THE STRUCTURE OF ELASTIC TISSUE AS STUDIED WITH THE ELECTRON MICROSCOPE*

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PLATES 43 TO 47

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In contrast to its near relative, collagen, elastic tissue has thus far received meager attention with respect to fine structure analysis. Both morphologically and chemically this tissue has in the past been generally considered to be a relatively homogeneous entity. The present study indicates this to be highly unlikely. At least two distinguishable entities have been observed.

Previous investigations revealed no distinctive patterns of structure either by x-ray diffraction (1, 8) or electron microscopy (17). Collagen, on the other hand, yielded x-ray patterns indicating a high degree of organization (1, 2, 9). A complex axial repeating period with considerable detail of intraperiod structure was demonstrated in the fibrils with the electron microscope (5, 13, 15).

Astbury (1) observed a typical collagen x-ray diffraction pattern in stretched ligamentum nuchae, which, however, was reduced to amorphous rings after autoclaving (to remove the large amount of collagen known to be present). W. J. Schmidt (12) described an increase in double refraction on stretching the ligamentum presumably due to orientation of fibrous units.

Wolpers (17) found it necessary to treat elastic tissue with pepsin in acid for 24 hours before he could obtain fibers of a size suitable for study with the electron microscope. This partially digested elastin[1] from ligamentum nuchae revealed large, branching, amorphous fibers varying in width from 2,500 A to 200 A, the very smallest seen being 80 A. No axial periodicity was noted. Fixation in osmic acid resulted in a fine longitudinal fibrillation which Wolpers ascribed to the action of the fixative and not intrinsic to the actual structure. Orcein, a stain considered relatively specific for elastic fibers, was observed to deposit on these fibers in the form of small flakes. The elastin of the mouse aorta, after acid pepsin digestion, osmic acid fixation, and sonic fragmentation, appeared as thin, fenestrated laminae with numerous, short, stubby fibers protruding from the surface.

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[1] The term "elastin" will be used synonymously with elastic tissue in this report and will not define a particular protein.

Meyer and Ferri (11) from thermoelastic considerations, deduced that elastin is probably made up of highly contorted polymeric chains bonded laterally by only few links. Stretching the fiber orients these units in an axial direction thereby reducing the entropy and increasing the internal energy. Thermal motion produces the elastic recoil. Jordan Lloyd (7) agreed with this interpretation and added that the structure differs from rubber in that the peptide linkages of the elastin polymeric chains have polar properties which, by cross-linking, serve to maintain the extended state in stretched and dried elastic fibers. Imbibition of water is necessary to spread the chains apart, thus permitting thermal contraction whereas rubber, being entirely apolar, will not "lock" in the extended state when dry at ordinary temperature. McCartney (10) observed that ligamentum nuchae when freed of its collagen by peptic digestion contracts linearly with increase in temperature and returns along the same straight line to its original length on cooling. This suggests no extensive alteration in structure when the tissue is heated to 100°C. in water.

Chemical analysis of ligamentum nuchae by Stein and Miller (16) indicated roughly one in thirty amino acid residues to be polar (excluding glycine) in contrast to about 50 per cent for collagen.

Methods

The swim bladder of the carp, the aorta of the adult rabbit, and 2 day old rat, that from a human being 5 years old, and ligamentum nuchae of the cow were used as sources of elastin in this study. The swim bladder[2] proved to be a particularly suitable source of material because the collagen (ichthyocol) was readily removed by heating to 60°C. in dilute acetic acid, although nearly all of it could be removed by the dilute acid without heat. Mild fragmentation procedures reduced the elastin fibers to suitable size. Elastic tissue of the ligament and aorta posed difficult problems of fragmentation.

Metal shadowing was found to be the most suitable preparative adjuvant although heavy metal staining (6) was also attempted. The same preparative procedures were used for all tissues except for modifications required by certain special characteristics of each material.

The elastin of the carp swim bladder was prepared in the following manner. The tunic was stripped from the fresh bladder, washed well with water, cut into small pieces, and allowed to stand overnight in 1 per cent acetic acid in the refrigerator. The collagen swells greatly forming a viscous solution which is readily filtered off through silk bolting cloth. The gelatinous residue was then placed in dilute acetic acid and fragmented for 10 seconds in a Waring blendor. The suspension, which is fairly viscous, was centrifuged to sediment the finely dispersed elastin. Repeated washing in the centrifuge in dilute acid removed practically all the "dissolved" collagen, leaving a residue which formed a fine, cloudy suspension on agitation. However, to be certain that no collagen filaments (14) were present to confuse the picture, the suspension was boiled for 30 minutes. Swim bladder collagen is readily gelatinized at this temperature. Heating greatly reduces the viscosity of the suspension and facilitates sedimentation of the elastin fibers. As will be described later, heat and dilute acid do not seem to affect the observed structure of intact elastin. The finely comminuted elastin was then washed several times in the centrifuge and resuspended in distilled water.

The aortic tissues and bovine ligamentum nuchae were prepared in a somewhat different fashion. The fresh, unfixed tissues were sectioned with the freezing microtome. One portion was boiled for 1 hour in 1 per cent acetic acid to remove the collagen, while a second portion was not heated so as to permit study of the relationship between collagen and elastin.

[2] Faure-Fremiet and Garrault (4) give an excellent histological description of the fish swim bladder.

In order to reduce the elastic fibers to a size suitable for electron microscopy preparations were further fragmented in a sonic (9 kc. magnetostriction type) oscillator.

The resulting suspensions were prepared for examination in the usual manner by depositing drops on supporting films of conventional nickel specimen grids and blotting them off with filter paper after about 2 minutes. Some preparations were examined without further treatment. Most were shadowed with 8 mg. of chromium or 10 mg. of uranium at an angle of 10° and a filament to specimen distance of 20 cm.

