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## SPONTANEOUS AORTIC LESIONS IN FALLOW DEER (DAMA DAMA L)

By

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POUNGHOMPOO, SOMLAK: *Spontaneous aortic lesions in fallow deer (Dama dama L)*. Acta vet. scand. 1985, 26, 549—562. — In 19 out of 22 aortas from fallow deer, 15 months to 5½ years, aortic lesions were found. Three types of lesions were observed, fatty streaks were seen in 2, fibrous plaques in 15 and fibrous plaques with complicated lesions (calcification and acid mucopolysaccharides) in 2 of the aortas. Elastic tissue degeneration of the inner two thirds of the tunica media was frequently found in the aorta of the animals > 3½—5½ years of age. There was no statistical evidence for a correlation between age and frequency ( $P \infty 0.10$ ) but a trend towards age dependence was seen. The percentage of involved surface was found to significantly increase ( $0.05 > P > 0.01$ ) with age. Lesions were found to start in the abdominal aorta in young animals and to extend cranially to the thoracic aorta with age. The percentage of involved surface in different affected regions, comprising all age groups were, in the posterior abdominal portion, 10.5 %, in the anterior abdominal portion, 4.3 % and in the posterior thoracic portion, 1.04 %. The influence of hemodynamic flow upon the localization of the aortic lesions, the endothelial cell population density and the endothelial nuclear patterns were discussed.

atherosclerosis; fatty streaks; complicated lesions; fibrous plaques.

Spontaneous aortic lesions found in roe deer (*Pongshompoo & Reh binder* 1983) and moose (*Pongshompoo & Reh binder* 1985) were similar to changes observed in man and other animals (*Robert et al.* 1959, *Gupta & Reh binder* 1981, *Skold & Getty* 1961). Age, sex, hemodynamic, stress, cold weather and starvation were thought to be factors involved in the formation of aortic lesions. As starvation was considered one factor in a multifactorial genesis of atherosclerosis in roe deer, the prevalence of aortic lesions in farmed fallow deer, regularly given a supplementary feed, were studied.

Farmed fallow deer live under quite similar circumstances as do roe deer and moose except for the supplementary feed supplied during winter time. Fallow deer are both grazers and browsers while roe deer are mainly grazers and moose are mainly browsers (Ahlen 1975). Considering the age, in a survey of wild and park fallow deer in Southern England, the oldest animals were found to be around 9 years (Armstrong *et al.* 1969). Thus fallow deer seem to reach the same age as roe deer (Borg 1970) but have a shorter life span moose (Wolf 1969).

The objective of this investigation has been to study the incidence and morphology of spontaneous aortic lesions and their association with age and sex in fallow deer.

#### MATERIAL AND METHODS

Aorta from 22 fallow deer (19 males and 3 females) 15 months to 5½ years of age were collected between September 22, 1982 and December 1, 1982. As the animals were eartagged as calves, age was determined from reading eartags. The material was divided into 3 age groups  $\leq 1\frac{1}{2}$  year,  $> 1\frac{1}{2}$ — $3\frac{1}{2}$  years, and  $> 3\frac{1}{2}$ — $5\frac{1}{2}$  years. The aortas were removed, opened longitudinally and fixed in 10 % buffered formalin.

The material was processed according to the methods described by Pongshompoo & Rehbinder (1983). In addition, Verhoeff's elastin stain for elastic fibres and PTAH stain for fibrin were added. Affected surfaces was measured by using a planimeter (Kontron, Messgerät GMBH).

Histological sections were examined and the lesions were graded. The lesions which occupied the greatest areas of a segment was considered the predominant lesion.

Statistical analysis was performed by means of regression analysis.

