

The Origin of Atherosclerosis

hardening

Atherosclerosis

a form of Arteriosclerosis

The monoclonal hypothesis, which holds that the proliferating cells of an atherosclerotic plaque all stem from one mutated cell, with suggests new lines of research on the causes of coronary disease

Atheroma - fatty degeneration and infiltration by lipids.

by Earl P. Benditt

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Cardiovascular disease has become the major cause of death in the U.S. and other Western countries not just because people are living longer but because some unknown aspects of modern life are increasing the incidence of atherosclerosis, the chronic arterial disorder that is the major cause of heart attacks and strokes. We cannot identify those aspects until we know the true nature of the characteristic atherosclerotic lesion. The atherosclerotic plaque, a lumpy thickening of the arterial wall, narrows the passageway and initiates the formation of a blood clot that can ultimately close down a critical artery.

In the past 15 years it has become clear that the plaque is characterized by an accumulation of smooth-muscle cells. The important question is: Why do the cells accumulate? Some of us now think the cells of a plaque are the progeny of a single mutated smooth-muscle cell from near the site of the plaque. If that is so, the plaque is comparable to a benign tumor of the artery wall. And if it is comparable, then the search for initiating factors should be directed toward the genetic and environmental factors that cause mutation—the same kinds of agents and conditions that transform cells and thus initiate cancers. Here I shall review the theories that have governed thinking about atherosclerosis until recently. Then I shall report some experiments that led to the new single-cell hypothesis and the evidence that tends to confirm it, and discuss some implications of the proposed new approach.

Arteries are conduits with a specialized cell composition and structure that enable them to conduct blood under pulsing pressure and to maintain and repair themselves. The artery wall has three layers: the intima, the media and the adventitia. Lining the inner surface of the intima is a single layer of endothelial cells, which hold the blood cells within the artery and modulate the passage of water and other substances from

the blood plasma into the tissues. The composition of the remainder of the intima varies. It may consist only of non-cellular connective-tissue fibers or it may include cells, depending on the type of artery and the age and sex of the individual; the intima of coronary arteries tends to be thicker in males than in females, and the thickness and the number of cells increase with age in both sexes. It is in the intima that atherosclerosis has its effect.

A prominent region of elastic tissue called the internal elastic membrane separates the intima from the media, the artery's main supporting layer. The cells of the media are smooth-muscle cells, so designated because their contractile fibers lack the striated pattern of skeletal-muscle cells. In elastic arteries such as the aorta the cells of the media are arranged in small groups that spiral between the coarse elastic fibers supporting the artery wall; arteries that supply organs, such as the coronary arteries, have a similar structure but less elastic tissue and relatively more cells. The fibrous proteins elastin and collagen and the carbohydrates (glycosaminoglycans) of the media are all secreted by the smooth-muscle cells.

The outermost layer of the artery wall, the adventitia, is composed of the cells called fibroblasts and their associated intercellular collagen and glycosaminoglycans. The adventitia carries the blood vessels that nourish the outer layers of the artery wall and also anchors the wall to the surrounding tissues.

Like any other tissue the arteries are subject to various disease processes such as the reaction to injuries and their repair, infections, inflammations and tumors. It has been difficult to categorize these disease processes because many of them look alike in their end stages and one is able to examine the minute structure of their lesions on only two occasions: at autopsy and, less frequently, during surgery. One can differentiate among various disease processes either

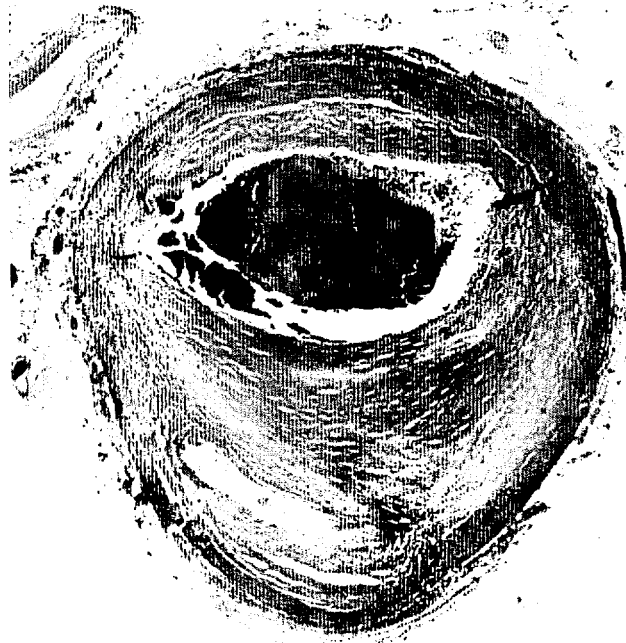
by following their development from their inception, examining tissue samples with a microscope, or by looking for special features that distinguish one process from another. That is done by studying animal diseases that mimic human ones. Much can be learned from animal models, but there is always the question of how well a particular model parallels the similar process in human beings.

The characteristic lesions of atherosclerosis, at least as they are seen at autopsy, are the raised fibrous plaques. They appear (in the dissected, undistended vessel) as discrete lumps, elevated above the unaffected regions of the intima and ranging in color from pearly gray to yellowish gray. The main cellular component of the plaque is, as I have indicated, a smooth-muscle cell very similar to the major cell of the normal artery wall; macrophages and other white blood cells also infiltrate the plaque's dense connective tissue, which consists largely of collagen fibers. The plaque usually contains glycosaminoglycans and sometimes elastin and such blood proteins as fibrinogen. Lipoproteins, the carriers of cholesterol in the blood, are found both inside and outside cells. Deep in the lesions there are debris from dead and dying cells and varying amounts of lipids (fats); crystals of cholesterol can sometimes be seen even with the unaided eye in the softened debris in advanced lesions. It is this fatty debris that suggested the name atherosclerosis, from the Greek *athera* (gruel) and *sclerosis* (hardening).

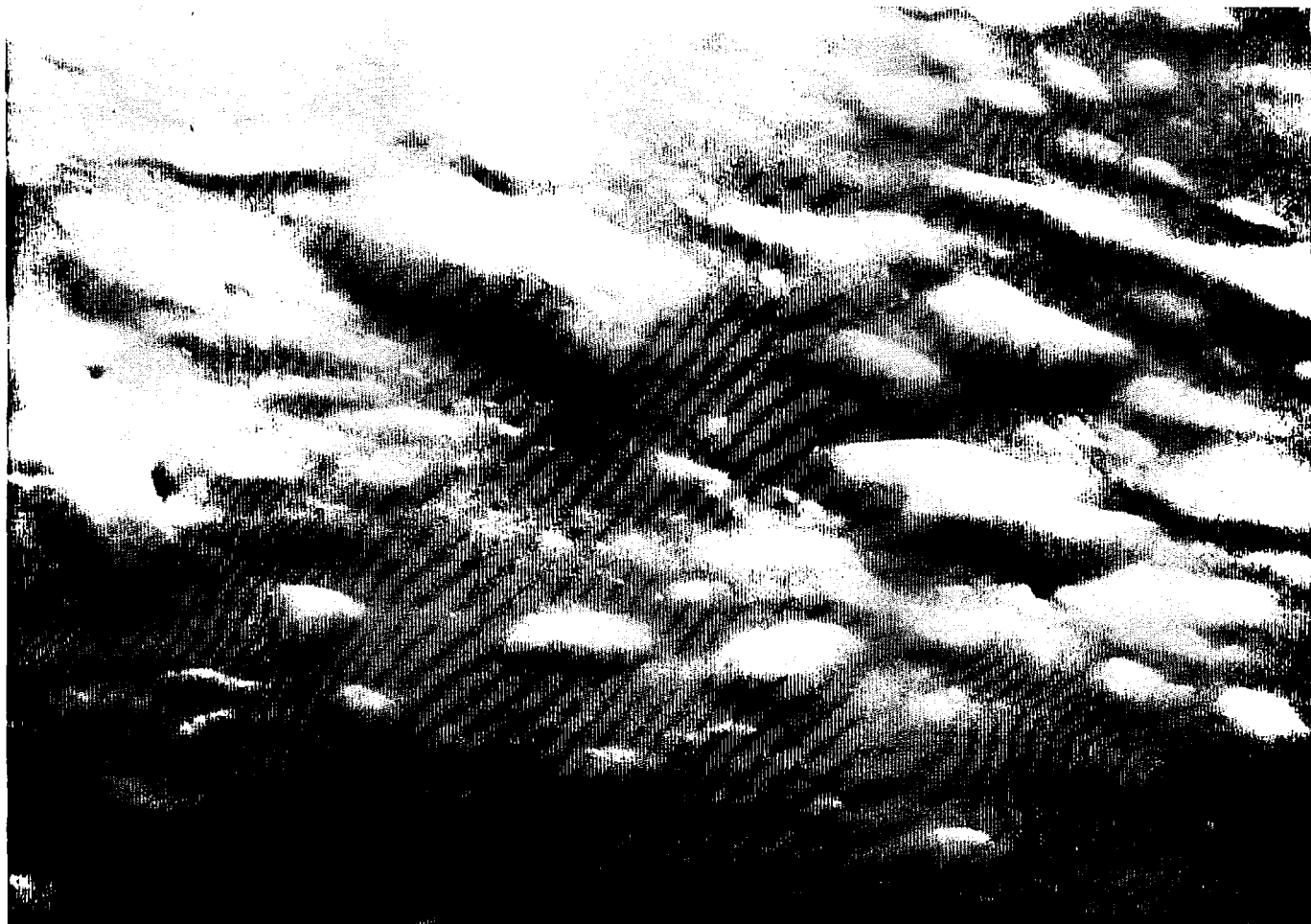
The atherosclerotic plaque can close down an artery by itself, but more often its harmful effect is to predispose the artery to occlusion by thrombosis, which represents an aberration in the delicately balanced blood-clotting system that is essential for temporarily patching leaks and injuries in blood vessels. A thrombus is a complex aggregation of blood platelets, white cells and red cells in a network of fibrin, the main



