ATHEROGENESIS AND PLASMA CONSTITUENTS

ROBERT H. MORE, M.D., AND M. DARIA HAUST, M.D.

From the Department of Pathology, Queen's University and Kingston General Hospital, Kingston, Ontario, Canada

Our previous observations indicated that the white plaque of arteriosclerosis is a prominent component of the arteriosclerotic lesion and that it represents the outcome of blood proteins deposited either on the intima as a mural thrombus or in its substance as an inflammatory exudate.[1-7] This field has been largely neglected, although McGill and colleagues [8] have more recently investigated it from another point of view. At this point it has seemed of interest to determine whether or not an atheroma had an origin similar to that of the white plaque. We have taken the term "atheroma" to refer to a local "pool" of largely extracellular lipids and tissue debris in the subendothelial connective tissue of the aortic and coronary artery intima. While this definition is generally accepted, some investigators [9] also include the fibrous component of arteriosclerotic lesions in this category. This is in accordance with the concept of Marchand.[10]

It is the purpose of this presentation to report on the morphologic features in the development of certain forms of atheroma and to evaluate their close association with unorganized remnants of some blood proteins deposited on or into the arterial wall in the course of the arteriosclerotic process.

MATERIAL AND METHODS

Material for this study consisted of 130 sections selected from 1,300 sections of arteriosclerotic lesions. These were obtained from 150 aortas and the coronary arteries of 100 hearts. The 130 sections chosen represented various stages of atheroma formation and contained lesions believed to be the precursors of atheromas.

Processing of the tissue was carried out as described elsewhere.[11] The following stains were utilized: hemalum, phloxine, saffron; pentachrome II (omitting elastica); elastica (Weigert-Hart, nuclear fast red, metanil yellow); Alcian blue, periodic acid-Schiff, hematoxylin, and orange G; phosphotungstic acid-hematein; Masson's trichrome; Fettrot and hematoxylin.

RESULTS

When the mural thrombus was small or the insudation into the intima was superficial, all of the blood protein substance was commonly "con-

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verted" to connective tissue elements by means of avascular organization progressing from the lumen. The organization was thus complete. When the thrombus, however, was of large size, two mechanisms of organization were encountered concomitantly. In this circumstance, avascular organization progressing from the lumen co-existed with orthodox vascular organizing tissue developing from the base of the thrombus, with capillaries growing in from the media (Fig. 1). When the organization by these two processes was complete, they were seen side by side. Frequently, however, remnants of fibrin remained between the two types of organization (Fig. 2). In addition, unorganized fibrin was present in the form of coalescent bands and clumps at the base of young connective tissue plaques. This was often seen in those situations where the thrombi were deposited on older, sclerotic fibrous plaques (Fig. 3) and probably represented a failure on the part of the latter to initiate organization.

Similar in nature but different in distribution was the appearance of fibrin remnants deep in the sero-fibrinous insudate within the intimal substance. Thus, the fibrin threads and strands when unorganized in the depth of the intimal substance formed numerous small clumps (Fig. 4) or became more coalescent and aggregated to form dense bands (Fig. 5).

In early lesions, scattered macrophages appeared in both insudative and thrombotic lesions. They were closely applied to the clumps and bands of fibrin remnants, and some of them had the typical appearance of lipophages (Fig. 6). The lipophages were numerous in advanced lesions. In addition, they were also seen to accumulate at the base of entirely unorganized thrombi which in turn were covered by one or more layers of recent thrombus deposit (Fig. 7). In more advanced lesions the lipophages disintegrated, thus becoming the nidus for atheroma, while others could be seen applied closely to the inconspicuous remnants of fibrin (Fig. 8). It was clear that the intra- and extracellular lipids accumulated as the fibrin and other plasma constituents disappeared. The outcome of this process was the familiar pattern of typical atheroma containing remnants of fibrin and covered by a fibrous cap (Fig. 9). The latter represented the superficial part of the thrombus which had been organized in avascular manner from the direction of the lumen. Often, small narrow bands of unorganized fibrin were also present within the fibrous cap, and a few lipophages applied to these bands signaled the development of an atheroma at this site (Fig. 9).

