

## THE ELASTIC FIBER

### I. The Separation and Partial Characterization of its Macromolecular Components

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#### ABSTRACT

The two morphologically different constituents of the mature elastic fiber, the central amorphous and the peripheral microfibrillar components, have been separated and partially characterized. A pure preparation of elastic fibers was obtained from fetal bovine ligamentum nuchae by extraction of the homogenized ligament with 5 M guanidine followed by digestion with collagenase. The resultant preparation consisted of elastic fibers which were morphologically identical with those seen in vivo. The microfibrillar components of these elastic fibers were removed either by proteolytic enzymes or by reduction of disulfide bonds with dithioerythritol in 5 M guanidine. The microfibrils solubilized by both methods were rich in polar, hydroxy, and sulfur-containing amino acids and contained less glycine, valine, and proline than the amorphous component of the elastic fiber. In contrast, the amino acid composition of the amorphous component was identical with that previously described for elastin. This component demonstrated selective susceptibility to elastase digestion, but was relatively resistant to the action of other proteolytic enzymes and to reduction. These observations establish that the microfibrils consist of a different connective tissue protein (or proteins) that is neither collagen nor elastin. During embryologic development the microfibrils form an aggregate structure before the amorphous component is secreted. These microfibrils may therefore play a primary role in the morphogenesis of the elastic fiber.

#### INTRODUCTION

Elastic fibers prepared for electron microscopy by routine methods have been shown to consist of two morphologically different components: centrally located amorphous structures surrounded by tubular-appearing microfibrils (110 Å diameter). Both of these components have been observed in mature elastic fibers from fetal and newborn calf ligamentum nuchae (1-4), developing rat tendon (5), and various other tissues (6-17). In a recent study, Greenlee et al. (1) noted that each of these structures has different and charac-

teristic staining properties. Microfibrils demonstrate marked affinity for both cationic lead and uranyl acetate, in contrast to the amorphous component which stains with anionic phosphotungstic acid. These staining properties were interpreted to indicate that each structure has a different surface charge, which permits it to interact with the particular stain used.

Several investigations have shown that the earliest recognizable elastic fibers appear as a homogeneous collection of only one of the com-

ponents, the 110 Å microfibrils, arranged in aggregates and usually oriented in parallel array (1, 3, 5, 18). The amorphous component increases in amount with increasing fetal age so that by term the elastic fiber is several microns in diameter and consists largely of this component.

These observations led us to propose that these morphologic entities were separate but integral components of the elastic fiber. The two components could represent different proteins, only one of which is elastin, or different forms of elastin in which the centrally located amorphous material results from an alteration of the microfibrils. The resolution of these problems required the separation and characterization of the morphologic components of the elastic fiber. This report presents initial studies in which it has been possible to separate and partially characterize these two structures.

#### MATERIALS AND METHODS

The ligamentum nuchae from fetal calves ranging in age from 2 to 9 months was removed by careful dissection at the abattoir. Each ligament was wrapped and frozen until used. The age of the fetus was determined on the basis of forehead-to-rump length as noted by Sandberg (19).

#### Procedure for Obtaining Elastic Fibers

Fig. 1 summarizes the procedures used in the separation of the components of the elastic fiber. Approximately 4 g (wet weight) of tissue obtained from term fetal ligaments were employed for each experiment. The tissue was finely minced with scissors and homogenized in a glass-glass homogenizer in 5 M guanidine hydrochloride (brought to pH 7.0 with 0.1 N sodium hydroxide), until a fine suspension of fibers was obtained. Collagen and other contaminating substances were extracted by a modification of the method of Miller and Fullmer (20). The suspension was placed in additional 5 M guanidine to provide a total of 80 ml and stirred for 24 hr at room temperature. It was then centrifuged at approximately 2,000 rpm, the supernatant discarded, and the remaining pellet resuspended in the same volume of fresh 5 M guanidine. This procedure was repeated three times for a total period of 72 hr. The suspension was then centrifuged, the supernatant discarded, and the pellet washed three times in 0.2 M Tris buffer (pH 7.4) and three times in distilled water. Collagenase (collagenase-CLSPA, Worthington) (1% of the dry weight of the starting material) was added to the pellet in suspension in 0.2 M Tris buffer (pH 7.4) containing 0.5% penicillin-streptomycin, and the suspension was incubated at 37°C for 24 hr in a shaker bath. This suspension was centrifuged, the supernatant discarded, and the pellet washed as

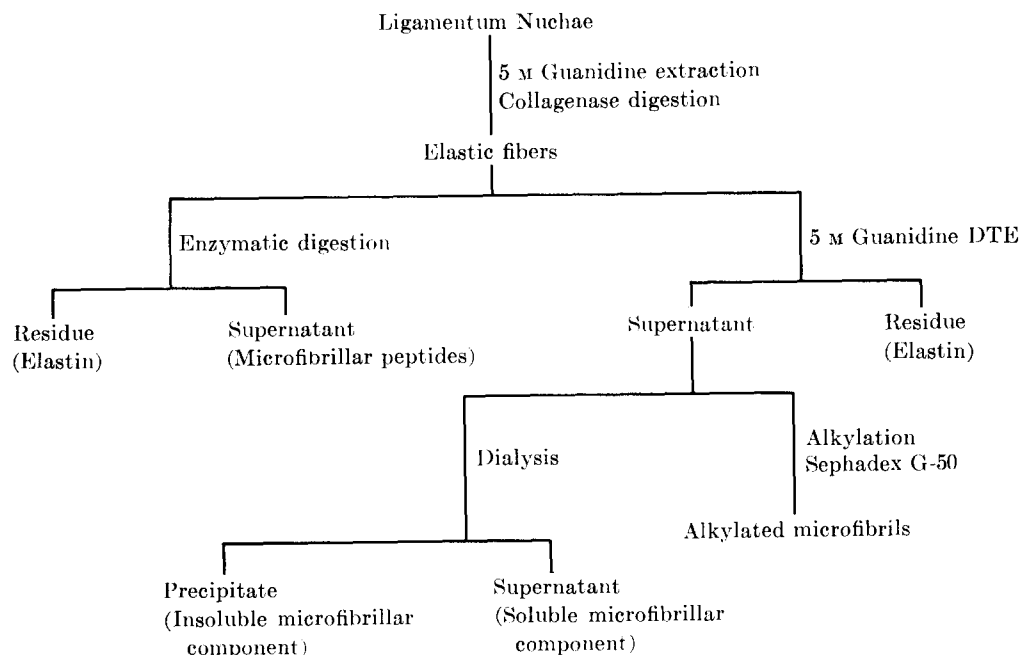


FIGURE 1 Procedures used to separate the macromolecular components of the elastic fiber.

before. Before the last distilled water wash, a representative aliquot was taken of the fibers suspended in distilled water, centrifuged to form a pellet, and subsequently prepared for electron microscopy. The remainder of the suspension was centrifuged into pellets which were used for the experiments described below.

### *Enzymatic Digestions*

#### TRYPSIN AND CHYMOTRYPSIN

A series of digestions with  $\alpha$ -chymotrypsin (3 $\times$  crystallized, Worthington Biochemical Corp., Freehold, N.J.), trypsin (2 $\times$  crystallized, Worthington), or both, were performed in 0.1 M  $\text{NH}_4\text{HCO}_3$  (pH 7.8) for 24 hr at 37°C; either 0.5% penicillin-streptomycin or a drop of toluene was used to retard bacterial growth. The quantity of enzyme used was 1% of the dry weight of the microfibrillar component of the elastic fiber. The microfibrils were estimated to constitute 5–10% of the dry weight of the elastic fibers in earlier experiments on dried, weighed, and rehydrated preparations of elastic fibers with known amounts of enzyme.