ATHEROSCLEROTIC PLAQUE narrows the lumen, or passageway, of a human coronary artery, enlarged 19 diameters in this photomicrograph made by the author. The plaque is a thickening of the artery wall composed mainly of connective tissue and smooth-muscle cells, with a region of cellular and fatty debris (*lighter gray areas*).

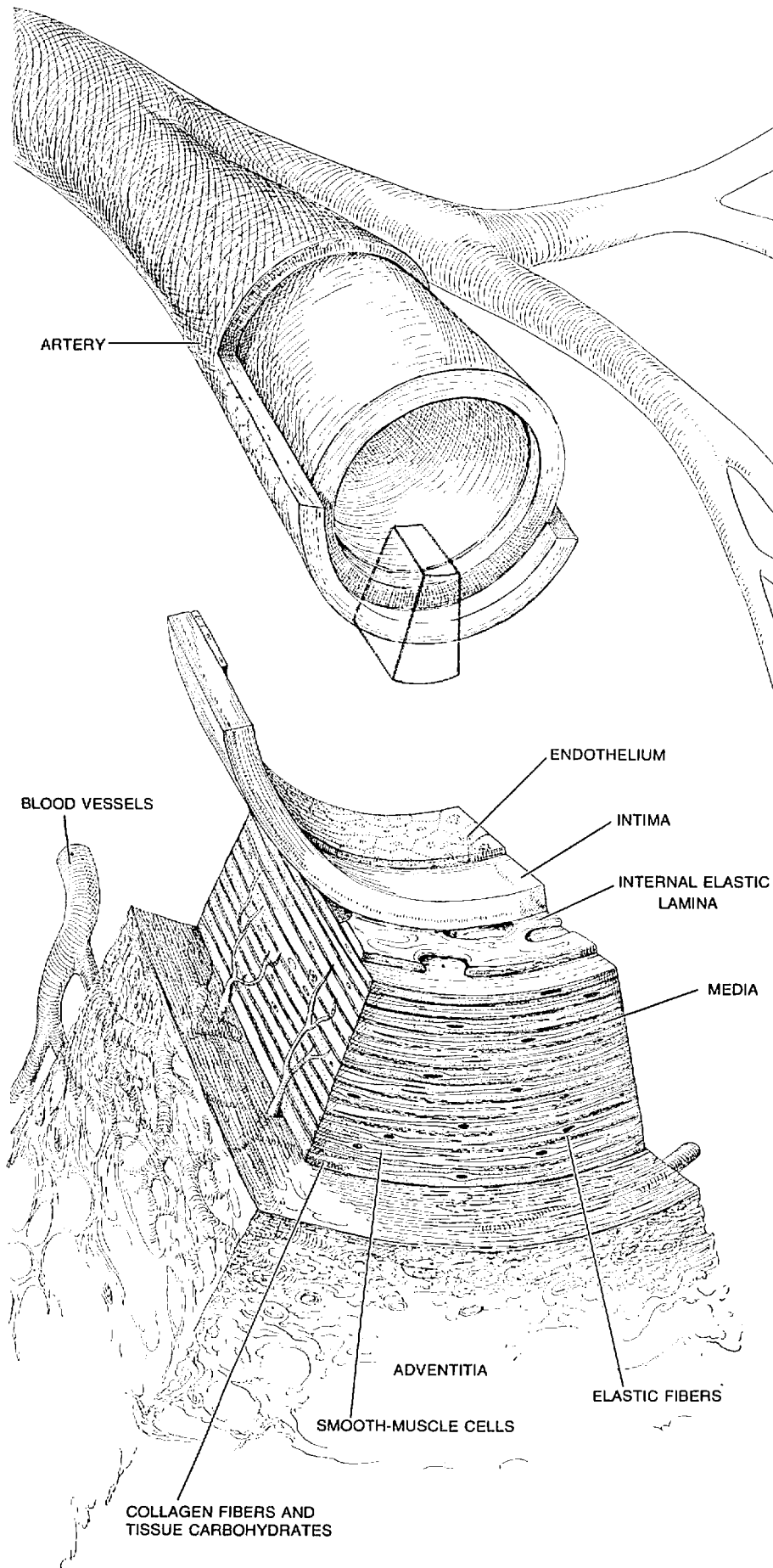


THROMBUS that formed just downstream of the narrowing caused by an atherosclerotic plaque blocked a coronary artery, shutting off the blood flow to part of the heart muscle and leading to a fatal heart attack. The darker regions of the thrombus are composed of red blood cells and the lighter regions contain white blood cells and platelets.



ATHEROSCLEROTIC INNER SURFACE of an artery is seen at about two and a half times natural size in this photograph of a hu-

man-aorta segment that has been slit open. The plaques are seen to be discrete lumps that bulge into the lumen of the dissected artery.

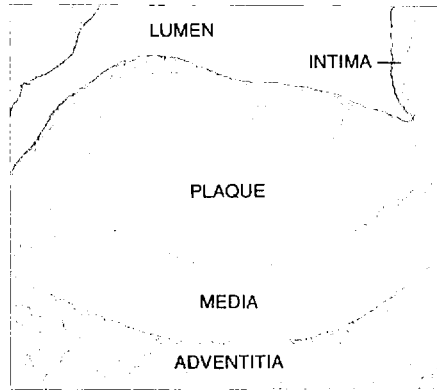


ARTERY WALL is built up of several layers, whose precise composition varies with the particular artery. A segment of a major elastic artery of the type of the human aorta is diagrammed.

clotting protein. Thrombosis is common in the veins of the leg, but for a thrombus to form in the rapidly flowing bloodstream of an artery it appears that special conditions are required: either a slowing of the flow or a region of turbulence in it; injury to the inner lining of the vessel, causing platelets to stick to it, and perhaps increased stickiness of the platelets themselves. Such conditions may be found on the downstream side of a plaque, where there is a region of turbulence and sometimes an ulcerated endothelial surface. Platelets stick to the surface and aggregate into a mass that appears to successively recruit white blood cells, initiate the formation of fibrin from fibrinogen in the plasma and then recruit more platelets as well as red cells. The layered mass thus built up may remain a relatively flat mural thrombus or may grow to become an occluding thrombus that blocks the passage of blood.

The theories that have guided the investigation of atherosclerosis until recently are versions of two basic approaches with origins in the 19th century. The dominant theory is associated with the German pathologist Rudolf Virchow. It holds that the infiltration of fatty substances from the bloodstream into the artery wall gives rise to deposits of cholesterol that act as an irritant, causing inflammation and the proliferation of cells. This insudation theory would appear to be supported by the increased rate of coronary heart disease among people with higher than normal levels of cholesterol in their blood. Elevated cholesterol levels have in turn been correlated geographically with diets that are high in food fats and cholesterol, a finding that has given rise to efforts to prevent atherosclerosis by regulating the diet. The insudation theory seemed to be further reinforced by the experiments of the Russian investigator N. N. Anitschkow early in this century. He found that a disease resembling human atherosclerosis could be produced in rabbits by adding egg yolks or cholesterol to their diet and hence raising their blood levels of fat and cholesterol. In the experimental rabbit disease, it should be noted, the lipids appear in the lesions early and can be shown to arise from the blood lipids. The rabbit model and its data have strongly influenced investigators' perceptions of the human disease.

