

## **Marfan syndrome. Demonstration of abnormal elastin in aorta.**

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### **Research Article**

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# Marfan Syndrome

## DEMONSTRATION OF ABNORMAL ELASTIN IN AORTA

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**ABSTRACT** Aortae from three patients with classic presentation of Marfan syndrome, who died of vascular complications, were subjected to biochemical analyses of the connective tissue; for comparison, aortae from eight age-matched controls, without evidence of connective tissue abnormalities, were examined. Elastin was prepared from the aortae by two techniques. First, the tissues were extracted with 5 M guanidine-HCl, bacterial collagenase digestion and reduction with dithiothreitol (elastin I preparation). Secondly, this material was further purified by extraction with 0.1 M NaOH at 99°C (elastin II preparation). Amino acid analyses of both elastin preparations indicated that the values for desmosine and isodesmosine were reduced in Marfan cases to approximately one-half of the control values. A corresponding increase in lysyl residues was noted in elastin II preparations. Also, the concentration of elastin per milligram dry weight of tissue was reduced in Marfan cases. The hydroxyproline content of elastin was increased in two cases with the Marfan syndrome. Recoveries indicated that the alkali treatment solubilized 46.2% of the elastin I preparations in Marfan aortae compared with 23.7% in controls. In contrast to elastin, the concentration and solubility of collagen were unchanged; the amino acid composition and the genetic types of insoluble collagen isolated by limited pepsin proteolysis were the same in both Marfan and control aortae. The results of our study demonstrate that the cross-linking of aortic elastin is reduced in the three patients with

Marfan syndrome. Thus, a defect in elastin could explain the vascular fragility observed clinically in these patients.

### INTRODUCTION

Marfan syndrome, a prototype of heritable disorders of connective tissue (1, 2), clearly involves aberrations in the extracellular matrix. Clinically, several organs, including the ocular, cutaneous, skeletal, and cardiovascular systems are affected. The most severe complications of this syndrome are due to weakness of the aortic structures, manifested by aneurysms, dissection and rupture of the aorta, which frequently lead to premature demise of the patients.

Previous biochemical studies have suggested collagen aberrations, including abnormal cross-linking (3), increased solubility (4-6), elevated urinary excretion of hydroxyproline (7), and accelerated collagen synthesis and turnover (8). Also, in one isolated case, an abnormality in the  $\alpha 2$  (I)-chain of type I collagen has been reported (9, 10). Histopathologic and ultrastructural observations have indicated, however, that the major pathologic feature of the aorta in Marfan syndrome resides in the elastic tissues (11), but no biochemical abnormality has been demonstrated in elastin in these patients. Recently, assay of urinary desmosines, representing breakdown products of elastin, has revealed markedly reduced values in patients with Marfan syndrome, as compared with controls (12), suggesting that the metabolism of elastin may be altered in these patients. Also, recent studies have indicated that insoluble elastin isolated from aortae of two patients with Marfan syndrome is more susceptible to digestion by pancreatic elastase than control elastin (13).

In this study, we have examined the biochemical composition of aortic connective tissue in three patients who died from complications of the Marfan syn-

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A preliminary report of this study was given at the 66th Annual Meeting of the Federation of American Societies for Experimental Biology, New Orleans, 15-23 April 1982 and appeared in abstract form in 1982. (*Fed. Proc.* 41: 852a.)

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drome. The results indicate that the concentration of elastin is significantly reduced in the aortae of these patients, as compared with age-matched control subjects. Also, the content of desmosines in elastin isolated from Marfan aortae was diminished. No abnormalities were found in aortic collagen in these patients. Thus, a structural abnormality in elastin may be the underlying defect in some patients with the Marfan syndrome.

## METHODS

### *Clinical studies*

Three patients with Marfan syndrome were included in the study; they all had a classic form of the disease as judged by clinical, genetic, and pathologic observations (see below). The aortic tissues were obtained at autopsy within a few hours of death. For comparison, aortae were obtained from eight age-matched control subjects who died from nonvascular causes. None of the control patients had clinical or histopathologic evidence of Marfan syndrome or other heritable disorders of connective tissue. Detailed clinical features of the three patients with Marfan syndrome are as follows:

**Patient 1.** This 34-yr-old male, who was 197 cm tall, had arachnodactyly, scoliosis, and pectus excavatum. His father had died at age 37 yr from cardiovascular problems, and a brother, alive at age 28, had a diagnosis of Marfan syndrome. The patient had a replacement of the aortic valve with a Bjork-Shiley prosthesis and repair of an acute aortic dissection at age 30. A week before his terminal admission, the patient developed fever, and blood cultures yielded *Staphylococcus epidermidis*. In spite of appropriate antibiotic therapy the fever continued and he died 1 wk later in ventricular fibrillation. Autopsy showed vegetative endocarditis on the prosthetic aortic valve yielding *Candida albicans*, and dehiscence of the prosthesis, as well as recent infarcts of the spleen, kidneys, and myocardium due to emboli. At autopsy, several dissecting aneurysms of thoracic aorta and right subclavian artery, which had developed multiple saccular segments, were noted. Microscopic examination of the aorta showed loss and fragmentation of the elastic fibers (Fig. 1). Also, accumulation of metachromatic extracellular matrix was noted.