At the end of the extraction the suspension was centrifuged at 9,750 *g*, the supernatant frozen, and the pellet washed three times in 0.1 M  $\text{NH}_4\text{HCO}_3$  (pH 7.8), and three times in distilled water. After the final wash, the pellet was frozen and dried to constant weight, then rehydrated and suspended in distilled water. Part of this suspension was separated and centrifuged into a small pellet for electron microscopy. The remainder was centrifuged into another pellet and lyophilized in preparation for amino acid analysis. Control digestions were performed with similar amounts of enzyme inactivated in boiling water for 10 min before use.

#### HYALURONIDASE

A 0.05% solution of hyaluronidase (Worthington) in 0.1 M phosphate buffer (pH 5.3) was incubated with elastic fibers for 1 hr at 37°C. Controls consisting of boiled enzyme were used as above.

#### BETA-GLUCURONIDASE

A 0.2% solution of beta-glucuronidase (Worthington), in 0.1 M Na acetate buffer (pH 4.5), was incubated with elastic fibers for 1 hr at 37°C. In a control experiment a solution containing only acetate buffer and the elastic fiber preparation was used.

#### ELASTASE

A 0.01% solution of elastase (Worthington Code, ESFF) in 0.2 M Tris buffer (pH 8.8) or 0.1 M  $\text{NH}_4\text{HCO}_3$  (pH 7.8) was used for digestion of elastic fiber preparations for either 15, 30, 45 min or 1 hr at

37°C. Buffer without the enzyme was used as a control.

### *Solubilization of Microfibrils by Reduction of Disulfide Bonds*

A 0.05 M solution of dithioerythritol (DTE-crystalline, Mann Research Laboratories) (21) was prepared in 5 M guanidine containing 0.1 M Tris buffer (pH 8.5) and 0.1% EDTA. A known weight of elastic fibers was suspended in 15 ml of this solution and stirred in an atmosphere of nitrogen at 37°C for 24 hr. This suspension was centrifuged at 35,000 *g* for 20 min at 15°C, and the supernatant was divided into equal parts. One half was exhaustively dialyzed at 4°C against water. A fine precipitate formed which was separated by centrifugation. Both precipitate and supernatant were lyophilized. To the remaining half of the original supernatant was added iodoacetate in a fourfold molar excess over DTE. The pH was rapidly readjusted to 8.5 and the solution was incubated in the dark at room temperature for 45 min. A fivefold molar excess of mercaptoethanol over iodoacetate was then added. This solution was chromatographed on Sephadex G-50 (fine beads, Pharmacia Fine Chemicals Inc., New Market, N.J.), equilibrated, and eluted with 0.1 N acetic acid. The effluent was monitored with a Gilford (Gilford Instrument Company, Oberlin, Ohio) recording spectrophotometer at 280 m $\mu$ . The fractions comprising a peak eluting at the excluded volume of the column were pooled and lyophilized.

### *Electron Microscopy*

Preparations to be examined by electron microscopy were fixed for 1 hr in 2% osmium tetroxide buffered with *s*-collidine (pH 7.4), then fixed for ½ hr in 10% neutral buffered formalin, and were routinely dehydrated and embedded in epoxy resin (Epon 812). 1  $\mu$  sections were stained with azure II-methylene blue for examination by light microscopy to determine the homogeneity of preparations. Thin sections were stained with uranyl acetate followed by lead tartrate and examined in either an RCA EMU-3G or an AEI EM6B electron microscope.

### *Amino Acid Analysis*

Samples for amino acid analysis were hydrolyzed under nitrogen in redistilled, constant hydrochloric acid boiling at 108°C for 24 hr (microfibrils), or for 72 hr (amorphous component). Analyses were performed with a Beckman 120 C analyzer (Beckman Instruments Inc., Fullerton, Calif.) modified for high speed single column gradient elution by a slight modification of the method described by Miller and

TABLE I  
Amino Acid Composition of the Elastic Fiber and its Components\*

	Amorphous Component (Elastin)			Microfibrils			
	Elastic Fiber	After Enzymatic Digestion†	After DTE§	Enzymatic Digest	Alkylated¶	Reduced Dialyzed; Precipitate**	Reduced Dialyzed; Supernatant‡‡
CM-cysteine	—	—	—	—	72.3	—	—
Hydroxyproline	16.4	11.3	10.7	1.7	n.c.	—	—
Aspartic acid	16.1	5.4	6.4	92.5	114	114	120
Threonine	13.8	8.6	8.9	47.3	55.1	55.9	56.1
Serine	16.1	9.4	9.9	52.8	58.9	62.2	65.4
Glutamic acid	24.6	14.7	15.0	98.3	111	114	117
Proline	90.2	110	120	73.5	70.4	63.5	68.9
Glycine	305	324	324	142	120	110	123
Alanine	212	223	223	82.6	58.9	65.1	49.2
Cystine/2	10.2	5.0	4.1	56.3	8.0	48.2	88.1
Valine	130	140	135	69.7	54.1	56.3	46.2
Methionine	0.7	—	—	13.0	15.8	15.6	15.1
Isoleucine	29.6	26.2	25.5	43.8	45.2	47.7	46.6
Leucine	60.3	67.8	61.1	65.5	57.2	68.6	50.3
Tyrosine	9.4	8.2	7.1	27.6	30.0	36.0	31.0
Phenylalanine	34.4	30.9	30.1	32.8	32.1	37.7	29.6
Isodesmosine/4	3.9	4.1	3.3	—	—	—	—
Desmosine/4	5.5	6.5	4.6	—	—	—	—
Hydroxylysine	—	—	—	0.7	—	—	—
Amide nitrogen	n.c.	n.c.	n.c.	n.c.	(116)	(118)	(132)
Lysine	11.9	7.6	7.4	36.7	36.9	45.0	30.7
Histidine	3.7	0.7	0.6	11.5	14.2	15.4	13.5
Tryptophan§§	n.c.	n.c.	n.c.	11.9	n.c.	n.c.	n.c.
Arginine	11.1	5.2	5.4	42.3	45.2	45.2	41.8

\* Values are given as residues per 1,000 and are the averages of 2 or more determinations, except for the analysis of the alkylated microfibrils. Analyses of the elastic fiber and its amorphous component were corrected for hydrolytic losses. Analyses of microfibrils are uncorrected. Methionine includes methionine sulfoxide, and in the alkylated sample, the degradation products of the carboxymethyl sulfonium salt of methionine. Cystine includes cysteic acid. A dash indicates that the amino acid was either entirely absent or present as less than 0.1 residue/1,000. n.c.: not calculated; CM-cysteine: carboxymethyl-cysteine.

† Obtained as a residue after digestion of microfibrils with chymotrypsin and trypsin.

§ Obtained as a residue after solubilization of microfibrils with DTE.

|| Soluble peptides obtained after chymotryptic and/or tryptic digestion of elastic fibers.

¶ Solubilized by reduction and alkylation.

\*\* Solubilized by reduction in 5 M guanidine followed by dialysis. Precipitate fraction.

‡‡ Solubilized by reduction in 5 M guanidine followed by dialysis. Supernatant fraction.

