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Review

Regression of Atherosclerosis in Primates

Herbert C. Stary

Department of Pathology, Louisiana State University School of Medicine, 1542 Tulane Avenue, New Orleans, LA 70112, USA

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Introduction

Studies published during the past decade claimed that, in nonhuman primates, proper manipulation of the diet could cause obstructive atherosclerotic lesions to shrink, and nonobstructive atherosclerotic lesions (fatty streaks) to disappear. Those reports contradicted the findings from earlier studies dealing with the question of atherosclerosis regression in animal species phylogenetically further removed from man than nonhuman primates. The earliest studies, in rabbits, had suggested that although the lipid component of atherosclerotic lesions could decrease, that decrease was accompanied by transformation to more complicated fibrous plaques without apparent reduction in the size of the lesions (Anitschkow, 1928, 1967). Subsequent studies in rabbits were equally discouraging (Constantinides et al., 1960; Prior and Ziegler, 1965; Adams et al., 1973).

In the 1950s and 1960s, nonhuman primates became the preferred model for atherosclerosis research in America because of close anatomic, biochemical, and metabolic similarities between man and some of the other primates, and because several investigators (Mann and Andrus, 1956; Taylor et al., 1962) had observed that various primate species could develop atherosclerosis that resembled the lesions in man. The availability of primate atherosclerosis models and intense clinical interest in preventing coronary heart disease and other complications of atherosclerosis probably gave the impetus to reexamine the regression question experimentally.

The recent claims of atherosclerosis regression in primates aroused great interest and hope, and predictably, they also raised critical questions. Perhaps the most valid question was whether or not the therapeutic measures to which

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monkeys had been subjected would also cause lesion regression in humans. The lesions that had been regressed in monkeys did not reflect the entire morphological spectrum of human atherosclerosis, and the dietary manipulations had been drastic.

Here I will attempt to organize and analyze the available data on atherosclerosis regression in primates and address some of the critical questions, particularly those raised in connection with the significance of the nonhuman primate data to man, and those concerned with the methodology used in the experiments. Primate atherosclerosis in general was reviewed in several recent publications (Strong, 1976; Gresham, 1976; Wissler and Vesselinovitch, 1977). Armstrong (1976) and Wissler (1978) reviewed the earlier studies of atherosclerosis regression in various animal species, and two separate volumes that deal with various aspects of atherosclerosis regression were also published recently (Hauss et al., 1978; Schettler et al., 1978).

Regression With Diets Very Low in Fat and Cholesterol

Armstrong et al. (1970) published convincing evidence that stenosis of arteries by atherosclerotic plaques can be significantly diminished by dietary manipulation alone. In their experiments, relatively advanced lesions containing lipid and connective tissue components were produced in rhesus monkeys that were fed an atherogenic diet (1.2% cholesterol, 40% eggyolk fat), thereby raising the serum cholesterol to about 700 mg/dl. After 17 months on this diet a group of monkeys was killed. Coronary artery lesions were measured and aortic lesions chemically analyzed. The food of the remaining monkeys was then changed either to a cholesterol-free, low-fat diet, or to a cholesterol-free, linoleate (corn oil)-rich diet. When the monkeys were killed 40 months after diet change, measurement of the coronary arteries showed that overall luminal narrowing at all sites had decreased to 17% and 25% respectively in the two groups from the 58% narrowing found after 17 months on the atherogenic diet. Aortic cholesteryl esters decreased 69% and free cholesterol by 53% (Armstrong and Megan, 1972). The findings in two monkeys killed 20 months earlier suggest that the cholesterol decline had occurred during the first 20 months after diet change. In a separate experiment, Armstrong and Megan (1975) showed that the collagen content was increased in the aortas of cynomolgus monkeys after 17 months on an atherogenic diet, and that subsequent removal of the cholesterol from this diet for up to 20 months caused a moderate decrease in this collagen increment.

Thus Armstrong and his colleagues showed that by withdrawal of the atherogenic stimulus from the diet, lipid could be mobilized and that some types of collagen fibers could probably break down and be removed from advanced lesions. However, lesions did not disappear and stenosis was not completely removed even after $3^1/_2$ years. In our own laboratory, we therefore tested in two consecutive experiments whether or not there was a stage in the development of atherosclerosis at which lesions could be made to dissolve completely. Our two experiments were identical in every respect except for the number

of weeks during which animals received dietary treatment. First we produced hypercholesterolemia and arterial lesions in male rhesus monkeys by feeding them an atherogenic diet consisting of commercial primate food supplemented with butter, beef tallow, and 0.4% cholesterol. During the 12-week period of the special diet, serum cholesterol elevations ranged from a mean of 230 mg/dl in the lowest responding monkey to 640 mg/dl in the highest responder. At the end of the 12 weeks we killed a group of monkeys with a representative range of serum cholesterol elevations to determine the characteristics of lesions that presumably were present also in the remaining monkeys in which regression was to be studied. We changed the diet to unsupplemented commercial primate food that was very low in fat and cholesterol, and killed groups of the remaining monkeys at intervals of 2, 3, 4, 8, 12, 16, 24, 32, 40, 64, and 128 weeks after the diet change. Serum cholesterol declined to normal levels (110–165 mg/dl) in most animals within 4 to 8 weeks after diet change. Gross sudanophilic lesions in the aorta were only half as extensive 32 weeks after diet change compared with those in the baseline group, and the lesions further decreased at 64 weeks (Eggen et al., 1974; Strong et al., 1976). Results of chemical lipid analysis of the aortic intima-media paralleled the gross measurements. During the 12-week period while animals were receiving the atherogenic diet, free and, to a greater extent, esterified cholesterol increased to more than twice normal. Seventy-three percent of the free and 85% of the esterified cholesterol increase had disappeared by 32 weeks after start of the regression diet. The remainder of the increment decreased more slowly. After 64 weeks the aortic free and ester fractions were still slightly higher than those of control monkeys that had always received only a low fat, low cholesterol diet (Eggen et al., 1974; Kokatnur et al., 1975).

