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Development of Elastin Layers in the Aortic Wall of Human Fetuses

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The presence of elastin layers in the aortic walls of twelve human fetuses was confirmed with scanning electron microscope pictures after hot alkali treatment and histochemical examination. In addition, the number of elastin layers in aortic walls of 5 different segments were compared in fetuses of varying ages. Aldehyde fuchsin stained slides of elastin ascending aortas showed a range between 27 and 55 layers of elastin in fetuses of 8 weeks to 32 weeks. However, in the lower abdominal aortas, elastin layers decreased from 28 to only 3 layers for fetuses of the same age. Furthermore, as elastin layers decreased from ascending aorta to abdominal aorta with the progression of fetal life, similar changes in the elastin lamellae were observed. These results suggest that while aortas grow rapidly in length, the medial elastin thickens slowly, perhaps due to slow development of hydrodynamic forces and pressures. Also the adventitial elastin appears to lose out gradually along the length from ascending aorta to abdominal aorta.

Key Words: Elastin layer, aortic wall, human fetus, 5 segments

The aortic wall in humans is known to be comprised of elastin, collagens, vascular smooth muscle cells and proteoglycans (Bertelsen and Jensen 1960). Elastin in the aortic wall affects hemodynamics and the overall function of cardiovascular systems since the mechanical properties of the wall are governed by the amount of elastin and collagens in the wall and their relative proportions (Dobrin 1959).

Aged arteries are known to show more stiffness than young arteries (Roach and Burton 1959), and this stiffness may lead to vicious hypertension and/or arteriosclerosis (Spinal *et al.* 2983; Wellman and Edwards 1950). Although elastin may contribute significantly in the pathophysiology of the cardiovascular system, due to a lack of information about the development of elastin in humans has made it difficult to fully understand its role. At present it is not yet fully appreciated on how elastin does and why.

To demonstrate the elastin membrane histologically, histochemical staining and scanning electron microscope pictures are used. The prime histochemical stains used are; Masson's or Gomori trichrome and Gomori aldehyde fuchsin. The scanning electron microscope (SEM) helped to elucidate a three-dimensional model of elastin (Song and Roach 1984), which has a membranous rather than fibrous structure. Also, fenestrations of various sizes have been quantified using SEM in the aortic intima of adult animals (Roach and Song 1988; Song and Roach 1983, 1984).

In our present studies, we have attempted to show the organization and development of the aortic elastin layers in human fetuses aged 8 to 32 weeks old using both histochemical methods and SEM pictures after hot alkali treatment. Finally, quantitative changes in the aortic elastin laminae both spatially and temporally during fetal life from 8 to 32 weeks can also be established.

MATERIALS AND METHODS

We collected a dozen fetuses whose ages ranged

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between 8 weeks and 32 weeks and physical details of the subjects are presented in Table 1. These fetuses were released to our laboratory with parental consensus and the reasons for termination were mostly due to psychiatric problems from maternal side. Therefore, the subjects were regarded as healthy and normally developed for their particular age. These experiments were approved by a committee of medical ethics at Yonsei University College of Medicine.

All the subjects, except 8 week fetus, had been pretreated with pressurized fixation through ventricular lumen with 10% formalin solution as embalming process at 100 mmHg. They were preserved in the fixative for at least 2 weeks except the one for SEM. The whole lengh of aorta from aortic valve to the iliac bifurcations was dissected out (Fig. 1) and 5 different segments were cut for histological sections. The dissection levels were 1) ascending aorta (AA; about 5 mm from the origin of aorta and before the arch), 2) upper thoracic (UT; beginning of the descending aorta and the second intercostal arteries), 3) lower thoracic (LT; above the diaphragm), 4) upper abdominal (UA; between the diaphragm level and the renal arteries), and 5) lower abdominal (LA; above the bifurcation of common iliac arteries).

Preparation of histological sections and counting elastin layers

After paraffin embeding, 3 to 6 sections were selected from each segments and tried for histochemical stains; hematoxylin and eosin, Gomori trichrome and aldehyde fuchsin methods (Gomori 1950).