§§ Determined spectrophotometrically.

Piez (22). Analyses of the amorphous component (elastin) were corrected for hydrolytic losses (23). All of the amino acids listed in Table I, except for carboxymethyl cysteine and hydroxyproline, were adequately separated. Separation of methionine sulfoxide, cysteic acid, homoserine, homoserine lactone, and carboxymethyl homocysteine was also achieved. Analyses for tryptophan were performed by the spectrophotometric method of Edelhoch (24).

## RESULTS

### Appearance of Elastic Fibers *in Vivo*

Fetal calves originally examined as sources of ligamentum nuchae ranged in age from 2 to 9 months. A region typical of an intact ligament from a 5½-month-old fetus is illustrated in Fig. 2. In this micrograph part of a fibroblast

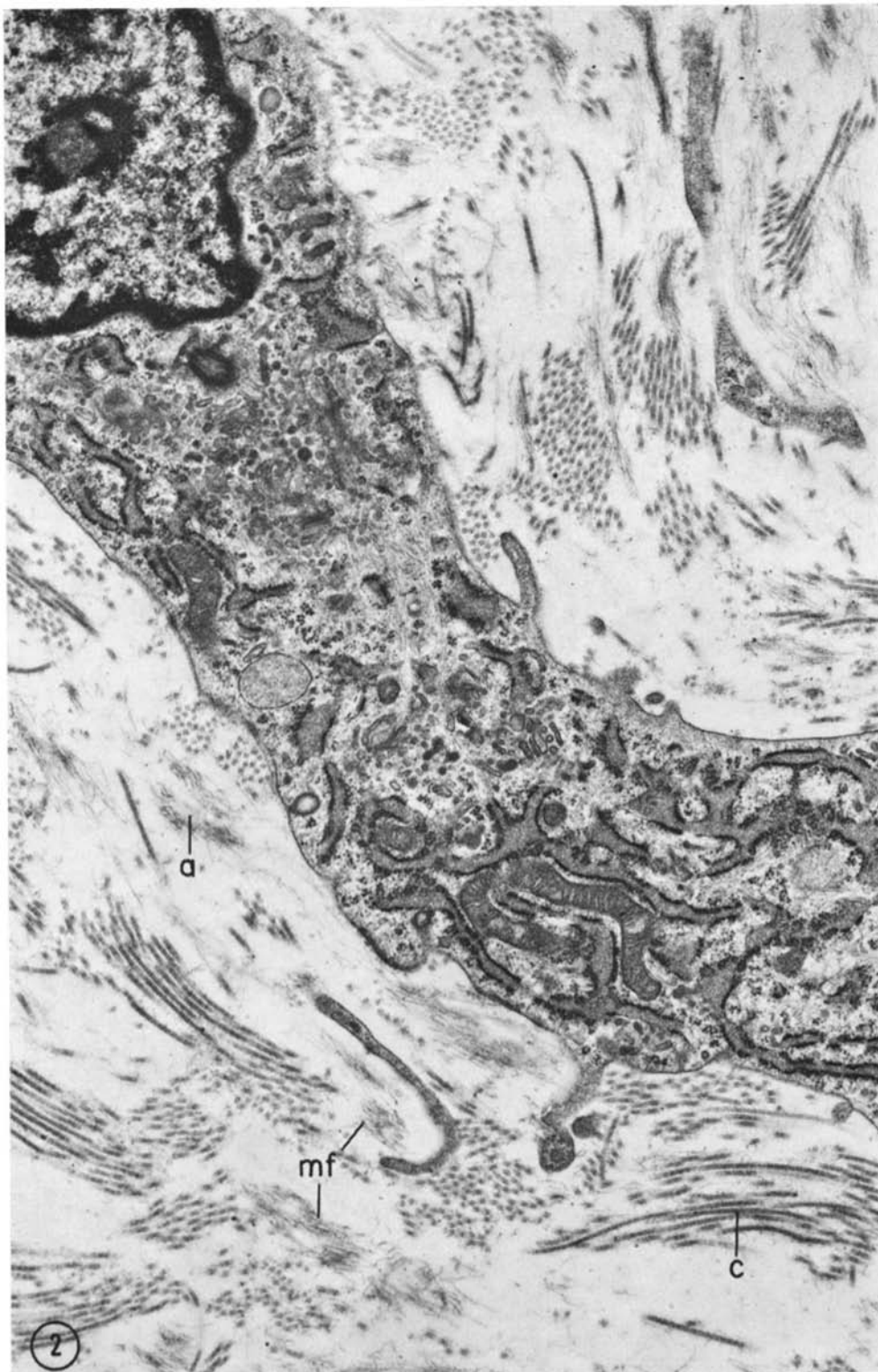


FIGURE 2 This micrograph demonstrates a portion of a 5½-month-old fetal ligamentum nuchae in which part of a fibroblast with its extensive rough endoplasmic reticulum and Golgi complex can be seen. The extracellular matrix consists of collagen fibrils (*c*) and elastic fibers, in which two structures, microfibrils (*mf*) and a central amorphous component (*a*), are evident.  $\times 13,000$ .

can be seen together with the developing connective tissue matrix which consists of collagen fibrils and many small elastic fibers. Both morphologic components of the relatively immature elastic fibers are visible at this stage of development.

The first recognizable elastic fibers in very young fetuses consist of only a single component, the tubular-appearing microfibrils. These fibrils are usually grouped in bundles, in parallel array, and individually may appear as a string of beads or vesicles (Fig. 3a). With increasing fetal age the elastic fibers contain a second morphologic component, an amorphous-appearing substance usually surrounded by the microfibrils. Fig. 3b demonstrates part of an elastic fiber from a 4½-month-old fetal calf in which this arrangement of the two components of the elastic fiber can be seen. At term (9 months) most of the elastic fibers are quite large (2–6μ diameter) (Fig. 4). In these fibers the predominant structure is the central amorphous component. In Fig. 4 the elastic fiber has been sectioned on a bias, permitting the peripheral envelope or mantle of microfibrils to be more easily visualized. 9-month fetal ligaments were used as the source of preparations for all of the studies which follow.

#### *Appearance of Purified Elastic Fiber Preparations*

The elastic fiber preparations obtained after guanidine-collagenase extraction were free of collagen fibrils, and the elastic fibers were morphologically identical with those seen in the intact ligament (compare Figs. 4, 5, and 6). The fibers illustrated in Figs. 5 and 6 are typical of those present in the starting material utilized in the experiments described below.

#### *Enzyme Digestion Studies*

##### TRYPSIN AND CHYMOTRYPSIN

After digestion with trypsin and/or chymotrypsin the microfibrils were missing from the surface of each elastic fiber. Fig. 7 demonstrates an elastic fiber preparation after digestion with chymotrypsin. Fig. 8 shows a control specimen of a similar digest in which the enzyme was boiled for ten min before use. Comparison of Figs. 6 and 8 with Fig. 7 demonstrates that the microfibrils were selectively removed by chymotrypsin.

##### HYALURONIDASE AND β-GLUCURONIDASE

Digestion with either hyaluronidase or β-glucuronidase did not alter the morphology of either component of the elastic fiber.

##### ELASTASE

The appearance of the elastic fibers was markedly altered after digestion with elastase. Examination of elastase-treated elastic fibers after 15, 30, and 45 min demonstrated increasing, selective lysis of the amorphous component. In contrast, the microfibrils were not affected by elastase after 15 and 30 min (Fig. 9). However, partial digestion was observed after 45 min.

