Atherosclerosis

Regression in Nonhuman Primates

M.R. Malinow

RESULTS of Anitschkow's early experiments (1927) suggesting that arterial lesions induced in rabbits by high cholesterol diets decrease in size and in fat content after cessation of cholesterol feeding have been confirmed in numerous species of animals, including rabbits, chickens, dogs, pigeons, pigs, and nonhuman primates (see references in Malinow, in press). This review deals with the studies performed in monkeys, their relevance to human atherosclerosis, and possible mechanisms of the observed arterial changes.

Definition of Regression of Atherosclerosis

In this paper, regression indicates anatomical changes observed in the arteries of monkeys after a drastic reduction in marked hypercholesterolemia induced by diet. At the microscopic level, regression includes (1) restored integrity of the endothelium lining the plaques; (2) arrest of intimal cell proliferation; and (3) a decrease in the number of cells, in the amount of intracellular and interstitial lipid, and in the extent of necrotic and calcific foci in the plaques (see below for references). Shrinkage of the lesions increases the lumen diameter previously narrowed by atheromatous plaques, but there is residual fibrosis in the regressed plaques, an indication of more collagen in the regressed arteries than in normal ones. Knowledge about the regression of experimental atherosclerosis needs to be expanded through studies directed at intramural hemorrhage and luminal thrombosis, since these processes are associated with ischemic episodes in humans. Some of the controversial aspects of regression as well as data supporting its existence, have been dealt with extensively (Prichard, 1974; Armstrong, 1976; Wissler and Vesselinovitch, 1976, 1977; Weber, 1978; Blankenhorn, 1978; Wissler, 1979; Stary, 1979; Malinow, in press); in addition, many aspects have been discussed in two recent books (Hauss et al., 1978; Schettler et al., 1978).

Methods Used to Study Regression of Experimental Atherosclerosis in Monkeys

Postmortem Studies

The method used most extensively in regression studies consists of feeding animals atherogenic, cholesterol-containing foods during an induction period and then killing several animals representative of the experimental population. The arterial findings are compared to data on the remaining animals, which receive cholesterol-free food or are subjected to other putatively regression-inducing regimens during the succeeding months or years. This method has several limitations. First, when the evolution of atheromatous plaques is considered by comparisons at two times separated by months or years, the possible existence of intermediate shorter periods of regression or progression or of slowing of progression cannot be established. (Fig. 1). Second, there is a wide variation in the extent of cholesterolemia and atherosclerosis within groups of monkeys maintained on a given atherogenic diet when other variables such as caging conditions, sex, and "age" are similar (Fig. 2). Third, the ages of monkeys obtained commercially and of animals caught in the wild are generally unknown; they can be estimated only through certain parameters such as weight, general appearance, state of dentition, bone and sexual development, and dry weight of the lens (Haigh and Scott, 1965; Malinow et al., 1966; Lusted et al., 1966; Malinow and Corcoran, 1966; Stahl et al., 1968). Whether age influences the regression of experimental atherosclerosis in monkeys is not...
Atherosclerosis

FIGURE 1 Schematic evolution of atherogenesis between two time intervals (T1, T2). Baseline = 1; accelerated atherogenesis = 2; progression with intermittent regression = 4; arrest of progression = 5; regression with intermittent progression = 6; continuous regression = 7. For the sake of simplicity, 2, 3, 5, and 7 have been represented as a continuous line although intermittent progression or regression could be present.

known, but it is an important factor in atherogenesis in humans (McGill, 1968). Fourth, in such studies, large groups of difficult-to-obtain animals may be required to obtain statistical significance.

In Vivo Studies

One may avoid the problems of postmortem comparisons between groups of animals by following the evolution of the atherosclerotic plaques in the same animals. For instance, gross serial inspection after aortotomy or after surgical exposure of the appropriate arterial segments has revealed that atherosclerotic lesions in two rhesus macaques decreased in size or disappeared about 1 year after the monkeys had returned to a chow* diet (DePalma et al., 1972). Potentially more informative procedures, such as serial arteriotomies or biopsies, would certainly modify the natural evolution of the plaques under study, and they are not generally used in regression studies.

Few investigators have used serial contrast angiography to document atherogenesis in monkeys (DePalma et al., 1972; Kramsch et al., 1979). Foremost are methodological factors to assess radiological changes on the basis of measurements from films taken at various intervals. The limitations of serial angiography in humans have been discussed by Barndt et al. (1977) and Blankenhorn and Sanmarco (1979). Potentially more informative procedures, such as serial arteriotomies or biopsies, would certainly modify the natural evolution of the plaques under study, and they are not generally used in regression studies.

