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Smooth muscle cell – reticulin lamellar units of 13.2 μm thickness composing the aortic intima

Richard E. Tracy[1], **Grace E. Kissling**[2], **and Mary B. Curtis**[1]

[1] LSUMC, Department of Pathology, 1901 Perdido Street, New Orleans, LA 70112, USA
[2] Department of Mathematics, University of North Carolina at Greensboro, Greensboro, NC 27412-1393, USA

Summary. Smooth muscle cells of the aortic intima are generally of two forms, spindle and stellate. Spindle cells are typically axial in their orientation, while stellate cells lie parallel to the luminal surface; cell processes do not characteristically extend in the radial direction through the intimal thickness. Evidence is given here to suggest that these cells are clustered in layers of about 13.2 μm thickness which are separated by condensations of reticulin fibers. These layered clusters may extend as much as 1 cm to 2 cm in the axial direction. The numbers of layers appear to increase during growth and maturation to a stable value of about 12 by age 30–40. With further aging and growth of fibrous plaques, the layers seem to become thicker and to merge, obliterating their boundaries, to become 15 to 35 μm or more in average thickness. This expansion and merging of lamellar units precedes atheronecrosis and appears to represent an important precursor of the necrotic core. The greatest growth of fibrous plaques, at ages 40 to 60, takes place after the stabilization of cell numbers at ages 30 to 40, and is almost as likely to happen in the least cellular as in the most cellular places. Hence, these data suggest that smooth muscle cell numbers are not important determinants of the locations nor of the growth rates of fibrous plaques in the lateral thoracic aorta.

Key words: Atherosclerosis – Aging – Aorta

Introduction

The intima of the human thoracic aorta grows thicker with age. The smooth muscle cells which propel the thickening are seeded into the intima in greatest numbers during the ages 10 to 25. Previous evidence has suggested that the increase of cell numbers is concluded by age 30 to 40, and that subsequent intimal thickening is due to expansion of the volume of connective tissue occupied by an essentially fixed number of cells (Tracy et al. 1985a, b). Hence, the period of life in which atherosclerotic plaques are becoming abundant (age 40 to 60) is the period which was found to have nearly unchanging cell numbers. Moreover, within the same aorta, thinner and thicker intima tended to have similar numbers of cells. These results seem to contradict the suggestion that cells proliferate during the growth of fibrous plaques (Benditt 1977; Ross 1983). Although such proliferation has been shown in a variety of experimental models, data of this sort on adult humans are lacking (Orekhov 1983). Additional ways to examine this issue were therefore undertaken, and the results are reported here.

The approach taken here is to count intimal cells in histologic sections, and to relate the counts to the volume of tissue that the cells occupy. The average volume of space occupied by each smooth muscle cell proved to be a useful concept. This average volume is called the "realm" of the average cell. In the course of the investigation, the realm was found to have some remarkable properties, which may bear upon the earliest stages of evolution of the atherosclerotic plaque.

Offprint requests to: R.E. Tracy

Materials and methods

Selection of cases. The 686 aortas used for this study are taken from a previously described series (Tracy et al. 1979; Tracy et al. 1986). They represent aortas from men and women aged 15 to 69 from Bogota, Durban (Bantu and Indian), Manila, Mexico, New Orleans (white and black) and Sao Paulo (whites only).

Processing of tissue. The left and right lateral walls of the thoracic aorta from the 4th to the 12th intercostal ostia were cut into segments, decalcified and blocked in paraffin.

Cell densities. H & E stained 11 μm sections were marked at 20 equally spaced positions on the left and right, 40 positions in all. The interval of separation between evaluated positions is 1/20 of the distance from the 4th to the 12th intercostal ostia, which is about 0.7 cm in most aortas. The points in each aorta that had atheronecrotic cores were considered censored, that is, unavailable for observation. The number of such points is N. Fibroplastic intimal thickness (F_1) was measured and numbers of cells in a 100 μm wide band from intima to media (C_T) were counted in the 40-N uncensored points. Cell density is $C_D = C_T/F_1$. Averaged over 40-N observations, these variables are $\bar{C}_D$, $\bar{C}_T$, and $\bar{F}_1$.

40-Points study. In a randomly chosen set of 200 aortas, intimal thickness and cell numbers were assessed at different depths in each of the 40-N nonnecrotic positions, where N is the number of points excluded because atheronecrosis was encountered. Thickness (F_1) was measured with an eyepiece ruler using the 10× objective lens. With an eyepiece grid, under the 40× objective lens, areas of 100 μm square were marked off from the surface to the intima-media boundary, with a "remainder" of less than a complete square at the deepest level. Cell "densities" (C_D) were obtained by counting the numbers of spindle cell nuclear profiles in each square, and dividing by the thickness of the microregion to adjust the deepest "remainder" for being an incomplete square. The sum of the counts at all levels is the total cellularity (C_T). The precise position for counting was often shifted slightly to avoid microregions affected by foam cell-monocyte infiltrates.

9-Columns study. Eleven aortas having technically superior preparations and representing the full range of intimal thicknesses were chosen for detailed inspection. On the left or right sample (whichever was better prepared) the twenty marked positions (minus those with atheronecrosis) were divided into nine adjacent "columns" of 100 μm width. In addition to the assessment of cell density at different 100 μm levels within column one, as done for all 200 aortas in the 40-points study, the total cell numbers were counted in each of the nine columns. This gave 9 replicate counts of cells at each position.

