# PRODUCTION OF ATHEROMATOSIS IN THE AORTA OF THE BIRD BY THE ADMINISTRATION OF DIETHYLSTILBESTROL*‡

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PLATES 17 TO 21

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Although the concentration of lipids in the blood and liver of the male and immature female bird does not differ greatly from the range found in other species such as man and dog, avian lipid metabolism differs from mammalian in being under the control of ovarian hormones (1, 2). Thus, in the bird actively engaged in egg-laying there occurs a rise in the levels of fat, phospholipid, and cholesterol of the blood; in this state total lipids of the blood may rise as high as 4000 mg. per 100 cc., as compared with values of 500 mg. in the non-laying state. This rise in the various lipid constituents of the blood could be reproduced in the immature female or male bird by the injection of such estrogenic compounds as estrone, estradiol, and stilbestrol. In stilbestrol-treated birds, values for plasma lipids as high as 10,000 mg. per cent have been observed (3). the response of the plasma lipids to this estrogen does not depend upon the fat content of the diet; it was shown to occur in starved birds as well as in those fed a fat-free diet (3).

This distinctive feature of the lipid metabolism of the bird made possible the development of a new method for the study of experimental atherosclerosis. It is shown here that atheromatosis can be induced in the bird by the administration of diethylstilbestrol, a procedure that results in a sustained hyperlipemia. An extensive study of this estrogen-induced lesion is presented here. In addition, a comparison has been made between it and the atheromatosis induced in the bird by the feeding of cholesterol.

## *Materials and Methods*

Thirty-six single comb White Leghorn cockerels reared on the low fat Poultry Division stock, diet (1) were divided at 3.3 months of age into 3 groups. Pellets of fused diethylstilbestrol, each weighing approximately 25 mg., were implanted subcutaneously in the birds of one group; each bird received a single pellet at the start of the experiment and another 30, 70, and 95 days later. The birds of a second group were fed cholesterol at a level of 2 per cent in the diet.

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An amount of cottonseed oil sufficient to dissolve the cholesterol was used and mixed with the Poultry Division stock diet. The third group of birds was maintained on the stock diet as controls.

The birds were weighed at intervals, and from time to time blood samples were removed from the alar vein. Blood was drawn into heparinized syringes, centrifuged, and the plasma separated. The concentration of cholesterol, phospholipids, and total fatty acids in this plasma was determined by a procedure described elsewhere (4). Free cholesterol was determined in an acetone solution from which phospholipids had been precipitated.

The birds were sacrificed by exsanguination. The aorta and major aortic branches were removed together. After fixation in 10 per cent solution of formaldehyde U.S.P., the vascular apparatus was opened longitudinally and its gross appearance noted. Blocks of tissue were removed from each aorta at the following levels: apex of arch, interadrenal region, and about 1 cm. above the aortic bifurcation. Contiguous frozen sections cut from each block were stained with Sudan IV and hematoxylin (5) and Nile blue (6). An unstained frozen section was used for examination with polarized light. These blocks were then embedded in paraffin, and contiguous sections treated with hematoxylin and eosin stain, Laidlaw's connective tissue stain, and a combined Verhoeff-van Gieson stain (6).

### *Arteriosclerosis in Untreated Male Birds: the Control Group*

Spontaneous arteriosclerosis has repeatedly been observed in the bird (7-12). The gross and microscopic appearance of the aortas of the 12 control male birds studied here is summarized in Table I.

#### *Thoracic Aorta.*—

Although grossly visible lesions occurred rarely in the thoracic aortas of our male birds (Table I), a minimal amount of lipid material was, however, visible microscopically in the intima of this portion of the aorta in 4 birds (Fig. 1). This minimal lesion, which is shown in Fig. 1, was unaccompanied by fibrous thickening. It consisted of deposits of fine lipid droplets in the connective tissue cells of the intima and adjacent portions of the media. As judged by examination with polarized light, cholesterol was absent in this lesion.

The thoracic aortas of 8 of the birds were free of stainable lipid material (Table I). An example is shown in Fig. 2.

#### *Abdominal Aorta.*—

Nine birds of this group (Table I) showed either gross and/or microscopic evidence of intimal thickening in the lower part of the abdominal aorta in the area just above the bifurcation. The lesion consisted of longitudinal ridge-like plaques 1 to 2 mm. wide and 7 to 15 mm. long. The gray or white color of this lesion indicates its fibrous nature. The earliest lesion was usually found on the anterior wall of the artery, whereas the more advanced plaques were found on both anterior and posterior walls.

Early and late stages of this type of spontaneous arterial disease are shown in Figs. 3 to 8. The larger or more advanced lesion (Figs. 5 to 8) consisted of

dense, wavy, hyalinized connective tissue fibers running parallel to the endothelium covering the plaque (Fig. 6). The fibroblasts between the collagenous fibers were enlarged (Fig. 6) and contained numerous small lipid droplets, particularly in the deep portions of the plaques (Fig. 7). Fig. 8, which is a view

TABLE I

*The Spontaneous Lesion Found in the Aorta of Control Male Birds*

(All birds were 3.3 months old at start of experiment)

| Bird | Age when sacrificed | Thoracic aorta | | | | Lower abdominal aorta | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Number of grossly visible plaques | Microscopic examination | | | Grossly visible plaques | | | Microscopic examination | | |
| | | | Intimal thickening | Lipids* | Cholesterol‡ | Number | Size | Color | Intimal thickening | Lipids* | Cholesterol‡ |
| | *mos.* | | | | | | *mm.* | | | | |
| 15-02 | 11 | 0 | 0 | 0 | 0 | 0 | | | 1+ | 1+ | 0 |
| 15-27 | 8 | 0 | 0 | 1+ | 0 | 0 | | | 0 | 0 | 0 |
| 15-28 | 7 | 3§ | 0 | 2+ | 0 | 0 | | | 0 | 0 | 0 |
| 15-55 | 11 | 0 | 0 | 0 | 0 | 0 | | | 1+ | 1+ | 0 |
| 15-79 | 11 | 0 | 0 | 0 | 0 | 1 | 1 × 5 | White | 3+ | 2+ | 1+ |
| 15-81 | 8 | 0 | 0 | 1+ | 0 | 0 | | | 2+ | 1+ | 1+ |
| 15-82 | 11 | 0 | 0 | 0 | 0 | 0 | | | 2+ | 2+ | 0 |
| 15-90 | 11 | 0 | 0 | 0 | 0 | 2 | 2 × 10<br>1 × 10 | Gray<br>White | 4+ | 2+ | 0 |
| 15-91 | 11 | 0 | 0 | 0 | 0 | 2 | 2 × 15<br>1 × 12 | Yellow<br>Gray | 4+ | 2+ | 1+ |
| 15-94 | 11 | 0 | 0 | 1+ | 0 | 1 | 1 × 7 | Grayish white | 4+ | 2+ | 1+ |
| 15-99 | 11 | 0 | 0 | 0 | 0 | 0 | | | 2+ | 2+ | 0 |
| 15-88 | 11 | 0 | 0 | 0 | 0 | 0 | | | 0 | 0 | 0 |

* As judged by Sudan IV and Nile blue stains.

‡ Estimated by polarized light and Nile blue stain.

§ Each plaque measured 0.5 × 0.5 mm. and was yellow in color.

Histologic grading of lipid or cholesterol in artery:

1+ = minimal amounts in intima only.

2+ = present in media as well as intima but not extensively.

3+ = moderate amounts in intima and media.

4+ = abundant in intima and media.

of a lesion with polarized light, demonstrates that some of this lipid is cholesterol.

Although the above description refers only to the abdominal lesion in young male birds, this same lesion has also been observed in the female bird (12).

The difference between the spontaneous lesions found in the thoracic and abdominal portions of the aorta is quite striking. Whereas that in the thoracic aorta consisted entirely of lipid material, the abdominal one, on the other hand, contained small amounts of lipids and appeared primarily fibrous in nature.