The short intervals at which we killed groups of animals allowed us to study the sequence in which various lesion components regressed in aortic and coronary artery lesions. The sequence of events within lesions as observed with the electron microscope is summarized in Fig. 1 (Stary et al., 1977). Lipid-containing macrophages (foam cells), which had accumulated in intimal lesions during the hypercholesterolemic period but had not yet died, died during regression. Many foam cells were dead at all early intervals of lesion regression as, simultaneously, their overall number was decreasing. We saw no evidence of migration of foam cells from the vessel wall during lesion regression, a mechanism that has been suggested by others as a possible means whereby foam cells might remove arterial lipid. Tritiated thymidine radioautography of the aorta showed that, during the same period, the increased proliferative activity of endothelial cells, intimal smooth muscle cells, and foam cells observed during hypercholesterolemia, returned to the low proliferative activity of control animals (Stary, 1974 a, b). Foam cell death combined with the cessation of increased proliferative activity caused a dramatic decrease in lesion cellularity. After 24 to 32 weeks, that is, by the time that most of the aortic cholesterol increment had disappeared chemically, regressing lesions contained only infrequent, isolated foam cells.

Extracellular lipid and debris neither decreased nor increased but remained stable while the number of intact foam cells was decreasing. This morphological observation indicates that a continuous turnover and removal from the arterial