**Patient 2.** This 41-yr-old male, who was 193 cm tall, had a family history of the Marfan syndrome. His mother had died at age 28 yr, and his sister at age 49, both of aortic hemorrhages. The patient had had bilateral inguinal herniorrhaphy at age 19. At age 36, chest pain had led to cardiac catheterization that showed a dilated aorta. 2 yr later, another severe episode of chest pain led to an aortogram that showed dissection from the left subclavian to the iliac arteries. About 7-cm segment of proximal descending thoracic aorta was resected and replaced by a Dacron graft. Microscopic examination of the resected wall showed cystic medial necrosis of Erdheim. 6 mo later intermittent lower back and chest pain led to discovery of increased dilatation of the descending aorta. New dissections of the left subclavian artery and the descending thoracic and abdominal aorta were revealed a month later. It was elected to treat him medically, but ~2 yr later increased chest pain and a murmur of aortic insufficiency required surgical intervention. A Dacron graft

was placed within the aorta below the earlier anastomosis. The patient, however, died from surgical complications. Autopsy showed a markedly dilated ascending aorta (10-cm circumference). A 2 × 2-cm dissecting aneurysm of the proximal left subclavian artery had two ostia into the lumen. The dissection continued throughout the abdominal aorta to its bifurcation and was connected by four small ostia to the lumen of the aorta. The left renal artery opened directly from the aneurysm. Microscopic examination of the aortic tissues showed stretching of medial elastic fibers and deposition of amorphous metachromatic extracellular material.

**Patient 3.** This 36-yr-old male, who was 194 cm tall, had severe kyphoscoliosis. He had undergone bilateral cataract extractions at age 9, bilateral inguinal herniorrhaphy at age 22 and had suffered a right retinal detachment at age 32. When this was repaired in 1973, a diagnosis of the Marfan syndrome was first made. He had a family history strongly suggestive of the Marfan syndrome. His father (182 cm tall) had died of unknown cause at age 35; a sister (188 cm tall) had died of a "heart attack" at age 34; another sister and a brother (188 cm tall) were blind; this sister had seven children, two of whom had very poor vision. 1 yr after the initial diagnosis of the Marfan syndrome the patient had bouts of supraventricular and ventricular arrhythmias and hypertension. Cardiac catheterization showed ascending aortic dilatation and aortic insufficiency, and there was evidence of aortic dissection. He was admitted for aortic valve replacement and a dissection of the ascending aorta was discovered and resected. A Dacron graft was placed from 2.5 cm above the aortic root to the proximal edge of the innominate artery ostium. Massive bleeding, however, followed immediately after the surgery and the patient died a short time later. Autopsy showed extension of the sutured aortic perforation with hemopericardium and there was a 4-mm extension of the aortic tear. Microscopic examination of the aorta and left common carotid artery showed disruption of the elastic laminae and small cystlike collections of extracellular matrix.

### *Preparation of tissues*

The outline for preparing collagen and elastin from aortae is shown in Fig. 2. First, 4-mm punch biopsies were taken from random sites through the intima and media of frozen aortae taking care to avoid any atherosclerotic plaques. The aortae were then weighed, homogenized with a Polytron tissue homogenizer in 0.05 M Tris-HCl buffer, pH 7.5, containing 1.0 M NaCl, 20 mM Na<sub>2</sub>EDTA, 5 mM N-ethylmaleimide (NEM), 1 mM phenylmethylsulfonyl fluoride (PMSF) and 50 mM  $\epsilon$ -aminocaproic acid, at 4°C. The homogenate was extracted for 18 h and then centrifuged at 27,000 g for 60 min. Aliquots of the supernatant were taken for assay of soluble protein, hydroxyproline, and uronic acid (see below). The rest of the supernatant was adjusted to 4.4 M NaCl to precipitate collagenous polypeptides. After centrifugation, part of the precipitate was dialyzed against 0.5 M acetic acid at 4°C, and digested with pepsin (300  $\mu$ g/ml) for 3 h at 25°C. Another part of the precipitate was dialyzed vs. 0.15 M NaCl, 5 mM CaCl<sub>2</sub>, and 50 mM Tris-HCl, pH 7.6, and digested with highly purified collagenase (Sigma type VI, Sigma Chemical Co., St. Louis, MO) for 3 h and 25°C. The enzyme digests and an undigested sample of the precipitate were heated at 100°C for 5 min in the presence of 10% sodium dodecyl sulfate (SDS) and 4 M urea and analyzed by SDS-polyacrylamide slab gel electrophoresis using 8% gels (14).

