Revised concepts of atherogenesis

A review

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More than 20 years ago Page[1] reported on the supposed causes of atherosclerosis. Most of the investigators in this field are no longer "new and quite shiny." They have toiled long and diligently and communicated with each other profusely, and yet "cures" for atherosclerosis have not been proclaimed at least twice a year as Page had expected. That is not to say that knowledge has not advanced in the past 2 decades. The recognition of the major importance of this disease process has attracted many new investigators. Publications have been prolific and newer technology has added sophistication to old experiments. Yet few advances have been of sufficient magnitude to excite the attention of the clinician, surgeon, or pathologist with other areas of interest. Instead, advances in understanding atherosclerosis have been subtle and complex, as is the disease itself.

There are several reasons why this should be so. For one, arteries tend to react to a wide variety of injurious or inflammatory stimuli with a rather uniform set of responses, so that divining the nature of the stimulus from an examination of the lesion, always a hazardous process for the pathologist, no longer has any credibility in degenerative arterial disease. For another,

atherosclerosis is a disease which many assume takes a lifetime to develop to the stage where clinically significant occlusion of an artery occurs (*Fig. 1*). During that lifetime, the artery may have been subjected to many stimuli. Even if a full history of these influences were available, attempts to differentiate injurious stimuli from those which are relatively harmless would be speculative at best. So investigators have relied on experimental work, often of only a few minutes, weeks or months, to find keys to human problems which may have developed over much longer periods of time. Third, atherosclerosis is commonly held to be a multifactorial disease. If there is a "mosaic" theory of hypertension[2] and many potential "causes" of neoplasia,[3] then atherosclerosis can surely claim to have a risk for all seasons. At the last count there were at least 35 documented epidemiologic factors which predispose towards atherosclerotic heart disease.[4] The complexity of the interrelationships between these factors and atherogenesis seems limited only by our own human need to simplify epidemiology in order to comprehend it.

One simplification which many have made is to grade risk factors on the strength of their primary associations, and to ignore factors whose statistical associations with enhanced arterial disease are small. The separation of major from minor risk factors in this way is rational because it cannot be said with certainty that the correlations between atherosclerotic

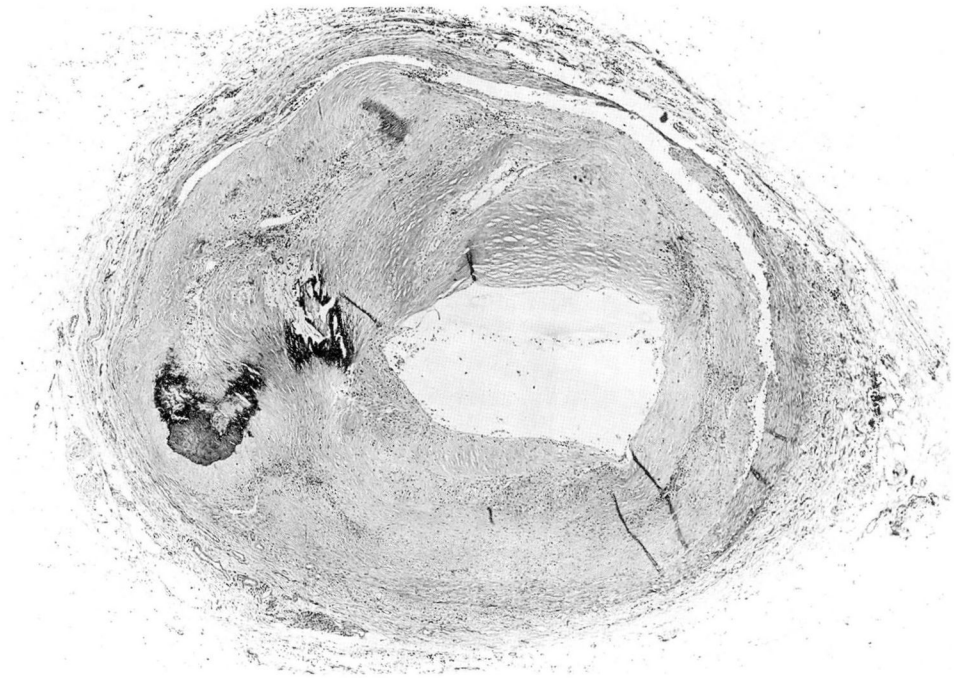

Fig. 1. Fibrous stenosis of an extramural human coronary artery in a patient who died with myocardial infarction. Normal vascular architecture has been destroyed and replaced by dense fibrous connective tissue. Areas of calcification and lymphocytic infiltration are also visible (hematoxylin and eosin stain, ×32).

disease and the latter have any pathogenetic meaning. On the other hand, those factors whose associations with atherosclerotic disease are strongest are unlikely to be exerting their influence indirectly. Presumably, they are related to the progression of the disease by virtue of some casual relationship to it.

Epidemiologic studies

Much of this graded classification of risk has come about through tedious but often highly successful epidemiologic studies. Of these, the most noteworthy is the Framingham Study,[5] which since 1949 has assembled and monitored a cohort of 2,176 men and 2,669 women. The results from Framingham unequivocally demonstrate that the risk of suffering a major atherosclerotic event (coronary heart disease, atherothrombotic brain infarction or intermittent claudication) is strongly related to age, blood pressure, serum cholesterol, cigarette smoking, relative weight, glucose intolerance, and an electrocardiogram indicative of left ventricular hypertrophy. The relationships between these risk factors and the development of atherosclerotic disease are such that single and multiple regression equations can be calculated which describe the incidence in terms of these factors. The highly significant regression coefficients imply that the average population incidence is *dependent* upon the levels of these risk factors. This is as close to a demonstration of etiologic connection between these factors and atherosclerotic disease as any epidemiologic study is capable of achieving.

A fascinating aspect of the data from Framingham is that the risk of suffering a fatal coronary attack can be calculated on a personal level. Thus a 50-year-old man who smokes cigarettes, has a normal glucose tolerance and electrocardiogram, a systolic blood pressure of 135 mm Hg, and a serum cholesterol of 235 mg/dl, has a probability of 23/1000 of developing coronary heart disease within 2 years and a probability of 108/1000 within 8 years.[5] This 10.8% chance would be decreased by almost 4% if he did not smoke cigarettes and conversely, it would be increased by a similar amount if his serum cholesterol level was 50 mg/dl higher.