That an atheroma may indeed originate within a mural thrombus, notably at its base where organization fails to develop, was indicated by the unequivocal polypoid shape of some of the lesions containing atheromas. Thus, in a section of such a lesion the base was filled with

atheromatous substance while the portion next to the lumen consisted of an avascular fibrous connective tissue. The connective tissue elements were not sharply delineated from the atheromatous substance but blended imperceptibly with it. This showed that the deeper portion of the thrombus which did not become organized underwent alterations producing typical atheromatous material. The atheroma and the avascular organizing tissue overlying it unmistakably retained the shape of the original polypoid thrombotic implant (Fig. 10). An additional hallmark of thrombotic origin was the presence of fibrin within the lesion (Figs. 10 and 11).

An eccentric plaque encountered frequently, especially in coronary arteries, was often composed of several "layers" of sclerotic tissue (Fig. 12). The genesis of each of these layers is best explained as indicative of an episode of insudation or thrombus deposition followed by organization. Between some of the fibrous layers, small or large atheromas could be seen. Careful examination revealed that these atheromas were present at the bases of broad and thick fibrous layers. The shallow, narrow fibrous layers were commonly unassociated with atheromas since they were completely organized. This was in contradistinction to the thicker and more massive protein deposits.

Discussion

Historically, four principal theories have been advanced to explain the pathogenesis of atheroma. Each of these has received support and amplification by various workers. Rokitansky[12] considered the atheroma to result from the degeneration of plasma constituents, principally fibrin, deposited on the intima of the vessel wall. This has been referred to by British workers as "encrustation."[13,14] Virchow[15] doubted that subendothelial accumulations could be the result of surface deposition and considered that the atheroma reflected degeneration following an inflammatory process within the intima. He based this concept upon the observation of a mucinous change in the intima preceding the formation of atheroma. He believed that the atheroma then served as a stimulus to connective tissue proliferation resulting in the formation of fibrous caps. Another theory and the one most widely favored has been that of Marchand.[10] He considered that there was selective imbibition of lipids into the arterial intima, with the formation of lipid pools and a resulting connective tissue proliferation. Finally, Winternitz, Thomas and LeCompte[16] postulated another theory, based on the original observations of Paterson,[17,18] who described vascularization and hemorrhage in the arteriosclerotic intima of coronary arteries. According to them an atheroma developed on the basis of a hemorrhage from intimal capillaries.

They observed that at the site of hemorrhage there was a gradual accumulation of lipoid substance and so-called "cholesterin clefts" culminating in atheroma formation.

The common feature in the theories advanced by Rokitansky, Virchow, and Winternitz and co-workers was the recognition of the role of blood proteins in atheroma formation. In previous publications [1-6] we have shown that the fibrous tissue associated with arteriosclerosis arises principally by the organization of accumulated blood proteins often rich in fibrin, deposited either on the intimal surface or in the subendothelial tissue. The observations reported here have shown that atheromatous as well as connective tissue plaques may develop from the accumulations of blood proteins. This is probably the result of degenerative changes following incomplete organization of inflammatory exudates or fibrin deposits on the endothelial surface. These observations thus incorporate elements of both Rokitansky's encrustation theory [12] and Virchow's intimal inflammation theory [15] and serve to re-emphasize the fundamental role of blood accumulations in the genesis of both the atheroma and the fibrous plaque. This work supports the findings of Clark, Graef and Chasis,[19] Duguid [14] and Crawford and Levene,[13] showing that in man, mural thrombosis represents a stage in the genesis of some atheromas. The experimental work of McLetchie [20] also supports this mechanism. In his experiments mural thrombosis of pulmonary arteries was induced by the injection of Russell's viper venom into rabbits. Failure of complete organization of the mural thrombi was associated with the accumulation of lipids. Thus, while the superficial (lumen) layers of these thrombi underwent organization, the deeper portions underwent fatty changes; the resulting lesions resembled those of human arteriosclerosis.

These observations establish the sequential relation between the accumulation of blood proteins in and on the intima and the genesis of atheroma. They, however, do not provide information on the factors which promote this sequence. Aside from the size of thrombi and the amount of insudate, there is no explanation in this or previous studies [1-6,21,22] indicating why one mass of intimal protein was completely organized while in another instance this was only partially accomplished. Furthermore, the observations provided no evidence on which to judge whether or not some disturbance in the metabolism of the body lipids may have played a role in certain stages of the progressive changes observed. In all samples of blood, lipids are demonstrable chromatographically on electrophoretic strips. The failure to demonstrate lipids readily in all protein deposits by some of the techniques used poses a problem in the interpretation of the pathogenesis of atheroma from the accumu-

lation of blood proteins. This may be a matter of the degree of lipids binding to proteins. However, it poses the question of whether or not there is some source of the accumulated lipids other than their derivation from the deposited proteins. Whatever the source of the lipids, these investigations indicate that atheromas as well as fibrous plaques may arise from mural thrombi or inflammatory exudates or both. The possibility that some atheromas may originate in a different way cannot be dismissed.