Nigrosin stain. The surfaces of cells and of elastic lamellae of the aortic media are invested with fine collagenous fibers which also form a loose mesh between these structures (Clark and Glagov 1985; Berry et al. 1974a). These fibers can be stained by silver reticulin methods, and also by neutral alcoholic preparations of nigrosin (Tracy et al. 1979). We developed the following procedure for demonstrating the reticulin. Reagent A: 0.2 mg Nigrosin B (spirit soluble, Solvent black 5, Matheson, Coleman and Bell), 100 ml isopropanol (60%), pH 7.0. Rinse in 60% isopropanol; Reagent A, 2 h; quick dip in 60% isopropanol; carry through the usual routine hematoxylin – eosin.

Measuring and counting reticulin lamellae. Thirty-four of the aortas with superior technical quality of preparation and representative of all ages and intimal thicknesses were processed through the nigrosin procedure. In each aorta, all regions of average intimal thickness in which lamellation could be easily discerned were examined (eg. region "d" in Fig. 3). In the oldest specimens with atheronecrosis, only regions that had thinner than average intima could be observed, because the "average" region was censored by complex advanced change. In other aortas, however, many easily evaluated sites could be found and the recorded observations are considered to be representative of average regions in those specimens. At the selected sites, intimal thickness was measured and numbers of layers were averaged. The ratio of the intimal thickness to the number of layers represents the average thickness of the lamellae.

The realm of a cell. Given C_D cells in a 100×100 μm square area of an 11 μm section, the average "realm" of a cell can be described as the rectangular solid of volume $K \times K \times (G \cdot K)$, where K is the width and $G \cdot K$ is the length, i.e., G is the proportion of the length to the width. The expression, $K = \sqrt[3]{(170000/GC_D)}$, was previously derived by assuming that the space sampled in an 11 μm section for observing nuclei of about 6 μm thickness should be about $(6/2 + 6/2 + 11) \times 100 \times 100$. The value, $G = 3.17 = 19/6$, is adopted using data from Thomas et al. (1979) that nuclei of aortic cells in section average about 19×6 μm. The quantity, K, computed from these assumptions, is the width of the realm, and constitutes a variable of interest to this study.

Results

Cells and thickness in a random pool of 200 aortas

Of the $200 \times 40 = 8000$ (aortas × positions) observed points, 503 had atheronecrosis, leaving 7497 nonnecrotic points. Figure 1 is a plot of cell numbers (C_T) versus intimal thickness (F_1) for these nonnecrotic points. Visual inspection of this plot might suggest no association between these two variables. The correlation, however, is statistically significant. A cubic regression equation yielded the curve plotted in Fig. 1 (multiple $R = 0.382$). This curve shows that the correlation is best in the least thickened points.

Extremes of cellularity and thickness

In the randomly selected set of 200 aortas, 138 specimens had no atheronecrotic cores. The number of spindle cells in the thickest of 40 observed points was greater than average in 115 of these aortas, and tended to be about 50% greater than average in most specimens. The point with the most spindle cells had thicker intima than average in 129 aortas, and tended to be about 50% thicker in most specimens. Hence, a tendency for large values of thickness and cellularity to coexist is indicated by these findings, again contradicting the apparent lack of association between these two variables. In three of the aortas, the most cellular point was also the thickest. All three of these were microscopically unusual, as exemplified by Fig. 2.

Increasing the amount of sampled tissue

Enumerations of cells in a larger sample of tissue at each site helped to improve correlations, but the association between intimal thickness and cell

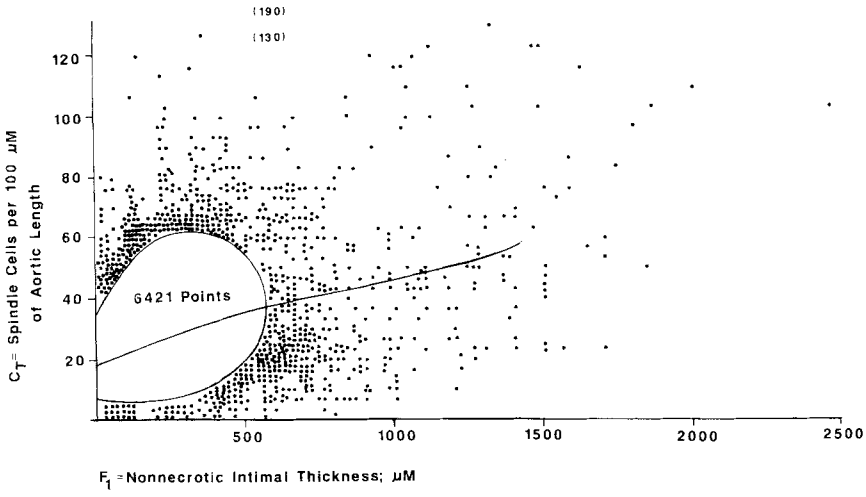

Fig. 1. Measurements at 7497 nonnecrotic points from 200 aortas are shown. Two off scale points are in parentheses. The cubic regression equation is drawn. The oval region is an artistic device used to show the location of 6421 points which would, if plotted individually, smudge together into a black region and obliterate the regression line