The effect of digestion with crystalline trypsin (Armour) was studied by incubating portions of each material, boiled and not heated, in 0.1 per cent enzyme buffered to pH 8–8.4 with $NaHCO_3$ at 37°C. for periods ranging from 2½ to 24 hours under toluene. These suspensions were then washed in the refrigerated centrifuge at 4,000 R.P.M. for 1 hour with distilled water to remove the dissolved solids. Specimens were prepared for electron microscopy as described, except that the drops were allowed to remain on the grids for at least 10 minutes before blotting. Buffered controls without enzyme were also examined.

The influence of temperature on the fibrous elements released by tryptic digestion was investigated by heating portions of the washed, trypsinized suspensions in a water bath at temperature intervals of about 10°C. ranging from 40° to 100° for 30 minutes.

Pure crystalline trypsin solutions were examined to rule out possible artifacts from this source.

The effect of pH on the washed, trypsinized suspensions was studied in the range 2.5 to 10.5 at nearly unit intervals and room temperature. Unbuffered acetic acid and ammonium hydroxide were used to make up the pH series. This experiment was not performed on the rat aorta.

To observe the effect of formalin fixation portions of boiled ligamentum nuchae elastic tissues were fixed in 10 per cent neutral formalin for 7 days, incubated in trypsin, and prepared for electron microscopy as described.

An RCA type EMU electron microscope was used in this study.

RESULTS

Tissue of the Fish Swim Bladder.—The elastic tissue of the swim bladder tunic is extremely friable, requiring only 10 seconds in the Waring blendor to reduce it to a fine, cloudy suspension. Electron microscopy revealed the suspended particles to be long, contorted, branching fibers ranging from about 300 A to 5,000 A in width and often measuring many microns in length. In addition to the fibers there were numerous clumps of coarse, granular material. Excessive blending destroyed most of the fibers leaving only granular masses. In some preparations which had not been heated but only washed in dilute acetic acid, extremely fine filaments were present in the background which disappeared on heating to 40°C.; these were most likely ichthyocol (14). No intact collagen fibrils were found in boiled preparations. The following descriptions are based on electron micrographs of chromium-shadowed specimens.

The fibers appeared to be irregularly flattened on the supporting film (possibly a drying effect) and usually showed a roughly parallel and often coarsely interlaced fibrillation. These fibrillar units ranged in width from about 300 A to 1,000 A and appeared to be embedded in an amorphous matrix. The latter was observed to flatten out at the edges and often assumed a finely packed texture (Fig. 1). The "grain" of this texture, which was not always observ-

able, ran both parallel and perpendicular to the long axis in different fibers. Branching of a fiber was characterized by a separation of the fibrils (see Text-fig. 1) with a stretching and thinning of the binding matrix at the bifurcation. Occasionally a frayed fiber would show the presence of fibrous units no more than 100 A in width. There was no evidence of a true axial periodicity.

Boiling for 30 minutes in 1 per cent acetic acid had no effect on the observable structure.

Observations of unshadowed fibers added nothing to the pattern and the limited number of experiments with osmic and phosphotungstic acid staining were unproductive.

Aorta of the Adult Rabbit, Rat, and Human Being.—Boiling frozen sections of aorta in 1 per cent acetic acid destroys the collagen and most probably all other structural elements except the elastin. Fragmentation by freeze-sectioning and high speed homogenization produced very few tissue fragments small enough for electron microscopy. However, strong sonic vibrations disrupted the strongly coherent elastic membranes, producing fibers of size suitable for study (Fig. 4). These closely resembled the elastic fibers found in the tissue of the swim bladder. Whether the fibers observed were part of the lamina or actually represented the interlamellar fibers could not be determined. Considerable amounts of granular material were also present. No fine filaments were found. Large numbers of typically striated collagen fibrils were associated with the elastic fibers in the unheated preparations and often appeared to protrude from the fragments of elastin.

Ligamentum Nuchae of the Cow.—The long, thick fibers of this tissue were extremely resistant to fragmentation, more so than the fibers of the aortic elastica. Prolonged treatment with 9 kc. sonic waves was required to produce even a few suitable fibers. The fragments were usually small irregular chunks rather than fibrous units. Apparently these fibers are as strongly coherent in the lateral direction as they are longitudinally. The few fibers obtained were too thick and dense to reveal any detailed structure. In one rare case of a much flattened, frayed elastic fiber, numerous fine filaments about 100 A in width were found lying parallel with the fiber axis.

The Effect of Trypsin.—The most striking result of tryptic digestion was the appearance of many fine threads of constant cross-section in all fields examined and in preparations of all three tissue types. They were barely visible in unshadowed preparations. Threads released from the three different types of elastic tissue were indistinguishable with regard to their morphology (Figs. 6, 7, and 9).

In particularly clean preparations two morphological forms of threads were observed. One type found in varying numbers in different preparations of the same tissues was a tightly and evenly coiled double helix formed by the twining of two thin, apparently smooth filaments. In the same fields many uncoiled

filaments could be found usually lying in closely associated, parallel pairs and usually much longer than the coiled forms (Figs. 8 to 10). There were many instances observed in which a coiled thread was transformed abruptly into a parallel pair of smooth filaments, and in some cases, as shown in Fig. 7, only a single filament would project beyond the coil. Occasionally both ends of a coiled thread would continue as a pair of parallel filaments. The widths (actually height above the supporting film) of 350 filaments and threads obtained from ligamentum nuchae were determined from the shadow length, the only selection factor being the perpendicularity of the long axis of the thread to the direction of the shadow. A characteristic width of about 120 A was calculated for the coiled threads and approximately 70 A for the individual filaments. These figures apply as well to the threads and filaments obtained from the other tissues studied. Direct measurements of widths on an unshadowed preparation (which is not entirely satisfactory because of low contrast) roughly confirmed these figures.

The pitch of the helix in 120 measured threads from ligamentum nuchae ranged from 470 A to 580 A with a peak at about 530 A. Again, these figures were approximated by those for the aortic threads. These fibrous elements were usually straight or gently curved but never acutely kinked. No discrete axial periodicity was resolved other than that produced by the observed coiling.

Threads and filaments were never found in preparations not digested by trypsin.

