

Some segmental morphological and morphometrical features of the intima and media of the aortic wall in *Chinchilla lanigera*

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Background: The aim of this study is to describe the morphology, morphometry and ultrastructure of segments of the thoracic and abdominal aorta portions in *Chinchilla lanigera*. Thickness measurements of the tunica intima and media complex of the aorta were taken.

Materials and methods: In all observed specimens, the thickness values for the tunica intima and media complex of the cranial thoracic aorta were significantly higher (mean: 702.19 μm) when compared to the values of other analysed aortic segments (means: 354.18 μm ; 243.55 μm). Complex statistical methods were used to assess the differences between various aortic segments.

Results and Conclusions: The components of the vessel walls show variations in structure and thickness, presumably due to an adaptation to functional demand. (Folia Morphol 2019; 78, 4: 729–737)

Key words: arteries, chinchilla, aorta, quantitative histological, qualitative histological, media, intima, *post hoc*

INTRODUCTION

Chinchilla lanigera is a rodent species belonging to the Chinchillidae family, originating from the central Andes in Chile, Peru and Bolivia. Chinchillas are mainly kept for fur, but also as pets [33] or, lately, as laboratory animals. They are used in experimental medicine in the study on middle otitis or parasitology [1, 13–15, 18].

The structural description of the aortic wall has been published for several species, like humans [8], common and laboratory rat [2, 16, 36], dog [27], goat [24, 25], paca [10], opossum [19], capucin, chicken [23] and gerbil [26], but, to the best of our

knowledge, such a description has never been made for chinchilla. More than that, there is no significant combined morphological and morphometrical description of the layers of the arteries in this species.

Aorta, in all mammals, is the main vessel, part of the greater circulation, which ensures the distribution of blood from the heart to most of the body. It originates at the base of the heart — ascending aorta (*aorta ascendens*), passing through the thoracic and abdominal cavities, giving off visceral and parietal branches [3]. A set of vessel are directed towards the head, neck and forelimb, originating from the level of the aortic arch (*aorta ascendens-arcus aortae*) while

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within the thoracic cavity (*aorta descendens-aorta thoracica*), a series of branches are distributed by means of parietal and visceral branches. The aorta crosses the diaphragmatic hiatus to distribute both visceral and parietal branches within the abdominal cavity (*aorta descendens, aorta abdominalis*).

The macroscopic pattern of distribution of some aorta-originating branches has been described lately in a series of studies in chinchilla [20, 29–31] or other rodents [5, 34].

Structurally, the arterial segments have been classified into elastic, transitional and muscular types based on the histological characteristics and organisation of connective tissue fibres and smooth muscle cells in three distinct tunics: *tunica intima*, *tunica media* and *tunica adventitia* [9].

The intima is the most innermost layer that comes in direct contact with the blood flow and it comprises a flattened endothelial layer placed on a basal membrane, a subendothelial layer formed by conjunctive tissue (elastic fibres, reticular fibres and collagenous fibres) and an internal elastic limiting membrane. The media consists of muscular cells arranged in a circular direction with a certain amount of elastic and collagenous fibres interposed. The adventice (outer layer) contains elastic and collagen fibres along with some muscular fibres in a relatively little organised form.

The main objective of this study is to describe the structure of the aortic segments and other morphological features, including the morphometrical data of the intima and media, as observed in light microscope in chinchilla. More than that, an advanced statistical approach is used to check whether the method can be used as indicator for some fine differences that cannot be perceived by a regular histological investigation.

MATERIALS AND METHODS

The biological material was represented by 15 adult chinchilla individuals, originating from a private breeder, commercially slaughtered for fur. After the skinning, the carcasses were anatomically dissected, following the regular procedure. The aortic segments were identified, following the abdominal wall and sternum dissection. The aorta was fixed by slow 4% formalin perfusion through the ventricular cavity as the right atrium was sectioned to allow blood outflow. The dissection of the aorta followed, with the proper removal of parts of the four mentioned fragments (*ascending aorta, arcus aortic, tho-*

racic and *abdominal aorta*). Collected fragments were further fixed in 4% formalin and followed the routine histological procedure. Fixation was performed in Stieve fixative fluid for 24 h, dehydrated in ethyl alcohol (2 times for 2 h succeeding immersions) followed by another passage in absolute ethanol for another 1.5 h. The last tissue clearing was performed with butyl alcohol (3 times, 24 h) and further on paraffin included. Serial sections of 5 μm were cut and the trichrome Goldner-Verhoeff staining method was applied on the samples [17].