The typical elastin layers thus obtained are shown in Fig. 2 (AA) and 3 (LA). These enabled us to count

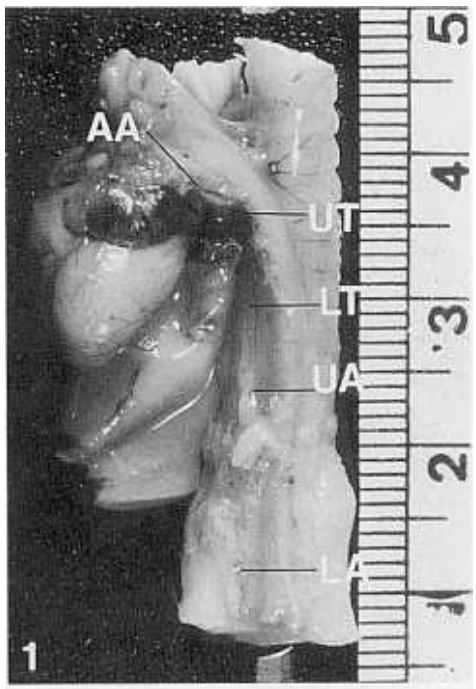

Fig. 1. A photograph of a whole length of aorta dissected from a fetus of 14 weeks old exposing the 5 segments (AA, UT, LT, UA, LA) from aortic origin at the heart to the iliac bifurcation.

Table 1. Physical characteristics of subjects

Groups	Fetus No.	Sex	Crown rump length (mm)	Foot length (mm)	Fetal age
	E141	-	-	-	8 weeks
	F10-1	-	61	10	10 weeks
	F12-1	-	80	13	12 weeks
2	F14-1	-	115	22	14 weeks
	F18-1	-	160	33	18 weeks
3	F20-1	Female	200	38	20 weeks
	F20-2	Male	200	36	20 weeks
	F23-1	Male	205	45	23 weeks
4	F27-1	Male	260	59	27 weeks
	F28-1	Male	-	61	28 weeks
	F30-1	Female	280	71	30 weeks
	F8M-1	Female	295	69	32 weeks

the numbers of lamellae in the aortic walls. All the counting was done from microphotographs or directly from high power views of microscope (over × 100). Two independent observers (authors) counted separately and numbers agree within a standard error limit.

Having chosen 4 different quadrants (3, 6, 9, and 12 o'clock sites) of the aortic rings from 5 segments, we counted the number of elastin layers in 2 different ways. Assuming that elastin layers from concentric tunnel structures, we first drew a line from the center of the aortic ring to the adventitial and counted the solid elastin layers crossing the line from the lumenal side, intimal elastin lamella (IEL). Secondly, we counted the solid elastin lines while avoiding empty spaces and zigzagging from IEL to-

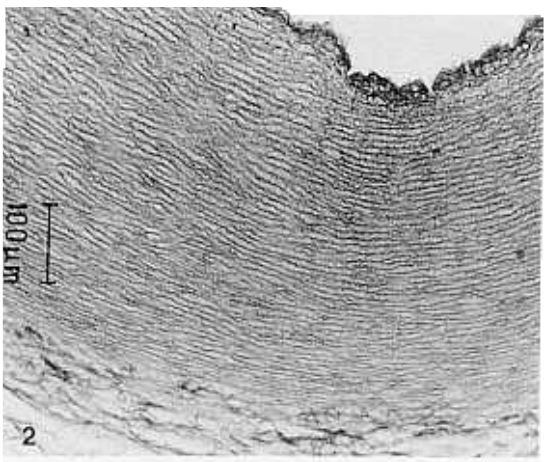

Fig. 2. A photomicrograph of aortic wall cross-sectioned at AA of 14 weeks old fetus showing about 50 layers of elastin stained with aldehyde fuchsin.

