ATHEROSCLEROSIS IN RUMINANTS

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ATHEROSCLEROSIS is a principal cause of death and morbidity in humans in the United States. Because of the ruminant's unique digestive and metabolic characteristics, it may be a very useful research animal for study of this disease. There have been, however, only limited reports of atherosclerosis in ruminants. Likar, Likar and Robinson (1966) found spontaneous gross lesions in 53.5% of the aortas from 200 Massachusetts cows. Skold, Jacobson and Getty (1967) reported spontaneous atherosclerotic plaques in the abdominal aortas of Iowa cattle. Stehbens (1965) observed spontaneous intimal proliferation and lipid deposition in the cerebral arteries of Australian sheep and steers. Roser and Magarey (1964) found lipid staining areas in 75% of the aortas or coronary arteries of lambs; the incidence rose to 97% in aged sheep. Jennings, Jennings and Burton (1969) found cardiovascular lesions in all of 19 male Red deer taken from an island near Scotland. Stout and Bohenquez (1969) reported atherosclerosis in Cervidae kept in zoos. Atherosclerosis in the African buffalo was reported by McKinney (1968).

The purpose of our present study was to determine the incidence of spontaneous atherosclerosis in selected domestic and wild ruminants under various environmental conditions, to appraise the atherosclerotic lesions histologically and to determine plasma and liver cholesterol levels in some of the animals.

Materials and Methods

Aortas from six 6-month-old calves, 11 aged ewes, 10 goats (five males and five females), 35 wild native white-tailed deer (24 males and 11 females) and nine wild Alaskan caribou (six males and three females) were examined grossly and histologically. The goats, deer and caribou ranged in age from young to old. Four of the calves (two males and two females) received whole milk, by nipple, for 180 days; the other two calves (males) were fed a concentrate mixture and a limited amount of milk for 31 days and the concentrate mixture from 31 to 180 days. In the young ruminant ingestion of milk by sucking causes closure of the esophageal groove thereby bypassing the rumen and directing liquid to the abomasum (Wester, 1930; Wise and Anderson, 1939; Hegland et al., 1957; Ørskov and Benzie, 1968). This reflex can be maintained until the animal is a year or more of age (Wester, 1930; Ørskov and Benzie, 1968). All calf diets were supplemented with vitamins and minerals. The complete dietary history of the other animals was not known.

All aortas were fixed in 10% neutral buffered formalin. Gross detection of lipid was determined by staining with Sudan IV (Holman et al., 1958). Blocks of tissue were taken from fatty streaks, lesions and grossly "normal" areas for preparation of slides for light microscopy.

A Technicon Auto-Analyzer was used to determine total cholesterol in plasma and liver extracts. The latter were prepared by homogenization of about 750 mg lyophilized liver for 45 seconds in isopropanol with a teflon tissue grinder. The homogenate and rinse were poured into 125 ml Erlenmeyer flasks and sealed with Parafilm and rubber stoppers. The samples were then put on a wrist-action shaker for at least 24 hours. After removal from the shaker, samples were transferred to 250-ml centrifuge bottles. The flasks were rinsed with isopropanol, and the rinsings were also added to the centrifuge bottles. The samples then were centrifuged. The supernatant from each sample was decanted into a 200-ml beaker and reduced to a volume of about 5 ml in a high velocity ex-
transferred to a 10-ml volumetric flask; the
haust hood. Subsequently, the extract was
beaker was rinsed with isopropanol, and the
rinsings were added to the flask. Isopropanol
was added to increase total volume to 10 milli-
liters. Five-tenths milliliters of this extract
was then extracted in the same manner as
suggested by Technicon (1965).

Results and Discussion

Sudanophilic streaking was noted grossly in
the aortas of three of four milk calves, but
not in the calves fed limited-milk and grain.
All ewes, five goats, six deer and three caribou
had gross sudanophilic lesions. The aortas
from all animals had some degree of intimal
thickening. This observation of intimal thick-
ening in the very young and in such animals
as the nomadic caribou that have liberal ex-
ercise would lend support to the hypothesis
that intimal thickening is a normal physio-
logical process that varies in different blood
vessels as well as with age and species (Le-
vene, 1956a; Stehbens, 1965; Getty, 1966; Ta-
ther and Berg, 1969). Prior and Jones (1952)
found a positive correlation between intimal
thickenings in human infants and atheroscler-
osis in adults. Fangman and Hellwig (1947),
Prior and Jones (1952), Moon (1957), Le-
vene (1956b) and Stehbens (1964) suggest
that intimal thickening may be an integral
precursor in the pathogenesis of atherosclero-

Calcium deposition as evidenced by Von
Kossa calcium stain was observed in aortas of
one milk-fed calf, eight ewes, one goat, three
of six deer examined and two caribou. The
nature of the calcium deposits in the calf aorta
is shown in figure 1; some ewe aortas showed
similar deposits. Most of the calcium deposits
in the aortas of the ewes, however, were as
shown in figure 2; i.e., large deposits of cal-
cium in the tunica media. In one of the deer
(a 200-pound 5½-year-old female), calcium
was deposited at the junction of the intima
and media as well as at the junction of the
adventitia and media (figure 3). In the other
two deer, calcium was deposited only at the
junction of the intima and media. Jennings,
Jennings and Burton (1969) observed “mas-
sive calcium deposits” in the media of three
stags. Roser and Magarey (1964) and Kni-
eriem, Kao and Wissler (1968) reported in-
timal calcification in ovine and bovine, respec-
tively. In our present study, histological
observations of sections stained with Oil-Red-

Figure 1. Abdominal aorta from milk-fed Hol-
stein calf. Note stippled calcium deposition ad-
Jacent to the internal elastic membrane (arrows)
between the tunica intima (I) and tunica media
(M). Von Kossa calcium stain. Line scale=50μ.