All the histological material was analysed and photographically documented by using an optic microscope Olympus BX 41 device with a digital Olympus E330 camera. The captured images were furthermore processed with Gimp or ImageJ® software for the histological measurements. Several measurements were made on the vascular tunics and on the elastic laminae.

In order to highlight the possible statistical differences among the studied segments, a series of advanced statistical tests were performed on the available data series for the taken measurements.

Due to unequal sample series, the *post hoc* tests seem suitable for the comparison of the distribution of the mean values. Out of the typical tests, the Tukey, Scheffe, Holm and Bonferroni tests were used and their results were compared. The statistical tests were performed in accordance to: http://astatsa.com/OneWay_Anova_with_TukeyHSD/.

Statistical analysis

For each studied morphological entity, the series of statistical tests was performed (one way ANOVA). The next tests were applied based on the F-value which had been provided by the previous tests ($F \leq 0.005$).

RESULTS

Qualitative analysis — general data

Ascending aorta. The media of this segment is very thick, with a relatively ordered structure, the elastic component clearly prevailing. In this layer, the circularly placed elastic laminae are very clearly visible on Verhoeff staining (Fig. 1). The interlaminae spaces were larger in the inner half of the media while the outermost half seems to present narrower interlaminae spaces, due to the accentuated coarseness of the thinner elastic laminae. The interlaminae spaces present circular bundles of smooth muscle cells embedded in connective tissue in a peculiar arrange-

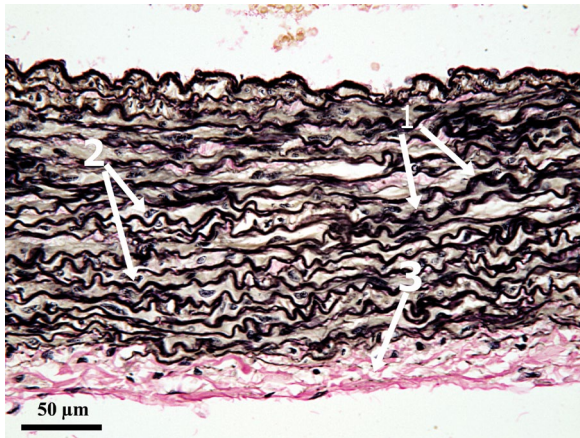


Figure 1. Ascending aorta (Verhoeff); 1 — elastic laminae; 2 — smooth muscle cell nuclei; 3 — adventice.

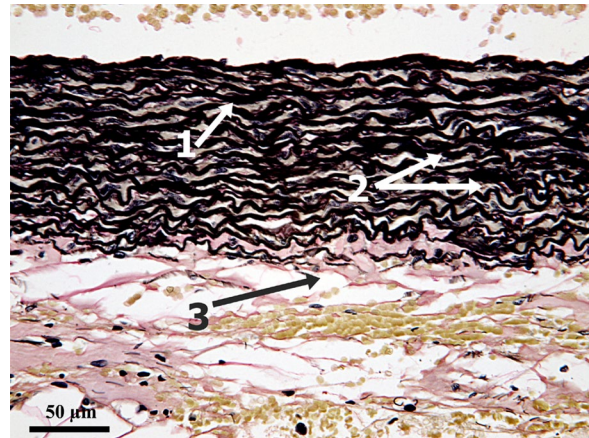


Figure 3. Aortic cross (Verhoeff); 1 — elastic laminae; 2 — smooth muscle cells; 3 — adventice.

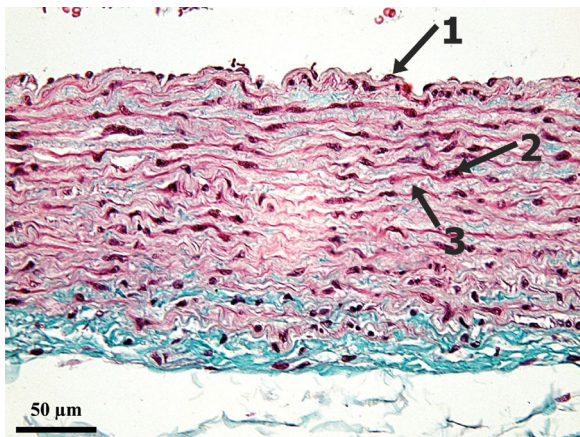


Figure 2. Ascending aorta (Trichrome Goldner); 1 — intima; 2 — connective tissue of media; 3 — muscle cells of the media; 4 — adventice.