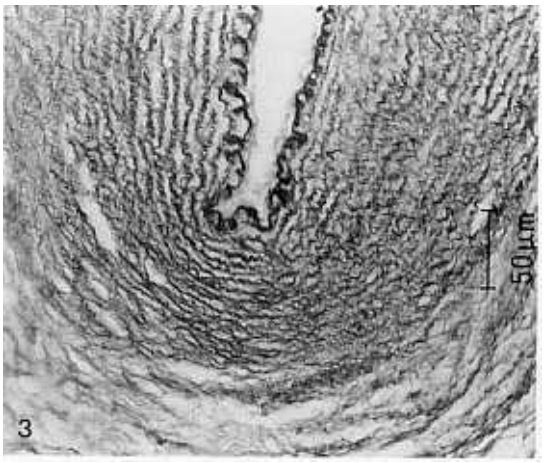

Fig. 3. A photomicrograph of aorti wall cross-section at LA of 14 weeks old fetus showing about 15 layers of elastin staied with aldehyde fuchsin.

Table 2. Comparison of results between 2 different methods to count the lamellae units in 4 sites of each aortic rings

	AA		UT		LT		UA		LA	
	1st/2nd		1st/2nd		1st/2nd		1st/2nd		1st/2nd	
			44/44		35/37					
			33/33		35/35					
			27/31		39/47					
			26/35		32/39					
			38/53		34/36					
			36/52		29/33					
			26/26		38/34					
			49/49		33/39					
			48/48		32/40					
			33/47		33/32					
			30/30		37/34					
			30/36		36/40					
Mean	53.3	54.1	35.8	40.3	34.4	37.2	33.0	33.9	28.4	29.3
±SD	±7.2	±6.8	±8.2	±9.5	±2.8	±4.2	±2.7	±2.3	±4.1	±4.9

1st method; counting elastin layers following the straight line.
2nd methods; counting elastin layers voiding empty spaces obliquely.

ward the adventitia. We found that the two methods yielded the same numbers and were not statistically different (Table 2). The mean values from 24 measurements are presented in Table 3 of the results.

SEM methods

In addition to histological methods, we tried to purify elastin exclusively from the extracellular matrix of aortic walls and to observe them stereologically for the identification of the elastin laminae in the media of aortas. We chosed a 20 weeks old aorta and treated it with 0.1 N NaOH at 70℃ for 2 to 5 hours. Next, it was freeze-dried with Dura Dry (FTS systems Inc.) in order to dry the sample at −700 mmHg and −80℃ simultaneously. The sample was then sputter-coated with Au (gold) in an Eiko (IB 3) coater at 1400 V and 6 mAmp which could coat at 50 A/min for 2 min only. The SEM pictures were taken with Hitachi S450 (15 KV) and were shown in Figure 4.

RESULTS

We have conveniently grouped the subjects into 5 groups as shown in Table 1 according to age and development. Apparent sex were not disernable in groups 1 and 2, but we could measure the lengths of both CRL (crown rump length) and FL (foot length) in all subjects except those 8 weeks old. These figures provided an estimated parameter for fetal growth and later, related to TL (total length) and thickness of the aortic wall, which is also a determinant of medial elastin layers in Table 3.

In Table 2, we compared 2 mean values from 12 measurements listed at the bottom of the table. The mean number of elastin layers in Table 3 were calculated from 24 measurements in each segment from individual aortas. It is obvious that both the thickness and the length of aortas increase with respect to age as the length from aortic origin at the