TABLE II

*Lesions Found in the Aortas of Stilbestrol-Treated Male Birds*

(All birds were 3.3 months old at start of experiment)

| Bird | Age when sacrificed | Thoracic aorta | | | | | | Lower abdominal aorta | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Grossly visible plaques | | | Microscopic examination | | | Grossly visible plaques | | | Microscopic examination | | |
| | | Number | Size | Color | Intimal thickening | Lipids | Cholesterol | Number | Size | Color | Intimal thickening | Lipids | Cholesterol |
| | *mos.* | | *mm.* | | | | | | *mm.* | | | | |
| 15-08 | 11 | 2 | 15 × 1<br>0.5 × 0.5 | Yellow<br>" | 3+<br>1+ | 3+ | 2+ | 0 | | | 0 | 0 | 0 |
| 15-10 | 11 | 1 | 2 × 2 | " | 3+ | 3+ | 1+ | 0 | | | 1+ | 1+ | 0 |
| 15-24 | 6 | 0 | | | 0 | 1+ | 0 | Not examined | | | | | |
| 15-26 | 11 | 4 | 12 × 2<br>4 × 2<br>1 × 1<br>1 × 1 | "<br>"<br>"<br>" | 3+ | 2+ | 2+ | 1 | 12 × 1 | Yellow | 3+ | 3+ | 2+ |
| 15-30 | 10 | 5 | All 2 × 2 | " | 2+ | 2+ | 1+ | Not examined | | | | | |
| 15-35 | 8* | 3 | 0.5 × 0.5 | Orange | 3+ | 3+ | 0 | Not examined | | | | | |
| 15-36 | 9 | 0 | | | 2+ | 2+ | 1+ | 1 | 1 × 5 | " | 2+ | 3+ | 2+ |
| 15-45 | 11 | 1 | 1 × 1 | Yellow | 2+ | 2+ | 2+ | 0 | | | 1+ | 3+ | 0 |
| 15-48 | 11 | Numerous‡ | Coalescing | " | 4+ | 4+ | 4+ | 0 | | | 1+ | 1+ | 1+ |
| 15-58 | 6 | 0 | | | 0 | 1+ | 0 | 0 | | | 1+ | 1+ | 0 |
| 15-65 | 11 | 0 | | | 0 | 2+ | 2+ | 2 | 0.5 × 0.5<br>1 × 7 | "<br>" | 4+ | 4+ | 2+ |
| 15-85 | 8 | 0 | | | 0 | 3+ | 3+ | Not examined | | | | | |

* One pale orange plaque 1 × 1 mm. was found in right brachiocephalic artery.

‡ The entire thoracic aorta was involved. Numerous longitudinal lesions were also found in both brachiocephalic arteries.

## *Arteriosclerosis in Stilbestrol-Treated Male Birds*

*Thoracic Aorta.—*

A considerable deposition of lipids was observed in the thoracic aortas of a l 12 birds of this group (Table II). As noted above, this condition was found in 4 of the 12 control birds, but only to a minor degree.

*Gross Appearance of the Thoracic Lesion.*—Grossly visible lesions were found in 7 of the 12 injected birds. In 4 of them (15-08, 15-10, 15-26, and 15-45) one or more ovoid, slightly elevated intimal plaques were found on the convex side of the aortic arch about 1 cm. from the aortic valve. These lesions were lemon yellow in color, and measured from 1 to 2 mm. in width and as much as 15 mm. in length. When more numerous, they had a tendency to coalesce. This was particularly evident in bird 15-48, in which many coalescing elevated yellow intimal lesions were present in the brachiocephalic arteries and throughout the aorta. In

bird 15-35, which was sacrificed 8 months after the injection of stilbestrol was begun, the most prominent lesion was a single plaque in the left brachiocephalic artery. In still another bird (15-30) small areas of the intima of the thoracic aorta were found to have a lemon yellow color, but these areas were neither elevated nor plaque-like.

*Microscopic Appearance of the Thoracic Lesion.*—The significant microscopic finding was a lipid infiltration in the connective tissue cells of the intima and adjacent media (Figs. 10, 12, 16, 17). The lipids were present as small droplets within the cytoplasm (Figs. 12, 17), and the bulk of it, when stained with Nile blue, was light pink in color. Moderately abundant, refractile, rod-shaped, or rectangular crystals were visible with polarized light in these layers (Figs. 13, 18). These lipid-infiltrated cells had a vacuolated cytoplasm (Fig. 15) when stained with hematoxylin and eosin. The lining endothelial cells were often swollen and vacuolated, and lipid globules could be demonstrated with specific stains.

Microscopic examination revealed the presence of thick intimal plaques in the aortas of 8 of the birds (Figs. 9, 14). The thickened intima consisted of large vesicular fibroblastic cells, supported by reticulum and collagenous fibers running perpendicular to the endothelial surface (Fig. 11). The more deeply situated fibers were collagenous and condensed. In addition, these intimal plaques contained distinct foam cells of the macrophage type. The newly formed intimal connective tissue cells likewise were filled with lipid material. There was no mitotic evidence of fibrous cellular proliferation. In 8 of the 12 birds small to moderate numbers of refractile rod-shaped or rectangular crystals were seen with polarized light (Fig. 13). In a single stilbestrol-treated bird (15-48) greater amounts of cholesterol were discernible with polarized light than in any of the cholesterol-fed birds (Fig. 18). No alteration of the elastic tissue in the thoracic aorta was found in any of the stilbestrol-treated birds.

### *Upper Portion of Abdominal Aorta.*—

*Gross Appearance.*—In 5 birds, grossly visible lesions were found in the upper portion of the abdominal aorta at the level of the adrenal glands. In one bird (15–48) the lesion resembled that seen in the aortic arch. In the other 4, the lesions were small and few in number. They consisted of slightly elevated, rounded pale yellow or orange plaques which measured less than 1 mm. in diameter.

*Microscopic Appearance.*—In 8 birds, intimal thickening was demonstrated. In 5 birds this was minimal and consisted of a thin layer of vacuolated fibroblastic cells supported by coarse reticular fibers lying between the endothelium and the media. These cells, as well as some of the adjacent medial cells, contained lipid droplets. In only one bird (15-26), however, was it possible to demonstrate with polarized light a few spindle-shaped refractile crystals. In 3 birds, the intimal thickening was more prominent, usually leading to formation of flattened plaques on the anterior or posterior walls. In general, they consisted of fibroblastic cells supported by collagenous and reticulum fibers which tended to run parallel to the endothelial surface. The fibroblastic nuclei were flattened and lay in clear fusiform spaces, which were enclosed by connective tissue fibers and contained lipid droplets. In the larger plaques the deeper portions were more cellular, and these cells contained more lipid substance. In addition, the supporting reticulum and collagenous fibers in the larger plaques tended to run perpendicular to their surfaces. In the largest intimal plaques found in 3 birds, spindle-shaped and rectangular refractile crystals were abundant. In birds in which intimal lipid deposition was abundant, moderate amounts of lipid substances were also present in the media. Most of this was intracellular, but some apparently lay in extracellular situations. Occasionally groups of lipid-containing foam cells of fibroblastic type were present in the medial layer beneath the intimal plaques. There was no alteration of the elastic tissue, and the endothelial cells were not remarkable.

### *Lower Portion of the Abdominal Aorta.—*

*Gross Appearance.*—The lower portion of the aorta above the bifurcation was examined in only 8 birds (Table II). Three showed a ridge-like plaque on the anterior wall. The lesions of all 3 were distinctly lemon-yellow in color. These plaques measured approximately 1 mm. in width and between 7 and 12 mm. in length.

*Microscopic Appearance.*—Microscopically demonstrable intimal lesions were found in 7 birds (Table II; Figs. 19 to 23). Such lesions were composed of fibroblastic cells with compressed nuclei lying in clear spaces between wavy compact collagenous fibers. The latter tended to run parallel to the endothelial surface. Within the connective tissue cells were numerous lipid droplets. Only minimal amounts of refractile crystalline material were seen with polarized light in these microscopic lesions. In the birds not showing intimal lesions the media contained moderately abundant intracellular lipid globules.

The grossly visible ridge-like plaques that were found on the anterior arterial wall of 3 birds were almost large enough to obliterate the lumen (Fig. 19). In these birds the intima of the posterior and lateral walls was only mildly thickened and consisted of vesicular fibroblastic cells containing lipid and supported by circumferentially arranged collagenous fibers.

The large anterior plaques referred to above resembled the spontaneous lesion but showed, in addition, the following changes. The deeper portions were relatively acellular and consisted of dense hyalinized collagenous fibers enclosing fusiform spaces (Fig. 20). The latter contained vesicular fibroblastic cells. The long axis of the cells and fibers ran transversely. The fibers in the central portions of these plaques were swollen and homogeneous. Pools of vacuolated, protein-containing fluid lay between many fibers. Near the periphery were small calcific granules. The portion of the plaque adjacent to the endothelium was more cellular and consisted of vesicular foam cells of the fibroblastic type (Fig. 21). These were supported by delicate reticulum fibers tending to run perpendicular to the endothelium. The latter was intact, and the cells were not altered. Sudan IV stain revealed abundant bright orange lipid globules in the plaque, most numerously in the deeper and central portions (Fig. 22). In the latter situation, large pools of extracellular lipid material were evident. Considerable amounts of lipid substance were also present in the medial layer. Polarized light revealed moderate amounts of refractile rod-shaped or spindle-shaped or flat rectangular crystalline material in the plaque as well as in the thickened intima (Fig. 23). Beneath these large plaques the internal elastic membrane was thin.