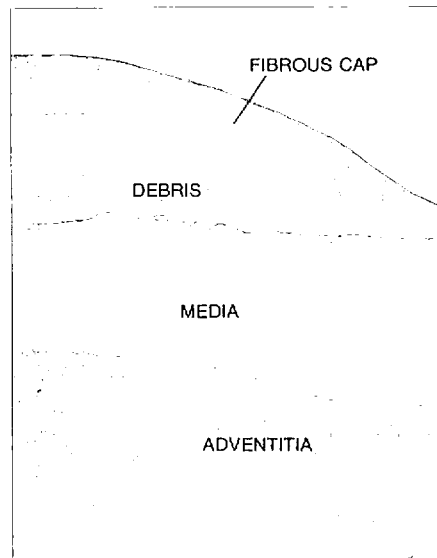
The fact that autopsies of infants and young children show small fatty deposits in the major blood vessels has given further credence to the lipid-insudation theory. It has also led people to assume that the natural history of atherosclerosis involves an evolution from the fatty streaks of childhood to the fully developed fibrous plaques of the adult disease. The trouble with such an assumption is that the fatty streaks appear to be



CROSS SECTION OF PLAQUE in a human coronary artery is enlarged 24 diameters in this photomicrograph, which is mapped at the right. The roughly oval atherosclerotic plaque, stained pink, is a thickening in the intima of the artery. The next layer is the media, stained brownish black. The outer layer, the adventitia, contains connective collagen and fatty tissue.

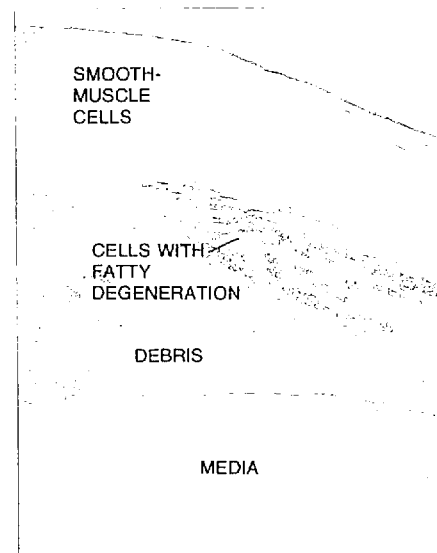
present to about the same extent in children of all populations, whereas adult atherosclerosis is rare in some populations and common in others.

The other basic concept of the origin of atherosclerosis, the encrustation theory, holds that the plaque begins with a small mural thrombus that is converted into a mass of tissue in the intima as arterial-wall cells migrate to it, multiply and then secrete the characteristic extracellular substances. Localized injuries to the arterial wall in experimental animals lead to a repair process of that kind, and substances that pass from the plasma into the wall, along with debris from dead and disintegrating elements of the thrombus, do cause such experimental lesions to resemble atherosclerotic plaques in many ways. It is postulated that in human atherosclerosis such lesions grow large enough to obstruct an artery as the result of repeated episodes of injury, each followed by the reparative clotting, migration and secretion.



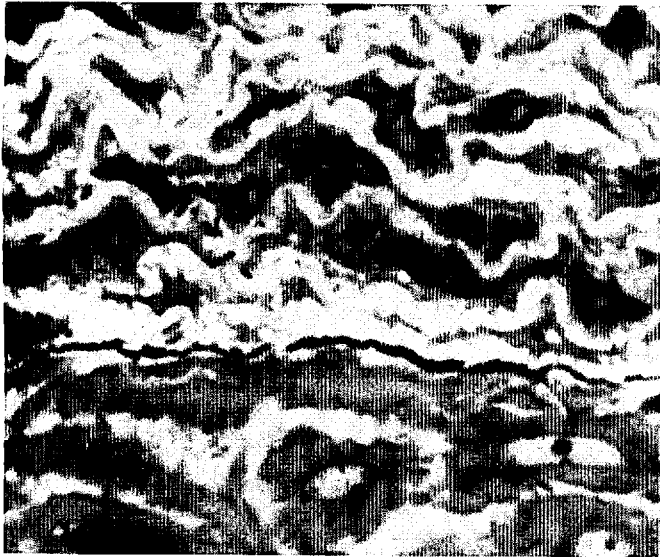
PORTION OF PLAQUE in the human aorta is enlarged 27 diameters. The fibrous cap of the plaque, a mass of cells embedded in a matrix of collagen and other extracellular material, is underlain by debris. The plaque is elevated above the adjacent intimal surface of the vessel.

Ideas about the nature and origin of atherosclerosis began to change when the electron microscope revealed the cellular composition of the plaque. The fibrous, cellular cap covering the cholesterol-rich debris turned out not to contain ordinary fibroblasts, the cells that proliferate to heal a skin wound, but rather to contain smooth-muscle cells similar to those of the normal artery wall. Then it was discovered that in its early stages the human plaque does not contain much lipid, which implied that lipid insudation is not the primary initiating factor. By now the major current theories of the genesis of atherosclerosis share the belief that the lesions begin as localized excessive accumulations of smooth-muscle cells in the intima. The current debate is about what initiates those accumulations and what makes them grow.

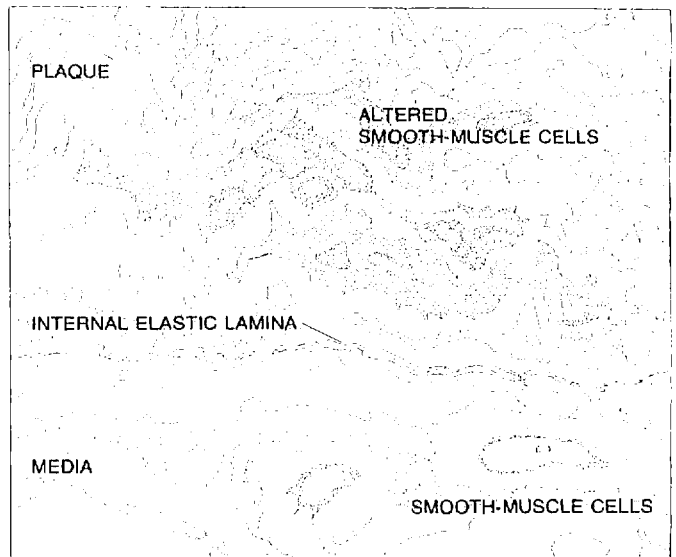


PLAQUE IN THE AORTA is further enlarged to 60 diameters and stained for cell nuclei (blue) and fat (orange). The cap is clearly composed of many cells embedded in an unstained fibrous matrix. The debris includes cells that contain fat and, below them, extracellular fat.