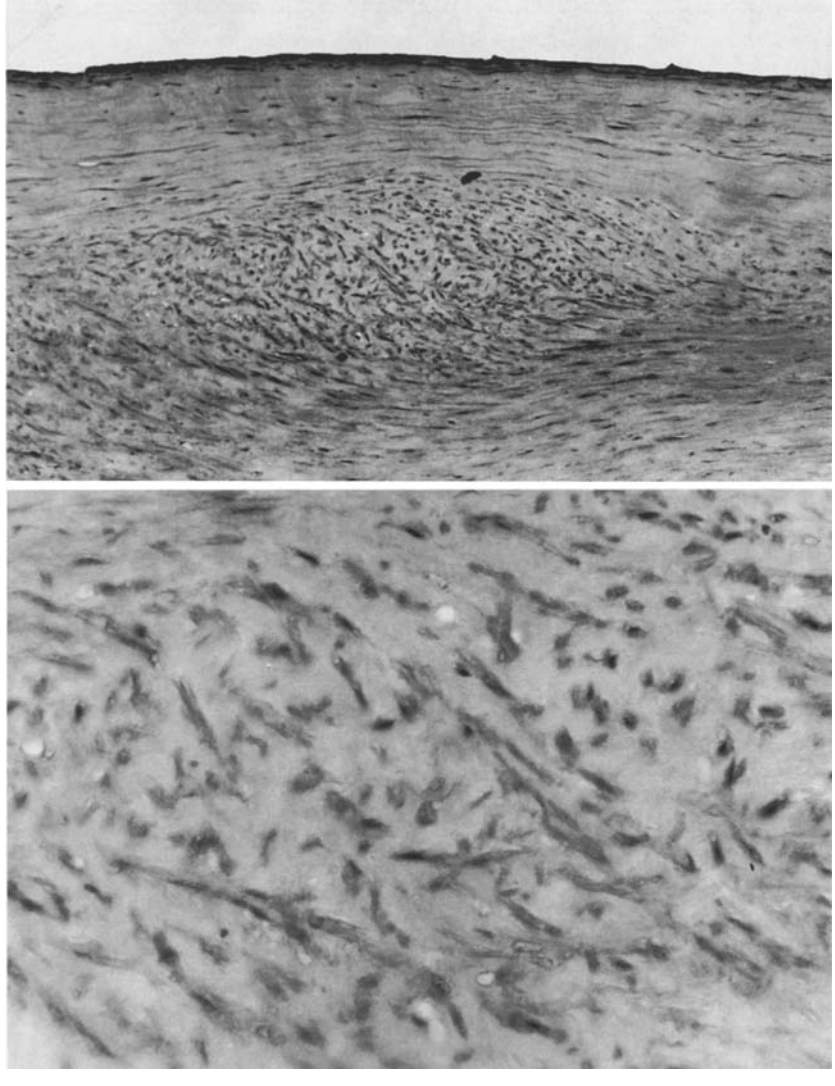

Fig. 2. A rare appearance, exemplifying all three of such points observed in 138 aortas, is presented. This aorta had mean thickness of 276 μm and mean cell numbers of 14.9 per 100 μm aortic length. The most cellular of 40 points, shown in these photographs, had thickness of 1500 μm. At this photographed position, a 100 μm wide column of intima had 130 cells, although 77 were found in the objectively defined column at this position

Table 1. Correlation coefficients relating intimal thickness to cell numbers using 9 measurements from adjacent "Columns" in 11 aortas

Aorta	Age	Mean[a]		Correlations using				Numbers of measurements
		Thickness	Cells	Means of measures[b]		Means of correlations[c]		
				R	R^2	R	R^2	
a	16	58	10.4	0.800	0.64	0.662	0.44	20
b	24	95	16.6	0.738	0.54	0.581	0.34	20
c	40	101	19.1	0.156	0.02	0.110	0.01	19
d	35	147	17.4	0.504	0.25	0.368	0.14	19
e	40	154	24.5	0.751	0.56	0.546	0.30	20
f	50	267	25.1	0.065	0.00	0.054	0.00	18
g	37	313	32.4	0.256	0.07	0.213	0.05	20
h	55	347	31.2	−0.012	−0.00	−0.013	−0.00	20
i	59	396	31.5	0.330	0.11	0.299	0.09	11
j	64	506	28.6	0.374	0.14	0.290	0.08	19
k	65	572	27.6	0.413	0.17	0.360	0.13	17
Combined through Fisher's Z-transform				0.443	0.196	0.333	0.110	

[a] Thickness in μm; Cells ($\bar{C}_T$) in nuclear profiles in a 100 μm band from luminal surface to intima-media boundary
[b] 9 Measures of cell numbers were combined into a mean for correlation with thickness
[c] 9 correlations of cell numbers with thickness were averaged into a mean correlation coefficient through Fisher's Z transformations

Table 2. Correlation coefficients between cell numbers in 100 μm wide "columns" separated by various axial intervals in 11 aortas of varying intimal thickness