Effects of Temperature, Formalin Fixation, and pH.—Heating threads and filaments of each of the three forms of elastic tissue suspended in distilled water resulted in their disappearance in the temperature range 70–85°C. Boiling the tissue in dilute acid prior to tryptic digestion did not alter the critical temperature.

Formalin-fixed ligamentum nuchae which had been boiled in dilute acid prior to fixation was digested in trypsin in a manner identical with that described for the fresh material. Typical threads and filaments were found. No further characterization was attempted at this time.

The influence of pH on the state of aggregation and morphology of the threads obtained from ligamentum nuchae was studied. At pH 3.0 only amorphous clumps of material often resembling small, flat discs were observed. At about pH 3.6 these clumps were larger, flatter, more irregular, and more granular (Fig. 13). At pH 5.1 the aggregates appeared coarsely reticular with short, stubby, nodular threads protruding from the edges. No obvious coiling was observed but there seemed to be a fine, granular debris covering everything which obscured detail (Fig. 12). No free filaments were observed. At increasingly higher pH the threads were increasingly longer and finer. At pH 5.7 some free threads were observed, plus many loose aggregates. At pH 6.2 very little aggregation was observed and characteristic threads and filaments

were present. Coiled forms were found in large numbers in the pH range 6.2 to 9.1 (Figs. 7 and 8). If a suspension of threads were allowed to stand for about 24 hours at neutral pH, clumping was also observed.

The ratio of coiled threads to smooth filaments diminished with increasing alkalinity; at pH 9.8 no coiled threads could be found (Fig. 11). Increase in pH had no observable influence on the pitch of the helices; they were either coiled with the characteristic pitch or completely uncoiled. Boiling the tissue in dilute acid prior to digestion did not alter the pH effects. Less detailed experiments performed on the threads of rabbit aorta and fish swim bladder indicated a similar behavior pattern.

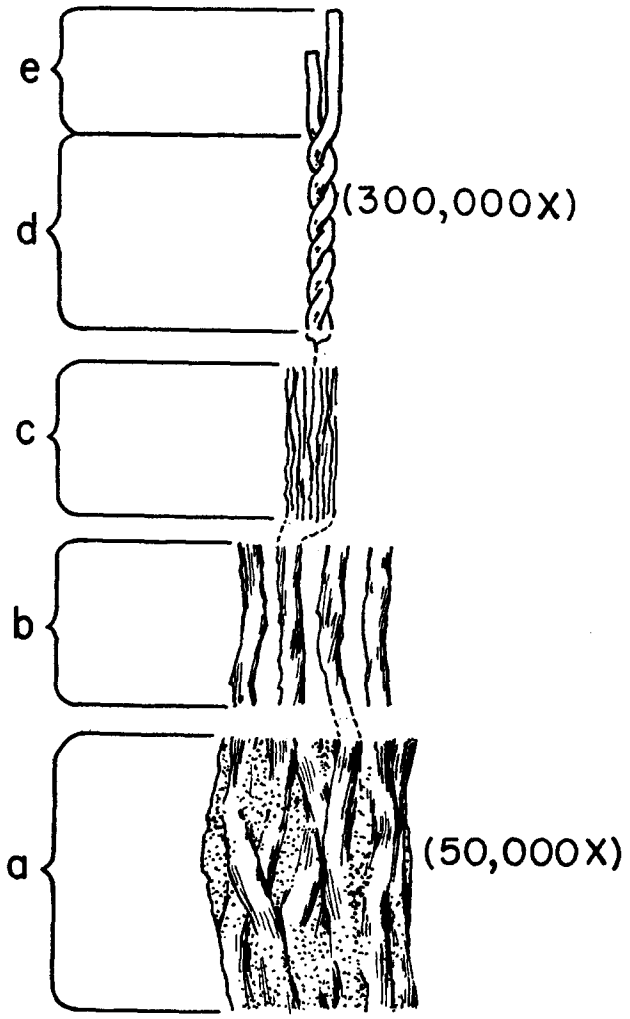
Partially Digested Fibers.—Trypsinized elastin of the swim bladder tunic and aorta revealed numerous fibers of a size well suited for electron microscopy. Many of the partially digested swim bladder fibers appeared to be flattened, amorphous, and moth-eaten (Fig. 3). In many cases, however, the amorphous matrix seemed to have been partially stripped away, revealing nearly naked fibrils (Figs. 2 and 5). In rare instances partial fragmentation of these fibrils revaled thinner units of about the size of the free threads. However, even here there seemed to be enough amorphous material present to obscure any details such as coiling. The characteristic coiled threads have not yet been observed in either intact or partially digested fibers.

DISCUSSION

From the data at hand one may construct a tentative model of the architecture of the elastic fiber (see Text-fig. 1). At least two distinct chemical and morphological components, namely the threads and the amorphous binding substance, are associated to form a fiber which is capable of long range elasticity, great mechanical strength, and refractiveness to boiling water, dilute acids, or alkalis. Collagen is present as an incidental component, probably incorporated in the fiber extracellularly during its formation as suggested in Bloom's study of elastic fibers in tissue culture (3). The threads are arranged in long, roughly parallel bundles, such as observed in partially digested fibers, which may or may not course the full length of the fiber. These bundles are probably both infiltrated with and embedded in the trypsin-sensitive, amorphous binding matrix. The relative proportions of matrix and threads cannot readily be determined by electron microscopy.

Matrix.—Whether this substance is amorphous in a rigorous sense or is actually a tangled mass of long macromolecular chains like rubber, remains to be determined. The matrix substance is apparently responsible for the heat resistance of the intact fiber since the naked threads (released by trypsin) are relatively heat-labile. The matrix is probably bound to the filaments in such a way as to prevent their destruction by heat. Probably the great difference in friability between the intact fibers of the different elastic tissues is determined by the tensile strength of the matrix.

It is interesting to note that formalin fixation apparently does not prevent the digestion of the matrix material by trypsin. This fact may be of significance in considering the chemical nature of the matrix.