Table 3. Number of medial lamellar units by cases

Fetus No. IL(mm)[1]	AA[2]	UT	LT	UA	LA
E141	−/27.0±4.5/100%	−/11.0±2.2/40.7%	−/ 7.9±1.4/29.3%	−/ 4.7±0.8/17.4%	−/ 3.1±0.7/11.5%
F10-1 25	2/45.8±3.2/100%	6/22.0±4.9/48.0%	10/19.8±1.1/43.2%	16/12.8±1.4/27.9%	22/ 9.3±1.4/20.3%
F12-1 35	3/37.2±2.3[3]/100%	8/22.4±2.8/60.2%	15/21.9±1.9/58.9%	22/10.8±1.6/29.0%	30± 5.3±0.8[4]/14.2%
F14-1 40	5/49.6±5.1/100%	15/26.0±3.6/52.4%	20/27.4±2.0/55.2%	25/21.8±4.9/44.0%	35/12.6±4.4/25.4%
F18-1 55	5/49.5±7.6/100%	15/27.8±1.9/56.2%	35/22.0±3.2/44.4%	45/22.7±2.7/45.9%	50/21.8±2.4/44.0%
F20-1 65	5/49.0±8.3/100%	15/28.7±3.1/58.6%	35/28.5±3.2/58.2%	45/22.6±3.6/46.1%	60/10.7±1.2/21.8%
F23-1 75	5/51.1±4.9/100%	20/32.6±4.9/63.8%	35/30.7±5.0/60.1%	55/24.2±3.4/47.4%	70/19.5±3.2/38.2%
F27-1	5/55.9±9.1/100%	30/36.5±3.6/65.3%	40/32.0±4.6/57.2%	50/28.8±4.0/51.5%	-
F28-1 95	5/44.8±1.9/100%	30/32.5±6.0/72.5%	45/28.1±3.3/62.7%	75/28.1±3.2/62.7%	90/23.1±4.4/51.6%
F30-1 100	5/51.5±4.8/100%	25/39.2±8.4/76.1%	45/39.2±4.7/76.1%	75/28.8±3.7/55.9%	95/25.8±4.8/50.1%
F8M-1 105	5/53.7±6.9/100%	30/37.7±9.1/70.2%	50/35.8±3.8/66.7%	80/33.5±2.5/62.4%	100/28.8±4.4/53.6%

[1] total length of aorta

[2] data format is distance from the origin of aorta/mean±standard deviation/percentile of AA.

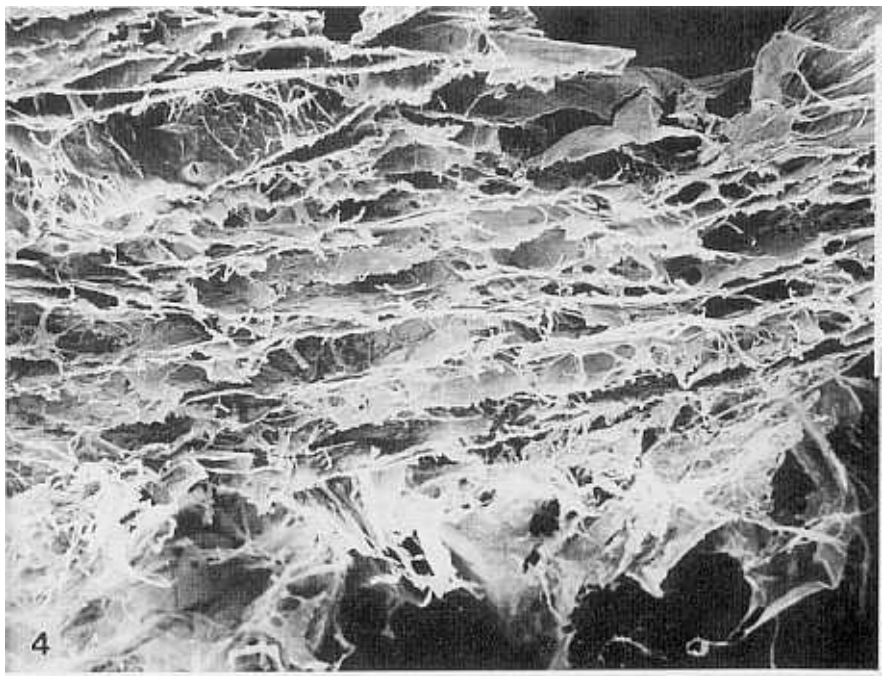

Fig. 4. A SEM picture of aortic wall cur to show elastin layers from 20 weeks old fetus (male) after hot alkali treatment. White bar on the right side represents 500 um.

aorto-cardiac junctions to the iliac bifurcation enlarges from 25 mm at 10 weeks to 105 mm at 32 weeks. This increase occurs in each aortic segment ie. AA, UT, LT, UA, and LA. Therefore, we have also indicated the lengths of each segment where the histological sections were made.