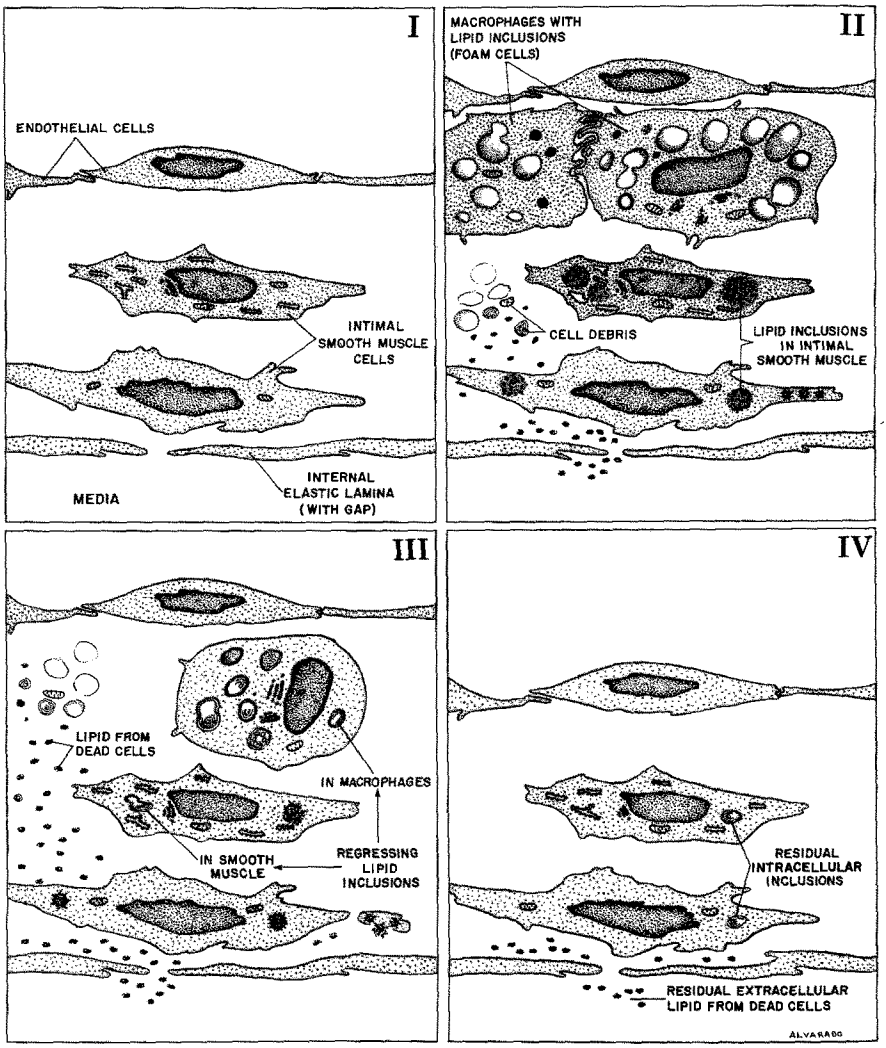

Fig. 1 I-IV. Schematic drawings summarizing the main fine structural features in the development and in the regression of a small atherosclerotic lesion (fatty streak). **I** Normal intima. Smooth muscle cells, some rich in rough-surfaced endoplasmic reticulum and others rich in myofilaments, are frequent. Macrophages are rare. No lipid either intra- or extracellularly. **II** Fatty streak produced by dietary elevation of the serum cholesterol (400–600 mg/dl) for 12 weeks. Intimal thickness is increased primarily due to the accumulation of macrophages with lipid inclusions (foam cells). Intimal smooth muscle cells also contain lipid inclusions. Many cells are dead. Debris and lipid from dead cells accumulate in the intima and extend to the media through gaps in the internal elastic lamina. **III** Fatty streak in early regression, about 16–20 weeks after elevated serum cholesterol has been lowered (110–180 mg/dl). The number and size of viable foam cells is much smaller. Many cells are dead. The inclusions of the remaining foam cells and of smooth muscle cells have characteristically changed into secondary lysosomes. The amount of debris in the interstitial space has remained constant. **IV** Residual of a fatty streak (1–2 years after serum cholesterol has been lowered). Cellularity of the intima is normal. Macrophages are infrequent. The cytoplasm of a few intimal smooth muscle cells contains the residuals of larger lipid droplets. Extracellular lipid and debris from dead cells is not present or minimal

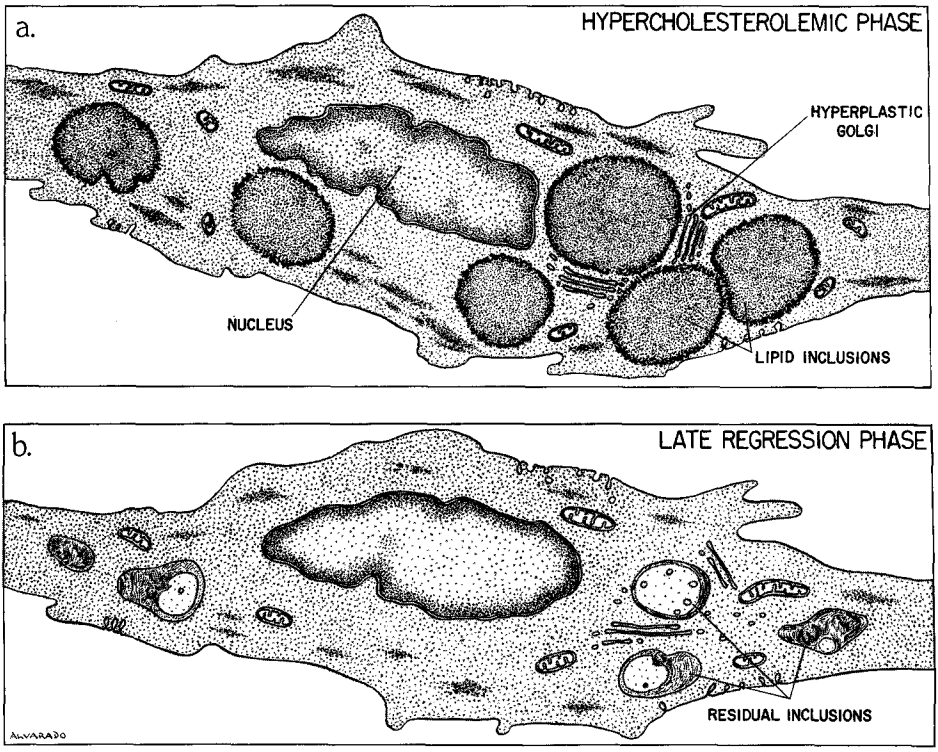

Fig. 2a and b. Schematic drawings depicting the fine structure of a smooth muscle cell during development and regression of a fatty streak. **a** Hypercholesterolemic phase. Free lipid droplets appear in the cytoplasm, increasing both in size and in number with the degree and the duration of the serum cholesterol elevation. Golgi apparatus and vesicles of the endoplasmic reticulum are hyperplastic. **b** Late regression phase (one year after drastic reduction of hypercholesterolemia). The free lipid droplets have changed into smaller membrane-bound inclusions or reduced further to pleomorphic structures consisting of finely laminated arrays of membranes (tertiary lysosomes). The total number of inclusions is smaller. Golgi apparatus and endoplasmic reticulum are less prominent. Drawing modified after Stary (1977)

wall must have been occurring since the decomposing foam cells were continually adding partly digested lipid and debris to the extracellular space. After foam cells were no longer present there was a gradual net loss of extracellular lipid and debris. After 128 weeks, trace amounts were visible by means of electron microscopy only in those animals that had had the highest serum cholesterol elevations (Stary, 1978). As opposed to foam cells, intimal and medial smooth muscle cells with lipid showed a great ability to survive. We saw dead smooth muscle cells only rarely once hypercholesterolemia was drastically reduced. A slowly progressive decrease in the size of their lipid droplets became apparent about 24 weeks after diet change. After 64 and 128 weeks, only a small number of smooth muscle cells contained inclusions, indicating that many smooth muscle cells had metabolized inclusions completely. When present, residual inclusions were residual bodies (telolysosomes) with probably very low enzyme activity, much smaller than the earlier lipid droplets (Fig. 2) (Stary, 1977).