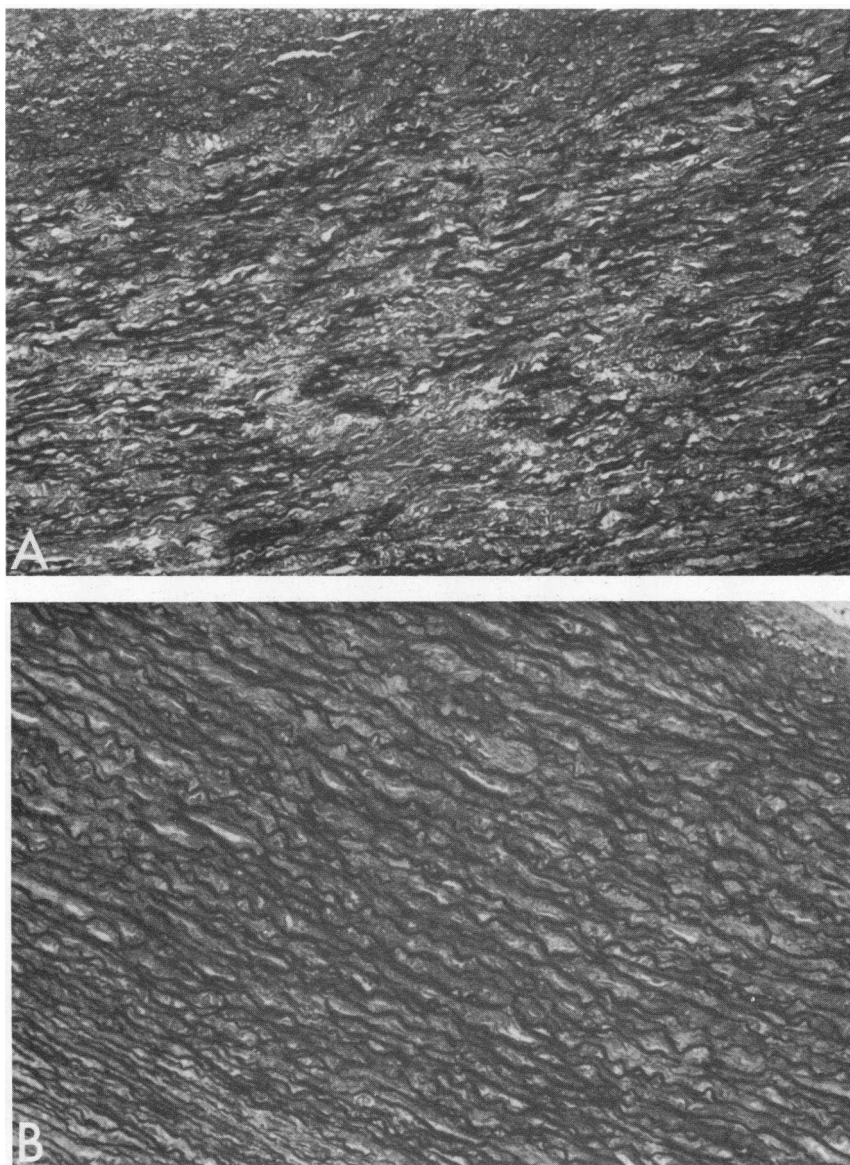


FIGURE 1 Demonstration of abnormal elastic fibers in aorta of a patient with Marfan syndrome (case 1). In diseased aorta (A) fragmentation and patchy loss of elastic fibers can be noted when compared with normal aorta (B). (Verhoeff-van Gieson stain; original magnification  $\times 10$ .)

The supernatant of the 4.4 M NaCl precipitation was adjusted to 60% saturation of ammonium sulfate (390 mg/ml). After 18 h precipitation at 4°C, the samples were centrifuged at 27,000 *g* for 60 min and the precipitate was dialyzed against 0.1 M ammonium formate containing the protease inhibitors indicated above. A mixture of propanol and butanol (1.5:2.5, vol/vol) was added dropwise at 4°C (15). The samples were centrifuged, and the supernatants were evaporated and prepared for SDS-polyacrylamide gel electrophoresis, as above. The proteins were stained with Coomassie Brilliant Blue and their mobilities were compared with that

of 72,000-D tropoelastin prepared from aorta of copper-deficient pigs.