In Table 3, there are certain facts which indicate that the number of elastin layers change with age (temporally) and with aortic segment (spatially). However, the AA had near fixed mean values of elastin numbers between group 3 and 5, and thereafter elastin layers decreased along the segments to LA. In addition, the decrease in number was greater in younger fetuses than older fetuses. Because the changes were relative to the initial number of elatin layers in AA, we plotted the decrease in elastin numbers as a percentile of AA in individual subjects, as shown in Figure 5.

The SEM picture of pure elastin layers in Figure 4 illustrates that elastin layers can be distinguished as a solid membrane among other connective tissues and gives perfect indications for the growth of aortic wall. There are also profuse microfibrils or smaller interlamellar fibers between elastin lamellae. Since, other than elastin, even cellular structures

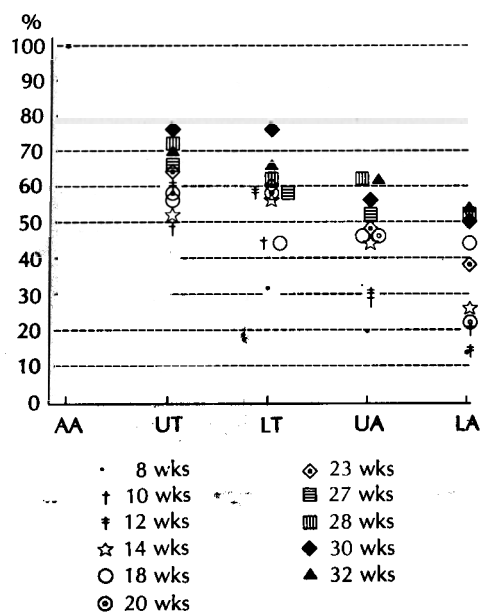

Fig. 5. Percentile changes of elastin layers at each segments of aortas (UT, LT, UA, LA) compared with AA in every fetuses.

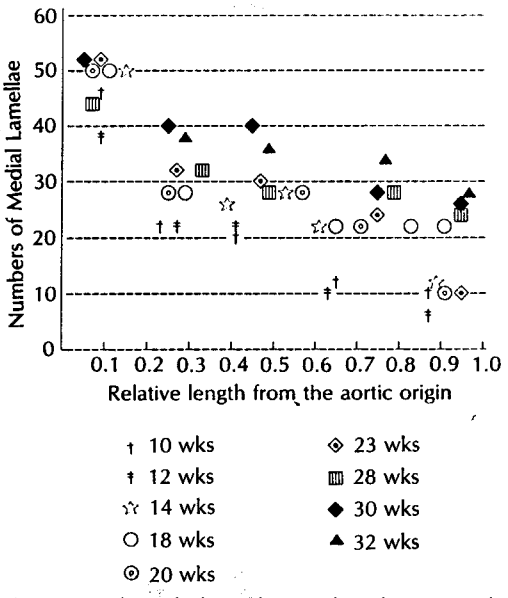

Fig. 6. *Number of elastin layers plotted against relative lengths of segments from the aortic origin, showing increase of elastin laysers while aging of fetuses.*

are eliminated, it is not easy to depict from the picture the relationship between smooth muscle cells and extracellular matrix in the aortic media. At the bottom of the Figure 4, it is clear that adventitial elastin membranes do not form the concentric tunnel surrounding the aortic wall. The interlamellar space may be between 10 and 50 um in the length and is not uniform in this picture which shows sufficient spaces for vascular smooth muscle cells and fibroblasts.