Of course, the population of Framingham, Massachusetts is not necessarily that of Cleveland, Ohio or Bristol, England, and caution must be exercised in extrapolating the Framingham data to the rest of the United States or to other countries. Similar care should be used in evaluating other local prospective studies, and yet substantial agreement on the major coronary risk factors has been achieved through national[6,7] and international[8] cooperative studies of cardiovascular disease. First, atherosclerosis is a disease which dramatically increases in severity with age and tends to be more severe in men than in women of the same age. These two primary associations are probably crucial in fully understanding the disease, yet of less immediate concern to the patient, since he or she can do nothing to alter them. If we accept for the moment the presence of these two primary associations, the most important of the remaining risk factors for coronary heart disease can be listed as (not necessarily in order of importance): (1) hyperlipoproteinemia, (2) hypertension, (3) cigarette smoking, and (4) impaired glucose tolerance.

This assumes that other factors such as obesity and left ventricular hypertrophy are important by virtue of their relationship to one of these four, which admittedly may not be the case. But few would doubt the likelihood of a rapid acceleration of coronary heart disease in the presence of these four factors.

The major risk factors cited constitute a primary framework within which any modern theory of atherogenesis must be constructed. It is no longer sufficient to suggest that occlusive atherosclerosis develops in some and not others by a random lottery. What has evolved from the epidemiologic work is that there are now clear-cut circumstances under which we know accelerated atherogenesis occurs. It is extremely important that a modern theory of atherogenesis before all else should postulate a mechanism which explains these relationships.

In recent years a considerable amount of new information has been obtained about fundamental aspects of arterial behaviour. It is worthwhile to consider whether such new information brings us any closer to defining the exact causes of atherosclerosis. Much of this recent evidence has subtly altered mechanistic concepts, so that it is now possible to state that none of the classic pathways of atherogenesis (lipoprotein infiltration theory, thrombogenic hypothesis and injury-repair concept) is sufficient on its own to answer the question of how atherosclerosis is caused. What is needed is a unified theory which can rationally incorporate the relevant parts of older theories.

Changes in our concepts of atherogenesis in the past 2 decades are best illustrated by reference to specific problems. The discussion below focuses on certain important facets of atherogenesis which I believe must be understood and incorporated in any unifying theory of atherosclerosis.

The entry of lipoproteins and lipids into the arterial intima

It is axiomatic that there is an accumulation of lipid in atherosclerotic lesions. In practice, the extent of this lipid accumulation varies with the nature of the plaque, being severe in fatty streaks and frank atheroma and less so in fibrous plaques. It is not helpful to characterize all atherosclerotic lesions as fatty, for there are many cases of severe intimal fibrosis where virtually no lipid can be demonstrated histologically within the lesion (*Fig. 1*). There are also fibrous plaques which contain large masses of amorphous lipid at their centers (*Fig. 2*). The distinction between these different forms of the atherosclerotic lesion and the amount and quality of lipid which they contain has been painstakingly presented by Smith et al.[9–12] Some of the results from their laboratory studies are presented in *Figure 3*. The absence of morphologically recognizable lipid from many human fibrous plaques is mirrored by a cholesterol concentration in the lesion no different from that of normal intima. Of those lesions which can be regarded as fatty, there are two quite distinct types, fatty streaks in which most of the lipid is intracellular, and fibrofatty plaques in which most of the lipid is in amorphous extracellular pools. A remarkable finding of Smith et al, amply confirmed by others, is that the lipid in foam cells in fatty streaks has a bizarre fatty acid composition unlike that of plasma, whereas the

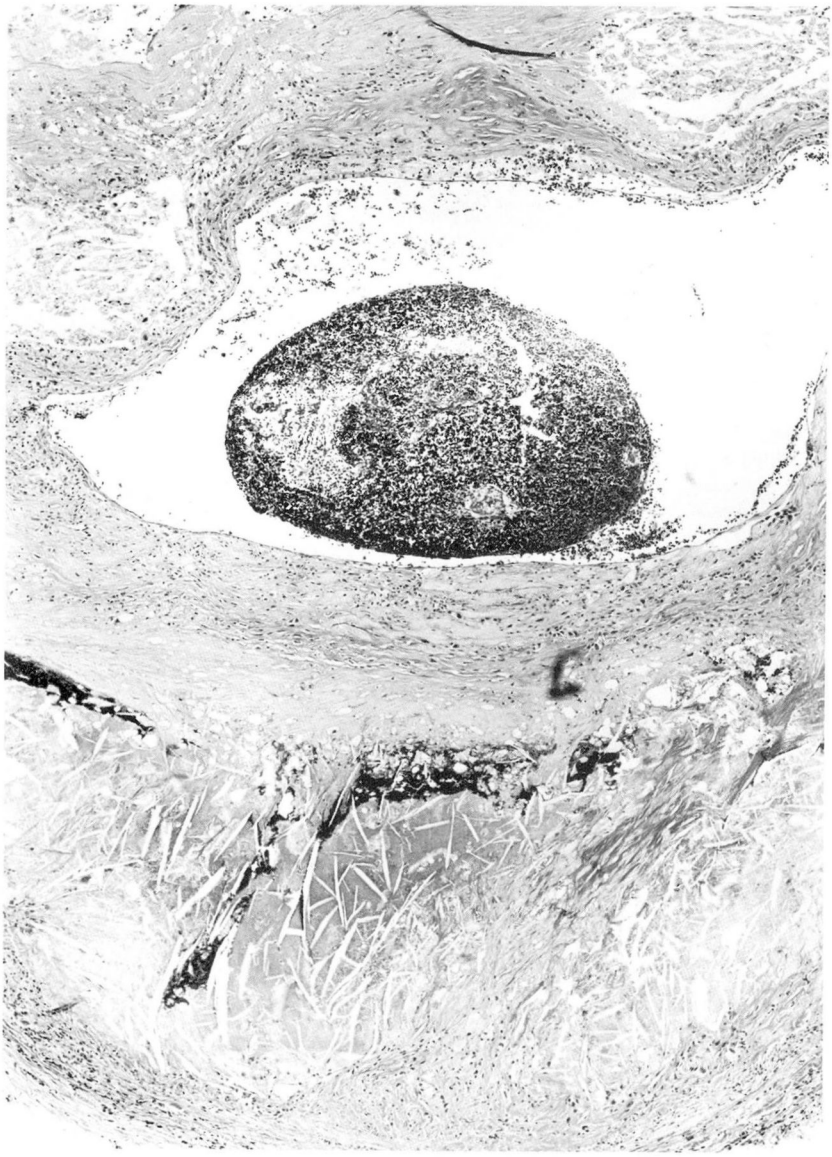

Fig. 2. Human coronary artery. Complex atherosclerosis. A large pool of amorphous and crystalline lipid is present in the thickened intima and is surrounded by hyaline connective tissue. The tail of an obdurating thrombus is present in the lumen (hematoxylin and eosin stain, ×52).

