HYPOTHESIS ON PATHOGENESIS OF HUMAN ATHEROSCLEROSIS

Some of the histological and functional characteristics of the intimal lining of the arteries, particularly the presence of different cell types in a ground substance matrix and the absence of a direct blood supply (see page 24), have helped one to understand the histogenesis of spontaneous atheromas, often initiated, in man, shortly after birth.

The well-developed atherosclerotic plaque, resulting from the interplay of inflammatory and reparative processes, is a complex lesion, containing extracellular deposits of calcium salts, blood components, cholesterol crystals, and acid mucopolysaccharides. The initial changes, however, seem to occur at the cellular level; electron microscopy has shown that they are often accompanied by an abnormal intracellular storage of lipids, particularly cholesterol esters, fatty acids, and lipoprotein complexes. These findings have strengthened the thesis that lipid infiltration from the bloodstream may be a significant factor in the growth of the atheromatous plaque. Furthermore, since lipids may be recognized by cytotoxic techniques, they have been used as helpful indicators of abnormal cell behavior, independent of their role in the etiology of atheroma.

Because of the severity and frequent occurrence of atherosclerosis, the use of short-term organ-culture techniques for the isolation of human arterial intimal cells or intimacies has been applied to the identification of susceptible cell populations, based on their response to incorporation of homologous-serum lipids in vitro. Intimacies from arteries, with and without histological evidence of atherosclerosis, have shown, respectively, two different types of cells or atheroïds—one which is genetically highly susceptible to intracellular lipid accumulation, the other genetically resistant.

Based on these findings, the following hypothesis is advanced: Susceptible atheroïds, in the presence of increased perfusion rates of plasma lipoproteins due to local hemodynamic changes, hypertension, elevated serum lipids, or changes in the permeability of the endothelial surface (trauma, fibrin deposition, platelet aggregation), will be transformed into lipoid-laden atheroïds. The secondary release of intracellular lipids from these cells will induce surrounding atheroïds to incorporate them, creating a self-fueling process with cytological changes that increase metabolic requirements, including oxygen-consumption rates, which, if not met, result in increased permeability of the cell membrane to lipids. These events set up a vicious circle that favors further expansion of focal atherosclerotic changes. The latter include the laying down of collagen fibers by fibroblasts that are then transformed into fibrocytes surrounded by acid mucopolysaccharide deposits; this eventually results in a characteristic intimal hyperplasia, following ground substance changes. These histological lesions are self-perpetuating, resulting in the replacement of intimacies by an acellular area of arterial intima that stimulates further surface involvement of the vascular wall in this interplay between deposition, inflammatory response, and scarring, ending with the eventual production of a typical atherosclerotic plaque.

In contrast, genetically resistant atheroïds, even in the presence of elevated serum-lipid levels, do not respond (or respond only in a limited fashion) to an increased perfusion of plasma lipids. If some atheroïds appear, they are few in number, and only superficial fatty streaks are developed, resulting in minimal intimal elevations with few fibrocytes and the absence of a well-organized plaque. This type of cytological response seems also less susceptible to the clinical complications of atherosclerosis (particularly thrombosis or rupture) owing to the limited involvement of the vascular wall.

This concept of the pathogenesis of human atherosclerosis emphasizes the clinical significance of identifying, at the earliest possible time, those individuals whose arteries are susceptible to atheroma, in an effort to modify or prevent those environmental conditions, for the cells of the arterial intima, which favor the acceleration of atherogenesis. This hypothesis also stresses the importance of local factors in the localization of the lesions, suggesting possible therapeutic approaches for their arrest or inhibition.
Factors in Etiology of Atherosclerosis

In terms of mortality, certainly the most important single problem facing the more highly developed countries is atherosclerosis of the cardiac and cerebral blood vessels. In the past 2 decades it has become increasingly evident that there is no single cause of atherosclerosis (see also Ciba Collection, Vol. 4, pages 212 and 213), and this is why Page, years ago, labeled it a "multifaceted disease" and a "disease of regulation." Atherosclerosis seems to reflect the culmination of many factors acting over a lifetime, and it usually becomes clinically manifest only when complications, such as thrombosis or aneurysm, occur.

All students of the subject are agreed that it has a strong hereditary background. So far, no one has been able to alter this aspect. The environment also may have an important contribution to make, but specific factors in it have not been positively identified. The hardness of the water is said by some to be correlated with the incidence of atherosclerosis, but, as yet, there is no general acceptance of this thesis.