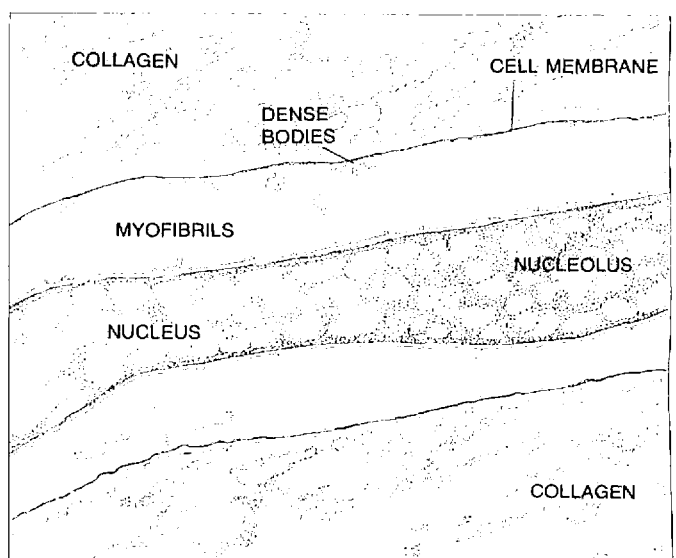
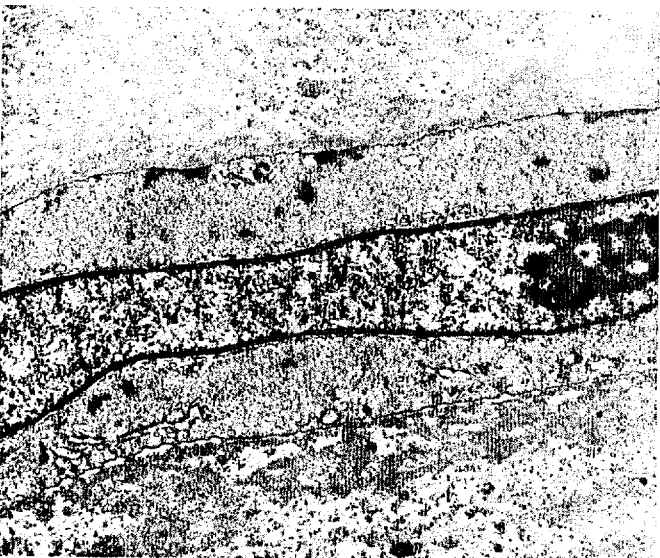
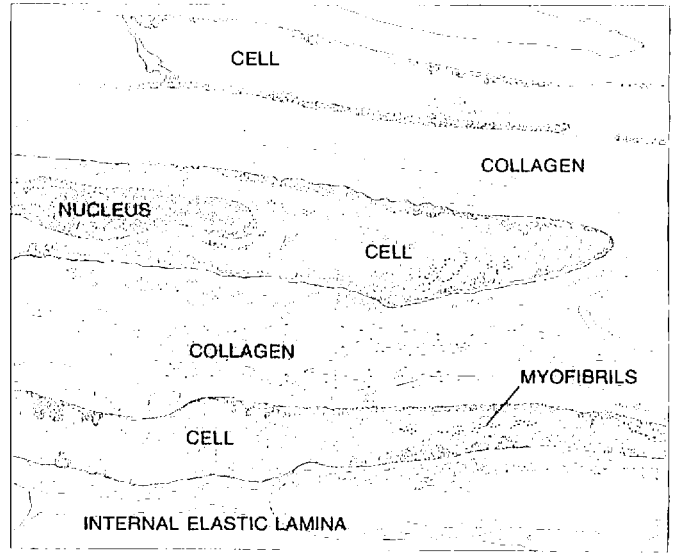
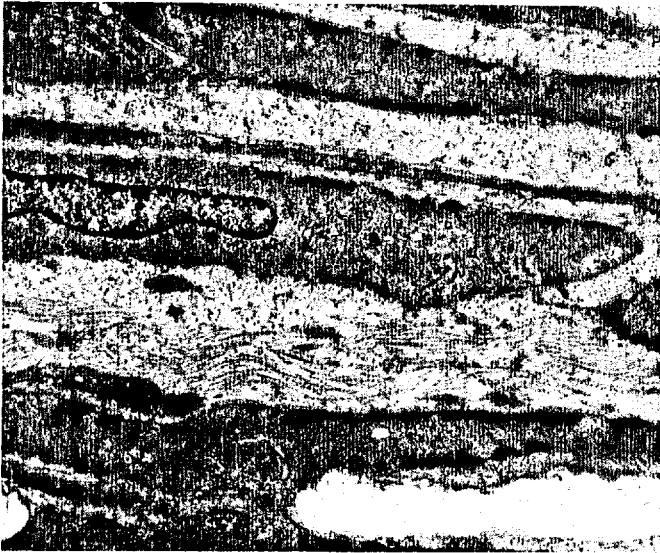
After having worked with various animal models of atherosclerosis in our laboratory at the University of Washington School of Medicine and having found them unsatisfactory, in 1967 I began, along with Ned S. Moss, to study atherosclerosis in chickens. That was the animal in which Ruth Pick, Jeremiah Stamler and Louis N. Katz of the Michael Reese Hospital had discovered that the development of fatty lesions in chicken blood vessels after cholesterol feeding could be prevented by treatment with a form of the female hormone estrogen; their finding had led to trials in which estrogen was administered to some male coronary patients in an effort to prevent a second occlusion. Moss and I fed cholesterol and administered estrogen to chickens and examined their vessels with the electron microscope. We found, first of all, that the lesions that cholesterol induced in the chickens did not resemble human atherosclerotic



CELLULAR NATURE of an early atherosclerotic plaque is demonstrated in this photomicrograph. A section of the media and the

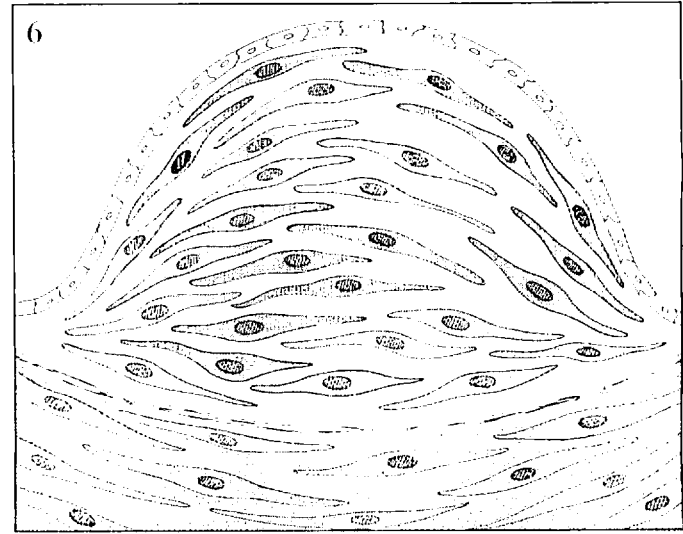
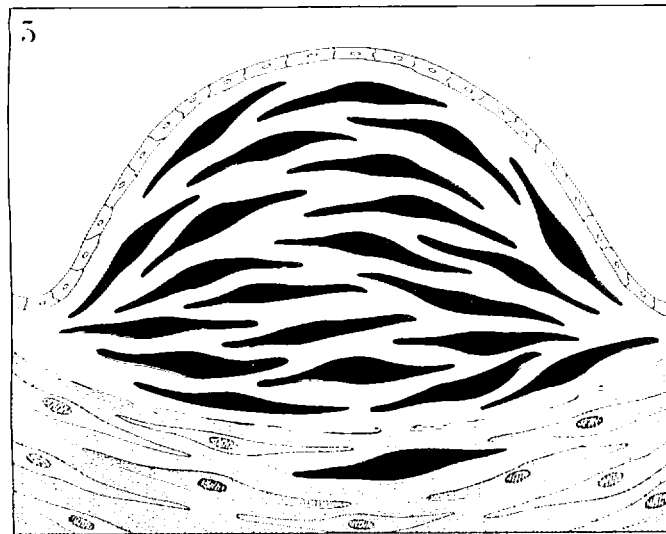
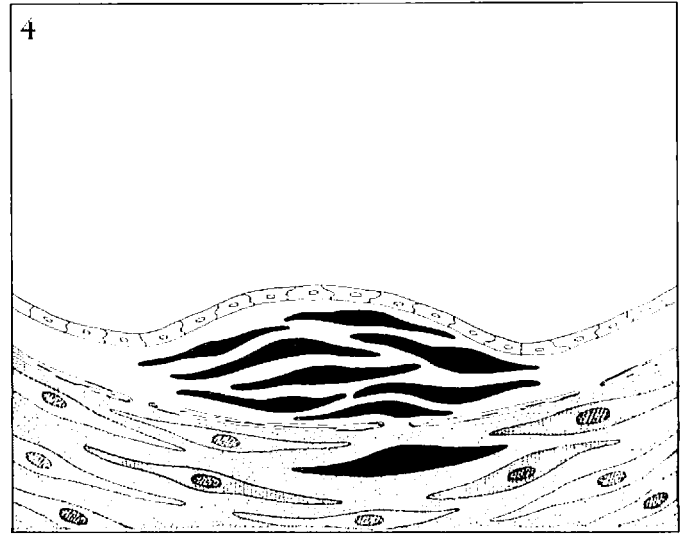
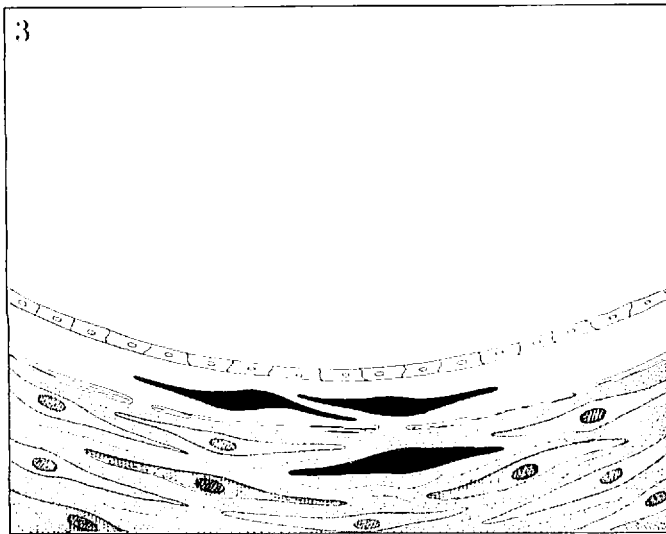
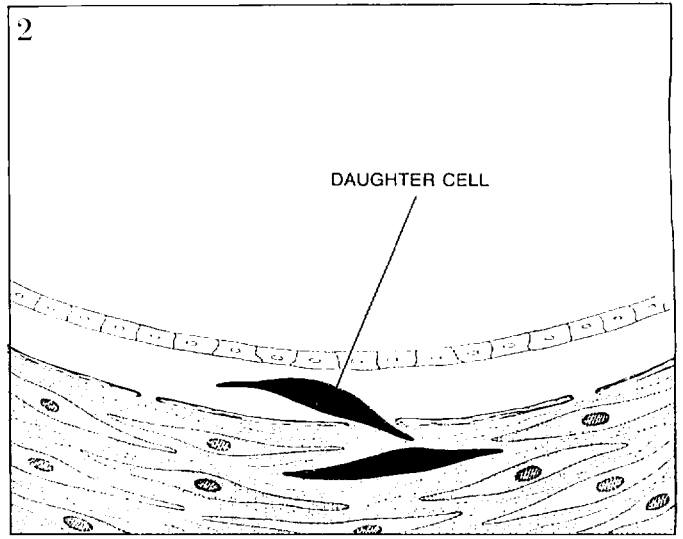
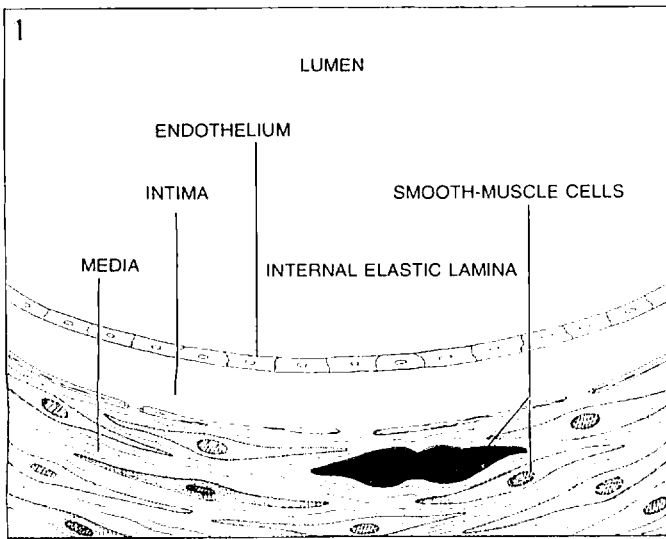


