Chapter 44

Some "Schools" of Thought on IHD Etiology and on IHD Acute Fatal Events

Our intention in this chapter is to describe some concepts which have influenced how we think about Hypothesis-2 --- especially about its second part, which will be the focus of Chapters 45 and 46.

Although it is an over-simplification, we will say that historically, scientists working on the problem of coronary atherosclerosis have tended to belong to two "schools." The "Endothelial Injury" school regards injury by various agents, to the endothelial layer of the coronaries, as the first step in the whole process. The "Lipid" school regards entrance of certain types of lipoproteins, into the intima from the circulating blood, as the first step in the whole process. Both schools are consistent with an inflammation model of atherogenesis, and so we begin with a few words about inflammation.

• Part 1. The Inflammatory Response and the Role of Smooth Muscle Cells

Inflammation is a basic response of the body to local injury. The exact reaction varies according to the injurious agent and other specifics. The general function of the inflammatory response is to bring plasma proteins and white cells to the injured site, so that foreign bodies can be destroyed or isolated, and the site prepared for repair. In other words, overlap occurs between "the inflammatory response" and various "immune-system responses." Repair --- generally accompanied by local proliferation of collagen-secreting fibroblasts --- is the final stage of the inflammatory process. Under some circumstances, total repair is not possible, and the final stage of the inflammatory response is containment of the problem (for instance, by formation of an abcess or of a granuloma). Calcium-deposition and growth of a new blood supply at the site are also frequent features of the inflammatory response.

1a. Smooth Muscle Cells in the Arterial Inflammatory Response

The similarity between the inflammatory process and atherogenesis was noticed as early as 1823 by P. Rayer (Rayer 1823), according to Munro and Cotran (Munro 1988, p.249). Munro and Cotran point out (Munro 1988, p.254):

"Inflammation outside the arteries is often characterized by proliferation of fibroblasts, which then deposit collagen and other substances. In the arterial intima, it is the smooth muscle cell which serves this role. Smooth muscle cell proliferation is a critical event in the development of an atheromatous plaque. Smooth muscle cells are present in fatty streaks, although they are overshadowed by macrophage-derived foam cells; in the fibrous cap of the well-developed fibro-fatty plaque, they appear to be the predominant cell type. It is evident that the smooth muscle cells synthesize the bulk of the connective tissue matrix, including collagen, elastic tissue, and the proteoglycans (Kramsch 1971, + Murata 1986, + Yla-Herttuala 1986). In addition, they can accumulate lipid and become foam cells."

It should be noted here that smooth muscle cells are multifunctional cells, capable of modulating their function, as needed, from the function almost exclusively of contraction, to the function almost exclusively of synthesis of elastic fibres, mucopolysaccharides, and myosin (Campbell 1988).

In their very interesting paper, Munro and Cotran review the several "biochemical signals which underlie smooth muscle proliferation" (p.255), and conclude (p.256) that "... there is no dearth of mechanisms to account for smooth muscle proliferation in the vessel wall in atherosclerosis."

1b. A Marker for Inflammation Found to Predict Myocardial Infarction

In 1997, Paul M. Ridker and co-workers published the results of a study of 1,100 apparently healthy men from whom a single base-line measurement of plasma C-reactive protein had been made years earlier. "C-reactive protein is an acute-phase reactant that is a marker for underlying systemic inflammation" (Ridker 1997, p.973).

These workers found a positive and significant dose-response: "The relative risk of first myocardial infarction increased significantly with each increasing quartile of base-line concentrations of C-reactive protein (P for trend across quartiles, <0.001), in such a way that the men in the highest quartile had a risk of future myocardial infarction almost three times that among those in the lowest quartile (relative risk, 2.9; 95 percent confidence interval, 1.8 to 4.6; P<0.001)." And (Ridker 1997, p.978): "C-reactive protein is not simply a short-term marker of risk, as has previously been demonstrated in patients with unstable angina (Liuzzo 1994), but is also a long-term marker of risk, even for events occurring six or more years later." Ridker also reports positive findings from a smaller female study-sample (Ridker 1998).

Within an excellent update on systemic responses to inflammation, Gabay and Kushner comment (Gabay 1999, p.452) that such findings "may reflect the presence of low-grade inflammation in coronary arteries or elsewhere, or alternatively, they may reflect pro-inflammatory or pro-thrombotic effects of C-reactive protein itself [two references]." Elsewhere (p.451), they offer an important reminder that inflammatory responses can have both beneficial and detrimental aspects.

• Part 2. The "Endothelial Injury" and the "Infection" Hypotheses

Among investigations within the "Endothelial Injury" school of atherogenesis, attention has naturally focused quite often on identifying what may injure the endothelium in the first place. We will begin with a list of candidates from Ross 1977, and then focus briefly on one of them: Infectious agents. Infection as a cause of atherosclerosis was suggested by William Osler (Osler 1908) and others, and has been under consideration for almost a century.

2a. Initial Injurant: List of Suspects from Russell Ross et al

In the 1970s, Russell Ross and his colleagues in the Department of Pathology at the University of Washington School of Medicine, produced a series of often-cited papers on the "Endothelial Injury" hypothesis (Ross 1973 + 1976 + 1977) --- also called "Response to Injury" hypothesis. The Ross 1977 paper is well summarized by its abstract (Ross 1977, p.675):

"We postulate that the lesions of atherosclerosis arise as a result of some form of 'injury' to arterial endothelium. This injury somehow results in alteration in endothelial cell-cell attachment or endothelial cell-connective tissue attachment, so that forces such as those derived from the shear in the flow of blood result in focal desquamation [tearing away] of endothelium. This is followed by adherence, aggregation, and release of platelets at the sites of local injury." And: "During the process of release, a mitogenic factor [an agent which stimulates cell-division] is secreted from the platelets which, together with plasma constituents, gains entry into the artery wall, resulting in focal intimal proliferation of smooth muscle cells. This intimal proliferation is accompanied by the synthesis of new connective tissue matrix proteins and often by the deposition of intracellular and extracellular lipids."

In the text, Ross lists all of the following as "potential sources of injury" to the intima's endothelial cells and as contributors to "their desquamation" (Ross 1977, p.676): "Chronic hyperlipidemia (Ross 1976-b), various chemical factors such as homocystine (Harker 1974; Harker 1976), uremia, metabolites, infections, immunologic injury (Minick 1973; Hardin 1973), and mechanical factors (Ross 1973, + Stemerman 1972, + Bjorkerud 1971, + Moore 1973, + Helin 1971). Mechanical injury may occur at particular anatomic sites as a result of the increased shear stress

applied to the endothelial cells from the flow of the blood at these sites (Glagov 1972, + Caro 1973)."

Ross's Update in 1993

In 1993, updating the "response-to-injury hypothesis of atherosclerosis," Russell Ross writes (Ross 1993, p.801): "Atherosclerosis is not merely a disease in its own right, but a process that is the principal contributor to the pathogenesis of myocardial and cerebral infarction, gangrene and loss of function in the extremities. The process, in normal circumstances a protective response to insults to the endothelium and smooth muscle cells of the wall of the artery, consists of the formation of fibrofatty and fibrous lesions, preceded and accompanied by inflammation. The advanced lesions of atherosclerosis, which, when excessive, become the disease and which may occlude the artery concerned, result from an excessive inflammatory-fibroproliferative response to numerous different forms of insult." And (Ross 1993, p.804):

"Central to the response-to-injury hypothesis (Ross 1986 + 1973 + 1976 + 1976-b + 1981) is the proposal that the different risk factors somehow lead to endothelial dysfunction, which can elicit a series of cellular interactions that culminate in the lesions of atherosclerosis. The initial injurious events do not necessarily lead to endothelial denudation. In fact, with better modes of tissue preservation, it is clear that the early lesions develop at sites of morphologically intact endothelium (Faggiotto 1984 + 1984-b; Masuda 1990 + 1990-b; Rosenfeld 1987 + 1987-b)."

2b. Some Recent Insights about the Endothelium

In 1997, Parmley noted (p.11) that "The endothelial cells lining vessels such as the coronary arteries were once thought to be relatively inert, functioning primarily as a barrier between the blood and the underlying vessel wall." Those days are past. Munro 1988 comments (p.251):

"Recent research on vascular endothelium, sparked to a considerable degree by the response to injury hypothesis, has established endothelial cells as active cells which perform a variety of critical functions." Both Parmley and Munro list such functions (and Munro provides numerous references). We will use a similar list of functions from Ross (Ross 1993, p.804):

(1) provision of a nonthrombogenic surface; (2) a permeability barrier through which there is exchange and active transport of substances into the artery wall; (3) maintenance of vascular tone by release of small molecules such as nitric oxide, prostacyclin, and endothelin that modulate vasodilation or vasoconstriction, respectively; (4) formation and secretion of growth-regulatory molecules and cytokines; (5) maintenance of the basement membrane collagen and proteoglycans upon which they rest; (6) provision of a nonadherent surface for leukocytes; and (7) ability to modify (oxidize) lipoproteins as they are transported into the artery wall." And (Ross 1993, p.804-805):

"Changes in one or more of these properties may represent the earliest manifestations of endothelial dysfunction. Evidence has accumulated to suggest that oxLDL [oxidized Low-Density Lipoprotein] is a key component in endothelial injury (Cathcart 1985; Rosenfeld 1990; Steinbrecher 1990; Steinberg 1991). Once formed by the endothelium, oxLDL may directly injure the endothelium and play an initial role in the increased adherence and migration of monocytes and T lymphocytes into the subendothelial space ..."

Parmley offers the opinion (Parmley 1997, p.11) that "It is clear that the endothelial cells have a major role in determining vascular reactivity, the potential for thrombosis, and perhaps, represent the key element in the development of atherosclerosis."

Variable permeability of the endothelial layer to solutes in the bloodstream --- to lipoproteins, for instance --- is an issue to which we return in Part 5 of this chapter.

2c. Injury by Viral Agents: The 1990 Concept of Melnick et al

The hypothesis, that viruses may play a causal role in Ischemic Heart Disease, has been around for quite a while --- with work centered on the herpes simplex virus and the cytomegalovirus (see, for example, Fabricant 1978, + Gyorkey 1984, + Adam 1987, + Hajjar 1988, + Yamashiroya 1988, + McDonald 1989).

In 1990, Joseph L. Melnick, Ervin Adam, and Michael E. DeBakey, at the Baylor College of Medicine in Houston, Texas in 1990, produced a "special communication" in the Journal of the

American Medical Association in which they reviewed existing evidence on this hypothesis (Melnick 1990). Near the outset of their review, they state (Melnick 1990, p.2204):

"Recent work in this area has been stimulated by the finding that atherosclerotic lesions, strikingly similar to those in human disease, were reproducibly induced in chickens by an avian herpesvirus ... Our studies at Baylor College of Medicine (Melnick 1983, + Petrie 1987, + Adam 1987, + Gyorkey 1984, + Petrie 1988) were stimulated by the avian model (Fabricant 1978). We looked for evidence of involvement of cytomegalovirus (CMV) and herpes simplex virus (HSV) in human arterial tissue. These agents belong to the same herpesvirus family. They are very common and cause primary infections in infants and young children in every country ..." On the same page, Melnick et al note that "... in the United States, the prevalence of antibodies to CMV is about 10% to 15% in the adolescent population, rises to more than 50% by age 35 years, and exceeds 70% in adults older than 64 years." Describing part of their own work, Melnick et al write (Melnick 1990, p.2204):

"In the arterial samples we studied, we were unable to detect markers of HSV infection. However, CMV-infected cells were detected in samples taken from a number of lesions taken from patients with atherosclerosis and also in biopsy specimens of apparently uninvolved aorta taken from such patients. Infectious virus was not recovered, but CMV antigen and viral DNA were detected. In addition, epidemiologic studies were conducted in which viral antibodies were measured in patients with atherosclerosis and in matched control patients. High levels of antibodies to CMV were associated with clinically manifest atherosclerotic disease."

The body of their paper goes into detail about the research at Baylor, and describes related findings at the University of Washington, the University of Illinois, the University of Limburg, Stanford University, and the University of Minnesota. Near the end of their paper, they conclude (Melnick 1990, p.2207):

"There is solid evidence that a member of the herpesvirus family can cause atherosclerosis in chickens ... The evidence for involvement of one or more members of the herpesvirus family in human atherosclerosis is much more circumstantial, but it is increasing. The findings of CMV and HSV antigens and nucleic acid sequences in arterial smooth muscle cells suggest that virus infection of the arterial wall may be common in patients with severe atherosclerosis. Although certainly suggestive, these findings by themselves do not demonstrate a role of viruses in the pathogenesis of atherosclerosis, but they lead to an attractive working hypothesis of the steps involved (Table 3). Of special importance are the recent findings that heart transplant recipients who are immunosuppressed and become infected with CMV are particularly prone to develop severe atherosclerosis in the transplanted organ." (They refer to Grattan 1989 and to McDonald 1989.)

The Melnick Recommendation

The final sentence in Melnick 1990 is: "... the studies reviewed herein should provide a basis for further investigation of the role of viruses in human atherogenesis and of their control by means of vaccination or chemotherapy" (p.2207).

Speir et al on Inhibition of Normal p53 Protein by a CMV Product

In 1994, Edith Speir, of the National Institutes of Health, and several co-workers published work entitled "Potential Role of Human Cytomegalovirus and p53 Interaction in Coronary Restenosis" (Speir 1994). The p53 protein (encoded by the p53 gene) is known as a "tumor suppressor" because one of its normal functions is to activate a gene which blocks a cell from completing division. The abstract of Speir 1994 is short (Speir 1994, p.391):

"A subset of patients who have undergone coronary angioplasty develop restenosis, a vessel renarrowing characterized by excessive proliferation of smooth muscle cells (SMCs). Of 60 human restenosis lesions examined, 23 (38 percent) were found to have accumulated high amounts of the tumor suppressor protein p53, and this correlated with the presence of human cytomegalovirus (HCMV) in the lesions. SMCs grown from the lesions expressed HCMV protein IE84 and high amounts of p53. HCMV infection of cultured SMCs enhanced p53 accumulation, which correlated temporally with IE84 expression. IE84 also bound to p53 and abolished its ability to transcriptionally activate a reporter gene. Thus, HCMV, and IE84-mediated inhibition of p53 function, may contribute to the development of restenosis."

We note that Speir and co-workers established to their satisfaction that the p53 protein in their positive restenosis samples was normal (Speir 1994, p.392). In other words, they are not proposing

that the virus is mutating the p53 gene which codes for the p53 protein. They are proposing that the p53 protein is disabled by binding to the IE84 protein produced by the re-activated virus. Comments on their paper and related work include Epstein 1994, + Finkel 1995, + Zhou 1996, + Smith 1997, + Kol 1997, + Zhou 1997.

Speir and co-workers also examined specimens from 20 primary atherosclerotic lesions, taken from atherectomy patients who had never had angioplasty (Speir 1994, p.391). None of the 20 primary specimens were found p53-immunopositive (p.392). Their work as of 1994 appears not to be applicable to the pathogenesis of primary atherosclerotic lesions.

