VASCULAR LESIONS IN THE DOG FOLLOWING THYROIDECTOMY AND VIOSTEROL FEEDING*

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Introduction

For over twenty years it has been known that overdosage of viosterol produces widespread arterial lesions of a chronic type in the experimental animal, particularly in the thyroidectomized dog.\(^5\)\(^7\) Investigators have described calcification of the media of the aorta as the principal change produced,\(^5\)\(^9\)\(^12\)\(^18\) and many have observed in these same animals a fragmentation or marked distortion of the internal elastic lamella accompanied by the deposition, on or in it, of more or less calcium. This latter change was not so constant nor so widespread as was that in the aorta and usually affected only its larger musculo-elastic branches. Only rarely were proliferative intimal lesions described.\(^5\)\(^9\)\(^4\)

Our interest in these lesions was stimulated by the work of Steiner and Kendall\(^7\) who reported having produced arteriosclerosis in dogs fed thiouracil and cholesterol for seventeen to twenty months, and also by a report of Dr. John Peters\(^4\) of a study of a number of hyperthyroid patients who had received long courses of treatment with thioureia. In some of these individuals widespread acute arterial necroses were found at autopsy. Although allergy to the thioureia and iodine was of course suggested as an explanation for these lesions, it was also suggested that the hypothyroid state resulting from the prolonged treatment with thioureia might have had something to do with their production.

The working hypothesis for the present investigation was that there might be antecedent acute arterial lesions in viosterol-fed dogs, particularly if thyroidectomized, similar to those observed clinically by Dr. Peters, and also that there might be a relationship between these acute lesions and the usually described chronic medial calcification. Further, the changed metabolism as expressed by the altered lipid pattern of the blood might alter their morphogenesis.

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Methods

Twenty mongrel dogs of both sexes were thyroidectomized. Two or more parathyroid glands were preserved with extreme care, and the animals were not given viosterol until at least a week later. If tetany developed, the dog was sacrificed and discarded. Commercial viosterol* in doses averaging 50,000 units per kilogram of body weight was added daily to a diet consisting of about a pound and a half of pig kidney. Kidney was fed as the cheapest form of lean meat available in quantity, and it had no special significance in the experiment. Animals which were thyroidectomized but did not receive viosterol, and normal animals fed the same amounts of viosterol served as controls.

Free and total cholesterol levels were determined by the method of Schoenheimer and Sperry,1⁵ phospholipid phosphorus by a modified Youngburg method,4 serum calcium by the method of Roe and Kahn,9 serum inorganic phosphorus by the method of Lowry and Lopez,1¹ and blood nonprotein nitrogen by micro-Kjeldahl digestion and steam distillation of the ammonia formed. Blood for these determinations was collected under oil (after the animals had been fasted for 24 hours) before, and one week after, thyroidectomy and at frequent intervals after viosterol feeding was instituted.

Results

No gross vascular changes were observed during the first 10 to 14 days of viosterol feeding, but after that arterial lesions were regularly produced by this regimen. The earliest lesions were focal and rather widely scattered, later they were more widespread and tended to become confluent. In fact, the changes were more widespread than was anticipated from the literature, and with time, there developed a definite distributional pattern not described by others, see Table 1.

Aorta. During the first 10 days of viosterol feeding following thyroidectomy, only a marked edema of the inner third of the media is seen histologically. This is a constant finding in the aortas of the viosterol-treated animals. Even months later it is prominent in those portions of the vessel which are apparently normal on gross examination.