The word "chow" is a registered trademark of the Ralston-Purina Company. It is considered loosely synonymous with "natural-ingredient diets."

Regression of Atherosclerosis in Monkeys

In spite of methodological limitations, several well-documented observations have demonstrated the occurrence of regression of atherosclerosis in monkeys and have established the morphological and biochemical changes associated with it. Studies reported in the 1970s (summarized in Table 1†) mostly used two species—rhesus (Macaca mulatta) and cynomolgus (Macaca fascicularis) macaques—probably because of the availability of these animals and the relatively short time neces-

†Table 1 does not include data on other species such as Saimiri sciureus (Maruffo and Portman, 1968; Hayes et al., 1972) or Macaca arctoides (Pick et al., 1978), which agree in the main with the data on rhesus and cynomolgus macaques, nor does it include the observations of an important group of investigators communicated in several abstracts (Bond et al., 1976; Wagner et al., 1976; Bond et al., 1978), whose findings at the end of two different induction periods have been reported recently (Clarkson et al., 1979).
Abbreviations: RH = rhesus macaques; Cyn = cynomolgus macaques; A = group comparison of postmortem findings at end of regression period to end of induction period; B = angiography, aortotomy, and direct inspection of femoral arteries; N.R., not reported.

Regression Studies in Rhesus and Cynomolgus Macaques

<table>
<thead>
<tr>
<th>Species</th>
<th>Dietary cholesterol level (g/100 g food)</th>
<th>Length of induction period (mo.)</th>
<th>Length of regression period (mo.)</th>
<th>Representative plasma or serum cholesterol level (mg/dl)</th>
<th>Method of assessing regression</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rh</td>
<td>1.2</td>
<td>14 to 24</td>
<td>9, 12</td>
<td>300–800</td>
<td>A</td>
<td>Armstrong et al., 1970</td>
</tr>
<tr>
<td>Rh</td>
<td>0.37</td>
<td>2</td>
<td>4</td>
<td>1000</td>
<td>A</td>
<td>Tucker et al., 1971</td>
</tr>
<tr>
<td>Rh</td>
<td>0.35</td>
<td>3</td>
<td>8, 16</td>
<td>100–200</td>
<td>B</td>
<td>DePalma et al., 1972</td>
</tr>
<tr>
<td>Cyn</td>
<td>1.2</td>
<td>14 to 24</td>
<td>9, 12</td>
<td>400</td>
<td>A</td>
<td>Eggen et al., 1974; Kokatmur et al., 1975; Strong et al., 1976</td>
</tr>
<tr>
<td>Rh</td>
<td>0.37</td>
<td>3</td>
<td>0.5 thru 16</td>
<td>260–830</td>
<td>A</td>
<td>Stary and Strong, 1976</td>
</tr>
<tr>
<td>Cyn</td>
<td>0.5</td>
<td>6</td>
<td>10</td>
<td>700</td>
<td>A</td>
<td>Malinow et al., 1976</td>
</tr>
<tr>
<td>Rh</td>
<td>2.0</td>
<td>18</td>
<td>18</td>
<td>840</td>
<td>A</td>
<td>Vesselinovitch et al., 1976</td>
</tr>
<tr>
<td>Rh</td>
<td>2.0</td>
<td>12</td>
<td>12*</td>
<td>250</td>
<td>A</td>
<td>Weber et al., 1977</td>
</tr>
<tr>
<td>Rh</td>
<td>0.5 g/animal per day†</td>
<td>5</td>
<td>5</td>
<td>160</td>
<td>A</td>
<td>Chakravarti et al., 1977</td>
</tr>
<tr>
<td>Cyn</td>
<td>0.5</td>
<td>6</td>
<td>18‡</td>
<td>830</td>
<td>A</td>
<td>Malinow et al., 1978b</td>
</tr>
<tr>
<td>Cyn</td>
<td>0.5</td>
<td>6</td>
<td>18§</td>
<td>830</td>
<td>A</td>
<td>Malinow et al., 1978a</td>
</tr>
<tr>
<td>Rh</td>
<td>2.0</td>
<td>14</td>
<td>14</td>
<td>900</td>
<td>A</td>
<td>Vesselinovitch et al., in press</td>
</tr>
<tr>
<td>Rh</td>
<td>2.0</td>
<td>5</td>
<td>12</td>
<td>700</td>
<td>A</td>
<td>Hollander et al., 1979</td>
</tr>
</tbody>
</table>

* Dietary cholesterol, ~0 or 2% with and without cholestyramine.
† Plus intravenous adrenaline on alternate days.
‡ During regression: dietary cholesterol ~0 or 0.1% with and without alfalfa meal.
§ During regression: dietary cholesterol 0.1% plus cholestyramine, dextrothyroxine, or Wy 13,364.