DISCUSSION

In the literature, the presence of elastin in the aortic walls has been well recognized not only by histologists (Gillman 1959), but also by physiologists and biophysicists. This was because the nature of arteries was known to be viscoelastic and rubbery (Dobrin 1959; Roach and Burton 1957). Elastin was known in the past to fibrous and stained differently from collagens and other ground substances, such as mucopolysaccharides. Also, it had a positive reaction to Schiff's reagent due to free aldehyde groups and sulfonic acid radicals in the elastin molecules. Almost all of the sudies in the past were performed with either light microscopes (LM) (Banga *et al.* 1956) or transmission electron microscopes (TEM) (Hall *et al.* 1955), which required very thin sections so the even membranes may appear to be fibers. However, the recent application of SEM has brought about the concept of membranous lamellar structures of elastin in the aortic walls (Roach and Song 1988).

Our SEM picture shown in Figure 4 illustrates branched lamellae of elastin layers rather than simple fibrous or flat membranes, which complicates the architecture of the aortic media. Since these specimens were treated previously with not alkali (0.1 N NaOH at 70℃) for more than 2 hours, we are confident that other than elastin, extracellular matrix components and vascular smooth muscle cells have been destroyed, leaving only pure elastin structures (Starcher and Galione 1976). At present, we can not elucidate the relationship between elastin structures and vascular smooth muscle cells which are supposed to synthesize elastin in the wall. Even if Clark and Glagov (1985) disagreed with the proposals made by Wolinsky and Glagov (1967) that the medial lamella unit (MLU) may represent the elastin content in the aortic wall, our quantitative and qualitative data definitely show that the number of elastin layers in the aortic wall varies with segment (spatially) and age (temporally).

As presented in Table 2, we vigorously tested the methods of quantifying elastin layers histologically. The reason why we tried to compare the two different methods was to avoid any errors resulting from fragmentation or fenestrations which can show empty or vacant layers in the absence of elastin. We have also excluded a few, 2 or 3 adventitial elastin layers that did not form concentric tunnels from the total number of layers in Table 3.

Our data listed in Table 3 represent the first quantification of elastin in the aortic media of human fetuses. It is clear that in group 2 to 5, while aging from 14 weeks to 32 weeks, not only did the aortic lengths increase from 40 to 105 mm, but also increase total number of medial layers of elastin in each segment; UT, LT, UA and LA (except AA which increased gradually). This trend is well reflected in Figure 5. We assume that group 1, especially 8 weeks old embryos, represents a transient period when the elastin layers appear suddenly and thereafter at 5 mm from the origin of aorta. A fixed number of elastin layers are maintained until a sudden physiological change occurs. There may be errors in estimating the numbers of elastin layers

when non-pressurized aortas were used (Wolinsky and Glagov 1964), such as subjects in group 1. The descriptions made by Jensen and Bertelsen (1961) and Gillman (1959) may be quite true, but without pressure-fixation it is hard to quantify the elastin layers (Wolinsky and Glagov 1964).

At pressent, our data implies that while the length grows, and a fixed number of elastin layers are formed at the aortic origin (AA), the segments elongate with fewer elastin layers that gradually add to newly generated media from adventitia. This increment may enhance greatly after birth as reported by Scarselli (1961), but unlike ours, his analysis was not done spatially. Eventually a complete formation of aortic elastin is made at the term.

The lambs at full term (van Baardwijk and Roach 1983) may not be comparable to human fetuses at young ages with respect to segmental change. Our data suggest the gradual increase in elastin layers of abdominal aortas during maturation. The results shown in Figures 5 and 6 suggest that while in AA growth rate in elastin layers is slower than other segments, the lower segments elongate more and grow thicker in elastin layers of aortic media while developing. It may be predicatable that during developmental processes between 8 weeks and full term, reduplication, remodelling, regeneration, repair, and degeneration of elastin membranes can occure as descried by Gillman (1959), although our present data may be inconclusive. However, there is always individual variation in development and aging in fetuses.

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