After two or more weeks on the viosterol regimen, focal necrosis of the inner half of the media of the aorta becomes increasingly common, particularly at its root and in its thoracic portion. The elastic fibers in the center of the lesions are destroyed or fragmented, and there is a surrounding acute, purulent, inflammatory reaction (Figs. 1 and 2). The wall of the vessel adjacent to such a purulent focus is extremely edematous, causing considerable distortion of the normal elastic fiber pattern. Intimal proliferation (Fig. 3) becomes notable only after the necrotic center of the lesion

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* Kindly furnished by the International Vitamin Division of the Ives-Cameron Co., Inc., Brooklyn, N. Y. Viosterol in oil, 1 gram = 10,000 units vitamin D, U.S.P.
begins to liquefy. Concurrently, new capillaries which invade the outer media are formed by budding of endothelial cells at the medio-adventitial junction (Fig. 4). Lipid stains (Sudan IV, Sudan black, and treatment with osmic acid) demonstrate that stainable material is found scattered about the necrotic mass in the media and in the zone of reaction in the adventitia, distributed in the form of fine extracellular droplets. Weeks or months later salts, predominantly calcium, are found to have been deposited

### Table 1

**DISTRIBUTION OF VASCULAR LESIONS IN VIOSTEROL FED DOGS**

|                      | Coronary arteries | Peripheral arteries |
|----------------------|-------------------|---------------------|
|                       | Acute panarteritis| Intimal proliferation|
|                       | Foam cells        | Carotid             |
|                       | Subclavian        | Iliac and femoral   |
|                       | Mesenteric        | Renal               |
| Number of dogs        | Root of aorta     | Pulmonary artery    |
|                      | Thoracic aorta    | Pulmonary artery    |
|                      | Abdominal aorta   | Pulmonary artery    |
|                      | Pulmonary artery  | Left auricular      |
|                      | Endocardium       | Thrombi and location|
|                      |                   |                     |
| Fed viosterol 4 weeks or less | 8 | 1 |
|                       | 6                  | 0                   |
|                       | 4                  | 3                   |
|                       | 1                  | 4                   |
|                       | 7                  | 5                   |
|                       | 6                  | 0                   |
|                       | 2                  | 0                   |
|                       | 1 l. auricle       | 3                   |
|                       | 4                  | 4                   |
|                       | 0                  | 5                   |
|                       | 3                  | 5                   |
|                       | 4                  | 0                   |
|                       | 5                  | 0                   |
|                       | 0                  | 0                   |
| Fed viosterol 4-10 weeks | 6 | 0 |
|                       | 6                  | 5                   |
|                       | 4                  | 4                   |
|                       | 6                  | 5                   |
|                       | 5                  | 6                   |
|                       | 1 pul. artery      | 1                   |
|                       | 0                  | 5                   |
|                       | 5                  | 4                   |
|                       | 5                  | 6                   |
|                       | 1                  | 1                   |
| Fed viosterol 10 or more weeks | 6 | 0 |
|                       | 6                  | 4                   |
|                       | 5                  | 4                   |
|                       | 5                  | 5                   |
|                       | 4                  | 2                   |
|                       | 1 aorta organized  | 2                   |
|                       | 0                  | 4                   |
|                       | 6                  | 4                   |
|                       | 4                  | 5                   |
|                       | 5                  | 2                   |
|                       | 2                  | 2                   |
| Totals ................| 20                 | 15                  |
|                       | 18                 | 11                  |
|                       | 14                 | 13                  |
|                       | 10                 | 16                  |
|                       | 18                 | 3                   |
|                       | 15                 | 3                   |
|                       | 4                  | 3                   |
| Normal Dogs .......... | 8                  | 3                   |
|                       | 4                  | 3                   |
|                       | 2                  | 5                   |
|                       | 1                  | 0                   |
|                       | 6                  | 0                   |
|                       | 2                  | 0                   |
|                       | 0                  | 0                   |

in such lesions (Fig. 5), and there may be no evidence of an inflammatory reaction or of intimal thickening. Under these conditions, stainable lipid is also lacking. However, a subacute intimal inflammatory process may be superimposed on such an apparently healed medial lesion (Fig. 6). When such is the case, fine extracellular droplets of lipid are easily demonstrated. A small organizing mural thrombus was attached to the newly proliferated intima of one such lesion.