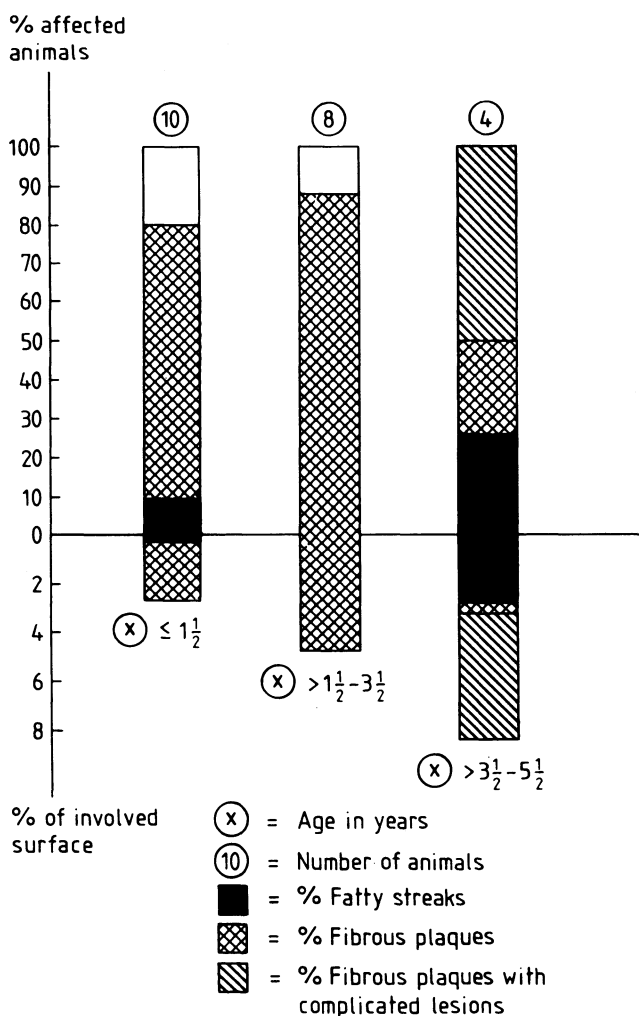
#### RESULTS

In 19 out of 22 examined animals, 3 types of lesions were found, fatty streaks, fibrous plaques and fibrous plaques with complicated lesions (calcification and acid mucopolysaccharides). The distribution is shown in Table 1.

The lesions showed no correlation between age and frequency ( $P \infty 0.10$ ), but the percentage of involved surface appeared to have a slightly significant ( $0.005 > P > 0.02$ ) increase with age

**Table 1.** Number of fallow deer and distribution of different kinds of aortic lesions.

Age in years	Number of animals	Aortic lesions			Total number lesions	% affected
		Fatty streaks	Fibrous plaques	Fibrous plaques with complicated		
$\leq 1\frac{1}{2}$	10	1	7	—	8	80
$> 1\frac{1}{2}-3\frac{1}{2}$	8	—	7	—	7	87.5
$> 3\frac{1}{2}-5\frac{1}{2}$	4	1	1	2	4	100
<b>Total number</b>	<b>22</b>	<b>2</b>	<b>15</b>	<b>2</b>	<b>19</b>	



**Figure 1.** Percentage of affected fallow deer in different age groups and percentage of involved surface affected by different kinds of aortic lesions.

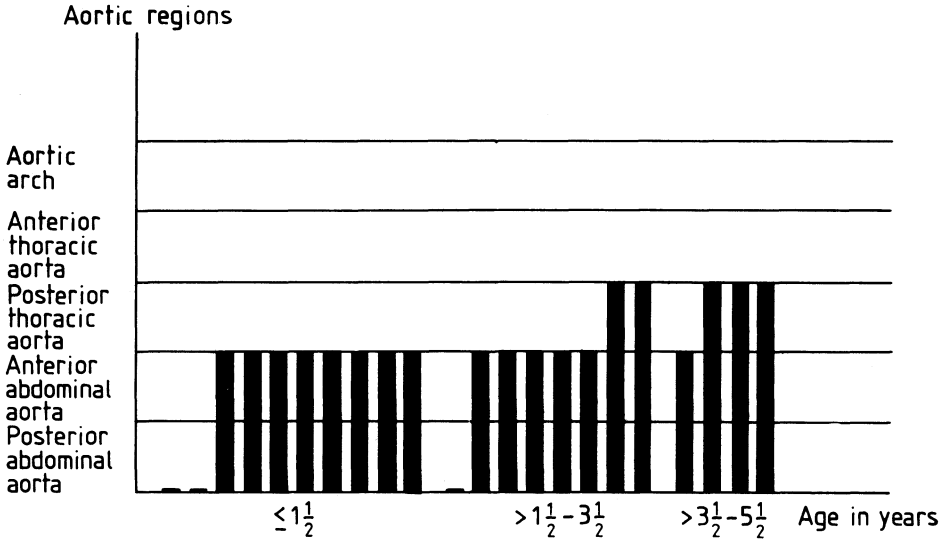


Figure 2. Distribution of aortic lesions of the individual animal in 5 different anatomical regions.

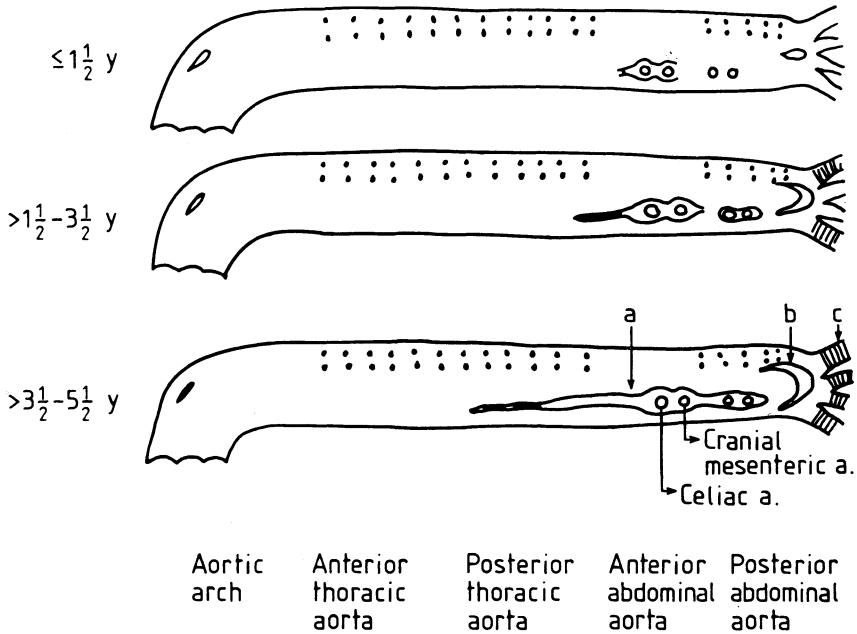


Figure 3. Distribution of aortic lesions. Fibrous plaques, are seen as two parallel elevated ridges running cranially from the orifices of the celiac and cranial mesenteric arteries (a). An ovoid or a crescent shaped ridge also occurred at the bifurcation (b). Circular wavy surfaces and intimal thickenings of the external and internal iliac arteries were observed (c). Involved surface and regions increase with age.

