Atherosclerosis—an epilogue

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Certain major risk factors of atherosclerosis are recognized and methods for their early detection and regulation should result in a decreased incidence of deaths from atherosclerotic complications.

Our studies, while considering the multifaceted nature of the disease process, have concentrated on the relation of lipoproteins to atherosclerosis, and factors regulating serum lipid and lipoprotein levels including genetic, physiologic, environmental, and immunologic mechanisms.

Animal studies have included comparative physiologic investigations to try to explain why one species such as dogs are relatively resistant, while another, man, spontaneously develops atherosclerosis.

Studies on human beings have evaluated serum lipoproteins of a large population segment of healthy subjects of the Cleveland area, of groups of distinctive ethnic and genetic background from central Africa; natives of St. Kitts, British West Indies; Indians of Peru and Arizona; and Alaskan Eskimos. Detailed investigations were made of a selected group of subjects studied individually over relatively long periods.

The concept as originally elaborated so clearly by Page[1] in his Connor lecture that atherosclerosis is a disease resulting from interaction of many

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factors still probably most accurately describes the etiology of atherosclerosis. This was elaborated into the Atherosclerotic Mosaic Theory. Studies described below, carried out at the Cleveland Clinic for the past quarter century provide additional blocks to support the "multifaceted" concept as do works from many centers around the world.

Experimental animals

Lipoprotein patterns of sera of different animal species and strains were studied to determine whether any characteristics were common to those which develop spontaneous atherosclerosis or which are resistant to the disease.[2] These were the first of many investigations to determine what factors are important in the development of atherosclerosis.

In dogs that are quite resistant to the development of atherosclerosis, most of the lipid is normally carried as α-lipoprotein, high density lipoprotein (HDL). An exact characterization of high density lipoproteins of serum of normal dogs has been made, p = 1.063 to 1.21 g/ml.[3] Canine HDL is a relatively homogeneous class with a molecular weight of about 230,000 and properties similar to those of human HDL. Ninety percent of the apoprotein consisted of a protein with molecular weight of 28,000, with aspartic acid the NH_2 terminal amino acid as in human A-1 polypeptide. Two other proteins in small amounts, one with a molecular weight of 55,000 and the other with a molecular weight of 8000, were also present.

Canine serum lipoprotein patterns may be modified by a variety of factors. Dogs with malignant hypertension produced by Page's[4] method of wrapping the kidneys in cellophane showed marked changes in lipoprotein and protein electrophoretic patterns including a large increase in concentration of low density lipoprotein (LDL) and very low density lipoprotein (VLDL) and in γ-globulin. The dogs had high mean blood pressure levels which were consistently greater than 190 mm Hg. Complications accompanying the hypertension included detached retina and occasionally evidence of stroke. In contrast, dogs with neurogenic hypertension by debuffering, with blood pressures varying from 200 to 260 mm Hg, usually showed no significant change in their serum lipoprotein levels or protein electrophoretic pattern and no evidence of malignant hypertension.

Atherosclerotic lesions were produced in dogs by feeding the Malmros-type atherogenic diet,[5] which is high in cholesterol and saturated fat and deficient in essential fatty acids. Lesions were observed after the animals had been ingesting the diet for 12 to 18 months.[6] The dogs' serum cholesterol levels frequently exceeded 800 mg/dl, and the LDL, VLDL, and HDL fractions were increased at various times during the diet. Usually after 8 to 10 months the -S 0-5 HDL concentration tended to decrease while the -S 5–10 and less dense fractions increased. The LDL and VLDL levels at this time were high. When the diet was supplemented with polyunsaturated fatty acid, development of lesions was delayed and the lesions were minimal. The serum lipoprotein abnormalities were also less marked.

When dogs were protein-depleted by a combination of plasmapheresis and feeding of a protein-free diet, increased levels of serum cholesterol were found. The HDL, protein-rich lipoprotein fraction, as in well-nourished dogs, was the

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primary lipid transport mechanism, despite the extreme decrease of concentration of serum albumin and some other protein fractions.[7] In dogs, any available protein seems to be preferentially used for maintenance of HDL for lipid transport.