extracellular lipid from fibrous plaques has similarities to that of plasma β-lipoprotein. This difference is exemplified in *Figure 3* by the highly unusual oleate:linoleate (18:1/18:2) ratio in the cholesteryl esters of fatty streaks. This suggests that the lipid in some of the most cellular lesions is derived from local synthesis, or at least that plasma lipid is subject to sizeable metabolic alteration or selective influx. Put another way, lipid accumulation in fatty streaks appears to be a result of atherosclerotic

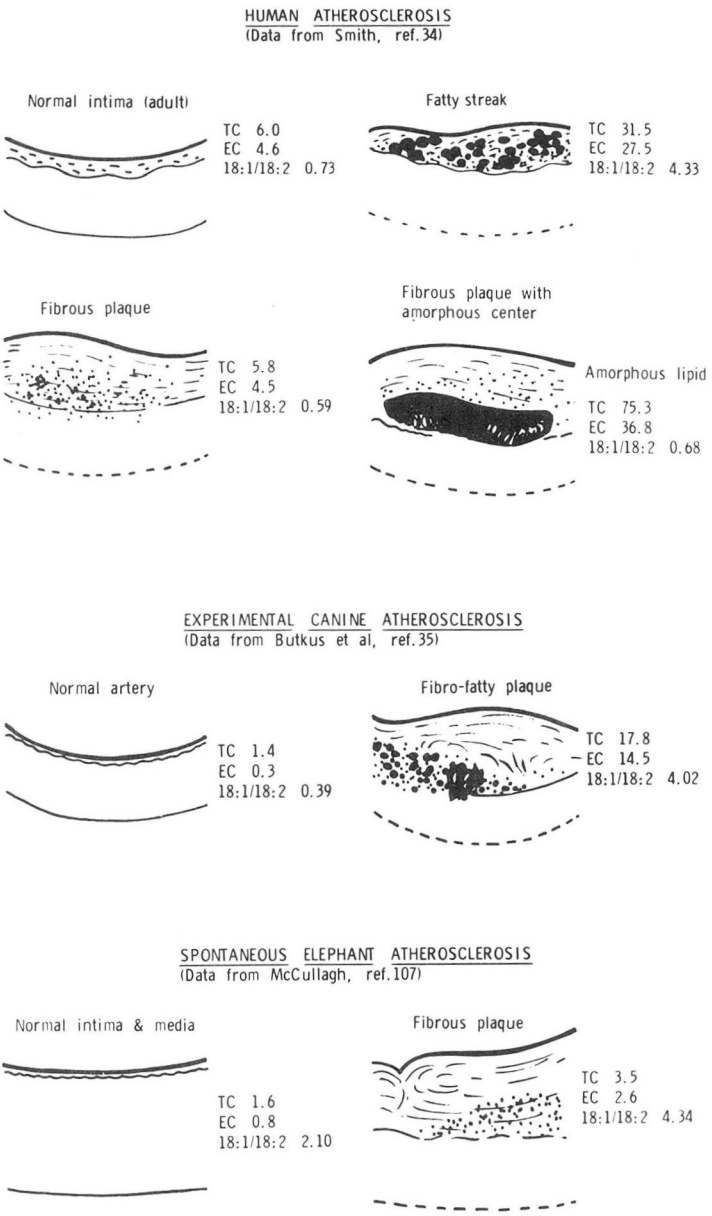

Fig. 3. Diagrammatic representation of the typical amounts of cholesterol and its esters present within different types of atherosclerotic lesion. The data have been selected from the sources shown to illustrate the unique characteristics of the human fibrous plaque. Lipid and cholesterol are shown in black. TC—total cholesterol concentration in mg/100 mg dry tissue; EC—esterified cholesterol concentration in mg/100 mg dry tissue; 18:1/18:2-weight ratio of cholesteryl oleate to cholesteryl linoleate.

change rather than a cause of it.

It will be seen from the data in *Figure 3* that although there are large quantities of cholesterol in the amorphous centers of "atheromatous" plaques, the cholesteryl oleate-lino-

leate ratio is similar to that of normal intima and dissimilar to that of fatty streak. This argues strongly in favor of a different pathogenesis for the two types of lesions. Unfortunately, the issue has become confused by analysis of experimental lesions (illustrated in *Figure 3*) by reference to a morphologically impressive, induced atherosclerosis in dogs,[13] which have invariably revealed cholesteryl ester fatty acid patterns similar to those of human fatty streak. It is therefore possible that the morphologic similarity of many such experimental lesions to advanced human atherosclerosis is fortuitous and that they may not provide particularly accurate models of lipid metabolism in human fibrous plaque and atheromatous lesions. Neither are spontaneous animal lesions, occurring in the absence of hyperlipidemia, reliable indicators of the processes involved in lipid accumulation in human fibrous plaques. The elephant plaques illustrated diagrammatically in *Figure 3* display a rather bizarre fatty acid pattern which at first sight seems inconsistent with their ordered and relatively acellular structure.

One of the predominant lipids to accumulate in human, experimental and spontaneous mammalian atherosclerotic lesions is cholesteryl oleate and in experimental animals at least, most of this ester accumulates from plasma.[14] Kinetic studies of nonesterified cholesterol in atheromata have also shown that despite the presence of local cholesterol synthesis,[15] the bulk of the lesion nonesterified cholesterol is derived from plasma.[16] The important question which bears on the pathogenesis of atherosclerosis is how does this influx of lipid occur? Is it carried into the intima as part of intact lipoprotein molecules, or is there a transfer of lipid from plasma lipoprotein to arterial cell independent of lipoprotein flux.