(Table 1 and Fig. 1). Furthermore, the histological lesions, appeared more advanced in older animals i.e. vascularization and complicated lesions. The distribution of each group is shown in Figs. 2 and 3. In the  $\leq 1\frac{1}{2}$  year group, lesions occurred in the posterior and anterior abdominal aorta. In animals of the  $>1\frac{1}{2}$ — $3\frac{1}{2}$  year group as well as in the animals of the  $>3\frac{1}{2}$ — $5\frac{1}{2}$  year group, lesions occurred also in the posterior thoracic aorta. The frequency of involved surfaces in affected regions, comprising all age groups, were in the posterior abdominal portion, 10.5 %, in the anterior abdominal portion, 4.3 % and in the posterior thoracic portion, 1.4 % (Fig. 4).

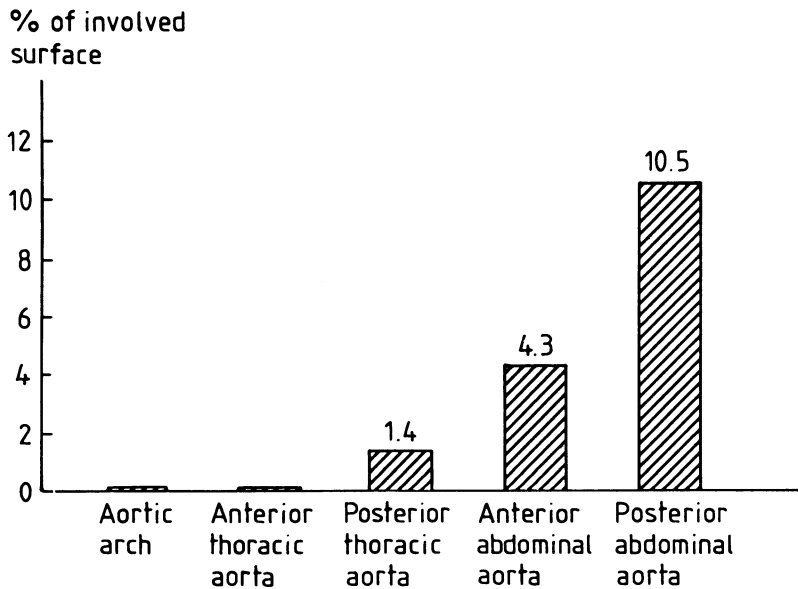


Figure 4. Percentage of involved surface in different affected regions, comprising all age group.

#### *Fatty streaks*

Gross staining with Sudan IV revealed yellowish, soft, flat or slightly elevated areas, being intimal fatty streaks, varying between  $0.1-0.4 \times 0.5-7.5$  cm. In the posterior thoracic and anterior thoracic and anterior abdominal portions, the lesions were principally parallel, linear or longitudinal streaks localized at the ventral mid-line of the aorta. In the posterior abdominal portion, fatty streaks appeared as small irregular areas, mostly

located near the mid-line, ventrally or dorsally, close to the iliac bifurcation. In the external and internal iliac arteries, the fatty streaks were characterized by stained circular wavy surfaces.

The histological investigation revealed, that the intima of fatty streaks or spots was 2—8 times thicker than that of a normal intima. In connection with the intimal changes the media was also regularly 1½—2 times thickened. The thickened part of the tunica intima comprised a single layer of endothelial cells, fibroblasts, fibrocytes, elastic fibers, collagen fibers, a few mononuclear cells and a few smooth muscle cells. The components were separated by edema fluid. Most intimal smooth muscle cells were oriented parallel to the long axis but some of them appeared without definite orientation. The internal elastic lamina showed a multitude of changes, as edema, fragmentation, undulation, dissolution and reduplication. In the tunica media, the lesions were characterized by varying degree of edema, elastolysis, vacuolation and disorientation of smooth muscle cells, the later being a prominent feature. Smooth muscle cells in the inner third of the media were often oblique or perpendicular to the long axis of the aorta. Clusters of disoriented smooth muscle cells were regularly found facing a gap of the internal elastic lamina (Fig. 5).

Frozen sections of fatty streaks revealed lesions consisting of cells with droplets of lipids in their cytoplasm and a variable amount of fine droplets of lipids in the extracellular space. Areas of lipid deposition either intracellular or extracellular or both, were regularly found in the inner half of the intima, especially close to the interstitial elastic lamina (Fig. 5). Lipid droplets in the tunica media were rare.

