In a previous study, it was shown that the development of arteriosclerosis in the abdominal aortas of dogs followed localized aortic irradiation with 50-kv. x-rays. The arteriosclerotic lesions observed were similar in all respects to those that occur naturally in dogs, particularly in older animals. It was proposed that irradiation may have caused a selective injury of the internal elastic membrane, and that this degenerative process had been followed by development of intimal fibrosis, plaque formation, and minimal lipid infiltration.

The present report deals with the development of pronounced abdominal aortic arteriosclerosis in 15 dogs after high-voltage irradiation of the distal aortic segment with an electron beam produced by a van de Graaff electron accelerator. It is shown here that arteriosclerosis induced in the canine aorta by irradiation with electrons is similar to that observed after x-irradiation and to the naturally occurring process.

Methods

SURGICAL PROCEDURE

Young adult, mongrel, female dogs, weighing between 7 and 15 Kg., were used in this experiment. Under anesthesia with intravenous sodium pentobarbital (U.S.P., 65 mg./Kg.), the abdominal aorta was exposed through a lower abdominal midline incision. The abdominal aorta was mobilized from the level of the renal arteries to just below the aortic bifurcation by division and ligation of the lumbar and inferior mesenteric arteries. A block of lucite, measuring 6.0 by 6.0 by 2.0 cm., was placed between the mobilized abdominal aorta and the vertebral column. A tubular lucite collimator, measuring 12.0 cm. in length with an inside diameter of 4.3 cm., was placed over the aorta against the lucite block beneath and extended ventrally to approximately 6.0 cm. above the level of the abdominal skin. The caudal edge of the collimator lay at the aortic bifurcation. The lower edge of the collimator was grooved, allowing the aorta to lie in the groove without compression. Skin clips tightened the skin about the collimator and held it in place.

IRRADIATION

The radiation was performed with 1.5-Mev electrons obtained with a 2-Mev van de Graaff apparatus. All radiations were delivered in a one-minute interval. The entire dog was shielded by a sheet of lucite measuring 2.0 cm. in thickness. A central hole in the lucite sheet fitted the top of the collimator, and the radiation beam was directed through the hole in the lucite shield and through the collimator to the aorta beneath. The dosages at the ventral surface of the aorta were as indicated in table 1. The depth dose curve is given in figure 1. It may be seen that the relative dose increased as the thickness increased to about 150 mg. per sq. cm., then decreased to a dose lower than that at the ventral surface of the aorta. The outside diameter of the abdominal aorta ranged from 8.0 to 9.0 mm., and the thickness of the wall from 1.5 to 2.0 mm. During the irradiation, elevation of the aorta by the lucite block beneath compressed the vessel so that its dorsal-ventral diameter was approximately 0.5 cm. Following irradiation, the lucite block and collimator were removed and the abdominal wound was closed with silk sutures. Each dog was given penicillin, 1,000,000 units, postoperatively. Three dogs, which served as controls, were subjected to the same operative procedures and were placed beneath the electron accelerator, but received no irradiation.

PREPARATION OF TISSUES

The abdominal aortas, with the attached iliac arteries, were removed at autopsy, opened longi-
TABLE 1
Histological Grade and Classification of Aortic Arteriosclerosis in the Dog After Electron Irradiation

<table>
<thead>
<tr>
<th>Dog number</th>
<th>Interval</th>
<th>Dose in rads</th>
<th>Grading*</th>
<th>Character* of plaques</th>
</tr>
</thead>
<tbody>
<tr>
<td>178</td>
<td>2 weeks</td>
<td>5000</td>
<td>XXXX</td>
<td>Late</td>
</tr>
<tr>
<td>177</td>
<td>3 weeks</td>
<td>4000</td>
<td>XXXX</td>
<td>Early</td>
</tr>
<tr>
<td>608</td>
<td>4 weeks</td>
<td>5000</td>
<td>XX</td>
<td>Early</td>
</tr>
<tr>
<td>618</td>
<td>3 months</td>
<td>9500</td>
<td>XXXXX</td>
<td>Late</td>
</tr>
<tr>
<td>619</td>
<td>3 months</td>
<td>4750</td>
<td>XXXXX</td>
<td>Late</td>
</tr>
<tr>
<td>171</td>
<td>17 months</td>
<td>4000</td>
<td>XXXX</td>
<td>Late</td>
</tr>
<tr>
<td>174</td>
<td>17 months</td>
<td>3000</td>
<td>XXXX</td>
<td>Late</td>
</tr>
<tr>
<td>180</td>
<td>17 months</td>
<td>3000</td>
<td>XXXX</td>
<td>Late</td>
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<tr>
<td>120</td>
<td>17 months</td>
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<td>Late</td>
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<tr>
<td>181</td>
<td>17 months</td>
<td>2000</td>
<td>XXXX</td>
<td>Late</td>
</tr>
<tr>
<td>182</td>
<td>17 months</td>
<td>2000</td>
<td>XX</td>
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<td>17 months</td>
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<td>173</td>
<td>17 months</td>
<td>1000</td>
<td>XXXX</td>
<td>Late</td>
</tr>
<tr>
<td>175</td>
<td>17 months</td>
<td>1000</td>
<td>XXXX</td>
<td>Late</td>
</tr>
</tbody>
</table>

*Maximum lesions.