Recently, cigarette smoking has been found to be closely correlated with the incidence of coronary atherosclerosis and, as a result, cigarettes are being widely, if not altogether successfully, interdicted. Lack of exercise is also believed to be one of the deficiencies associated with myocardial infarction and coronary atherosclerosis. Some believe that the function of exercise is to improve collateral circulation and to increase the efficiency of oxygenation of the myocardium. It is being widely recommended for victims of infarction.

Excessive saturated fat in the diet is still another factor believed to be involved importantly in atherosclerosis. Countries in which the fat content of the diet approaches 40 percent of the total calories have an inordinately high incidence of coronary heart disease. The high-fat diets are believed to achieve their deleterious effects by increasing the levels of the blood lipids. Some consider that the cholesterol content of the blood is the most important constituent, while others stress triglycerides. Probably both are concerned through mediation of the lipoproteins, which act as the primary transport mechanism for lipids in the blood. Cholesterol is a convenient clinical measure of certain types of lipoproteins.

Strong evidence suggests that unsaturated fatty acids with two or more double bonds aid in reducing cholesterol blood levels when their relationship to saturated fatty acids is increased. These are the bases for the current thinking regarding dietary control of atherosclerosis.

Currently there are two main streams of thought about atherosclerosis: (1) that it is chiefly due to infiltration of the blood vessel by fat from the bloodstream, and (2) that it is due to breaking down of fibrin and/or platelet aggregates on the surface endothelium, followed by overgrowth of the latter to form a plaque; lipid infiltration is then a secondary phenomenon. Doubtless, both are correct and both simplistic.

The first hypothesis has led to an enormous increase in the study of the metabolism of cholesterol and other lipids that are found associated in atherosclerotic lesions. It has also led to a frantic search for drugs that lower blood lipids, because of the repetitive demonstration of the close association between cholesterol levels and the incidence of heart attacks. Currently de-thyroxine, nicotinic acid, Atromid-S®, and estrogen are under the most intensive study. Estrogens were introduced largely because it was found that women, before the menopause, were about one fourth as susceptible to myocardial infarction as men.

The second hypothesis also has many adherents. Attempts have been made to associate the "stickiness" of the platelets to their aggregation as well as to injury of the blood-vessel endothelium. The conversion of fibrinogen to insoluble fibrin is also important in providing a matrix for surface thrombi. Capillary hemorrhage within the blood-vessel wall is usually considered to be secondary to the primary facets of atherosclerosis, but any hemorrhage large enough to reduce the lumen of a coronary artery is potentially dangerous.

The endocrine glands have been closely associated with atherosclerosis through a variety of mechanisms. The pituitary gland seems to exert some effect indirectly, through its regulatory arteries, on other endocrine glands. Reduced thyroid secretion is believed to accelerate atherosclerosis. There has been no specific implication of the suprarenal corticoids but the catecholamines have been widely suspected. One view is that their effects are chiefly on the oxygenation of the myocardium, while others suggest that they mediate the effects of emotional strain and tobacco. There is clearly no unanimity on the mechanism of their participation. The influence of “femaleness” and the estrogen has already been mentioned. "Malesness" and the androgens may accentuate atherosclerosis, but the proof for this effect is less convincing. The pancreas has been associated with atherosclerosis because of the part it plays in diabetes and carbohydrate metabolism. One of the major complications of this disease is atherosclerosis; it appears earlier and is more severe than in healthy persons.

Obesity may or may not be associated with endocrine disturbance, but it is associated, to some degree, with increased atherosclerosis, although probably not as importantly as it formerly was supposed.

The clinical effects of atherosclerosis are often determined by secondary factors. The anatomy of the blood supply to an organ may be critical, especially in the pattern of the coronary vessels. A strategically located atherosclerotic plaque may do far more damage than extensive, diffuse atherosclerosis. The formation of a clot, either on the surface of a plaque or by rupture of an atheromatous ulcer, may be the first clinical evidence of the presence of atherosclerosis. The effects of the latter have even been seen by the appearance of cholesterol crystals in the vessels of the eye.

Much emphasis has been given recently to identifying what is known as the "coronary profile." These are persons believed to be highly susceptible to coronary atherosclerosis and myocardial infarction. They are characterized by being male, short, stocky and muscular, and excessively aggressive, smoking large numbers of cigarettes, being obese, taking little exercise, being under emotional pressure, and having elevated blood cholesterol and triglyceride levels and, often, abnormal glucose tolerance. Friedman and Byers place more emphasis on the psychological responsiveness as a means of characterizing the people. While the "coronary profile" is a useful clinical generality, it often is inept in its predictions.