The work which supports the lipoprotein infiltration theory by providing evidence for the presence of serum lipoproteins in atheromatous tissues is primarily histochemical. Utilizing fluorescent antibodies of varying purity, Watts,[17] Kao and Wissler,[18] Woolf and Pilkington,[19] Walton and Williamson[20] and Hoff et al[21] have demonstrated material immunologically identical to serum β-lipoprotein in atherosclerotic lesions. The specificity of the reaction is a crucial issue here, for insufficiently purified fluorescent antibody may cross react with smooth muscle material. However, if we assume that the identification of β-lipoprotein derived material by this technique is correct, it would appear that although β-lipoprotein can be detected in atherosclerotic lesions there is no clear association between the amount of lipoprotein and the severity of the lesion. In addition, fluorescence is not normally seen in undiseased arteries. Similar conclusions are reached by immunoelectrophoretic studies of lipoproteins extracted from the intima.[22–24]

The situation can be summarized by saying that the bulk of the lesion cholesterol appears to be derived from plasma, and that some but not all of this cholesterol can be re-extracted from the arterial wall in the form of lipoprotein molecules which are similar in flotation and immunologic characteristic to the low density lipoproteins of plasma. It is tempting to regard the matter of lipoprotein influx as proven, but we cannot say to what extent entry of intact lipoprotein contributes to the buildup of the sequestered atheroma lipid. We

know from other studies that lipoprotein lipid, dissociated from the apoprotein, may be taken up by atherosclerotic intima and it may be that this is quantitatively a more important process than infiltration of entire lipoprotein molecules.

Endothelial permeability and endothelial damage

The endothelium lining the aorta and its major branches, including the coronary arteries, normally acts as a barrier to the passage of proteins and other large molecules. The cells of the endothelium are separated from one another by tortuous intercellular channels that are too small to allow the passage of any but the smallest of plasma proteins.[25] Recent observations with the electron microscope on the passage of ultrastructural marker proteins have shown that this barrier is made more impermeable by the presence of complete tight junctions in cerebral arteries[26] and by partial or incomplete tight junctions in other large arteries.[27] Extensive membrane appositions, or gap junctions have also recently been described in aortic endothelium.[27,28] Where a gap is present between opposed cell membranes in these regions it appears to be only 20 to 40Å wide. It is quite irrational to propose that under normal physiologic conditions molecules of the size of low density lipoprotein could pass into the subendothelium by this route. This is emphasized by *Figure 4* which is a proportional scale diagram illustrating the relative sizes of gaps and particles.

There is evidence which suggests that endothelial junctions are somewhat labile and that under conditions of hypertension[29] there may be a reversible opening of tight junctions without cell separation. The opening appears to be sufficient to allow the passage of horseradish peroxidase

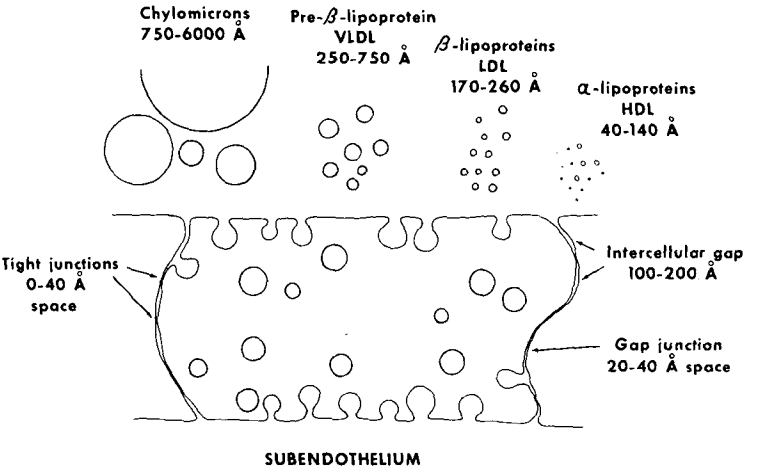

Fig. 4. Diagrammatic representation of normal arterial endothelium drawn to a scale of 25 nm (250 Å) = 1 mm. The drawing shows normal intercellular channels containing focal tight junctions and extensive gap junctions. Plasmalemmal vesicles vary from 600 to 1000 Å in diameter, but the neck opening of surface vesicles is rarely larger than 300 Å. Plasma lipoprotein particles are drawn to a similar scale. The endothelial cell nucleus, mitochondria and other organelles have been omitted for clarity. The basement membrane-like material often seen in the neck of membrane attached vesicles is also not shown. The drawing is equivalent to an electron micrograph of magnification ×40,000.

(mol wt 40,000) but not ferritin (mol wt 500,000). Such physiological lability does not appear to be sufficient to allow passage of plasma low density lipoprotein (mol wt 3,000,000). However, it has recently been claimed[30] that tight junctions in arterial endothelium are reversibly dilated by physiologic concentrations of angiotensin, and that the increase in permeability is sufficient to allow transport of lipoprotein. Increases in pore size of this magnitude have not been demonstrated unequivocally in normal continuous endothelium, and this view is not in agreement with other evidence.[29] For the time being it is fair to conclude that interendothelial passage of lipoprotein is unlikely to occur provided the endothelial cells are undamaged.

There is another route by which large molecules may traverse the endothelium, and that is within the plasmalemmal vesicles that are continually forming from the cell surface, travelling randomly in the cell interior, and refusing with the membrane either on the same or another side of the cell (*Fig. 4*). These vesicles are about 700 Å in diameter[31] and could, therefore, accommodate normal low density lipoproteins which have molecular diameters of 170 to 260 Å.[32] It is accepted that this "large pore system" can act as a route of transendothelial passage of plasma proteins, but with much smaller molecules than plasma low density lipoprotein there is considerable molecular sieving.[31] It may be that quantitatively this route allows the entry of only insignificant amounts of lipoprotein, but the experiments which would enable us to make such a statement with confidence have not been performed.

A fair appraisal of the recent work on arterial endothelium and lipoprotein transport would be that under normal conditions the endothelium acts as an incomplete but pretty effective barrier to the passage of plasma lipoproteins. Rather than stress that lipoproteins normally percolate through the arterial wall from lumen to lymph vessel, it would be better to emphasize the relative *impermeability* of normal intact endothelium.