Figures 7 and 8 are photographs of the aortas of two thyroidectomized animals fed viosterol for 23 and 17 days respectively. The similarity in dis-
tribution between these lesions and those of early human arteriosclerosis in the aorta is apparent.

**Pulmonary artery.** In the main pulmonary artery the earliest and most severe lesions are found in that portion which crosses over the aorta. The changes are similar in every respect to those described in the aorta with the exception that hemorrhage associated with the acute inflammatory reaction is a more frequent finding (Figs. 9 and 10). After several weeks of viosterol treatment the whole circumference of the pulmonary artery is involved. The vessel becomes dilated, and the intima roughened by many raised irregular plaques. No lesions are observed in the smaller intrapulmonary branches.

**Left auricle.** After three or more weeks of viosterol feeding, lesions of that portion of the left auricular endocardium extending upward from the base of the anterior leaflet of the mitral valve were found in 15 of the thyroidectomized dogs. As in the aorta, the earliest change is an acute inflammatory reaction in the subendocardial tissue. At first, such lesions are hemorrhagic (Fig. 11), and in three animals organizing thrombi were attached to the roughened endocardium (Figs. 11 and 12). With more prolonged treatment with viosterol, calcium salts are deposited in the subendocardial tissue, so that whitish linear streaks run perpendicular to the attachment of the mitral leaflet and upward toward the right pulmonary vein. Scattered but similar lesions are also found above the posterior mitral leaflet (Fig. 11), but the characteristic site is that described.

**Coronary arteries.** Acute panarteritis, characterized by focal fibrinoid necrosis of the arterial media, local destruction of the elastic fibers of the internal elastic lamella, and marked intimal and adventitial proliferation was observed in one thyroidectomized dog fed viosterol for three weeks (Figs. 13 and 14). However, the changes usually observed after this period of treatment are much less severe. There is edema of the muscular wall of the main coronary arteries and of their larger subepicardial branches with distortion and fragmentation of the internal elastic lamella. Concomitantly, fibrous tissue is laid down in the zone of damage forming an elevated intimal plaque. An advanced lesion of this type is shown in Figure 15. Lipid stains of such a vessel are negative.

With more prolonged (10-20 weeks) viosterol treatment after thyroidectomy, plaques are found regularly in the larger coronary arteries. However, lipid-containing foam cells histologically identical with those seen in early human arteriosclerosis are now present in some of the lesions. Usually the foam cells lie between the intimal lining of the vessel and the internal
elastic lamella (Fig. 16), but if that structure has been fractured, they also infiltrate between the muscle cells of the arterial media beneath the break. (Figs. 17 and 18). The lipid in these cells is contained in large droplets which are osmophilic and sudanophilic and which are birefringent under crossed Nicol prisms. If a frozen section of a plaque containing foam cells is heated gently, and light pressure applied to the coverslip, droplets are released which show characteristic Maltese crosses under polarized light. When there are many foam cells in the intimal plaques of the coronary arteries, very few, if any, are present in the other vascular lesions and the cells of the reticuloendothelial system are normal.

One cannot predict with certainty from blood lipid levels whether foam cells will be present in the plaques in the coronary arteries. For example, Figure 16 shows the coronary artery of a thyroidectomized dog whose total cholesterol level averaged only 230 mgm.% during the 10 weeks it was fed viosterol. Conversely, in another thyroidectomized dog with persistent blood cholesterol levels three times as high, only fibrous plaques were found after 13 weeks of viosterol treatment. The levels of the other lipids were comparable in the two animals. There is, however, a definite correlation between the presence of foam cells and the level of blood lipids in most cases.