Mean intimal thickness	Correlation between "Columns" separated by x μm							
	$x=0$	100	200	300	400	500	600	700
58	0.649	0.720	0.661	0.683	0.531	0.567	0.589	0.435
94	0.644	0.577	0.554	0.522	0.535	0.477	0.571	0.464
101	0.383	0.304	0.266	0.148[a]	0.113[a]	0.132[a]	0.019[a]	0.366
147	0.548	0.432	0.467	0.379	0.482	0.487	0.441	0.400
154	0.566	0.459	0.430	0.383	0.373	0.425	0.526	0.645
267	0.703	0.665	0.599	0.564	0.534	0.403	0.417	0.514
313	0.632	0.668	0.622	0.588	0.619	0.555	0.527	0.561
347	0.637	0.583	0.554	0.543	0.484	0.470	0.528	0.617
396	0.869	0.826	0.849	0.777	0.792	0.720	0.663	0.766
506	0.610	0.594	0.629	0.617	0.506	0.469	0.502	0.343
572	0.800	0.780	0.746	0.754	0.650	0.768	0.757	0.712
"Within" matrix pooled estimate	0.6868	0.6396	0.6396	0.5877	0.5512	0.5349	0.5462	0.5558

[a] Not significantly different from zero; $\alpha=0.05$

numbers was still weak. This was shown by expanding 9-fold the amount of tissue to examine at each of the 20−N nonnecrotic points in 11 half aortas (Table 1). Correlations of intimal thickness with cell count ranged from −0.01 to 0.80. An average correlation of $r=0.443$ was obtained through averaging Fisher's Z transforms. This compares with 0.333 when only one-ninth as much tissue was used at each point in these 11 aortas, and 0.382 for the cubic relationship in Fig. 1, also using one-ninth as much tissue. Aortas with little intimal thickening (a to e in Table 1) had an average correlation of $r=0.632$; those of greater thickness (f to k) had $r=0.243$. (The corresponding R^2 are 0.40 and 0.06, ie 40% and 6% respectively).

Correlations of cell numbers across varying axial distances

Columns of tissue of 100 μm width that were adjacent to each other in the lengthwise direction of the aorta tended to have similar cell counts. In 11 half aortas, the correlations ranged from 0.38 to 0.86, with a combined estimate of 0.68 (Table 2). Columns separated by 700 μm had an average correlations of 0.55. Columns separated by 7000 μm

Table 3. Correlation coefficients relating positions separated by 1, 2, 3, or 4 intervals of axial distance for intimal *thickness* and numbers of *cells* in 148 half aortas having no atheronecrosis, grouped by mean intimal thickness

Interval	$\bar{F}_1$ = Mean intimal thickness in half aorta; μm					
	<100	101–160	161–220	221–280	281–360	>360
Thickness						
1	0.392[a]	0.578[a]	0.503[a]	0.414[a]	0.298[a]	0.540[a]
2	0.176[a]	0.335[a]	0.197[a]	0.176[a]	0.158[a]	0.276[a]
3	0.063	0.105[a]	0.045	0.095	0.032	0.249[a]
4	0.000	−0.055	−0.055	0.000	−0.063	0.100
Cells						
1	0.173[a]	0.344[a]	0.383[a]	0.114[a]	0.239[a]	0.184[a]
2	0.032	0.148[a]	0.100	0.114[a]	0.118[a]	0.071
3	−0.032	0.000	−0.055	0.077	0.110[a]	0.071
4	−0.134[a]	−0.158[a]	−0.045	0.000	0.032	−0.077
Numbers of aortas						
	29	37	17	23	29	13

Intervals are 1/20 of the distance from the 4th to the 12th intercostal ostia, and are about 0.7 cm in most aortas
Notice that when adjacent positions are consistently similar, the distant positions must be consistently different, thus generating positive correlations at short intervals and negative correlations at a larger distance

[a] Correlation coefficients are significantly different from zero ($\alpha = 0.05$)

Table 4. Correlation coefficients relating paired measurements of spindle cell densities separated by various radial distances in groups of aortas with various intimal thicknesses measured in 100 μm thick levels. (Numbers of data pairs in parentheses)

Aortas having	Separation between measured regions; μm				
	0	100	200	300	400
2 Levels	0.148[a] (158)				
3 Levels	0.124[a] (267)	−0.154 (116)			
4 Levels	0.200[a] (351)	0.133[a] (206)	0.112 (78)		
5 Levels	0.168[a] (383)	0.034 (229)	−0.042 (98)	0.152 (28)	
6 Levels	0.363[a] (455)	0.314[a] (312)	0.275* (182)	−0.062 (95)	−0.319 (34)

[a] Significantly different from zero; $\alpha = 0.05$

could be observed in 148 aortas having no necrosis in the right half sample (Table 3). Correlations ranged from 0.11 to 0.38 in different assortments of aortas. At separations of 21 000 μm, correlations were usually not significantly different from zero.

Correlations of cell numbers across varying radial distances

Adjacent layers of tissue of 100 μm thickness in the radial direction from lumen to intima-media boundary tended to resemble each other only weakly in cell numbers (Table 4). Correlations ranged from 0.12 to 0.36 in different assortments of aortas. The correlations were generally not significantly different from zero at separations of 100 μm or more.