2d. Injury by Bacterial Agents: Chlamydia and Helicobacter Pylori

During the past decade, multiple investigating teams have looked at a variety of Chlamydia bacteria and Helicobacter pylori as possible atherogens. For example:

Thom et al, 1992

In 1992, Thom et al reported on a case-control study designed to evaluate "the association between prior infection with Chlamydia pneumoniae, as measured by IgG antibody, and coronary artery disease" (Thom 1992, p.68). To qualify as a CAD patient, individuals had to have "at least one coronary artery lesion occupying 50% or more of the luminal diameter" (p.68), ascertained by diagnostic coronary angiography. A positive association was found only in cigarette smokers (Thom 1992, p.68, p.71, p.72). Association has been reported, too, by Davidson (Davidson 1998).

Mendall et al, 1994

In 1994, Mendall et al reported on their pilot study, undertaken "to determine whether Helicobacter pylori, a childhood acquired chronic bacterial infection, is associated with an increased risk of coronary heart disease later in life" (Mendall 1994, p.437). In this case-control study of white males, Mendall et al found a positive association between documented Coronary Heart Disease (CHD) and seropositivity for H-pylori-specific IgG antibodies (Odds Ratio = 2.15, p = 0.03), after adjustment for other variables. They speculate that "H pylori could influence the development of CHD through the systemic effects of chronic active inflammation in a large viscus" or by infection-induced alterations in levels of fibrinogen and apolipoprotein-a (p.438-439). They conclude (Mendall 1994, p.439): "The association between H pylori and CHD reported here needs confirmation by other more rigorously controlled epidemiological studies ... A causal link between H pylori and CHD would be of major public health importance because the infection can be eradicated with a single course of antibiotics with little chance of reinfection."

Patel et al, 1995

In 1995, Patel, Mendall and co-workers reported their findings from a larger study of white males "to investigate the relation between seropositivity to chronic infections with Helicobacter pylori and Chlamydia pneumoniae and both coronary heart disease and cardiovascular risk factors" (Patel 1995, p.711). They found the Odds Ratios for electrocardiographic evidence of ischemia or infarction to be 3.82 (95% confidence interval 1.60 to 9.10) and 3.06 (1.33 to 7.01) "in men seropositive for H pylori and C pneumoniae, respectively, after adjustment for a range of socioeconomic indicators and risk factors for coronary heart disease ... Possible mechanisms [for the relationship] include an increase in risk factor levels due to a low grade chronic inflammatory response" (Patel 1995, p.711).

Patel 1995 reports, "Cardiovascular risk factors that were independently associated with seropositivity to H pylori included fibrinogen concentration and total leukocyte count. Seropositivity to C pneumoniae was independently associated with raised fibrinogen and malondialdehyde concentrations" (p.711). The authors conclude (Patel 1995, p.714): "We have shown an association of the prevalence of coronary heart disease with potentially treatable infections which are common in the general population. Our figures imply that between one third and one half of current coronary heart disease in this population was statistically attributable to either or both infections. What are required now are well conducted prospective studies and eradication trials to evaluate the causal relation of these infections to haemostatic function, progression of atherosclerosis, and cardiovascular morbidity and mortality." A related study is reported by Gupta (Gupta 1997).

Muhlestein et al, 1996

In 1996, Muhlestein et al reported the results from their study in which they tested for "an association between Chlamydia and atherosclerosis by comparing the incidence of the pathogen found within atherosclerotic plaques in patients undergoing directional coronary atherectomy with a variety of control specimens and comparing the clinical features between the groups ... Coronary specimens from

90 symptomatic patients undergoing coronary atherectomy were tested for the presence of Chlamydia species using direct immunofluorescence. Control specimens from 24 subjects without atherosclerosis (12 normal coronary specimens and 12 coronary specimens from cardiac transplant recipients with subsequent transplant-induced coronary disease) were also examined" (Muhlestein 1996, p.1555). The abstract states the results well (Muhlestein 1996, p.1555):

"Coronary atherectomy specimens were definitely positive in 66 (73%) and equivocally positive in 5 (6%), resulting in 79% of specimens showing evidence for the presence of Chlamydia species within the atherosclerotic tissue. In contrast, only 1 (4%) of 24 nonatherosclerotic coronary specimens showed any evidence of Chlamydia. The statistical significance of this difference is a p-value <0.001. Transmission electron microscopy was used to confirm the presence of appropriate organisms in three of five positive specimens. No clinical factors except the presence of a primary non-restenotic lesion (odds ratio 3.0, p=0.057) predicted the presence of Chlamydia. CONCLUSIONS. This high incidence of Chlamydia only in coronary arteries diseased by atherosclerosis suggests an etiologic role for Chlamydia infection in the development of coronary atherosclerosis that should be further studied."

Toward the end of Muhlestein 1996 (p.1559), the authors acknowledge that Chlamydia might be present in the lesions only as an "innocent bystander, finding fertile ground to grow within the diseased atherosclerotic wall." They reason that "it should also be found within the walls of arteries diseased by processes other than atherosclerosis," and they argue that its ABSENCE "within diseased coronary segments of transplanted hearts appears to increase the likelihood that Chlamydia plays an active role in the pathogenesis of natural atherosclerosis." Returning to this topic (on p.1560), they state: "It is still possible that atherosclerotic plaque is merely a more fertile ground for Chlamydia to be deposited and grow. If this were the case, the presence of the pathogens would be a result rather than a cause."

Meier et al, 1999

In early 1999, Meier et al reported findings consistent with an association between certain bacterial infectious agents and risk of acute myocardial infarction (Meier 1999). Commenting in the same JAMA issue on the hypothesis of a causal role for bacterial infections in myocardial infarction, Folsom writes (Folsom 1999, p.461): "Hypothesized mechanisms by which infection could cause vascular disease include direct infection of the arterial wall, systemic infection leading to endotoxin injury of the endothelium, autoimmunity ..., systemic inflammation, or increases in inflammatory mediators, such as C-reactive protein, fibrinogen, and white blood cell count (Danesh 1997, + Libby 1997)."

• Part 3. The "Lipid Hypothesis": Gradual Acceptance of a Causal Role

Almost wholly, lipids (including free cholesterol, esterified cholesterol, fatty acids, triglycerides, and phospholipids) are transported in the bloodstream by combination with proteins --- hence, "lipoproteins."

In contrast to the "Response to Injury Hypothesis," the "Lipid Hypothesis" of Atherogenesis proposes that the entrance of certain types of plasma lipoproteins, into the intima from the circulating blood, is the initial step in the whole atherosclerotic process, and that atherosclerosis develops more quickly and more seriously when blood concentrations of these lipoproteins are high than when they are low. The lipoproteins have no physiologic function between the endothelium and the internal elastic membrane. They are "out of place," and unless they exit rapidly while soluble, they become a "foreign substance" which elicits efforts at removal or isolation --- the inflammatory response.

3a. What Is Our Goal with Respect to the Lipid Hypothesis?

During 1949-1950, our group at the Donner Laboratory (University of California, Berkeley) showed that numerous species of lipoproteins exist (Part 3b, below). The immediate question became: "Which lipoprotein species (if any) have causal roles in atherosclerosis?" In terms of the Lipid Hypothesis, one requirement was that the offending lipoproteins must be able to penetrate into the intima. The implication: Size would probably matter.

With respect to disease, a prime reason to STUDY causation is to PRACTICE prevention. Therefore, our group had many questions:

• (A) Which lipoprotein species (if any) are atherogenic, and can we identify ALL of the independently atherogenic species? (Parts 3h and 3i). If one misses half of the independently atherogenic species, one may miss a large share of the opportunities to prevent the disease.

• (B) Can reducing the concentration of SOME lipoprotein species, in the blood, cause elevation in the plasma concentrations of OTHER species on a steady-state basis? The answer is yes (Nichols 1957, + Gofman 1958).

• (C) Do certain regimes, which successfully reduce the blood-levels of some ATHEROGENIC species, elevate levels of OTHER atherogenic species? By 1957, our evidence --- from altered diets --- provided an affirmative answer (Appendix F). We continue to worry that some medically-prescribed low-fat, high-carbohydrate diets may even produce a net INCREASE in atherosclerosis risk for a possibly large segment of the population.

• (D) Are some other regimes converting NON-atherogenic lipoproteins into ATHEROGENIC species? For example, do some substances which seem to reduce the blood-measurement called "total triglyceride" do so by hydrolyzing a component of a non-atherogenic species --- and does this action produce a net increase in the blood-concentrations of atherogenic species? This question deserves resolution.

• (E) Is it possible to rank the atherogenic types of lipoproteins by the degree of their menace? My own opinion: Not yet.

3b. What We Never Expected: Such a Variety of Plasma Lipoproteins

By 1949, chemical analysis had divided lipoproteins into two parts: Alpha and Beta (Cohn 1946, + Oncley 1947, + Gurd 1949). But in 1950, there was only "limited interest in plasma lipoproteins," as Donald Fredrickson has reported (Fredrickson 1993, p.III-1).

In 1950, my colleagues and I wrote (Gofman 1950-b, p.161): "For many years, it has been suspected that the blood lipids might in some way be related to the pathogenesis of human atherosclerosis. All the major blood lipid constituents, including cholesterol and its esters, phospholipids and neutral fats, have been investigated without leading to definitive conclusions. Cholesteremia itself has received much attention..." And (Gofman 1950-b, p.161):

"... the low relationship manifested between the extent of atherosclerosis and serum cholesterol level has thus cast some doubt in the minds of many investigators upon the significance of serum cholesterol level, per se, in the pathogenesis of this disease."

The inconclusive, and ostensibly conflicting, results which existed, regarding the relationship between blood lipids and human atherosclerosis, were partly or largely a result of measuring only the lipid components of lipoproteins (Gofman 1950-a, p.171, p.186). Prior to 1949, the extensive diversity of the lipoprotein molecules was not known.

That situation changed abruptly in 1949 and 1950. At the Donner Laboratory, my colleagues and I were able to discover that Beta lipoprotein really consisted of a great many species, distinct in size, density, weight, and chemical composition. Not only do the lipid components vary, but the protein components vary too. We were entering a new world of diverse lipoproteins (Gofman 1949, + Lindgren 1950, + Gofman 1950-a, + Gofman 1950-b, + Lindgren 1951). Some views on these breakthroughs include Fredrickson 1993, + Gofman 1996-b (FASEB's "Milestones" series).

A Series of "Giant Molecules" : Boxes 1 and 2.

Entry into this new world occurred thanks to two ultracentrifuges (the analytic and the preparative), engineered and made available to humanity through the genius of Dr. Edward G. Pickels (Pickels 1942 + 1943; Gofman 1990-b). The variety of lipoproteins could be visualized in the optical diagrams made during the ultracentrifugation of serum (or plasma). By adjusting the density of the solution, we were able to segregate various classes of molecules from each other, in nearly their native states. The optical (schlieren) diagrams were useful in quantifying the concentration of various lipoprotein classes in the circulating blood plasma.

We were not expecting to find the large number of species of lipoproteins, all undergoing flotation in a solution of density, 1.063 grams per milliliter. Consider going from "Beta lipoprotein" to at least 9 discrete species and possibly as many as 100 or more either discrete species or a continuum of species. We initially referred to the entire complex of species as "Low-Density Lipoproteins (LDL)," because all underwent flotation in a solution of 1.063 gms/ml. As the work progressed, a number of separate classes of Low-Density Lipoproteins were identified, differing from each other either by their flotation rates (in a solution of density 1.063 grams/ml) or by their buoyant density (Boxes 1 and 2).

We described the lipoproteins as a series of macro-molecules ("giants"), because even the smallest ones are very large (Box 1).

3c. The Sf Unit: A Major Lipoprotein Identifier

For the lipoproteins of density < 1.063 gms/ml, it was found that the larger the lipoprotein size, the lower is the density. The very largest and least dense lipoproteins are called the chylomicrons.

Density and size together determined the speed of migration, occurring against centrifugal force in a solution of density 1.063 gms/ml --- with speed measured in Svedberg flotation units, or Sf units (Gofman 1950-a, p.168). The Svedberg unit, and the Standardized Svedberg Sf unit (Std Sf unit), are defined in Box 3 of this chapter.

The lower the density of the lipoprotein, the faster the migration (Box 2). At equal molecular size, the higher the density of the lipoprotein, the slower the migration.

Flotation rates, for the lipoproteins of density < 1.063 gms/ml, range from near Sf 0 up through approximately Sf 40,000 (the chylomicrons). The following entities are commonly discussed in the literature: Sf 0-12, Sf 12-20, Sf 20-100, and Sf 100-400. Combinations are also commonly discussed, e.g., the plasma concentration of Sf 0-20 (the combined concentration of Sf 0-12 and Sf 12-20 lipoprotein classes), or Sf 20-400 (the combined concentration of Sf20-100 and Sf 100-400 lipoprotein classes). And a particular investigator might use other cutting points, for reasons indigenous to his or her own studies.

These divisions of flotation rates are not arbitrary. Each group embraces lipoproteins which generally share some distinct chemical, biochemical, and metabolic properties (Box 1, bottom). Moreover, flotation rates are very highly correlated with the relative size of these molecules, in their native state. The body itself is not likely to "overlook" the DISTINCTIONS, in size and in the various size-correlated properties of these lipoproteins, in their native state. So we, too, heed the distinctions, as we endeavor to learn which lipoproteins are atherogenic and which are not (Part 3h).

3d. LDL, IDL, and VLDL, with Their Corresponding "Sf" Ranges

Currently, Sf values are combined under the following names (Krauss 1987, p.62):

- LDL: Std Sf 0-12 lipoproteins are described as "Low-Density Lipoproteins."
- IDL: Std Sf 12-20 lipoproteins are described as "Intermediate-Density Lipoproteins."
- VLDL: Std Sf 20-400 lipoproteins are described as "Very-Low Density Lipoproteins."

The lipoproteins of still higher flotation rates are also referred to as "Very-Low Density" by some investigators. We suggest that the use of flotation rates rather than the term "very-low density," for these Std Sf 400-40,000 classes, will prevent potential confusion with the Std Sf 20-400 lipoproteins.

3e. HDL: High-Density Lipoproteins and "Small, Dense LDL Particles"

The smallest and most dense lipoproteins are called High-Density Lipoproteins: HDL-1, HDL-2, HDL-3. Their hydrated densities (below) are from DeLalla 1954-b, p.333.

HDL-2 (density 1.075 g/ml) and HDL-3 (density 1.145 g/ml) belong roughly in the category formerly called "Alpha" lipoprotein (Lindgren 1951, p.83). Some unesterfied fatty acids are bound in protein complexes of even higher density than the HDL-3 lipoproteins. Because HDL-2 and HDL-3

sink (rather than float) in a salt solution of 1.063 g/ml, they acquired the name "High-Density" Lipoproteins.

HDL (HDL 2 + 3) is often called "the good cholesterol" in popular media, because of its purported protective effect against atherosclerosis. Appendix-G discusses that claim.