### *Fibrous plaques*

Fibrous plaques were found to be nonsudanophilic, grey-white, firm, mound, round or elongated lesions in the intima varying in size from 0.2—1.0 × 0.2—7.5 cm. Calcified fibrous plaques were considered as fibrous plaques with complicated lesions. In the posterior thoracic aorta and anterior abdominal aorta, fibrous plaques were seen as two parallel elevated ridges running cranially from the orifices of celiac and cranial mesenteric arteries. These areas often revealed also fatty streaks. In the posterior abdominal aorta, fibrous plaques occurred near the bifurcation and occasionally in the internal and external iliac

arteries. It was obvious that fibrous plaques gradually extended from a primary lesion with age, i.e. from an oval shaped to a crescent shaped lesion at the bifurcation, or from moundlike fibrous plaques, at the orifices of the celiac and cranial mesenteric arteries, to parallel ridges extending cranially into the posterior thoracic aorta (Fig. 3).

The microscopic features of fibrous plaques were more variable than those of fatty streaks. The intima was regularly 10—50 times thicker than that of a normal intima and the intimal thickening was much more pronounced than that of the media. Uncomplicated fibrous plaques occurred in different regions and varied in shape and composition (Figs. 6, 7 and 8). Fibrous plaques were generally covered by a single layer of endothelial cells or a few layers of proliferating endothelial cells forming a cap. The nuclei of the endothelial cells varied in their appearances being flattened or big and dense or necrotized. At the ostia, an increase in the number of the endothelial cells with big dense and round nuclei was found (Fig. 9). The polarity of the intimal cells, below the endothelial cap, divided the intima into 2—3 different layers. The cells of the outer  $\frac{1}{3}$  to  $\frac{2}{3}$  were rounded, lacking polarity. The cells of the middle third were disoriented and the cells in the inner third were generally oriented with their long axis horizontal to the lumen of the aorta. The internal elastic lamina underneath the fibrous plaques showed a combination of changes to a higher degree than found in fatty streaks i.e. fragmentation, undulation, dissolution and reduplication. The changes in the tunica media were similar to that of fatty streaks except for the higher degree of vacuolated cells, increased acid mucopolysaccharides and elastic tissue degeneration. Elastic tissue degeneration in the inner two thirds was frequently found in the aorta of animals  $3\frac{1}{2}$ — $5\frac{1}{2}$  years old. Clusters of medial smooth muscle cells were often observed to pass through ruptures of the internal elastic lamina into the intima of fibrous plaques and in fatty streaks.

#### *Fibrous plaques with complicated lesions*

Fibrous plaques with complicated lesions were characterized by calcification and deposits of acid mucopolysaccharides. Two mild cases of calcification were observed. Lesions generally appeared as fine or coarse granules deposited either intracellularly or extracellularly in the inner  $\frac{1}{3}$  to  $\frac{2}{3}$  of the media but also

sparingly in multiple foci in the intima. Calcium deposits were more pronounced in the intracellular space, ground substance and interlamellar space (Fig. 10). Calcifications were usually found in combination with an increase of acid mucopolysaccharides in the ground substance. Large amounts of a positive Alcian blue material accumulated as a thick band were occasionally observed along the fibrous cap in advanced plaques.

Vascularization was a common change in fibrous plaques and seemed to be proportional to the thickness of the fibrous plaques. A varying number of capillaries were seen in the inner third of the tunica media (Fig. 11). In a well developed case, branches of vessels were seen close to internal elastic lamina. It was apparent that these capillaries arose from the vasa vasorum of the tunica adventitia.

#### DISCUSSION

The fatty streaks, fibrous plaques and complicated lesions found in the aortas of farmed fallow deer were in most respects similar to those in man (*Holman et al.* 1958, *WHO-Study Group on Atherosclerosis* 1958, *Wissler* 1976) and other animals (*Skold & Getty* 1961, *Pongshompoo & Rehbinder* 1983, 1985).

In fallow deer the surface involved by aortic lesions increased with age. An increase in incidence of fibrous plaques in aging animals indicated that fatty streaks, also in this species, may change into fibrous plaques similar to findings in previous investigations in roe deer and moose (*Pongshompoo & Rehbinder* 1983, 1985).

In this investigation, it was not possible to perform a comparative study between sexes as females were kept for reproductive purposes. The number of older animals obtained was limited as most males were shot when they reached slaughter weight.

The incidence of affected animals 86.4 % (19/22) has to be considered quite high but the aortic lesions found were generally mild, i.e. fatty streaks and calcification. In roe deer and moose (*Pongshompoo & Rehbinder* 1983, 1985), starvation in combination with cold exposure during time, were considered factors involved in fat depositions in the aortic wall. It has been demonstrated that lipo-protein-lipase activity in plasma (*Sandhoefer et al.* 1962) and in different organs (*Hollenberg* 1959, 1960, *Robinson* 1960) changes with the state of nutrition. More-



over, in fasting rats (*Borensztajn et al.* 1970) and in rats exposed to cold (*Mallov* 1963) higher lipase activities and free fatty acid levels in plasma were found than in control animals. The effects of chronic hyperlipidemia are complex. The condition can result not only in the deposition of lipids in the atheromatous lesion but it may also produce the primary endothelial injury initiating the atherosclerosis process (*Ross & Harker* 1976). The investigated fallow deer were during winter exposed to cold weather, but they did not suffer from food shortage. It is also doubtful if the animals do suffer from cold weather as they are protected by a well isolating fur. Thus food shortage and cold weather have to be considered factors of doubtful or no importance in the development of aortic lesions in the investigated fallow deer.