Demonstration of reticulin lamellae

Figure 3 represents an example of a commonplace intimal lamellar architecture often seen in longitudinal sections of the middle aged lateral thoracic aorta using the nigrosin stain. In subjects aged 30 to 50, intimal thickness tends to be about 100 μm to 300 μm with cell densities of 10 to 20 cells per 10000 μm^2. The intima-media boundary is usually clearly demarcated by a prominent internal elastic lamella (*a* in Fig. 3). However, that lamella sometimes has large gaps (*b*) and at other times has apparent duplications which create layers that cannot be unambiguously assigned to intima or media (*c*). Much of the intima often appears to be constructed of layers of approximately the dimensions of the medial lamellae, but typically lacking elastin and having fewer cells whose orientation is longitudinal (*d*). Individual intimal "reticulin lamellae" can often be traced for long distances, sometimes as much as 1 to 2 cm (*e*), but these lamellae are usually interrupted from place to place which requires interpolating of missing zones to carry out the tracing (*f*).

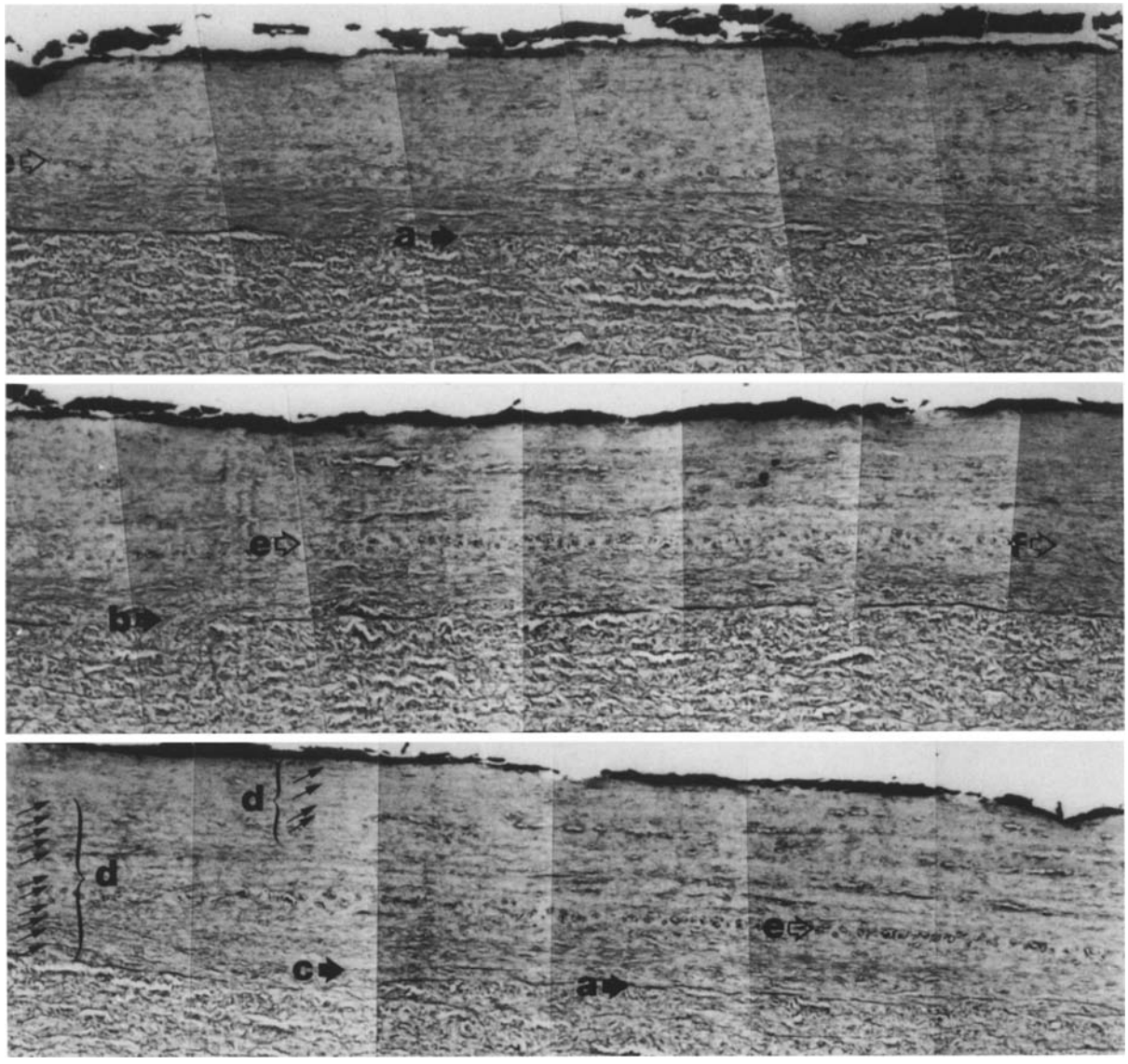

Fig. 3. A length of sample from the thoracic aorta is segmented so that the caudal end is at the upper left and the cephalic at the lower right. This example of commonplace intimal features seen in paraffin sections was chosen because it offers an exceptionally clear example of the layer "e", a layer which can be traced for great distances (Age 51, intimal thickness 250–300 μm, Nigrosin-H & E, ×100)

Among young aortas of differing intimal thickness, the layers are of about the same size but occur in varying numbers (Fig. 4). However, with advanced age, the layers appear to grow thicker and fewer, and are generally not visible in a specimen that has extensive atheronecrosis.