base of an early plaque in the aorta of a chicken is enlarged 1,200 diameters. The cells of the plaque are altered smooth-muscle cells.



INDIVIDUAL CELLS of plaques appear in these electron micrographs made by Ned S. Moss. Cells from the base of an early lesion in a chicken artery are enlarged 15,000 diameters (top). Collagen fibers

are seen head on and from the side, running between smooth-muscle cells. A single smooth-muscle cell is enlarged 40,000 diameters in a micrograph of a plaque from a human coronary artery (bottom).



GENESIS OF A PLAQUE as proposed by the monoclonal hypothesis is traced in these highly schematic drawings. The process begins in the inner media. There are two cell types. A single cell (*dark color*) has undergone a mutation that gives it a selective advantage, and some stimulus causes the mutated cell to divide (1). Its daughter cell migrates into the intima (2). The progeny of the mutated cell, having

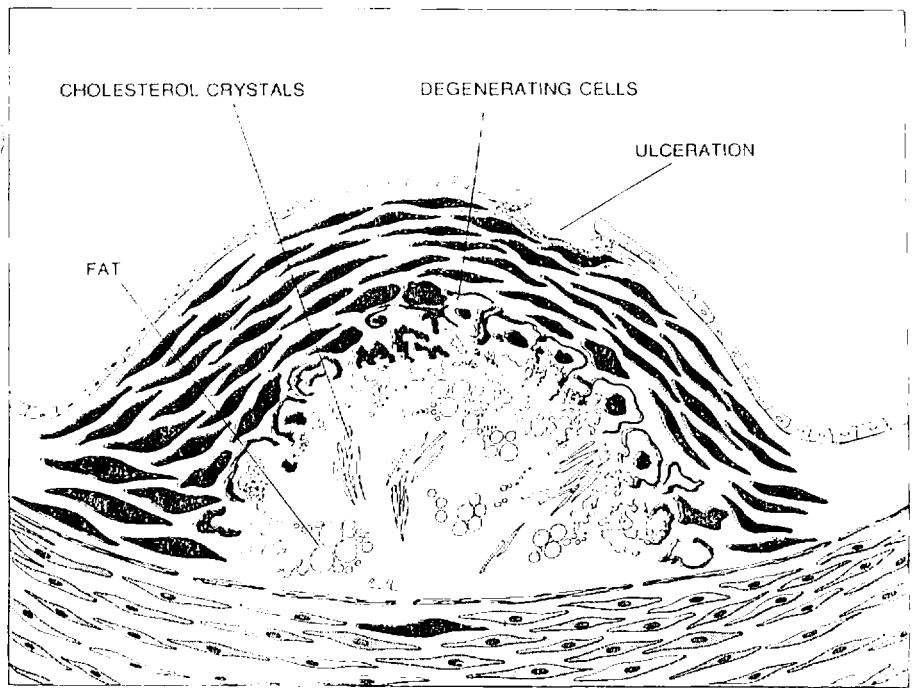
a selective advantage and perhaps somehow freed of some curb on proliferation, continue to multiply, thickening the intima (3, 4) and eventually forming a lumpy plaque (5) all of whose cells are progeny of the original cell that mutated. The last drawing (6) shows, by way of contrast, a polyclonal plaque, which is the kind that would arise from the migration and proliferation of many cells of both cell types.

plaques. They appeared to be composed entirely of fat-filled cells derived from blood macrophages; there was no evidence of significant smooth-muscle-cell proliferation. And none of the lesions evolved into the raised plaques characteristic of the human disease. We found, on the other hand, that chickens that were not given cholesterol developed an arterial disease whose lesions did bear a striking resemblance to the plaques of human coronary atherosclerosis. Actually this naturally occurring disease of chickens had been well described in the literature of comparative pathology, but we had all tended to ignore it when our attention was focused on the experimental disease produced by cholesterol.

Now we were able to observe the earliest stages of plaque formation in young, untreated chickens. The first sign of a naturally occurring plaque was a small group of cells in the intima, which is normally populated by only an occasional cell. As we reconstructed the sequence of events, one cell or possibly a few cells migrated from the media, proliferated and slowly gave rise to a mass of cells in the intima. There was no evidence of cholesterol deposits in these early lesions. Degenerated and dead cells, intercellular debris and cholesterol deposits did, however, appear in the later stages. Why? What was the precise source and nature of the cells that populated the plaque? What initiated their proliferation and what caused the continued multiplication and degeneration that produced the fully developed fibrous plaque?

We noted that the cells of the early spontaneous plaque were subtly different from normal artery-wall cells. They were arrayed differently, they appeared to be smaller and to have few or no intercellular junctions, and in contrast to the normal cells of the media they manufactured larger amounts of collagen than of elastin. Moreover, the early lesions developed fatty vacuoles (cavities) when the chickens were on cholesterol; nearby normal artery-wall cells did not develop such vacuoles. Perhaps some metabolic change stemming from the movement of the cells to a new location was responsible for the various differences between artery-wall and plaque cells in chickens.

We decided next to see for ourselves just what the cells of a normal artery wall look like in the course of injury and repair. John Poole (who was visiting our laboratory from the University of Oxford), Stephen Cromwell and I simulated an injury by putting a fine suture in the artery wall. A thrombus formed around the part of the loop that was in the flowing bloodstream, and smooth-muscle cells migrated from the media into the thrombus, where they multiplied. These cells that populated the



ADVANCED MONOCLONAL PLAQUE is often ulcerated at the surface where endothelial and smooth-muscle cells have died. The interior is filled with fatty debris, the result of cell death and degeneration as well as of migration of cholesterol and lipoproteins into the plaque.

thrombus had all the characteristics (apart from some positional distortions) of the normal smooth muscle cells of the media, including the formation of elastin and the development of intercellular junctions. In other words, they were in sharp contrast to the modified smooth-muscle cells we had seen in the spontaneous lesions in chickens.