HDL-1 lipoproteins have a density (1.05 g/ml) just below the density of a salt solution of 1.063 g/ml. The flotation rate of HDL-1 in such a solution is the lowest encountered in our work. On the basis of flotation in such a solution, HDL-1 lipoproteins are definitely LOW-density lipoproteins (approximately Sf 0-2), and they make a small contribution to the measured plasma concentration of the Sf 0-12 lipoproteins. Nonetheless, they acquired the name "HDL-1" because their own plasma concentration is best ascertained if they are centrifuged along with the High-Density Lipoproteins > 1.063 g/ml. Quantitative recovery of HDL-1 for analysis requires this special handling (Appendix-H, Parts 2 + 4b).

Currently, much attention is directed at the HDL-1 lipoproteins, under another name: Small, dense LDL particles. Appendix-H discusses the claim that they are especially atherogenic.

3f. Discrete Entities, vs. a Continuum

The High-Density Lipoproteins appear to be discrete or nearly discrete macromolecular species. In the Low-and-Intermediate Density spectrum (Std Sf 0-20), extensive studies by Frank Lindgren, Alex Nichols, and Norman Freeman led to identification of numerous nearly discrete entities on a density scale and flotation-rate scale. For lipoproteins with flotation rates above Std Sf 20, it is difficult to speak to the issue of discrete macromolecular entities versus a continuum of entities (Jones 1951, p.359). Between approximately Sf 400 and Sf 40,000, there is an apparent continuum, and as the flotation rates progressively increase, the densities progressively decrease. The largest molecules might be compared with protein sacks which can be filled with variable amounts of lipid. Triglyceride is the dominant cargo.

3g. "Cholesterol-Rich" vs. "Triglyceride-Rich" Lipoproteins: Box 1

Cholesterol is a constituent of all species of HDL, LDL, IDL and VLDL, but the amount of cholesterol per macromolecule, and its status as free or esterified cholesterol, differs extensively among the macromolecular lipoprotein entities. In addition to free or esterified cholesterol, the triglycerides (a variety of different ones), phospholipids, and fatty acids are constituents of the various lipoprotein entities, in varying amounts (Box 1, bottom).

The Std Sf 0-20 lipoproteins are commonly referred to as the "major cholesterol-bearing lipoproteins," or the "cholesterol-rich lipoproteins." And the Std Sf 20-400 lipoproteins are the "major triglyceride-bearing lipoproteins," or "triglyceride-rich lipoproteins." These are valid characterizations, but there is room for ambiguity with respect to the term triglyceride-rich. The lipids of the chylomicrons (not of the Sf 20-400 lipoproteins) are the richest in triglycerides, when richness is expressed as fraction of the lipid constituents within a macromolecule. However, in those blood samples where chylomicrons are scarce, the Sf 20-400 lipoproteins are the main source of the measured levels of triglyceride.

In general, in moving along the lipoprotein spectrum from Sf 400 toward Sf 0, the proportion of triglyceride versus other lipids per macromolecule decreases, and the proportion of cholesterol versus other lipids per macromolecule increases (Jones 1951, p.360, + Gofman 1952-c, p.282). As a result, high levels of TOTAL serum-cholesterol or TOTAL serum-triglyceride are, by themselves, uninformative about WHICH of the lipoproteins are elevated. Measurements which destroy the molecules, without first measuring them in their physiologically NATIVE state, do not identify the species (Lindgren 1951, p.80).

3h. The Atherogenic Lipoproteins, and the Non-Atherogenic Ones

Many of the historical and quantitative details, of how we come to know what we know (and what we do not yet know) about the Lipid Hypothesis, are provided in Appendices E, F, G, H, and I of this book.

Back in 1949-1950, we soon realized that it would be a monumental task to sort out any relationship of plasma "lipids" with atherosclerosis, because initially there was no overt reason to exclude ANY part of the vast lipoprotein spectrum --- fatty acid-protein complexes, HDL, Sf 0-40,000 lipoproteins. Appendix-E provides details about some of the studies which have led to our conclusion that the following lipoprotein classes have independent causal roles in atherogenesis:

- Standard Sf 0-12 lipoproteins.
- Standard Sf 12-20 lipoproteins.
- Standard Sf 20-100 lipoproteins.
- Standard Sf 100-400 lipoproteins, with the caveat that the upper limit for atherogenicity may lie well below Sf 400. The evidence of independent atherogenicity is weaker for the Sf 100-400 range than for the three other ranges. The relative weakness may reflect an upper limit on the size of blood lipoproteins which can penetrate into the intima. Or it may reflect the greater variability in blood-level measurements for Sf 100-400, compared with Sf 0-100 (Appendix E, Part 3). Or both.

Our studies did not make a direct endeavor to ascertain any atherogenic properties of Sf 400-Sf40,000 lipoproteins. It is likely that atherogenicity for these classes is limited by physical size. There are some who might not agree that these very large lipoproteins fail to gain access to the arterial intima. At this time, we consider the evidence to be otherwise. But we are, of course, always open to new evidence.

The following lipoprotein classes are non-causal in atheroclerosis:

- High-Density Lipoproteins-Two (HDL-2).
- High-Density Lipoproteins-Three (HDL-3).

HDL-1, and its purported special atherogenicity, are separately addressed in Appendix-H.

Our Livermore Lipoprotein Study at 10 years provided the first prospective evidence that the HDL-2 and HDL-3 lipoproteins might be inversely related to atherogenesis (Gofman 1966, pp.686-687). However, we are unable to say that this represents evidence for a protective role against atherosclerosis. The strong inverse relationship in cross-sectional studies, of the HDL(2+3) with the atherogenic Std Sf 0-20 and 20-400 lipoproteins (data in Appendix G), is undoubtedly the cause of some of, and maybe most of, the observed APPARENT protective effect.

Some have argued that a protective role for HDL-2+3 is biologically plausible, but we are not impressed yet by that argument. We remain skeptical that the (HDL-2 + HDL-3) lipoproteins truly do provide a protective effect against atherogenesis (Appendix-G).

3i. Acceptance for Part of the Lipid Hypothesis: From "Gofman's Heresy" to Current Wisdom?

Our concept, that certain classes of lipoproteins have a CAUSAL role in atherosclerosis (and that others do not) met with considerable skepticism. It was even suggested that anything related to cholesterol could not be deadly, because the human body needs and synthesizes cholesterol on its own, regardless of diet.

Gradually, as other workers also came to the conclusion that the primary cholesterol-bearing low-density lipoproteins are causally associated with Coronary (Ischemic) Heart Disease, this part of the Lipid Hypothesis became generally accepted --- not universally, of course. By 1983, in the Lyman Duff Memorial Lecture, Henry C. McGill, Jr., commented: "Gofman's heresy, that some lipoproteins are atherogenic and others are not, is now dogma" (McGill 1984, p.443). In 1993, the former head of the National Institutes of Health, Donald S. Fredrickson, wrote: "Today, there is no quarrel over the measurement of LDL rather than total serum cholesterol as the more powerful indicator of coronary risk" (Fredrickson 1993, p.III-3).

By contrast, there is not yet general acceptance of our findings that the triglyceride-rich Sf 20-100 and some of the Sf 100-400 lipoproteins are also atherogenic. The lack of acceptance does not reflect an inability by others to replicate our findings. Instead, debate has been raging for 30 years over whether, or not, "triglyceride" is atherogenic. The controversy is linked with the erroneous assumption that, in studies of atherogenesis, a chemical triglyceride measurement is the equivalent of

an ultracentrifugal measurement of Std Sf 20-100 lipoproteins, in their nearly native states. This error has persisted for decades, and is only now being corrected hesitantly in the medical literature. We comment on the "triglyceride" controversy (and why it is important to resolve it) in Part 4.

• Part 4. High-Carbohydrate Diets and Triglycerides: A Lipoprotein Pitfall?

In 1957-1958 (Nichols 1957, + Gofman 1958), we showed that a high-carbohydrate diet can INCREASE steady-state blood-levels of the "triglyceride-rich" lipoproteins --- Std Sf 20-400. Results are shown in Appendix-F. This finding is consistent with the fact that the metabolism of carbohydrates ("sugar") and the metabolism of lipids are closely linked. This linkage was not as well known in the past as it is today. A dramatic reminder, related to diabetes mellitus, is briefly provided in Part 4a.

4a. Intersection of Carbohydrate Metabolism and Lipid Metabolism

Diabetes mellitus is simultaneously a disease of carbohydrate metabolism and lipoprotein metabolism. In 1952, Dr. Felix Kolb, Dr. Oliver DeLalla, and I were lucky to have the opportunity to learn what happened to the spectrum of lipoproteins circulating in the blood of a diabetic patient in severe acidosis and nearly in coma, as the patient moved from essentially full decontrol of diabetes mellitus toward control of the crisis, by treatment with electrolyte-balancing and insulin.

Our observations are reported in the journal Metabolism (Kolb 1955) and are also presented in Box 3.

What occurred were orderly, non-random changes in the blood-concentrations of lipoprotein-classes. Initially (June 19, 1952), the plasma was exceedingly cloudy, almost certainly due to high concentrations of lipoproteins in the Sf 400 to chylomicron range. In the Sf 0-400 range, concentrations the Sf 100-400 lipoproteins were massively elevated: 3,739 mg per 100 ml. The dominant class in terms of concentration changed progressively over time to smaller lipoprotein molecules with lower triglyceride content. As the concentration of one class decreased, the concentration of progressively smaller, more dense lipoprotein molecules increased. And as these declined in concentration, the concentration of the even smaller macromolecules increased. The columns in Box 3 show clearly how it occurred.

We witnessed the essence of the steps in lipid metabolism, in relation to the change from decontrolled to controlled carbohydrate metabolism. As Box 3 indicates at the bottom, we also witnessed, on the patient's skin, the disappearance of the "eruptive" xanthomas --- lesions which are filled with lipids.

Of course, we do not imply that diabetics in acidosis are a model for the general population --- but we do emphasize that linkage, between carbohydrate metabolism and blood-levels of various lipoproteins, occurs within the general population, too.

4b. High-Carbohydrate Diets and the Unsettled "Triglyceride" Issue

For decades, high-carbohydrate diets have been commonly recommended as "heart healthy." At the same time, we know that such diets elevate blood-levels of "triglyceride-rich" lipoproteins (Std Sf 20-400 lipoproteins), for some segment of such dieters --- perhaps for most of them (Appendix F). A high-carbohydrate diet may turn out to be optimal for some people, but very unhealthy for others. Therefore, an urgent obligation exists finally to SETTLE the question:

Do elevated levels of some, most, or all, of the Std Sf 20-400 "triglyceride-rich" plasma lipoproteins make independent, causal contributions to atherogenesis?

In the next attempts to answer this question, the first principle should be to measure the levels of the lipoproteins at issue, instead of measuring something ELSE --- namely, the levels of triglyceride. Measurements of serum triglyceride-levels are simply NOT informative about the serum levels of the Std Sf 20-100 lipoproteins and the separate serum levels of the Std Sf 100-400

lipoproteins --- because EVERY lipoprotein in the spectrum, from HDL to chylomicroms, can contribute triglyceride to such a measurement (Box 1; also Appendix-E, Boxes 1 + 2). Under these circumstances, it is difficult to explain how anyone could expect to learn WHICH groups of serum lipoproteins are atherogenic, by studying serum-levels of "total triglyceride."

4c. A "Shortcut" Which Fogs the Lens: A Self-Inflicted Handicap

Long ago, perhaps due to the time and cost involved in ultracentrifugal analysis of an individual's lipoprotein patterns, ultracentrifugal analysis of blood was virtually discarded in favor of chemical analysis. This is the "shortcut" which produces measurements like "total cholesterol," "total triglyceride," and a variety of chemical ratios. Clearly, "total cholesterol" and "total triglyceride" are measurements which do not reveal the distribution of the various lipoproteins which were carrying those lipids in the bloodstream.

To our amazement, the recent decades have produced study after study using only chemical determination of triglycerides, with no indication what percentages of the measurement come from (Std) Sf 0-20, Sf 20-100, Sf 100-400, and Sf 400-40,000 lipoproteins. Substituting "total triglyceride" measurements for ultracentrifugal analysis of serum lipoprotein patterns, in research into the atherogenicity of particular segments of the lipoprotein spectrum, has been like fogging the lens through which investigators look at such questions. It is a self-inflicted handicap which could be remedied at will.

No wonder that, 45 years beyond opening of the world of lipoproteins, contradictory findings exist on whether OR NOT elevated serum levels of "total triglyceride" (hypertriglyceridemia) are an independent cause of Ischemic Heart Disease, or just a "marker" for a real cause (for instance, see Bierman 1992, p.647, + Blankenhorn 1994, p.185, + Avins 1989, 1997, + Hokanson 1996, + Enas 1998, + Lamarche 1998). In 1996, succinctly describing the confusion, Meir Stampfer and co-workers wrote (Stampfer 1996, p.882; with the capitalization from the original):

"WHETHER TRIGLYCERIDE level is an independent risk factor has been controversial for many years."

4d. Why It Is Important to Learn the Atherogenicity of Every Lipoprotein Class

Why is it important firmly to establish either the atherogenic potency or the protective potency of every class of lipoprotein?

- First, the knowledge is essential in order to guide progress in limiting Ischemic Heart Disease, by modifying the steady-state serum-levels of such classes. If the "triglyceride-rich" Std Sf 20-400 lipoproteins are atherogens and we fail to recognize them as such, then a major opportunity to limit IHD is ignored --- an abhorrent situation.

- Second, if the Std Sf 20-400 lipoproteins are atherogens and we fail to recognize them as such, then the possibility of causing net HARM with specific types of intervention is real (Part 3a) --- an intolerable development. We think that evidence of atherogenicity in the Std Sf 20-100 segment of the spectrum was already strong more than 30 years ago, and that the health of a great many people may have been harmed by failure to resolve the doubts.

It is heartening to observe a growing sense of urgency, expressed in the literature, about the need to settle the so-called "triglyceride" issue.

• Part 5. Healthy Intima: Balanced Entry and Exit of Lipoproteins

Many of us who were working on the atherosclerosis problem in the 1950s considered it plausible that plasma lipoproteins filtered into the intima by penetration through an INTACT endothelial layer. The problem, as we saw it then, was getting the lipoproteins out of the arterial wall, before they underwent denaturation with loss of solubility. Wei Young and I wrote in 1963, "Clearly, a major effort is still required to understand the fate of lipoproteins in the region BETWEEN the endothelium and the internal elastic membrane" (Gofman 1963, p.217).

Major efforts were made and are still being made. Many are described in the 1988 review by Munro and Cotran. Today, workers on blood lipoproteins (for example, Young 1994) show prominent enthusiasm over the possibility that oxidation of lipoproteins, either before or after entry into the arterial intima, may be the key change in low-density lipoproteins to make them atherogenic or more atherogenic. (See also Ross 1993, p.805; + Fuster 1994, p.2128; + Libby 1995, p.2849.) Whether the oxidation story is correct or not, the lipoprotein-atherosclerosis causal relationship itself is unaffected. It has withstood plenty of scrutiny for a half-century --- because it is scientifically solid.