Other peripheral arteries. Dilatation and medial calcification, often segmental, like that seen in human disease of the Monckeberg type, are regularly produced in the iliac, femoral, subclavian, and carotid arteries of the thyroidectomized dog fed viosterol (Fig. 7). Beneath a greatly distorted and elongated internal elastic lamella, groups of smooth muscle cells of the arterial media are seen to be calcified without other apparent histological change. Rarely, small fibrous intimal plaques are formed when dilatation of the vessel causes a portion of one of these calcified foci to project into the lumen. Similarly, calcification of the internal elastic lamella was observed histologically in the large intrarenal and mesenteric arteries of three animals. Renal arteriolosclerosis was not produced on this regimen, but focal calcification of the basement membrane of the secretory tubular epithelium, atrophy, and calcification of the epithelial cells were regularly observed histologically. Numerous calcified casts were also present in the collecting tubules.

Metabolic effects. Much experimental work has been done to determine the toxic effects of excessive doses of viosterol upon laboratory animals. In the normal dog there is produced a marked metabolic disturbance characterized by hypercalcemia and hyperphosphatemia, and by elevation of all the blood lipid levels. In addition, the blood nonprotein nitrogen rises, and
there is hemoconcentration. These alterations are marked at the end of only one week of viosterol treatment, and although certain adjustments take place with time, they become even more pronounced after one month, Table 2.

**Table 2**

**Blood Findings in Four Representative Experimental Dogs**

| Dog B108 Normal Control fed viosterol. |  |
|-----------------|--------|
| Control period | 46/116/11.5/11.4/12.0/4.8/28.2/38 |
| Seven days after viosterol | 44/96/14.3/15.0/13.8/4.9/53/39.2 |
| 50,000 units U.S.P. per kg. |  |
| 21 days after viosterol | 134/300/22.5/21.6/21.8/8.1/86/53.2 |
| 60 days after viosterol | 176/420/22.1/17.6/17.8/7.2/74/51 |
| 70 days after viosterol | 200/390/21.0/20/22.6/5.6/54/46 |

| Dog B89 Thyroidectomized. |  |
|-----------------|--------|
| Control period | 67.5/120/12/13.8/11.5/5.0/30 |
| Seven days after thyroidectomy | 83.0/144/18.6/19/11.6/5.6/35 |
| Seven days after viosterol | 167.0/410/27.6/28.8/19.6/6.6/70.7 |
| 17 days after viosterol | 137.0/276/24.5/28.4/27.9/6.6/105 |

| Dog B97 Thyroidectomized. |  |
|-----------------|--------|
| Control period | 48/70/17.6/17.2/13.7/5.3/35.0/48 |
| Seven days after thyroidectomy | 55/110/19.3/18.8/13.8/5.0/44.8/49 |
| Six days after viosterol | 167/548/32.8/34.4/15.9/7.1/46.9/48 |
| 30 days after viosterol | 411/688/36.7/38.8/20.2/5.9/46.2/48 |
| 60 days after viosterol | 250/648/32.0/40.4/14.7/4.4/35.9/39 |
| 90 days after viosterol | 222/480/30.0/31.6/16.5/6.2/53.2/43 |
| 120 days after viosterol | 264/840/37.0/38.0/14.0/6.1/56.0/46 |
| 140 days after viosterol | 397/872/35.2/44.0/24.6/5.4/34.3/50 |

| Dog B86 Thyroidectomized |  |
|-----------------|--------|
| Control period | 35.0/100/15.1/12.0/15.0/6.2/40 |
| Seven days after thyroidectomy | 57.0/128/14.6/18.4/7.8/30.8/37 |
| 60 days after thyroidectomy | 78.0/196/17.5/17.6/12.6/7.8/31.5/36 |
| 120 days after thyroidectomy | 76.5/220/17.7/15.6/12.2/7.4/28.7/35 |
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All the metabolic changes are more profound when the dog is thyroidec- tomized before viosterol overdosage. As will be seen from Table 2, however, the greatest effect of thyroidectomy is upon the lipid metabolism. Blood cholesterol levels were always doubled, and often increased four-fold or more. Similarly, serum levels of phospholipid phosphorus and the fatty acid titre were at least doubled. Thyroidectomy alone produces no such profound metabolic effects (Table 2), and the hematocrit and nonprotein nitrogen levels remain within normal limits. However, both of these are persistently elevated when the dog is given toxic doses of viosterol. What may be the effect of the meat diet and the hemocoagulation upon the blood level of nonprotein nitrogenous material cannot be determined from these experiments.