Results

GROSS DESCRIPTIONS
In the distal segments of the abdominal aortas of the three dogs examined between two and four weeks after irradiation, small, round, white fibrous intimal plaques were observed. These averaged 0.5 mm. in diameter, and some coalesced. In one dog, 1.0-mm., white, transverse, linear plaques were present. All plaques lay in the most distal 4.0-cm. segment of the abdominal aorta.

In the other 12 dogs which were examined at intervals of 3 and 17 months after irradiation, the distal abdominal aortic segments from the trifurcation proximally showed evidence of pronounced disease. These lower segments appeared sharply demarcated from the segments above, which were entirely normal without evidence of intimal disease. The abnormal aortic segments in these dogs ranged from 2.3 to 4.3 cm. (mean 3.2 cm.) after formaldehyde fixation, and clearly represented the area of irradiation within the collimator, which had an inside diameter of 4.3 cm. These lower segments were slightly contracted and narrowed. The adventitial layers were fibrous and indurated. In most instances, the intima appeared diffusely thickened, faintly granular and roughened, and delicately wrinkled. In the majority of dogs, the irradiated segment was distinctly paler.

In all 12 dogs, multiple white fibrous plaques were observed in the intimas of the irradiated segments, mainly on the posterior walls. These ranged from 1.0 to 17.0 mm. in diameter, although the majority were 1.0 to 2.0 mm. in diameter (fig. 2). Some lay transversely, but the majority were arranged longitudinally. The larger plaques appeared to be coalescing. None had yellow pigmentation suggesting lipid infiltration. There were no intimal plaques above the sharp demarcating line in the abdominal aortas. The thoracic aortas of all dogs were free of intimal lesions.
Depth dose curve of electron irradiation.

FIGURE 1

No plaques or other intimal lesions were found in the aortas of the three control, sham-irradiated dogs.

MICROSCOPIC DESCRIPTIONS

Considerable alteration of the internal elastic lamina had occurred in much of the circumference of the irradiated aortic segment. Although changes of a similar character were found in the nonirradiated segments above, they were minimal and focal. The elastic layer displayed focal beading and fragmentation. Here the layer had lost its refractile quality and appeared swollen and granular. In most of these areas, regeneration of newly formed elastic layers had occurred, seeming to have been derived from the supporting ground substance. Wherever the membrane was disrupted, and particularly where considerable regeneration had occurred, accumulations of mucopolysaccharide surrounded the elastic layers, caused elevation of the endothelium, and thus caused focal intimal thickening or plaque formation. Other abnormalities of the endothelial cells were not demonstrated.

Further diffuse and focal intimal thickening then resulted from proliferation of plump fibroblasts that appeared to be derived from the endothelium. The intercellular substance in the smaller, and presumably early, plaques consisted of acid mucopolysaccharide substance, only small amounts of which could be removed by treatment of the tissue with testicular hyaluronidase. As the plaques enlarged and matured (fig. 3), delicate reticulum, collagen, and elastic fibers appeared first in the mucoid ground substance close to the fibroblasts (fig. 4). Gradually, these fibers became more abundant and coarse (fig. 5), eventually replacing the mucoid ground substance. As the intimal thickening progressed, or as the plaques enlarged and became more collagenous, fewer fibrocytes remained (fig. 6).

In the earlier plaques, the fibroblasts and intercellular fibers assumed a position perpendicular to the endothelial surface. As they became more numerous, their arrangement was haphazard, and in the largest plaques,
Both the cells and fibers were arranged circumferentially (fig. 7).

Some plaques were distinctly layered, with the deeper layers appearing to be condensed and collagenous, and the superficial and lateral portions consisting of proliferating fibroblasts and abundant mucopolysaccharide substance (fig. 8). Wide segments of the internal elastic membrane had disappeared beneath some of the larger plaques (figs. 4 and 9). Except in one dog, intimal thickening and the development of intimal plaques were significantly more pronounced on the dorsal, as compared with the ventral and lateral, aortic walls.

Table 1 shows that the intimal thickening, or plaques classified as "early" (cellular with abundant mucopolysaccharide), was found mainly within three months after irradiation, whereas larger, mature, collagenous plaques, designated as "late," were observed mainly three months or more after irradiation. The finding of "late" plaques two weeks after irradiation and "early" plaques 17 months after irradiation in several instances suggests variable growth sequences in some of these arteriosclerotic lesions. The grading of the severest lesions in each aorta is also shown in table 1. Although grade 3 and 4 lesions were found in the aortas of many dogs, the same aortas also contained smaller intimal lesions of lower grades of severity.