Abbreviations: RH = rhesus macaques; Cyn = cynomolgus macaques; A = group comparison of postmortem findings at end of regression period to end of induction period; B = angiography, aortotomy, and direct inspection of femoral arteries; N.R., not reported.

It is likely that the rate of regression of atherosclerosis differs among nonhuman primates, but this may be a difference of degree, and regression served after relatively short periods (14 months) of such basal cholesterolemia (Vesselinovitch et al., in press). The question of regression with higher plasma cholesterol levels (around 300 mg/dl) in rhesus macaques is being addressed by a team of investigators at the Bowman Gray School of Medicine; the studies are still in progress. However, one unique group of rhesus macaques receiving 2.5% cholestyramine has shown regression even with cholesterolemia around 300 mg/dl (Vesselinovitch et al., in press). Stary (1978) has studied five rhesus macaques maintained on diets supplemented with 0.04% cholesterol during a 24-week period; terminal plasma cholesterol ranged from 150 to 360 mg/dl. Lesions were smaller than expected when compared with suitable controls; electron microscopic features of both regression and progression were present. The authors have concluded that the lesions progressed at a slower pace than expected.

Anatomical Changes during Regression

The results observed in monkeys suggest that long intervals of low plasma cholesterol (i.e., 100–200 mg/dl) may be required to obtain almost complete normalization of atherosclerotic plaques (Stary and Strong, 1976) whereas partial regression—which could still be substantial—can be observed after relatively short periods (14 months) of such basal cholesterolemia (Vesselinovitch et al., in press). The question of regression with higher plasma cholesterol levels (around 300 mg/dl) in rhesus macaques is being addressed by a team of investigators at the Bowman Gray School of Medicine; the studies are still in progress. However, one unique group of rhesus macaques receiving 2.5% cholestyramine has shown regression even with cholesterolemia around 300 mg/dl (Vesselinovitch et al., in press). Stary (1978) has studied five rhesus macaques maintained on diets supplemented with 0.04% cholesterol during a 24-week period; terminal plasma cholesterol ranged from 150 to 360 mg/dl. Lesions were smaller than expected when compared with suitable controls; electron microscopic features of both regression and progression were present. The authors have concluded that the lesions progressed at a slower pace than expected.

Anatomical Changes during Regression

It is likely that the rate of regression of atherosclerosis differs among nonhuman primates, but this may be a difference of degree, and regression served after relatively short periods (14 months) of such basal cholesterolemia (Vesselinovitch et al., in press). The question of regression with higher plasma cholesterol levels (around 300 mg/dl) in rhesus macaques is being addressed by a team of investigators at the Bowman Gray School of Medicine; the studies are still in progress. However, one unique group of rhesus macaques receiving 2.5% cholestyramine has shown regression even with cholesterolemia around 300 mg/dl (Vesselinovitch et al., in press). Stary (1978) has studied five rhesus macaques maintained on diets supplemented with 0.04% cholesterol during a 24-week period; terminal plasma cholesterol ranged from 150 to 360 mg/dl. Lesions were smaller than expected when compared with suitable controls; electron microscopic features of both regression and progression were present. The authors have concluded that the lesions progressed at a slower pace than expected.
Aortic and coronary atherosclerosis in Macaca fascicularis given semipurified foods containing varied cholesterol supplements. The monkeys were given high fat, high cholesterol foods for 6 months and similar foods with 2.5% corn oil and a cholesterol content adjusted to a level of 0.34 mg/Kcal during the following 18 months. The diet also contained 50% alfalfa meal or 5% cholestyramine during the last 18 months of the experiment. Each point represents the average (± SE) of 18 monkeys. The graphs demonstrate the positive influence of alfalfa meal and cholestyramine on regression of atherosclerosis. (Details are in Malinow et al., 1978a, 1978b; modified from Malinow, 1979.)

has been observed in several nonhuman primate species. Regression probably does not proceed equally in all arterial territories. For instance, it seems more complete in the aortas than in the coronary arteries of cynomolgus macaques (Fig. 3), but methodological factors in the assessment of the lesions may be responsible for these apparent differences.