The concurrence of foam cell loss ultrastructurally with the disappearance of most of the arterial cholesterol increment grossly and chemically after 32 weeks indicates that grossly and chemically demonstrable cholesterol is intracellular and located in macrophage-derived foam cells. At this point in time smooth muscle cell inclusions had decreased only moderately and much extracellular lipid and debris persisted. With the electron microscope we did not see an increase in interstitial collagen while cells and lipid were regressing from fatty streaks.

The results of our experiments are encouraging in that they show that drastic dietary treatment can completely remove lipid and proliferated cells without conversion to fibrous thickening. The findings are, however, also somewhat discouraging because they show that changes induced in arteries by a high cholesterol diet given for only three months required a much longer time than that to disappear. Portman et al. (1967) and Maruffo and Portman (1968) studied the fate of 3-month-old fatty streaks in squirrel monkeys but their results were not clearcut, probably because their animals were killed and studied after a relatively short (5-month) period of treatment with a low cholesterol diet.

Regression With Diets Moderately Low in Fat and Cholesterol

From the experimental evidence reviewed thus far, at least some types of human lesions apparently could be reduced in size by lowering very high serum cholesterol to the low levels possible in nonhuman primates. Important questions that remained unanswered were whether or not an only moderate decline of high serum cholesterol levels would reduce lesion size, improve lesion morphology, or slacken lesion progression. That knowledge is important because in most human subjects the serum cholesterol level cannot be reduced to as low a level as that of nonhuman primates, or even to that normal for the general population. In the Coronary Drug Project (The Coronary Drug Project Research Group, 1975) moderately high serum cholesterol levels were reduced by 6 to 10%. In another study (Leren and Hjermann, 1977), somewhat higher serum cholesterol levels were reduced 15 to 17.6%. The effect of such a relatively moderate reduction in the serum cholesterol level on the patient's arteries has remained controversial although some studies suggest that the incidence of clinical episodes may have decreased (Leren and Hjermann, 1977).

With this question in mind, a team of investigators at Bowman Gray School of Medicine used nearly 260 rhesus monkeys in one large experiment to study whether or not modest reductions in serum cholesterol from a very high level would decrease lesion size and subsequently decelerate lesion progression. An atherogenic diet (0.5% cholesterol) was fed for either 19 or 38 months to two large groups of monkeys, raising the serum cholesterol to a level of about 800 mg/dl. After some monkeys from each group were killed to measure the size of the lesions produced, the diet was individually adjusted by means of five diets with different cholesterol contents to lower the serum cholesterol to a range of 180 to 220 mg/dl in some subgroups and to 280 to 320 mg/dl in others. The animals were maintained at those two levels for either 24 or

48 months and then killed. Results from animals in which progression was 19 months are available. Chemical analysis of abdominal aortas showed that total cholesterol decreased from about 10 mg/g at 19 months to about 5 mg/g in animals maintained at the 300-mg/dl level, and to 2 mg/g in animals maintained at the 200-mg/dl level for the additional 24-month period (Wagner et al., 1976). The collagen concentration, however, had increased after 24 months, but it increased less at the 200-mg/dl than at the 300-mg/dl level. Gross evaluation of the aortic lesions showed striking regression of fatty streaks in animals changed to the 200-mg/dl level when compared either with those of animals killed at the end of 19 months or with those of animals changed to the 300-mg/dl level (Bond et al., 1977). On the other hand, regression of fibrous plaques did not occur in either of the groups kept alive for the additional 24 months.

In our own laboratory we studied the effect of moderate reductions in the serum cholesterol level on lesions by electron microscopy (Stary et al., 1978). By at first feeding our standard atherogenic diet for 12 weeks, we produced serum cholesterol elevations ranging up to a mean of 610 mg/dl. We then completely removed the saturated fat and cholesterol supplement from the diet of 5 monkeys, reduced the cholesterol supplement to 0.04% in the diet of another 5 monkeys, and fed those two diets for an additional 24 weeks. Serum cholesterol declined to normal levels in all animals receiving the diet from which the saturated fat and cholesterol supplement had been removed completely. Animals in which the cholesterol supplement had been reduced partially, had serum cholesterol levels which ranged from 150 to 360 mg/dl when they were killed at the end of the 24 weeks. This unexpected individual variability of the animals in response to a partial decrease in dietary cholesterol made it possible to study the effect of various degrees of serum cholesterol reduction on the morphology of lesions. All animals had lesions that were smaller than one would have expected to find when considering their initial serum cholesterol elevation. The size corresponded to the serum cholesterol level established with the new diet, and with the electron microscope features of both regression and progression were present. The experiments indicate that any lasting decline from a high serum cholesterol level decreases the size of lipid-rich lesions, that the reduction corresponds to the extent of the serum cholesterol decline, and that subsequent lesion progression proceeds at a slower pace.

Atherosclerosis Regression With Drugs

Drugs have been added to atherogenic diets to test whether or not they would retard progression of lesions in the presence of very high serum cholesterol levels. Drugs and special nutrients were also tested in combination with moderately atherogenic diets to determine whether they would reduce lesions in the presence of moderate serum cholesterol elevations similar to those prevalent in a large portion of the population. And finally, drugs were added to low cholesterol regression diets to determine whether they would accelerate lesion regression beyond that possible with the diets alone.