Arterial blood pressure was studied in 10 of the animals. In none was there a real hypertension. One normal animal fed viosterol for 10 weeks had a mean systolic pressure of 160 mm. of mercury at the end of the experiment.

Control animals. Eight normal dogs were fed on the same viosterol schedule as were those which were thyroidectomized. Vascular lesions having the same distribution as those in the thyroidectomized dogs resulted, but in every instance they were fewer, less acute, and less extensive. Moreover, the period of feeding of the toxic doses of viosterol had to be extended considerably as there were no grossly obvious lesions until the dog had been under treatment for six weeks or more. No foam cells were observed in the vessels of two animals treated for 70 days.

No vascular lesions occurred in 120 days in those dogs which were thyroidectomized but not fed viosterol.

Discussion

Since the work of Goldblatt, it has been known that in the dog persistent hypertension when associated with renal failure is followed by focal, acute necroses in the systemic arterioles. Acute lesions of large vessels, including particularly the pulmonary artery and aorta have been observed in this laboratory in association with experimental renal ischemia. Furthermore, the work of Holman, involving severe renal damage and the feeding of special diets containing a high percentage of lipid, disclosed lesions having the same morphology and distribution as those described in this communication. It is noteworthy that there was in our experimental animals some
evidence of azotemia. This may have had a role in the production of the vascular changes described.

Other investigators who have studied the toxic effects of viosterol upon the experimental animal have used different routes for administration of the vitamin. They have given it in single or multiple doses over varying periods of time, and different laboratory animals have been used. It is difficult, therefore, to draw conclusions between their results and ours. However, certain factors are worthy of comment. Ham and Ham and Lewis report that in rats following a single massive parenteral dose of viosterol, calcification of the muscle fibers of the arterial media occurs without previous anatomical change having taken place in the fibres. With this, Duguid, who also studied the changes in rats, disagrees. He believes, on the basis of histological evidence, that the primary lesion is a degeneration of the arterial smooth muscle, and that calcification follows this change. Both of these authors agree that the intimal reaction is secondary to the medial lesion.

Under the conditions of the present experiments, one of two types of change always preceded the more chronic vascular lesions. The first of these was medial necrosis accompanied by an acute inflammatory reaction. The other was fracture, disruption, or other histologically obvious distortion of the internal elastic lamella of the smaller arteries, or of the elastic fibers in the larger arteries or in the subendocardial tissues of the left auricle. If neither of these changes was present, edema of the media, almost certainly a reversible process, was the only alteration observed, and none of the later changes was produced. Intimal plaques may or may not form in response to the medial lesions (Figs. 5 and 6), but intimal proliferation was not observed in these experiments without underlying medial change.

The role of the thyroid is difficult to assess. Vascular lesions did not follow thyroidectomy alone, but they were produced more quickly and were greater in extent in those dogs whose metabolism was already altered by thyroidectomy. Moreover, lipid-containing foam cells were not observed, even with prolonged viosterol treatment, in the coronary artery lesions of the normal dogs. Menne, Beeman, and Labby observed that there was greater lipophage deposition in the aortas of thyroidectomized rabbits fed cholesterol than in normal or thyroid-treated animals kept on the same diet for the same period of time. However, it must be emphasized that arteriosclerosis is a focal disease, and that local as well as metabolic factors must therefore be sought to explain the focal involvement of the vessel wall. In
Fig. 1. Focal necrosis with acute inflammatory reaction in inner third of media of aorta. Thyroidectomized Dog B89 fed viosterol for 17 days. x 52. Note edema of wall of aorta.

