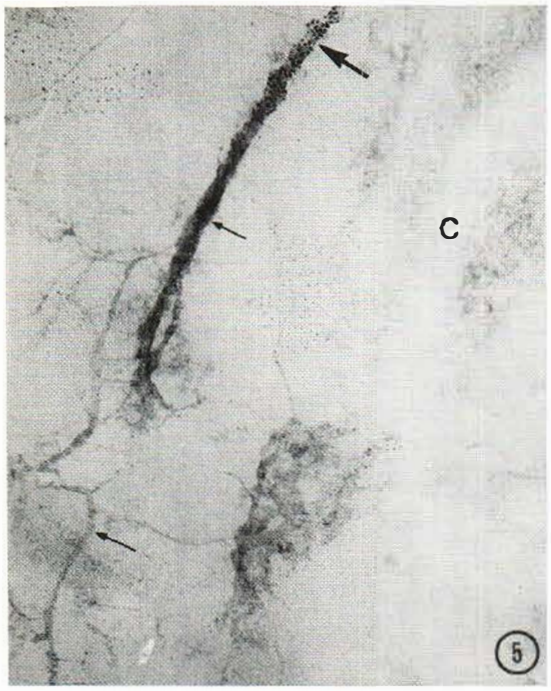


Fig. 5. Thread-like microfibrils show better contrast than collagen fibrils (*C*). Thin arrows indicate dense spots along to the microfibrils. Thick arrow indicates granules on a microfibril. Lead citrate stain. × 84 000.

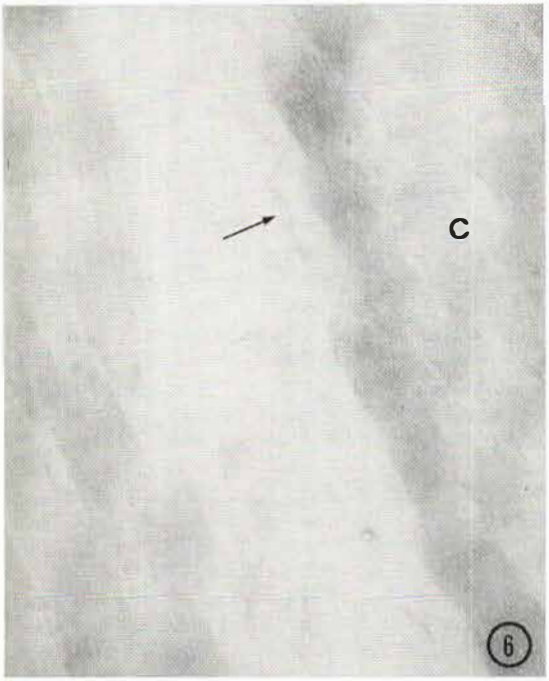
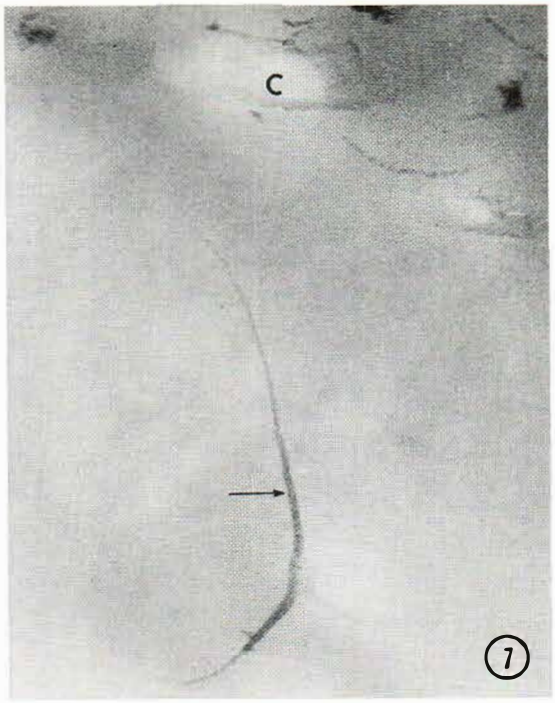
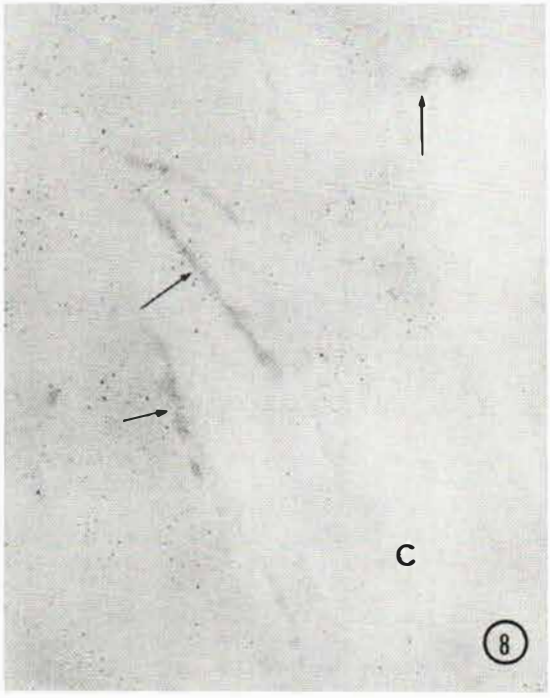
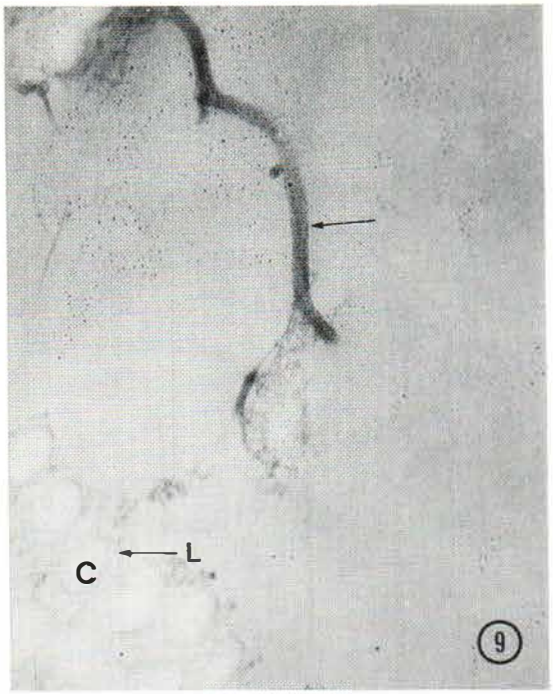