Wissler and his coworkers (1975) found that cholestyramine, when added to an atherogenic diet, retarded lesion progression. In this experiment the diet (2% cholesterol, 12.5% butter, 12.5% coconut oil) had been given to rhesus monkeys for 24 months, with 2.5% cholestyramine added to the diet of one group of the animals for the last 12 months. Pick and Glick (1977) added propranolol, monoxidil, or clofibrate to the atherogenic diet of stumptail macaques (*M. arctoides*). Over the 6-month period of the experiment, animals eating the diet alone, and those eating the drug-supplemented diets, had similar serum cholesterol elevations (500 to 800 mg/dl). Measurement of coronary artery atherosclerosis showed that monkeys receiving the clofibrate supplement had the least amount of arterial narrowing, even though those monkeys did not have lower serum cholesterol levels than the others. Although the beneficial effect of clofibrate on lesions in this experiment was not related to an effect on serum cholesterol, evidence from an experiment in miniature swine indicates that clofibrate reduces the size of lesions by lowering serum cholesterol levels (Jarmolych et al., 1978; Augustyn et al., 1978).

Cholestyramine in combination with a "prudent" diet was tested by Vesselinovitch et al. (1977, 1978). At first, atherosclerosis was produced in rhesus monkeys by feeding them an atherogenic diet (2% cholesterol, 12.5% butter, 12.5% coconut oil) for 14 months. Regression of lesions was then attempted by feeding the prudent diet (0.25% cholesterol, 15% corn oil) with and without cholestyramine, for an additional 14 months. The prudent diet with or without cholestyramine exerted a beneficial effect on atherosclerosis. The combined therapy produced an additional decrease of some lesion components. Malinow et al. (1978a) also were successful in reducing lesions by adding cholestyramine to a diet moderately high in fat and cholesterol. This work, some of which is still in progress, involves 263 female cynomolgus monkeys (*M. fascicularis*). An atherogenic diet (0.5% cholesterol, 25% butter) was fed for an initial 6 months to raise the serum cholesterol (830 mg/dl) and to induce lesions. The atherogenicity of the diet was then reduced (0.1% cholesterol, 24% butter) and either cholestyramine or dextrothyroxine or Wy-14463 was added. The cholesterol levels declined most in animals receiving cholestyramine (160 mg/dl) and least in those on Wy-14463 (370 mg/dl). The size of established lesions was reduced with cholestyramine but not with Wy-14463. Nor were lesions decreased in animals receiving dextrothyroxine, which had reduced serum cholesterol midway between that of the other two drugs. In other groups (Malinow et al., 1978b), after a 6-month period on the above atherogenic diet, the atherogenicity of the diet was reduced (0.06% cholesterol, 16% butter) and 51% alfalfa was added. This intervention diet was fed for 18 months, lowering the serum cholesterol from about 700 mg/dl to 170 mg/dl and causing a decrease of aortic and coronary atherosclerosis. A similar diet without alfalfa caused reduction of the serum cholesterol to 290 mg/dl and although it failed to cause significant lesion shrinkage it was effective in preventing lesion progression.

The drug W-1372 was tested in squirrel monkeys in which serum cholesterol levels had been raised to about 490 mg/dl by an atherogenic diet (Berger et al., 1969). After seven months, W-1372 was added to the atherogenic diet of one group of monkeys causing the serum cholesterol to decline to a mean of 330 mg/

dl. At autopsy at the end of nine months, the aortas of monkeys who had received the drug for the final two months had only half the fatty deposits of monkeys that had not received the drug supplement. Vesselinovitch et al. (1976) studied W-1372 in rhesus monkeys. They produced lesions by feeding their standard atherogenic diet for 18 months, subsequently switching to low-cholesterol food for an additional 18 months. W-1372 added to the low-cholesterol food did not produce more lesion regression than the low-cholesterol food alone.

The experimental evidence obtained so far favors cholestyramine as the currently most practical drug that can actually decrease the size of arterial lesions. The mechanism whereby some of the drugs have a positive effect on retarding or regressing lesions remains unexplained. Most act by enhancing declines in serum cholesterol, but a direct effect of some on the tissue cannot be ruled out.

Atherosclerosis Regression by Reduction of the Blood Pressure

Epidemiological evidence that hypertension accelerates and aggravates atherosclerosis (Robertson and Strong, 1968) and coronary heart disease (Kannel et al., 1969) in man has been confirmed experimentally in two primate species. In stumptail macaques (*M. speciosa*) surgically induced hypertension, superimposed on dietary hypercholesterolemia, caused more extensive coronary and aortic atherosclerosis than did hypercholesterolemia alone (Pick et al., 1974). The extent of vessels involved with lesions was greater, but the size of individual lesions and the amount of lipid within lesions was not. These results differ somewhat from those obtained in cynomolgus monkeys (*M. fascicularis*) by Hollander et al. (1976). Monkeys with hypercholesterolemia and hypertension had larger coronary artery lesions (more severe narrowing of the lumen) than did monkeys with hypercholesterolemia alone.