Mechanisms of modifying serum lipoproteins of dogs in addition to renal injury and diet included alteration in hormonal levels. Thus, administration of growth hormone at high dosage to normal dogs resulted in large increases in free fatty acid (FFA) levels and after 10 to 14 days of treatment, increased serum cholesterol, HDL, and LDL levels.[8] Hypothyroid function produced either by thyroidectomy or by injection of ^{131}I was accompanied by large increase in concentration of serum cholesterol and HDL, LDL, and VLDL.[4] Following treatment of the dogs with thyroxin, the patterns returned to normal. These dogs were not examined for atherosclerosis when hypothyroid, but it is recognized that hypothyroid function when supplemented with dietary manipulation accelerates development of atherosclerosis.[9]

Since neurologic factors may greatly influence metabolic functions, the effect of high spinal cord transection in dogs on serum lipid and lipoprotein levels was studied.[10] For the first month after cord transection at level C-6, the serum cholesterol and HDL levels decreased, but after appetite returned and body weight was maintained, the patterns returned almost to preoperative levels.

Initially, plasma FFA levels were low and glucose normal, but after several months plasma FFA levels were consistently high and glucose levels became progressively lower. Unlike normal dogs when exposed to excitement or cold stress, in which plasma FFA levels and glucose increase, the level of both components decreased in the animal with high spinal cord transection. It is thus evident that the plasma lipoprotein levels in the carefully cared for animal with high spinal cord transection can be maintained. The animal is, however, unable to respond adequately to stress to maintain energy-rich FFA or glucose at optimum levels. Dogs with high spinal cord transection maintained in good condition for as long as 6 years showed no evidence of atherosclerosis.

In contrast with dogs, pigs spontaneously developing atherosclerosis have a lipoprotein pattern with nearly equivalent amounts of LDL and HDL.[11] Certain select strains of pigs have pronounced differences in serum lipid levels and also lipoprotein distribution. Thus, short-fat miniature pigs had significantly higher cholesterol and LDL levels than long-lean miniature swine fed the same diet. Albumin and total protein concentration of serum were also higher in the short-fat (endomorphic) than in the long-lean (ectomorphic) type. During the year of study, which included the period of rapid growth and development, the serum cholesterol, LDL, VLDL, and albumin concentrations were significantly higher in the short-fat than in the long-lean animals. Even though they received the same diet, which at certain times was regulated so that the weight gain of the two groups was equal, the differences in serum composition persisted. No evidence of atherosclerosis was found in any of the 15-month-old animals at the termination of the study. Appreciable numbers of swine not especially bred show atherosclerosis, approximately 13% at 1 to 2 years of age and 25% at 2 to 3 years. Whether the miniature swine would have shown lesions had they been stud-

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ied at an older age is not known.

The lipoproteins of cats of mixed breed[2] showed a wide variation in concentration and frequently showed both double LDL and HDL peaks. Comparatively little work on atherogenesis has been carried out on this species, but recently Howard[12] has found that lesions can be induced in cats by feeding them a diet containing 2% cholesterol; a diet containing 0.5% was ineffective.

A simple and unique lipoprotein pattern was observed in the serum of guinea pigs when studied at a density of 1.21 in the analytical ultracentrifuge.[2] Only one lipoprotein, an LDL, -S 20–40, was demonstrated. All other sera of mammalian species thus far studied contain at least some HDL, as well as LDL. The guinea pigs' response to a diet high in cholesterol is also different from other species. When the guinea pigs were fed a diet containing 1% cholesterol, Sardet et al[13] found that hemolytic anemia developed after 4 to 6 months. Naito[14] has found that this process is accelerated if the fat content of the diet is increased. Early atherosclerotic-type lesions may also occur in the guinea pig on this dietary program.

Monkeys have lipid and lipoprotein patterns similar to those of young human beings, or of older people with lipids at the lower level of normal for their age. By feeding monkeys an atherogenic-type diet, Taylor et al[15] produced atherosclerosis in 1958. We had an opportunity to study sera of these animals and found increased levels of VLDL and LDL. Injury to the arterial wall by freezing greatly accelerated the development of lesions.

The serum lipoprotein pattern of rats on a regular chow diet has both HDL and LDL components, but shows no measurable amount of VLDL, i.e., pre-β fraction. The HDL is resolved as a broad band when studied by paper electrophoresis, and as two or three bands by acrylamide gel. In the analytical ultracentrifuge the fraction appears as a broad band with flotation rate -S 0–20.[2, 7] This contrasts with the pattern of normal human and dog sera where the HDL fraction has more homogeneous properties.

When rats were fed an atherogenic diet high in fat and cholesterol content and containing thiouracil and cholic acid described by Hartcroft, they developed extremely high serum VLDL, LDL, cholesterol, and total lipid levels. After 15 months on the diet they failed to show atherosclerotic lesions.[16] While the strain of rats used was similar to that used by Hartcroft in his study, it appears that this particular group of rats was unusually resistant to development of atherosclerosis and that hyperlipidemia alone was not sufficient to produce it. Exercise significantly reduced serum total lipid and cholesterol levels of our Hartcroft-diet rats, but did not affect their tissue lipid levels. When serum lipid concentrations were high due to diet, they were reduced by exercise.