Summary

Atheroma formation has been found to be closely associated with unorganized remnants of mural thrombi on the one hand or deep sero-fibrinous intimal insudates on the other.

Atheromas appeared to develop from a few lipophages closely applied to fibrin remnants; in advancing lesions these accumulated and subsequently disintegrated. Although fibrin remnants became diminished, a few strands and bands were often found in typical atheromas.

The predilective sites for atheroma formation were found to be at the bases of mural thrombi where organization failed to occur, and within a layer of unorganized thrombus on which more recent thrombi were superimposed. In other instances this was observed between two types of organizing tissue (avascular, stemming from the lumen, and conventional vascularized tissue from the vessel wall) which failed to meet, thus leaving a layer of unorganized fibrinous material between them. Additional sites were encountered at the bases of thrombi deposited on densely sclerotic plaques and in the depth of the intima where massive sero-fibrinous insudate failed to organize.

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[*Illustrations follow*]

Legends for Figures

Fig. 1. Coronary artery. The eccentric thickening is made up of organizing thrombus. The organization from the lumen is avascular (homogeneous light gray in the photograph) whereas that at the base is orthodox in type with numerous capillaries and collagen fibers developing in a criss-cross fashion. Remnants of thrombotic material are seen as black clumps between the two types of organization. Pentachrome II stain (elastica omitted). × 25.

Fig. 2. Higher power view of the area between the two types of organization (avascular at the top) with clumps of thrombotic substance intervening (black in photograph, orange-yellow in section). Stain as in Figure 1. × 82.

Fig. 3. Coronary artery. A young connective tissue plaque (top, light gray) overlying an old sclerotic one (lower right corner). A considerable amount of thrombotic remnant is seen between the two plaques. The organizing tissue at the base contains capillaries, while the part of the plaque adjacent to the lumen is avascular. Hemalum, phloxine, saffron stain. × 77.

Fig. 4. Aorta. Massive repeated sero-fibrinous insudation into the intima. Fibrin threads predominate in the middle and upper third of the intima, representing a more recent insudation. Fibrin clumps and bands dominate the lower third, representing a previous insudation. Note the swollen (light gray) ground substance and distorted fragments of connective tissue fibers. The latter are absent in the upper part of the intima. Lipophages are seen in association with some fibrin clumps. Phosphotungstic acid-hematein stain. × 102.

Fig. 5. Aorta. Dense masses of fibrinous substance deep in the intima represent the remnants of an unorganized fibrinous insudation. In addition, the intimal surface is covered by mural thrombi over an extensive area. Hemalum, phloxine, saffron stain. × 77.

Fig. 6. A high power view of the intimal fibrin remnants in Figure 5. Macrophages are scattered in the area and a few lipophages are applied to the fibrin masses. Stain as in Figure 5. × 244.