5a. Concept of a Constant Passage of Fluid and Solutes

In 1963, in contrast to claims that injured endothelium must be required to initiate atherosclerosis, Wei Young and I built on earlier work by others to describe a model for ingress and egress of lipoproteins with respect to the intima (Gofman 1963, p.198):

"Anitschkow 1933, following many years of highly productive investigation of atherosclerosis, held the opinion that the normal artery experiences a constant passage of fluid through its wall in the direction from arterial lumen to adventitia. Further, some of the constituents of such fluid were considered to pass on through, along with the fluid vehicle, thus leading to no pathological consequence whatever. Other constituents of the fluid vehicle ... tend to REMAIN WITHIN the wall of the artery ... Atheroma, it was reasoned, developed, its extent being conditioned by (a) the nature of the substances remaining behind in the arterial wall, and (b) the over-all 'responsiveness' of the arterial tissue to these substances. During the last decade, opportunity for critical evaluation of the Anitschkow concept has greatly increased."

Much of the work involved the passage of materials to extravascular spaces from the interior of intact CAPILLARIES (Pappenheimer 1953, + Kellner 1954, + Courtice and Garlick 1962). Courtice and Garlick found evidence supporting the concept that the transfer-rate of lipoprotein molecules across the capillary wall varied with variation in the size of such molecules. As of 1963, the mechanism of macromolecule transport across normal endothelium was still speculative. We wrote (Gofman 1963, p.208):

"It has been suggested that such passage may occur (a) via junction regions between endothelial cells (Chambers and Zweifach, 1947), (b) via some of the 'pores' which constitute part of the endothelial lining (Pappenheimer 1953), or (c) via vesicles of the endothelial cells, revealed by electron microscope studies (Palade 1956, + Bennett 1956) as an active transport process." As for egress of the plasma lipoproteins from the intima, we speculated (Gofman 1963, p.200):

"Presumably, in health, the fenestra provide an adequately competent path for removal of fluid and solutes, including such giant solutes as lipoprotein macromolecules. If, for any reason or set of reasons, the transfer of lipoproteins be impeded through such fenestra RELATIVE to their influx via the endothelial lining, the stage would be set for an accumulation of lipoproteins within the subintimal space (i.e., between the endothelium and internal elastic membrance). The entrapped lipoproteins could result in several of what are probably later manifestations of fully developed atherosclerotic lesions."

Below, we will refer to a balance of ingress and egress as the "equilibrium concept."

5b. Elspeth Smith on (a) Normal Endothelium and (b) Impeded Egress

By the 1970s, evidence was appearing which supports the concepts that (a) plasma lipoproteins pass from the lumen into the intima through uninjured endothelium, and (b) imbalance between ingress and normal egress is the key to lipid accumulation in the intima. For instance, Elspeth B. Smith, in the Department of Chemical Pathology at the University of Aberdeen (Scotland) was investigating these issues. Smith wrote (Smith 1977, p.672):

"Normal endothelium is PERMEABLE to plasma macromolecules even in healthy young animals (Bell 1974-a + 1974-b), but no appreciable amount seems to ACCUMULATE in the absence of diffuse intimal thickening (Smith 1974). Gelatinous lesions are characterized by a massive increase in the content of plasma macromolocules, but we do not know if they are initiated by increased endothelial permeability, so that the rate of entry exceeds the rate of clearance, or by a primary change in the retentiveness of the intima." And earlier in the same paper (Smith 1977, p.667):

"Both our steady-state concentration data (Smith 1974) and permeability data (Bell 1974-a + 1974-b) suggest that a rather constant volume of whole plasma enters normal intima. This concept of a PACKAGE OF PLASMA is also supported by the constancy of the relation between the concentrations of plasma proteins in intima when expressed in terms of plasma volumes (Smith 1976, + Smith 1975)." And (Smith 1977, p.667):

"In intima there is much greater accumulation of LDL [low-density lipoprotein] than of albumin relative to their concentrations in plasma (Smith 1974, + Smith 1972); together with the concept of a package of plasma, this suggests that the steady-state concentrations may be largely determined by rate of egress of the macromolecules, and there has been much speculation that glycosaminoglycans (GAG) are involved in retention of LDL either by formation of specific ionic complexes (Tracy 1965, + Bihari-Varga 1967, + Srinivasan 1972) or by molecular sieving in the GAG gel (Laurent 1963, + Iverius 1973)." The 1977 Smith paper also discusses other processes which may cause irreversible precipitation of lipoproteins in the intima, or may split the lipids off the proteins.

5c. Some Current Wisdom on Endothelial Injury: Munro, Ross, Brown

The concept that extracellular lipid in atherosclerotic plaques arrives within the intima from the lumen, without endothelial injury, is now clearly accepted by workers such as Munro and Cotran (Munro 1988, p.257, Figure 2), Ross, and Brown. In Ross's current model (Ross 1993, p.804-805), the endothelial cells have the "ability to modify (oxidize) lipoproteins as they are transported into the artery wall" (Ross 1993, p.804). See also Part 2a, above.

B. Greg Brown and colleagues, referring to mature plaques, comment (Brown 1993, p.1785): "Lipid may enter the core region of the fibrous plaque by transmural flux (Fry 1987) of its more mobile forms --- lipoprotein particles, droplets, and vesicles (Guyton 1985, + Smith 1967) --- or it may be deposited there during foam cell necrosis (Stary 1987, + Haust 1971)."

5d. Some Current Wisdom on Equilibrium: Fry, Munro, Fuster

With respect to the equilibrium concept, the concept is fundamental to an interesting mathematical model presented by Donald L. Fry in "Mass Transport, Atherogenesis, and Risk" (Fry 1987). Fry's model allows him to "play" with various factors which are thought to affect the balance between ingress and egress of atherogens in the intimal wall: "Endothelial gap fractional area" (increased endothelial permeability), elevated blood pressure, elevated serum concentration of the atherogen, internal elastica fenestration, and pre-existing intimal thickening. It is worth noting that his model, like some earlier ones, predicts that an "extraordinary increase in the apparent diffusive permeability of the endothelial surface ... occurs with only slight opening of the endothelial junctions" (Fry 1987, p.93).

Munro and Cotran explicitly endorse the equilibrium concept with respect to cholesterol in the atherosclerotic lesions (Munro 1988, p.257): "Cholesterol accumulation in the plaque should be viewed as reflecting imbalance between influx and efflux ..." The equilibrium concept is also embraced by Valentin Fuster, for instance, in his Lewis A. Conner Memorial Lecture at the American Heart Association's 1993 National Meeting (Fuster 1994). Discussing the progression of atherosclerosis, from early lesions to the troublesome lesions, he states (Fuster 1994, p.2127):

"The potential for clinical problems, however, begins when the process continues. That is, if the influx of lipids and/or accumulation into the vessel wall continues and is more significant than their efflux, then the process evolves with a continuously slow progression of these lesions."

• Part 6. Evidence of Fewer Acute IHD Events after Lipid-Lowering

In contrast to chronic Ischemic Heart Disease of various degrees, the ACUTE syndromes or "events" are myocardial infarction (not always fatal), unstable angina, and ischemic sudden death.

The causal role of the low-density lipoproteins in the acute IHD events is apparently affirmed by recent demonstrations that dramatically fewer acute IHD events occur after various regimes which lower the serum levels of LDL cholesterol and total triglyceride. Several lipid-lowering trials are referenced in Box 4.

Such evidence has caused expert panels in the United States (ExpertPan 1993) and in Europe (TaskForce 1994) to recommend use of regimes to reduce serum levels of LDL cholesterol "as one of the fundamental preventive measures to reduce mortality from coronary heart disease" (Pedersen 1995, p.1350). In the same editorial of the New England Journal of Medicine, Pedersen adds (p.1351): "The benefits of reducing cholesterol are now established beyond any reasonable doubt." A year later, in a review article entitled "Advances in Coronary Angioplasty," John W. Bittl ends by recommending "intensive efforts to lower lipid levels" (Bittl 1996, p.1300).

But dissent remains. Box 4 includes some critics of the Lipid Hypothesis --- such as Uffe Ravnskov (1991, 1992, 1993, 1994, 1995-a, 1995-b), who suggests that the positive clinical benefits are due to some properties of the regimes OTHER THAN their lipid-lowering properties.

Of all the lipid-lowering studies in Box 4, we shall describe only three here. All three involve large randomized trials with a "statin" drug. Simvastatin and pravastatin are HMG-CoA reductase inhibitors (HMG-CoA: 3-hydroxy-3-methylglutaryl co-enzyme A).

6a. Scandinavian Simvastatin Survival Study (4S), 1994

The first is the "4S Study" --- the Scandinavian Simvastatin Survival Study, published in the Lancet (Scandinavian 1994).

In this randomized double-blind study, 2,223 patients were in the placebo group and 2,221 were in the simvastatin group (p.1384). The patients enrolled were patients (81% males; 51% age 60+) with angina pectoris or previous myocardial infarction and initial serum cholesterol levels in the range 5.5 to 8.0 mmol/L on a lipid-lowering diet. All the baseline characteristics of the two groups are shown in the paper's Table 1. The median follow-up period was 5.4 years. The primary endpoint of the study was total mortality. The secondary endpoint was major coronary events, "which comprised coronary deaths, definite or probable hospital-verified non-fatal acute MI [myocardial infarction], resuscitated cardiac arrest, and definite silent MI verified by electrocardiogram" (p.1384).

"Over the whole course of the study, in the simvastatin group, the mean changes from baseline in Total, LDL, and HDL cholesterol, and serum triglycerides, were -25%, -35%, +8%, and -10% respectively. The corresponding values in the placebo group were +1%, +1%, +1%, and +7% respectively" (p.1385).

Results (p.1385). The relative risk of death from any cause was 0.70 with simvastatin ($p = 0.0003$). "The relative risk of coronary death was 0.58 (95% confidence interval, 0.46 to 0.73) with simvastatin. This 42% reduction in the risk of coronary death accounts for the improvement in survival." And (p.1387): "With the exclusion of silent MI, the risk of coronary death plus nonfatal MI was reduced by 37% over the whole study, by 26% in the first 2 years, and by 46% thereafter." And (p.1388): "The improvement in survival produced by simvastatin was achieved without any suggestion of an increase in non-CHD mortality, including deaths due to violence and cancer, which have raised concern in some overviews of cholesterol-lowering trials (Oliver 1992, + Smith 1992, + Muldoon 1990, + Rossouw 1990, + Ravnskov 1992)."

6b. West of Scotland Coronary Prevention Study (WOSCOP), 1995

"WOSCOP" (the West Scotland Coronary Prevention Study, published in the New England Journal of Medicine) is entitled "Prevention of Coronary Heart Disease with Pravastatin in Men with Hypercholesterolemia" (Shepherd 1995).

In this randomized, double-blind study, 3,293 men were in the placebo group and 3,302 in the pravastatin group (p.1302). The patients enrolled were men (average age, 55 years) "with moderate hypercholesterolemia and no history of myocardial infarction" (p.1301). All the baseline characteristics of the two groups are shown in the paper's Table 1. The average follow-up period was 4.9 years. The primary endpoint of the study was the occurrence of nonfatal myocardial infarction or death from coronary heart disease as a first event (p.1302).

"When the data were analyzed according to the treatment actually received, pravastatin was found to have lowered plasma levels of cholesterol by 20 percent, LDL cholesterol by 26 percent, and

triglycerides by 12 percent, whereas HDL cholesterol was increased by 5 percent. There were no such changes with placebo" (p.1304).

Results (p.1303, Table 2). Pravastatin produced a 31% reduction in the risk of definite nonfatal myocardial infarction and CHD death (95% confidence interval, 17 to 43 percent; $p < 0.001$). For definite CHD death by itself, there was a 28% reduction (95% confidence interval of -10 to 52; $p = 0.13$). And (p.1306) "the benefit of pravastatin therapy with respect to fatal coronary events and the absence of any increase in the number of deaths from other causes led to a 22 percent reduction in the relative risk of death from any cause ($p = 0.051$)." The pravastatin group and placebo group showed no significant difference during the study in deaths from cancer, suicide, or trauma (p.1305). In their discussion, the authors also comment (Shepherd 1995, p.1306):

"The relative reductions in risk attributable to pravastatin therapy were not affected by age (<55 years vs. >= 55 years) or smoking status. Furthermore, a significant treatment effect was seen in the subgroup without multiple risk factors and the subgroup without pre-existing vascular disease. Thus, it is possible to conclude that in the subjects who might be considered to fall strictly into the primary prevention category, pravastatin therapy produced a significant reduction in the relative risk of a coronary event."

6c. Cholesterol and Recurrent Events Study (CARE), 1996

"CARE" (the Cholesterol And Recurrent Events Study, published in the New England Journal of Medicine) is entitled "Effect of Pravastatin on Coronary Events after Myocardial Infarction in Patients with Average Cholesterol Levels" (Sacks 1996).

In this study, 2,078 patients (290 females) were in the placebo group, and 2,081 (286 females) in the treatment group. The median follow-up time was 5 years. After treatment, comparison of the treated group with the placebo group showed decreases in the pravastatin group of 20% in total cholesterol, 28% in LDL cholesterol, 14% in triglycerides, and an increase of 5% in HDL cholesterol (Sacks 1996, p.1003). Coronary deaths were reduced 20% ($P = 0.10$), fatal plus nonfatal coronary events were reduced by 24% ($P = 0.003$), and fatal plus nonfatal strokes were reduced by 31% ($P = 0.03$) (from Sacks 1996, p.1003, Table 2). Hebert and co-workers (Hebert 1997, pp.318-319) suggest that the reduction in stroke may be due to the reduction in myocardial infarction, a risk-factor for stroke. The 4S study found a significant 28% reduction in stroke in the simvastatin-treated patients, too.

A Notable Result in the CARE Study: Excess Breast Cancer

Hebert and co-workers did a meta-analysis of lipid-lowering trials involving statin therapy ALONE (Hebert 1997). They looked at fatal cancers and total cancers in the 13 trials which reported these events, and found a relative risk in the treated groups combined of 0.95 for cancer deaths, and 1.03 for all cancer. The confidence intervals suggest probably no difference between treated and placebo groups (Hebert 1997, p.320, Table 8).

But notable is that, in the CARE Study, there were 12 cases of BREAST cancer (all nonfatal) in the pravastatin group, compared with only 1 case (a fatal recurrence) in the placebo group of equal size. Of the 12 cases in the pravastatin group, "3 occurred in patients who had previously had breast cancer, 1 was ductal carcinoma in situ, and 1 occurred in a patient who took pravastatin for only six weeks" (Sacks 1996, p.1006).

The authors write (Sacks 1996, p.1007): "In evaluating this finding, it should be noted that although there was one case of breast cancer among the women given placebo, five cases would have been expected on the basis of the rate of breast cancer in the general population for women of similar race and age." In other words, the small-numbers problem may account for much of the difference. We note that the same study showed an excess of colo-rectal cancer in the placebo group (21 cases) compared with the pravastatin group (12 cases). The authors also report (Sacks 1996, p.1007):

"Importantly, interim results of the Long-Term Intervention with Pravastatin in Ischemic Disease trial (LIPID 1995), from four years of treatment of 1,508 women, show no increase in breast cancer (Barter P., Safety and Data Monitoring Committee, LIPID Study; personal communication).

The totality of evidence suggests that these findings [excess breast cancer] in the CARE trial could be an anomaly ..." Let us hope so. Additional follow-up is imperative.