The use of silver stains affirms that the intimal lamellae are related to the "reticulin" of classical histochemistry. After this procedure, the intima and media differ from each other in the absence of prominent repeating elastic lamellae in the intima, but resemble each other in the continuation of the repeating layers of reticulin through both intima and media. Typically, the same kind of layering is seen in the intima as in the media, except that the elastin is omitted (Fig. 5).

Quantification of reticulin lamellae

As the intima thickens with age, the numbers and thicknesses of layers tend to increase (Fig. 4 and 6). From an average number of lamellae of between 4 and 12 at age 20 to between 7 and 27 at age 60, the numbers increase quickly from age

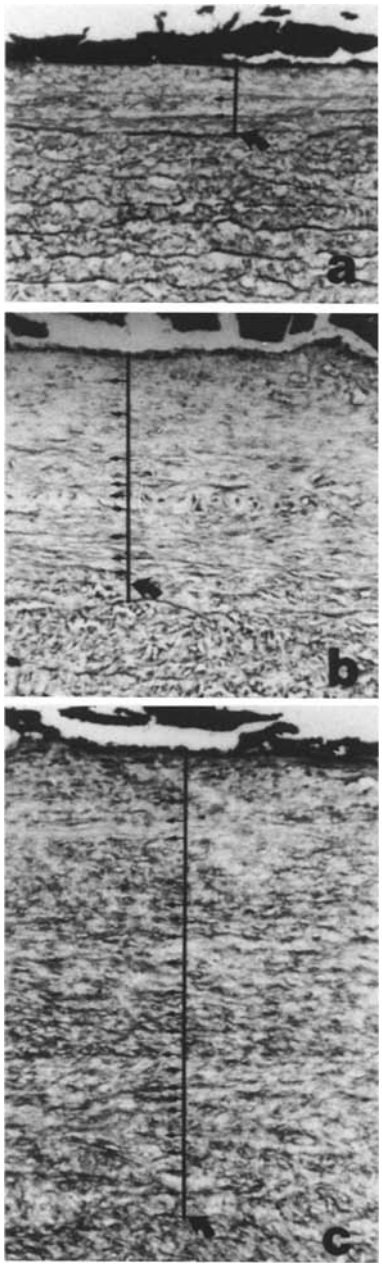

Fig. 4. Three specimens to illustrate a range of intimal thicknesses were chosen to show here because the reticulin lamellae (small straight arrows) were exceptionally well defined. Curved arrows mark the intima-media boundary. Ages and intimal thicknesses (μm) are (**a**) 29 & 70; (**b**) 51 & 230; (**c**) 51 & 400. Nigrosin-H & E, ×140

18 to 30 and remain fairly constant thereafter. From an average thickness of between 10 and 20 μm at age 20 (averaging near the value of 13.2) to between 15 and 35 at age 60, the thicknesses are nearly constant from age 18 to 30 and increase quickly thereafter.

Width of the smooth muscle cell realms

The patterns of change in reticulin lamellae are paralleled by the changes in the numbers and sizes of the realms of smooth muscle cells, determined by enumerating spindle cell nuclear profiles. In Fig. 6, these measurements are shown in solid lines for basal cause of death cases and dashed lines for athero-related cause of death cases, computed for overlapping 10-year age groups (sliding average). The number of realms increases rapidly along with the number of layers in youth and both stabilized after maturity. The sizes of realms are about constant in youth, as are thicknesses of layers, and both subsequently rise steadily in late life. Moreover, the thicknesses and numbers of reticulin lamellae correspond to the sizes and numbers of the smooth muscle cell realms respectively. Although the correspondence is not perfect, the similarities in the trends are striking.

Discussion

Intimal thickness of the lateral thoracic aorta tends to have a weak but statistically significant correlation with the number of smooth muscle cells. The correlation is better in young subjects with thin intima and weaker in older subjects with thick intima (curve in Fig. 1). This result is not greatly different when the amount of observed tissue is increased nine-fold (Table 1). The generally poor correlation between these two variables in the older, more thickened aortas seems to be biologically real and not just an artifact of inadequate sampling.

In weanling pigs, intimal growth is associated with increasing numbers of smooth muscle cells which arise by local proliferation in the abdominal aorta (Kim et al. 1985a). A similar process might account in part for intimal growth in the human aorta up to 200 μm of thickness and 30 years of age. Thereafter, further growth occurs by accrual of more connective tissue per cell. In growing bovine bulls, intimal cells obtained from "fibrous plaques" tended to synthesize more collagen than those from "normal segments" or "fatty streaks", and this tendency was retained through 5 passages in culture (Stavenow 1984). Perhaps the life history of intimal cells is to proliferate during growth of the organ and to synthesize connective tissue thereafter. A stimulus of diet-induced hypercholesteremia, with blood cholesterols of 400 mg/dl and up, caused mitoses and 8-fold increases of intimal cell numbers in growing pigs (Kim et al. 1985a). It can be speculated that no such result would be ob-