Aortic changes (Table 2). Vesselinovitch et al. (1976, in press) found that the percentage of surface involved in the rhesus aorta decreased to less than one-half after 14 or 18 months of a normal diet and that the involvement was even less if cholestyramine was given. After 10 or 18 months of regression, the average aortic involvement also decreased in cynomolgus macaques, and it was then between 1+ (no atherosclerosis) and 2+ (minimal lesions) (Malinow et al., 1978a, 1978b). Hollander et al. (1979), however, did not observe a decrease in the size of aortic lesions in cynomolgus macaques given a chow diet for 12 months after 5 months of an induction diet containing 2% cholesterol; the cholesterol concentration in the aortas did decrease (see below). Lesions observed microscopically after regression showed less calcium, less necrosis (Vesselinovitch et al., in press), and less stainable lipids than those observed before regression (Vesselinovitch et al., 1976, in press; Malinow et al., 1978b). Scanning electron micrographs showed that, in contrast to the intimal surfaces observed at the end of induction, the intimal surfaces of the aortic plaques observed after regression were covered by a continuous endothelium; transmission electron microscopy showed tight junctions and basement membrane reduplication, probably signs of repair (Weber et al., 1977). After regression, the intima showed mild residual thickening and minimal intracellular lipids; the remaining lipids were close to the internal elastic lamina (Vesselinovitch et al., 1976). The intima also showed an accumulation of colloidal iron-reactive material and collagen, but "foam cells" were less abundant than at the end of induction (Malinow et al., 1978b).

Coronary arteries. In regression studies involving several animals per group, it usually is difficult to avoid the variability introduced by the different stages of lesions observable in a single animal, and an arbitrarily selected lesion—for instance, the

Table 2 Regression of Aortic Atherosclerosis in Monkeys

<table>
<thead>
<tr>
<th>Macaque species</th>
<th>Length interval (mo.)</th>
<th>Gross surface involved</th>
<th>Grading method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>End of induction</td>
<td>End of regression†</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhesus</td>
<td>18</td>
<td>81 ± 7.2 (5)</td>
<td>Percentage of surface involved</td>
<td>Vesselinovitch et al., 1976</td>
</tr>
<tr>
<td>Cynomolgus</td>
<td>6 10</td>
<td>3.3 ± 0.4 (11)</td>
<td>Arbitrary grades 1+ to 5+</td>
<td>Malinow et al., 1976</td>
</tr>
<tr>
<td>Cynomolgus</td>
<td>6 18</td>
<td>3.0 ± 0.3 (18)</td>
<td>Arbitrary grades 1+ to 5+</td>
<td>Malinow et al., 1978b</td>
</tr>
<tr>
<td>Rhesus</td>
<td>14 14</td>
<td>68 ± 3.9 (5)</td>
<td>Percentage of surface involved</td>
<td>Vesselinovitch et al., in press</td>
</tr>
<tr>
<td>Cynomolgus</td>
<td>5 12</td>
<td>50 ± 7.0 (8)</td>
<td>Percentage of surface involved</td>
<td>Hollander et al., 1979</td>
</tr>
</tbody>
</table>

* Mean ± SE; number of animals in parentheses.
† Monkeys fed low cholesterol diet.
most advanced one—may be considered representative of any given territory (Malinow et al., 1978b). A unique approach by Stary and Strong (1976) utilized a reproducible location, i.e., the initial bifurcation (or trifurcation) of the left coronary artery in rhesus macaques fed a diet supplemented with butter, beef tallow, and 0.4% cholesterol for 12 weeks; serum cholesterol ranged from 230 mg/dl to 640 mg/dl. The first signs of regression of early coronary atherosclerosis occurred about 4 weeks after the serum cholesterol levels had returned to normal. Proliferation of foam cells (as determined by tritiated thymidine) ceased, necrotic foam cells became numerous, the number of foam cells progressively decreased, the intracellular lipid inclusions shrunk, and cell debris accumulated in the interstitial spaces of the intima. The lipid-laden smooth muscle cells lost most of their lipids, and the intima returned to an almost normal appearance, probably (as Stary and Strong suggested) through clearance of intracellular lipid and cell debris from the necrotic foam cells via the medial and adventitial lymphatics (Stary and Strong, 1976). Regression of more advanced coronary atherosclerosis in monkeys with plasma cholesterol levels maintained around 140 to 200 mg/dl during the intervention period has been reported by several investigators (Armstrong et al., 1970; Vesselinovitch et al., 1976, in press; Malinow et al., 1978a, 1978b). Although the duration of induction and regression, as well as the methods of measurement, varied, the extent of the lesions in all experiments decreased to one-third or less than present after induction (Table 3). Regression was observed even in monkeys receiving food containing saturated fat and cholesterol, provided that cholestyramine or alfalfa meal was also given (Wissler et al., 1975; Malinow et al., 1978a, 1978b; Vesselinovitch et al., in press). In the "regressed" lesions there were fewer intra- and extracellular lipids, narrower acellular spaces, more condensed elastic fibers, and reoriented, more densely packed cells than in the lesions observed after induction. Although the lumen became wider, the fibrotic intima was thicker than normal (Malinow et al., 1978b). The medial damage seemed to diminish more than the intimal changes in cynomolgus macaques (Malinow et al., 1978b), but not in rhesus macaques (Armstrong, 1976). The fat-laden cells in the adventitia—commonly seen in cynomolgus macaques at the end of induction—largely disappeared (Malinow et al., 1978b).