#### *Nonenzymatic Solubilization*

Elastic fiber preparations treated with dithioerythritol in 5 M guanidine were morphologically identical with those seen after either trypsin or chymotrypsin digestion (compare Fig. 10 with Fig. 7). The microfibrils were selectively solubilized by this process, leaving the amorphous core of the elastic fiber as an insoluble residue.

#### *Amino Acid Analyses*

The ability, by two different approaches, to selectively remove the microfibrils from the surface of the elastic fibers provides a means of obtaining relatively pure preparations of each major component of the elastic fiber for amino acid analysis.

##### AMORPHOUS COMPONENTS

The amino acid analyses (Table I) of the residue (the amorphous component) obtained either after enzymatic digestion of elastic fibers or solubilization of microfibrils with DTE demonstrate a composition virtually identical with that previously determined for elastin by other investigators (19, 25–27). Characteristically, this material contains approximately 1% hydroxyproline, is relatively rich in proline, glycine, alanine, and valine, relatively poor in aspartic acid, threonine, serine, cystine, and glutamic acid, and contains desmosine and isodesmosine.

##### INTACT ELASTIC FIBER

The intact elastic fibers (containing both components) possess a composition which is similar

to that of the amorphous component but is richer in polar, hydroxy, and sulfur-containing amino acids, suggesting the presence of an additional protein or proteins (Table I).

#### MICROFIBRILS

Extraction of a lyophilized, rehydrated pellet of intact elastic fibers with enzymes, or guanidine and DTE, resulted in a supernatant containing

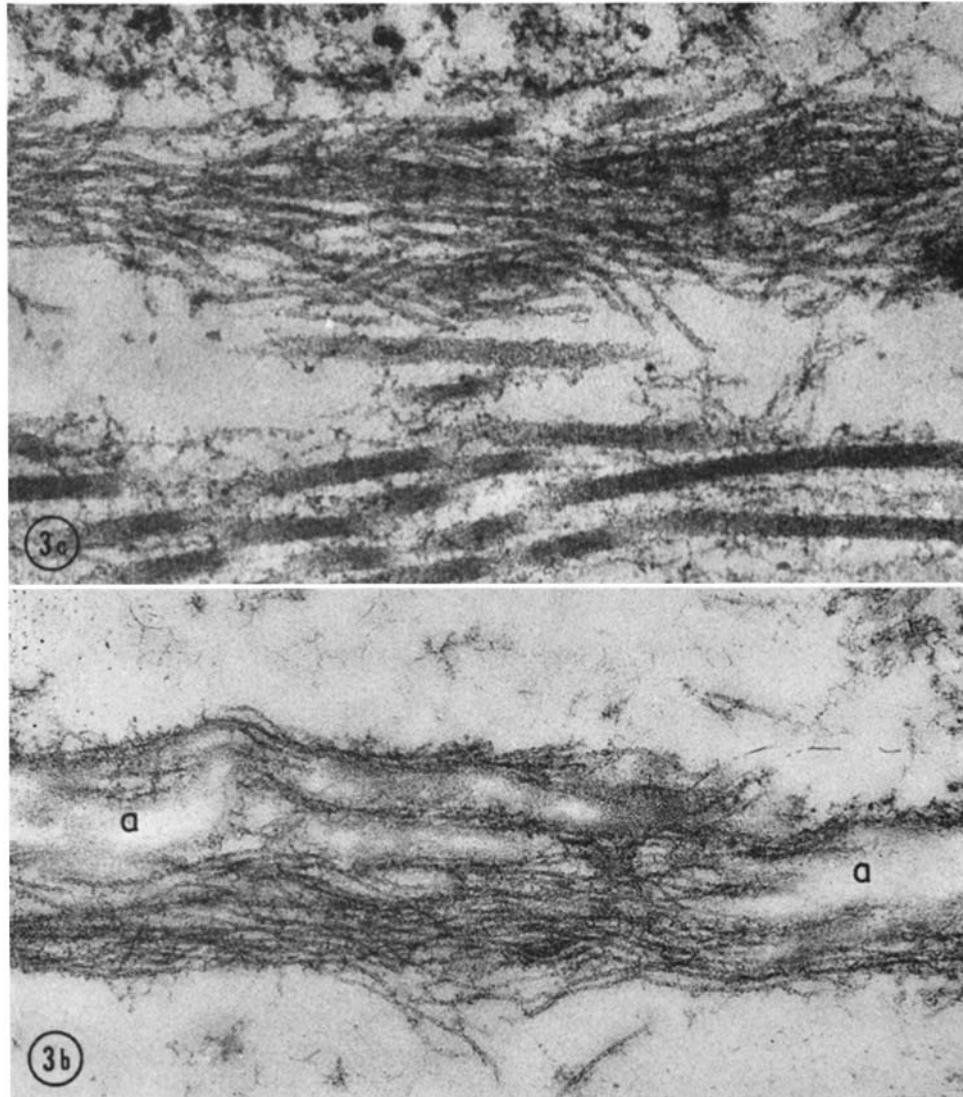


FIGURE 3a Part of an immature elastic fiber from a 3-month-old fetal calf ligament together with several collagen fibrils are present in this micrograph. The beaded appearance of the microfibrils is apparent in both this figure and in Fig. 3b. In contrast to Fig. 3b, the most immature elastic fibers consist of a single component, a homogeneous aggregate of the microfibrils.  $\times 79,000$ .

FIGURE 3b This micrograph contains part of an elastic fiber from a  $4\frac{1}{2}$ -month-old fetal calf ligamentum nuchae in which both components of this fiber are visible. At this time the microfibrils surround and are located between a second component, the amorphous constituent of the elastic fiber (a). Note that this amorphous component has relatively little affinity for the lead and uranyl acetate with which this section has been stained.  $\times 42,000$ .