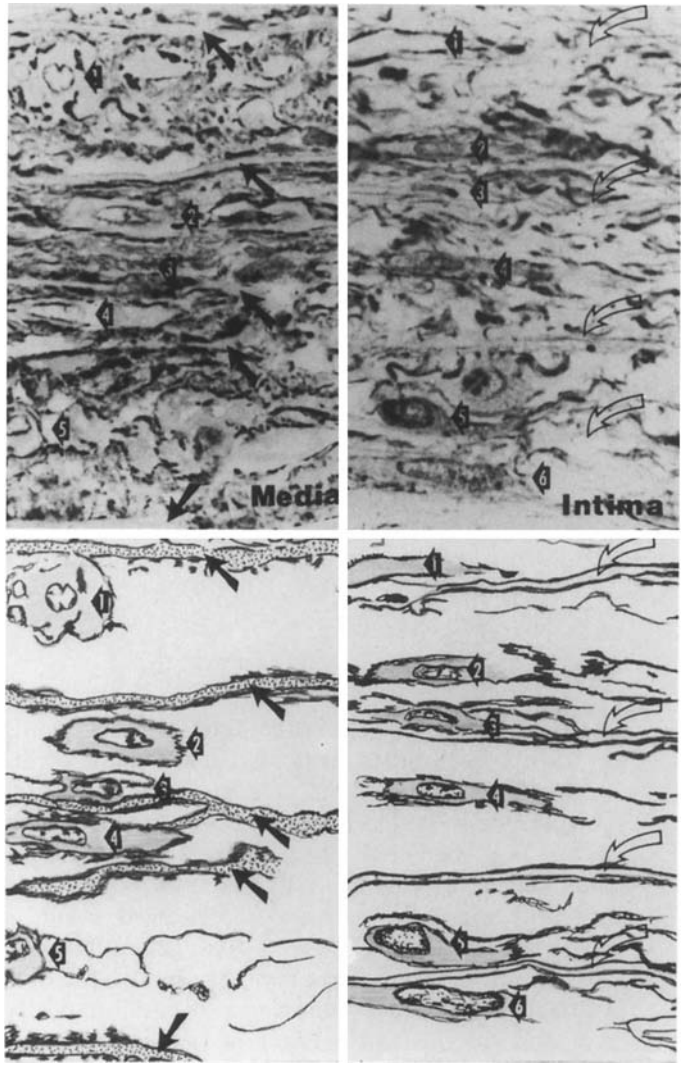

Fig. 5. Representative areas of media and intima from the lateral thoracic aorta of a 40 year man are stained for reticulin. This plastic embedded section is for demonstration purposes and does not represent the kind of technique used routinely on the tissue throughout this study. Numbered arrowheads denote nuclei of smooth muscle cells, except for (1) on the right in which the nucleus is not visible. Solid arrows (*left*) mark elastic lamellae; these are unstained in this procedure, except for the surface investment with reticulin which stains black. Open arrows (*right*) mark "reticulin lamellae" which can be shown by other techniques to be typically devoid of elastin. *The ruler* is calibrated in micrometers. 3 μm JB-4 section, Gridley's reticulin, × 740

tained in mature animals after the cessation of growth.

It has been suggested that plaques can arise as neoplasms in the arterial intima (Benditt 1977). We encountered three examples suggestive of this possibility (Fig. 2). An abundance of cells in a whorled pattern disrupted the usual intimal layering. The appearance is reminiscent of the leiomyomata sometimes seen in the submucosa of the intestinal tract. These examples were rare. The typical intimal thickenings were not rich in cells nor disorderly in their layered patterns.

A striking difference in the arrangement of cells has been observed between the axial and radial directions within the intima of the lateral thoracic aorta. In the radial dimension of the artery, cell numbers are weakly correlated between adjacent levels of the intima and generally uncorrelated between levels with separations of 100 μm or more. In contrast, along the axial dimension, cell numbers are strongly correlated between adjacent "columns" of 100 μm width, and this correlation persists for great distances, remaining significantly different from zero even at separations of about 1.4 cm. Thus, the cells appear to be clustered in the axial dimension over a distance on the order of 100 to 1000 times greater than the clustering distance in the radial dimension. This result suggests that the intima is composed of layers.

A layered intimal structure has been seen in youthful aortas with the use of reticulin stains (Fig. 3–5). The thicknesses of these layers are close to the value of 13.2 μm which has previously been predicted from theoretical considerations.

Suppose that layers of cells are seeded into the intima during growth and maturation, obtaining half of their mature numbers by age 15 to 19 and all of their mature numbers by age 40–49. The

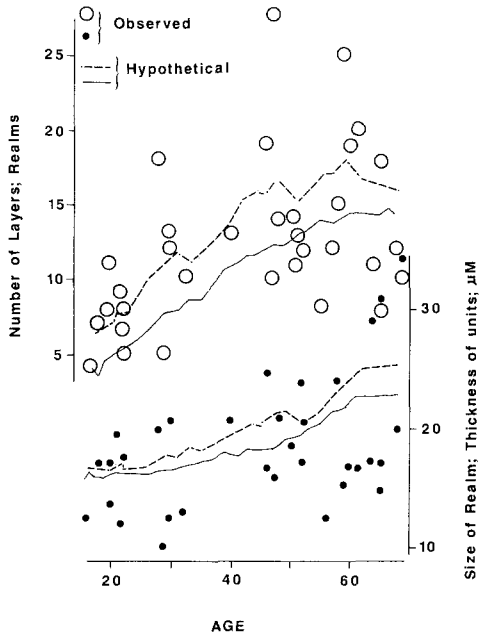

Fig. 6. Numbers (*open circles*) and average thicknesses (*closed circles*) of reticulin lamellae like those in Figure 2 are shown for 34 aortas. Numbers and sizes of average smooth muscle cell realms are shown for overlapping 10-year age groups for 212 atherosclerosis-related (*dashed lines*) and 474 basal aortas (*solid lines*)