### Biochemical Changes during Regression

In experiments conducted in rhesus macaques, free and esterified cholesterol decreased in the aorta after 32 weeks of regression (Kokatnur et al., 1975). Results were similar in cynomolgus macaques, even though the experimental design was somewhat different (G. S. Berenson et al., unpublished data). Decreases in aortic free, esterified, and total cholesterol were also observed by Hollander et al. (1979) in cynomolgus macaques that had been maintained for 12 months on a chow diet after a 5-month induction period, although no gross morphological evidence of regression was detected.

An increase in substances with the tinctorial characteristics of collagen and elastin in atherosclerotic plaques has been recognized since the earlier anatomical descriptions (Anitschkow, 1967). Moreover, lesions of regressed arteries show an accumulation of these fibrous proteins, which remain more abundant than in normal arteries (Anitschkow, 1967; Malinow et al., 1978b). The methodological difficulties involved in chemically analyzing fibrous proteins in arterial lesions— lucidly discussed by Armstrong in 1976—were recognized by Armstrong and Megan in their 1975 experiments.

### Table 3 Regression of Coronary Atherosclerosis in Monkeys*

<table>
<thead>
<tr>
<th>Macaque species</th>
<th>Length interval (mo.)</th>
<th>Coronary involvement</th>
<th>Grading method</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Induction</td>
<td>Regression</td>
<td>End of induction</td>
<td>End of regression</td>
</tr>
<tr>
<td>Rhesus</td>
<td>17</td>
<td>40</td>
<td>60 ± 8 (10)</td>
<td>17 ± 4 (10)</td>
</tr>
<tr>
<td>Rhesus</td>
<td>18</td>
<td>18</td>
<td>23 ± 2.5 (5)</td>
<td>9 ± 1.5 (5)</td>
</tr>
<tr>
<td>Cynomolgus</td>
<td>6</td>
<td>18</td>
<td>23.0 ± 5.1 (18)</td>
<td>5.4 ± 1.3 (18)</td>
</tr>
<tr>
<td>Cynomolgus</td>
<td>6</td>
<td>18</td>
<td>idem</td>
<td>7.6 ± 1.3 (18)</td>
</tr>
<tr>
<td>Cynomolgus</td>
<td>6</td>
<td>18</td>
<td>idem</td>
<td>4.1 ± 0.8 (18)</td>
</tr>
<tr>
<td>Rhesus</td>
<td>14</td>
<td>14</td>
<td>45 ± 4.5 (5)</td>
<td>16 ± 2.9 (5)</td>
</tr>
<tr>
<td>Cynomolgus</td>
<td>5</td>
<td>12</td>
<td>36 ± 7 (8)</td>
<td>34 ± 8 (8)</td>
</tr>
</tbody>
</table>

* Mean ± SE; number of animals in parentheses.

† Monkeys fed low cholesterol diet with the exception of those on lines 3 and 5 that included, in addition to high cholesterol diet, alfalfa meal and cholestyramine, respectively.
with cynomolgus macaques; it is difficult to isolate effects in small lesions from the "diluting" effects of the rest of the arterial wall, and to express the data in terms of a change per unit of a changing baseline. Chemical analysis showed an increase in the collagen and elastin content induced by atherosclerosis in the aortas and coronary arteries of the monkeys; the regressed arteries had a greater amount of the fibrous proteins than the control arteries—confirmation of the previously mentioned histological findings (Anitschkow, 1967; Malinow et al., 1978b)—and only slightly less collagen than arteries examined at the end of induction. However, Hollander et al. (1979) found that collagen and elastin contents in the thoracic and abdominal aortas of cynomolgus macaques were greater after regression on a 12-month chow diet than at the end of a 5-month induction period.

