AORTIC LESIONS IN GOATS INFECTED WITH *MYCOBACTERIUM JOHNEI*

By

S. Majeed and J. Goudswaard

Departments of Pathology and Bacteriology, Veterinary Faculty, Utrecht, The Netherlands

INTRODUCTION

Aortic lesions in small ruminants have only been described occasionally. Butler (1966) and Majeed and van der Kamp (1970) reported alterations causing aortic rupture in ewes, while Nakamatsu, Fujimoto and Satoh (1968) found lesions in the aortic wall in goats suffering from Johne's disease. More attention has been paid to this pathological condition in cattle which may be associated with diseases not primarily located in the circulatory system. Cows suffering from disturbed mineral metabolism (Arnold, 1954), vitamin D_3 intoxication (Greig, 1963) and Johne's disease (Alibasoglu, Dunne and Guss, 1962), showed aortic lesions, while a direct relation between the aorta and the parasite *Onchocerca armillata* has been found in cattle as well as in sheep (Ford, 1907).

In the present paper aortic lesions in goats affected with Johne's disease are studied and compared with those found in cattle.

MATERIALS AND METHODS

Aortas were available from 18 naturally and experimentally infected goats, killed in an advanced stage of Johne's disease as well as aortas from 4 non-infected goats. Tissue blocks from these aortas were fixed in 10 per cent. buffered formalin (pH 6·5 to 6·9). Sections from paraplast embedded material as well as frozen tissue were stained with haematoxylin and eosin, Weigert's resorcin-fuchsin, Azan, the silver impregnation method for reticulin fibres, periodic acid Schiff, Alcian blue, Von Kossa, Perl's and Fett Rot (Pearse, 1968).

An attempt was made to find polarising and autofluorescing material. Tissue blocks were taken at several levels and at regular distances from the thoracic and the abdominal areas from the non-infected animals (1 to 5 years of age): in addition, sections were taken from the digestive tracts, the organs and most of the lymph nodes from the infected animals.

RESULTS

Apart from insignificant and superficial irregularities of the intimal surface, no macroscopic lesions were found in the aortas of 13 of the goats with Johne's disease, but the remaining 5 showed rough, more or less square patches related to the efferent orifices of the aorta (Fig. 1). These incrustations, due to calcium deposits, were present mostly in the thoracic part. The projections were accentuated by contraction of the aorta after longitudinal cutting. No abnormal changes in the colour of the intima that might indicate fatty deposits could be detected, nor could changes be seen in the endocardium of any animal.

All transverse sections showed a spotted appearance due to irregular distribution of elastic fibres and smooth muscle cells (Fig. 2). The changes were predominantly in the media, which could be divided into an "inner" and an "outer"

zone (Fig. 2). The outer zone or adventitial part of all the aortas was characterised by the presence of smooth muscle bundles arranged concentrically or longitudinally alternating with regions of concentrically arranged elastic fibres. Between the smooth muscle cells there were a few very thin or fragmented elastic fibres. The inner zone was composed of concentrically arranged elastic fibres which were not interrupted by smooth muscle cell bundles, but a small number of single layered smooth muscle cells were found in this area. Most elastic fibres ran straighter than those of the outer zone. The latter had a wavy appearance, indicating that this part of the media was more contracted than the one located in the centre. The situation was different in the 5 aortas showing macroscopic lesions. In the smooth muscle cell regions of the outer zone vacuolation was present while nuclei were absent, suggesting degenerating processes (Fig. 3).

In general, there was more collagen between elastic fibres than between muscle cells. However, more PAS and Alcian blue material was found in the latter. Many elastic fibres contained brown pigmented granules and rods (Fig. 4) which were negative to PAS and Perl's stain; they did not polarise or show autofluorescence. In the elastic regions a few small foci were present consisting of material positive to Alcian blue.

In the inner zone there were well-delineated sheets composed of obviously stretched elastic fibres (Fig. 5). They were embedded in more Alcian blue positive material than in other parts of the media. The elastic fibres in these sheets showed fragmentation or were encrusted with calcium and occasionally also with iron. These degenerated medial sheets sometimes contained minor foci of proliferating fibroblasts and minor accumulations of hyalinized collagenous tissue. In these sheets, however, other more prominent focal lesions occurred consisting of focal necrosis surrounded by a collagenous capsule. The capsule was composed of hyalinized collagen and was covered at the inner side by a layer of material positive to Alcian blue. It was vascularized and contained fragmented elastic fibres and sometimes a foamy and crystallized lipid material which was polarizing.

Scattered over the media were small haemorrhages interspersed between elastic fibres and smooth muscle cells. There were also capillaries and arterioles throughout the media, especially in the outer zone and in the vicinity of the capsulated necrotic foci. The endothelial cells of some vasa vasorum showed hyperplasia. Small groups of mononuclear cells and lymphocytes were occasionally seen. There were very thin subintimal fibrotic plaques (Fig. 6) situated at random along the aorta or covering the sheetlike or encapsulated lesions.