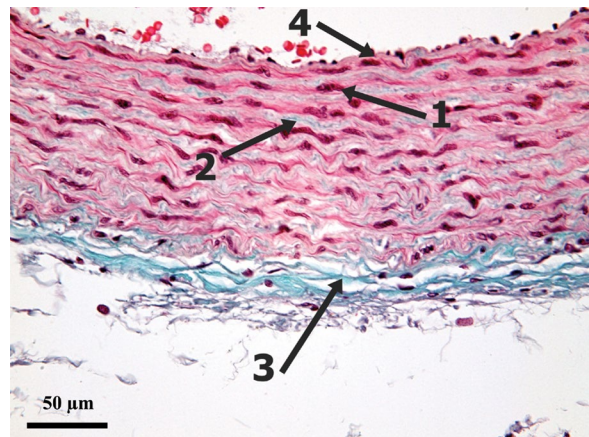


Figure 4. Aortic cross (Trichrome Goldner); 1 — muscle cells of the media; 2 — connective tissue of the media; 3 — adventice; 4 — intima.

ment, forming a single row of muscular cells flanked by two elastic neighbouring laminae (Fig. 2). The internal limiting membrane appears thin, but clearly visible on trichrome staining. The external limiting membrane seems difficult to identify.

Aortic cross. This sector has lot of the features found on the ascending aorta. The internal limiting membrane is less distinctive as in the ascending aorta. Media prevails, as the structure is predominantly elastic as well. The number of elastic laminae is similar with the one recorded in the case of the ascending aorta, but a slight difference is to be noted when shape and disposition is taken in consideration. The elastic laminae are slightly thicker and less folded, except for the anterior third of the aortic cross where these differences are not distinguishable (Fig. 3). The

muscular cells are ordered, showing a one single cell row arrangement intermingled in the medial layer of the studied samples (Fig. 4), the external limiting membrane not being evidenced.

Thoracic aorta. The thoracic aorta shows again similar aspects with the ones described previously, with elastic laminae disposed in a similar manner, with a slightly increased width when compared with the aortic cross segment (Fig. 5). In terms of number of laminae, there is a progressive decrease (14.43). The interlaminae spaces are visibly larger in the internal part of the artery, while the outermost medial part maintains a similar aspect with the previous aortic sectors. Although the interlaminae spaces are larger, the muscular component suffers no changes in terms of the described arrangement in the other aortic sec-

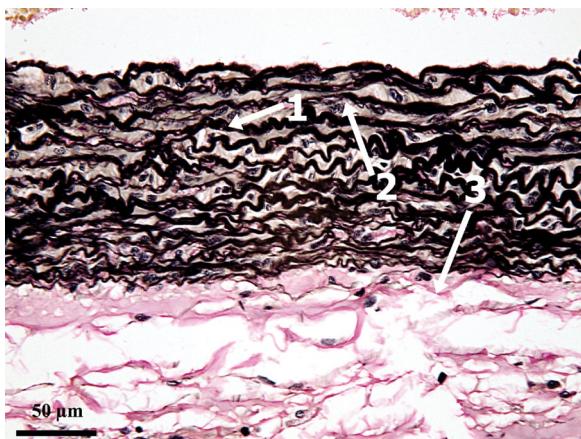


Figure 5. Thoracic aorta (Verhoeff); 1 — elastic laminae; 2 — smooth muscle cells of the media; 3 — adventice.