The question of collagen changes after regression also has been studied by a semiquantitative morphological method in which an ocular grid or a transparency is superimposed on histological slides (Vesselinovitch et al., in press). Areas of structures specifically stained were evaluated after the points covering the component had been counted. Rhesus monkeys were studied after 14 months of induction and after 14 months of regression. The average collagen content per average lesion size increased in the aorta and coronary arteries in all groups of animals after regression. However, since the lesions had shrunk after regression, the values were related to the average lesion size observed at the end of induction (relative collagen content). Using this approach, Vesselinovitch et al. (in press) found no differences in the relative collagen contents of the aortas of animals treated with a "prudent diet" or the aortas of animals maintained on an atherogenic diet with added cholestyramine, but there was a reduction of about 17% in animals receiving cholestyramine and the prudent diet. In the coronary arteries, the relative collagen values were unchanged in monkeys receiving the prudent diet, but they were increased by about 37% in monkeys receiving cholestyramine with the prudent diet or cholestyramine with the atherogenic diet. Thus, these exact morphological measurements do not demonstrate decreases in newly deposited collagen after regression.

Regression also has been associated with other biochemical arterial changes. The amount of acid mucopolysaccharides (estimated histochemically) was greater in the coronary arteries of cynomolgus macaques that had experienced regression than in the arteries of normal animals (Malinow et al., 1978a, 1978b). However, dermatan sulfate and condroitin-6-sulfate values were less than postinduction values, and hyaluronic acid and heparin sulfate increased in the aortas of rhesus macaques after regression (Radhakrishnamurthy et al., 1975).

Acid lipase activity in aortic homogenates from rhesus macaques was less after regression than after induction; the enzymatic activity was correlated with the concentration of aortic cholesterol (Vesselinovitch et al., in press).

The calcium content was greater in the thoracic and abdominal aortas of cynomolgus macaques after a 12-month chow diet that followed a 5-month induction period than after induction, although the net cholesterol and lipoprotein fluxes were reduced (Hollander et al., 1979). However, the morphometric method of Vesselinovitch et al. (in press) demonstrated a reduction in calcification and necrosis in rhesus monkeys subjected to regression for 14 months.

Relevance of Regression Studies in Monkeys to Atherogenesis in Humans

As is true in all animal experiments, extrapolation of results across species lines is not justified as a logical process since investigators cannot assume that experimental findings in one species will be valid in a population of another species. (However, it is well known that pathogenic mechanisms or therapy for certain conditions may be similar in both animals and humans.) Moreover, extrapolations from cholesterol-fed monkeys may be applicable only to those individuals in which atherosclerosis is related to, or is aggravated by, exogenous cholesterol or hypercholesterolemia, and the effects of ingested cholesterol on plasma cholesterol and lipoproteins in monkeys overshadows the effects of saturated fats (Malinow, 1979), whereas in humans ingested saturated fat is a much more important factor in the regulation of cholesterolemia (Jacobs et al., 1979). Finally, the high level of plasma cholesterol attained in monkeys during relatively short induction periods somewhat limits the applicability of these findings to humans, in whom atherosclerosis proceeds at lower plasma cholesterol levels, presumably over long periods of time (Kannel et al., 1971).

Regression of atherosclerosis has been observed in monkeys, usually in those with plasma cholesterol levels drastically reduced to values between 100 and 200 mg/dl (see Table 1), although in a few instances regression or slower progression has been observed when very high cholesterol levels have been reduced to around 300 mg/dl. If regression of atherosclerosis, then, is more likely to occur in humans when plasma cholesterol levels are kept below 200 mg/dl, the limited success of most clinical trials may be explained by the fact that plasma cholesterol has not fallen to these levels (Committee of Principal Investigators, 1978).

Observations in monkeys have demonstrated regression of "young" plaques that lack the extensive calcification, fibrosis, ulceration, and thrombosis characteristic of advanced lesions in humans. It is unlikely that regression of "young" plaques and regression of "old" plaques are similar.
Mechanisms of Regression of Atherosclerosis

Mechanisms involved in the regression of atherosclerosis occurring after cessation of cholesterol feeding or after interference with the intestinal absorption of cholesterol are largely unknown. Moreover, many interpretations of regression are based on observations of normal arteries or on observations conducted during early experimental atherosclerosis. The lack of pathogenic studies during regression severely limits such an approach.

Figure 4 shows a simplified model of an artery depicting exchanges between three pools: 1, blood; 2, arterial wall; and 3, lymph. The term model is used here not to relate observed data to an hypothesis under consideration, but to point out areas in which the generation of data is needed. The vectors denote transport of a substance into and out of the pool and also its chemical synthesis and degradation. Flow rates are indicated as \( Q_{mn} \), i.e., the amount of substance transferred per unit time from \( n \) to \( m \); a more detailed mathematical analysis of transport in the arterial wall is discussed lucidly by Bratzzler et al. (1977).