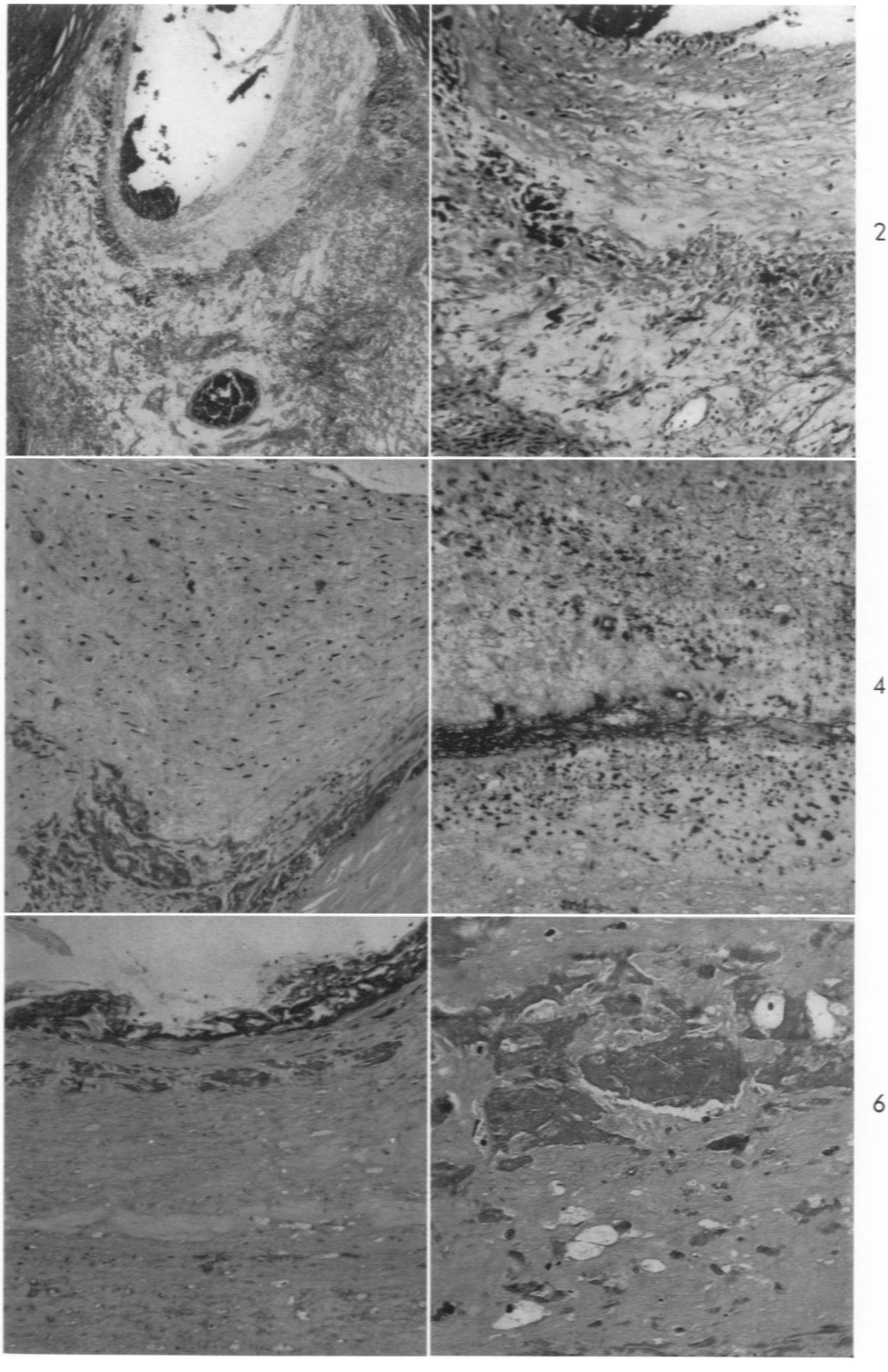


FIG. 7. Aorta. Three different layers of thrombus deposition (B, C, D) overlie the intima (A). Clusters of lipophages are seen within the oldest thrombus deposit (B) immediately overlying the intima. In some areas the accumulated lipophages have disintegrated, leaving a small pool of lipid (light) within the thrombotic coalescent mass. Phosphotungstic acid hematein stain. × 102.

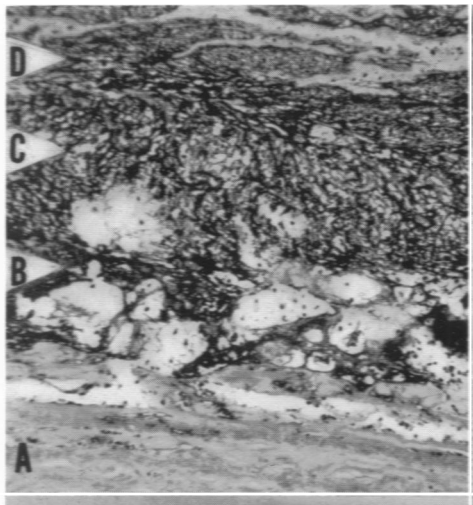
FIG. 8. Aorta. The intima (A) is covered by a plaque (B) which in turn is covered by layers of thrombi. The oldest thrombus (C) blends with the substance of the plaque. Remnants of unorganized fibrin within the plaque are closely associated with lipophages. Accumulation and disintegration of the latter signals an atheromatous nidus (D). Masson's trichrome stain. × 77.

FIG. 9. Aorta. An atheroma (bottom) containing remnants of unorganized fibrin (black) is covered by a fibrous plaque containing numerous cells. A few clumps of fibrin remain in the plaque to the left. Note how intimately the connective tissue of the plaque blends with the atheroma. Hemalum, phloxine, saffron stain. × 36.