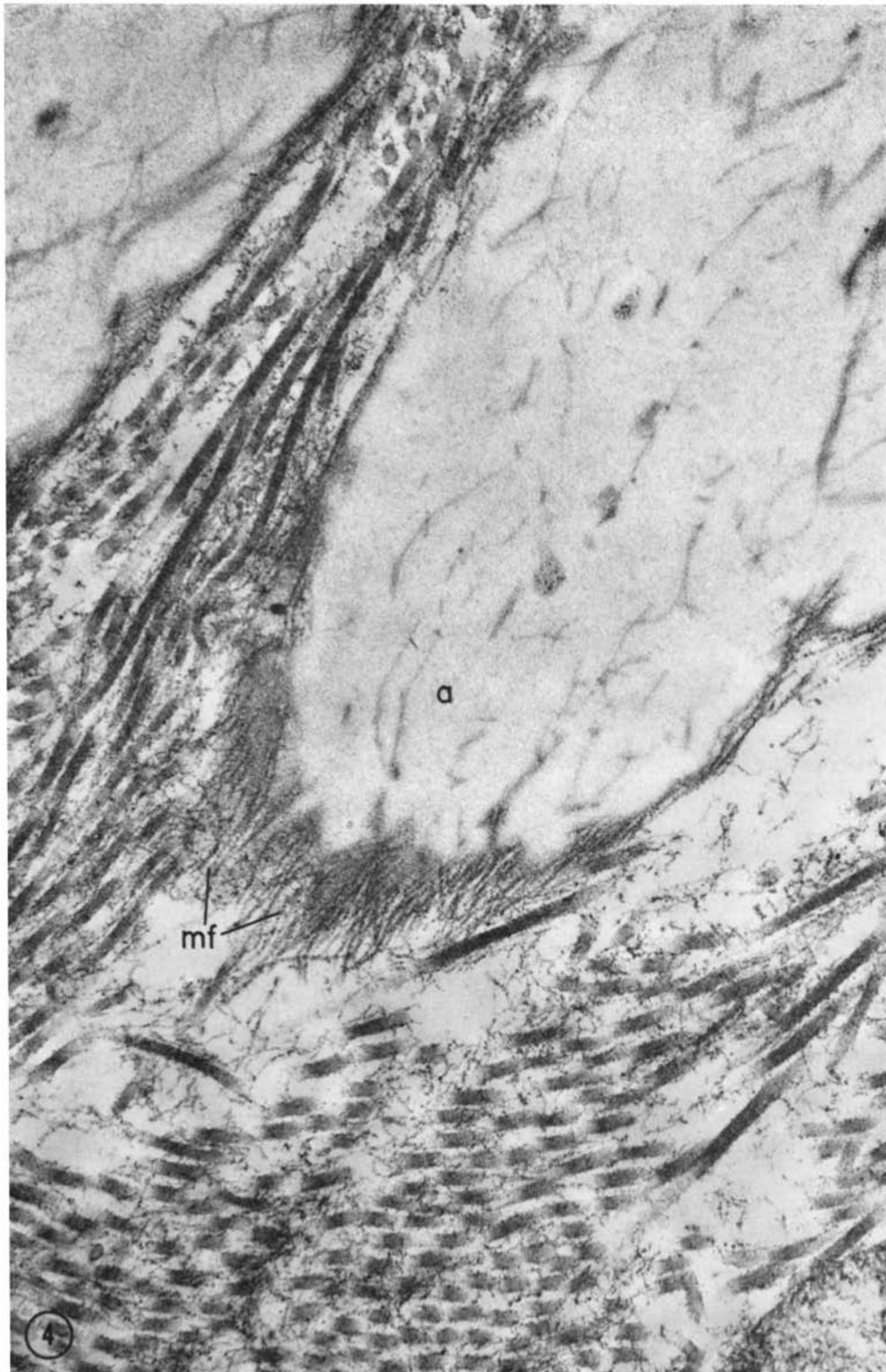


FIGURE 4 Part of a 9-month-old fetal ligament in which the collagen fibrils and the large elastic fibers characteristic of this age are apparent. By this time the elastic fibers are quite large ( $2-6\mu$  diameter) and the major constituent of the elastic fiber is the amorphous component (*a*) which does not stain with lead or uranyl acetate. This fiber is cut somewhat on a bias so that the peripheral envelope or mantle of microfibrils (*mf*) can be seen at the cut edge of this fiber. Ligaments of this age served as the starting material for the elastic fiber preparations used in the remainder of this paper.  $\times 41,000$ .



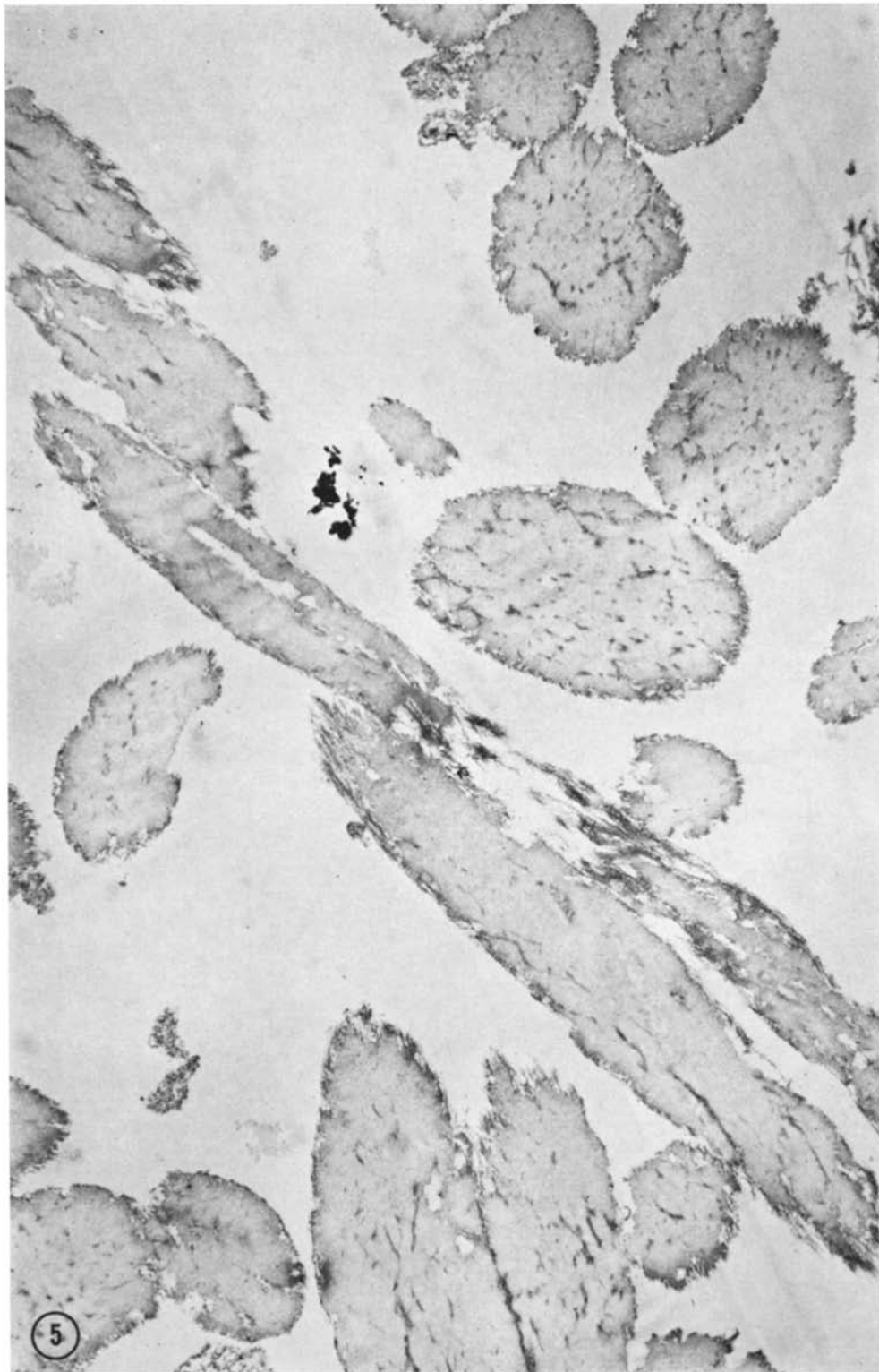


FIGURE 5 This micrograph contains a representative portion of a preparation of elastic fibers obtained from fetal ligaments such as those seen in Fig. 4. The elastic fibers have been sectioned both transversely and longitudinally, and both the microfibrillar component and the amorphous constituent are present. These intact fibers served as starting material for the isolation of both the microfibrils and the amorphous component.  $\times 15,000$ .