Lesions were found predominantly in the posterior abdominal region. It was obvious that the lesions gradually extended cranially from the orifices of the celiac and cranial mesenteric arteries with age. In addition, the percentage of involved surface, in different affected regions, comprising all age groups, was largest in the abdominal aorta. Similar findings were previously reported from roe deer and moose (*Poungshompoo & Reh binder* 1983, 1985). A predominance for spontaneous atherosclerosis lesions in the abdominal aorta has also been reported in man (*Dow* 1952, *Robert et al.* 1959) and in swine (*Getty* 1965).

According to *Fry* (1968), an increase in blood velocity, a surface shearing stress, by an accelerated blood flow as short as one hour, resulted in a marked cytoplasmic swelling, cell disintegration and finally dissolution and erosion of the endothelial surface. *Moore & Thnatowycz* (1978), showed that a repeated endothelial injury caused lesions rich in lipid in animals on a normal diet, except for severely thrombocytopenic animals. They concluded that repeated lacerations of the neo-intima favoured lipid deposition.

Several investigators have pointed out that fatty changes in thrombi are identical to atherosclerotic lesions (*Duguid* 1946, *Crawford & Levene* 1952, *Still* 1966). *Poole et al.* (1958) showed that when a rapid blood flow was maintained, only very few and small deposits were found on the areas experimentally freed from endothelium and there was no progressive thrombosis. The arterial blood flow and blood pressure in the thoracic aorta is relatively greater than that of the abdominal aorta as blood pres-

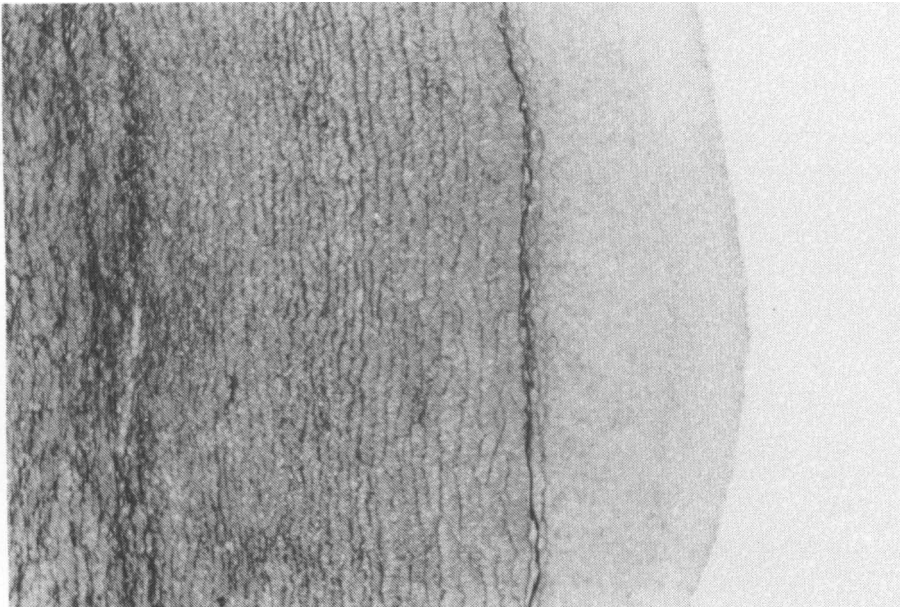
sure and rapidity of flow decreases and becomes more constant correlated to the distance from the heart (*Junqueira et al. 1977*). Although, the thoracic aorta has a greater permeability to lipid than the abdominal aorta (*Bell et al. 1977, Day et al. 1974*), it can not maintain mural thrombi and thus thickening of the aortic intima and progressive fatty changes due to rapid blood flow does not occur with the same frequency as in the abdominal aorta.

Fibrous plaques were also frequently situated at the bifurcation where the blood stream from the abdominal aorta changes its direction flowing through the external and internal iliac arteries (Fig. 3). *Sako (1962)* studied the effect of an increased blood flow upon atherogenesis in cholesterol fed dogs with an iliac-venous fistula. The atheromas produced were confined to the iliac artery proximal to the fistula. The lesions found in fallow deer appeared similar in localization and morphology. *Texton et al. (1965)* showed that atherosclerotic lesions whether occurring naturally or induced by altering the hemodynamics, were consistently found or produced at sites of diminished lateral pressure. He demonstrated that the lateral pressure was reduced at narrow portions where the velocity was increased. Predilection areas for atherosclerosis were thus; curvature, bifurcation, branching and tapering. Consequently, hemodynamic changes in connection with diminished lateral pressure in areas proximal to the orifices and bifurcations have to be considered important factors in the development of atherosclerotic lesions in fallow deer.