But, as we have seen, many atherosclerotic lesions, both human and animal, contain large amounts of low density lipoprotein which appears to be indistinguishable from that of plasma. If it is assumed that the majority of this lipoprotein is derived directly from plasma, it is obviously necessary to postulate that the endothelial barrier over the plaque is or has been breached. Until recently the morphologic evidence for this has been equivocal. Endothelial cells surmounting plaques are often abnormal in being highly vacuolated and possessing cytoplasmic alterations,[33,34] but frank changes in intercellular gaps have been difficult to demonstrate. However, recent examination of "en face" preparations of arterial endothelium by light microscopy[35] and scanning electron microscopy[36] have revealed distortion and loss of endothelial cells overlying intimal plaques. In addition, many investigators have shown that endothelial loss or damage can result from a wide variety of chemical, physical, and immunologic injuries. Such results need be as minor as a local variation in blood flow or the presence of a circulatory toxin such as carbon monoxide.

The loss of endothelial integrity appears to be the crux of the matter

of lipid accumulation in atherogenesis. While the endothelial lining of an artery remains intact it functions as a remarkable protective barrier, not only excluding plasma lipoproteins but also shielding the delicate metabolic balance of the tunica intima from the perturbing influences of other plasma proteins. Once damaged or lost, it allows a series of events to take place involving serum influx, increased lipid absorption by intimal cells, esterification of cholesterol, blocking of diffusion channels in the intercellular matrix, degeneration of intimal cells, and the recruitment and proliferation of connective tissue cells in the intima.

However, the regenerative capacity of endothelium is not insignificant. A single traumatic event is unlikely on its own to result in a permanent loss of endothelial integrity. Neither is it likely that the small intimal elevations which result from a single injury persist indefinitely. For the progressive degeneration and thickening of the intima which characterizes atherosclerosis, it makes sense to suggest that there must be a constant or at least repetitive endothelial insult.

If such a sustained endothelial injury is accepted as an inciter of atherosclerosis, it is clearly necessary to demonstrate that such injuries can result from the action of major risk factors. Fortunately, the question of whether hypertension, cigarette smoking, and hyperlipoproteinemia can cause endothelial injury is amenable to investigation. For example, recent studies have associated the atherogenic effects of tobacco smoke with its carbon monoxide content.[37] Experiments in rabbits[38] and primates[39] exposed to relatively small concentrations of carbon monoxide (0.0009–0.025 vol %) for periods of a few weeks have shown that the initial reaction is one of endothelial injury. The endothelial cells swell and become disorganized, the interendothelial gaps appear widened, and there is considerable edema of the subendothelium. These changes have also been seen in the umbilical veins of infants born to smoking mothers but not in those of nonsmoking mothers.[40]

Experimental work has also underlined the sensitivity of the endothelium to high blood flow rates, increased shear stress, and heightened pulse pressure,[41] all components of the hypertensive situation. Endothelial cells have been shown to become disorganized and even detached if the wall shear stress is high.[42] Pressure related stresses on the endothelial layer may lower the threshold to shear damage. If excess vasoactive agents circulate in the hypertensive state, it is possible that they contribute to endothelial damage.

The reaction of the endothelium to hyperlipidemia is more contentious, because it is difficult to separate the direct effects on endothelial cells from the secondary response of the intima to deposited lipid. However, high concentrations of low density lipoprotein appear to be toxic to arterial cells in culture,[43] and they may well be so to endothelial cells *in vivo*. However, it is not necessary to postulate direct endothelial toxicity in hyperlipoproteinemia, for the heightened blood viscosity occurring in this situation is likely to bring about cell disruption by causing increased drag effects on the endothelium.

Finally, since the initial observations by Goldenberg et al[44] that endo-

thelial proliferation is a characteristic feature of diabetic angiopathy, it has become increasingly clear that vascular endothelium is highly susceptible to damage in diabetes mellitus[45] and that endothelial injury may be the initiating event in the widespread vascular complications of this disease.[46,47] It remains unclear whether endothelial damage in diabetes is secondary to abnormally high levels of circulating metabolites such as glucose or lactate, or whether there is an integral alteration in cell metabolism resulting in a lowered resistance to injury and premature senescence. Whichever is the case, such effects may also be present in persons who have varying degrees of glucose intolerance without overt diabetes, thus accounting for the significance of this metabolic abnormality in the epidemiology of atherosclerotic disease.

Endothelial injury therefore appears to represent a common pathway by which each of the major risk factors might contribute to the initiation of atherosclerosis. This view is supported by a number of recent investigations in which changes in intimal morphology have been studied after artificial endothelial injury. Although atherogenesis is undoubtedly a complicated process in which many aspects of intimal morphology and metabolism are disturbed, loss of endothelial integrity seems paramount. I suggest that in many respects atherosclerosis may be regarded as a *chronic endotheliopathy*.

Proliferation and fibrosis within the intima

If a careful microscopic examination is made of the diseased segments of the coronary artery that are removed surgically from patients undergoing coronary reconstructive surgery, or of those that are dissected postmortem from patients who have died from occlusive coronary disease, it will be seen that the bulk of the occluding lesion is usually composed of either dense fibrous connective tissue similar to that shown in *Figures 1 and 2* or of thrombotic material similar to that blocking the lumen in *Figure 5*. Even when sudden thrombosis occurs, it invariably does so in an artery that is already narrowed by fibrous connective tissue (*Fig. 5*). Thus, a large part of the clinical syndrome of reduced coronary blood flow appears to be a result of chronic stenosing fibrous plaques within the coronary arterial tree.[48] This is not to say that the lesions show no lipid accumulation, nor that thrombosis may not produce the precipitating crisis, but rather that we have tended to disregard the fibrosis which is so characteristic of these lesions.

What causes such profound intimal fibrosis? There are two schools of thought. One is that the presence of cholesterol, and certain saturated lipids stimulate the proliferation of smooth muscle cells in the intima and that these cells are "modified" in the process into active connective tissue synthesizing cells. The other school of thought is based on the pronounced tendency of human atherosclerotic lesions to rupture and split, producing areas of intramural hemorrhage, mural thrombosis necrosis and calcification.[49] The fibrosis which ensues can be regarded as a repair reaction to rupture and necrosis, or as a foreign body reaction, or as an organization of mural hemorrhage and thrombosis. This second view has received less support from the atherosclerosis community than