We realized then that various forms of the insudation-irritation theories had continued to dominate thinking about the cellular proliferation observed in plaques. At one time it had been assumed that the cells were wound-healing fibroblasts, and this fitted the idea of a response to irritation. Then electron microscopy had revealed that the cells were smooth muscle cells, not fibroblasts, and yet no one had seriously questioned that the cells were there in response to some kind of irritation.

The study of the spontaneous lesions and the suture-induced lesions emphasized the unusual nature of the spontaneous-plaque cells and pointed to one of two origins for them: they could come from some small cell population that is present but not ordinarily observed in the normal media and is caused to proliferate by a stimulant of some kind, perhaps one connected with injury, or they could be smooth muscle cells that have been altered by mutation. If they are genetically altered cells, then the cells of a given plaque would be expected to be monoclonal: to have been derived, like the cells of a benign tumor, from a single mutated cell. If, however, the plaque

cells arise in response to injury or some other stimulus, the stimulus would presumably have its effect on many normal cells at many places, in which case the plaque cells should be polyclonal. The question—monoclonal or polyclonal—is susceptible to experimental testing in humans by a genetic technique.

The technique, originally applied by the geneticist A. H. Sturtevant to the study of tissue development in fruit flies, is based on the fact that an individual animal may be a "mosaic" composed of two distinguishable cell populations. As Mary F. Lyon of the Medical Research Council Radiobiology Unit at Harwell, England, first postulated, this is true of all human females. In female cells there are two *X* chromosomes, one derived from the father and one from the mother, only one of which is active in adult cells; the other is inactivated early in embryonic development and remains in the nucleus as a dense bit of chromatin known as the Barr body. The inactivation in any one cell is apparently random, so that either the maternal *X* chromosome or the paternal one may remain active in a given cell and in all the progeny of that cell. If there is a "marker" gene, such as one for a particular enzyme, in the maternal chromosome that is different from the corresponding gene in the paternal chromosome, one can distinguish between cell populations in which one or the other chromosome is active. There are several such polymorphic genes on the *X* chromosome. One of them codes for the enzyme glucose-6-phosphate dehydrogenase (G-6-

PD), and its two forms code for two major enzyme types that can be distinguished by electrophoresis: when subjected to an electric field, one type (*A*) moves faster than the other type (*B*).

As in the well-known case of the sickle-cell hemoglobin trait, a subset of the *A* type of G-6-PD confers resistance to malaria and is relatively common in the U.S. black population, which originated in malarious areas of Africa and continued to live for a long time in malarious areas of the U.S. About 40 percent of black females are heterozygous for the G-6-PD gene, that is, some of their cells carry each of the two enzyme types. R. G. Davidson, H. M. Nitowsky and Barton Childs of the Johns Hopkins University School of Medicine capitalized on the presence of the two enzyme types to establish the fact that once a particular *X* chromosome is inactivated in a human cell it remains inactivated. They cultured bits of skin from black women who had the two enzyme types. The fibroblast populations that grew in culture manufactured both enzymes, showing that the cells were of both types. When the investigators isolated single cells from the mixed population and cultured them, however, each clone (the progeny of a single cell) exhibited only one enzyme type, *A* or *B*; repeated subculturing of cells from a single clone continued to yield the same enzyme type. With the stability of the two cell populations' enzyme production thus established, it became possible to turn the process around: to analyze extracts of tissue samples for their G-6-PD composition by electrophoresis, and so to determine their cell mixture. For exam-

ple, at the University of Washington School of Medicine, David Linder and Stanley M. Gartler examined tumors of uterine smooth muscle and found that each tumor was of one cell type; Philip J. Fialkow and others extended the studies to leukemia and other tumors.

In 1973 we applied this analytic technique to atherosclerotic plaques and normal artery-wall tissue from vessels obtained at autopsy. The first case yielded 15 plaques that could be analyzed. Four produced only the *A* type of the enzyme and eight produced only the *B* type: three showed a mixed cell population. On the other hand, of 27 artery-wall samples that did not exhibit lesions, 25 had a rather even mixture of the two types of cells. (The two samples with a single enzyme pattern may have contained small plaques, but we could not be sure.) Analysis of more cases bore out those first exciting findings: it was clear that an atherosclerotic plaque frequently consists of cells of just one type. In 1975 nearly identical results were reported, based on a larger number of cases, by T. A. Pearson, A. Wang, Kim Solez and Robert H. Heptinstall of the Johns Hopkins School of Medicine. Similar results continue to be obtained as the observations are extended in these laboratories and in others. I should point out that it is not surprising that some atherosclerotic lesions appear not to be monoclonal, since several sources of normally mixed cell populations may be in or close to a plaque, for example contaminating blood cells or ingrowths of adjacent artery-wall connective tissue. Indeed, I had expected

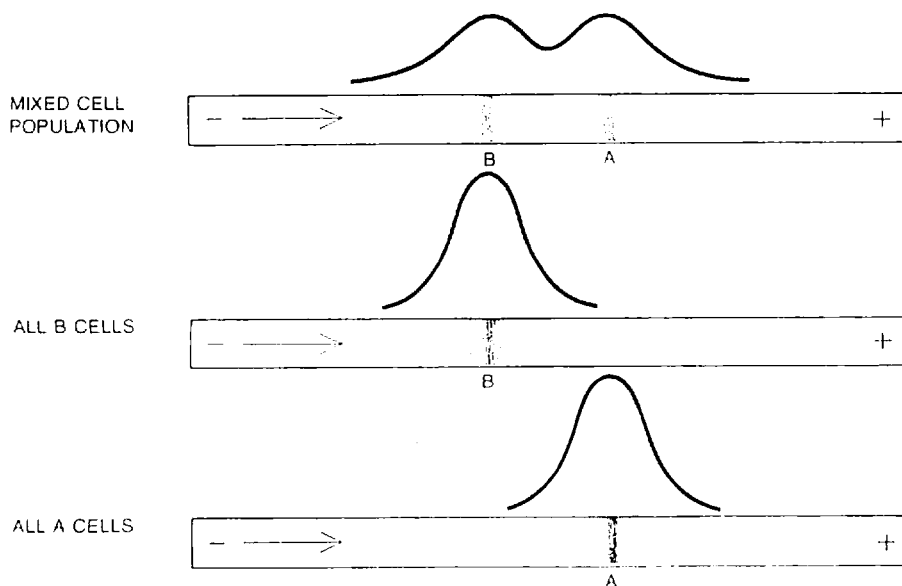
that blood-cell contamination might prevent our seeing any clear distinctions, and the data were therefore surprisingly unequivocal.

Before one can interpret the presence of only one cell type in a particular cell growth in a mosaic organism as being evidence of monoclonal origin, however, several issues need to be settled. First, one must establish that the size of the patches of cells of one type that can be found in normal tissue (patches that are presumably the result of accidents of cell growth and mixing during the formation of the embryo) is very small compared with the size of the putatively monoclonal growth. We did this by taking more than 1,000 samples of the smallest possible size from the inner portions of normal artery walls and analyzing the statistical variation in their cell populations. Our data suggest that the volume of a patch of cells of only one enzyme type in normal tissue is about a ten-thousandth of a cubic millimeter, which probably means it contains about 10 cells. A typical plaque, in contrast, has a volume of many cubic millimeters.

Second, one must exclude the possibility that the usual proliferative response seen in healing processes stems from a single cell. There are several indications that such is not the case. Wounds are quite generally observed to heal by the simultaneous proliferation of cells all around the periphery; more specifically, we found that many cells around a small injury to the aorta of experimental animals divided simultaneously. Moreover, examination of the thickened intima seen in middle-aged and elderly people, which is thought to be the result of repeated small injuries, reveals that the thickened areas are composed of mixed cell populations.

Some investigators have held that the presence of just one cell type in atherosclerotic plaques may be due to some process that favors the proliferation of one of the two cell types present in a mosaic individual. The fact that we regularly find both lesions composed of type-*A* cells and lesions composed of type-*B* cells in the same individual indicates, however, that neither enzyme type is producing a selective advantage or disadvantage.