TEXT-FIG. 1. Schematic representation of the structure of the elastic fiber (collagen fibrils omitted). (*a*) Undigested fiber showing fibrils imbedded in amorphous matrix (see Fig. 4). (*b*) Partially digested fiber showing fibrils stripped of matrix (see Figs. 2 and 5). (*c*) Single fibril, enlarged, revealing it to be a bundle of threads. (*d*) Single coiled thread, greatly enlarged (see Figs. 8 and 9). (*e*) Individual filaments which, when twined, form the thread (see Figs. 9 to 11).

The Fibrous Component.—The threads obtained from the different elastic tissues are similar in many respects; *e.g.*, morphology, heat sensitivity, and

reaction to pH. This suggests that these characteristic units are a uniform constituent of elastic tissue.

The tendency to aggregate on the acid side and to fray into finer threads at higher pH suggests an isoelectric point in the acid range. Stein and Miller (16) from electrophoretic studies of purified, finely comminuted, whole elastic tissue estimated an isoelectric point of 4.8. At the present time there are no further chemical data to characterize these structures more fully.

The coiling of these fine fibrous elements is of great interest because of its possible rôle in elastic and contractile processes. Indeed, the structural pattern described here has all the components of the molecular model for elastin postulated from thermodynamic considerations by Meyer and Ferri (11); *i.e.*, a compressed, coiled spring held under tension by a stretched elastic band. If this analogy were pursued, one would expect that the observed coiled threads are compressed in the native state and that tryptic digestion frees them from the restraining force of the matrix, thus permitting a large increase in pitch. It is therefore important to demonstrate conclusively whether the coiled structure is characteristic of the intact tissue and not a result of manipulation. The chance that the coiled threads are artifacts produced by the axial rotation of adjacent filaments while in suspension is remote. The coils are always tight and regular, and show little variation in pitch even in different forms of elastic tissue; moreover intermediary stages of coiling have never been observed. Another bit of evidence is the appearance of large numbers of filaments and the disappearance of coiled threads with a rise in pH and the inability to reunite these threads into coils on lowering the pH. Only haphazard lateral aggregation with some irregular twining occurs. The assumption here is that the smooth, thin filaments are produced by uncoiling of the threads and are not a separate species.

The large numbers of parallel pairs of filaments which sometimes abruptly twine to form a short segment of typically coiled thread suggest that two filaments form a single thread. Because the individual uncoiled filaments are nearly always very much longer than the coiled threads—longer than one would expect to result from simple uncoiling of the thread—there exists the possibility of a much finer, molecular coiling in the filament which is also smoothed out when the thread uncoils. It is evident, however, that considerably more data are needed to answer conclusively the basic questions as to the morphology of the threads in the native state and the respective rôles played by the two components in elastic behavior.

Further experimentation with the electron microscope and physical chemical techniques on the liberated threads, and also x-ray diffraction and polarized light studies on the intact tissue, are needed to provide the essential evidence. With regard to early polarized light studies (12) the correlation between the observed isotropy of relaxed elastic tissue and the structures described here is obscure at present.

Stein and Miller (16) have made an amino acid analysis of "purified" elastin. However, they have treated this substance as a single homogeneous protein even to the extent of working out the amino acid "frequencies" according to the Bergmann-Niemann "periodicity theory." The results described here demonstrate the presence of at least two components. It is therefore highly desirable that new amino acid analyses be made on the individual components. This should now be feasible since the present work indicates procedures by which the two fractions may be separated.

The author wishes to thank Mrs. Mary Frances Simmons and Mr. James Wilson for their valuable technical assistance.

CONCLUSIONS

Electron microscope examination of fragmented elastic tissue obtained from fish swim bladder, bovine ligamentum nuchae, and aortas of various mammals, including man, reveals characteristically formed fibers and much amorphous material. Boiling in dilute acid destroys the associated collagen but does not obviously alter the elastic tissue.

Digestion in crystalline trypsin of either boiled or unheated tissue from any of the above-mentioned sources causes the release of thin threads ranging in length from 0.1 μ to many microns. A large proportion of these threads are evenly and tightly coiled double helices formed from at least two interlacing filaments and measuring about 120 A in width. The distance between coils ranges from about 470 to 590 A. The individual smooth filaments, many of which are present in parallel pairs, measure approximately 70 A in width.

Raising the pH of a neutral suspension of threads from ligamentum nuchae lowers the ratio of helical threads to uncoiled filaments, whereas lowering the pH with acetic acid results in clumping of threads with complete loss of identity at about pH 3.6.

Threads and filaments obtained from all sources studied were destroyed in the temperature range 75–85°C. at pH 7.

It is concluded that the elastic fiber is a two component system composed of bundles of trypsin-resistant threads of characteristic form and size plus a trypsin-sensitive, heat-resistant "amorphous" binding matrix.

The possible relationship of this structure to the elastic properties of the tissue is discussed.

BIBLIOGRAPHY

1. Astbury, W. T., *J. Internat. Soc. Leather Trades Chem.*, 1940, **24**, 69.
2. Bear, R. S., *J. Am. Chem. Soc.*, 1944, **66**, 1297.
3. Bloom, B., *Arch. exp. Zellforsch.*, 1929, **9**, 6.
4. Faure-Fremiet, E., and Garrault, H., *Arch. anat. micr.*, 1937, **33**, 81.
5. Gross, J., and Schmitt, F. O., *J. Exp. Med.*, 1948, **88**, 555.
6. Hall, C. E., Jakus, M. A., and Schmitt, F. O., *J. Appl. Physics*, 1945, **16**, 459.

7. Jordan, L. D., and Garrod, M., *Tr. Faraday Soc.*, 1948, **64,** 441.
8. Kolpak, H., *Kolloid. Z.*, 1935, **73,** 129.
9. Kratky, O., and Sekora, A., *J. makromol. Chem.*, 1943, **1,** 113.
10. McCartney, J. E., *Quart. J. Physiol.*, 1913, **7,** 103.
11. Meyer, K. H., and Ferri, C., *Arch. ges. Physiol.*, 1936, **238,** 78.
12. Schmidt, W. J., Die Doppelbrechung von Karyoplasma, Zytoplasma und Metaplasma, Berlin, Borntraeger, 1937.
13. Schmitt, F. O., and Gross, J., *J. Am. Leather Chem. Assn.*, 1948, **63,** 658.
14. Schmitt, F. O., Gross, J., and Salo, T., unpublished data.
15. Schmitt, F. O., Hall, C. E., and Jakus, M. A., *J. Cell. and Comp. Physiol.*, 1942, **20,** 11.
16. Stein, W. H., and Miller, E. G., Jr., *J. Biol. Chem.*, 1938, **125,** 599.
17. Wolpers, C., *Klin. Woch.*, 1944, **23,** 169.