For analyses of collagen, the insoluble residue, after extraction with 1.0 M NaCl, was digested with pancreatic elastase at a 1:100 enzyme to substrate ratio, at 37°C for 20 h (16). The sample was centrifuged at 27,000 *g* for 60 min, and the pellet was redigested with elastase. After recentrifugation, the pellet was washed with 0.15 M NaCl, 50 mM Tris-HCl, pH 7.5, and resuspended in 0.5 M acetic acid. After dialysis against the same buffer, an aliquot was prepared for amino acid analysis (see below). Another aliquot

## PREPARATION OF ELASTIN AND COLLAGEN FROM HUMAN AORTA

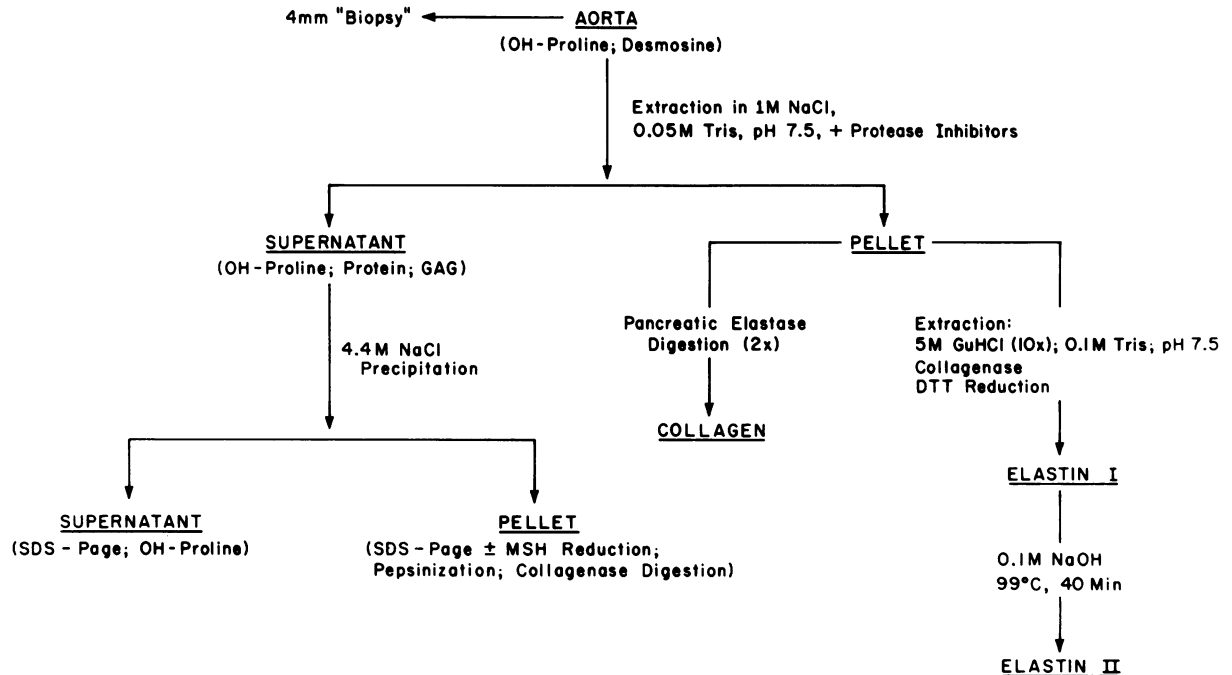


FIGURE 2 Preparation of collagen and elastin from human aorta. GAG, glycosaminoglycans; DTT, dithiothreitol; MSH, mercaptoethanol; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

was subjected to digestion with pepsin (17). The solubilized collagen was examined by SDS-polyacrylamide gel electrophoresis with and without reduction with 2-mercaptoethanol. Part of the solubilized collagen was also fractionated into genetically distinct types using 1.7, 2.6, 3.6, and 4.4 M NaCl concentrations at neutral pH (18).