6d. Large Clinical Benefits, but Small Decrease in Stenosis

Today, with respect to acute IHD events, B. Greg Brown and colleagues have provided evidence that the large clinical benefits, from lowering the serum low-density lipoproteins, are associated with only minimal reduction of stenosis in the angiographically measured plaques. Brown et al analyzed results from nine lipid-lowering trials, all of which demonstrated a benefit from treatment, and conclude (Brown 1993, p.1784):

"As seen in Table 2, averaged estimates of disease severity per patient worsened (progressed) by about 3% stenosis among the control subjects, whereas they improved (regressed) by 1-2% stenosis among the treated patients. When these results are expressed in terms of absolute change in arterial narrowing, they appear to be remarkably small." In view of such evidence, various analysts reason that lipid-lowering must achieve its benefits by "stabilizing" atherosclerotic plaques (for example, Fuster 1994, p.2138, + Libby 1995, p.2849). Parmley comments (Parmley 1997, p.12):

"As one looks at all of the lipid-lowering studies where quantitative coronary arteriography has been used to judge efficacy, it is clear that there are only minor regressive changes in lipid lesions over a several year period. On the other hand, there is a dramatic reduction in clinical events, suggesting that somehow, lipid-lowering has stabilized the plaque so that it is not as easy to break down and cause unstable angina or acute myocardial infarction."

The recent observations, of major health benefits from only minor reductions in degree of stenosis, resemble a prediction we made in 1969 about Coronary Heart Disease: "A marked drop in CHD risk and incidence might be achieved if degree of coronary atherosclerosis is kept to values just below the steeply rising portion of the CHD risk curve" (Gofman 1969, p.38). Our view was based on our analysis of preliminary results from the International Atherosclerosis Project (Strong 1966), from which we had concluded (Gofman 1969, pp.37-38) that the curve --- relating risk of Coronary Heart Disease with extent of coronary atherosclerosis --- must be sigmoid rather than linear (x = degree of atherosclerosis, y = risk of IHD).

While the analysis in 1969 correctly predicted large benefits from only small reductions in degree of atherosclerosis, we did not imagine then where some of the explanation might lie --- a topic discussed in Part 7.

• Part 7. Plaque Rupture: Proximal Cause of a Large Share of IHD Deaths

Today, many leading figures in IHD research regard thrombus formation DUE TO PLAQUE RUPTURE OR FISSURE as the proximal cause of an important share of acute IHD events, including death. See, for instance: Davies 1985, p.364, p.370, + Brown 1993, p.1785, p.1787, + Nicod 1993, p.1749, + Blankenhorn 1994, p.178, + Fuster 1994, p.2129, + Libby 1995, p.2848, + Parmley 1997, p.12, + Burke 1999, Table 1, p.922. Plaque erosion, instead of rupture, may characterize sudden cardiac death in premenopausal women (Burke 1998). In addition, there are investigators who regard plaque ruptures which are nonfatal --- and which are followed by fibrotic organization of the rupture-induced thrombus and its incorporation as part of the plaque --- as the explanation for the sometimes very rapid and unpredictable growth, angiographically, of specific plaques. (See, for instance: Davies 1985, p.366, + Brown 1993, p.1785, + Fuster 1994, p.2126, p.2127, 2128, 2132, + Libby 1995, p.2844).

Awareness of plaque rupture, fissure, ulceration, and erosion, is not new, and papers by both Davies 1985 and Fuster 1994 include fine historical context.

Below, we begin by quoting from a particularly lucid presentation by Peter Libby, M.D., on the important role of plaque rupture in the acute IHD events.

7a. Reassessment of a "Central Dogma in Clinical Cardiology": Severe Stenosis

"From Bench to Bedside" is the mini-title for the paper entitled "Molecular Bases of the Acute

Coronary Syndromes," by Peter Libby, M.D., of the Vascular Medicine and Atherosclerosis Unit, Department of Medicine, at Boston's Brigham and Women's Hospital (Libby 1995). Near the outset, Libby states (Libby 1995, p.2844):

"Much of the basis of contemporary cardiology and cardiac surgery rests on the axiom: the greater the stenosis, the greater the risk of a clinical event such as myocardial infarction or unstable angina pectoris. However, data emerging from clinical and pathological studies over the past decade have occasioned a reassessment of this central dogma of clinical cardiology (Fuster 1994)." And (Libby 1995, p.2844):

"First, the use of thrombolytic therapy in acute myocardial infarction became widespread in the wake of the GISSI study in 1986 (Gruppo 1986). Angiographic studies performed after thrombolysis during acute myocardial infarction led to the surprising finding that the atherosclerotic lesions that gave rise to the occlusive thrombus did not cause high-grade stenoses in many cases. Assessment of angiograms obtained before acute myocardial infarctions and those obtained during the infarction corroborated this concept that the lesions most likely to precipitate an infarct-provoking thrombosis often did not appear highly stenotic by angiography (Hackett 1988, + Ambrose 1988, + Nobuyoshi 1991, + Giroud 1992)." And (Libby 1995, p.2844):

"Another line of clinical evidence suggested dissociation between the degree of stenosis and coronary events. In the past decade, a number of 'regression trials' tested the hypothesis that lipid-lowering regimens would reduce the degree of high-grade coronary stenoses. The angiographic results showed disappointingly minimal effects on established stenotic lesions. Yet these studies revealed a consistent and resounding decrease in acute clinical coronary events (Blankenhorn 1994, + Brown 1993)." And (Libby 1995, p.2844):

"Meanwhile, state-of-the-art pathological studies using perfusion fixation of freshly obtained material provided new evidence buttressing the concept that rupture of atherosclerotic plaques precipitates the formation of the occluding thrombus that causes acute myocardial infarction (Davies 1985). The elegant pathological studies of Davies and colleagues (Davies 1985 + 1990) also sought evidence for plaque disruption in hearts from patients dying of noncardiac causes. They documented evidence for plaque disruption in these patients even without overt symptoms of coronary disease or acute myocardial infarction. These results suggested that not all disruptions of atherosclerotic plaques lead to clinically apparent or symptomatic events. Such subclinical episodes of plaque disruption with local thrombin activation and subsequent healing may indeed represent a major pathway for progression of atherosclerotic lesions."

And Libby's Key Summary (Libby 1995, p.2844-45)

"Taken together, these new results suggest that while angiographically severe coronary artery disease clearly correlates with the propensity to develop or succumb from acute myocardial infarction, the presence of the severe stenoses may merely serve as a marker for the presence of angiographically modest or even inapparent, non-critically stenotic plaques more prone to precipitate acute myocardial infarction." And at this point, Libby reminds readers that plaques grow away from the lumen for a long time before they encroach on the lumen itself (see Chapter 43, Part 3e).

The evidence seems convincing that acute IHD events are most often precipitated by plaques associated (not long before the event) with mild to moderate degrees of stenosis, rather than with severe degrees of stenosis. (Some details from Brown 1993 are provided in Chapter 46, Part 3.) Among the figures who either generate or embrace such evidence are Ambrose 1986, 1988, + Brown 1986, 1993, + Little 1988, + Blankenhorn 1994, + Fuster 1994, + Libby 1995, + Bittl 1996, + Parmley 1997.

It is worth noting that the relationship of stenotic degree with acute IHD events is not identical with the issue of plaque rupture as the proximal cause of acute events (Parts 7b and 7c).

7b. "Thrombogenic Lipid-Cores" as Catastrophes-in-Waiting

Libby (1995, p.2845) reminds readers that the lipid-imbibing foam cells in the core of the "typical [atherosclerotic] lesion" can "produce large amounts of tissue factor, a powerful pro-coagulant

that potently stimulates thrombus formation when in contact with blood (Wilcox 1989)." Fuster emphatically agrees (Fuster 1994, p.2133):

"Fernandez-Ortiz et al (1994) studied the thrombogenicity of various human atherosclerotic plaques, including fatty streaks (lesions types II and III), atheromatous plaques or lipid-rich plaques with abundant cholesterol crystals (lesions types IV and Va), and fibrotic plaques with collagen-rich matrix (lesions types Vb and Vc). The lipid core exposed in atheromatous lipid-rich plaques was the most thrombogenic, with thrombus formation fourfold to sixfold greater than that on all other substrates."

7c. Crucial Role of a Plaque's Fibrous Cap

Libby continues his exposition by explaining the crucial role of a plaque's fibrous cap (Libby 1995, p.2845):

"... the integrity of the fibrous cap overlying this lipid-rich core fundamentally determines the stability of an atherosclerotic plaque. Rupture-prone plaques tend to have thin, friable fibrous caps (Richardson 1989, + Loree 1992). Plaques not liable to precipitate acute myocardial events tend to have thicker caps that protect the blood compartment in the arterial lumen from potentially disastrous contact with the underlying thrombogenic lipid core ..." And (Libby 1995, p.2845):

"Biomechanical analyses demonstrate maxima of circumferential stress at sites of plaques prone to rupture (Richardson 1989, + Cheng 1993). Thus, mechanical forces concentrate on the fibrous cap, which must resist these high stresses to avoid rupture and the attendant risk of developing an acute coronary event. This stress-laden fibrous cap is all that stands between the blood and the thrombogenic lipid core of the lesion."

B. Greg Brown and colleagues report (1993, p.1789): "Fissuring is predicted by a large accumulation of core lipid in the plaque and by a high density of lipid-laden macrophages in its thinned fibrous cap. Lesions with these characteristics constitute only 10-20% of the overall lesion population but account for 80-90% of the acute clinical events."

According to Celermajer (1998, p.2014), Magnetic Resonance Imaging (MRI) may hold promise --- despite cardiac and respiratory motion --- for in vivo visualization of the fibrous cap, core, and other features which might permit identification of the rupture-prone plaques in coronary arteries, prior to rupture.

7d. Massive Shift in Thinking about Smooth Muscle Cells in Acute Events

We credit Libby with helping to create a massive shift in our own thinking --- and probably in the thinking of others --- about the role of smooth muscle cells in the acute and deadly events of IHD.

For instance, in his paper, "Molecular Bases of the Acute Coronary Syndromes," Libby identifies a molecule (interferon-gamma) which can inhibit smooth muscle cells in plaque from producing collagen. After describing several lines of evidence, Libby states (Libby 1995, p.2846):

"Taken together, these results concordantly suggest that chronic immune stimulation within atheroma leads to elaboration of interferon-gamma from T cells, inhibiting collagen synthesis in vulnerable regions of the plaque's fibrous cap. This mechanism provides a molecular explanation for impaired maintenance and repair of the collagenous meshwork in vulnerable plaques, rendering it [meshwork] weak and prone to rupture in the critical region of the plaque. Intact ability to synthesize collagen may sustain the ability of the fibrous cap to resist the concentration of mechanical forces in STABLE plaques." (Emphasis added.)

Libby's T-cell explanation for plaque rupture seems reasonable, but incomplete. Chronic immune stimulation and T cells are typically present in plaques, but only some plaques rupture. On the other hand, some contribution by T-cells to plaque rupture seems reasonable. We continue quoting Libby on another very important point (Libby 1995, pp.2846-2847):

"Curiously, proliferation of smooth muscle cells has dominated our thinking about the pathogenesis of atherosclerosis for decades. Smooth muscle cell growth may indeed contribute

importantly to earlier phases of lesion development. Yet the present data, summarized above, suggested that the aspects of the biology of atheroma that actually lead to ACUTE clinical manifestations, depend on IMPAIRED smooth muscle cell growth and matrix elaboration, rather than the contrary." (Emphasis added.) And Libby warns (Libby 1995, p.2847):

"This concept warrants consideration by those embarking on therapeutic quests seeking inhibitors of smooth muscle cell proliferation as treatments for atherosclerosis, on the basis of relatively short-term experiments using simple animal models. Inhibition of smooth muscle cell proliferation in human patients might produce the undesired effect of destabilizing vulnerable regions of atherosclerotic plaques by the mechanisms described above."

7e. Plaque Rupture and Exercise: "Black's Crack in the Plaque"

In 1997, the New England Journal of Medicine carried an important exchange entitled "More on Coronary-Plaque Rupture Triggered by Snow Shoveling," in the correspondence section. Both paragraphs by Paul D. Thompson merit presentation here (Thompson 1997, p.1678):

"Hammoudeh and Haft (Hammoudeh 1996) report on 15 patients in whom acute coronary syndromes developed during or immediately after shoveling snow. They suggest that evidence of rupture of coronary plaque and acute thrombosis associated with physical exertion has not previously been observed in living patients. On the contrary, Ciampricotti et al (1989), using coronary angiography, found irregular coronary lesions 'consistent with' plaque rupture in 8 of 13 patients who had myocardial infarction or cardiac arrest within an hour of vigorous athletic activity." And (Thompson 1997, p.1678):

"Furthermore, in 1975 Black et al (Black 1975) reported autopsy, angiographic, or clinical evidence of acute plaque rupture in 13 patients with acute coronary syndromes related to vigorous exertion. In what now reads like a very clairvoyant discussion, Black et al suggested that the increased 'twisting and bending' of coronary arteries during vigorous exertion increased the frequency of plaque rupture and that 'Black's crack in the plaque' was responsible for most exertion-related acute coronary events. Once again, we are reminded that what seems new in medicine is often a rediscovery of what we knew, but forgot."

Haft and Hammoudeh, in reply (Haft 1997, pp.1678-79), begin with a recommendation: "Men and women in the age group that places them at increased risk of coronary events should be warned against shoveling snow ..." They also write that the concept of plaque rupture as a proximal cause of myocardial infarction "has been with us for some time (Daoud 1963), but its importance and frequency have been appreciated only since modern high-resolution coronary arteriography became available (Ambrose 1985)." Haft and Hammoudeh end with a comment (p.1679): "As with many pathophysiologic phenomena that originally appear to be rare, plaque rupture is emerging as the common mechanism in triggered myocardial infarction and other acute coronary events."

Others might not go that far. The frequency of fatal plaque rupture seems far lower at rest than after exercise (Burke 1999, p.922). In a New England Journal of Medicine editorial, Attilio Maseri (Maseri 1997, p.1015) states his view that fissuring of plaque is one of possibly "multiple causes" of acute ischemic events: "The search for the multiple pathogenic components of acute ischemia is a major challenge."

Meanwhile (during the search for additional proximal causes), we shall accept the existing evidence that disruption of atherosclerotic plaque explains some large share --- not yet quantified --- of deaths from Ischemic Heart Disease.

7f. Coming Full Circle: Lipids from Start to Finish?

According to the American Heart Association (1995, p.9), "In 48 percent of men and 63 percent of women who died suddenly of coronary heart disease, there were no previous symptoms of this disease."

This is consistent with the fact that atherosclerotic plaques can already be large before they ever intrude into the lumen, and with the observations described above (Part 7a) that the lesions most likely to precipitate an infarct-provoking thrombus often do not appear highly stenotic by angiography.