## *Arteriosclerosis in Cholesterol-Fed Male Birds*

### *Thoracic Aorta.—*

*Gross Appearance.*—In all 12 birds of this group numerous intimal plaques were found in all portions of the aorta (Table III). In the thoracic portion of all but one (bird 15-89) these appeared as small, elevated, longitudinal streaks or pinpoint deposits. These lesions were most numerous on the convex surface of the intima, where they tended to coalesce. In most of the birds, the lesions were pale yellowish tan in color, although in two of them the plaques were yellowish white or yellowish orange in color. Similar intimal lesions were often visible in the cusps of the aortic valves, in sinuses of Valsalva, and in the brachiocephalic, carotid, subclavian, and femoral arteries.

*Microscopic Appearance.*—All birds showed microscopic atheromatous lesions in the thoracic aorta (Fig. 24). There was a generalized intimal thickening with an increase in the number of intimal cells, most marked in those zones where grossly visible intimal plaques were present. Between the plaques were deep indentations. The endothelium was usually intact, but in one instance had ulcerated and a small mural thrombus had formed. The endothelial cells were as

a rule enlarged and often had vesicular cytoplasm in which lipid droplets could be demonstrated. The thickened intima consisted mainly of large cells of the fibroblastic type with oval, pale, vesicular nuclei and rather abundant clear, or occasionally granular, cytoplasm. Connective tissue fibers lay between these cells and tended to run perpendicular to the endothelial surface (Fig. 25). Near the latter, they had a fine texture and corresponded to reticulum. In the deeper intimal layer they were coarse and collagenous. In one bird there was a zone of fibrocartilagenous and hyalin-cartilagenous metaplasia of a portion of the thickened intimal con-

TABLE III

*Lesions Found in Aortas of Cholesterol-Fed Male Birds*

(All birds were 3.3 months old at start of experiment)

| Bird | Age when sacrificed | Thoracic aorta | | | | | | Lower abdominal aorta | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Grossly visible plaques | | | Microscopic examination | | | Grossly visible plaques | | | Microscopic examination | | |
| | | Number | Size | Color | Intimal thickening | Lipids | Cholesterol | Number | Size | Color | Intimal thickening | Lipids | Cholesterol |
| | *mos.* | | | | | | | | *mm.* | | | | |
| 15-07 | 11 | Numerous | Coalescing | Tan | 3+ | 3+ | 2+ | One* | 1 × 6 | Yellow-tan | 4+ | 4+ | 4+ |
| 15-18 | 10 | " | " | " | 3+ | 2+ | 3+ | " | 1 × 43‡ | Yellow-tan | 2+ | 2+ | 3+ |
| 15-19 | 8 | " | " | " | 2+ | 2+ | 1+ | 2 | Each 1 × 4 | Tan | 2+ | 3+ | 3+ |
| 15-33 | 8 | " | " | " | 1+ | 2+ | 2+ | 2 | Each 1 × 5 | " | 2+ | 3+ | 1+ |
| 15-59 | 11 | " | " | Orange | 3+ | 4+ | 2+ | Numerous | 1 × 6 | Yellow-white | 4+ | 4+ | 4+ |
| 15-60 | 9 | " | " | Tan | 3+ | 3+ | 3+ | One* | 2 × 7 | Tan | 3+ | 3+ | 4+ |
| 15-67 | 8 | " | " | " | 3+ | 3+ | 3+ | " * | 1 × 5 | Orange | 2+ | 3+ | 3+ |
| 15-72 | 10 | " | " | " | 3+ | 3+ | 3+ | " * | 1 × 8 | White | 4+ | 4+ | 4+ |
| 15-84 | 11 | " | " | " | 3+ | 3+ | 2+ | " * | 3 × 15 | Yellow | 4+ | 4+ | 4+ |
| 15-86 | 11 | " | " | " | 3+ | 3+ | 3+ | " * | 1 × 10 | Tan | 4+ | 4+ | 4+ |
| 15-89 | 11 | 0 | | | 1+ | 1+ | 1+ | " | 2 × 7 | Grey and yellow | 4+ | 2+ | 2+ |
| 16-00 | 8 | Numerous | Coalescing | Tan | 3+ | 4+ | 3+ | " * | 2 × 6 | Tan | 4+ | 4+ | 2+ |

* Both thoracic and abdominal portions contained small coalescing tan intimal plaques in addition to the single ridge.

‡ This single plaque extended from the level of the adrenal glands to the bifurcation.

nective tissue. The thickened intima contained no elastic tissue. A few of the intimal cells were macrophages which contained lipid material; these cells had small compact rounded nuclei. A few lymphocytes were present. When stained with Sudan IV, all the intimal cells and many of the medial connective tissue cells were shown to be richly supplied with bright orange lipid droplets which filled the cytoplasm (Figs. 26, 27). Smaller amounts of lipid material lay in extracellular situations. The amount of fatty material decreased toward the outer portion of the media, but was present as far as the adventitia. Large masses of refractile crystals were visible with polarized light in the intima and adjacent media (Fig. 28); these were either spindle-shaped or flat, notched, and rectangular. There was no alteration of the medial elastic tissue of the thoracic aorta.

### *Upper Portion of the Abdominal Aorta.—*

*Gross Appearance.*—This portion of the aorta contained small tan plaques similar to those found in the thoracic aorta. They were less numerous and smaller than those in the thoracic aorta and hence showed less tendency to coalesce.

*Microscopic Appearance: The Intima.*—Sections from the aorta at the level of the adrenal glands showed a thickened intima composed mainly of enlarged lipid-containing fibroblasts which were supported by a delicate collagenous and reticulum network. These fibers ran perpendicular to the endothelial surface and were usually more abundant and compact at the periphery of the intimal plaques. Occasional lipid-filled macrophages were noted. Several aortic branches were occluded by a similar intimal fibrous thickening. A few small lipid droplets were present in and between the muscle cells of the adjacent media. There were numerous refractile rods in the thickened intima when viewed with polarized light.

Beneath the widened intima, the internal elastic membrane was narrowed or absent in some areas.

*The Media.*—The media was narrowed and small intimal calcific masses were present in those areas where the intimal thickening was particularly marked.

*The Plaque.*—In several birds the anterior wall at this aortic level was the site of an intimal plaque which histologically appeared similar to the spontaneous abdominal lesions described for the control group. These were composed of moderately dense connective tissue fibers, lying parallel to the circumference of the vessel and enclosing compressed fibrocytic nuclei. The latter were surrounded by clear fusiform spaces filled with lipid droplets which stained bright orange with the Sudan IV and blue to pink with the nile blue. A few lipid droplets lay also in the muscular cells of the adjacent media. A moderate amount of refractile crystalline material was present in these intimal plaques. In several instances, the inner portion of the plaque adjacent to the lumen was composed of large, lipid-filled, fibroblastic foam cells supported by a delicate collagenous and reticulum stroma. These cells were abundantly supplied with lipids including refractile material, and were identical with the intimal cells of the thoracic aorta. In a few birds, the intimal alteration at the level of the adrenal glands approached in severity that seen in the lower aorta above the bifurcation.

### *Lower Portion of the Abdominal Aorta.—*

*Gross Appearance.*—As already noted, the intimal plaques of this area were less numerous, smaller, and showed less tendency to coalesce than those described above. In the abdominal aorta of all birds in this group, there were large longitudinal, ridge-like plaques in the interrenal region. These plaques most frequently lay on the anterior wall and measured 1 to 2 mm. in width and 4 to 8 mm. in length. Infrequently, the posterior wall was similarly involved, though here the plaques were multiple, tended to fuse, and were tan-colored.

The anteriorly situated plaques were usually yellowish, yellowish orange, or white in color; a few, however, were tan-colored.

As a result of such extensive plaque formation, the lower abdominal aorta was usually quite rigid.

*Microscopic Appearance.*—Striking changes were observed just above the aortic bifurcation. The thickening of the intima on the anterior wall was usually extensive, forming a large plaque which almost occluded the lumen of the vessel (Figs. 29 to 32). The posterior intima was also thickened (Fig. 29) and consisted of fibroblastic cells with abundant clear cytoplasm. These cells were supported by moderately dense, edematous, collagenous and reticulum fibers. The long axis of the nuclei and the intercellular fibers tended to run perpendicular to the endothelial lining of the vessel. This thickened intimal tissue extended laterally on both sides, reaching the lateral portions of the large plaque lying in the anterior wall.