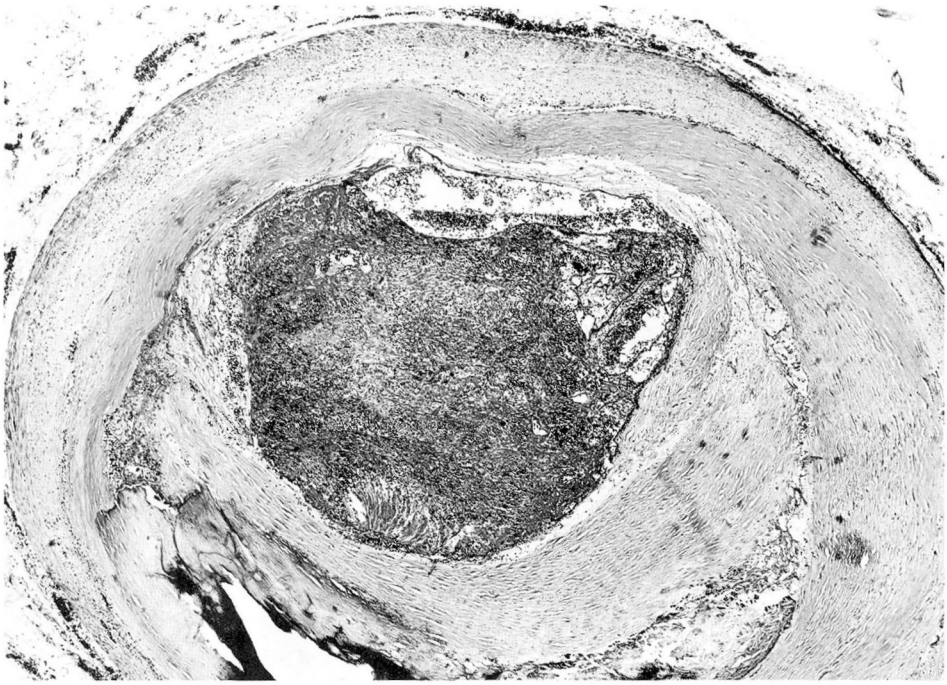

Fig. 5. Human coronary artery. Recent thrombotic occlusion of an artery already stenosed by advanced fibrous atherosclerosis (hematoxylin and eosin stain, ×40).

would be expected from an examination of human lesions, partly because hemorrhage within plaques is uncommon in experimental animal lesions.

I see no reason to separate these events. Fibrosis is characterized in all locations by a dramatic accumulation of fibrous collagen in the intercellular matrix. The critical question is what is the mechanism which controls collagen production and deposition in pathologic sites and how is it activated by these different stimuli.

Recent studies with aortic smooth muscle cells in culture suggest that lipoproteins, isolated from hyperlipoproteinemic serum, can directly stimulate cell proliferation.[50,51] However, increased numbers of smooth muscle cells do not inevitably imply increased collagen biosynthesis. So far, serum lipoproteins have *not* been shown to initiate increases in arterial collagen synthesis,[52] although dramatic elevations in the rate of collagen formation have been observed in experimental atherosclerotic lesions produced under hyperlipoproteinemic conditions.[53–55] The *Table* summarizes much of the evidence for enhanced collagen synthesis in atherosclerotic lesions and compares rates of synthesis and levels of enzyme stimulation within diseased arterial segments with values obtained from adjacent normal segments. Theoretically, it seems reasonable to propose that activators of fibrosis exist within atherosclerotic plaques, although their nature and origin are presently obscure. Some investigators[56] suggest that the enzyme prolyl hydroxylase is an important regulatory step in collagen biosynthesis. Increased prolyl hydroxylase activity has been demon-

Table. Increases in aortic collagen synthesis in experimental atherosclerosis

Species	Atherogenic regimen	Elevation in rate of collagen synthesis. Ratio of atherosclerotic tissue to normal	Assay procedure
Dog [53]	HC diet	5.2	^{14}C-hypro
Rabbit[54]	HC diet	1.7	^{14}C-hypro
Rabbit[57]	Epinephrinethyroxine	5.2	PH activity
Rabbit[58]	Mechanical injury	2.6	PH activity
Pig[59]	HC diet	3.0	PH activity
Pig[59]	Norepinephrinethyroxine	2.8	PH activity
Pigeon[55]	HC diet	3.3	^{14}C-hypro
Pigeon[60]	HC diet	1.0	PH activity

HC diet = hypercholesterolemic diet; ^{14}C-hypro = collagen synthesis assayed by measuring the incorporation of ^{14}C-proline into peptide bound ^{14}C-hydroxyproline; PH activity = prolyl hydroxylase activity assayed in the tissue. Rates of collagen synthesis are assumed to parallel changes in the activity of this enzyme in tissues.

strated in several different models of experimental atherosclerosis.[57–59] The cholesterol and lard fed pigeon appears at present to be anomalous[60] (*Table*). Prolyl hydroxylase is similarly stimulated in fibrosing liver, and chemical activators of the enzyme have been isolated from this tissue,[61] but no such activator has yet been identified in atherosclerotic tissues.

Progression and regression of atherosclerotic lesions

Stemerman and Ross[62] showed recently in an interesting experiment with macaques, that if the abdominal aorta and iliac arteries are denuded of their endothelium with an intraarterial catheter, there is immediate influx of plasma proteins, degeneration of subendothelial intimal cells, and migration of smooth muscle cells from the media into the damaged intima. The result is the formation of a fibroproliferative thickening of the intima. It is interesting that after about 6 months these intimal thickenings are no longer visible. The intima reverts to its normal width of a few cells, the endothelium is regenerated, and there is apparently little indication of the previous injury.[63] However, if a sustained hypercholesterolemia is produced by feeding the animals a high fat diet throughout the experiment, the lesions do not regress but develop into lipid-rich fibrous plaques similar to those seen in man.[63]

Two conclusions can be drawn from these experiments. First, that even with an exceptionally severe single intimal injury, reparative intimal thickening is followed by a phase of regression and regeneration; and second, that lipid influx is a potent stimulus to the development of fibrous plaques. The importance of hyperlipoproteinemia has already been discussed, but the capacity of the arterial wall for regeneration is another matter. It suggests that lesions produced under natural conditions may also be susceptible to modification when the causative influences are removed. It implies a certain amount of optimism in the moderating effects of reducing risk factor levels in individuals and in the population as a whole.