For preparation of elastin, the 1 M NaCl insoluble residue was extracted with 0.1 M Tris-HCl, pH 7.4, containing 5 M guanidine-HCl, for 18 h at 4°C. The extraction was repeated 10 times, and the insoluble residue was further extracted 2 times with 0.1 M Tris-HCl, 50 mM dithiothreitol, and 0.1% Na<sub>2</sub>EDTA. The final residue was digested with bacterial collagenase, at a 1:100 enzyme/substrate ratio, in 50 mM Tris-HCl, pH 7.5, containing 0.15 M NaCl and 10 mM CaCl<sub>2</sub>, at 37°C for 24 h. The collagenase-resistant material was washed twice with 5 M guanidine-HCl; this material is referred to as elastin I preparation. This preparation was further subjected to extraction with 0.1 M NaOH at 99°C for 40 min (20). The extraction was repeated three times; this material is referred to as elastin II preparation. Both elastin I and elastin II preparations were dialyzed against distilled water, lyophilized, and subjected to amino acid analyses.

### Assay procedures

Hydroxyproline was determined by a specific colorimetric assay according to Kivirikko et al. (21). Uronic acid was assayed by the method of Bitter and Muir (22), and proteins were determined according to Lowry et al. (23). SDS-poly-

acrylamide gel electrophoresis was carried out as outlined by Laemmli (14), and in some cases a delayed reduction of disulfide bonds by 2-mercaptoethanol was used (24). Amino acid analyses were performed on a Beckman 119 CL amino acid analyzer (Beckman Instruments, Inc., Fullerton, CA).

### Calculations

The concentrations of elastin in the aortae were calculated on the basis of desmosine determinations (25). Specifically, the number of micromoles desmosine per microgram dry weight of aorta was divided by the same value obtained for elastin I preparation in the same aortic specimen, as indicated above.

The concentrations of collagen in the aortae were calculated from the hydroxyproline analyses of the tissue. The amount of hydroxyproline in elastin, as determined by amino acid analyses, was first subtracted from the total hydroxyproline values. The concentration of collagen was then obtained by multiplying the hydroxyproline values by a factor of 8.2.

The statistical calculations were performed using Student's *t* test.

## RESULTS

The aortae from three patients with definitive Marfan syndrome and from a total of eight age-matched con-

trols were subjected to biochemical analyses. First, solubilities of total protein, collagen (measured as hydroxyproline), and proteoglycans (measured as uronic acid) were determined; no differences in these parameters were noted. Specifically, the concentration of soluble proteins in two Marfan patients was 6.8 and 10.2 mg/100 mg dry weight of aorta, as compared with  $6.4 \pm 0.8$  (mean  $\pm$  SD) in seven controls. The amount of collagen solubilized by 1 M NaCl in three patients with Marfan syndrome was  $1.06 \pm 0.42\%$  of the total collagen, as compared with  $0.96 \pm 0.18\%$  in eight controls. The concentration of extractable uronic acid was 0.92 and 0.89 mg/g dry weight of aorta in two patients, as compared with  $1.10 \pm 0.37$  in seven controls. Further analyses of the 1 M NaCl soluble collagen by SDS-polyacrylamide gel electrophoresis, with and without reduction of the disulfide bonds, revealed only type I collagen, and there was no evidence of collagen precursor forms. Furthermore, the propanol-butanol extraction, followed by polyacrylamide gel electrophoresis, of the 1 M NaCl soluble fraction did not reveal any 72,000-D tropoelastin.

The insoluble residue from the 1 M NaCl extraction was subjected to several different isolation procedures (Fig. 2). First, elastin was prepared by successive steps including extractions in 5 M guanidine-HCl, digestion with bacterial collagenase, and disulfide bond reduction by dithiothreitol (elastin I; Methods). Amino acid analyses of elastin I preparations indicated that the contents of desmosine and isodesmosine were low in all three patients with the Marfan syndrome (Table I). The content of hydroxyproline was exceptionally high in two patients with Marfan syndrome (cases 1 and 3), while the value for the third case (case 2) was within the limits of control values (Table I). In general, the content of acidic amino acids tended to be elevated in elastin I prepared from Marfan aortae, as compared with controls.

The elastin I preparations were further subjected to extraction with 0.1 M NaOH at 99°C for 40 min. This procedure solubilized an average of 46.2% of the elastin I preparation in the Marfan samples, while in the controls only 23.7% was solubilized. Amino acid analyses of the final insoluble residue, elastin II, still dem-