In the central portions of the large anterior plaque, the connective tissue adjacent to both endothelium and media consisted of a thin compact layer of flattened fibroblasts and collagenous fibers running parallel to the circumference of the vessel. This resembled the connective tissue arrangement of the spontaneous abdominal lesions. The endothelium was intact throughout, though the lumen was narrowed, slit-like, and crescentic.

Beneath the large anterior plaque the media was atrophic and mildly fibrotic (Figs. 29, 32). In some instances the media was absent, and masses of foam cells were found extending into the adventitia. The internal elastic membrane was absent, and the external one was thin and fragmented (Figs. 29, 32).

The bulk of the plaque was composed of pale, eosinophilic, fibrillary, almost mucoid, connective tissue containing very few flattened fibroblastic cells. Many of these were vacuolated (Fig. 33). Some of these cells were degenerating and enclosed numerous large clear fusiform spaces often arranged in a transverse herringbone manner (Figs. 30, 35). Small calcific granular masses were present at the periphery of the plaques (Figs. 32, 35). Occasionally the central portion of the plaque contained condensed fibrillary fibrin. Beneath the condensed connective tissue adjacent to the endothelium were moderate numbers of macrophages with abundant vesicular cytoplasm (Fig. 34). These were not enveloped by reticulum fibers. In the degenerated central portion of the large plaques, the reticulum and collagenous fibers were frayed, split, and often fragmented. Lipid material (Fig. 30) was abundantly present in the connective tissue cells of the thickened intima, posteriorly and anteriorly overlying the large plaque. Within the central portion of the latter, lipid droplets lay between the fusiform spaces and were largely in extracellular situations.

Refractile material, as demonstrated with polarized light, varied in its distribution (Fig. 31). That in the thickened intima appeared as rods or flat rectangular plates. In the central portion of the large anterior plaque, there were many long needle-like crystals which at times were segmented. In one bird (15-89) the anterior plaque resembled more closely the spontaneous fibrous lesion, though lipid-containing foam cells of the fibroblastic type were seen at its lateral angles.

### *Relation of the Lipids Deposited in the Aorta to Those in Plasma*

The concentrations of cholesterol, total fatty acids, and phospholipids found in the plasma of control, cholesterol-fed, and stilbestrol-injected birds are recorded in Tables IV–VI. They show that the 2 latter groups of birds differed not only in the total amounts of lipids contained in their plasma but also in the composition of these lipids (*i.e.*, proportions of cholesterol, triglycerides, and phospholipids present). Thus in the stilbestrol-treated birds, values for total lipids well above 10,000 mg. per cent were not uncommon—the highest value was 17,700 mg.—, whereas in the cholesterol-fed birds the values for total lipids did not exceed 5000 mg. Although the concentration of plasma cholesterol was in most cases higher in the stilbestrol-treated birds than in those fed cholesterol, this lipid constituent did not account for the difference in the total lipids observed in the 2 groups. It was the triglycerides and phospholipid components that accounted for the amazingly high concentrations of lipids in the plasma of the stilbestrol-treated birds. Values for phospholipids between 3000 and 5000 mg. and values for neutral fat in the neighborhood of 9 to 10,000 mg. per 100 cc. of plasma were not uncommonly found in the estrogen-injected birds.

In those fed cholesterol, on the other hand, the highest concentrations of phospholipids and neutral fat were respectively 645 and 3900 mg.

As judged from reactions to stains and from examination with polarized light, the composition of the lipids deposited in the arterial walls differed in the cholesterol-fed and stilbestrol-injected birds. In the aorta of the former, cholesterol constituted the bulk of the lipids deposited, whereas in the stilbestrol-treated birds lipids other than cholesterol predominated. This finding is somewhat

TABLE IV

*Plasma Lipids of Control (Untreated) Male Birds*

(All lipid values are expressed as mg. per 100 cc.)

| Bird | Interval after start of experiment | Cholesterol | | | Total fatty acids | Phospho-lipids | Total lipids |
|---|---|---|---|---|---|---|---|
| | | Total | Free | Ester | | | |
| | *mos.* | | | | | | |
| 15-02 | 6.5 | 134 | 44 | 90 | 227 | 187 | 361 |
| | 0 | 107 | 16 | 91 | 280 | 183 | 387 |
| 15-27 | 1.5 | 88 | 22 | 66 | 310 | 171 | 398 |
| | 3.0 | 105 | 25 | 80 | 352 | 126 | 457 |
| 15-79 | 6.5 | 86 | 30 | 46 | 282 | 125 | 368 |
| | 0 | 92 | 25 | 67 | 380 | 223 | 372 |
| 15-81 | 1.5 | 81 | 34 | 47 | 315 | 157 | 396 |
| | 3.0 | 104 | 29 | 75 | 380 | 158 | 484 |
| 15-82 | 6.5 | 67 | 22 | 45 | 253 | 137 | 321 |
| 15-88 | 6.5 | 107 | 32 | 85 | 272 | 189 | 379 |
| 15-90 | 6.5 | 102 | 35 | 67 | 288 | 176 | 390 |
| 15-91 | 6.5 | 99 | 27 | 72 | 257 | 148 | 356 |
| 15-99 | 6.5 | 55 | 19 | 36 | 267 | 118 | 322 |

surprising, for, as noted above, the concentration of cholesterol in plasma of the estrogen-treated birds was as great as, and sometimes greater than, that of the cholesterol-fed birds. The excessive amounts of phospholipids and triglycerides contained in the plasma of the estrogen-treated birds would appear to have influenced cholesterol deposition in the aortas. The extent to which plasma cholesterol accumulates in the aorta may depend not only on the absolute concentration of cholesterol in plasma, but also on the relative proportions of the various lipid constituents present in plasma. In other words, cholesterol may compete with other plasma lipids for space in the arterial wall.

TABLE V

*Plasma Lipids of Stilbestrol-Injected Male Birds*

(All lipid values are expressed as mg. per 100 cc.)

| Bird | Interval after first injection | Cholesterol | | | Total fatty acids | Phospho-lipids | Total lipids |
|---|---|---|---|---|---|---|---|
| | | Total | Free | Ester | | | |
| | *mos.* | | | | | | |
| 15-08 | 1 | 114 | 26 | 88 | 508 | 276 | 622 |
| | 2.5 | 741 | 557 | 184 | 10900 | 4300 | 10700 |
| | 4.5 | 694 | 481 | 213 | 10100 | 3000 | 10700 |
| | 6.5 | 444 | 307 | 137 | 7270 | 2200 | 7710 |
| 15-10 | 2.5 | 575 | 518 | 257 | 11300 | 3100 | 11800 |
| | 4.5 | 758 | 532 | 226 | 10500 | 3180 | 11200 |
| | 6.5 | 532 | 316 | 216 | 12400 | 3200 | 12900 |
| 15-24 | 1 | 234 | 67 | 167 | 939 | 537 | 1170 |
| 15-26 | 1 | 99 | 52 | 47 | 1340 | 438 | 1440 |
| | 2.5 | 570 | 314 | 256 | 6400 | 3200 | 7020 |
| | 4.5 | 920 | 535 | 385 | 11600 | 3240 | 12000 |
| | 6.5 | 119 | 37 | 82 | 272 | 204 | 391 |
| 15-30 | 1 | 214 | 41 | 173 | 755 | 360 | 969 |
| | 2.5 | 780 | 605 | 175 | 9300 | 3100 | 10100 |
| | 4.5 | 480 | 440 | 40 | 12900 | 4180 | 13300 |
| 15-35 | 1 | 129 | 38 | 91 | 597 | 295 | 726 |
| | 2.5 | 327 | 129 | 198 | 2190 | 580 | 2510 |
| 15-36 | 1 | 172 | 56 | 116 | 597 | 141 | 769 |
| | 2.5 | 585 | 368 | 217 | 14400 | 3330 | 14900 |
| | 3.0 | 735 | 222 | 513 | 16900 | 4930 | 17600 |
| 15-45 | 1 | 116 | 45 | 71 | 382 | 205 | 498 |
| | 2.5 | 96 | 34 | 62 | 387 | 197 | 487 |
| | 4.5 | 762 | 620 | 142 | 10900 | 2680 | 11600 |
| | 6.5 | 515 | 312 | 203 | 11400 | 2830 | 11900 |
| 15-48 | 1 | 218 | 72 | 146 | 925 | 546 | 1140 |
| | 2.5 | 511 | 195 | 316 | 2430 | 758 | 2940 |
| | 4.5 | 885 | 510 | 375 | 9680 | 2840 | 10500 |
| | 6.5 | 381 | 232 | 149 | 4320 | 1370 | 4700 |
| 15-58 | 0 | 180 | 64 | 116 | 680 | 428 | 860 |
| | 1.5 | 341 | 230 | 111 | 3080 | 977 | 3420 |
| 15-65 | 0 | 101 | 39 | 62 | 497 | 302 | 598 |
| | 1.5 | 440 | 302 | 138 | 6050 | 1970 | 6490 |
| | 2.5 | 296 | 207 | 89 | 3030 | 1070 | 3320 |
| | 6.5 | 99 | 31 | 68 | 710 | 250 | 809 |
| 15-85 | 1 | 151 | 47 | 104 | 386 | 106 | 537 |
| | 2.5 | 565 | 368 | 197 | 13900 | 3300 | 14400 |
| | 3.0 | 615 | 224 | 391 | 17100 | 5200 | 17700 |