The same teams of investigators studied the question of whether or not treatment of hypertension would slow the rate of atherosclerosis progression or would promote regression. In the study by Hollander et al. (1976) blood pressure levels were increased to an average value of 180/120 mm Hg by coarctation of the aorta, and serum cholesterol levels to about 510 mg/dl by feeding an atherogenic diet (2% cholesterol, 10% butter) for 6 months. The atherogenic diet was then withdrawn and subgroups of monkeys were given a low cholesterol diet for an additional 12 months, reducing serum cholesterol to about 140 mg/dl. In addition, one of the subgroups received antihypertensive therapy which reduced the blood pressure to an average value of 130/80 mm Hg over the 12-month treatment period. Combined treatment of hyperlipidemia and hypertension was more effective than treatment of hyperlipidemia alone. Narrowing of the coronary artery lumen by plaques had averaged 68% in a group of monkeys killed at the end of the 6-month period of hypertension and hypercholesterolemia. In the treated groups killed after the additional 12 months, it averaged 54% with diet alone, and 42% with combined dietary and antihypertensive treatment.

The regression study by Pick et al. (1978) again used stumptail macaques to test the effect of antihypertensive drugs. Lesions were induced by feeding an atherogenic diet (2% cholesterol, 12.5% peanut oil, 12.5% coconut oil) and by constriction of the renal artery. Serum cholesterols increased to levels somewhat higher than those in the study by Hollander et al. but blood pressures were less elevated. After 30–36 weeks on the diet (24 weeks after renal artery narrowing), groups of monkeys received antihypertensive therapy for an additional 24 weeks, either in combination with the atherogenic diet or with a low cholesterol diet. A third group was given the low cholesterol diet without drugs. Coronary artery luminal narrowing was less in both groups in which the atherogenic diet was changed to a low cholesterol diet. Reduction of the systolic hypertension did not cause an additional decrease in coronary narrowing. The failure of lesions to regress more may have been due to the short duration of the antihypertension treatment. Nevertheless, the treatment may have caused some positive changes that may have remained undetectable with the methods used.

In their evaluation of lesions these studies relied on gross and light microscopic methods. Biochemical analyses for collagen, elastin, and lipid content of arteries or lesions, which are a better measure of subtle changes, were not made. Nor have the cellular or intercellular changes been studied with the electron microscope. There is therefore little information on how hypertension aggravates diet-induced atherosclerosis, and on how a lesion's components regress when antihypertensive and dietary therapy are combined. In the study by Hollander et al. (1976) in which atherosclerosis regression was enhanced by treatment of hypertension, the differences in the degree to which lesions regressed were reported to be quantitative rather than qualitative.

Some information about changes in arterial wall components, and thus about mechanisms, comes from studies in rhesus monkeys having induced renal hypertension alone compared with rhesus monkeys having dietary hypercholesterolemia alone (Wolinsky et al., 1975). Experimental hypertension and hypercholesteremia each caused similar increases in coronary and aortic collagen, in elastic tissue, and in two lysosomal enzymes. The difference was in arterial and aortic lipid content, which was high in the hypercholesterolemic monkeys but which remained low in those with hypertension. Studies of induced hypertension in other animal species showed increased proliferation of aortic and arterial smooth muscle cells, primarily in the media (Schmitt et al., 1970; Bevan et al., 1976). In hypercholesterolemia, on the other hand, increased cell division occurred primarily in the intima (McMillan and Stary, 1968; Stary, 1974a).

The concensus from presently available evidence seems to be that the number of medial smooth muscle cells and the amount of collagen and elastin in medium sized and in elastic arteries depends on the blood pressure and that, when the serum cholesterol reaches a certain minimum threshold (Bretherton et al., 1975), blood pressure becomes one of the determinants of the rate at which lipid will deposit in the intima. When the blood pressure is reduced the rate at which lipid is deposited is reduced. Furthermore, there is hope that the increase in some other vascular components caused by hypertension possibly might regress. Wolinsky (1971) discovered that increases in arterial and aortic

noncollagenous proteins produced in rats by a 10-week period of hypertension regressed after a subsequent 10-week period of normotension.

Problems and Methodology of Regression Experiments

The Type of Lesions Studied

Experimental lesions successfully reduced in primates were those rich in lipid, comparable to the plaques associated in man with high serum cholesterol levels as seen in some lipoprotein abnormalities. It is to individuals with such lesions that recent experimental work in primates promises particular hope. Some obstructive human lesions, however, consist predominantly of fibrous tissue, being relatively acellular and containing little lipid. Whether the modest decrease of collagen, shown to take place in lipid-rich lesions, will occur to a greater extent in fibrous plaques and cause a significant reduction in size remains to be seen. The possibility of regression of connective tissue has been reviewed by Armstrong (1978). A related, unanswered question is whether lipid and debris sequestered at the inner core of acellular fibrous plaques can be mobilized by dietary measures within a reasonable time span. Experiments attempting to produce and then to regress such lesions are in their nature very long-term studies. To produce realistic replicas of human fibrous plaques, breeding colonies of nonhuman primates were established by the National Heart, Lung, and Blood Institute. These colonies have been exposed to risk factors of atherosclerosis and hypertension, carefully controlled and measured from birth. The animals are now being made available to investigators of regression.