The serum lipoprotein pattern of rats made nephrotic by injection of antirat kidney antisera showed greatly increased concentration of chylomicra, VLDL, and LDL; serum albumin level was very low.[17] Similarly, old rats, protein-depleted by a protein-free diet showed increased VLDL and LDL, and a slight decrease in HDL concentration. In contrast, young rats fed a protein-free diet showed little increase in chylomicra, VLDL, or LDL, but elevated levels of -S 10–20 HDL. Both groups showed low serum total protein and albumin levels. No atherosclerotic lesions were found in either old or young rats on the deficient diets.[7]

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Humans

Serum lipoproteins in healthy populations

Geographic and genetic variation. The serum lipoprotein pattern in man has been studied by numerous techniques, and as a result of combined efforts of physical chemists, chemists, immunologists, and electron microscopists, a basic understanding of structure and composition of the major lipoproteins in serum has been obtained.[18]

The two major lipoprotein fractions present in fasting serum of the healthy adult are α- and β-lipoprotein, i.e., HDL and LDL. A relatively small concentration of pre-β (VLDL) may also be present.[19] In the sample collected a few hours after eating, chylomicra may also be present.

Study of large segments of the population in a collaborative investigation showed that the serum cholesterol levels increased from puberty to age 60 in males, after which they declined slightly. In females who had lower levels than males in the early age bracket, cholesterol levels continued to rise throughout the age group studied: 18 to more than 70 years.[20] The LDL concentration of American men was higher than that of women, and the concentration of HDL was lower.

The lipoprotein patterns of different geographic and ethnic groups were studied to obtain information concerning the relative importance of genetic background, mode of life, physical activity, and diet in determining the lipoprotein distribution and its possible relation to the occurrence of atherosclerosis.

The serum lipoprotein patterns of Navajo and Peruvian Indian men showed serum cholesterol levels slightly lower than American Caucasians of similar ages in the Cleveland area.[21, 22] Their lipoprotein patterns showed lower LDL and higher HDL concentrations than that of the Cleveland men. A group of Negro native St. Kittitians in a rural area showed serum cholesterol and LDL levels significantly lower than the levels of Clevelanders.[23] Unlike women in the United States, the St. Kittitians showed no postmenopausal increase in cholesterol or in any lipoprotein level. The VLDL levels in the sera of the women at all ages were about the same and similar to that of the Clevelanders. In contrast, the lipoprotein pattern of Alaskan Eskimos of Pt. Hope[24] had VLDL and triglyceride levels significantly lower than Americans; their other serum lipid and lipoprotein levels were not remarkable. Both serum LDL and VLDL levels of the Masai of southern Kenya[25] were significantly lower; their HDL levels were similar to Americans.

All of the above groups except the Clevelanders had a history of great physical activity. As summarized in the *Table,* their diets varied greatly, but in none of the groups was excessive body weight a characteristic. The incidence of atherosclerotic heart disease in each of these groups was very low in comparison with that in the Cleveland population. It is thus evident that, despite ingestion by some groups as the Eskimo and Masai of diets high in cholesterol or of a diet relatively high in salt and carbohydrate as the St. Kittitian, high serum cholesterol and lipoprotein levels do not necessarily develop. Whether this is primarily dependent upon their basic genetic pattern or is modified by physical activity and limited caloric intake has not been determined. The fact that the low levels of VLDL observed in the Alaskan natives are significantly increased when they eat a diet high in carbohydrate[26] suggests that at least in

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Table. Diet composition of populations studied

	Total calories, kcal	Calories, %			Cholesterol mg/day
		Fat	Carbohydrates	Protein	
American*	2400 (1500–3000)	42.4	42.6	14.2	663 (525–720)
St. Kittitian	1639	17	69.8	13.2	200
Masai	3000	66	14–18	15–20	500–1000
Alaskan Eskimo	3000 (2300–4500)	50	15–20	30–35	420–1650
Navajo Indian similar to American					
Peruvian Indian similar to St. Kittitian					

* Data obtained in same decade as these data are calculated from Stamler J: Lectures on Preventive Cardiology, Table 10, p 88, New York, Grune and Stratton, 1967. These data are based on estimate of food ingested for United States adult males in six studies, not including Diet Heart Data.

some dietary carbohydrate may be a dominant factor.