TABLE VI

*Plasma Lipids of Cholesterol-Fed Male Birds*

(All lipid values expressed as mg. per 100 cc.)

| Bird | Period fed cholesterol | Cholesterol | | | Total fatty acids | Phospho-lipids | Total lipids |
|---|---|---|---|---|---|---|---|
| | | Total | Free | Ester | | | |
| | *mos.* | | | | | | |
| | 1 | 400 | 98 | 302 | 610 | 215 | 1010 |
| | 1.5 | 322 | 70 | 252 | 560 | 197 | 882 |
| 15-07 | 2.5 | 251 | 101 | 150 | 768 | 253 | 1010 |
| | 3.0 | 271 | 108 | 163 | 819 | 294 | 1090 |
| | 6.5 | 403 | 296 | 107 | 2240 | 505 | 2640 |
| | 1 | 185 | 81 | 104 | 1140 | 238 | 1330 |
| 15-18 | 2.5 | 230 | 147 | 83 | 1200 | 383 | 1430 |
| | 5.5 | 172 | 146 | 26 | 1870 | 537 | 2040 |
| 15-19 | 1 | 227 | 139 | 88 | 3270 | 518 | 3500 |
| | 2.5 | 226 | 138 | 88 | 2730 | 492 | 2960 |
| | 1 | 184 | 82 | 102 | 2650 | 485 | 2830 |
| 15-38 | 2.5 | 590 | 318 | 272 | 2500 | 380 | 3090 |
| | 3.0 | 775 | 238 | 537 | 3330 | 517 | 4100 |
| 15-59 | 2.5 | 236 | 114 | 122 | 2380 | 415 | 2620 |
| | 6.5 | 392 | 306 | 86 | 4340 | 575 | 4730 |
| | 1 | 251 | 148 | 103 | 2610 | 518 | 2860 |
| 15-60 | 2.5 | 425 | 191 | 234 | 3520 | 592 | 3940 |
| | 4.5 | 158 | 139 | 19 | 2480 | 645 | 2650 |
| 15-67 | 2.5 | 255 | 129 | 126 | 3020 | 387 | 3270 |
| | 1.5 | 566 | 340 | 226 | 2280 | 369 | 2840 |
| 15-72 | 3.0 | 387 | 338 | 40 | 2720 | 335 | 3090 |
| | 4.5 | 458 | 247 | 211 | 3160 | 558 | 3620 |
| | 6.5 | 555 | 340 | 215 | 2300 | 404 | 2850 |
| 15-84 | 2.5 | 264 | 110 | 154 | 595 | 545 | 859 |
| | 6.5 | 154 | 98 | 56 | 468 | 547 | 722 |
| | 1 | 187 | 79 | 108 | 1210 | 216 | 1400 |
| 15-86 | 2.5 | 234 | 110 | 124 | 1720 | 332 | 1960 |
| | 4.5 | 338 | 130 | 208 | 3450 | 376 | 3970 |
| | 6.5 | 377 | 290 | 87 | 3050 | 440 | 3430 |
| 15-89 | 2.5 | 175 | 53 | 122 | 287 | 120 | 462 |
| | 6.5 | 254 | 135 | 119 | 2520 | 217 | 2770 |
| | 1 | 357 | 177 | 180 | 1590 | 225 | 1940 |
| 16-00 | 2.5 | 560 | 360 | 200 | 2030 | 356 | 2590 |
| | 3.0 | 720 | 318 | 402 | 3260 | 517 | 3980 |

It is of interest to note that the plasma of the stilbestrol-treated birds was milky, opaque, and of the same lemon yellow color as the intimal lesions in the aortas of these birds. The plasma of the cholesterol-fed birds, however, was tan-colored and resembled the color seen in their intimal lesions.

## DISCUSSION

Three forms of arterial disease in the bird are described here: the spontaneously occurring one, that induced by stilbestrol injections, and that brought about by cholesterol feeding. These 3 lesions are fundamentally similar in nature. In the thoracic aorta, all 3 are characterized by lipid deposition within the intima and the adjacent media. They differ, however, in 2 important aspects: (1) the degree of lipid infiltration and (2) the proportion of the various lipids that infiltrate the wall of the artery.

When present, the spontaneous *thoracic* lesion of the rooster was visible only by microscopic examination and consisted entirely of a lipid infiltration of the intima, none of which was identifiable as cholesterol. The degree of lipid infiltration in the intima and adjacent media of the thoracic aorta induced by stilbestrol and cholesterol feeding was great enough to produce grossly visible lesions which were accompanied by fibrous proliferation. Although cholesterol was identified in both of the experimentally induced thoracic lesions, the bulk of the lipid in the stilbestrol-induced lesion was not cholesterol, whereas in the cholesterol-fed bird cholesterol formed the bulk of the lipid infiltration.

The basic *abdominal* lesion that occurs in all 3 types is fibrous in nature and appears finally as an elongated longitudinal plaque. In the untreated birds the plaque contained small amounts of lipid material, some of which was cholesterol. In the stilbestrol-treated birds the spontaneous fibrous plaque was heavily infiltrated with lipids, part of which was cholesterol. In those fed cholesterol, the spontaneous plaque was greatly modified by a heavy deposition of cholesterol.

In both the cholesterol-fed and the stilbestrol-treated birds, more lipids were deposited in the spontaneous fibrous plaque than in the neighboring normal vascular tissue. A similar phenomenon is found in man suffering from syphilitis aortitis; atheromatous deposition is most pronounced in those portions of the aorta which have been altered by the syphilitic fibrosing process. These findings suggest that the fibrotic intima is more permeable to lipids than the normal.

Two types of spontaneous arteriosclerosis are found in the bird, and, according to Dauber (12), one of these, namely the lipid infiltration of the intima of the thoracic aorta, occurs only in the female bird. A minimal degree of lipid infiltration, however, was found also in the thoracic aortas of our control group of roosters. This same thoracic lipid lesion was also observed by us in several roosters and capons over 5 years of age. Thus, while this lesion occurs in both roosters and hens, it is obviously more extensive in hens, probably as a result of

the periodic lipemia associated wirh egg laying (1). With the artificially induced lipemia of cholesterol feeding or stilbestrol implantation, this lipid infiltration of the thoracic aorta of the rooster is accentuated, and, when extensive enough, is associated with proliferation of fibroblastic foam cells. This process tends to involve the entire aorta, but is most striking in the thoracic portion.

The second type of spontaneous arteriosclerosis seen in the bird is the ridge-like intimal thickening of the abdominal aorta. This occurs in both the hen and the rooster, but in the former, according to Dauber (12), lipid is more abundant, again probably the result of the physiological lipemia associated with egg laying. The etiology of this fibrotic lesion is still obscure. In both sexes the lesion is fundamentally a fibrosing process in which lipid material gradually accumulates, so that greater amounts are seen in older birds. Prolonged lipemia produced by cholesterol feeding or by stilbestrol implantation modifies this lesion by depositing in it excessive amounts of lipids.

The lesion produced by stilbestrol implantation more nearly resembled the spontaneous lesion than did the one occurring after cholesterol feeding. The cholesterol-induced lesion is striking because of its high content of cholesterol crystals. This type of arterial lesion should be considered part of a widespread cholesterol storage process and therefore in the nature of an artificial arterial disease.