In summary, it is now clear that atherosclerosis results from intimal and medial cellular defects that depend on the cellular genetic makeup interacting over long periods of time with exogenous and endogenous environmental factors. This is why it has been called a "multifaceted disease of regulation."
Structure of Coronary Arteries

In man, the coronary arteries are susceptible to atherosclerosis as well as to its complications, particularly intravascular thrombosis, often resulting in myocardial infarction. Atherosclerosis is a form of arteriosclerosis characterized by initial involvement of the inner layer of the arterial wall or intima; the intimal localization of early atheromas helps to differentiate this form from other types of arteriosclerosis, such as Mönckeberg's medial sclerosis or peripheral arteritis nodosa, that primarily involve the muscular or adventitial layers.

Recent histochemical, organ-culture, and electron microscopic studies have brought forth evidence of the complexity of the arterial wall. As shown, a medium-sized muscular artery, such as a main coronary vessel, consists of a series of concentric tubes or coaxial coils of differentiated cellular and extracellular components in three layers: tunica intima, tunica media or muscular, and tunica adventitia. In the intima the innermost cell layer in direct contact with the bloodstream consists of a sheet of polygonal endothelial cells, usually less than a micron thick, except at the site of the cell nucleus, and elongated in a direction parallel to the vessel's axis. Many of these endothelial cells have pinocytotic vesicles of "cavolins" in their cytoplasm, abundant mitochondria, well-developed granular endoplasmic reticulum, and Golgi complex. The areas of contact between endothelial cells, under the electron microscope, vary from simple mutual contact of the cell membranes to well-defined intercellular bridges or desmosomes, corresponding to the so-called intercellular cement lines described in earlier light microscopic studies. A distinct basement membrane separates these endothelial cells from the subendothelial space, which varies in thickness not only because of the size of the artery under study but also according to the age of the subject. In fetal life and shortly after birth, the endothelium in the coronary arteries lies in direct contact with the internal elastic membrane and lamina, without the subendothelial space that appears by the end of the first decade of life. In adulthood the intima consists of a matrix of ground substance containing small amounts of acid mucopolysaccharides and elastic and collagen fibers separating scattered intimal cells or intimacies.

Because of morphological and cytological differences in the intima, their response to in vitro incorporation of serum lipids has been used to identify them. Some intimacies or atheromata incorporate lipid rapidly and show ultrastructural characteristics of modified smooth-muscle cells, with typical bundles of myofilaments in their cytoplasm, pinocytotic vesicles, and portions of limiting basement membrane on the cell surface. On the other hand, other cells, fibroblasts, are spindle-shaped, have fingerlike cytoplasmic projections, an absence of basement membrane, and few pinocytotic vesicles. Occasionally, large mononuclear ovoid cells appear. These contain cytoplasmic inclusions with "single-unit" acid phosphatase positive granules or lysosomes, resembling macrophages. Separating the lamina proprius from the underlying smooth-muscle cells of the media is usually a well-developed internal elastic membrane consisting of a tenacious matrix containing fibrils approximately 500 angstroms in diameter and with abundant fenestrations. This internal elastic membrane or lamina is usually wavy in cross sections, and the fenestrations are oval or rounded openings extending across its surface.

The underlying tunica media is characterized by concentric layers of smooth-muscle cells measuring from 10 to 25 microns in length, oriented transversely to the main axis of the artery. Individual smooth-muscle cells are surrounded by a network of collagenous and elastic fibers, which continue without transition into both the internal and external elastic membranes. The external elastic membrane separates the media from the adventitia and is characterized by the presence of loosely packed collagen and elastic fibers. Small blood vessels or vasa vasorum as well as both sympathetic and parasympathetic nerve fibers from the autonomic nervous system are also found.

These morphologic and functional characteristics of the coronary arteries are modified early in the presence of the intimal changes characteristic of atherosclerosis (see page 215). The most important clinical difference between coronary and aortic atherosclerosis is the high incidence of acute thrombosis in coronary vessels, resulting, through infarction, in irreversible damage to the underlying myocardium.
Pathologic Changes in
Coronary-Artery Disease

Disregarding the various theories concerning the etiology and pathogenesis of athere-
orsclerosis (Caire's disease), the alterations produced by this glance at the present
is one that may be of interest to those who have studied the effects of various
vascular systems. The appearance of this lesion is similar to that seen in many of the experimentally
produced vascular lesions. Another apparently
early phenomenon, difficult to correlate and possibly not related, is a subintimal deposit
of collagen that often is primitive and hence, rich in mucopolysaccharides.
Lesions of this type shadow atherosclerosis wherever it occurs and, as previously described,
can involve the peripheral arteries of the body. The relationship of this vascular
lesion to the kind seen in Oriental races has not been elucidated, but this disorder
does occur in that racial group also. A further complication of this lesion is the presence of fibrin on or within its
superficial layers. As a consequence, the
complicating theories of zonal layers of thrombosis have crept into speculations concerning
evolution of the atheroma. Problems arise in
relating this collagenous to atherosclerosis.