Libby's paper expounds extremely well about several molecules which could weaken the fibrous cap of a plaque --- any plaque. At the end, Libby comes to the ultimate issue: How can the risk of acute myocardial infarction be reduced? Citing the review paper by MacIsaac et al (1993), Libby writes (p.2849): "To reduce the risk of acute myocardial infarction, one must stabilize lesions to prevent their disruption, particularly the less stenotic plaques." Then Libby asks (Libby 1995, p.2849):

"How might one achieve such a goal? The results of recent lipid-lowering trials provide a hint. The reduction in clinical events without substantial change in the degree of luminal stenosis could reflect a stabilization of the non-critically stenotic lesions (Blankenhorn 1994, + Brown 1993). This stabilization might result from reducing the inflammatory stimuli provided by modified lipoproteins that could contribute to activation of lesional foam cells and T lymphocytes ..." (See also Buja 1994, + DeLorgeril 1994-b, + Fuster 1994, + Moreno 1994, + Van de Wal 1994.)

And so, we "come full circle," with the blood lipoproteins having a key causal role at the beginning, middle, and thrombogenic end of the Ischemic Heart Disease story. Therefore, if Hypothesis-2 is also valid, it must be consistent with the lipoprotein facts.

• Part 8. Introduction of a Tumor Hypothesis by the Benditts in 1973

Earl P. Benditt makes a very important statement near the outset of the same paper from which we quoted in Chapter 43, Part 3a. He states (Benditt 1976, p.96):

"Frequently, we tend to confuse the lesions induced in animals with the real lesion. Our ultimate goal is the answer to the question, 'What is the nature and origin of HUMAN atherosclerosis?' Because the lesion has a mass of cells as a major feature, we can rephrase the question as follows: What is the nature of the cellular proliferation involved in the 'new formation,' the atherosclerotic plaque?" And (Benditt 1976, p.97):

"Specifically, we can ask, 'Is it of multicellular or of monoclonal origin?' The importance of this distinction lies in the fact that many neoplasms have been found to be of monoclonal origin. [A clone is a group of genetically identical cells descended from the same progenitor cell.] On the other hand, ordinary cell proliferations seen in embryogenesis, maintenance, and repair seem to be multicellular in character." And (Benditt 1976, p.97):

"It becomes immediately apparent, when one asks the question in the form indicated, that on the answer depends the direction of our search for factors that are responsible for the disease. Phrased this way, the question takes on a new significance, because there is now a basis for obtaining an answer, and the methods involved are applicable to human tissues and to human lesions."

8a. Female Mosaicism: The Basis of Benditt's Method

Benditt summarized the method as follows (Benditt 1976, p.97):

"The method of analysis, applicable to study of proliferated masses of cells and for distinguishing the origin of these cells from one or from many precursor cells, requires individual organisms that are mosaics, that is, that comprise a mixture of two or more distinctive cell types. According to the concept of Lyon (1968), all human females are mosaics, composed of two phenotypically distinct cell types. This situation is due to the fact that early in embryonic development, there is a random inactivation in each somatic cell of one or the other of the two X-chromosomes." And (Benditt 1976, p.97):

"Once inactivation has occurred, each cell reproduces true to type, and all daughter cells of a particular cell exhibit the activity of the single same X-chromosomal genes. The stability of this state has been shown by cultivating single cells from connective tissues of human mosaic donors (Davidson 1963). Given a stable mosaic population, it is possible to ask questions with regard to cell population origins in embryogenesis and as to whether a pathologic new formation is derived from one or from many cells." And (Benditt 1976, p.97):

"The enzyme glucose 6-phosphate dehydrogenase (G-6-PD) is a polymorphic X-linked gene product. Particularly interesting ... are the common B form and the A form, which migrates more rapidly in an electric field. The A form of G-6-PD is present in a substantial proportion of the Black

population. In our series, 40% of 115 cases of Black females are heterozygous, and their tissues comprise two cell types, each of which produces one or the other enzyme form (Beutler 1962). This property of females has been used to assess the origin of cell populations in several tumors (Fialkow 1974), including benign smooth muscle tumors [called leiomyoma] of the uterus (Linder 1965)."

8b. Findings of Benditt's Team and Pearson's Team

Benditt's first series (Benditt and Benditt 1973) involved three women, from whom 30 atherosclerotic plaques from the aorta were analyzed (table on Benditts' p.1754). In addition, the paper presented data on 59 samples of uninvolved (healthy) aorta. Writing in 1976, Benditt comments (p.97) that the plaques "revealed the startling fact that most discrete plaques appear to be monoclonal; that is, they comprise a monotypic cell population ... Pearson et al (1975) have recently published data on material examined in a similar way, and report almost the identical result." The Benditts' work was in the Department of Pathology at the University of Washington School of Medicine, and Pearson's team was in the Department of Pathology at the Johns Hopkins University School of Medicine.

Benditt presents his own results and Pearson's 1975 results in his Table 1 (Benditt 1976, p.97), which we reproduce nearby. Samples in the AB column have both cell-types present. Samples in the A column are monotypic; they have A-producing cells, but not B-producers. Samples in the B column are also monotypic; they have B-producing cells, but not A-producers.

Table 1 from Benditt 1976, p.97: "G-6-PD Phenotypes of Cell Populations of Human Atherosclerotic Plaques Compared to NonPlaque Artery Samples."

	<--- NON-PLAQUES --->				<--- PLAQUES --->			
	AB	A	B	Total	AB	A	B	Total
Benditt: 4 cases	57	0	2	59	6	8	16	30
Pearson 16 cases	99	1	1	101	3	17	9	29
Total	156	1	3	160	9	25	25	59

The difference is dramatic, between non-plaque and plaque samples. Only 2.5 % of non-plaque samples are monotypic (4 out of 160). But 85 % of the plaque samples are monotypic (50 out of 59). Monotypic A-lesions and monotypic-B lesions occur separately within a single vessel (Benditt 1976, p.98). And (Benditt 1976, pp.97-98):

"The fact that the bulk of raised atherosclerotic lesions are monotypic with regard to cell type, as indicated by isoenzyme pattern, does not immediately yield the strong inference that lesions of atherosclerosis are monoclonal." Benditt evaluates three possibilities, other than monoclonality, which might explain the observed difference in his Table 1 between healthy samples and atherosclerotic samples. He concludes (Benditt 1976, p.99):

"When all of the data are considered together, the reasons are strong for believing that raised lesions are monoclonal."

8c. Twelve Years Later: Benditt's View on Monoclonality in 1988

Benditt did not change his mind. In 1988, he wrote a paper entitled, "Origins of Human Atherosclerotic Plaques," in which he discusses monoclonality again. With reference to his own findings (1973, 1974), he says (Benditt 1988, p.998) that "Two other laboratories have reported essentially the same findings," and he cites Pearson 1977 and Thomas 1979. Benditt discusses Thomas's dissenting interpretation (Thomas 1983), cell-proliferation in plaque, and Ross's

hypothesis (Part 2) that platelet-derived growth factor plays a key role in such cell proliferation. And (Benditt 1988, p.999):

"The role of oncogenes and related growth factors has surged to the forefront of research in neoplasia, proliferation of cells in atherosclerotic plaques, and embryologic development. Platelet-derived growth factor (PDGF) has been proposed by Ross (Ross 1986) to be a key element in inducing proliferation in atherosclerotic lesions ... The clonality of the atherosclerotic lesions raises the possibility that there is a change in the pattern of growth factor or oncogene expression characteristic of the smooth-muscle cell populations of plaques. We [his group at the University of Washington] have been examining this possibility." At the end of his paper, Benditt refers to "benign smooth muscle tumor" and he concludes (Benditt 1988, pp.999-1000):

"... while it is quite reasonable to look for alternatives to the inference of monoclonality, no substantiated alternative has appeared ... At the moment, our belief is that abnormal expression of these growth-related genes does not account for the abnormal cell growth in either the benign smooth muscle tumor or the plaque cells."

8d. Benditt on Potential CAUSES of Arterial Tumors: Silence on Radiation

In Benditt's 1976 paper (p.99), he speculates about various "factors" which may be "the causes of monoclonal proliferation." He says, "Prominent among these factors is the role of chemical mutagens (or premutagens) derived from the environment. Another possibility is the role of viral agents, such as that which causes the common wart, a monoclonal lesion." Benditt properly regards cigarette smoking as an environmental factor, meaning an exogenous factor. He continues (Benditt 1976, pp.99-100):

"Cigarette smoking is well established as a 'risk factor' [for IHD]. Burning of cigarettes produces aryl hydrocarbons, and some of these (benzo[a]pyrene) hydrocarbons are well-known precarcinogens. It is easy to believe that hydrocarbons from cigarette smoke affect the lung; can they also affect distant arteries? ... Aryl hydrocarbon hydroxylase, an inducible enzyme system, is elevated enormously in the placentas of women who are heavy smokers and to an intermediate extent in women of average smoking habits, when compared with enzyme levels in nonsmokers (Juchau 1974). Clearly, the route must be from the lung via the blood to the placenta." And (Benditt 1976, p.100):

"In addition to this finding, it has been shown that low-density lipoproteins are the main carriers of benzo[a]pyrene in the bloodstream (Margolis 1969). Coupling this with the now established fact (Bierman 1976) that low-density lipoproteins are preferentially taken up by smooth muscle cells derived from the arterial wall, we then have a system entirely compatible with the initiation of cellular alteration that leads to monoclonal growth."

Benditt's comments on smoking are fully compatible with the second part of Hypothesis-2 --- that medical radiation induces mutations in cells of the arterial wall. With respect not only to cancer but also to monoclonal mini-tumors in coronary arteries, medical xrays and chemical carcinogens can work as co-actors. Indeed, it is very likely that no single mutation in a somatic cell is clinically potent as a tumor-maker unless it receives "help" at some time from additional mutations and promoting agents in the same cell.

In 1976, the Benditt paper is silent about ionizing radiation as a mutagen. It mentions only chemicals and viral agents. By contrast, near the end of a paper in 1985, Benditt and colleagues write: "The origin of certain atheromata may be linked to the presence of chemical and physical mutagens and viruses in our diet or environment" (Majesky 1985, p.3453). Ionizing radiation is a "physical mutagen," of course. In 1988, ionizing radiation is mentioned neither indirectly nor by name, when Benditt and colleagues state that "chemical mutagens or viruses" are potential causes of the monoclonal plaque cells (Benditt 1988, p.999).

An undisputed fact deserves repeating: Ionizing radiation is a potent mutagen (Chapter 42, Part 2).

8e. Some of the Additional Contributions by Pearson et al, 1978 and 1983

In 1978, Pearson and colleagues contributed additional evidence of monoclonality in

atherosclerotic plaques (Pearson 1978-b). At the outset (pp.93-94), they explain that, in heterozygous women: "Normal tissue will consist of a mosaic of patches of cells expressing the same isoenzyme [either A-type or B-type]. However, these patches are so minute that the assay of even very small bits of normal tissue will yield both isoenzymes. Monoclonal lesions, on the other hand, will contain only one isoenzyme type in the heterozygous female (Linder 1965, + Fialkow 1974)."

The new evidence in Pearson 1978-b comes from 10 aortic plaques (from 6 deceased heterozygous women), which Pearson and co-workers divided into 45 portions. In this inquiry, they compared the upper and lower layers of the plaques. Pearson et al found (Pearson 1978-b, pp.96-97):

- 15 (33%) were monoclonal in both layers.
- 9 (20%) were monoclonal in one layer (upper).
- 9 (20%) were monoclonal in the other layer (lower).
- 12 (27%) were not monoclonal in either layer.

Pearson and colleagues state: "The monoclonal nature of atherosclerotic fibrous plaques has been demonstrated by independent investigators with unequivocal results (Benditt 1973, + Pearson 1975, + Pearson 1977). Although the laboratory observations have been firmly established, a variety of interpretations have been made as to the mechanism by which monoclonal populations arise" (Pearson 1978-b, p.99, followed by their discussion).

In 1983, Thomas and Kim state (Thomas 1983, p.247) that the Benditts' observations "have been amply confirmed by Pearson et al (1975, 1978) in Baltimore and by Thomas et al (1979) in Albany ... However, Thomas et al ... have serious doubts regarding the validity of the monoclonal theory of origin of atherosclerotic lesions." And (Thomas 1983, p.247): "To date, we have studied more than 600 lesions [from 44 aortas]. Normal aortic tissue from these aortas showed both A and B forms of G-6-PD (ditypism). Among 25 of these aortas, one or more samples of atherosclerotic lesions showed a single G-6-PD type (monotypism). Among the 469 lesions sampled in these aortas, 160 [34%] yielded at least one monotypic sample. All remaining lesions (440 of the original 600) yielded only ditypic samples. Furthermore, when multiple samples were taken from the lesions with one or more monotypic samples, ditypic samples were also obtained, frequently in greater numbers than monotypic samples."

Monoclonal Characteristics of Fatty Streaks and Thickened Intima

In 1983, Pearson and co-workers published a study in which they looked for monoclonality in human samples of NON-plaque segments of normal aortic media (237 samples), thickened intima (133 samples), and fatty streaks (58 samples) --- all from 13 heterozygous black females. The observed frequency of monoclonality in these NON-plaque samples of (Pearson 1983-a, p.36):

In media, zero; in thickened intima, 2 out of 133 (1.5%); in fatty streaks, 1 out of 58 (1.7%). All of these frequencies are dramatically distinct from the frequency found in atherosclerotic plaques (Parts 8b and directly above). An interesting observation is that Pearson et al found the distribution of isoenzyme values from fatty streaks to be "markedly different" from the distribution in the thickened intima and in media --- as measured by "percent B Isoenzyme."

8f. An Excellent Review-Paper in 1990

In 1990, Mutation Research presented a fine review by Arthur Penn of the Benditts' "monoclonal hypothesis." Entitled "Mutational Events in the Etiology of Arteriosclerotic Plaques," Penn's paper begins (Penn 1990, p.149): "In this review, evidence is provided in support of the 'monoclonal' hypothesis of arteriosclerotic plaque formation. Experimental and clinical data, collected over the last 16 years, are presented that are consistent with the view that environmental mutagens, including viruses and chemical carcinogens, play a key role in plaque etiology."

We shall return to some of Penn's own work in Part 9c of this chapter. As indicated above, he, too, overlooks ionizing radiation as a proven mutagen to which people are commonly exposed.

• Part 9. Other Thinkers about Benditt's Tumor Hypothesis

The Benditts' proposal, of monoclonality and a tumor etiology for the process of atherosclerosis, came in June 1973. In July 1975, Martell linked (a) the Benditt tumor hypothesis, (b) the clear tumor-producing power of alpha-particle ionizing radiation, and (c) the evidence that smoking is a cause of coronary heart disease, to produce his own hypothesis about a cause of atherosclerosis.

9a. Martell's Hypothesis on a Cause of Atherogenesis

Martell states (Martell 1975, p.404):

"Alpha interactions with chromosomes of cells surrounding insoluble radioactive smoke particles may cause cancer and contribute to early atherosclerosis development in cigarette smokers."

There is no doubt whatsoever that cigarette smoking is a cause of coronary heart disease. In 1975, Martell is proposing WHY it is. He is proposing that the alpha-particle emitters in mainstream smoke eventually get to the arterial wall through the blood and lymph stream, after their initial deposition in the lungs.