The results presented here clearly establish that a sustained hyperlipemia, whether of endogenous origin (stilbestrol-injected birds) or of exogenous origin (cholesterol-fed birds), can result in an infiltration of lipids in the aortic wall. While cholesterol forms a striking component of the experimentally induced atherosclerotic lesions studied here, it does so mainly because of its insoluble nature. It would appear that lipids other than cholesterol also infiltrate the vascular wall, but these can subsequently diffuse out of the arterial wall. Cholesterol, on the other hand, after its deposition in the arterial wall, makes its appearance finally as large crystalline masses which are no longer movable.

The late fibrotic *abdominal* plaque in the bird, particularly after modification by deposition of large amounts of cholesterol and other lipids, resembles the late atherosclerotic lesion found in man, for both are fibrous plaques containing cholesterol and other lipids. In the normal male bird, however, the large abdominal aortic plaque is present long before lipid deposits become abundant, whereas in man the lipid deposit precedes fibrous scarring. It is evident therefore that, despite their similarity in appearance, the mechanism resulting in the formation of the abdominal lesion in the bird differs from that which produces human atherosclerosis.

## SUMMARY

A new experimental procedure for the production of arteriosclerosis in the bird is described. The subcutaneous implantation of diethylstilbestrol by

means of which a sustained increase in the concentration of cholesterol, phospholipid, and neutral fat can be readily established, is shown to induce atherosclerosis of the aorta.

The atherosclerosis has been compared with that artificially induced in the bird by the prolonged feeding of cholesterol and also with that occurring spontaneously. The stilbestrol-induced lesion more closely resembled the spontaneously occurring one in the bird than did that produced by cholesterol feeding. But all 3 lesions were fundamentally similar, differing only in the amounts and proportions of the various lipid constituents present.

The concentrations of cholesterol in plasma of the stilbestrol-treated and cholesterol-fed birds were of the same order. Yet cholesterol constituted a greater proportion of the lipids deposited in the arterial wall of the cholesterol-fed than in that of the stilbestrol-treated birds. This finding suggests that the cholesterol content of the vascular lesion depends not only on the absolute concentration of cholesterol in plasma, but also on the proportion of cholesterol to other lipid constituents in plasma.

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## EXPLANATION OF PLATES

*Control Group*

PLATE 17

FIG. 1. Bird 15-28. Thoracic aorta showing minimal lipid infiltration of intima and adjacent media. Sudan IV and hematoxylin stain. × 78.

FIG. 2. Bird 15-90. Thoracic aorta showing absence of lipid deposition. Sudan IV and hematoxylin stain. × 78.

FIG. 3. Bird 15-02. Abdominal aorta showing minimal intimal thickening without lipid infiltration. Sudan IV and hematoxylin stain. × 78.

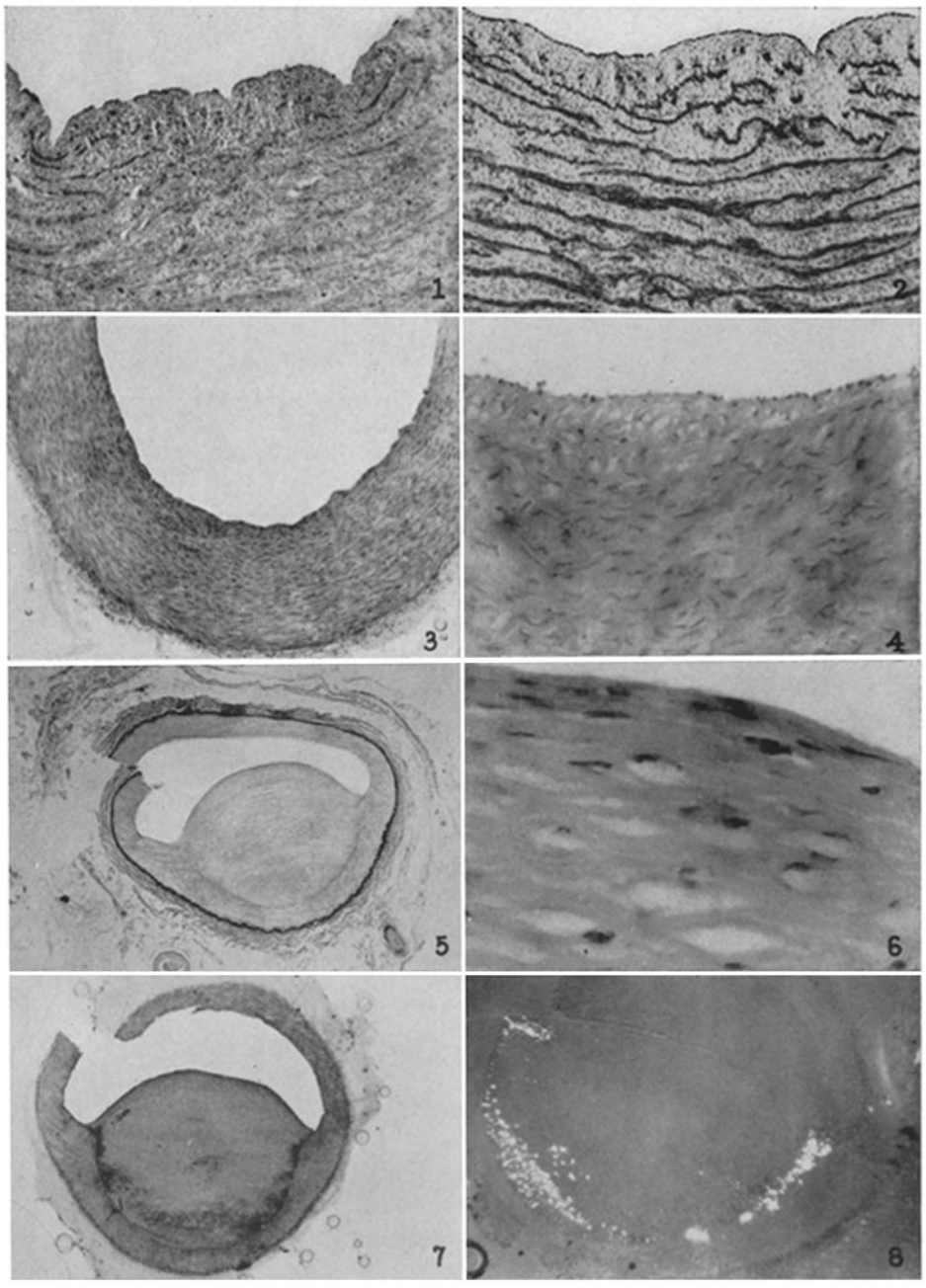
FIG. 4. Bird 15-02. Abdominal aorta showing early intimal fibrous thickening. Hematoxylin and eosin stain. × 210.

FIG. 5. Bird 15-90. Abdominal aorta showing large fibrous abdominal plaque. Verhoeff-van Gieson stain. × 23.

FIG. 6. Bird 15-90. Abdominal aorta showing transverse arrangement of cells and fibers of the large abdominal plaque. Hematoxylin and eosin stain. × 820.

FIG. 7. Bird 15-90. Abdominal aorta showing large intimal plaque with lipid deposition in the deeper portions. Sudan IV and hematoxylin stain. × 23.

FIG. 8. Bird 15-91. Abdominal aorta showing large intimal plaque with cholesterol in the sites of lipid deposition. Polarized light. × 35.




(Chaikoff *et al.*: Atheromatosis in aorta of bird)

*Stilbestrol Group*

PLATE 18

FIG. 9. Bird 15-26. Thoracic aorta showing intimal thickening. Hematoxylin and eosin stain. × 78.

FIG. 10. Bird 15-26. Thoracic aorta showing lipid infiltration of the intima and adjacent media. Sudan IV and hematoxylin stain. × 78.

FIG. 11. Bird 15-26. Thoracic aorta showing intimal thickening with vertical arrangement of reticulum fibers. Laidlaw stain. × 78.

FIG. 12. Bird 15-26. Thoracic aorta intima showing lipid droplets in connective tissue cells. Sudan IV and hematoxylin stain. × 210.

FIG. 13. Bird 15-26. Thoracic aorta showing deposition of cholesterol in intima and adjacent media. Polarized light. × 35.