In fallow deer, lesions around the orifices showed not only an increase in intimal thickening but also an increase in the number of endothelial cells with big dense nuclei and occasionally disoriented nuclei. *Flaherty et al. (1972)* found in uniform vessel segments i.e. middle and lower descending aorta of dogs, the endothelial nuclei to be oriented parallel to the axis of the blood vessel. The ratio parallel to non parallel nuclei was, however, large in the orifices of many major arteries. The highest density of endothelial nuclei occurred in the orifice of the celiac artery. The same authors showed that altered flow patterns did change the pattern of nuclear orientation within 10 days. It seems clear that hemodynamic factors will influence upon the nuclear patterns, the number and the removal and regrowth of endothelial cells also in fallow deer.



**Figure 5.** Posterior abdominal aorta showing focal to patchy lipid accumulation in the inner half of the tunica intima near the internal elastic lamina. Note clusters of smooth muscle cells in the disrupted internal elastic lamina and disoriented smooth muscle cells in the inner half of the tunica media. Female fallow deer, 5 1/2-year-old (Sudan IV x 112).



**Figure 6.** Anterior abdominal aorta showing a fibrous plaque. Under the plaque is present undulated, duplicated internal elastic lamina. Degeneration of the medial elastic lamellas in the inner third of the tunica media is also observed. Male fallow deer, 1 year and 3 months old (van Gieson x 44.8).

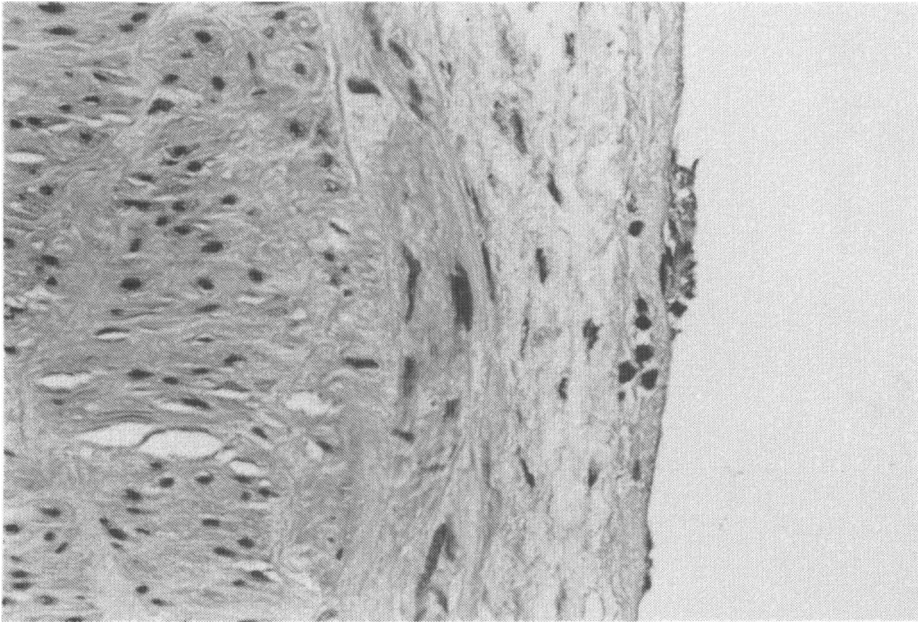


Figure 7. Section through fibrous plaque, at the bifurcation, illustrating mononuclear cell infiltration in the upper part of the tunica intima underneath or oblique to a fibrin mass attached to the luminal surface of the aorta. Note also disorientation of medial smooth muscle cells. male fallow deer, 2 years and 9 months old (H & E x 448).

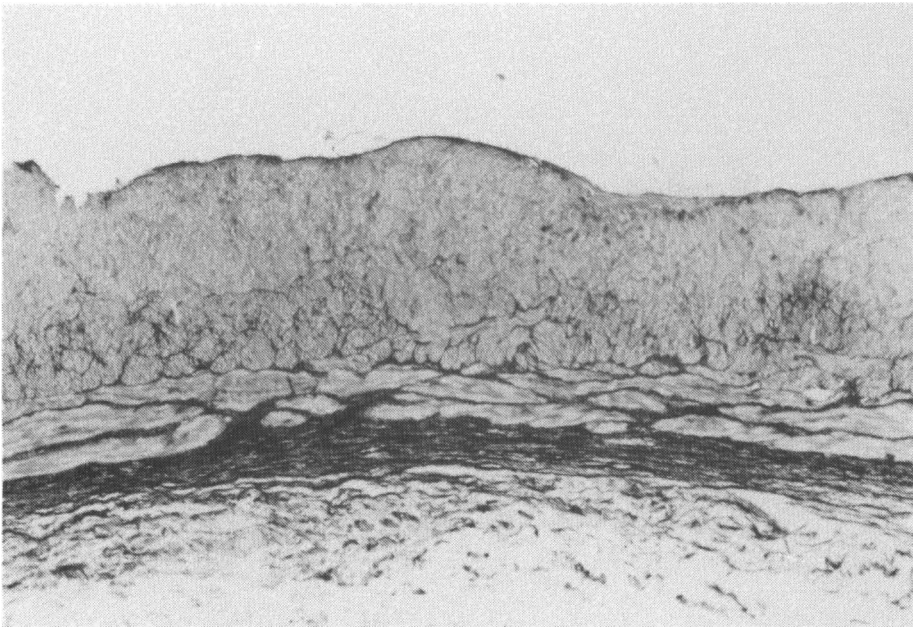


Figure 8. External iliac artery showing partial thickening and wavy surface of the tunica intima. Degeneration of the medial elastic tissue of the inner third of the tunica media is marked. Male fallow deer, 1 year and 10 months old (Verhoeff x 44.8).