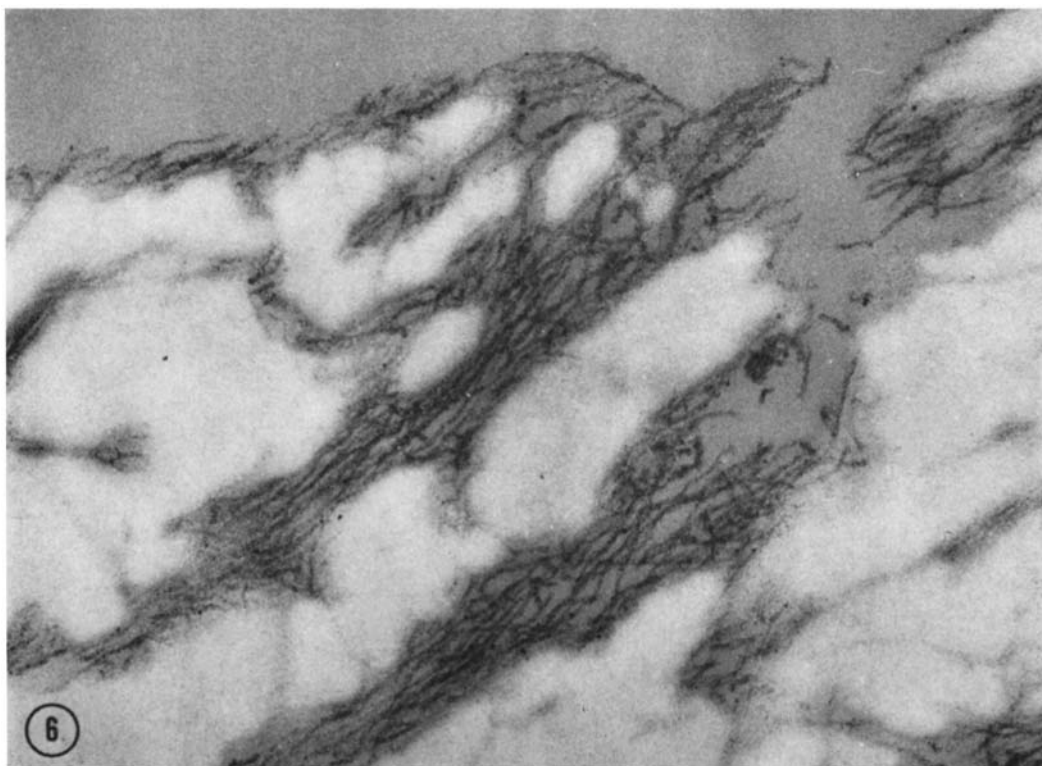


FIGURE 6 This micrograph demonstrates, at a higher magnification, a part of one of the elastic fibers seen in Fig. 5. The microfibrils surrounding the amorphous component of the mature elastic fiber are evident.  $\times 55,000$ .

either the degraded microfibrils or the undegraded solubilized microfibrillar protein, respectively (Fig. 1). The undegraded microfibrils were isolated by alkylation and molecular sieve chromatography of the solubilized protein, or by dialysis against water. In the latter instance, most of the material precipitated and was isolated by centrifugation.

The microfibrillar protein is markedly different from the amorphous component in amino acid composition (Table I). The most accurate analyses of the microfibrillar protein are probably those obtained after dialysis of DTE-solubilized material. These fractions, a precipitate and a supernatant, differ from each other in their content of cystine, alanine, valine, leucine, and lysine, and may contain different proteins. Both fractions differ from the amorphous component in containing far less glycine, valine, alanine, and proline and more polar, hydroxy, and sulfur-containing amino acids.

The microfibrillar preparations obtained as enzymatic digests or by alkylation differ somewhat in composition from those obtained by dialysis. It is likely that the enzymatic digest is contaminated by small amounts of peptides resulting from partial digestion of residual collagen and/or the amorphous component. The alkylated preparation appears to be a mixture of the soluble and insoluble microfibrillar fractions obtained after dialysis.

## DISCUSSION

### *The Central Amorphous Component*

The observations reported here establish that the central, amorphous component, which constitutes the major portion of the mature elastic fiber, is identical with elastin as defined by previous investigators (19, 25-27). This conclusion is indicated both by amino acid analyses and by

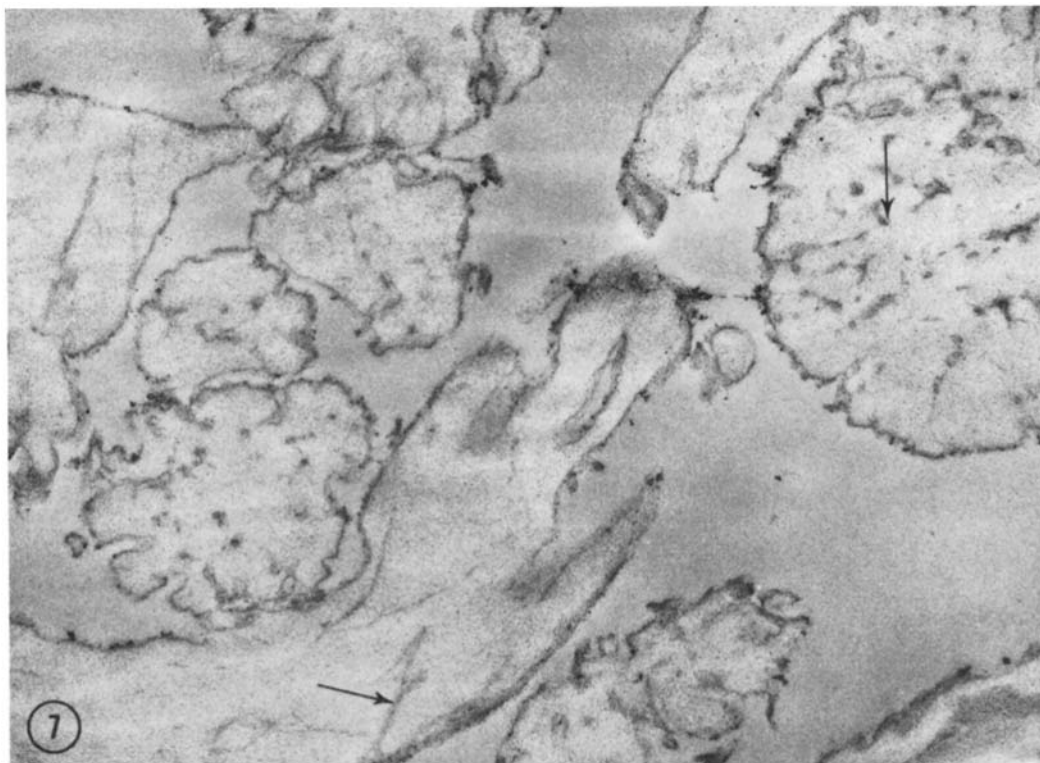


FIGURE 7 This micrograph contains a preparation of elastic fibers which were digested with chymotrypsin for 24 hr. The preparation was then centrifuged. The fibers in this micrograph are representative of the pellet. As compared with those seen in Fig. 6, the elastic fibers lack microfibrils and consist largely of the amorphous component. Some undigested microfibrils are embedded within the interstices of these fibers (arrows).  $\times 24,000$ .

the greater susceptibility of the amorphous component to elastase. It is further supported by the observation in the electron microscope that alkali-extracted elastic fibers (defined operationally as elastin) lack peripheral microfibrils and contain largely the amorphous component (R. Ross, unpublished observations). Since it is known that available elastase preparations contain both elastolytic and some nonspecific proteolytic activity, the eventual degradation of the microfibrils after prolonged digestion with elastase is to be expected.