A decrease in accumulated atheromatous materials (A, Fig. 4), implicit in regression, may result when the combined rates of the egress of these substances and their metabolic removal exceed the sum rates of influx and production of these materials.

\[
Q = \int \left[ (Q_{21} - Q_{12} - Q_{32}) + (Q_{AP} - Q_{MA}) \right] \, dt \quad (1)
\]

In the above formula, \( Q \) is the quantity of the atheromatous materials accumulated in the wall, and \( Q_{21} \), \( Q_{12} \), and \( Q_{32} \) denote their rates of influx, egress, production, and metabolic removal, respectively (modified from Chien, 1976); precursors (P) and metabolites (M) are also in equilibrium with the blood and lymph at influx and efflux rates \( Q_{21} \), \( Q_{12} \), \( Q_{32} \) and \( Q_{32} \). It seems likely that biochemical procedures similar to those performed on normal and atheromatous arteries, too numerous to be cited here, could be carried out with success in studies of regressing atheromatous plaques and thus could better define \( Q_{AP} \) and \( Q_{MA} \). Many other phenomena that might be implicated in the material balance of the arterial wall, such as the relationships of platelets and prostaglandins to endothelial function (Mason et al., 1977) or the subcellular distribution of lipids and enzymes in atheromatous plaques (deDuve, 1974) and their effects on regression need further study.

The transport \( Q_{21} \) of water, solutes, and macromolecules occurs across arterial wall boundaries, which probably include the intimal layer of arteries and intramural arterial branches, the vasa vasorum, and the vessels of neovascularized plaques; this transport has not been studied during regression. All of the transported substances most likely have different flux rates, which probably include ultrafiltration and diffusion through endothelial junctions and fenestrae, as well as diffusion and vesicular transport through the endothelial cells (Casley-Smith, 1967). The flux rate \( Q_{32} \) may also include transport occurring whenever the effective hydrostatic pressure is lower than the effective osmotic pressure. Such apparently unlikely transport of plasma ultrafiltrate moving back and forth across the endothelium synchronously with each heart beat, named “sloshing” by Kenyon (1979), was formulated on the basis of a mathematical model of water flux through nonatheromatous aortic tissue, but requires confirmation in vivo since it may be of importance during regression. Definition of flux rates (Fig. 4) probably will require the use of the thermodynamics of irreversible processes [formulated by Kedem and Katchalsky (1958) and Katchalsky and Kedem (1962) on the basis of Onsager’s ideas (1931)], which assumes that \( Q_{mn} \) for a given substance is influenced by all forces known to act on the system and that there may be interactions between all transported substances.

The postulated gradient of free fluid pressure that extends from the intima to the adventitia (Bader, 1963) may move substances by diffusion and percolation through the arterial walls, from the intima to the adventitia, where reabsorption through the lymphatics (\( Q_{32} \)) may occur much as in other organs (Intaglietta and Zweifach, 1974). Although Figure 4 depicts only ultrafiltration into lymph, \( Q_{32} \) may also include percolation and diffusional flow. Thus, substances may be transported from medial and adventitial interstitial spaces to periadventitial interstitial spaces and hence to lymphatic capillaries, since the extracellular space of the adventitia seems to merge with the periadventitial space without precise anatomical limits. Stein and Stein (1973) have hypothesized, on the basis of their own findings, previous electron microscopic observations (Bruns and Palade, 1968; Casley-Smith, 1969), and the results of chemical analyses...
in aortas of individuals of different ages (Smith, 1965), that under normal conditions, there is a brisk flow of lipoproteins through the arterial wall, suggesting a very efficient system for the removal of lipoprotein cholesterol; this removal probably involves the lymphatics and may be enhanced during regression. Consequently, to understand further the mechanisms of regression, we must measure ultrafiltrate flows in arterial lymphatics, as well as the concentrations of the different lipoproteins and their ratios to corresponding plasma levels. One may roughly estimate total lymphatic drainage from a monkey aorta if one assumes that the figure for total lymph body flow in humans (120 ml/hr (Guyton, 1976)), applies to monkeys and that lymph flow is proportional to body weight and to the relative mass of the aorta (0.45 g/kg of body weight in monkeys (Maruffo and Malinow, 1966; Stahl et al., 1968), i.e., aortic lymph flow = 120 x 1/70 x 0.45/100 = 0.008 ml/hr per kg of body weight; the flow will be even lower in individual lymphatics. Predictably, though, technical problems are likely to be associated with the collection of fluids at the expected low flow rates.