Table 1 shows no apparent correlation between the dose of irradiation administered and the extent of intimal disease, since severe arteriosclerotic lesions were found in aortas that received both the lowest and highest doses of irradiation. No increase in disease with time was demonstrated.
Lipid deposits in the diseased segments of the aortas were minimal, but were seen in most dogs. In both the irradiated and non-irradiated portions of the abdominal aortas, fine lipid droplets were found in the ground substance adjacent to the fragmented, degenerating, internal elastic membrane. In the irradiated segments, similar finely dispersed Sudan IV-staining lipid droplets were also demonstrated in endothelial cells covering intimal plaques, as well as in the intercellular substance of the deepest portions of some plaques. None of this lipid was stainable with Nile blue or by the Schultz reaction for cholesterol, and none showed refractile qualities when observed with polarized light. None of the intimal lesions contained fibrin, as evidenced by staining with phosphotungstic acid hematoxylin.

There were no demonstrable lesions in the media of the irradiated or nonirradiated aortic segments in any of the dogs. Vasa vasorum were not demonstrated. The aortic adventitia of all dogs, however, was fibrotic, infiltrated with chronic inflammatory cells, and contained many vessels filled with organizing thrombi. These adventitial lesions had presumably resulted from surgical mobilization of the aorta.

Tiny intimal plaques consisting only of reduplicated internal elastic membrane and mucoid deposits were found in the upper non-irradiated aortic segment of only one irradiated dog, and in the upper abdominal aortic segment of one of the three sham-irradiated dogs. No other significant intimal lesions were observed in nonirradiated portions of the aortas of any of the 15 irradiated dogs or of the three sham-irradiated dogs.
Discussion

This study of the irradiated abdominal aorta of the dog suggests that the arteriosclerotic process was initiated by degeneration with fragmentation of the internal elastic lamina. This degenerative phenomenon was often followed by deposition of acid mucopolysaccharide substances, and eventually by fibrosis, leading to either diffuse intimal thickening or to the formation of intimal plaques. The mature arteriosclerotic lesions in the irradiated aorta were characterized by the development of collagenous, reticulum, and elastic fibers which replaced the earlier deposits of mucoid ground substance.

These lesions in the electron-irradiated aortas appeared identical, in their pathogenesis and histological structure, with those that occur naturally in the dog, and with those induced by x-irradiation in this species. Sparse lipid deposits in the intima were characteristic of both the experimental and naturally occurring lesions. The arteriosclerotic lesions induced by electron irradiation were generally more severe than those observed in the x-irradiated aortas, and almost all were on the dorsal aortic wall, although the radiation doses of electrons and x-rays delivered to the aortas were approximately the same. Naturally occurring arteriosclerosis in the dog is, as a rule, more pronounced on the dorsal than on the ventral aortic wall. There was no evidence that severity of arteriosclerosis was related to the dose of irradiation or was increased with time following irradiation.

In the present study, structural changes of degeneration and fragmentation of the internal elastic lamina which accompany aging and which lead to the development of arteriosclerosis appear to have been accelerated and accentuated by electron irradiation. It is proposed that the internal structure and configuration of the elastic macromolecular protein comprising the internal elastic lamina were disrupted by irradiation, possibly by the breaking of linkages responsible for its elastic qualities. Irradiation of other macromolecular materials has been shown to cause depolymerizing degradation of large molecules into dialyzable compounds by opening chemical bonds. It might be expected that high-energy electrons would have more pronounced effects in this respect than would low-voltage x-rays.

At least from the morphological standpoint, irradiation has now been shown to have the same effects on the internal elastic lamina as does the aging process. It remains to be determined whether similar chemical and functional alterations in the elastic tissue result from irradiation and from the aging process.

Whether injury of the internal elastic membrane is the result of aging, as in the naturally occurring disease, or is induced or accelerated by irradiation, as in the present experiment, the later sequences of mucoid accumulation, fibrosis, and at least some degree of lipid infiltration are similar in the two processes, and are consistent with the development of arteriosclerosis in the aorta; this development has already been delineated in several species of animals and in the human being.

Summary

The distal segments of the abdominal aortas of 15 dogs were irradiated with electrons in doses ranging from 1,000 to 9,500 rads and were examined histologically at intervals ranging from 2 weeks to 17 months following irradiation. Pronounced aortic arteriosclerosis developed and was sharply localized to the aortic segment irradiated. Except in one dog, radiation-induced arteriosclerosis was more pronounced on the dorsal than on the ventral aortic wall. There was no evidence that severity of aortic arteriosclerosis was related to the dose of irradiation or was increased with time following irradiation. The development of arteriosclerotic lesions of the aorta induced by electron irradiation was similar to that observed after x-irradiation and to that occurring naturally in old dogs. It is suggested that electron irradiation may have caused selective injury of the internal elastic lamina, which was then followed by intimal fibrous proliferation and formation of intimal plaques.
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References
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