THE EXPERIMENTAL PRODUCTION OF ARTERIOSCLEROSIS:
RESPONSE OF THE AVIAN ARtery TO INTRAMURAL CHOLESTEROL AND OTHER INSOLUBLE SUBSTANCES *

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Factors responsible for sclerosis of arteries may be different from those producing the fatty component of atherosclerosis. Plaques large enough to cause inadequate blood flow often contain more fibrous tissue than lipid. The production of the fibrous component in avian atherosclerotic plaques by the injection of cholesterol has been described previously. When a cholesterol suspension was injected into the wall of an avian artery, a foreign body reaction appeared at the site of injection. In addition, a plaque of proliferated collagenous connective tissue was formed beneath the intima. The development of plaques was not affected by adding cholesterol or various fats to the diet.

The present investigation has been undertaken to extend our observations on the response of the avian artery to cholesterol, and to determine whether the intimal proliferative lesions were caused merely by the presence of an insoluble substance or by the chemical action of cholesterol. Paraffin, an insoluble hydrocarbon, was chosen because it has some chemical similarity to cholesterol. Barium sulfate was used because it resembles cholesterol in its insolubility and particulate nature.

METHODS

The technique for intramural injection has been described. White Leghorn cockerels weighing 1 to 1.5 kg. were used. The cholesterol or barium sulfate was injected as a 20 per cent suspension in 0.9 per cent saline solution (0.2 ml.). The paraffin injections consisted of 0.2 ml. of slightly heated fluid paraffin which melted just above the body temperature of the chicken and became solid at 40° C. All injections were made directly into the adventitia or media of the left brachiocephalic artery.

The chickens were maintained on a diet of Purina Growing Mash throughout the experimental periods. Preliminary experiments with

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cholesterol and saline were carried out with 5 groups of 10 birds each; 2 birds from each group were killed at 2-week intervals to determine the best time for detailed study and the effect of diet. In later investigations, the chickens were divided into 4 groups of 10 birds each. These groups were: saline control, cholesterol injection, paraffin injection, and barium sulfate injection. All birds were killed after 6 weeks, and the right and left brachiocephalic arteries removed for examination. The right brachiocephalic artery served as a control for the spontaneous appearance of arteriosclerosis. The arteries were fixed in 10 per cent neutral formalin.

Frozen sections stained with Sudan IV were prepared from the arteries of 4 birds in each group. The remainder of the arteries were embedded in paraffin and cut in step sections at intervals of 200 µ. The staining techniques used were: hematoxylin and eosin, Sudan IV, periodic acid Schiff (PAS), Verhoeff's elastic tissue stain, phosphotungstic acid hematoxylin (PTAH), and Rinehart's stain for acid mucopolysaccharide.

RESULTS

Most injections were made into the adventitia. A small number were in the media, usually in the outermost portion but never in immediate proximity to the intima.

The intramural presence of cholesterol resulted in two major changes in the artery. A foreign body reaction was seen in the adventitia at the site of injection in every case; in addition, a plaque of connective tissue appeared beneath the intima at a short distance proximal or distal to this site in 75 per cent of the animals. The media itself usually was histologically intact. The foreign body reaction consisted of multinucleated giant cells, cholesterol slits, lymphocytic and mononuclear cells and increased amounts of connective tissue (Fig. 1). Sudan IV stains of the adventitial reaction to cholesterol revealed a large amount of neutral fat (Fig. 2); cholesterol is not sudanophilic. The fat was either free in the tissue spaces or in macrophages; it was not in the foreign body giant cells. In preliminary experiments, the lymphocytic and mononuclear cell response was greatest at 2 weeks, but then decreased. Giant cells and adventitial connective tissue were sparse during the first 2 weeks, but greatly increased thereafter.

The intimal plaques induced by the intramural injection of cholesterol in the adventitia appeared as masses of connective tissue beneath the intima. The commonest response was a small plaque of connective tissue protruding into the lumen (Fig. 3). Fibrin or other blood elements were not seen. The proliferation of connective tissue in some cases was so great that the arterial lumen was almost completely
obliterated (Fig. 4). The plaques gave negative reactions to the PAS and Rinehart stains, and did not contain elastic tissue or fat. The reaction with PTAH was characteristic of collagenous connective tissue. Endothelial cells were occasionally increased in number.

Barium sulfate caused proliferation of connective tissue at the site of injection. However, foreign-body giant cells were lacking, and the inflammatory response was minimal (Fig. 5). Crystals of the injected material were seen in the region of fibrotic reaction. It is noteworthy that the introduction of barium sulfate did not result in the appearance of sudanophilic substance. Only one of 10 birds receiving barium sulfate exhibited an intimal plaque. This plaque gave negative reactions to the PAS and Rinehart stains, did not contain elastic tissue or fat, and was small and tubular rather than focal. The reactive connective tissue here was morphologically different from that encountered in the cholesterol series. The connective tissue cells were concentrically arranged as opposed to a focal disorderly appearance in the reaction to cholesterol. Endothelial cells were not increased in number.

The intramural injection of paraffin elicited a still different reaction in the adventitia and media at the site of injection. There were no foreign-body giant cells. The reactive cells were predominantly lymphocytes accompanied by a few monocytes (Fig. 6). The site of the adventitial reaction contained spaces lined by connective tissue where the paraffin had washed out in the histologic preparation. Local destruction of muscle fibers associated with a lymphocytic response occurred where the paraffin had entered the media. The reaction to paraffin did not contain sudanophilic material. Intimal plaques were not seen.