TABLE I  
Amino Acid Compositions of Elastin Preparations from Control and Marfan Aortae

| Amino acid                 | Elastin I preparation* |         |       |       |                 | Elastin II preparation* |         |       |       |                 |
|----------------------------|------------------------|---------|-------|-------|-----------------|-------------------------|---------|-------|-------|-----------------|
|                            | Control†               | Marfan‡ |       |       |                 | Control†                | Marfan‡ |       |       |                 |
|                            |                        | 1       | 2     | 3     | Mean $\pm$ SD   |                         | 1       | 2     | 3     | Mean $\pm$ SD   |
| Hydroxyproline             | 11.3 $\pm$ 3.2         | 33.9    | 13.4  | 27.8  | 25.1 $\pm$ 10.6 | 7.5 $\pm$ 2.0           | 19.6    | 9.9   | 8.4   | 12.6 $\pm$ 6.0  |
| Aspartic acid              | 12.8 $\pm$ 1.3         | 13.0    | 19.8  | 16.1  | 16.3 $\pm$ 3.4  | 3.0 $\pm$ 1.3           | 6.0     | 5.0   | 4.9   | 5.3 $\pm$ 0.6   |
| Threonine                  | 18.0 $\pm$ 1.5         | 19.8    | 22.9  | 18.1  | 20.3 $\pm$ 2.4  | 2.5 $\pm$ 1.6           | 6.7     | 4.2   | 5.9   | 5.6 $\pm$ 1.3   |
| Serine                     | 14.9 $\pm$ 1.2         | 17.9    | 19.2  | 17.3  | 18.2 $\pm$ 1.0  | 2.8 $\pm$ 2.6           | 7.0     | 8.9   | 4.1   | 6.7 $\pm$ 2.4   |
| Glutamic acid              | 29.0 $\pm$ 4.2         | 39.7    | 38.0  | 33.9  | 37.2 $\pm$ 3.0  | 13.3 $\pm$ 2.7          | 17.8    | 11.9  | 14.1  | 14.9 $\pm$ 4.2  |
| Proline                    | 126.1 $\pm$ 8.2        | 111.1   | 111.0 | 117.0 | 113.1 $\pm$ 3.1 | 126.9 $\pm$ 2.8         | 125.7   | 131.3 | 123.9 | 126.9 $\pm$ 3.9 |
| Glycine                    | 283.1 $\pm$ 5.4        | 271.2   | 268.3 | 286.0 | 275.1 $\pm$ 9.2 | 298.3 $\pm$ 6.1         | 298.6   | 304.1 | 303.8 | 302.2 $\pm$ 3.1 |
| Alanine                    | 207.6 $\pm$ 8.3        | 205.7   | 200.1 | 191.5 | 199.1 $\pm$ 4.1 | 268.4 $\pm$ 4.5         | 247.9   | 248.6 | 258.2 | 251.6 $\pm$ 5.8 |
| Valine                     | 130.3 $\pm$ 2.5        | 122.9   | 128.3 | 128.6 | 126.6 $\pm$ 6.0 | 133.3 $\pm$ 9.7         | 130.8   | 130.6 | 135.3 | 132.2 $\pm$ 2.7 |
| Methionine                 | —                      | —       | —     | —     | —               | —                       | —       | —     | —     | —               |
| Isoleucine                 | 26.5 $\pm$ 1.3         | 29.1    | 27.9  | 29.5  | 28.8 $\pm$ 0.8  | 19.8 $\pm$ 4.0          | 18.8    | 20.5  | 19.1  | 19.5 $\pm$ 0.9  |
| Leucine                    | 62.9 $\pm$ 1.3         | 61.8    | 64.7  | 63.7  | 63.4 $\pm$ 1.0  | 62.5 $\pm$ 4.6          | 58.4    | 64.2  | 61.2  | 61.3 $\pm$ 2.9  |
| Tyrosine                   | 19.2 $\pm$ 0.3         | 15.2    | 20.0  | 20.6  | 18.6 $\pm$ 3.0  | 12.5 $\pm$ 1.3          | 14.2    | 12.3  | 13.0  | 13.3 $\pm$ 1.3  |
| Phenylalanine              | 26.6 $\pm$ 0.5         | 24.3    | 26.7  | 24.4  | 25.1 $\pm$ 0.9  | 26.4 $\pm$ 1.7          | 22.8    | 26.6  | 25.3  | 24.9 $\pm$ 1.9  |
| Hydroxylysine              | —                      | —       | —     | —     | —               | —                       | —       | —     | —     | —               |
| Lysine                     | 11.1 $\pm$ 1.3         | 14.0    | 15.3  | 13.3  | 14.2 $\pm$ 1.3  | 8.8 $\pm$ 1.9           | 15.1    | 12.5  | 13.9  | 13.8 $\pm$ 1.3  |
| Histidine                  | 3.0 $\pm$ 0.8          | 4.9     | 4.4   | 2.4   | 3.9 $\pm$ 1.4   | 1.6 $\pm$ 1.3           | 1.9     | 2.9   | 2.0   | 2.3 $\pm$ 0.6   |
| Arginine                   | 11.4 $\pm$ 4.4         | 12.5    | 16.3  | 6.9   | 11.9 $\pm$ 5.2  | 5.5 $\pm$ 0.9           | 5.7     | 3.2   | 3.5   | 4.1 $\pm$ 1.4   |
| Isodesmosine <sup>  </sup> | 2.6 $\pm$ 0.1          | 1.3     | 1.5   | 1.1   | 1.3 $\pm$ 0.2   | 2.6 $\pm$ 0.1           | 1.4     | 1.7   | 1.5   | 1.5 $\pm$ 0.2   |
| Desmosine <sup>  </sup>    | 3.9 $\pm$ 0.1          | 2.5     | 2.3   | 2.1   | 2.3 $\pm$ 0.2   | 4.2 $\pm$ 0.3           | 2.0     | 2.5   | 2.0   | 2.2 $\pm$ 0.3   |