As more lipid accumulates, an atheroma
plaque, which contains many constituents, is formed. This plaque may evolve only a segmental portion of the artery or can involve its entire circumference. (One
only admires the choice of the French
term in calling this grumous material
"putty"). When the macrophages die, their
contained lipid is released and serves as an irritant. Fibrin is accumulated, as are other
blood constituents, and the final reaction is
a stimulation of calcium deposition and the
proliferation of fibroblasts. How some of these lesions thicken is a matter of debate,
but many stenotic lesions appear to accumulate
more surface material by the deposition of superficial layers of thrombosis. That this
lesion, even though extensive, can regress is
demonstrated, in part, in individuals with
cirrhosis of the liver and in those who have
died of starvation.

Even a segmental atheroma can cause
atrophe of the underlying arterial wall, and the
concentric type seems to be associated with major destruction of the interna elastica
and portions of the media. Such changes
appear secondary to interference with the
nutrients permeating the intima. Careful
observation of these various stages of the
atheroma strongly leads to the belief that an inability to clear hemic constituents,
which have permeated the media, is a major
feature in the lesion's development. That
such a permeation is a most dynamic affair
can be demonstrated easily by perfusing isolated segments of living canine arteries and observing the quantity of lipid found in the vasa vasorum of the adventitia.

It is difficult to realize that, when the atheroma reaches the stage described above, it is still dynamic and still can undergo many changes. It can be dislodged from its moor-
ings and thereby serve as an embolus, or pieces of it may break off and embolize. The occurrence of hemorrhage into such a lesion has been described, and it is still debat-
able whether blood is dissecting into the plaque from the surface or by hemorrhage from the vasa vasorum; both routes probably contribute. As can be predicted, thrombosis is a complication that may completely occlude the artery. If this occurs and the individual survives, organiza-
tion of the fibrin clot progresses, with small new vessels
recanalizing the organized area. However, the ability of these small vessels to supply any appreciable volume of blood beyond the area of blockage is doubtful, and
this doubt is still further fortified by their appearance in arteriography. It also would seem possible that, on occa-
sion, a thrombus could completely disappear.

In addition to atheromatous and thrombotic blockage of the coronary arteries, emboli of all types can lodge within an otherwise-normal coronary artery. Such emboli can consist of bits of thrombi, valvular calcification, bits of tumor, and even, on occasion, small foreign bodies. Various kinds of inflammatory aortitis can involve the ves-
sels; syphilis of the aortic wall can occlude the ostia, and surgeons can inadvertently disturb the blood flow. All these events can result in severe myocardial difficulties.

The atheroma, however, is the main disorder, but it would probably not be significant if it were not for its distribution. In the case of the coronary arteries, there is usually diffuse involvement by the time anatomic stud-
ies are done, and the involvement seems to be predomi-
nantly in the "free" portions of the arteries, prior to their
branches entering the cardiac muscle. Those closest to the
ostia are the plaques usually seen and explained over;
since, logically, they would seem to be the ones most likely to interfere with cardiac circulation.
**Arteriosclerotic Heart Disease**

*Myocardial infarction* is the other side of the coin in the problem of coronary artery disease and, again, it exhibits an extensive panorama of changes. Death of the cardiac muscle may occur as a zonal variety or as multiple foci scattered diffusely throughout the heart. The latter can be found following suboptimal perfusion of the heart, utilizing a pump oxygenator, wherein all the minute necrotic foci are of the same age. More commonly, however, the multiple minute foci can be found as destroyed zones of varying ages. In the acute phases these microinfarcts may be extremely difficult to see and may be recognizable only microscopically as areas showing variable myofibril destruction and replacement by collagen. Chronically, these small foci may be minute white patches salted throughout the myocardium.