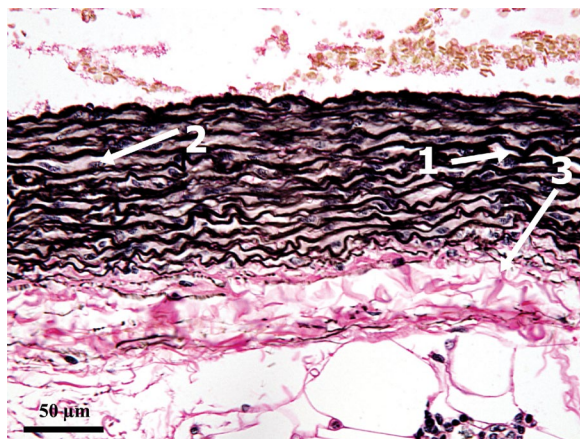


Figure 7. Abdominal aorta (Verhoeff); 1 — elastic laminae; 2 — smooth muscle cells; 3 — adventice.

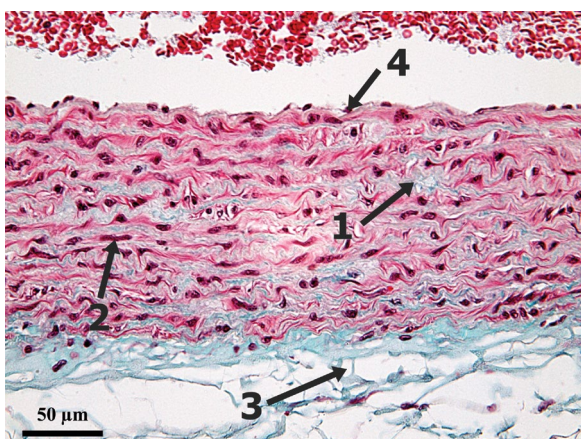


Figure 6. Thoracic aorta (Trichrome Goldner); 1 — connective tissue; 2 — smooth muscle cells; 3 — adventice; 4 — intima.

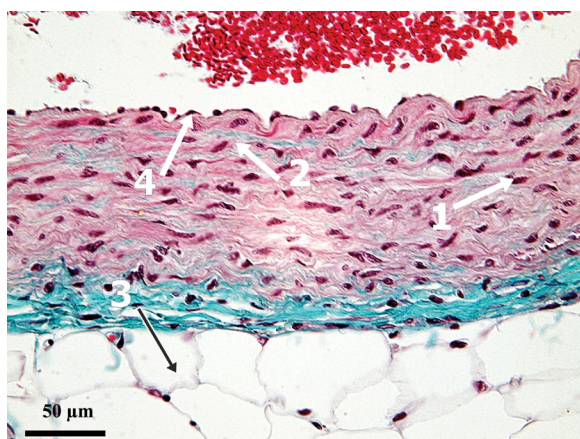


Figure 8. Abdominal aorta (Trichrome Goldner); 1 — smooth muscle cells of the media; 2 — connective tissue of the media; 3 — adventice; 4 — intima.

tors (Fig. 6). The internal limiting membrane cannot be differentiated from the other elastic laminae, the same standing for the external limiting membrane.

Abdominal aorta. In this sector, the width of the arterial wall is somehow reducing. The ratio between the three tunics is modified, with a slight decrease in the proportion of the media in favour of the adventice (Fig. 7).

The medial muscular cells are placed in rows between the elastic laminae in the scant interlamellar connective tissue (Fig. 8). The internal limiting membrane, still distinguishable, is not folded as in the previous aortic sectors, but seems a little thicker than the laminae deeply situated in the media. As in all sectors described so far, the external limiting membrane could not be identified.

Quantitative data

In order to evaluate the specific values, a series of microscopic measurements were taken in respect of the thickness of intima, media and the number of the elastic laminae for each of the sectors studied. The results (shown as average values and standard deviations, minimal and maximal values) are shown in Table 1.

Advanced statistics

The results were comparatively analysed in conjunction with the observed morphological data. Data recorded from all studied segments were compared, in the attempt of finding differential elements from this metrical perspective.