It seems reasonable to consider the single-cell plaque as being monoclonal in origin and to propose that some event provides a single cell with an advantage over its neighbors, and that the progeny of that altered cell dominates an ensuing process of normal replacement multiplication or some kind of stimulated multiplication. The commonly accepted reason for the appearance of such a selective advantage in a body cell is an alteration in its genetic apparatus: a mutation. We have proposed that there are three stages in the pathogenesis of athero-



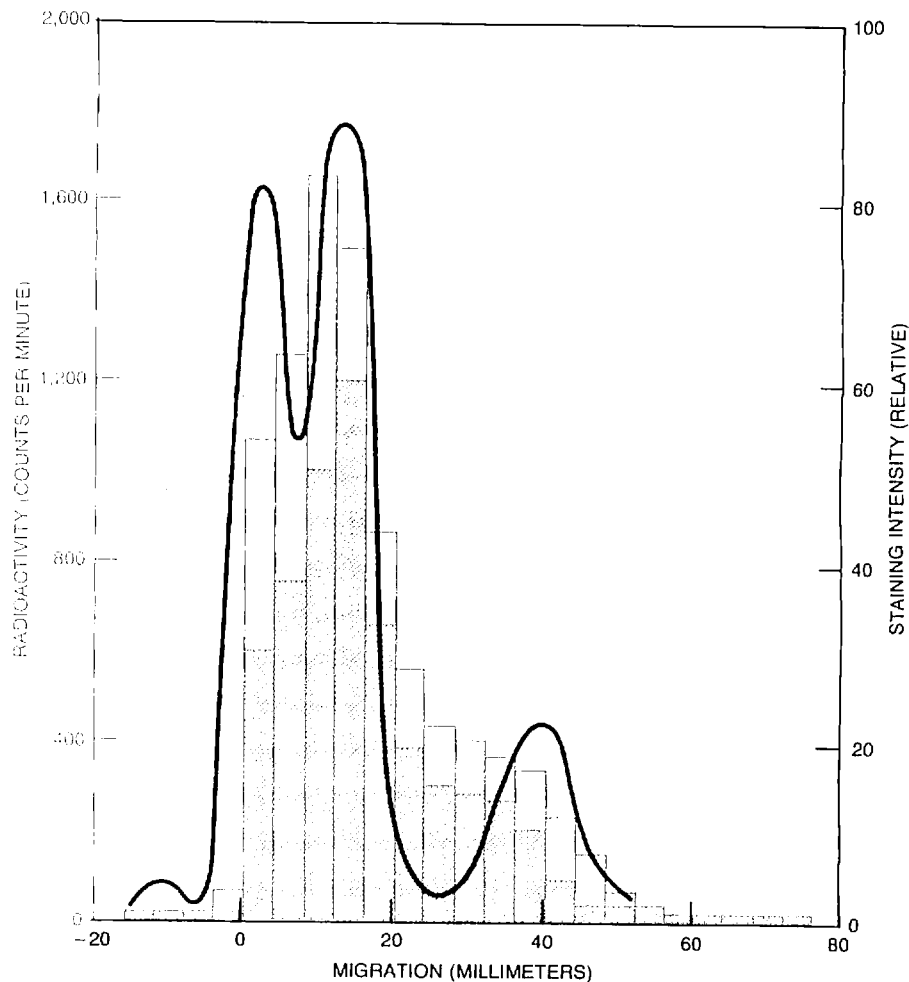
ELECTROPHORETIC PATTERN reveals the presence of two cell populations with two enzyme types. Enzyme solutions applied to a membrane migrate across it (arrows) under the influence of an electric current. After 50 minutes the position of the enzyme bands is visualized (color) by allowing the enzyme to react with its substrate, precipitating a dye. The presence of two separate bands shows there are two enzyme types; a photometric scan of the bands (black curves) allows one to estimate the proportions of the two enzyme types and thus of the cell types.

sclerosis. First comes an initiation stage during which there is mutation in an artery-wall cell. Then some factors or conditions promote the expression of the selective proliferative advantage conferred by the mutation, enlarging the mass of cells; the mutation, in other words, may never be expressed as gross plaque formation unless something promotes cell multiplication and gives the altered cells an opportunity to express their altered capability for growth. Finally, there is the stage of complication: the tendency of cells to degenerate and of lesions composed of those cells to break down and ulcerate is compatible with the presence of an altered cell population.

If this general proposal is correct, one ought to consider as causes of atherosclerosis the conditions and factors that cause mutation or facilitate the expression of advantageous mutations. Among the possible initiating factors are intrinsic genetic ones that lead to excessive mutations and such extrinsic ones as chemical mutagens, viruses and possibly ionizing radiation. The factors that might promote the subsequent development of atherosclerotic plaques are those that promote cell multiplication, such as certain chemical or physical injuries. The possible causes of the third stage, plaque degeneration, are not at all clear yet.

The primary value of the monoclonal hypothesis is that it provides a new framework within which one can ask new questions about the role of various risk factors. Cigarette smoking, dietary habits, changes in blood lipids (of genetic or dietary origin) and hypertension (high blood pressure) have all been implicated as contributing to the rising incidence of coronary disease and strokes. How does cigarette smoking, for example, fit into the monoclonal hypothesis?

The burning of cigarettes manufactures chemical substances that are known to be precursors of mutagens; among them are the aryl hydrocarbons, such as benzpyrene and methylcholanthrene. The fact that the enzyme aryl hydrocarbon hydroxylase, which converts these premutagens into mutagens, is induced (produced in extra amounts) in the liver and other tissues when the substances are administered shows that they are taken up by the blood and carried through the body. In what blood elements are these noxious substances carried? We have shown by electrophoresis that they are carried in the low-density and very-low-density lipoproteins, the same fraction of the blood-protein spectrum in which cholesterol is carried. It has also been noted that the higher the concentration of lipoproteins in the blood is, the more aryl hydrocarbons the blood will carry. Where do the aryl hydrocarbons go? Workers in several laboratories have established



POTENTIAL MUTAGENS are carried in the blood by the same blood proteins that carry cholesterol. The black curve shows the electrophoretic pattern of lipoproteins in human blood serum; the peaks at the left represent the chylomicrons and the low-density lipoproteins. Radioactively labeled cholesterol dissolved in serum migrated mainly with those lipoproteins (color bars). A radioactive sample of the premutagen methylcholanthrene migrated in the same lipoproteins (hatching in bars) as cholesterol, lipoproteins known to move to cells of artery wall.

that blood lipoproteins seem to be particularly good nutrients for culturing smooth-muscle cells from the human artery wall. Mont Juchau, James Bond and I have found that the aryl hydrocarbon hydroxylase enzyme system is present in the artery wall of rabbits, monkeys and human beings—and that the system in the wall is induced, or turned on, by aryl hydrocarbons.

What all of this means is that artery-wall cells are capable of converting into mutagens certain premutagenic substances that come from the environment and are transported to the cells by blood lipoproteins. Does that happen and does it cause atherosclerosis? Roy E. Albert and Martin Vanderlaan and their colleagues at the New York University School of Medicine administered two known mutagens, benzpyrene and dimethylbenzanthracene, to chickens. The mutagens gave rise to marked increases in the number of plaques in the aorta and in the rate at which the plaques developed, and they did so without there being any increase in the blood level of cholesterol. The mechanism of plaque

formation in the chickens has not been reconstructed, but the results are consistent with the monoclonal hypothesis.

Cholesterol, the centerpiece of the insudation theory, may also be found to fit the monoclonal hypothesis. M. F. Gray, T. D. V. Lawrie and C. J. W. Brooks of the University of Glasgow noted the presence of an epoxide derivative of cholesterol in human blood serum and found that the epoxide's level was elevated in people with high blood cholesterol. Cholesterol epoxide is known to be able to produce connective-tissue tumors in mice and rats. Perhaps it is because epoxides or other substances formed in the body from cholesterol are mutagens that cholesterol levels correlate with the incidence of coronary disease. Clearly it would be interesting to look for evidence of mutagens in the blood serum of populations that have high and low risks of atherosclerosis. We have begun to do that, working with the bacterial mutagen-screening system developed by Bruce N. Ames of the University of California at Berkeley. There are also mammalian-cell-culture

systems by means of which one can find substances that are injurious to DNA, the genetic material, and hence likely to be mutagenic for animal cells.