FIG. 10 Coronary artery. A large polypoid plaque almost completely occludes the lumen. Its shape unequivocally reflects its origin from a thrombus; this also holds true for the atheroma contained within its substance. A few remnants of thrombotic material are still present in the atheroma. Masson's trichrome stain. × 29.

FIG. 11. A higher power view of the fibrin remnants in the substance of the atheroma seen in Figure 10. Stain as in Figure 10. × 102.

FIG. 12. Coronary artery. An eccentric plaque is composed of several "layers" of sclerotic tissue, some being rich in elastic fibers (fine black fibers). The genesis of each of these layers is best explained as an episode of either insudation or thrombus deposition with subsequent organization. A small atheroma (whitish light gray) is present between the second and third layers from the lumen, just above the horizontal black line of elastic tissue. Weigert-Hart, nuclear fast red, metanil yellow stain. × 39.



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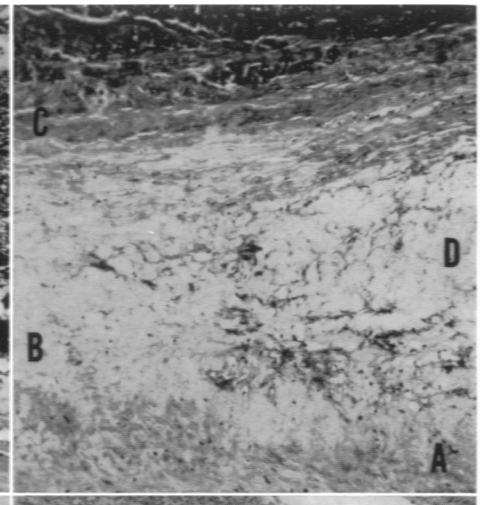

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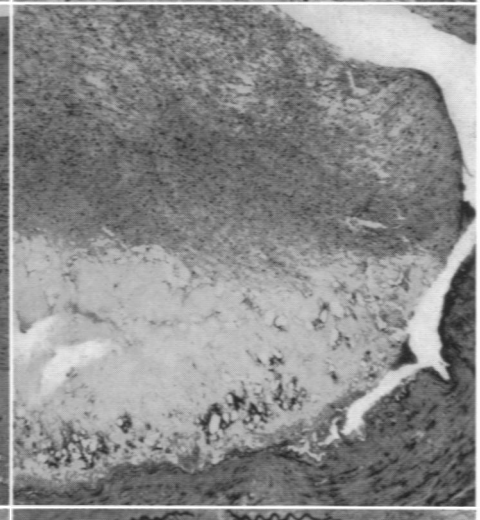

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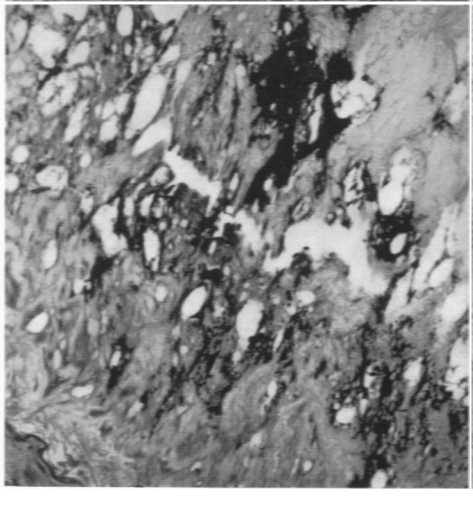

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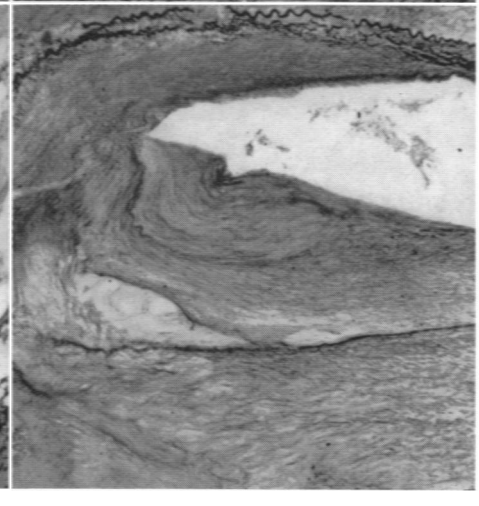

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