\* The values are expressed as residues/1,000.

† The values are mean  $\pm$  SD of four separate cases.

‡ The individual values are mean of 2–4 separate analyses.

<sup>||</sup> Expressed as lysine equivalents.

onstrated reduced desmosine and isodesmosine values (Table I). The relative content of hydroxyproline in elastin II was considerably reduced, as compared with elastin I preparations, but the hydroxyproline content of elastin II in case 1 still remained higher than the control values (Table I). It should be noted that neither elastin I nor elastin II preparations contained hydroxyllysine or methionine, indicating that they were not contaminated by collagen.

In further studies, collagen was isolated from the 1 M NaCl-insoluble material by digestion with pancreatic elastase (Fig. 2). The purified collagen was sol-

ubilized by limited pepsin proteolysis and examined by SDS-polyacrylamide gel electrophoresis and amino acid analyses. No differences in collagen between the Marfan patients and the controls could be demonstrated by these techniques. Specifically, the mean values for 4-hydroxyproline were 89.9 and 85.3 residues per 1,000 amino acids in the patients and the control group, respectively. No differences in other amino acids were noted. SDS-polyacrylamide gel electrophoresis revealed that type I collagen consisted of  $\alpha 1$  (I) and  $\alpha 2$  (I) chains in an apparent 2:1 ratio, and no evidence of abnormal  $\alpha$ -chains was noted.

To allow quantitation of elastin and collagen, 4-mm punch biopsy specimens were taken from different regions of each aorta before extraction with 1 M NaCl, and their desmosine and hydroxyproline contents were determined. The desmosine content was then used to calculate the content of elastin, based on the relative amino acid composition of elastin I preparations (Methods). Similarly, on the basis of hydroxyproline assay, the total content of collagen in aorta was calculated; the amount of hydroxyproline in elastin was taken into account in these calculations. The results indicated that the content of elastin was markedly reduced in the Marfan aortae as compared with controls, while the relative contents of collagen were unchanged (Fig. 3).

## DISCUSSION

In this study, we have demonstrated that the content of elastin in the aorta of three patients with definitive Marfan syndrome is reduced and that the elastin isolated from these tissues is deficient in desmosine cross-links. These findings correlate well with the histopathologic changes observed in aorta in the same patients, demonstrating fragmentation and loss of elastic fibers (Fig. 1). In other studies, the patients with Marfan syndrome have been shown to excrete consistently less desmosine in their urine than do the age-matched controls (12). Since the urinary desmosine levels reflect the overall turnover of cross-linked elastin (26), these observations suggest that aorta and possibly other tissues in Marfan syndrome patients may be deficient in elastic fibers. Furthermore, insoluble elastin isolated from aortae of patients with the Marfan syndrome has been found to be more susceptible to pancreatic elastase digestion as compared with control elastin obtained from normal arterial wall (13). In the same study, elastase-type activity was elevated in aortic tissue from one patient with the Marfan syndrome. This observation suggested that increased elastase activity might contribute to the weakening of the aortic wall.

The reasons for reduced desmosine content and loss of elastin in Marfan aorta, as demonstrated in our