In the aortas which did not show macroscopic lesions there was a varying degree of pigmentation of the elastic fibres. Vacuolation in smooth muscle cell bundles was noticed in most of these aortas and in 7 of them there were subintimal fibrotic plaques in the thoracic part of the aorta.

DISCUSSION

Changes seen in the aortas of goats with Johne's disease were mostly in the media and were characterized by degeneration and necrosis. It seems preferable, therefore, to call this lesion "focal medial degeneration and necrosis" rather than "arteriosclerosis", the term used by Alibasoglu *et al.* (1962). In cattle these authors described fibrosis, hyalinisation of collagen and calcification of elastic fibres in

the subendocardial regions together with similar lesions in restricted areas of the aortic wall. Furthermore there was an increase in smooth muscle cells and in elastic fibres. As stated by Simpson (1966) in an electron microscopic study of bovine material the most prominent features were fragmented and calcified elastic fibres rather than degenerated smooth muscle cells, as described in goats by Nakamatsu *et al.* (1968).

Degeneration of smooth muscle cells was, however, less pronounced although it occurred with regularity in both the inner and outer zone of the media. Hyperplasia of smooth muscle cells was not present. The formation of irregularily distributed smooth muscle cell bundles in the media was also part of the normal structure of the thoracic aorta in both young and old control goats. This irregularity was less obvious towards the diaphragm and disappeared in the abdominal part of the aorta to be replaced by a completely circular muscular coat. Uncomplicated fibrotic subintimal plaques were restricted to the 5-year-old controls in which structures were generally found in the abdominal part. The plaques in the thoracic aorta of the infected animals seemed to form part of a capsule surrounding a necrotic focus; in some cases they covered the sheets in the media but in others they were distributed at random. It was not possible to ascertain whether there was an increase in subintimal plaques in infected animals. The age of the infected goats showing these plaques did not exceed 3 years, while some were only one year old.

It was noted that degeneration of the aorta was chiefly restricted to the parts of the media where smooth muscle cell bundles did not occur and that the part played by smooth muscle cells in this degenerating process was very limited. On the other hand in sheep with aortic rupture Butler (1966) and Majeed and van der Kamp (1970) have described a lesion in which degeneration of smooth muscle cell bundles of the adventitial part of the media was prominent.

There were major changes in elastic fibres in which there were calcium and iron deposits. The presence of calcium suggested a situation similar to "Jamaica Wasting Disease" in cattle (Arnold, 1954) or to vitamin D_3 intoxication reported in cows (Greig, 1963). In these conditions hypercalcaemia was present. Johne's disease in cows, however, is not accompanied by hypercalcaemia (Patterson, Allen, Bennett, Ivins and Sweasey, 1968), nor is this believed to occur in infected goats. The observation that in goats suffering from Johne's disease equally severe lesions occur in emaciated and non-emaciated animals suggests that there is no significant association with wasting..

The incidence (67·7 per cent.) of aortic lesions in cows suffering from Johne's disease is very high (Alibasoglu *et al.*, 1962). A high proportion of the goats with Johne's disease also showed these aortic lesions. This suggests a close link between Johne's disease and focal medial degeneration and necrosis, the mechanism of which, however, is not understood. In respect of pathogenesis, it may be postulated that the lesions are purely local and are due either to disturbances in perfusion of the inner media or to circulatory disorders in the aortic wall, revealed by hyperplastic endothelial changes in the vasa vasorum. Identical lesions, associated with hypertension, were seen under experimental conditions in rats (Salgado, 1970) and also in man under natural conditions (Lothar and Röhrborn, 1970). It has not been proved, however, that hypertension occurs in goats with Johne's disease.

Remote processes might be primarily responsible and such agents as mediators released from the inflammatory reaction, might stimulate directly or indirectly the development of the lesions, as demonstrated by Merkal, Kopecky, Larsen and Ness (1970) in cows suffering from Johne's disease.

When the aortic lesions were compared with the tissue damage in the mesenteric lymph nodes and with the ulceration of the intestinal wall, which occurred infrequently in the animals studied, no similarity was found between the types of tissue reaction. In the aorta, destruction was restricted to elastic and muscular elements, while in the mesenteric lymph nodes epithelioid cell accumulations became necrotic. In the intestinal wall both epithelium and epithelioid cell formations were undergoing disintegration. The presence of iron incrustations and of calcium, might indicate a form of sensitisation as suggested by Seyle (1962) who showed that calcification occurs after tissue sensitisation by a systemic calcifying factor (e.g. parathyroid hormone or vitamin D) and by local application of a challenger (proteins, metallic salts). Blocking of the reticulo-endothelial system might arise and could be a cause of aortic lesions, as was proved in rats by Patek, Bernick and Frankel (1961). On the other hand there may be an association between aortic lesions and remote inflammatory processes, since a link of this nature was shown in rats after evoking cotton granuloma in the subcutaneous tissue under experimental conditions (Junge-Hülsing, 1967).