This suggests that the natural history of an atherosclerotic lesion is one

of proliferative response followed by stabilization and eventual remodeling in much the same way as a bone fracture heals by proliferative union, regeneration, and remodeling. This is true of minor intimal lesions such as fatty streaks. Mitchell and Schwartz[64] clearly documented this some years ago by demonstrating that although the number of fatty streaks in the human aorta may increase with age, their distribution may change. Furthermore, fibrous plaques do not necessarily develop in the sites where fatty streaking is most common at an early age. One must assume that many of the original fatty streaks regress spontaneously. It is more controversial to adopt a similar attitude to advanced fibrofatty lesions, but in other nonhuman primate experiments,[65] occlusive atheromatous lesions produced by feeding a high-fat diet for prolonged periods have been induced to regress to much smaller fibrous thickenings by a relatively short postexperimental period of low-fat feeding. It is possible that lengthening the regression period would bring about a comparable further reduction of the fibrous scar.

To some extent this argument is academic in that it is much more difficult to reduce effectively the levels of hyperlipoproteinemia, hypertension, and cigarette smoking in the human population than it is in the experimental monkey. One of the reasons for the steady progression of human atherosclerosis with age is that the inciting stimuli often remain active throughout, and the lesions are therefore never given the chance to remodel. What I have talked about as the *natural* history of the atherosclerotic plaque may be prevented from occurring under the high risk conditions of western society. Whether we can restore the natural pattern of regression by therapeutic means remains to be seen from the results of primary prevention trials.

Conclusion—a unified theory of premature atherosclerosis

If what has gone before is accepted, it is evident that there is no inherent biological reason why atherosclerosis should be a progressive disease. Arteries are capable of undergoing healing following an injury in a fashion similar to other tissues. Neither is there any immutable reason why occlusive arterial disease should be responsible for such a major share of the causes of human death in developed countries. It is not so in the Masai[66] and neither is it so in elephants.[67] Disease surveys in the latter species have shown that the development of a limited amount of fibrous atherosclerosis may be inevitable during a prolonged life. It is equally clear that the uncomplicated spontaneous atherosclerosis that occurs in this and other species is not often a cause of death in animals. Such lesions as occur spontaneously in animals have been referred to as prototypes of the uncomplicated human lesion.[68] With this in mind, the complex problems of human atherogenesis can be distilled into one rather demanding question. What is it which in many middle-aged people living in industrial societies converts a relatively harmless prototype lesion into a complicated occlusive "malignant" process which destroys the normal arterial structure and brings about premature death? Despite recent evidence of a monoclonal origin of atherosclerotic plaques[69] viral

transformation seems unlikely.

At our present state of knowledge, the most sensible answer to this question would appear to be a continued or often repeated abuse or insult to arterial endothelium. The major risk factors: hyperlipidemia, hypertension, glucose intolerance, and cigarette smoking must play a major part in this abuse. Each in its own way brings about and sustains endothelial injury, and this is probably of major importance in determining the fate of initially minor and uncomplicated lesions. *Figure 6* emphasizes and summarizes diagrammatically this concept of atherogenesis. The upper cycle of four pictures (A–D) represents the development of focal or diffuse intimal plaques or thickenings within coronary arteries in response to repetetive endotheliopathy. It is probably rational to suggest as Stehbens[70] has done that most initial injuries are hemodynamic, arising from normal "wear and tear" on the arterial wall, although there is no reason why primary lesions might not originate from toxic injuries to the endothelium or premature endothelial senescence.

Initial damage to the endothelium allows an influx of plasma lipoproteins, albumin, fibrinogen and other macromolecules, many of which become enmeshed in the hyaluronate gel of the intimal ground substance. The alteration of the microenvironment will directly affect many intimal cells, while the large numbers of sequestered plasma macromolecules will interfere with the diffusion of oxygen and nutrients through the tissue. This inevitably results in cellular degeneration in the intima and the liberation of inflammatory substances, metabolic activators, and mitogens. The metabolic and proliferative reaction to these events is depicted in diagram 6C. The plaque is formed from proliferation of activated intimal cells, possible recruitment and transformation of smooth muscle cells from the media, and enhanced synthesis and accumulation of intimal connective tissue. Lipid within the lesion is derived mainly from sequestered lipoproteins, but there is also an activation of several aspects of lipid metabolism in the newly proliferating intimal cells.

The endothelial reaction is likely to depend on the degree of injury: severely damaged cells will lose their attachment to the basement membrane and are lost; less damaged cells no doubt recover. Platelet thrombi form at areas of endothelial denudation and endothelial regeneration ensues. Failing further injury, the endothelial barrier is reestablished and entry of plasma macromolecules halts. From this point on, a phase of healing occurs which is characterized not only by endothelial integrity but also by reorganization of the reactively thickened intima. With time there is a migration of cells away from the lesion and partial catabolism of the newly synthesized connective tissue. Regression is unlikely to be complete, and yet connective tissue remodeling may occur to a greater extent than experimental work has yet indicated.

The critical stage in the atherogenetic process is the impairment of this normal process of healing and regeneration and its replacement by a continuous cycle of cell damage and metabolic stimulation. *Figure 6E* shows how sustained hyperlipidemia, hypertension, carboxyhemoglobinemia, or any other cause of continuous