Spontaneous arteriosclerotic lesions were never found in the control right brachiocephalic arteries.

**Discussion**

Winternitz, Thomas and LeCompte, in 1938, suggested that cholesterol and its esters, remaining after resorption of hemorrhages from the vasa vasorum, could cause intimal proliferation. Wartman and Laipple did not induce intimal alterations by injecting homologous whole blood into the wall of the femoral artery of dogs. Christianson produced intimal proliferative changes in the dog by the intramural injection of cholesterol dissolved in human fat. Interpretation of these observations is difficult, however, because of the complexity of the injected substances. Leary questioned the hypothesis of Winternitz and his co-workers because “atherosclerosis begins in the most superficial layers of the intima in the region farthest removed from the distribution of vasa vasorum.”
The adventitia of arteries does contain numerous vasa. On the basis of our experiments, it is evident that cholesterol in the adventitia is capable of inducing an intimal arteriosclerotic plaque. We have demonstrated that it was not necessary for the cholesterol crystals to be present in the media or subintima to elicit this phenomenon. Furthermore, the intimal plaques induced by intramural injection of cholesterol were frequently not adjacent in the longitudinal plane to the site of adventitial injection. The plaques were located either proximally or distally to the largest part of the adventitial foreign body response to cholesterol.

The large amount of free and intracellular sudanophilic material at the site of cholesterol deposit was of interest because of the frequent association of free fat and cholesterol crystals in human atherosclerotic plaques. Fat was not found, however, within the cholesterol-induced avian plaque. The ability of cholesterol to cause subintimal plaques when injected into the adventitia or media was not shared by barium sulfate or paraffin. The effect of cholesterol, therefore, was characteristic of this substance and was not merely the response of the artery to an insoluble or foreign compound. Fat was not seen at the sites of injection with either barium sulfate or paraffin.

The 3 coats of the arterial wall differed greatly in their biologic responses to the 3 injected substances. The adventitia had the most diverse reactions. Cholesterol caused a foreign-body reaction and a transient inflammatory reaction, proliferation of connective tissue and free fat; barium sulfate caused only an increase in connective tissue; whereas paraffin resulted in an inflammatory reaction, predominantly of lymphocytes.

By contrast, the media had little reactivity. Increased amounts of connective tissue rarely occurred as a result of the injections, and when present, appeared as extensions from the adventitia. Usually the media suffered a focal loss or interruption of elastic fibers, or was normal in appearance.

The intima had an even more stereotyped reaction. Plaques of connective tissue usually formed after the intramural introduction of cholesterol, and rarely or not at all after the introduction of barium sulfate. In the case of paraffin, the intima was not altered at all. Injury to the intima by other means results in formation of increased amounts of connective tissue. This has been shown by Duguid who passed threads through the arterial wall of dogs, and by Christianson, using intramural cholesterol dissolved in human fat.
SUMMARY

The injection of cholesterol into the adventitia of the avian artery caused a reaction consisting of foreign-body giant cells, cholesterol slits, and increased amounts of connective tissue. Free fat was abundant in the tissue spaces and within macrophages at the site of injection. Also induced was a focal proliferation of connective tissue in the intima in 75 per cent of the birds, even though the media was histologically intact. The plaques ranged in size from small elevations of the intima to almost complete occlusion of the arterial lumen.

The intramural injection of paraffin or barium sulfate induced a response at the site of injection which differed from that caused by cholesterol. The response was largely of lymphocytes when paraffin was injected, and of connective tissue with barium sulfate. Neither of these substances was accompanied by an accumulation of sudanophilic material. A small intimal plaque was seen in only one of 10 birds receiving the barium, and none occurred after paraffin injection. The response of the avian artery to intramural cholesterol, then, had some specificity, because plaque formation did not occur with the other compounds tested.

The 3 layers of the avian arterial wall differed greatly in their reaction to foreign substances inserted intramurally. The adventitia had diverse reactions; the media suffered only focal loss of elastic fibers. The intima appeared to be capable of only one reaction, the formation of a plaque of connective tissue, when the appropriate stimulus was delivered.

REFERENCES

LEGENDS FOR FIGURES

Fig. 1. An adventitial reaction to cholesterol, showing proliferation of connective tissue. The spaces in the upper part of the field are cholesterol slits surrounded by multinucleated giant cells. Hematoxylin and eosin stain. $\times 120$.

Fig. 2. An adventitial reaction to cholesterol. Masses of lipid appear in the tissue spaces and within macrophages. The lower part of the field contains a few muscle fibers within a fibrotic reaction. Sudan IV stain. $\times 90$.

Fig. 3. A small plaque (arrow) beneath the intima appearing in response to cholesterol injection. A typical adventitial reaction is present. Note the intact media. Hematoxylin and eosin stain. $\times 110$.

Fig. 4. Reaction to cholesterol. The adventitial response is massive. A subintimal plaque almost occludes the lumen. This is not thought to be an organized thrombus. Hematoxylin and eosin stain. $\times 20$. 

Fig. 5. Adventitial reaction to barium sulfate. Moderately dense connective tissue is present in the upper part of the field. A few muscle fibers have been elevated, but the media is otherwise intact. Crystals are present but are not shown in the photograph. Hematoxylin and eosin stain. × 120.

Fig. 6. Adventitial reaction to paraffin. The predominant cells are lymphocytes. Hematoxylin and eosin stain. × 480.