

Response of Arterial Wall to Intramural Cholesterol.*† (24180)

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Winternitz *et al.*(1) have suggested that hemorrhage from the vasa vasorum is the first step in pathogenesis of atherosclerosis. Cholesterol and cholesterol esters left behind after resorption of more soluble constituents of blood, were thought to be capable of inciting intimal proliferative changes. Wartman and Laipply(2) injected homologous whole blood into wall of femoral artery of dogs to establish the role of intramural hemorrhages in atherogenesis. Medial scarring was observed, but intimal changes were not found. Christianson(3) produced intimal proliferative changes in the dog by intramural injection of cholesterol dissolved in human fat. Interpretation of these findings is difficult, however, because of the complexity of the injected material.

Materials and method. White Leghorn cockerels of 1 to 1.5 kg were anesthetized with intravenous sodium pentobarbital (30 mg/kg). The cockerels were then intubated with a soft rubber catheter as endotracheal tube. Artificial respiration was maintained by means of pump with alternating positive pressure. An incision then was made between third and fourth ribs on left side. The heart, aortic arch and left brachiocephalic artery were exposed by retracting the ribs. This resulted in an orifice through which the brachiocephalic artery could easily be pulled up to insert a 20 gauge needle. The material injected was 0.2 ml of a 20% suspension of crystalline cholesterol in 0.9% saline solution. All injections were made directly into the adventitia or media of left brachiocephalic artery. Four groups of 10 birds each were handled in this manner. Diets of individual

groups were: 1) growing mash only; 2) growing mash plus 8% lard; 3) growing mash plus 8% corn oil; 4) growing mash plus 4% lard, 4% corn oil and 1% cholesterol. Two birds from each group were killed at 2 week intervals over a 10 week period and the right and left brachiocephalic arteries were removed for study. The right brachiocephalic artery served as control for spontaneous appearance of atherosclerosis. In addition, a group of 10 birds were fed only growing mash and were injected with 0.2 ml of 0.9% saline solution without cholesterol to determine the effect of trauma by needling. The arteries were embedded in paraffin and studied in step sections at 200 μ intervals. Histologic staining technics used were: hematoxylin and eosin, periodic acid-Schiff (P.A.S.), Verhoeff's elastic tissue stain, and phosphotungstic acid hematoxylin (P.T.A.H.).

Results. A foreign-body reaction to cholesterol was noted in adventitia or media at site of injection. This consisted of foreign-body giant cells, cholesterol slits, and increased amounts of connective tissue. Most injections were in the adventitia.

The most striking finding, however, was a focal proliferation of collagenous connective tissue in the intima (Fig. 1). Definite proliferative changes were found as early as 2 weeks after injection of cholesterol, but reached full development in a month. This reaction was present although often the media was anatomically intact. As a result of localized increase in connective tissue, the lumen was partially occluded and on occasion was almost completely obliterated. The plaques were P.A.S. negative and elastic tissue was not present. In the P.T.A.H. preparations, the reaction was that of collagenous connective tissue. The connective tissue plaques were often at a distance from the foreign-body reaction. Endothelial cells occasionally were increased in number. In the

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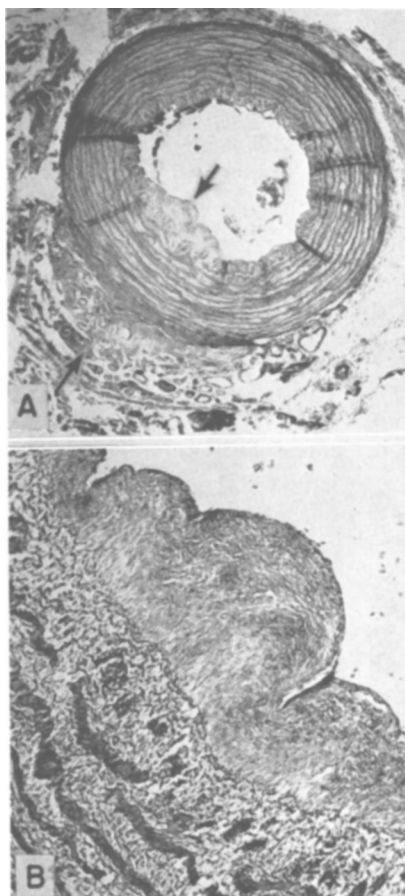


FIG. 1. (A) Cross-section of avian artery showing response to cholesterol in adventitia. A foreign-body reaction (lower arrow) is seen in the perivascular space, and an intimal plaque protrudes into the lumen (upper arrow). Note that the media is intact. Hematoxylin and eosin technic, $\times 15$. (B) Higher power view of proliferated connective tissue in the intimal plaque. Hematoxylin and eosin technic, $\times 110$.

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tive tissue plaques were often at a distance from the foreign-body reaction. Endothelial cells occasionally were increased in number. Spontaneous atherosclerosis was not found under the experimental conditions. The saline injected controls revealed only a needle tract. Connective tissue and intimal proliferation were not present.

Intimal plaques occurred in 75% of birds injected with cholesterol. This proportion was similar in each of the 4 dietary groups. The development of lesions, therefore, bore no relation to degree of saturation of ingested fat or to enrichment of diet with cholesterol. The lesions are therefore the result of local deposition of cholesterol and are not affected by the diet.

Summary. A technic is described for study of response of avian arteries to intramural injection of cholesterol. This material, when injected into the wall of the artery, causes a foreign body reaction at site of injection. In addition, a localized proliferation of collagenous connective tissue is found in the intima. These intimal plaques resemble some forms of human atherosclerosis. The development of the plaques is not affected by adding cholesterol or various fats to the diet. These findings suggest that the lesions are the result of the local metabolic alteration.

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