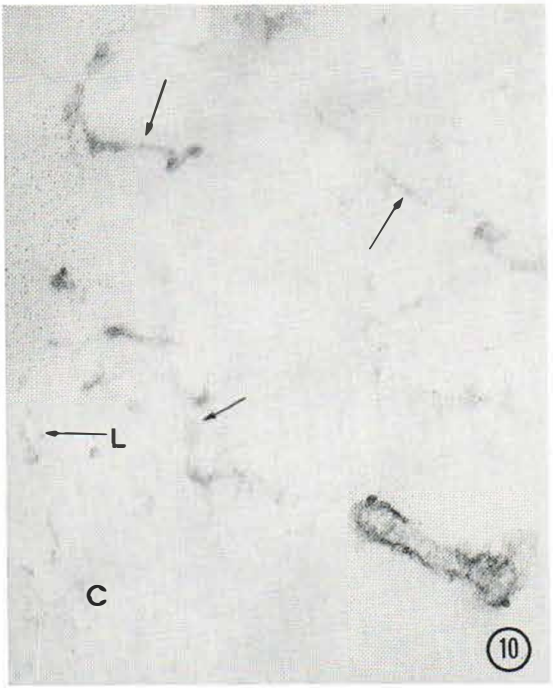
Fig. 6. Collagen fibrils (*C*) and microfibrils (*arrow*) show similar contrast after uranyl acetate stain. × 84 000.





Figs. 7 and *8.* Hyaluronidase influence on fresh skin. Fig. 7 shows control; Fig. 8 enzyme-influenced dermis. Microfibrils (*arrows*) show indistinct contours and reduced contrast after hyalurenidase. Collagen fibrils (*C*). Lead citrate stain. × 168 000.





Figs. **9** and *10.* Hyaluronidase effect on glutaraldehyde-fixed skin. Fig. 9 shows control; Fig. 10 enzyme-influenced dermis. Both thread-like and lattice-like microfibrils show indistinct contours and reduced contrast after digestion. Collagen fibrils (*C*); thread-like (*arrow*) and lattice-like (*arrow L*) microfibrils. Lead citrate stain. × 168 000.

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Received August 10, 1970

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