A larger myocardial infarct shows a variety of change that, in part, is dependent on the duration of the patient's survival following the episode of infarction. The earliest changes are associated with the resulting paralysis of vessel walls, engorgement of the capillaries, and migration of polymorphonuclear leukocytes. Some autolytic changes then occur, and the dead muscle fibers can be recognized as showing a loss of striations and an increase in eosinophilia, fragmentation, and the gradual disappearance of nuclei. The dead muscle, by this time, has become an irritant, and a larger number of polymorphonuclear leukocytes appear. Finally, the subsequent activities of repair include macrophage infiltration and replacement fibrosis. If the infarct is minute, such a sequence is of relatively short duration, but, if it is large, remnants of necrotic muscle may be found several years later. It appears that such dead tissue has been effectively sealed away from the physiologic activities.

The infarct may be named according to the area.

(Continued on page 219)
Lupus Erythematosus

Systemic lupus erythematosus (SLE) is a disease which predominantly involves the vascular system. The major clinical and pathologic features of this disease reflect the site of vascular injury. Renal glomeruli, which are the vascular structures most susceptible to the injurious effects of the circulating agents, are frequently damaged during the course of SLE. In many patients a necrotizing vasculitis, affecting small and medium-sized blood vessels, is observed also. Multiple visceral organs may be affected, with cardiac involvement a prominent feature of this syndrome.

The cardiac lesions may be considered in relation to damage involving the valves and mural endocardium, blood vessels, and connective tissue of the myocardium and the pericardium. Most patients with SLE have symptoms attributable to involvement of the heart in the disease process. It is usually difficult to differentiate the primary signs, which reflect endocarditis and myocarditis, from the secondary symptoms resulting from fever, hypertension, anemia, and concurrent renal and pulmonary disease. Pericarditis is the most frequent and one of the earliest signs of SLE. Endocarditis may be associated with systolic or diastolic murmurs which have no distinguishing characteristics. The vegetations do not embolize. Myocarditis is exceedingly difficult to recognize, because the lesions are usually mild and do not lead to cardiac dilatation or failure.

Numbacterial endocarditis, described by Libman and Sacks, was found originally in more than 50 to 60 percent of the hearts of the lupus erythematosus patients examined. The incidence of all cardiac lesions found at postmortem examination has changed radically since the institution of steroid therapy. The mitral and tricuspid valves are most frequently affected by single or mulberry-shaped excrescences, ranging in size from 1 to 4 millimeters. They occur in a random fashion, both on and away from the line of closure on both surfaces of the valve. Vegetations also may be found on the chordae tendineae, the papillary muscles, and the mural endocardium, usually at the base of the ventricles. Microscopically, the excrescences have a superficial layer of partially hyalinized platelet and fibrin thrombus. Deeper layers may show evidence of eosinophilic collagen degeneration and necrosis, with a variable infiltrate of neutrophilic and mononuclear cells (A). Bacteria have not been demonstrated. In some instances, fibrous thickening of the valve, indicative of previous episodes of endocarditis, may be present. In the region of the valve ring, the base of the valve and the valve-pocket proliferation of endothelial cells and myocytes may be prominent. Hematoxylin bodies may be found in the areas of endocardial inflammation.

Fibrinoid necrosis of the small and medium-sized arterioles may be associated with myocarditis. Endothelial proliferative and granular plugs of fibrin occlude the lumina of small vessels, showing necrosis of the wall. Infiltrations of neutrophils in the acute stage and mononuclear cells in the older lesions are prominent. Utilizing the fluorescent antibody technique, deposits of γ-globulin and the C3 component of complement have been demonstrated in the acute vascular lesion (B). In the late stages of vessel involvement, endothelial proliferation, thickening, and partial occlusion may be found.

Foci of myocardial inflammation, associated with interstitial edema and eosinophilic degeneration of collagen, may be prominent (C). Lymphocytes, plasma cells, and large histiocytic cells form the infiltrate. Hematoxylin bodies may be found in interstitial areas of inflammation. Degenerative changes in the myocardial fibers usually do not occur, and the myocarditis evident in SLE is usually not extensive, although areas of fibrosis may result.

Organizing fibrinous pericarditis, unassociated with uremia, is common, and fibrinoid necrosis of the pericardial connective tissue has also been observed. Serosanguinous effusions may accompany the pericarditis. Although fibrous adhesions may be seen, constrictive pericarditis does not occur.

The cardiac valvular lesions of SLE must be differentiated from those of rheumatic fever. Rheumatic vegetations occur on the atrial surface of the valve and have less tendency to undergo necrosis. The characteristic Aschoff nodule is absent in SLE.