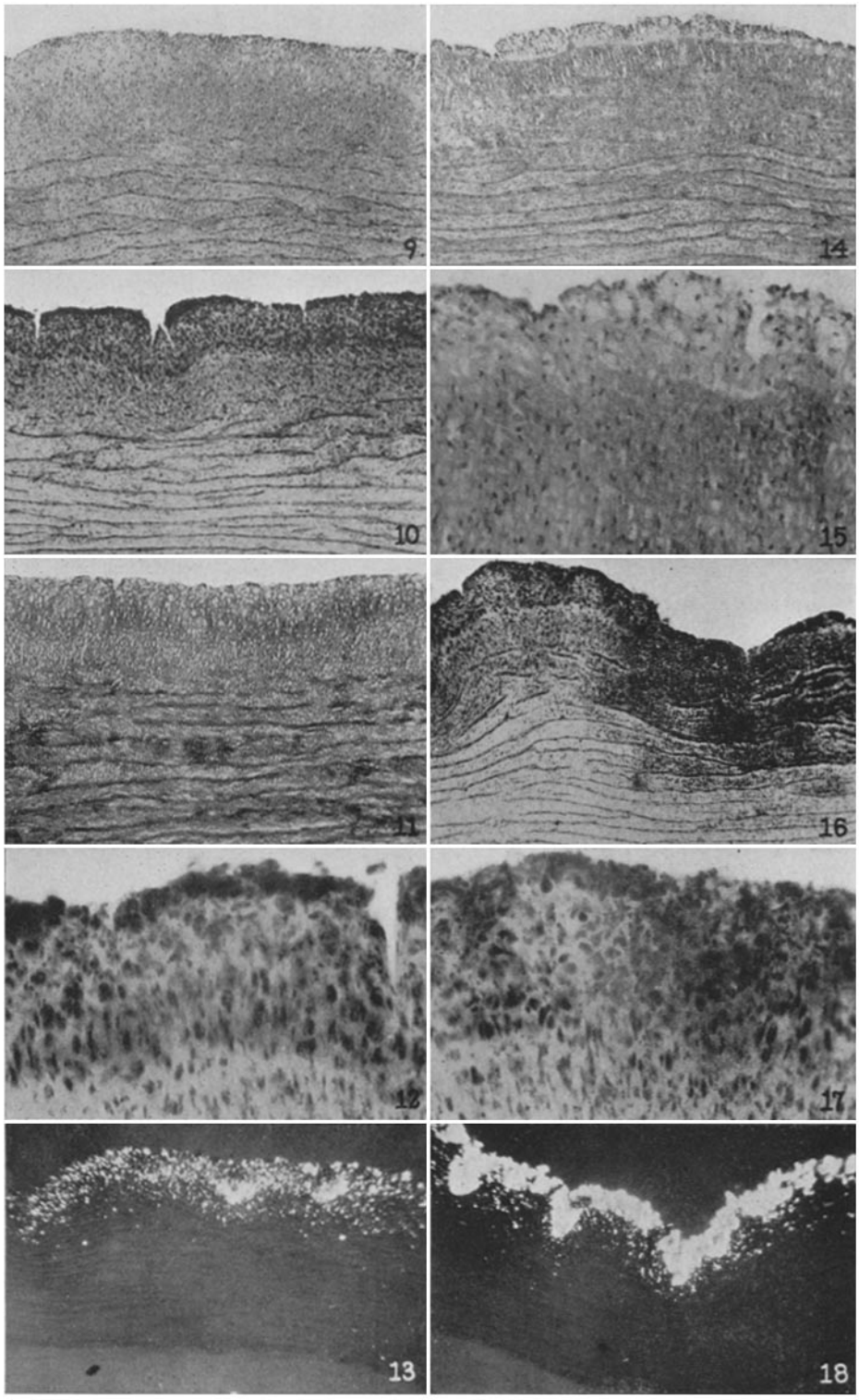
FIG. 14. Bird 15-48. Thoracic aorta showing intimal thickening. Hematoxylin and eosin stain. × 78.

FIG. 15. Bird 15-48. Thoracic aorta showing vacuolated connective tissue cells of thickened intima. Hematoxylin and eosin stain. × 78.

FIG. 16. Bird 15-48. Thoracic aorta showing extensive lipid deposits in the intima and adjacent media. Sudan IV and hematoxylin stain. × 78.

FIG. 17. Bird 15-48. Thoracic aortic intima showing lipid droplets in connective tissue cells. Sudan IV and hematoxylin stain. × 210.

FIG. 18. Bird 15-48. Thoracic aorta showing deposition of cholesterol in intima and adjacent media. Polarized light. × 35.




(Chaikoff *et al.*: Atheromatosis in aorta of bird)

*Stilbestrol Group*

PLATE 19

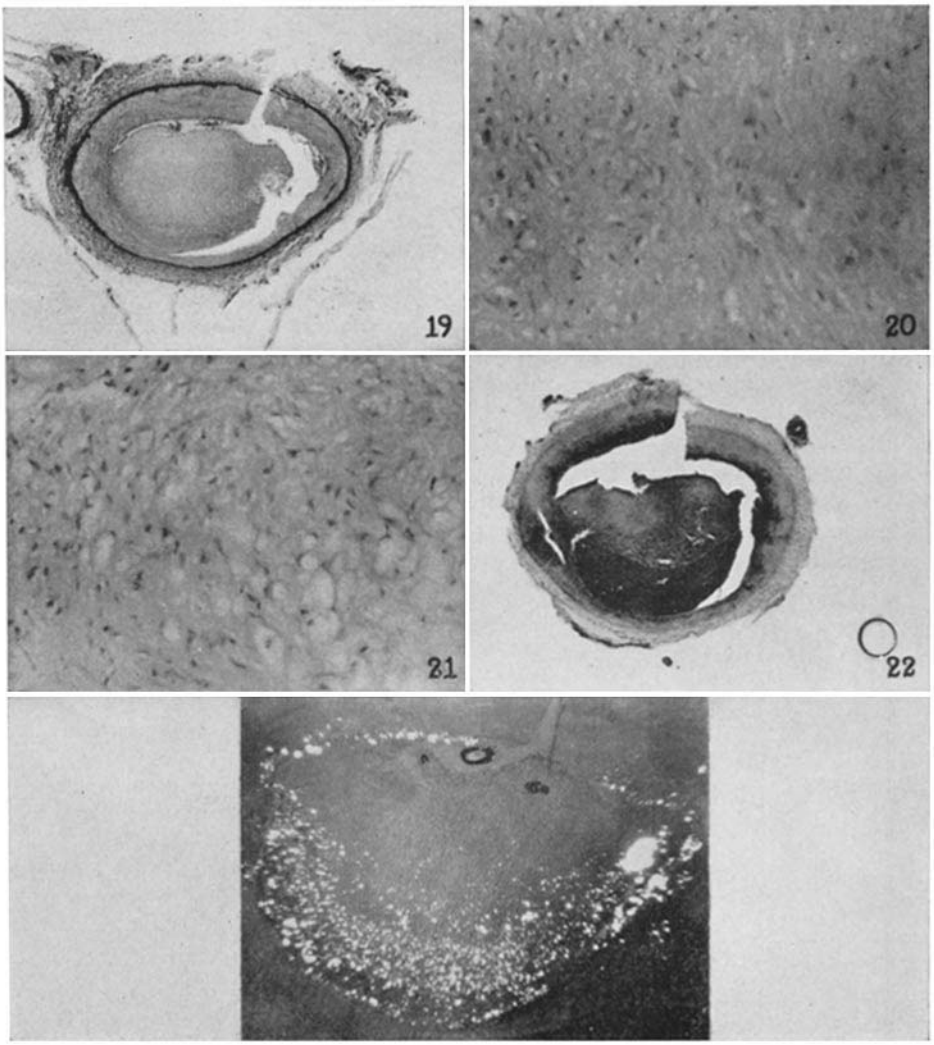
FIG. 19. Bird 15-65. Abdominal aorta showing large fibrous abdominal plaque. Verhoeff-van Gieson stain. × 23.

FIG. 20. Bird 15-65. Abdominal aortic plaque showing mild vacuolization of connective tissue cells. Hematoxylin and eosin stain. × 210.

FIG. 21. Bird 15-65. Abdominal aortic plaque showing marked vacuolization of connective tissue cells. Hematoxylin and eosin stain. × 210.

FIG. 22. Bird 15-65. Abdominal aorta showing large intimal plaque with lipid deposition in plaque and remainder of vascular wall. Sudan IV and hematoxylin stain. × 23. (Compare with Fig. 7.)

FIG. 23. Bird 15-65. Abdominal aorta showing large intimal plaque with cholesterol in sites of lipid deposition. Polarized light. × 35. (Compare with Fig. 8.)




(Chaikoff *et al.*: Atheromatosis in aorta of bird)

*Cholesterol Group*

PLATE 20

FIG. 24. **Bird 15-84.** Thoracic aorta showing intimal thickening. Hematoxylin and eosin stain. × 78.