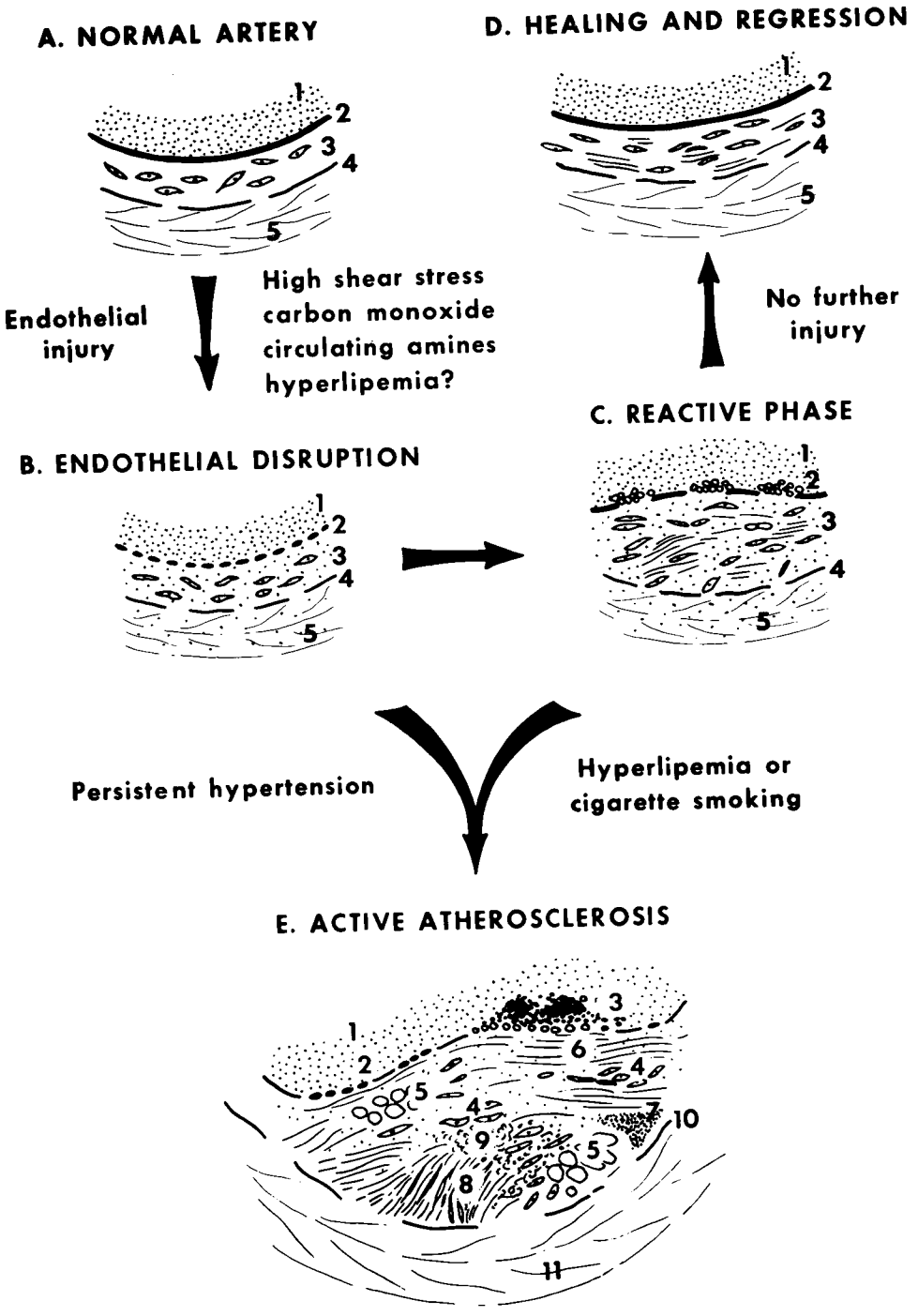

Fig. 6. Arterial reactions and atherogenesis. A-D stages in the normal cycle of intimal injury and repair.

A. 1. Plasma macromolecules; 2. intact continuous endothelium; 3. normal human intima containing fibroblasts, smooth muscle cells, macrophages and fine connective tissue stroma; 4. fenes-

injury is likely to prevent endothelial recovery and regeneration and encourage more profound mural thrombosis. Continued influx of plasma maintains the stimulus for plaque enlargement. Rapid impairment of diffusion within the plaque results in cell necrosis. Lipoprotein degeneration creates an abundance of insoluble lipid. Reactive fibrosis continues at a high rate, and the expanding bulk of the lesion encroaches on the arterial lumen.

This is admittedly a simplistic view of the atherogenic process and yet it represents a coherent theory. Each risk factor is viewed as contributing to endothelial injury, and plaque development is seen as primarily a reaction to changes in the intima brought about by endothelial damage. This theory has the advantage of not placing great emphasis on multiple pathways in the pathogenesis of the disease. The search for one basic pathway of atherogenesis may well be elusive, yet it is valuable for its demands that apparently opposing results be reconciled.

If the theory of atherosclerosis as a chronic arterial endotheliopathy is correct, some optimism may be permitted in viewing the potential development of control measures to prevent the disease. All of the presently recommended interventions for lowering major risk factors in individuals and in populations can be expected to reduce the incidence of endothelial injury and at least slow the rate of plaque development. A similar but more direct approach to prevention might be to search for agents which will protect the endothelium against low grade injury. Failing this, it might be feasible to diminish the response of subendothelial intimal tissue to endothelial damage. This might be achieved by inhibiting platelet function with drugs such as dipyr-

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trated internal elastic lamina; 5. medial smooth muscle, elastin and collagen arranged in concentric layers.

B. 1. Plasma macromolecules; 2. loss of endothelial integrity—cells swollen and intercellular junctions disrupted; 3. influx of plasma macromolecules into connective tissue of intima; 4. fenestrated internal elastic lamina; 5. some plasma constituents reach media, others remain enmeshed in intima.

C. 1. Plasma macromolecules; 2. platelet thrombi form at sites of injury; severely damaged endothelial cells are lost, regenerative changes occur in others; 3. reactive changes occur in intima: cells proliferate and/or become metabolically more active, smooth muscle cells migrate into intima from media and change to resemble fibroblasts; macrophages engulf trapped macromolecules, and there is heightened synthesis of connective tissue in the intima; 4. internal elastic lamina remains intact; 5. plasma constituents in media diffuse into lymphatics.

D. 1. Plasma macromolecules; 2. endothelial integrity restored; 3. majority of plasma components removed from intima, some low density lipoprotein and fibrinogen remain; many of the modified smooth muscle cells migrate from thickened intima, and much of excess intimal connective tissue is catabolized; 4. internal elastic lamina; 5. no residual changes in tunica media.

E. Progressive atherosclerotic lesion produced by continued intimal injury. 1. Plasma macromolecules; 2. continued injury prevents endothelial regeneration and increased permeability persists; 3. mural thrombi form where subendothelial tissue continues to be exposed; 4. "fibroblast-like" cells in intima are activated to secrete large amounts of connective tissue; 5. foam cells form and lipid metabolism is deranged in intimal cells; 6. connective tissue of intima becomes predominantly collagenous; 7. areas of hemorrhage within the plaque occur; 8. cholesterol crystals precipitate in plaque and form "clefts" in connective tissue; 9. lipid collects at base of lesion in amorphous "pools"; 10. necrosis of intimal cells and degeneration of internal elastic lamina; 11. anoxic degeneration occurs in some medial cells beneath the plaque.

idamole or aspirin, as suggested by Spaet,[71] or by inhibiting the proliferative and synthetic responses within the intima with agents which are specific and nontoxic. Urgent priority should be given to the development of such agents and to basic research which will further knowledge of the cellular and molecular causes of the disease. If the results of such research are directed towards a continued reassessment of current theories of atherogenesis, there is every reason to believe that functional aspects of plaque development will soon be understood and effective control of atherosclerotic disease brought nearer.

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