Thrombi, recent or organized, are sometimes a part of obstructive atherosclerotic lesions in man, particularly of those in the lower extremities. The possibility of regression of such combination lesions has, so far, not been explored, although Prathap (1973) studied the organization of arterial thrombi which he had induced in hypercholesterolemic Malaysian long tailed monkeys (*M. irus*). In that connection, we must caution against the tendency to speak of regression of advanced atherosclerosis when occlusive or mural thrombi decrease in size or disappear on sequential femoral angiograms (Blankenhorn, 1978; Hess, 1978). Present methods of routine angiography fail to distinguish between obstructive lesions of different etiology. We consider thrombosis as a process that sometimes occurs on the basis of atherosclerosis but that also occurs independently. Our usage of the term *atherosclerosis* does not include pure thrombi since both the morphology and the risk factors are different for the two conditions. In our use of the terminology, lysis, contraction, or recanalization of a thrombus cannot be taken to represent regression of an atherosclerotic plaque.

Determination of Changes in Lesions by Invasive Techniques

Determination of the morphological and biochemical characteristics of experimentally induced atherosclerotic lesions at more than one point, that is, both

during development and during regression, is unusually difficult. A direct invasive technique such as an arteriotomy permits visual inspection, but because of the incision, it alters the hemodynamics of the blood vessel, besides giving only limited information about a lesion. Biopsy of a lesion alters the life history of a lesion even more.

Sequential (serial) angiography has been the method of choice for observing changes in the size of human lesions. DePalma et al. (1972) used serial angiography in monkeys to demonstrate decreases in lesion size in the superior mesenteric artery and at the celiac axis after diet change and reduction of the serum cholesterol level. Since presently available angiography does not give sufficient information about the nature of lesions, a group of investigators has been developing computer-controlled image dissection of arterial angiograms (Blankenhorn, 1978; Crawford and Blankenhorn, 1978). The method measures the severity of atherosclerosis from the density profile of edges of the contrast-medium shadow and is capable of discriminating specific lesion types. This computer-estimated atherosclerosis (CEA) has been evaluated against pathological atherosclerosis assessment. So far, CEA in living patients treated for atherosclerosis revealed more patients with lesion regression than did visual evalution of angiograms. Patients who had accumulated atherosclerosis most rapidly showed the most rapid lesion regression.

Determination of Changes in Lesions by Noninvasive Techniques

An important current research priority of the National Heart, Lung, and Blood Institute is to further the development of noninvasive lesion imaging that would be simple enough to permit rapid screening of large populations and refined enough to give information not only about the presence or absence and size of atherosclerotic lesions but also about their nature and characteristics (Devices and Technology Branch, 1978). Knowledge of whether an obstructive lesion consists mainly of lipid, thrombus, or connective tissue would be immensely important for prognostic purposes and for determining the type of treatment to be applied (anticholesteremic, thrombolytic, or surgical). The systems being developed are also to be used in experimental animals for determining the effect of treatment on atherosclerotic lesions.

Most of the effort is concentrated on the development of high-resolution, real-time, ultrasonic imaging systems. Conventional pulse-echo imaging techniques are being refined for on-line analysis and three-dimensional graphics display of arterial lesions and blood flow characteristics. Success so far is limited to relatively superficial arteries such as the carotid and femoral arteries. Images of the carotid artery already being produced by a refined B-scan system are of equal or greater detail than those produced by invasive angiography, and the ultrasound scans are also superior in showing fine detail (Evans et al., 1978). In another system, ultrasound scans can resolve lesions 0.5 mm in size and by unique echo patterns show plaques largely constituted of calcium and cholesterol, and plaques of the hemorrhagic-fibrotic type. Confirmation was obtained by relating the computer image to the dissected artery at autopsy (Olinger and Nigam, 1978).

Determination of Changes in Lesions at Autopsy

Since it is imprudent to use invasive, and impossible to use noninvasive, methods to determine the chemical and structural characteristics of a specific lesion repeatedly, experimental studies of regression necessarily have followed indirect methods. All investigators began by producing lesions in a large group of monkeys by feeding an atherogenic diet and elevating serum cholesterol levels. At the end of the period of lesion induction they divided the animals into several groups, matched so that each group contained animals with a similar range of serum cholesterol elevations during the period on the atherogenic diet. They then killed and autopsied the animals in one group to measure and characterize the lesions (baseline lesions) that were to be regressed. Previous experiments had indicated that monkeys of the same species, age, and sex, eating the same atherogenic diet, developed lesions the size and characteristics of which largely reflected the degree and the duration of the serum cholesterol elevation. Therefore, the assumption was that lesions identical to those of the killed monkeys were also present in monkeys of the other groups. These groups were then treated with low cholesterol diets with or without drugs and killed after various additional time periods to study the fate of the baseline lesions.

Because the serum cholesterol level does not immediately return to normal when an atherogenic diet is changed to a treatment regimen, and because lesions may continue to increase rather than decrease during that period, we have, in our own experiments, also killed groups of monkeys after treatment had begun but while the serum cholesterol was still high, to obtain a more precise measure of the baseline lesions.

Most experiments included groups of animals that never had received an atherogenic diet. These were killed with the regression groups to determine the extent of lesions that might have gradually developed through risk factors other than the atherogenic diet. Without such controls, similar lesions if found in regression groups would have caused the efficacy of treatment to be underestimated. Success of treatment could also be overestimated if the increase in lumen, associated with the age-related increase in vessel size or with the segmental dilatations that sometimes occur in atherosclerotic arteries, were taken as plaque regression. Measurement of the arterial perimeter, in addition to the lumen, protects from this error.