SUMMARY

Aortic lesions occurring in goats infected both experimentally and spontaneously with *Mycobacterium johnei* were found in the inner zone of the media and were composed of necrotic foci situated in sheets of degenerated elastic fibres. These sheets showed fragmentation, pigmentation and incrustation with calcium and iron. It is postulated that there is a relationship between these lesions and the tissue damage produced elsewhere in the body.

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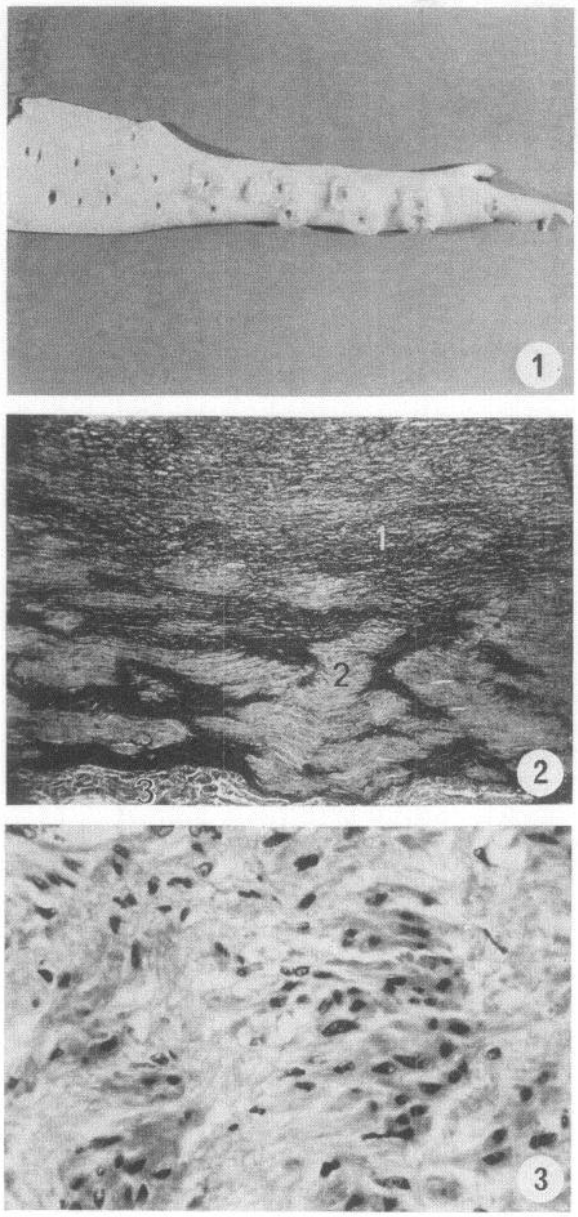

Fig. 1. Calcium incrustated areas of the inner media projecting into the lumen and related to the efferent orifices of smaller arteries of the thoracic aorta.

Fig. 2. Elastic inner zone (1); muscular outer zone (2); adventitia (3) of a transverse section of thoracic aorta. Weigerts Resorcin-fuchsin elastic stain. × 45.

Fig. 3. Focus of degenerated media (elastic fibres and smooth muscle cells); absence of nuclei. H. & E. × 230

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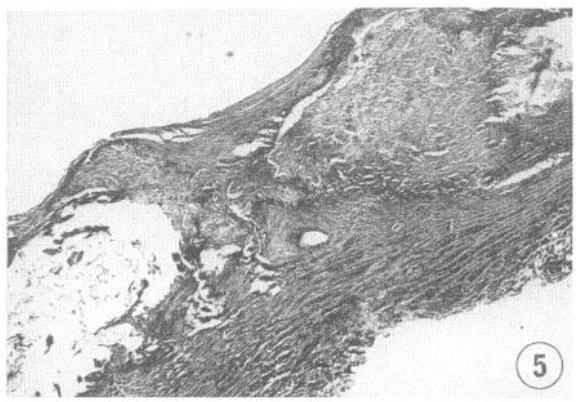

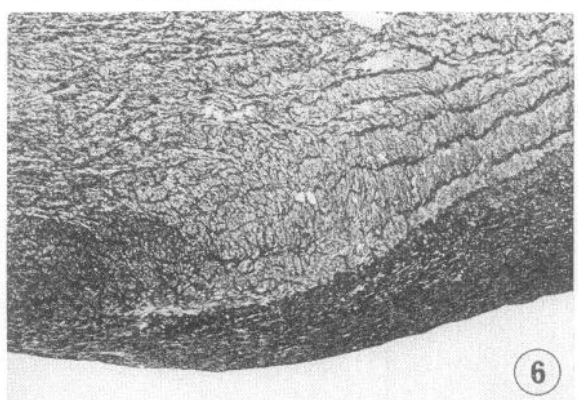

Fig. 4. Pigmentation granules in elastic fibres of aorta. H. & E. × 360.
Fig. 5. Necrotic foci in the inner zone of the media. H. & E. × 90.
Fig. 6. Fibrotic subintimal plaque. Weigerts Resorcin-fuchsin elastic stain. × 36.