It is clear, thanks to the work of Martell and others, that insoluble particles of radioactive lead-210 are present in cigarette smoke (our Chapter 48, Part 1c). Lead-210 (Pb-210) is a member of the uranium decay-series, and has a radioactive half-life of about 20 years. Lead-210 is a beta-emitting radio-nuclide which decays into radioactive bismuth-210, which is another beta-emitting radionuclide with a radioactive half-life of about 5 days. Bismuth-210 decays into polonium-210, which is an alpha-emitting radio-nuclide with a half-life of about 138 days. And finally, polonium-210 decays into stable lead-206. Lead-210 atoms retained in the body will continuously convert in this way to polonium-210 atoms, which are the alpha-emitters discussed by Martell (references in Chapter 48, Part 1c).

Martell cites the papers of Arthur Elkeles (1961, 1966, 1968) in support of his view that alpha particles are present at unusually high concentration in atherosclerotic plaques (Martell 1975, pp.409-410):

"The possibility that alpha radiation may be the mutagenic agent in atherosclerosis plaque formation is indicated by the results of Elkeles, who found anomalously high concentrations of alpha activity at the calcified plaque sites of atherosclerosis victims ... The high incidence of early coronaries among cigarette smokers may conceivably be explained by the accumulation of insoluble radioactive smoke particles at the plaque sites. Such a possibility should be experimentally evaluated."

Our View of the Martell Hypothesis --- and of Martell

We have no independent reason to question the observations by Elkeles, but we should point out that they may have an alternative interpretation.

Calcification is a process which can be contaminated with ions other than Ca++ within the insoluble crystalline material of the calcified lesion. So it is possible that lead-210, retained in the body from cigarette smoke, is co-precipitating in arterial lesions which are calcifying --- but this may not be evidence in favor of the polonium-210 as a CAUSE of arterial tumors. Unless there is contrary evidence of which we are unaware, we entertain the possibility that arrival of the lead-210 in the plaque, and the subsequent production there of extra alpha activity, may be a late and incidental event. Calcification, which often occurs in tubercular and syphilitic lesions too (not only in atherosclerotic lesions), and in breast lesions, is one of the body's common RESPONSES to a menacing lesion, rather than the lesion's cause.

On the other hand, Martell may have been on the right track. Alpha particles may well be one of the mutagens which INITIATE multiple mini-tumors in the coronary arterial walls. We think the idea deserves further attention.

Martell was a superb radio-chemist and fearless original thinker. His untimely death in July 1995 is a great loss for science and humanity. During his last year of life, I know from personal communications with him that he was absolutely convinced that alpha-emitters play an important role in the genesis of atherosclerosis.

9b. Trosko and Chang on the Role of Mutagens in Atherosclerosis and Cancer

In a 1980 issue of Medical Hypotheses, J.E. Trosko and C-c. Chang took note of Benditt's monoclonal hypothesis of cell-proliferation in human atherosclerosis, in their paper "An Integrative Hypothesis Linking Cancer, Diabetes and Atherosclerosis: The Role of Mutations and Epigenetic

Changes" (Trosko 1980, at p.456 and p.460). Unlike Benditt (1973, 1976, 1988), Trosko and Chang mention "radiations" as as one of the potential mutagens. Indeed, "radiations" are included even in the abstract (Trosko 1980, p.455):

"It appears that the disease states of cancer, atherosclerosis and diabetes might share a common etiology. These chronic diseases appear to be multi-staged in their progression, with genetic, nutritional, psycho-social, environmental and viral factors influencing their appearance. We offer a hypothesis (a "mutation theory of disease"), stating that these diseases can be described by initiation and promotion phases; initiation being the result of the production of mutated cells after unrepaired damaged DNA is replicated; promotion being the selective proliferation of the initiated cells to form clones of mutated cells. It is further postulated that promotion affects cell proliferation by altering a membrane-Ca++ regulatory system. Depending on the nature of the mutation in the clone of cells, specific disease states would result. The roles of radiations, chemicals, viruses, genes, nutrition and psycho-social stress are related to either the initiation (mutation production) or the promotion (cell proliferation) phase of these diseases."

Trosko and Chang hold the view that "mutagenesis is a necessary, but insufficient, step" for the pathogenesis of atherosclerosis (Trosko 1980, p.460). They repeatedly stress the need for an outside stimulus (promoter) to cause a mutated cell to divide, which of course it must do, if it is going to form a clone or tumor.

Like Benditt, Martell, and some others, Trosko and Chang deserve much credit for proposing --- long ago --- the likelihood that mutagens have a key role in the pathogenesis of atherosclerosis.

9c. Penn on Experimental Evidence for Benditt's Tumor Hypothesis

Like Martell, and Trosko, and Chang, Arthur Penn has appreciated the importance of the Benditts' monoclonal hypothesis, discussed in Part 8 above. Looking back, Penn writes in 1989 (p.190):

"An early prediction that arose from the monoclonal hypothesis was that viruses and chemical mutagens would be expected to play critical roles in plaque formation and development, just as they do in tumorigenesis. The earliest attempt at verifying this prediction came from Roy Albert and his co-workers at New York University's Department of Environmental Medicine. Weekly injections of the polycyclic aromatic hydrocarbon (PAH) carcinogens 7,12-dimethylbenz[a]anthracene (DMBA) or benzo[a]pyrene (BaP) resulted in large, proliferating plaques in the abdominal aorta in cockerels (Albert 1977). Plaques increased in size in a dose-dependent fashion (Penn 1981-a, with Bastatini + Albert) ..."

As techniques of molecular biology advanced, Penn and co-workers returned to exploring the tumor hypothesis in various ways. Among their many interesting findings, Penn and co-workers have shown (a) that DNA from the coronary-artery plaques of some patients can transform NIH 3T3 fibroblasts, which thereby acquire the power to produce tumors in nude mice (Penn 1986; plus similar findings based on arterial plaque from experimental animals, Penn 1989 + Penn 1991), and (b) that injection of experimental animals, with a variety of established chemical carcinogens and mutagens, promotes expansion of arterial plaques in such animals (Penn 1981-a + Penn 1988), and (c) that inhalation of cigarette smoke by cockerels accelerates development of arteriosclerotic plaques (Penn 1993, 1994, 1996).

Penn 1990 on Compatibility of Monoclonal and Injury Hypotheses

Penn et al have not been the only ones producing experimental evidence pertinent to the tumor hypothesis in development of arterial plaques. Benditt's group at the University of Washington, and others (for example, Yew 1989 + Ahmed 1990), also have done some work --- work which is referenced in Penn's various papers. Here, we limit ourselves to abbreviating Penn's ideas about the COMPATIBILITY of the "response to injury" and the "tumor" hypotheses of cell proliferation in human coronary-artery plaque. Penn writes in Mutation Research (Penn 1990, p.158):

"Although the 'injury' and 'monoclonal' hypotheses are generally regarded as being mutually exclusive, the available evidence points to possible roles for both injury and transformation in plaque etiology. The 'synthesis' ... can be summarized as follows: The primary events in plaque etiology

involve DNA damage and its fixation. Elaboration of this damage in a clinically or experimentally significant way may require injury to the arterial wall." And later (Penn 1990, p.160):

"As reviewed earlier in this article, there is abundant evidence that injury to the arterial wall can stimulate smooth-muscle cell [SMC] proliferation ... If genetically altered SMCs are the progenitor cells of plaques, if these cells are distributed randomly throughout the artery wall, as seems reasonable, and if injury plays a role in plaque formation, then the focal nature of plaques points to localized injury as a possible stimulus for the proliferation of the already transformed SMCs."

9d. The Benditts' Monoclonal Hypothesis: Still Alive and Respected

The Benditts' monoclonal tumor hypothesis has been neither discredited nor widely explored yet. In 1988, Munro and Cotran singled out the hypothesis as meriting respectful attention (Munro 1988, p.255). Indeed, the monoclonal hypothesis appears to have inspired recent work at the National Institutes of Health by Speir et al (Speir 1994). However, the work reported in Speir 1994 turned out to be no test whatsoever of the Benditts' monoclonal tumor hypothesis (details in Marx 1994, p.320).

• Part 10. Homocysteine as a Risk-Factor for Cardiovascular Mortality

The idea, that elevated blood-levels of homocysteine (an amino acid) may be causally involved in cardiovascular mortality, is credited by Nygard et al (Nygard 1997) to K.S. McCully (McCully 1969). The idea arose because premature vascular disease is characteristic of individuals with homocystinuria --- an inborn metabolic error which results in high blood-levels of homocysteine (>100 micromol/liter) and elevated levels in the urine. Untreated, about 50% of such individuals have thrombo-embolic events, and about 20% are dead before the age of 30, according to Nygard (1997, p.230). Thrombosis rather than atherosclerosis is the basis of their cardiovascular complications. Nygard comments (Nygard 1997, p.230):

"There is increasing evidence that homocysteine may affect the coagulation system and the resistance of endothelium to thrombosis (Malinow 1994) and that it may interfere with the vasodilator and anti-thrombotic functions of nitric oxide (Stamler 1996)."

Nygard cites five prospective, nested case-control studies which explore the relation between total homocysteine levels and the frequency of vascular disease: Alfthan 1994, + Stampfer 1992, + Arnesen 1995, + Perry 1995, + Verhoef 1994. According to Nygard, all except the Alfthan Study found a relationship. (Additional studies of interest include Ridker 1999.)

Nygard and colleagues undertook a prospective study of the relationship between plasma total homocysteine levels and mortality among 587 patients with angiographically confirmed coronary artery disease. Of these patients, 94 had single-vessel disease, 172 patients had 2-vessel disease, and 321 patients had 3-vessel disease at the outset (Nygard 1997, p.233, Table 1). At the outset, 337 of the 587 had already experienced a myocardial infarction, and 64 had already undergone coronary-artery bypass grafting (Nygard 1997, p.232).

Baseline measurements of homocysteine and other risk-factors for coronary heart disease were made in 1991 or 1992. Subsequently, 318 patients had bypass surgery, 120 patients had angioplasty, and 149 were treated medically (Nygard 1997, p.230). After a median follow-up period of 4.6 years, 64 patients had died --- 50 of them from cardiovascular disease, including 26 deaths from myocardial infarction (Nygard 1997, p.231).

Analysis shows a clear, strong, and positive dose-response between levels of plasma homocysteine at baseline, and relative risk of death (all causes combined). Patients were classed into four levels of "dose," measured in micromol/liter. The 130 patients with homocysteine below 9.0 served as the control group. After adjustment for age and sex, the results in terms of mortality ratios are:

Patients	Homo.Level	All causes	CVD only
n = 130	<9.0	1.00	1.00
n = 372	9.0-14.9	2.35	3.30
n = 59	15.0-19.9	5.75	6.30
n = 26	=> 20.0	7.04	9.90

As shown above, when death from cardiovascular disease (instead of from any cause) is evaluated, the dose-response is even stronger (Nygard 1997, p.234). By contrast, "The lipid-related factors showed either no relation or a much weaker relation to mortality [all causes] than the total homocysteine level" (Nygard 1997, p.234). The paper appears to be silent about what fraction of patients were treated with lipid-lowering regimes after the baseline measurements. Nygard and colleagues conclude their paper (Nygard 1997, p.236):

"This prospective study does not prove a causal relation between total homocysteine and mortality, but our results should serve as an additional strong incentive to the initiation of intervention trials with homocysteine-lowering therapy." We concur.

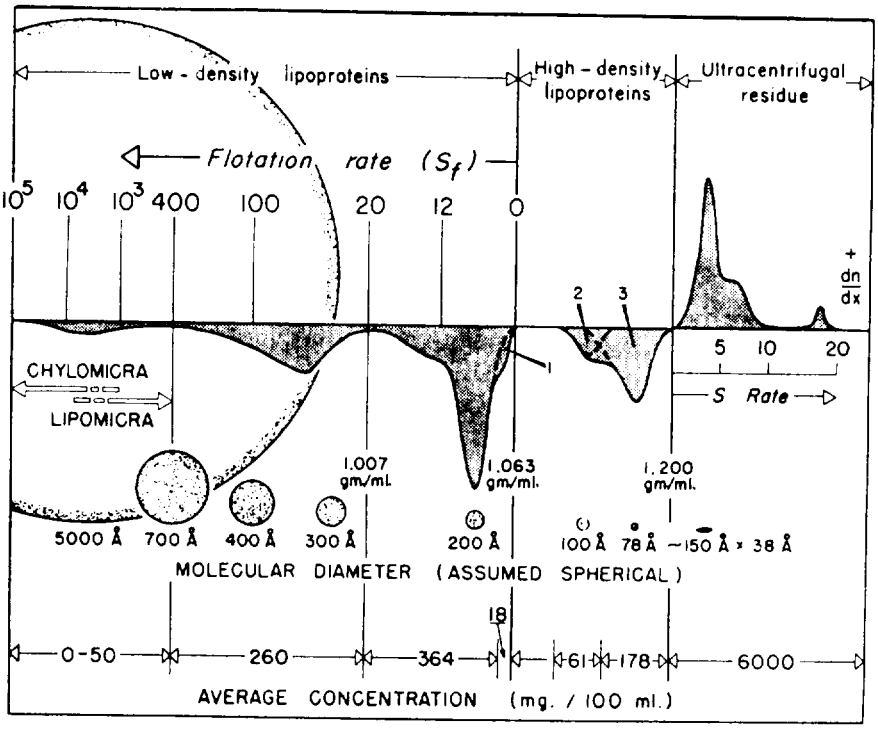
• Part 11. Other Aspects of IHD Etiology

In this chapter, we have barely mentioned, if at all, such established risk factors for Ischemic Heart Disease as heredity, age, gender, smoking, hypertension, diabetes mellitus, overweight, physical inactivity, and others. Of course they, too, are important aspects of IHD etiology. Here, however, we have focused on the concepts which have helped to shape our thinking in Chapters 45 and 46.

>>>>>>>>>>

Box 1 of Chap. 44

Plasma Lipoproteins: Size, Flotation Rates, Lipid-Composition, Protein Fractions.



Related text = Parts 3b + 3c.

• The figure above shows the ultracentrifugal composition of human serum (reproduced from Lindgren 1956; also in Lindgren 1957 + 1959). During the 1950s, the term "Low-Density Lipoproteins" embraced all lipoproteins which floated during ultracentrifugation in a solution of density 1.063 gm/ml. In the figure, "average concentrations" in mg/dl are for 45-year-old males at that time. The size and area of the gray inverted peaks (see Box 2) result from the different concentrations of lipoproteins having various Sf values (e.g., the Sf 20 lipoproteins are in relatively lower concentration than adjacent varieties). In the Low-Density continuum, the Sf 6-8 lipoproteins are relatively small in size. Nonetheless, their estimated molecular weights are 3 to 6 million units. (By comparison, serum albumin has a molecular weight in the neighborhood of 70 thousand.)