Tunica interna (thickness). For the series of dimensions recorded in all studied sectors of the inti-

Table 1. Quantitative data for the components of the aortic sectors (averages and standard deviation, n = number of measurements)

	Intima	Media	Elastic laminae width	Elastic laminae number (average)
Ascending aorta [μm]	3.77 ± 0.8 , (1.93–6.38), n = 69	179.93 ± 22.8 , (140.4–244.2), n = 74	2.60 ± 0.7 , (1.70–4.88), n = 68	18.86, n = 61
Aortic cross [μm]	3.82 ± 0.51 , (2.72–4.95), n = 35	112.21 ± 7.1 , (96.18–125.05), n = 66	2.76 ± 0.5 , (1.74–4.22), n = 39	18.71, n = 49
Thoracic aorta [μm]	2.83 ± 0.45 , (1.62–3.98), n = 41	123.80 ± 11.8 , (91.97–142.09), n = 55	3.41 ± 0.74 , (1.97–6.64), n = 129	14.43, n = 32
Abdominal aorta [μm]	3.24 ± 0.88 , (1.7–5.67), n = 61	115.73 ± 11.8 , (81.55–158.03), n = 47	3.11 ± 0.59 , (1.95–5.73), n = 81	19.71, n = 53

Table 2. Advanced statistics (*post hoc*) — intima measurements of different aortic sectors

Segment	Ascending aorta vs. aortic cross	Ascending aorta vs. thoracic aorta	Ascending aorta vs. abdominal aorta	Aortic cross vs. thoracic aorta	Aortic cross vs. abdominal aorta	Thoracic aorta vs. abdominal aorta
Tukey p-value	0.8999947	0.0010053	0.0021494	0.0010053	0.0010053	0.0440389
Tukey inference	Insignificant	** , p < 0.01	** , p < 0.01	** , p < 0.01	** , p < 0.01	* , p < 0.05
Scheffe p-value	0.987155	1.63e-06	0.053841	1.07e-07	0.001932	0.0762995
Scheffe inference	Insignificant	** , p < 0.01	** , p < 0.01	** , p < 0.01	** , p < 0.01	Insignificant
Bonferroni p-value	4.2738	3.32e-07	0.0022934	1.88e-08	0.0007186	0.0537212
Bonferroni inference	Insignificant	** , p < 0.01	** , p < 0.01	** , p < 0.01	** , p < 0.01	Insignificant
Holm p-value	0.7123111	2.77e-07	0.0011467	1.88e-08	0.0004791	0.0179071
Holm inference	Insignificant	** , p < 0.01	** , p < 0.01	** , p < 0.01	** , p < 0.01	* , p < 0.05

ma, the applied *post hoc* test shows that the p-value corresponding to the F-statistic of one-way ANOVA is lower than 0.01, which strongly suggests that one or more pairs of treatments are significantly different.

The Tukey test reveals (as shown in the Table 2) a significant difference (**, p < 0.01) between the following pairs:

- ascending aorta vs. thoracic aorta and abdominal aorta, as well as in between the aortic cross vs. thoracic and abdominal aorta;
- and a small significance was shown in the case of abdominal aorta vs. thoracic aorta (*, p < 0.05);
- no significant differentiation in the case of values recorded for intima of ascending aorta vs. aortic cross.

In the case of Scheffe, Bonferroni and Holm tests, the results are similar, except for the thickness of the

intima of thoracic and abdominal aorta, which seems to be statistically undifferentiated.

Tunica media (thickness). The p-value corresponding to the F-statistic of one-way ANOVA applied for the measurements of the media of studied sectors is lower than 0.05, suggests that the one or more groups are significantly different (Table 3).

The Tukey HSD test, Scheffé, Bonferroni and Holm multiple comparison tests follow. Based on the comparison of the series, results were computed:

Tables 1 and 2 shows degree of differentiation (**) in respect of the values of the media in the case of:

- ascending aorta vs. aortic cross (all tests), ascending aorta vs. thoracic aorta;
- aortic cross vs. thoracic and abdominal aorta (all tests).

Table 3. Advanced statistics (*post hoc*) — media measurements of different aortic sectors