These studies demonstrate that the elastic fiber is not homogeneous, but contains elastin and at least a second protein component, the microfibrils. The possibility that the amorphous component, elastin, consists of more than one macromolecular species should also be considered.

### *The Microfibrils*

The microfibrils of the elastic fiber were observed to be the first structures to appear in elastic fibers during embryogenesis (1-3, 5). Their different appearance and staining characteristics initially suggested that they might represent a different protein (1). Our investigations have provided a means of separating and partially characterizing these fibrils. An important adjunct to these investigations has been the ability to monitor each experiment by electron microscopy so that the biochemical effects upon the elastic fibers could be compared with the morphologic changes.

The selective degradation of elastic fiber microfibrils by chymotrypsin and trypsin makes it possible to determine their amino acid content and their relative contribution to the elastic fiber. In term fetal ligaments the microfibrils represent

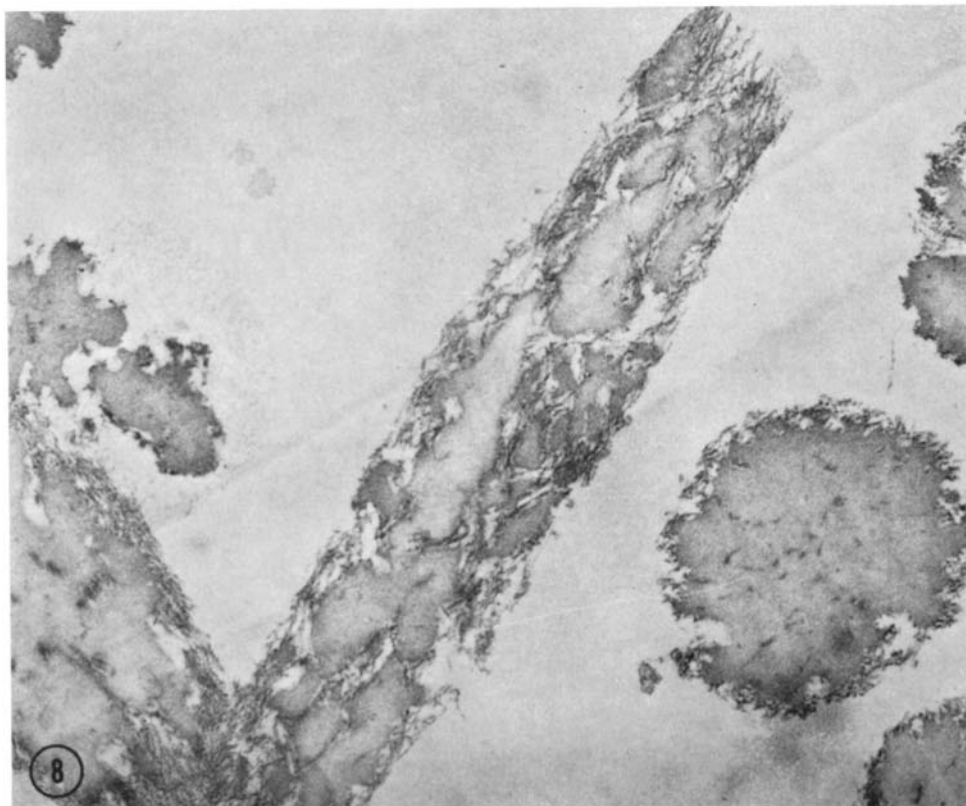


FIGURE 8 In this micrograph the elastic fibers were treated with heat-inactivated chymotrypsin. In this case, both components of the elastic fiber remain.  $\times 24,000$ .

approximately 5–10% of the dry weight of the elastic fibers. The composition of the preparation obtained after enzymatic digestion (Table I) indicates that the microfibrils differ markedly in amino acid content from the bulk of the elastic fiber. However, in view of the likelihood of contamination by both elastin fragments and residual collagen, a method for obtaining purer, undegraded preparations of microfibrils was sought.

The use of dithioerythritol in the presence of 5 M guanidine proved effective in selectively solubilizing the microfibrillar component of the elastic fiber. At least two components appear to be present, differing in solubility and amino acid composition (Table I). The analyses are in reasonably close agreement with those obtained after enzymatic treatment and indicate that the microfibrils represent a different connective tissue protein, or proteins, that is neither collagen nor elastin. The determination of the carbohydrate

content and the further characterization of these proteins are in progress.

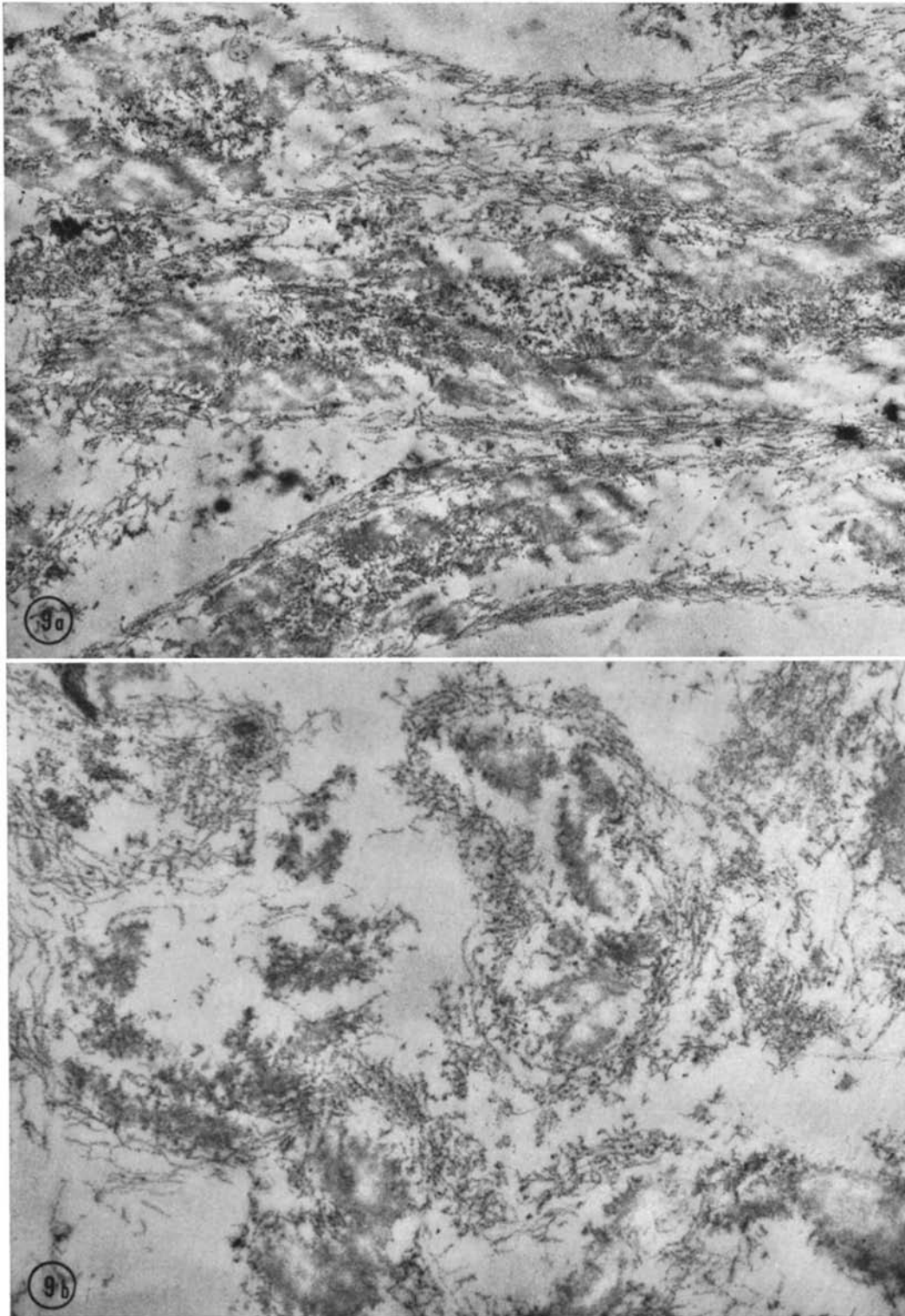
Cleary et al. (28) have also reported the composition of extracts from young fetal nuchal ligaments that contained relatively large amounts of polar amino acids and a lower content of glycine and valine. These preparations were probably impure and it is likely that they contained mixtures of microfibrillar protein, elastin, and other components.