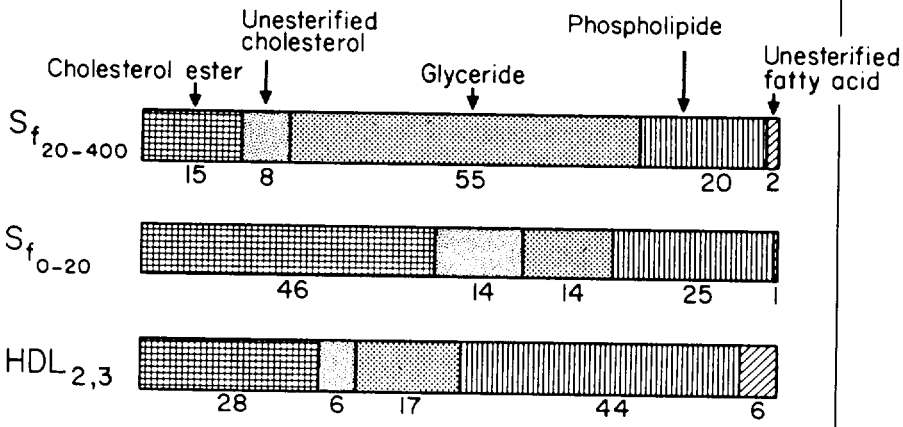
• In the lipoprotein segment called "High-Density," the figure indicates that HDL-3 is more abundant than HDL-2. HDLs also float --- when in solutions which have densities adequately greater than the HDLs' own densities. The "residue" segment on the right consists of the much denser plasma proteins (e.g., albumin, globulins) and "20" components --- which sink instead of floating in a solution of 1.20 gm/ml. (Their plot is on 30-fold reduced dn/dx scale.)

Protein & Lipid:
Approximate Shares per Molecule

	Percent Protein	Percent Lipid
Chylo.	2	98
Sf 20-400	13	87
Sf 0-20	25	75
HDL-1	30	70
HDL-2,3	55	45

Lindgren 1955 + 1956.
Bragdon 1956.

Composition of the Lipid Part: Avg Percentage.
Source: Appendix E, Box 2.



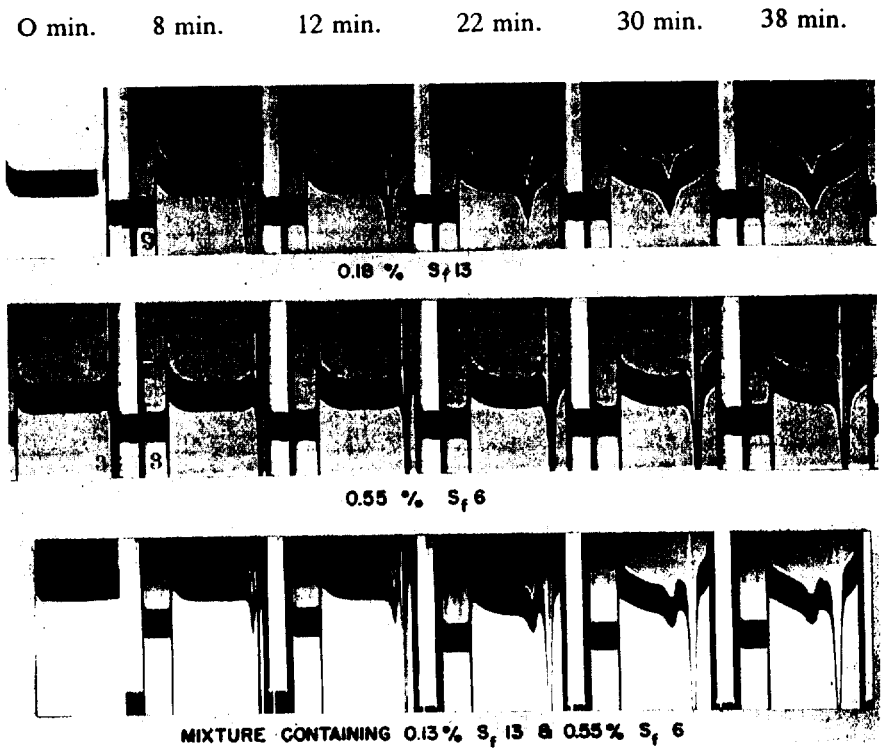
Two Discrete Low-Density Lipoproteins: Flotation Diagrams Separately and Combined.

Related text = Parts 3b + 3c.

• Below are optical (schlieren) flotation diagrams which illustrate the distinctly different behavior of the Sf 13 and the Sf 6 lipoproteins during ultra-centrifugation (from Lindgren 1951, p.86).

• Each strip of photographs was made while the rotor was spinning at full speed (52,640 revolutions per minute), by passing a light-beam through the solution to expose a film cassette. From left to right, the frames of each strip were taken at 0, 8, 12, 22, 30, and 38 minutes after the rotor attained full speed. The cell used had a fluid column of 12 mm.

• The direction of centrifugal force is from left to right. If a lipoprotein floats against this force, it will move progressively from the right side of the first frame, toward the left side in subsequent frames.



• In the FIRST strip, depicting behavior of Sf 13 lipoproteins, an inverted peak is noted at 8 minutes. This peak results from movement of the Sf 13 lipoproteins from right to left, against the centrifugal force. At time = 38 minutes, the inverted peak is far from the righthand edge of the frame.

• In the SECOND strip, depicting behavior of the Sf 6 lipoproteins, the movement is very clearly slower than in the Sf 13 strip. At equal time-intervals, the Sf 13 lipoproteins have traveled (floated) farther from the right side of the frame than have the Sf 6 lipoproteins. The Sf 13 lipoproteins move faster because they are larger and even less dense than the Sf 6 lipoproteins (see continuum in Box 1). The area of the peak under its baseline is proportional to concentration. The area is clearly larger for the Sf 6 lipoproteins, whose initial concentration was higher than the Sf 13's concentration.

• In the THIRD strip, a mixed solution of Sf 13 (0.13% concentration) and Sf 6 (0.55% concentration) is spun. Their mixture causes two distinct peaks, floating at different rates. The experiment indicates that the molecules are stable and retain their physical identities when mixed.

Box 3 of Chap.44

Serial Measurements of Lipoproteins in a Diabetic Woman, during Treatment of Acidosis.

See text, Part 4a.

Source: Felix O. Kolb + Oliver F. DeLalla + John W. Gofman, 1955, "The Hyperlipemias in Disorders of Carbohydrate Metabolism: Serial Lipoprotein Studies in Diabetic Acidosis with Xanthomatosis and in Glycogen Storage Disease." (Metabolism Vol.IV, No.4: 310-317, July 1955). Data are from Table 1, page 312.

• The four Low-Density Lipoprotein classes studied here are Sf 0-12, Sf 12-20, Sf 20-100, and Sf 100-400 . Sf 0-12 and Sf 12-20 are often combined as Sf 0-20. The Sf 20-100 and Sf 100-400 are often combined as Sf 20-400. The two High-Density Lipoprotein classes are HDL-2 and HDL-3. The HDL-1 class is special --- its flotation rate is the lowest encountered in solutions of density 1.063 gms/ml. So, while it is designated as a high-density lipoprotein, it actually is truly in the low-density class, defined by flotation in a solution of density, 1.063 gms/ml. A migration rate of 10^-13 cm per second per unit field of force equals a rate of one Svedberg: In sedimentation, one S unit; in flotation, one Sf unit. When all adjustments are made for temperature and concentration effects, the flotation rates are said to be in "Standard Sf units " (Std Sf units). The Svedberg unit honors Thé Svedberg, the great Swedish physical chemist who pioneered the ultracentrifuge itself (Svedberg 1940).

• The measurements below show the metabolic inter-relations of various lipoprotein classes in a 37-year-old diabetic woman, during treatment of acidosis. The general pattern of lipoprotein conversions is as follows:

Sf 100-400 ---> Sf 20-100
Sf 20-100 ---> Sf 12-20
Sf 12-20 ---> Sf 0-12

with concomitant, progressive loss of triglyceride catalyzed by Lipoprotein Lipase hydrolysis. Sugar-level in the urine falls from 4+ to 1+. The lipoprotein "spectrum" moves toward normality as the defective carbohydrate metabolism comes toward control.

• As the massive Sf 100-400 buildup declines (see second row) to 1530 from 3739---undoubtedly with hydrolysis of triglyceride--- the Sf 20-100 levels rise to a peak value of 1942, while the Sf 12-20 and Sf 0-12 also rise, but do not yet peak. With further therapy (see 3rd row), the Sf 100-400 levels fall even more to 685, the Sf 20-100 levels begine to decline, the Sf 12-20 levels reach their peak, and the Sf 0-12 levels are still building up . The Sf 0-12 levels are the last to peak. At the outset (Row 1), the HDL-1 massive elevation (168 mg per 100 ml is very high for HDL-1) is the same as that noted in chronic "essential hyperlipemia."

• The Lipoprotein and Cholesterol concentration measures are in units of milligrams per 100 ml.

Date of Study	Urinalysis Sugar	Acetone	Sf 0-12	Sf 12-20	Sf 20-100	Sf 100-400	HDL-1	HDL-2	HDL-3	Total Chole-sterol
6/19/52	4+	4+	195	155	1120	3739	168	29	167	1535
6/22/52	3+	1+	444	352	1942	1530	141	7	179	1280
6/28/52	2+	0	744	428	1277	685	66	7	179	806
7/03/52	2+	0	939	338	670	139	34	14	172	668
7/18/52	3+	0	614	134	493	228	41	33	203	377
8/01/52	2+	0	531	148	432	132	25	33	219	342
8/08/52	2+	0	616	150	332	152	19	19	192	377
8/14/52	2+	0	549	108	150	31	---	---	---	320
9/12/52	2+	0	444	186	668	423	---	---	---	---
10/22/52	1+	0	452	237	988	461	52	29	157	398

6/24/52 "Tuberose" and "Eruptive" Xanthomata of approximately 3 months duration.
8/14/52 Partial Clearing of the Xanthomata.
2/27/53 Complete Clearing of the Xanthomata.

Related text = Part 4a.

Box 4 of Chap. 44
Lipid-Lowering Clinical Trials and IHD: A List of Sources, by Years.

This list is provided as a convenience for readers; it is not meant to be complete.
C = Clinical Findings. D = Discussion. Our Ref.Entry = Entry in our Reference List.

Year	Our Ref. Entry	Popular Abbreviation, Topic, or Title.
1978–C.	ComPrinci 1978.	Committee of Principal Investigators ... Clofibrate Trial in Primary Prevention.
1984–C.	Brensike 1984.	NHLBI: Natl. Heart, Lung, Blood Institute ... Cholestyramine Therapy.
1984–C.	LipidRC 1984.	Lipid Research Clinics Coronary Primary Prevention ... Cholesterol-Lowering.
1985–D.	Consensus 1985.	Concensus Conference on Lowering Blood Cholesterol to Prevent Heart Disease.
1987–C.	Blankenhorn 1987.	CLAS: Cholesterol-Lowering Atherosclerosis Study (Cholestipol Niacin Therapy).
1987–C.	Frick 1987.	Helsinki Heart Study: Primary Prevention Trial (with Gemfibrozil).
1987–D.	European 1987.	Policy Statement of the European Atherosclerosis Society.
1990–C.	Brown 1990.	FATS: Familial Atherosclerosis Treatment Study ... Lipid-Lowering Therapy.
1990–C.	Buchwald 1990.	POSCH: Program on Surgical Control of the Hyperlipidemias.
1990–C.	Cashin-Hemphill 1990.	CLAS II: CLAS 1987, for 2 more years.
1990–C.	Kane 1990.	UC-SCOR: Univ.Calif.Specialized Ctr. of Research (Familial HyperCholesterolemia).
1990–C.	Ornish 1990.	Lifestyle: The Lifestyle Heart Trial.
1990–D.	Holme 1990.	An Analysis of Random Trials on the Effect of Cholesterol Reduction.
1990–D.	Joint 1990.	Joint Statement by the AHA and NHLBI: Cholesterol Facts.
1991–D.	Oliver 1991.	Might Treatment ... Increase Non-Cardiac Mortality?
1992–C.	Quinn 1992.	SCRIP: Stanford Coronary Risk Intervention Project.
1992–C.	Schuler 1992.	Heidelberg Study (Low-Fat Diet and Exercise).
1992–C.	Watts 1992.	STARS: St. Thomas Atherosclerosis Regression Study (Lipid-Lowering).
1992–D.	Smith (G.D.) 1992.	"Should There Be a Moratorium on Use of Cholesterol-Lowering Drugs?"
1992–D.	Hulley 1992.	"Health Policy on Blood Cholesterol: Time to Change Directions."
1993–C.	Blankenhorn 1993.	MARS: Monitored Atherosclerosis Regression Study (with Lovastatin).
1993–C.	Pravastatin 1993.	Pravastatin Multinational Study Group.
1993–D.	Brown 1993.	Lipid-Lowering and Plaque Regression.
1993–D.	ExpertPanel 1993.	Expert Panel on ... Evaluation & Treatment of High Blood Cholesterol.
1993–D.	Pearson 1993.	Rapid Reduction in Cardiac Events with Lipid-Lowering Therapy.
1993–D.	Ravnskov 1993.	"Reducing Serum Cholesterol ... of Doubtful Benefit to Anyone."
1994–C.	Furberg 1994.	Effect of Lovastatin ... on Cardiovascular Events.
1994–C.	Haskell 1994.	SCRIP: Stanford Coronary Risk Intervention Project.
1994–C.	MAAS 1994.	MAAS: Multicentre Anti-Atheroma Study with Simvastatin.
1994–C.	Pedersen 1994.	4S: Scandinavian Simvastatin Survival Study, on Cholesterol-Lowering.
1994–C.	Waters 1994.	CCAIT: Canadian Coronary Atherosclerosis Intervention Trial.
1994–D.	Blankenhorn 1994.	Arterial Imaging and Atherosclerosis Reversal.
1994–D.	TaskForce 1994.	European Task Force: Recommendations on Prevention of IHD.
1995–C.	Jukema 1995.	REGRESS: Regression Growth Evaluation Statin Study, with Pravastatin.
1995–C.	Shepherd 1995.	Prevention of CHD with Pravastatin ...
1995–C.	Pitt 1995.	PLAC I: Pravastatin Limitation of Atherosclerosis ...
1995–D.	Gould 1995.	"Cholesterol Reduction Yields Clinical Benefit: New Look at Old Data."
1995–D.	Havel 1995.	"Management of Primary Hyperlipidemia."
1995–D.	Pedersen 1995.	"Lowering Cholesterol with Drugs and Diet" (Editorial).
1995–D.	Ravnskov 1995-a.	"Beneficial Effects of Simvastatin May Be Due to Non-Lipid Actions."
1996–C.	Sacks 1996.	CARE: Cholesterol And Recurrent Events Trial: Persons w. Avg. Cholesterol.
1997–C.	PostCABG 1997.	Post CABG: Post Coronary Artery Bypass Graft & Lipid-Lowering.
1997–D.	Oliver 1997.	"The Low-Fat, Low Cholesterol Diet Is Ineffective."
1998–C.	Ornish 1998.	Intensive Lifestyle Changes in Reversal of Coronary Heart Disease.
1998–C.	Downs 1998.	Primary Prevention of Acute Coronary Events w. Lovastatin: Persons w. Avg. Chol.
1998–C.	Rosenson 1998.	Anti-Atherothrombotic Properties of Statins ... and CV Event-Reduction.
1998–D.	Pearson 1998.	"Lipid-Lowering Therapy in Low-Risk Patients" (Commentary).

Related text = Part 6.