Sampling for Comparisons of the Ultrastructure

For ultrastructural characterization of the lesions in each dietary group to be obtained, a large number of tissue samples must be studied. In addition, sites from which the samples are taken must be strictly standardized in animals from different dietary groups.

Previous studies determined that lesions localized predictably at certain points within the arterial system in monkeys of the same species, sex, and age. In the coronary arteries, the largest lesions inevitably developed at the

bifurcation of the left main coronary artery and in the adjacent part of the anterior descending branch. In our regression experiments, it became standard procedure, therefore, to cut this coronary segment into slender consecutive blocks from which semithin sections were prepared. Semithin sectioning of each block continued until intimal thickenings or lesions were found. These were then fine-sectioned and photographed.

The aorta of hypercholesterolemic monkeys is more diffusely involved with lesions than are the coronary arteries, but their locations are nevertheless predictable. To minimize the risk of missing the site of the largest lesion, we sampled, in each animal, four sites of predilection. We divided the tissue from each site into four blocks from which we cut semithin sections. From each of the four aortic locations we fine-sectioned and photographed the blocks with the largest lesions. When a larger lesion was grossly apparent outside the predetermined locations, that lesion was sampled in addition. Thus when comparisons between aortas from different dietary groups were made, electronmicrographs of lesions in four standard locations were compared, but larger lesions occurring outside those areas were also considered.

Measurement of the Blood Pressure

Although high blood pressure is one of the risk factors of atherosclerosis, blood pressure was not measured in many regression studies. The reason for the absence of routine measurements has been doubt about the accuracy and reliability of systolic and diastolic pressures when measured in tranquilized or anesthesized monkeys. Sernylan and ketamine, two related drugs in the tranquilizer family used to sedate or anesthesize nonhuman primates, cause the blood pressure to be inconsistent and also influence other functions such as glucose tolerance (Samuels et al., 1978). Without sedation, accurate and reproducible measurements can only be made in unrestrained monkeys that have been specially trained, or after instrumentation and a period of adjustment in monkeys that are on a tether (unrestrained but harnessed to a cable leading to the outside of the cage), or monkeys restrained in a chair, or by telemetry. All of those procedures are time-consuming, expensive, and difficult, particularly in long-term studies with large numbers of animals in which measurements must be obtained repeatedly. New methodology to measure blood pressure simply and reliably is being developed in several laboratories (Lehner and Greene, 1978; Samuels et al., 1978). The precise effect of ketamine and the possibility of avoiding undesirable effects continue to be studied by modifying dosage and mode and time of administration.

Conclusions

Results obtained by several different teams of investigators agree that lipid-rich atherosclerotic lesions, produced in primates by diets rich in cholesterol and

fat and by hypercholesterolemia, can be made to decrease in size. Early lesions can disappear completely whereas advanced ones shrink to a degree that results in significant increases in the lumen of arteries that had been severely obstructed. Lesion regression occurred in the aorta, and in coronary and other arteries. Only a drastic reduction in dietary cholesterol and fat caused the serum cholesterol to decline to a level that made lesion shrinkage possible. However, any decline from a high serum level was therapeutic, resulting in a slackening of lesion progression. Drugs added to low cholesterol diets enhanced the effect of the diets.

Correlative chemical, ultrastructural, and radioautographic methods explained some of the mechanisms whereby lesions regressed. Electron microscopy identified a morphological picture characteristic of regressing atherosclerotic plaques, different from that of progressing lesions. Regression occurred in two phases. During the initial 16–20 week period after serum cholesterol had declined to a level of 180 mg/dl or below, the combination of cessation of increased cell proliferation and death of damaged and short-lived cells resulted in a dramatic decrease of the cellularity. A second phase, consisting of a gradual net decrease of accumulated extracellular material – cell debris and lipid – followed; in the case of small fatty streaks, that phase was completed within one year, but it lasted considerably longer when lesions were large. Mechanisms similar to those that caused regression in nonhuman primates can be presumed to be also available in man. Regression of human lipid-rich lesions should therefore be possible.

A net loss of collagen or at least of some of its fractions accompanied regression of lipid from experimental primate lesions. The extent to which collagen can be mobilized from plaques that consist primarily of mature collagen is not known. Much current effort centers on the production in nonhuman primates of replicas of fibrous plaques that develop over a lifetime, becoming symptomatic in man during the sixth and seventh decade, and on their regression.

The positive effect that reducing the serum cholesterol level has on atherosclerotic lesions reiterates the role of dietary cholesterol as a risk factor. Since the time required to accomplish lesion regression is long and since fibrous plaques may not be as susceptible to treatment by serum cholesterol reductions as lipid-rich lesions, it seems reasonable to put immediate emphasis on preventive dietary measures beginning at adolescence rather than to await the appearance of symptoms.

How a decrease in blood pressure affects lesions requires further investigation but some evidence indicates that such a decrease may be beneficial. Treatment of other major risk factors of atherosclerosis, such as withdrawal of cigarette smoke or treatment of an excessive susceptibility to stress, has not been experimentally evaluated in primates.

Intensive efforts are under way to develop methodology that would aid in future studies of regression. Noninvasive ultrasound systems that will construct images of intraarterial lesions in man and experimental animals are already far advanced, and methods to measure blood pressure rapidly and reliably in nonhuman primates are under study in several centers.

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