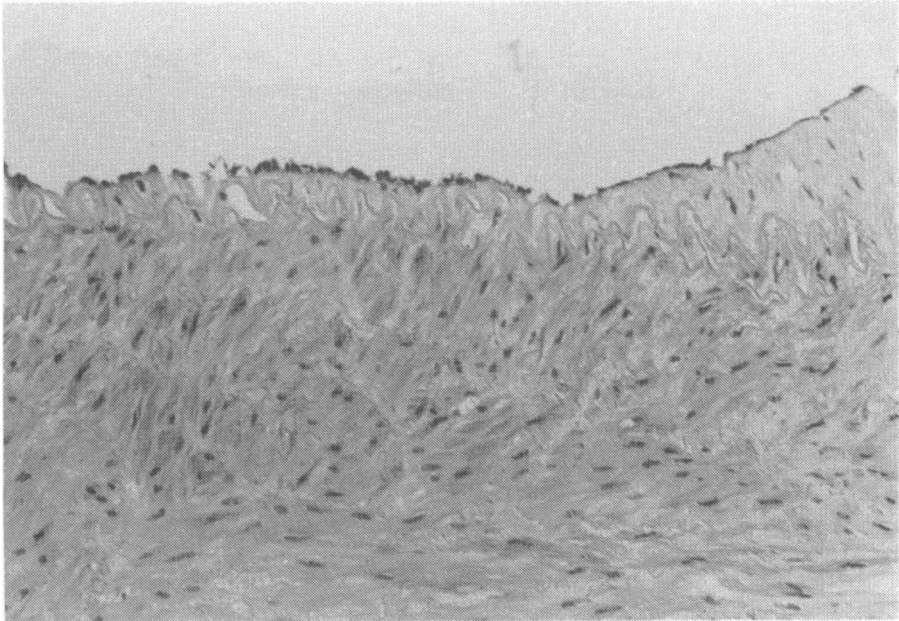


Figure 9. Ostium of cranial mesenteric artery of anterior abdominal aorta showing an intimal thickening (to the right) and an increase of a population density of endothelial cells with active big dense and round nuclei (to the left). Note, disorientation of medial smooth muscle cells. Male fallow deer, 1 year and 11 months old (H & E x 224).

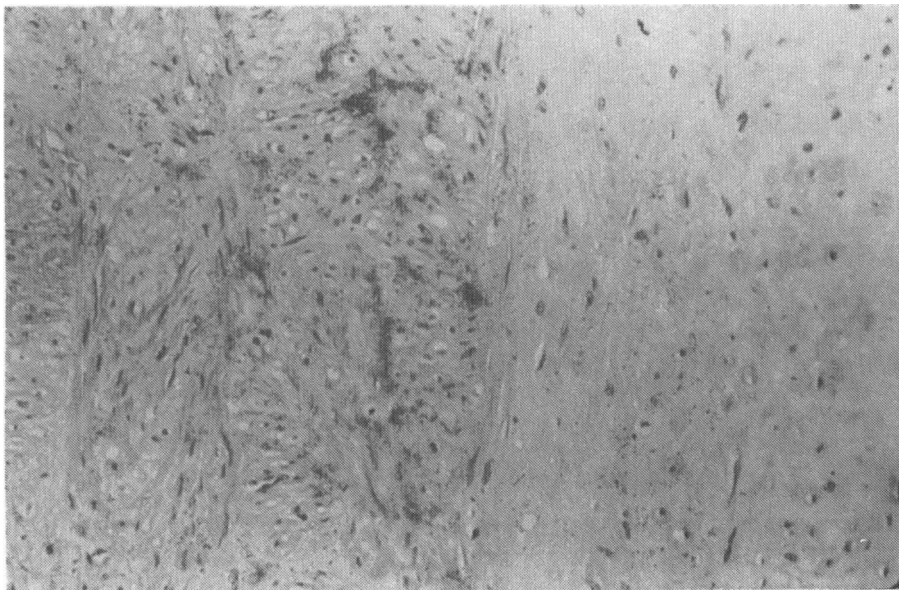


Figure 10. Multifocal calcium deposits intracellularly and extracellularly in the inner third of the tunica intima (to the right) and the tunica media (to the left) in the posterior abdominal aorta. male fallow deer, 4 1/2 years old (von Kossa x 224).