Fig. 2. Necrosis and purulent exudate in center of aortic lesion shown in Fig. 1. Dog B89. x 350.
Fig. 3. Larger necrotic focus in aorta of Dog B89 with beginning liquefaction in central portion of inflammatory lesion. There is early intimal proliferation and capillary buds are forming at junction of the media and adventitia. x 40.

Fig. 4. Endothelial cell knots and capillary buds invading media of aorta from adventitia. Dog B89. x 335.
FIG. 5. Healed "calcified" aortic lesions in a thyroidectomized dog after 75 days of viosterol feeding. Note lack of intimal reaction. Dog B106. x 37.

FIG. 6. Acute and subacute intimal reaction in aorta of thyroidectomized dog fed viosterol for 23 days. An older, "calcified" lesion is seen in the lower half of the photograph. Dog B90. x 320.
Fig. 7. Aorta of thyroidectomized Dog B90 fed viosterol for 23 days. There are many elevated plaques in both thoracic and abdominal segments. See also Figs. 6 and 12.

Fig. 8A. Thoracic portion of aorta of Dog B89 (see also Figs. 1, 2, 3, 4, and 11). Note similarity of distribution of these acute inflammatory lesions to that of early human arteriosclerosis of the aorta. Actual size.

Fig. 8b. Aorta of Dog B89. Photograph made at same scale as Fig. 7 for comparison.
Fig. 9. Main pulmonary artery of thyroidectomized Dog B93 after 12 days of viosterol feeding. There are acute inflammatory foci in the inner half of the edematous media. x 38.

Fig. 10. Higher magnification of inner half of same pulmonary artery. Early intimal proliferation overlying an acute inflammatory focus. B93. x 220.
Fig. 11. Left auricular endocardium of Dog B89. There is an acute, hemorrhagic inflammatory reaction in the subendocardial connective tissue above the base of the anterior mitral leaflet. A ball thrombus is attached to a similar lesion above the posterior leaflet. Note (arrow) the thickened coronary artery.

Fig. 12. Organizing thrombus attached to left auricular endocardium of Dog B90. Note the subendocardial inflammatory reaction. x 17.
Fig. 13. Left anterior descending coronary artery of Dog 319, x 57. There is an acute panarteritis with marked intimal and adventitial proliferation and focal necrosis of media. Viosterol feeding for 21 days after thyroidectomy.

Fig. 14. High power view of same coronary artery to show necrosis of media and disruption of internal elastic lamina. x 340.
Fig. 15. Marked intimal fibrosis of left main coronary artery. Thyroidectomized Dog B71. Viosterol feeding for 30 days. x 70.

Fig. 16. Subintimal lipid filled foam cells in coronary artery of Dog B59. x 50, Sudan Black stain. Thyroidectomized and fed viosterol for 70 days.
Fig. 17. A small intimal plaque from a coronary artery of Dog B10thyroidectomized and fed viosterol for 75 days. Most of the foam cells lie between the internal elastic lamella and the endothelium. Note that two or three foam cells have begun to spread out beneath the elastic lamina through a break in its continuity. There is edema of the arterial wall beneath the lesion. x 850.

Fig. 18. Intimal plaque containing larger foam cells from coronary artery of thyroidectomized Dog B103 fed viosterol for 92 days. x 425.
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this connection, it is interesting that fatty change did not occur generally in the cells of the reticuloendothelial system of the experimental animals.

Summary

Acute arterial and endocardial lesions are produced by feeding toxic quantities of viosterol to thyroidectomized dogs. Their distribution and pathogenesis are described.

These lesions are anatomically similar to those occurring in dogs with experimental renal disease. Their distribution in the aorta corresponds to that of early human arteriosclerosis.

Especially after prolonged hyperlipemia, lipid is deposited in the intima of the affected coronary arteries selectively at the sites of lesions.

Finally, a practical experimental method is described for producing in dogs arterial lesions that allow the study of the deposition and transport of lipid. This can be approached from the standpoint of the morphology of the vessel wall and by the physical and chemical composition of the blood.

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