EXPLANATION OF PLATES

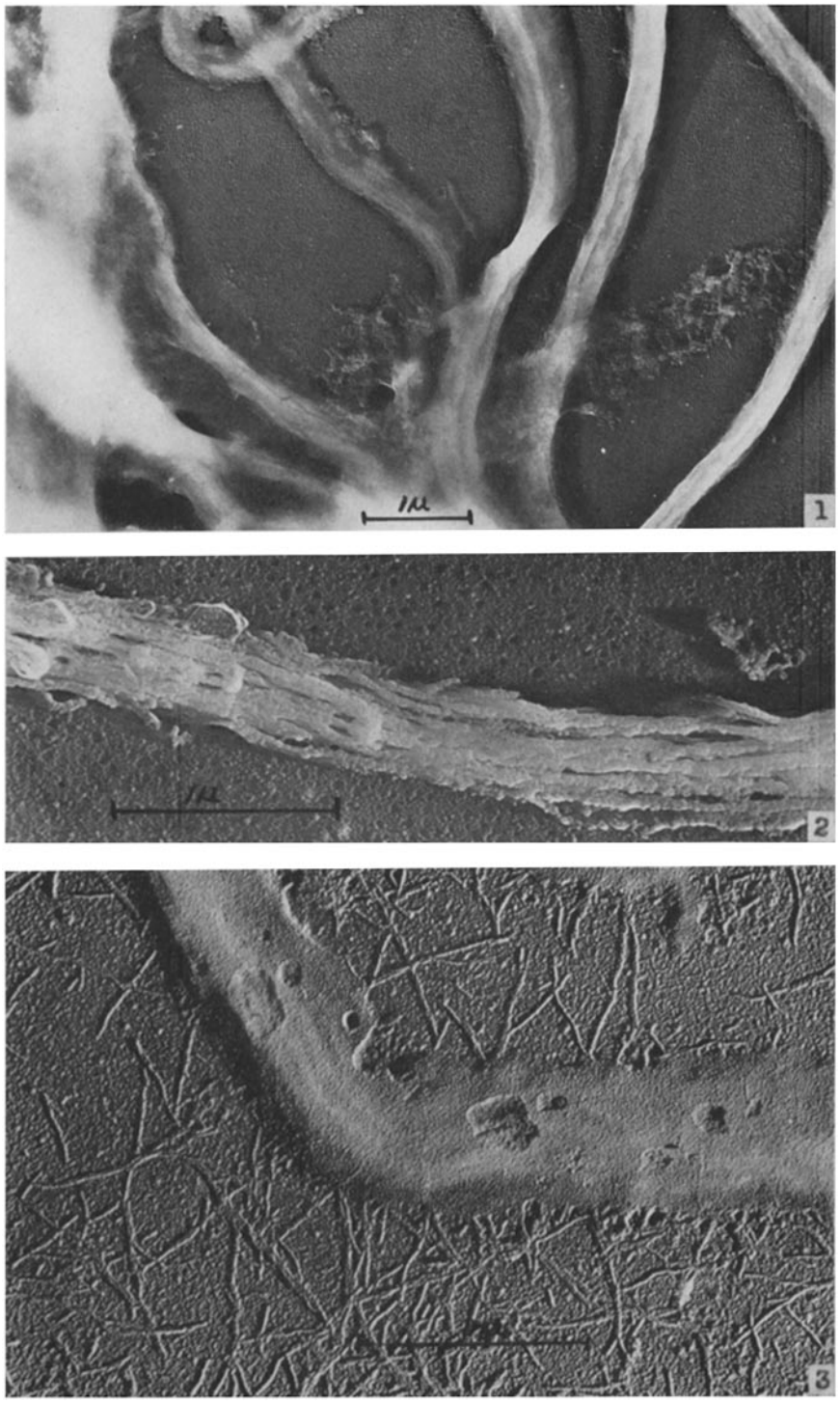
PLATE 43

Fish swim bladder elastin heated at 60°C. in dilute acid. Fragmented and washed. All specimens were chromium-shadowed.

FIG. 1. Fibers deposited on grid from aqueous suspension. × 14,000.

FIG. 2. Fiber which has been partially digested with crystalline trypsin. Much of the amorphous matrix has been removed revealing the fibrils. × 32,000.

FIG. 3. After digestion with trypsin filaments released by the enzyme can be seen. An amorphous fragment of a partially digested fiber remains. × 31,000.



(Gross: Structure of elastic tissue)