Table 5. Numbers of cases by probability of agreement with negative binomial distribution in 52 aortas censored and 138 not censored by atheronecrosis

Chi-square probability	Not censored		Censored	
	Observed	Expected	Observed	Expected
>0.9	9	13.8	6	5.2
0.75–0.90	21	20.7	12	7.8
0.50–0.74	38	34.5	11	13.0
0.25–0.49	37	34.5	15	13.0
0.10–0.24	23	20.7	4	7.8
<0.1	10	13.8	4	5.2

For each of the 138 not censored aortas, the distribution of spindle cells over 40 observed points was compared with the negative binomial distribution by Chi Square goodness-of-fit tests. For the 52 censored aortas, the distribution of spindle cells over 40-N nonnecrotic points was examined, where N is 1 to 15. The p-values from the Chi Square tests were used to tabulate the cases here

data suggest that these layers are about 11 μm to 17 μm thick and 14000 μm long, with uncertain circumferential size. The cells retain their propensity to build lamellae like those in the media, but stop short of completing the lamellae by not elaborating an elastic membrane, thus generating reticulin lamellae instead of elastic lamellae. The "reticulin lamellae" are much harder to observe by present day techniques than the "elastic lamellae", which makes their existence hard to demonstrate.

With aging, the layers seem to thicken and merge together to yield layers of 15 to 35 μm or more in thickness, until the layering is ultimately obliterated.

The repeating unit of structure of aortic *media* is phylogenetically old. Berry et al. (1974b) show photographs of dogfish shark and Crag lizard from which 15.9 and 17.8 μm are respectively obtained as the size of an average layer composing the ventral aorta of a fish and the thoracic aorta of a reptile. These dimensions are similar to those of the layers illustrated here for the *intima* of human thoracic aorta in Fig. 3–5. This coincidence raises the possibility whereby a ventral aortic structure adapted for the purposes of the fishes, may have been redesigned for the requirements of the larger mammals such as the human and the pig (Kim et al. 1985b). Perhaps the inner layers of larger mammalian arteries require an absence of "elastic membranes" to carry out their proper functions. Hence, the lamellar structure which is phylogenetically descended from the ancestral media seems to remain in the intima as a ghostly "virtual" pattern, often hard to demonstrate, but popping up here and there to remind us of its origins. Obliteration of this pattern appears to be an early feature of abnormal change leading toward atheronecrosis.

Conclusions

In aortas with mean intimal thickness less than 200 μm, representing ages 16 to 40, about 40% of the variation of thickness from place to place was "explained" by differences in cell numbers ($r^2 = 0.40$). In older aortas with mean thickness exceeding 200 μm, only 6% of the observed variance of thickness was "explained" by differences in cell numbers. Cell numbers increase up to age 30 to 40 and mean thicknesses of 200 μm, and thereafter change little as the intima continues to thicken. These results suggest that intimal growth and maturation occur at different times from place to place in an aorta. During the growth process, when some places are cellular and thickened while others are not, a strong correlation is found. When all places have become thickened and cellular, at the end of growth, the correlation nearly vanishes, and intimal thickness is generally independent of cell numbers. Further growth of intima into "fibrous plaques", according to this evidence, seems to take place after the smooth muscle cell populations have stabilized, and is almost as likely to happen

in the less cellular as in the more cellular places. These conclusions apply to the homogeneous set of points along the lateral thoracic aorta where differences are dispersed at random; they should not be generalized to sites that differ inherently because of intrinsic architecture.

Appendix

The positions on the lateral thoracic aorta are almost homogenous in their inherent propensities to fibroplastic thickening (Tracy et al. 1983) and spindle cell numbers (Tracy et al. 1985b). Cells and thicknesses can therefore be treated as practically random in their distribution over this sample of tissue. It is therefore reasonable to expect that the scattering of these features will obey some kind of probability distribution. Indeed, the negative binomial distribution has been found to offer a satisfactory description of both variables (Tracy et al. 1983, 1985b).

Numbers of smooth muscle cells cannot always be observed because the atheronecrotic core of some plaques obliterates these cells and causes them to be overgrown by a multitude of monocytes, macrophages and lymphocytes. Could it be that the obliteration by necrosis might select the most cellular or the least cellular places? If so, then the negative binomial form of the distribution of cells should be deformed by amputation of the upper or lower tail of the bell-shaped curve.

In 138 aortas without atheronecrosis, cells were distributed among 40 observed points in accordance with the negative binomial model. This was shown by fitting a negative binomial distribution to each aorta and computing a Chi square goodness of fit statistic. The *P*-values associated with the Chi square tests were distributed as expected under the null hypothesis that a negative binomial model is appropriate (Table 5). In the 52 aortas having between 1 and 15 atheronecrotic points (and therefore 25 to 39 uncensored observations), *P*-values for the Chi square tests comparing the observed with the fitted negative binomial models again were also distributed as expected under the null hypothesis (Table 5). These results suggest that cell numbers were of no measurable importance to the occurrence of atheronecrosis at particular points in an aorta. The necrosis did not censor the most cellular or least cellular places so as to distort the distribution; rather it censored at random with respect to cell numbers, insofar as this effect could be measured.

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