Figure 2. Abdominal aorta from an aged ewe.
Part of a large calcium deposit (arrows) in the
tunica media (M) has been dislodged during
sectioning. Much lipid is present in the tunica
intima (I) as evidenced by the red stain (black
in print). Mayer's Hematoxylin and Oil-Red-O.
Line scale=100μ.
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Figure 3. Abdominal aorta from 5½-year-old female deer. Note calcium deposition (arrows) at junction of tunica intima (I) and tunica media (M) and also at junction of tunica media and tunica adventitia (A). Von Kossa calcium stain. Line scale = 100μ.

In many instances, medial degeneration was noted subjacent to the plaque area (figure 4). Constantinides, Booth and Carlson (1960) made similar observations in the rabbit and suggested that medial degeneration may be initiated by medial hypoxia resulting from a local blockage of the lumen-to-media oxygen flow by the atheromatous mass. Kniereim, Kao and Wissler (1968), using an immunohistochemical method, demonstrated smooth muscle cells in the intima of the bovine aorta. Frequently, in our present study, there was a radial orientation of the smooth muscle nuclei at the point of disruption of the internal elastic membrane, as shown in the aorta of a milk-fed calf (figure 5). A migration of the smooth muscle cells into the tunica intima is evident. These smooth muscle nuclei were found in the tunica intima of all species examined. It is of interest to note that Antonius and Hill (1968) stated that proliferation of smooth muscle cells may be the initial anatomic lesion of atherosclerosis with lipid accumulation as a secondary and perhaps degenerative feature. Note the cellular infiltration (figure 6) of the intima in a caribou aorta which suggests degenerative changes preceding plaque formation. Lipid deposition and fibroelastic intimal thickenings were discernable with special stains.

Plasma and liver cholesterol levels were determined for some of the animals. At approximately 21 weeks of age the average (and range) of plasma cholesterol levels for the milk-fed calves were 197 (166 to 251) mg/100 ml and for the grain-fed calves 65 (56 to 74) mg/100 milliliters. The average (and

Figure 4. Abdominal aorta from 1½-year-old male deer. Note the absence of elastic fibers in the tunica media (M) beneath the plaque (P). I, tunica intima; A, tunica adventitia; L, lumen. Verhoeff’s elastic stain. Line scale = 1000μ.

Figure 5. Abdominal aorta from milk-fed Holstein calf. Observe smooth muscle cells migrating through disrupted internal elastic membrane at site of arrows. Mallory’s triple stain. Line scale = 100μ.

Figure 6. Thoracic aorta from caribou. Note cellular infiltration in area of developing plaque (arrows). H&E stain. Line scale = 500μ.
range) of plasma cholesterol levels for the goats were 66 (54 to 86) mg/100 ml and for the ewes were 73 (56 to 134) mg/100 milliliters. Average (and range) of liver cholesterol, on a dry matter basis, were 1.22 (1.09 to 1.49) % for the milk-fed calves and 0.86 (0.68 to 1.04) % for the grain-fed calves. The goat liver cholesterols expressed on a dry matter basis averaged 0.87% and ranged from 0.66 to 0.96%. The higher plasma and liver cholesterol levels in the milk-fed calves, in contrast to levels in the other animals, reflects their higher cholesterol, 11-14 mg/100 ml milk, (Homer and Virtanen, 1966, 1967) and fat intake. This is also related to the increased aortic sudanophilia seen in the milk-fed calves. Dietary fat (beef tallow) markedly increases total lipid. This is also related to the increased aortic sudanophilia seen in the milk-fed calves, grain-fed calves, ewes and goats, respectively, were 197, 65, 73 and 66 mg/100 milliliters. Liver cholesterol levels, on a dry matter basis, for milk-fed calves, grain-fed calves, and goats, respectively, were 1.22, 0.86 and 0.87%. Our observations suggest that whole milk increases plasma cholesterol and aortic sudanophilia in the ruminant. However, it appears that development of atherosclerosis in the older ruminant can occur in the absence of a diet rich in saturated fatty acids and of a high plasma cholesterol.

Summary

Atherosclerotic lesions were observed in the abdominal aortas of calves, sheep, goats, deer and caribou. Many histological similarities were seen between domestic and wild ruminants. Plaque formation, fatty streaks, lipid and calcium deposition, internal elastic membrane duplication and disruption and smooth muscle cells in the intima are quite similar to those observed in man and other animals.

Average plasma cholesterol levels for the milk-fed calves, grain-fed calves, ewes and goats, respectively, were 197, 65, 73 and 66 mg/100 milliliters. Liver cholesterol levels, on a dry matter basis, for milk-fed calves, grain-fed calves, and goats, respectively, were 1.22, 0.86 and 0.87%. Our observations suggest that whole milk increases plasma cholesterol and aortic sudanophilia in the ruminant. However, it appears that development of atherosclerosis in the older ruminant can occur in the absence of a diet rich in saturated fatty acids and of a high plasma cholesterol.

Literature Cited