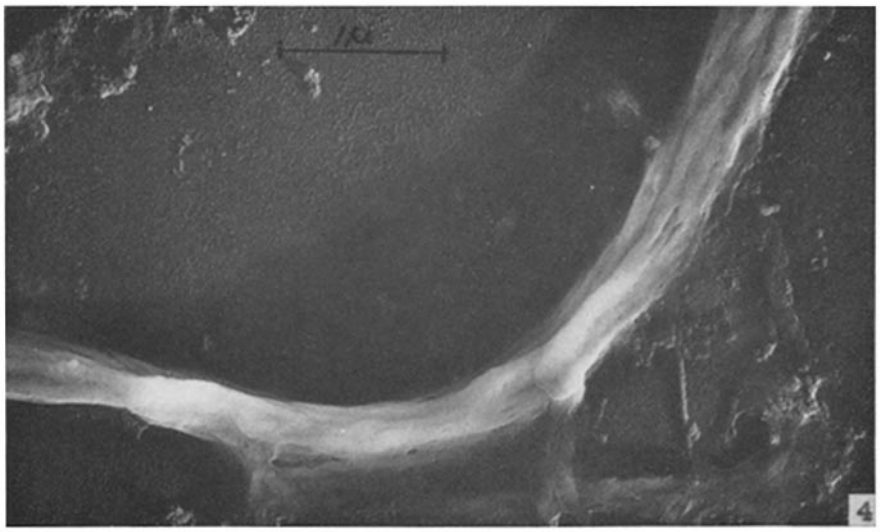
PLATE 44

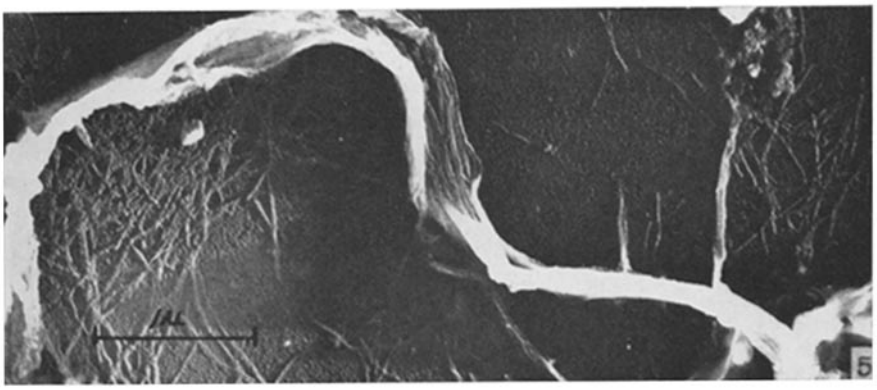
Adult rabbit aorta boiled in 1.0 per cent acetic acid, freeze-sectioned. All preparations were chromium-shadowed. × 22,000.

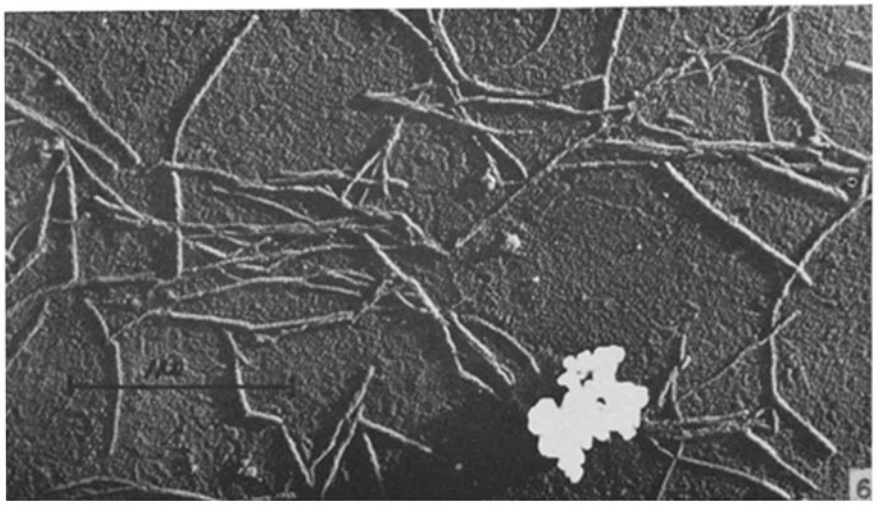
FIG. 4. Fiber from suspension produced by treating tissue sections with sonic oscillations. Coarse fibrillation of the structure is noted, plus considerable amounts of amorphous material. × 22,000.

FIG. 5. Partial tryptic digestion of an aortic elastin fiber, revealing the roughly parallel fibrils which are believed to be bundles of threads. Many free threads can be seen in the background. × 21,000.

FIG. 6. Aortic elastin threads released by trypsin. Characteristically coiled threads can be seen in addition to some uncoiled filaments. The large, dense lobular mass is an unidentifiable contamination. × 28,000.