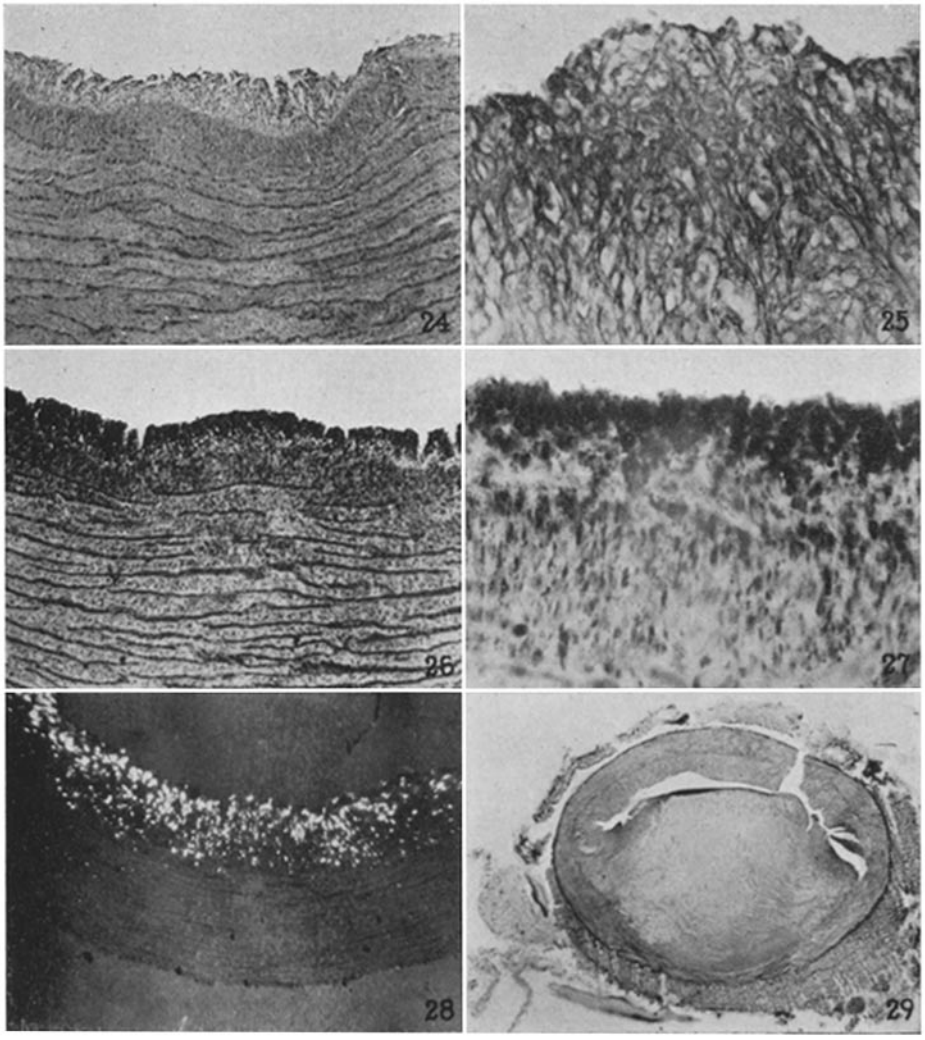
FIG. 25. **Bird 15-72.** Thoracic aortic intima showing perpendicular arrangement of reticulum fibers. Laidlaw stain. × 210.

FIG. 26. **Bird 15-26.** Thoracic aorta showing lipid infiltration of the intima and adjacent media. Sudan IV and hematoxylin stain. × 78.

FIG. 27. **Bird 15-84.** Thoracic aortic intima showing lipid droplets in connective tissue foam cells. Sudan IV and hematoxylin stain. × 210.

FIG. 28. **Bird 15-18.** Thoracic aorta showing deposition of cholesterol in intima and adjacent media. Polarized light. × 35. (Compare with Figs. 13 and 18.)

FIG. 29. **Bird 15-07.** Abdominal aorta showing large fibrous plaque and extensive intimal thickening. Verhoeff-van Gieson stain. × 23. (Compare with Figs. 5 and 19.)




(Chaikoff *et al.*: Atheromatosis in aorta of bird)

*Cholesterol Group*

PLATE 21

FIG. 30. Bird 15-07. Abdominal aorta showing large intimal plaque with lipid deposition in plaque and remainder of vascular wall. Sudan IV and hematoxylin stain. × 23. (Compare with Figs. 7 and 22.)

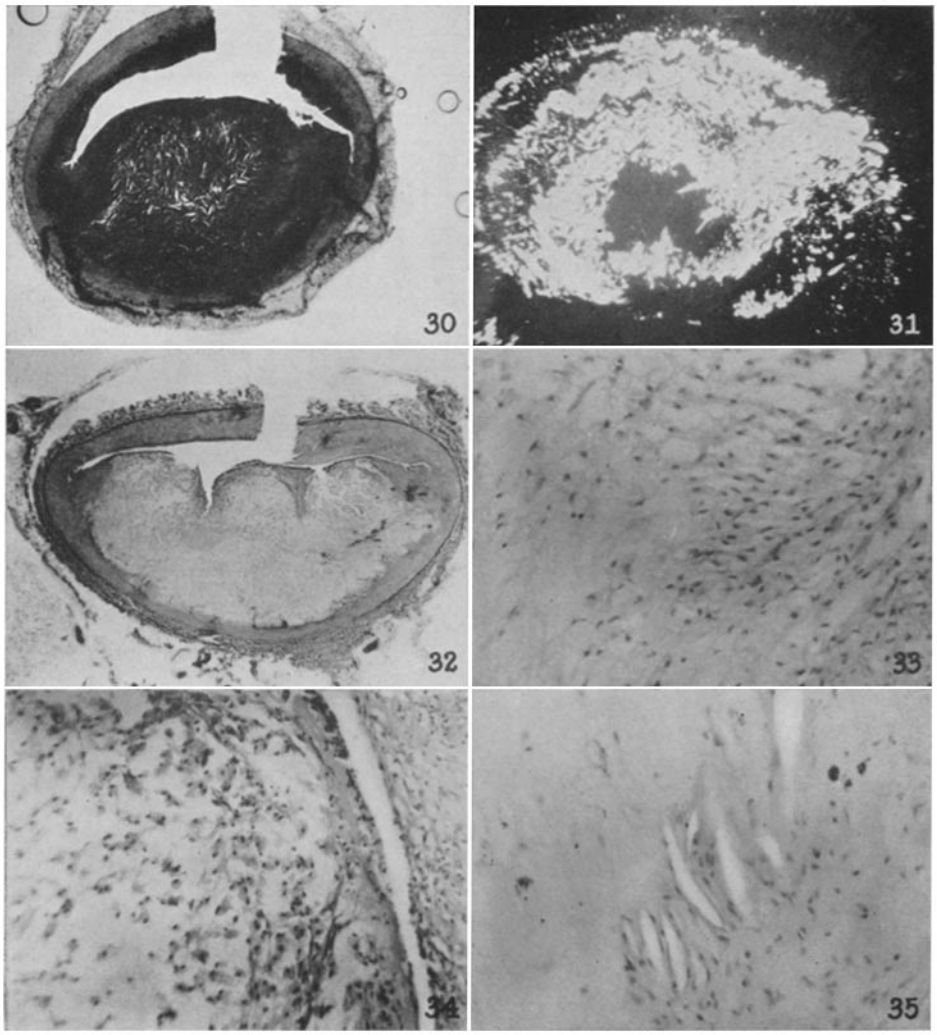
FIG. 31. Bird 15-07. Abdominal aorta showing large intimal plaque with abundant cholesterol in sites of lipid deposition. Polarized light. × 35. (Compare with Figs. 8 and 23.)

FIG. 32. Bird 15-84. Abdominal aorta showing large fibrous plaque and intimal thickening. Verhoeff-van Gieson stain. × 23. (Compare with Figs. 5 and 19.)

FIG. 33. Bird 15-84. Deeper portion of abdominal plaque showing vesicular fibroblasts. Hematoxylin and eosin stain. × 210.

FIG. 34. Bird 15-84. Superficial portion of plaque showing collections of macrophages. Hematoxylin and eosin stain. × 210.

FIG. 35. Bird 15-84. Abdominal plaque showing cholesterol clefts and calcific deposits. Hematoxylin and eosin stain. × 210.




(Chaikoff *et al.*: Atheromatosis in aorta of bird)