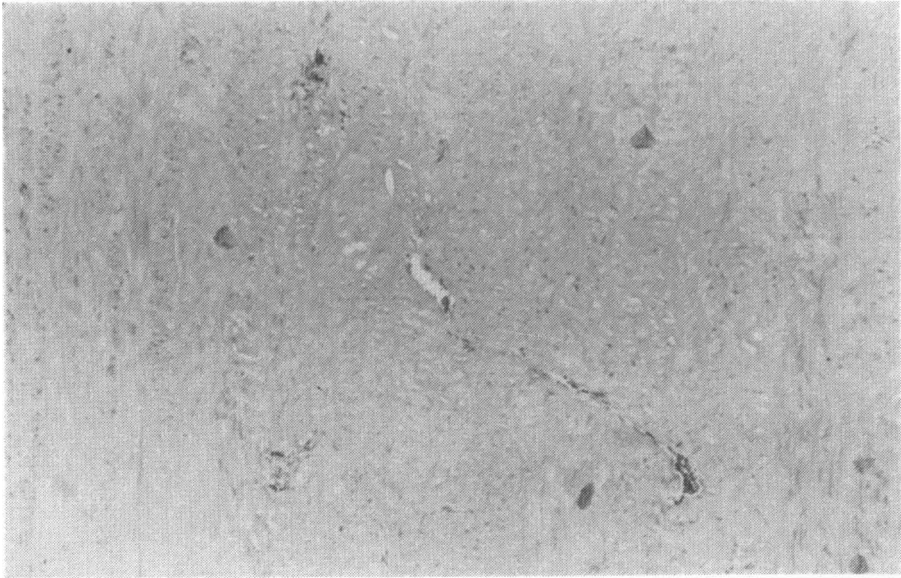


Figure 11. Posterior abdominal aorta showing vascularization in inner third of the tunica media underneath a thick fibrous plaque. Male fallow deer, 3½ years old (H & E x 112).

*Paterson et al.* (1957) stated that vascularization of the intima was an extremely early feature in the course of atherosclerosis. On the other hand, *Crawford & Levene* (1952), investigating spontaneous mural thrombi in human aortas, found a fountain of capillaries penetrating from the media into the unorganized pale staining remnants of a thrombus. In an electron microscopic study on the organization of experimental thromboemboli in the rabbits *Still* (1966) exhibited capillaries within the thrombus and in the area of attachment to the intima. Some of the capillaries were found externally to the internal elastic lamina and were therefore probably arising from the vessel wall. Permeability also for macromolecules into an induced thrombo-sclerotic plaque could be found in its very early stage, but not in later stages. Thus dye and lipid was seen to enter the plaque from small vessels newly derived from the adventitia (*Friedman & Byers* 1961, 1962). It is not certain whether an induced thrombosclerotic lesion is similar to a naturally occurring atherosclerotic plaque but at least both of them can probably get their blood supply direct from the aortic lumen and from new vessels arising from vasa vasorum (*Friedman & Byers* 1963, *Jaeger* 1964). The vascularization found in fallow deer is similar to the finding in roe deer and moose (*Poungshompoo & Reh binder* 1983, 1985) and even in man as it was found to arise from vasa vasorum. In cervidae high pressure capillaries were not found in intima as was seen in man.

### CONCLUSIONS

Age and hemodynamic stress are probably involved in the development of aortic lesions in fallow deer. Endothelial injury caused by hemodynamic stress may initiate the process of atherosclerosis. Fatty streaks can apparently gradually change into fibrous plaques. The plaques may receive blood supply from new blood capillaries which were found to arise from vasa vasorum of the tunica adventitia. Hemodynamic flow seems to influence upon the localization of the aortic lesions, the number of endothelial cells and the endothelial nuclear patterns.

In this investigation, the surfaces involved by the aortic lesions were found to increase with age. The aortic lesions apparently started in the abdominal aorta around the cranial mesenteric and celiac arteries and the bifurcation as seen in the

group of younger animals. The number of endothelial cells was higher around the arterial orifices.

#### ACKNOWLEDGEMENTS

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#### SAMMANFATTNING

##### *Spontana aortaskador hos dovhjort (Dama dama L).*

Hos 19 av 22 dovhjortar, 15 månader—5½ år gamla, påvisades skador i aorta. Tre olika typer av skador förelåg; stråk av fett i 2, fibrösa knutor i 15 och fibrösa knutor med komplicerande skador (förkalkning och sura mucopolysaccharider) i 2 aortor. Degeneration av elastisk vävnad, i de inre två tredjedelarna av tunica media, fanns frekvent i aorta från djur äldre än 3½ år. Det förelåg inget statistiskt säkerställt förhållande mellan ålder och frekvens ( $P \infty 0,10$ ) men däremot en tendens till åldersbetingelse. Procenten förändrad yta ökade signifikant ( $0,05 > P > 0,01$ ) med stigande ålder. Förändringarna befanns börja i abdominala aorta hos unga djur och med stigande ålder utbredde de sig till den thorakala aortan.

Frekvensen inbegripen yta i olika skadade delar, omfattande alla åldersgrupper var; bakre abdominala aortan 10,5 %, främre abdominala aortan 4,3 % och bakre thorakala delen av aorta 1,4 %. Flöde av hemodynamiskt påverkan vad avser lokalisationen av skadorna diskuteras liksom även tätheten av den endotheliala cellproduktionen och kärnornas positioner hos endothelcellerna.

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