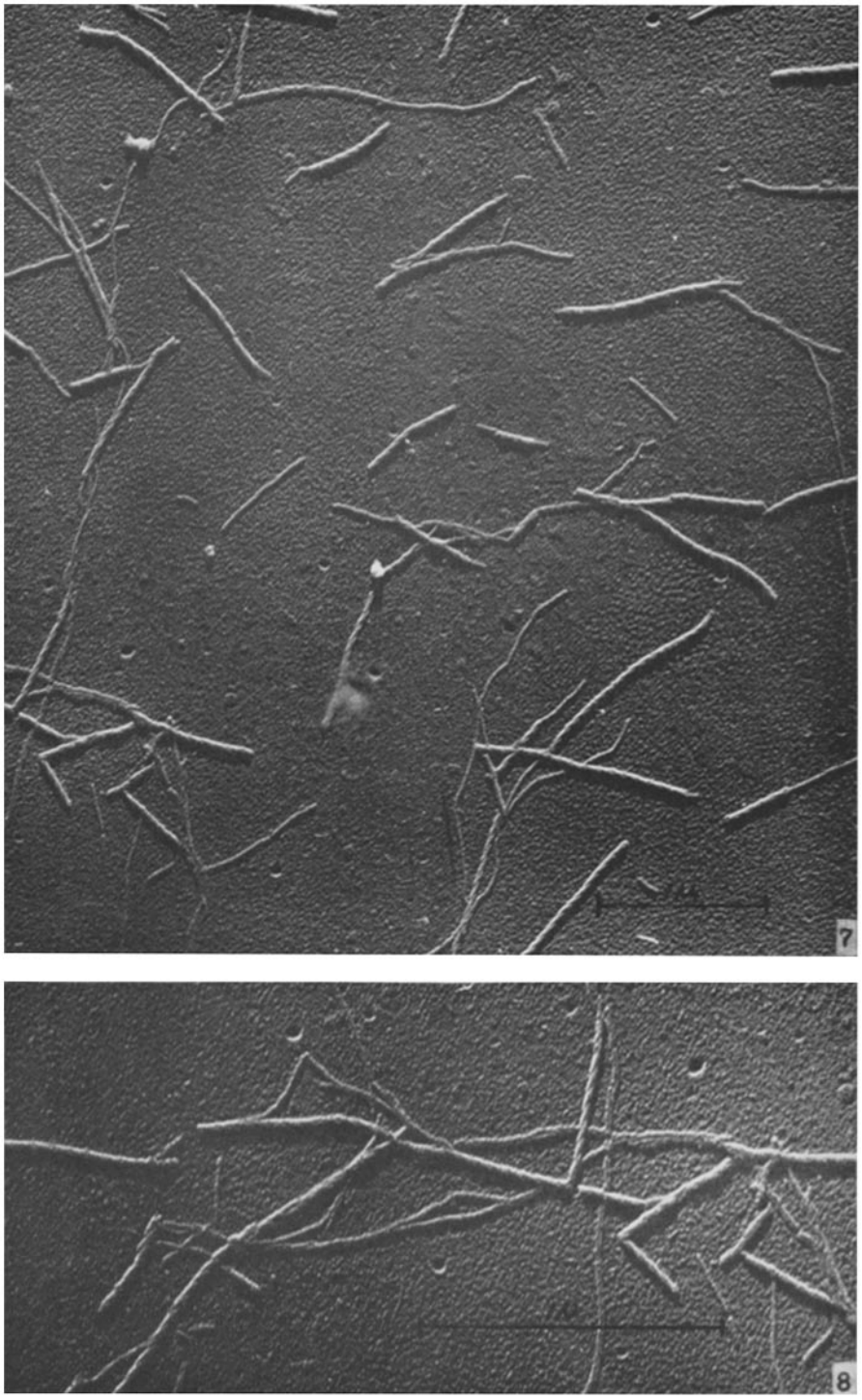


(Gross: Structure of elastic tissue)

PLATE 45

FIG. 7. Threads and filaments released from bovine ligamentum nuchae by tryptic digestion. Numerous coiled threads are observed, along with thinner uncoiled filaments which in some cases can be seen to project individually beyond the coiled regions. Shadowed with chromium. × 23,000.

FIG. 8. Higher magnification of coiled threads and filaments. Shadowed with chromium. × 42,000.

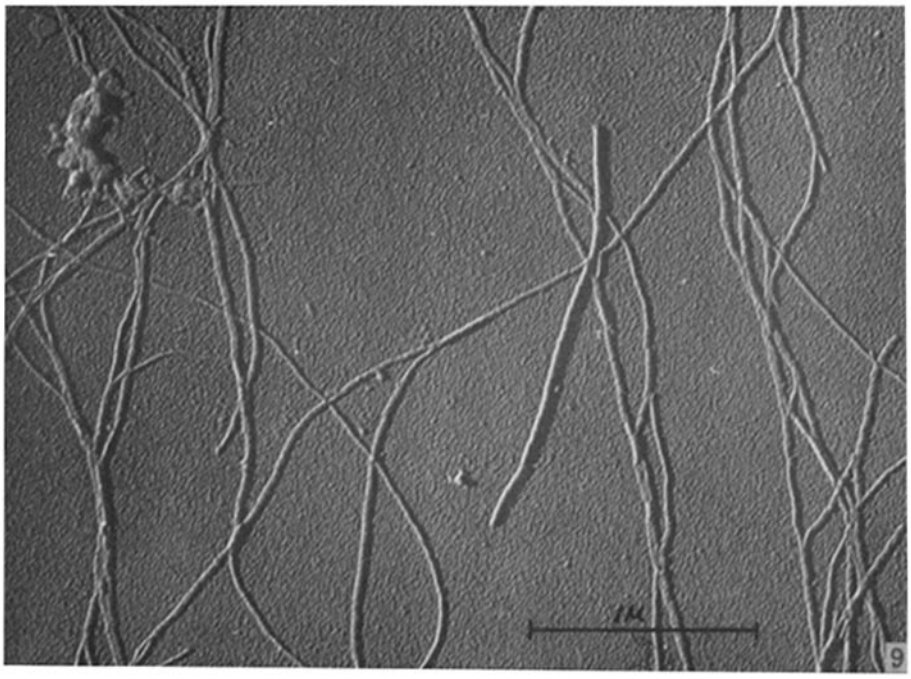


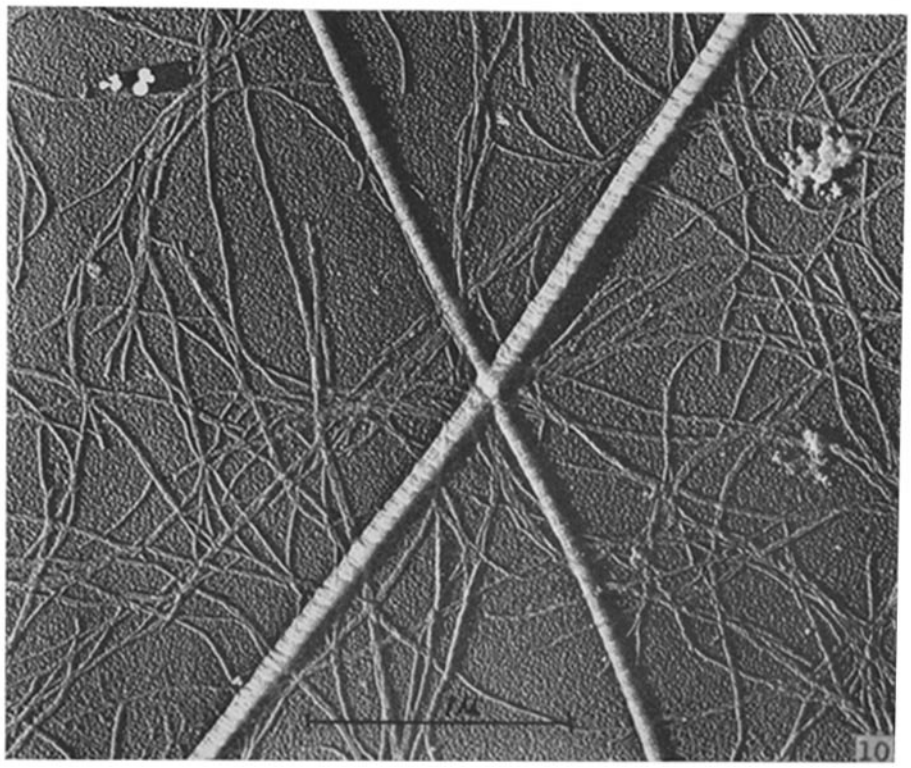
(Gross: Structure of elastic tissue)

PLATE 46

FIG. 9. Threads and filaments released by trypsin from the elastin of the fish swim bladder tunic. A single coiled thread is seen in this field. The filaments are much longer than the threads and in most cases are paired. Shadowed with chromium. × 29,200.