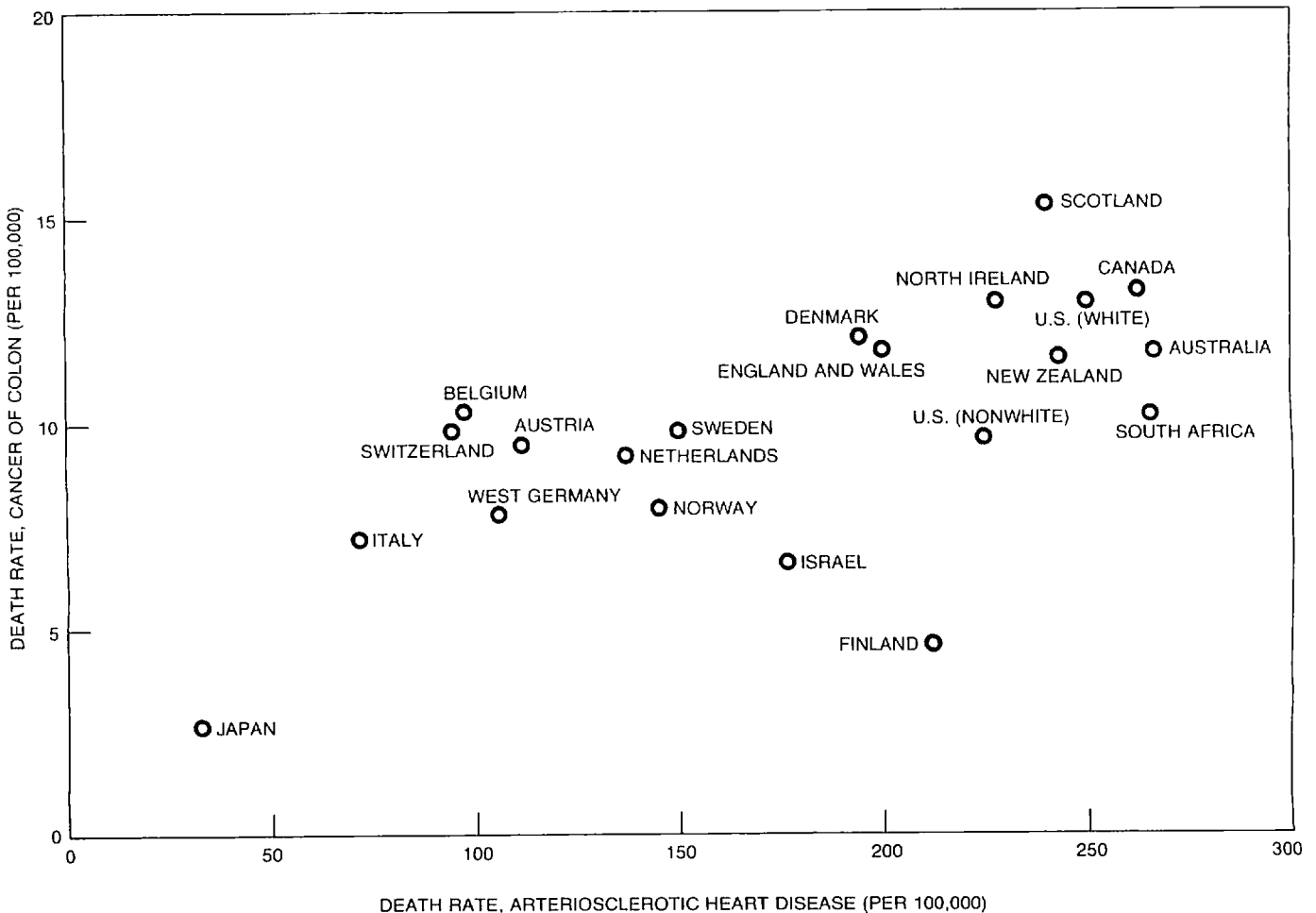
High blood pressure, which is known to increase the risk of atherosclerosis, may do so by exerting a chemical or even hydrodynamic effect that makes artery-wall cells multiply faster; in our laboratory Stephen Schwartz is trying to identify some such mechanism. Recently R. W. Pero and his colleagues at the University of Lund in Sweden found that the DNA of people with hypertension is more susceptible to breakage in cells by mutagens than the DNA of people with normal blood pressure. This finding seems to conform with evidence that the incidence of cancer is higher in individuals with hypertension. Is it possible that there are subsets of the human population with an increased propensity for mutation in particular tissues, such as the artery wall? An increased potential for mutation, if it is combined with exposure to substances in the environment that enhance the rate of mutation, could lead to a higher incidence of plaque formation.

The epidemiologist Ernest L. Wynder has pointed out an association on a geographical basis between the death rates for cancer of the colon and for arteriosclerotic heart disease [see illustration above]. Presumably there are either environmental or genetic factors, or both, that effect such an association. A connection has been established between at least one dietary factor and cancer as well as atherosclerosis: breast-cancer rates have been found to be closely correlated worldwide with dietary fat intake, a correlation not unlike that found for deaths from heart disease.

I have alluded to therapeutic trials of estrogens for preventing recurrent heart attacks in men. They were undertaken on the basis of experimental evidence in chickens and of the well-known observation that before menopause women have fewer heart attacks and less atherosclerosis than men, whereas after menopause the incidence in women of both coronary disease and atherosclerosis rises. When estrogen was tested in men in a large and carefully designed study, the Coronary Drug Project, no overall positive therapeutic effect of estrogen could be discerned. On the con-

trary, the overall death rate for the men given estrogen was somewhat higher than that for the control group, and so the treatment was discontinued. The estrogen group also showed an increased incidence of cancer. The monoclonal theory suggests a possible explanation for these effects of estrogen.

Some of the chickens to which Moss and I administered the potent synthetic estrogen diethylstilbestrol developed tumors of the lymphatic system, an effect that had been observed earlier. The atherosclerotic plaques also appeared to be worse in the diethylstilbestrol animals. And in the smooth-muscle cells of the plaques and artery wall of one of them we discovered virus particles, something we had not seen in a great many samples from many animals not treated with estrogen. In some of the earlier experiments at Michael Reese Hospital it had been noted that a combination of cholesterol feeding and estrogen administration caused the spontaneous plaques of chickens to ulcerate. It is well known that diethylstilbestrol can rapidly elicit the proliferation of latent tumor viruses in mice. These various observations suggest that the activation of latent



CORRELATION between the death rates for cancer of the colon and for arteriosclerotic heart disease, indicated by the roughly linear

arrangement of points on this scatter chart, suggests that cancer and atherosclerosis are likely to have some causative factors in common.

viruses may be a possible mechanism by which some of the complications of human atherosclerosis are induced.

The idea that atherosclerotic plaques may be some form of neoplasm, or abnormally proliferating tissue, is quite startling if one's concept of a neoplasm is limited to malignant cancers, which spread. Many tumors, however, are benign: they remain localized, grow slowly and may even regress. As a matter of fact, a current concept of how cancers originate postulates that several successive mutational steps are required before extreme loss of control allows the tumor to spread [see "The Cancer Problem," by John Cairns; *SCIENTIFIC AMERICAN*, November, 1975]; it appears that many more cells may undergo the first changes toward neoplasia than have up to now been considered. And even a small potential of cells for enhanced growth would obviously have more serious consequences in the narrow confines of an artery than, say, in the skin.

New information is emerging about the biology of the artery wall and its cells that should make it possible to understand how such loss of control may come about. At the University of Washington School of Medicine, Russell Ross and John A. Glomset and their co-workers have found a protein in blood platelets that promotes the multiplication of arterial smooth-muscle cells and other cells in culture; it seems to have a role in stimulating cell proliferation to repair an injury. One can envision the possibility that a step in the evolution of an atherosclerotic plaque is the loss by a cell of the need for this protein, so that the cell and its progeny divide when they should not and thus produce the mass of the plaque. Pursuing a different line of research, George M. Martin in our department has been studying the aging in culture of cells from animal and human artery walls. Cells from different arteries age at different rates; cells from segments of the aorta that are more prone to atherosclerosis tend to age more rapidly. This may be an important clue to what determines the unequal distribution of atherosclerotic plaques throughout the arterial system.

The multifactorial nature of atherosclerosis and its complications is evident. The monoclonal hypothesis does not immediately simplify the problem of identifying the causes of heart attacks and strokes. What it does do, I believe, is to enrich the information and the ideas that are available to investigators. It thereby puts us in a much better position from which to consider, test and identify the multiple factors, genetic and environmental, that are responsible for the current epidemic of these diseases, which not only shorten human life but also impair its quality in our steadily aging population.

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