Segment	Ascending aorta vs. aortic cross	Ascending aorta vs. thoracic aorta	Ascending aorta vs. abdominal aorta	Aortic cross vs. thoracic aorta	Aortic cross vs. abdominal aorta	Thoracic aorta vs. abdominal aorta
Tukey p-value	0.0010053	0.0010053	0.60170	0.0010053	0.0010053	0.0461158
Tukey inference	**p < 0.01	**p < 0.01	Insignificant	**p < 0.01	**p < 0.01	*p < 0.05
Scheffe p-value	1.1102e-16	0.0006922	0.6857347	1.1102e-16	1.1102e-16	0.0794786
Scheffe inference	**p < 0.01	**p < 0.01	Insignificant	**p < 0.01	**p < 0.01	Insignificant
Bonferroni p-value	0.0000e-00	0.0002275	1.3437801	0.0000e-00	0.0000e-00	0.0564001
Bonferroni inference	**p < 0.01	**p < 0.01	Insignificant	**p < 0.01	**p < 0.01	Insignificant
Holm p-value	0.0000e-00	0.0001137	0.2239633	0.0000e-00	0.0000e-00	0.018800
Holm inference	**p < 0.01	**p < 0.01	Insignificant	**p < 0.01	**p < 0.01	

In the case of the values of media of thoracic and abdominal aorta, no or very little differentiation (Holm test) is shown. Similarly, an insignificant difference is shown in the case of values of ascending aorta versus abdominal aorta.

Elastic laminae (thickness). The p-value corresponding to the F-statistic of one-way ANOVA is lower than 0.05, suggesting that the one or more treatments are significantly different.

The Tukey HSD test, Scheffé, Bonferroni and Holm multiple comparison tests

All three applied tests show the fact that statistically there seems no differentiation between the number of elastic laminae recorded at the level of the ascending aorta and the aortic cross, while on the other hand, slight differences between the segments are noticeable as we advance further from the initial aortic part (aortic cross vs. thoracic or abdominal aorta, ascending aorta vs. thoracic and abdominal aorta) (Table 4).

Regional variations of the tunics

Regional variations in tunica intima. The intima consists of the endothelial layer placed on the basal membrane, a subendothelial layer and the internal elastic lamina. The average values recorded for the thickness of the intima show a mentionable decrease in the case of the thoracic segment of the aorta (2.83 μm), with close values for the ascending aorta

(3.77 μm), aortic cross (3.82 μm), while the intima of abdominal aorta shows a small decrease only (3.24 μm).

The internal limiting membrane is thin, visible in the ascending aorta, but as we progress towards the abdominal aorta, the structure becomes less and less distinguishable, not being differentiated from other elastic laminae (thoracic and abdominal aorta).

The appearance of the layer seems relatively uniform in all segments, with another worth-mentioning feature that refers to the folding degree of the intima, in the abdominal sector a limited amount of folding being recorded when compared to the other segments.

The complex statistical analysis of the thickness figures shows an interesting feature of the data rows. The *post hoc* tests indicate a certain degree of differentiation for all compared sets, with significant differences among sectors. Although the values seem very similar in terms of averages, there is a significant statistical difference between ascending aorta, thoracic and abdominal computed aorta values.

Regional variations in tunica media. The medial layer the thickest coat of the three portions analysed—consists of elastic laminae, collagen fibres and smooth muscle cells in an interrelated arrangement.

The overall thickness (as average value) of the medial layer shows a decrease in dimensions from the ascending aorta towards the abdominal aorta.

The orientation of the elastic laminae maintains a predominantly circular arrangement in all studied.

Table 4. Advanced statistics (*post hoc*) — number of elastic laminae of different aortic sectors

Segment	Ascending aorta vs. aortic cross	Ascending aorta vs. thoracic aorta	Ascending aorta vs. abdominal aorta	Aortic cross vs. thoracic aorta	Aortic cross vs. abdominal aorta	Thoracic aorta vs. abdominal aorta
Tukey p-value	0.6018440	0.0010053	0.0093116	0.0010053	0.010053	0.0094489
Tukey inference	Insignificant	**p < 0.01	**p < 0.01	**p < 0.01	**p < 0.01	**p < 0.01
Scheffe p-value	0.6860750	1.5891e-08	0.0198735	5.8366e-09	0.0017750	0.0201326
Scheffe inference	Insignificant	**p < 0.01	*p < 0.05	**p < 0.01	**p < 0.01	*p < 0.05
Bonferroni p-value	1.3437624	2.4692e-09	0.0103576	8.6658e-10	0.0006446	0.0105179
Bonferroni inference	Insignificant	**p < 0.01	*p < 0.05	**p < 0.01	**p < 0.01	*p < 0.05
Holm p-value	0.2239604	2.0577e-09	0.0051788	8.6658e-10	0.0004297	0.0035060
Holm inference	Insignificant	**p < 0.01	**p < 0.01	**p < 0.01	**p < 0.01	

The number of laminae does not vary significantly in different aortic regions, the average number of fibres being 18–19 in ascending aorta, aortic cross and abdominal aorta, with a worth-mentioning exception in the case of thoracic aorta (14 lamellae).