FIG. 10. Human aorta from 5 year old girl. Not preheated. Digested with trypsin. pH raised to 10.0. Thin, uncoiled filaments are observed. Coiled threads have not thus far been found at this pH. Two typical intact collagen fibrils are also present. Shadowed with uranium. Pebbly background represents unusually coarse collodion film structure. × 36,100.





(Gross: Structure of elastic tissue)

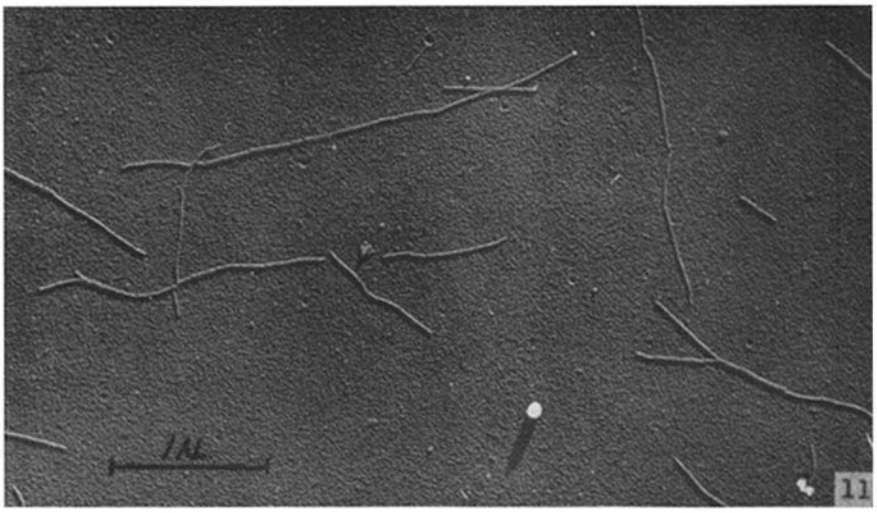
PLATE 47

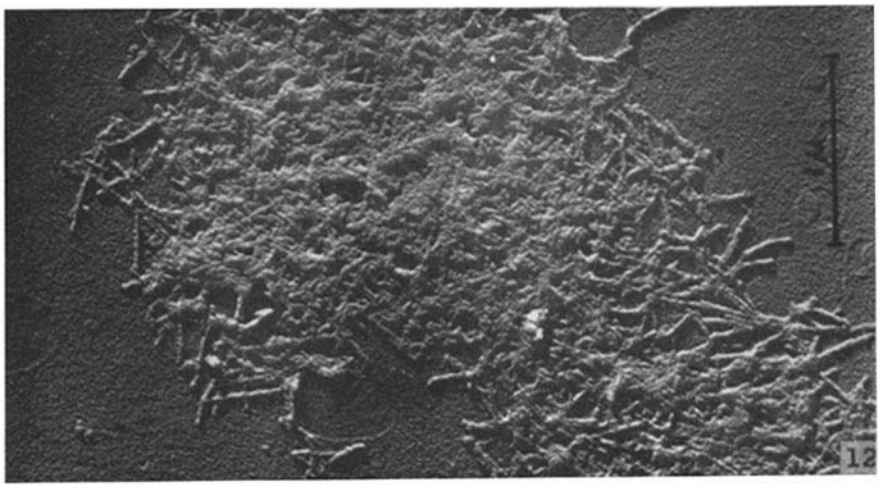
Influence of pH on ligamentum nuchae threads. All specimens were chromium-shadowed.

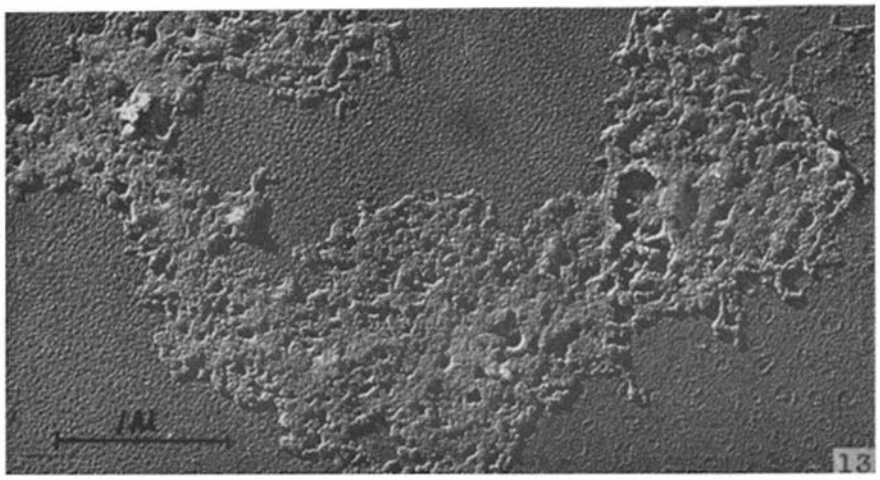
FIG. 11. pH 9.8. Only individual and occasionally paired filaments are observed. No coiled threads were seen. × 22,500.

FIG. 12. pH 5.1. Clump of short, irregularly nodular threads. Coiling was not resolved. × 24,750.

FIG. 13. pH 3.6. Granular mass in which individual threads are no longer identifiable. × 21,750.







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