The importance of the lymphatic efflux in regression has been suggested by observations performed in squirrel monkeys subjected to a regression regimen; aortic lesions regressed less than expected in animals showing periaortitis—probably with lymphatic obstruction—due to filarial parasites (Hayes et al., 1972). And Sims (1979) has found intimal thickenings indistinguishable from atherosclerosis in human arteries surrounded by neoplastic tissue—presumably with impaired outward flow.

The vasa vasorum, including the adventitial vessels, also constitute an important segment of the arterial microcirculation probably involved in regression. Measurements of exchanges in this vasculature are few (Stein and Stein, 1973), but flow in the vasa vasorum of the nonatherosclerotic aortas of dogs and in the atherosclerotic aortas of cynomolgus macaques has been measured recently (Heistad et al., 1978, 1979). It has now been established for the first time that vasa vasorum are responsive to adenosine infusion, hemorrhagic hypotension, acute hypertension, and atherosclerosis. This important application of the microsphere method to the study of arterial microcirculation suggests that similar studies could be performed during regression.

Techniques for studying the luminal-abluminal transport of macromolecules—effected by plasmalemmal vesicles in endothelia of normal vessels (Karnovsky, 1967; Bruns and Palade, 1968; Casley-Smith, 1969; Simionescu et al., 1973) and through transitory intercellular gaps (Robertson and Khairallah, 1973)—need to be adapted for abluminal-luminal transport studies. The results could throw light on the way in which atheromatous materials are carried away from diseased arteries; e.g., how insoluble or precipitated macromolecules are removed after being rendered soluble or adequately dispersed and transported by fluids circulating through the arterial wall. Moreover, study of the transport of atheromatous materials through cellular migration may require identification of contractile proteins in tissue cultures of cells from regressing atheromatous plaques.

The normal arterial intima is relatively impermeable to the penetration of macromolecules (Stein and Stein, 1973); this characteristic, which still needs to be defined in the other endothelia that may be involved in exchanges of the arterial wall (see above), probably changes during atherogenesis since, in damaged endothelium as in arterial "ballooning," selective permeability is lost and intimal proliferation ensues (Stemerman and Ross, 1972). There is morphological evidence of intimal repair during regression (Weber et al., 1977), and it seems likely that endothelial permeability is modified toward normality. Therefore, we need to study the restoration of endothelial barriers to further our knowledge of the mechanisms of regression.

In conclusion, this review suggests that prolonged lowering of the plasma cholesterol level after induction of atherosclerosis is associated with arterial changes that include net removal of atheromatous materials, arrest of intimal cell proliferation, cellular repair, and remodeling of the arterial wall. Yet to be defined is the way in which changes in lipoprotein levels may trigger arterial repair processes since the former have time constants on the order of days or weeks, whereas regression probably has time constants on the order of months or years. Research on regression is limited because regression occurs slowly and arterial observations are difficult in vivo. Furthermore, although arterial and periarterial lymphatics may be central to the pathogenesis of regression, observations on the function of these vessels are nonexistent. Thus, techniques to study flows and lipoprotein concentrations in arterial and periarterial lymphatics, as well as to measure convective and diffusive flows in arterial interstitial compartments, are sorely needed. In addition, it seems likely that progress will also depend on the development of new serial, nondestructive techniques that could define at short time intervals the biochemical, morphological, and hemodynamic changes that can now be detected only after months or years of regression.

References


Bond MG, Bullock BC, Lehner NDM, Clarkson TB (1976) Regression of primate carotid artery atherogenesis at 200 vs 300 mg/dl plasma cholesterol concentration (abstr). Stroke 9: 97-98.


Casley-Smith Jr (1969) The dimensions and numbers of small vesicles in cells, endothelial, and mesothelial and the significance of these for endothelial permeability. J Microsc (Oxf) 96: 255-258.


Physiol Rev 37: 405-426
Wissler RW, Vesselinovitch D, Borensztajn J, Hughes R (1975) Regression of severe atherosclerosis in cholestyramine-treated rhesus monkeys with or without a low-fat, low-cholesterol diet (abstr). Circulation 52: (suppl II): 16
Atherosclerosis. Regression in nonhuman primates.
M R Malinow

Circ Res. 1980;46:311-320
doi: 10.1161/01.RES.46.3.311

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/46/3/311.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation Research can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation Research is online at:
http://circres.ahajournals.org/subscriptions/