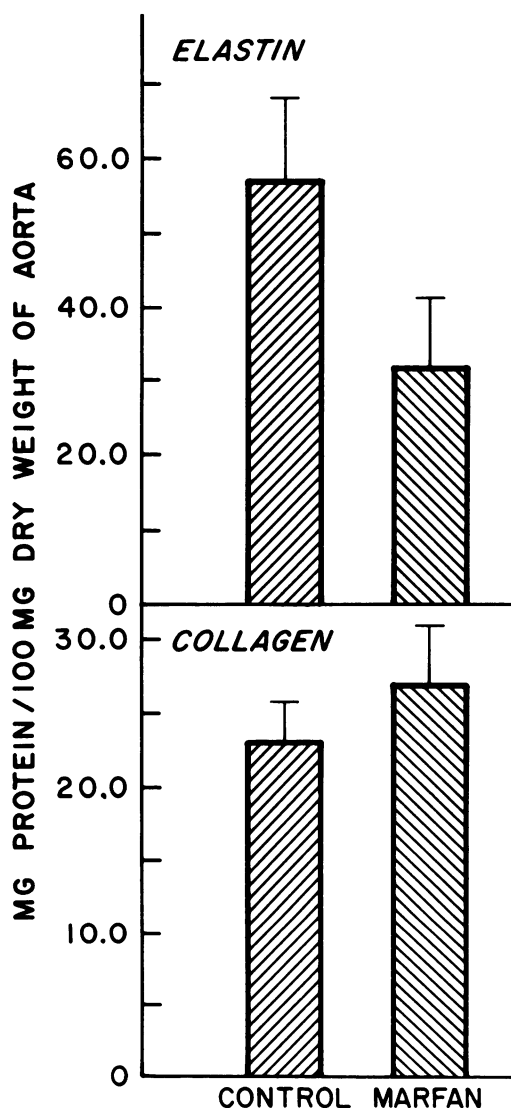


FIGURE 3 The concentrations of elastin and collagen in control and Marfan aortae. The values represent means  $\pm$  SD of three Marfan cases and four controls.

study, are not clear at this point. Desmosine, and its isomer, isodesmosine, are cross-link compounds not present in other mammalian proteins (27). The formation of desmosines involves several steps: First, selected lysyl residues are oxidatively deaminated by lysyl oxidase, a copper-requiring enzyme (28, 29). Subsequently, three aldehyde derivatives of lysine, allysines, fuse with a fourth unmodified lysyl residue to form the desmosine ring. The formation of desmosines is thus dependent on the activity of lysyl oxidase as well as on precise folding and alignment of the elastin polypeptides to bring the individual lysine residues into juxtaposition with one another. Previous investigators have demonstrated normal lysyl oxidase activity in skin fibroblast cultures derived from patients with the Marfan syndrome (30), but no information is available on the activity of this enzyme in Marfan aortae. Thus, it is unknown whether reduced lysyl oxidase activity might contribute to the reduced desmosine concentration in these patients. It should be noted that the increased lysine content in Marfan cases relative to the controls, in elastin II preparations, could account for the reduction in desmosine and isodesmosine. Thus, it is possible that the cross-linking defect is a result of deficient oxidation of lysyl residues. On the other hand, it may be that the reduced desmosine content reflects misalignment of allysine and lysine residues due to altered conformation of elastin. Such an alteration could result, for example, from a change in the primary structure of the polypeptides. This possibility would be compatible with autosomal dominant inheritance patterns noted in the patients with this syndrome. Alternatively, the abnormal conformation of elastin could reflect altered interactions between this protein and other components of the aortic wall. Such interactions could occur, for example, between elastin and the microfibrillar protein component of the elastic fibers. The increased contents of acidic amino acids in Marfan cases in elastin preparations may reflect unusually tight binding of these two macromolecular components.

Of considerable interest was the observation that the content of 4-hydroxyproline in elastin was increased in two patients with Marfan syndrome, as compared with the controls. The functional role of 4-hydroxyproline in elastin, if any, is currently unknown. Several observations with isolated cells or tissues have shown that the relative content of hydroxyproline in elastin can be highly variable without a significant effect on the intracellular synthesis or secretion of the elastin polypeptides (31, 32). It can be postulated, however, that in the extracellular space the hydroxyproline residues may interfere with alignment of the polypeptides during elastin fiber formation. Such a mechanism

could cause deficient desmosine formation as a result of displacement of critical lysyl residues or could interfere with stabilization of cross-link intermediates during desmosine formation.

Previous studies have demonstrated definite changes in collagen in patients with Marfan syndrome. In some patients, the solubility of collagen in skin and in skin fibroblast cultures has been shown to be increased (4-6), suggesting deficient cross-linking. In addition, chemically stable intermolecular cross-links of collagen were present in reduced quantities in several patients with the Marfan syndrome (3). In addition to these observations, an abnormal  $\alpha 2$ -chain of type I collagen has been demonstrated in an isolated case with clinical features of Marfan syndrome but no family history (9). Also, in another patient, an insufficient synthesis of type I collagen was suggested to be the prime cause of the connective tissue disorder (8). In our study, the quantity as well as the extractability of aortic collagen under nondenaturing conditions was unchanged, and no abnormality in the  $\alpha$ -chain composition of type I collagen was noted. It appears, therefore, that the Marfan syndrome may comprise a heterogeneous group of patients with diverse underlying molecular defects affecting either collagen, elastin, or other components of the extracellular matrix.

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