#### *Relationships Between Elastic Fiber*

##### *Microfibrils and Other Connective*

##### *Tissue Fibrils*

Several investigators have stated that the central amorphous component, elastin, consists of filamentous subunits of varying size (29–31), and some have proposed that this component consists of “fine fibrils and a dense, non-fibrous



FIGURES 9a and 9b These two micrographs are a low and a higher magnification of an elastic fiber preparation that was treated with elastase for 15 min. The appearance of the elastic fibers indicates that the centrally located amorphous component has been selectively removed. In some regions the outline of the elastic fiber is still discernible although relatively little of the amorphous component remains. The microfibrils which surround and are located within the interstices of the elastic fiber remain, indicating the greater susceptibility of the amorphous component to elastase digestion.  $\times 15,000$  (9a) and 28,000 (9b).

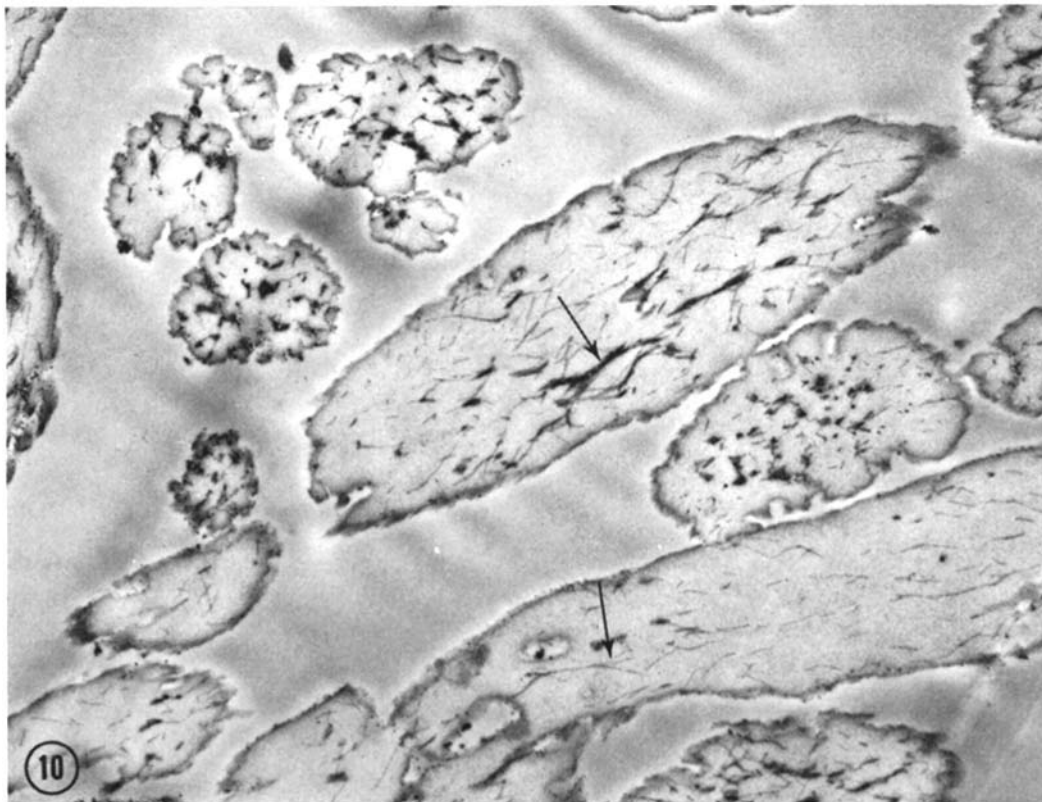


FIGURE 10 This preparation of elastic fibers was reduced with dithioerythritol in 5 M guanidine. The microfibrils have been selectively solubilized and removed from the outer surface of the elastic fibers, leaving the amorphous component as a residue. Those microfibrils located within the interstices of the elastic fiber remain (arrows).  $\times 12,000$ .

cement material" (14, 32). Others were unable to find a fibrous element in elastin (1-6, 33). The microfibrils studied in our investigations are unrelated to the various fibrillar elements described in the amorphous component, but rather represent a second component of the elastic fiber.

Relatively small extracellular fibrils of varying diameter (40-150 Å) have been described in the connective tissues of different organs. These fibrils have been found both free and in association with other structures, including elastic fibers, basement membranes, or laminae (also called boundary membranes by Low (34)), collagen (7, 8), lymphatic channels (35), and in close proximity to the surfaces of various cells (12-14).

A wide variety of hypotheses have been presented in attempts to establish the identity of one size category of these fibrils, the 110 Å micro-

fibrils. Haust (7) proposed that they represented a continuum within the connective tissue and stated that there is one organized morphologic precursor, the "microfibril," common to both collagen and elastic tissue. A somewhat similar notion had been previously proposed by Karrer (10) who presented some of the first definitive micrographs of these structures and suggested that they were an integral part of the elastic fiber. On the basis of the juxtaposition of the microfibrils of the elastic fiber with other connective tissue elements, it has also been proposed that they are collagen (6), elastin precursors (2, 17), or protein-polysaccharide (16, 36, 37). These microfibrils may be ubiquitous within the connective tissue and may be found to be associated with a variety of structures.



### *The Role of the Microfibrils in the Morphogenesis of Elastic Fibers*

The observation that the microfibrils form an aggregate structure before any elastin (the amorphous component) is visible (1, 3) suggests that they may play a role in determining the shape of elastic fibers in various tissues. In ligamentum nuchae and tendon these fibrils gather into small cylindrical aggregates, whereas in aorta they form a more or less flat sheet which follows the contour of the vessel.

The microfibrils appear to be under intimate control of the cell as they characteristically form in close apposition to its surface, and are often found in niches or infoldings of the cell (5). Elastin could then aggregate between and around the microfibrils and take the shape already assumed by the fibrils, thus leading to a cylindrical fiber in ligamentum nuchae and tendon, and a fenestrated sheet in aorta. The interactions between the microfibrils and elastin may be partly ionic since the two components differ in charge, as evidenced by their amino acid compositions and staining properties.

A consideration of the normal function and pathologic alterations of elastic tissues and of elastic fiber synthesis must consider both components of the elastic fiber. An understanding of the role played by the elastic fiber microfibrils should therefore be enhanced by the further characterization of the constituents of the fiber.

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