In terms of the diameter of the elastic laminae, one must notice the significant increase in the width of abdominal aorta (3.11 μm) and thoracic aorta (3.40 μm) in opposition to slightly increasing values recorded in ascending aorta (2.6 μm) and aortic cross (2.7 μm).

The muscle cells have a constant pattern of arrangement (row arrangement), with no significant changes from one sector to another, being slightly denser in the inner half of the arterial wall in all segments. The interlamellar spaces follow a similar pattern, with larger spaces in the inner half, while in the outermost half these spaces are narrower. This aspect is correlated with the slight increase on the prominence and folding of the elastic laminae within the media layer.

DISCUSSION

Based on the constituents, all investigated sectors of the aorta in chinchilla can be included in the elastic type, as described by a series of other authors [2, 4, 8, 28, 32].

The intimal layer's structure depends on the level of the hemodynamic stimulation [7, 11, 12, 25]. The folding degree of the intima and the underlying limit-

ing membrane, explained dynamically as a resistance mechanism to the dilatation during systolic activity and as a reserve of intima stretched by systole [25], seems to decrease in the abdominal sector of the aorta, maintaining a relatively uniform folding in the other sectors. This suggests a different pattern in forces distribution within this sector, but things should be regarded in conjunction to the fact that the internal limiting membrane is not distinguishable microscopically in the last part of the aorta. Another interesting fact is represented by the change in thickness of the intima in the thoracic sector. The values for this sector are reported as being somehow similar to the case of Guinea pig and rat, where a difference of more than 10% can be noticed [22], similarly with our data sets. The advanced statistical investigation shows a low differentiation for the first two initial sectors (ascending aorta and aortic cross), but clear differentiation between values of the next sectors (mainly thoracic and abdominal aorta).

The media plays the most important haemodynamic role (windkessel function) [35], related to the influence of the systolic pressure and turbulent blood flow during ventricular systole [11, 25].

The overall width of the media shows a decrease in width from the ascending aorta (170 μm) to a relatively constant dimension of 110–120 μm in the rest of the sectors, despite the fact that the advanced statistics indicates some kind of differentiation among sectors, with the exception of the thoracic aorta data.

The quantity of elastic laminae in different sectors of the aorta shows a significant decrease in number only in the area of the thoracic aorta (from 18–19 to 14). The data is partially confirmed by the advanced statistics in respect of the dimensions of elastic laminae, mainly in the case of differentiation of ascending aorta and descending aortic sectors; a situation that does not resemble the comparative data that was earlier published. As an example, in Guinea pig the number of laminae decreases from 29 in ascending sector to 15 in thoracic and 8 in abdominal sector, with a somehow similar decrease in rat (12 in ascending aorta, 9 thoracic aorta and 7 in abdominal aorta) [2, 22]. Opposed to this situation, in dog the number of elastic laminae in aortic segments is much higher (30–40 in ascending and thoracic descending aorta decreasing to 15 in the abdominal part) [21]. On the other hand, the number of elastic fibres in chinchilla's medial layer seems to be correlated with a change in width of the laminae in the case of the thoracic aorta. Here a reduction in the average number must be noticed, as a possible explanation for the slight physiological decrease in the aortic pressure in this sector and reduction in the mural resistance (as implied by Mello et al. [22]), possibly due to a limited number of collaterals emitted in the thoracic sector. Another explanation arises from the morphological observation on the interlamellar spaces [6] that are much larger than in the other sectors, filled with stronger bundles of muscular cells.

CONCLUSIONS

The observations of the present study show that the coats of the different sectors of the aorta in chinchilla display a series of interesting qualitative and quantitative regional variations, some perceivable by means of simple statistical parameters, some others by means of advanced statistical methods.

A series of functional correlations can be made on this basis, mainly related to haemodynamic factors. These observations may also serve as a starting point for understanding the differences in functional mechanics and physiology in different sectors and maybe some pathological phenomena that can be associated with these arterial segments.

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