Lipoprotein structure. Structure and composition of lipoprotein have been a major interest in many centers. One of the most challenging problems was the preparation of the apoproteins of the lipoproteins in an unmodified form suitable for analysis. Some of the earliest work on the human HDL apoprotein was carried out with Scanu.[27] He developed an efficient method of delipidizing the HDL and determined that the terminal amino group was aspartic acid. The electrophoretic mobility of the apoprotein was -5.00×10^{-5} sq cm/V/sec. Since that time he has continued to contribute extensively to the rapidly expanding knowledge of lipoprotein structure and function.

The intact lipoproteins have been isolated and their general physical and chemical properties determined. Preparation of the apolipoproteins of the various lipoproteins, chylomicron, VLDL, LDL, and HDL has been accomplished by the efforts of many groups throughout the world. A recent review on serum lipoproteins by Scanu et al[28] has summarized recent developments. The properties of the primary structures of the human HDL A-I and A-II apoproteins including molecular weight determinations and amino acid sequence have been worked out. A-II has a molecular weight of 17,400 and is composed of two identical polypeptide chains linked by a single disulfide bridge. The α-helix content of the human A-I dimer is about 60%, and for A-II nearly 40%. They behave as typical water-soluble proteins. In the lipid-free state these proteins are more susceptible to conformational alterations than when in intact HDL form. Recombination studies of apoprotein with lipids have progressed slowly and the results require careful interpretation because of the nature of the isolated apoproteins, the problems of solubility of the lipids and apoproteins, and difficulties of monitoring the process. Limited studies on reassembly[29, 30] of HDL_2 or the purified peptides with whole HDL lipid extracts indicated that each polypeptide seemed to have distinct affinity for lipids and could combine with either polar or nonpolar lipids independently.

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Hyperlipoproteinemias

Primary hyperlipoproteinemia. Primary hypercholesteremia has long been recognized as a genetically determined abnormality, and the occurrence of various types of abnormal lipoprotein patterns in d < 1.063 fractions in "healthy" people was appreciated at the time of the cooperative study on lipoproteins as predicators of coronary heart disease.[31] It was not, however, until the publications of Fredrickson et al[32] that a definitive classification of the types of essential hyperlipidemias was presented. This system simplified lipoprotein classification so that it could be understood much more widely. According to the original Fredrickson classification the most significant serum lipid and lipoprotein characteristics of the five types of hyperlipoproteinemia were: type I—increased triglyceride, increased chylomicron; type II—increased cholesterol, increased β-lipoprotein; type III—increased triglyceride and cholesterol, increased β-lipoprotein of low density and fast electrophoretic mobility; type IV—increased triglyceride, increased pre-β-lipoprotein; type V—increased triglyceride, increased pre-β and chylomicron. With the recognition of various types of lipoprotein abnormalities, methods of treating them were developed. At the Cleveland Clinic the serum lipid and lipoprotein changes that could be induced by diet were carefully studied in countless healthy subjects and in those with atherosclerosis at many stages of development. The results soon showed that patients with different types of lipoprotein abnormalities responded quite differently to different diets. Thus, for patients who had serum lipoprotein patterns with increased chylomicron levels, subsequently called type I or type V hyperlipoproteinemia, a low-fat diet was indicated; for those with type II or IV, a diet low in cholesterol and rich in polyunsaturated fat was most effective.[33] It also was obvious that some patients with hypercholesteremia also had some degree of hypertriglyceridemia, and showed not only elevated levels of serum cholesterol and LDL, but also increased triglyceride and VLDL levels. The hypercholesteremics with increased triglyceride[34] levels had significant reduction in serum lipid levels and frequently achieved a nearly normal lipid and lipoprotein pattern when adhering well to the vegetable oil food pattern. In contrast, those with only hypercholesteremia showed on the average, less reduction in serum cholesterol levels, and 20% failed to show significant response to diet.[34]

Because of the difference in the lipoprotein and lipid patterns of the two groups and their different responses to dietary management, it was suggested that the hypercholesteremic hyper-LD lipoproteinemics be placed in one group, type II, and the hypercholesteremics with increased triglyceride, LDL, and VLDL be placed in a second group, type VI.[35] The WHO committee which made recommendations for nomenclature of the lipoprotein types did not adopt the type VI terminology, but recommended that hypercholesteremia with hyper-LDL be called IIA and those with elevated triglycerides and VLDL (our type VI) be called IIB.[36]

Long-term studies have established that patients of type IIB or IV maintained lowered serum lipid and lipoprotein levels for as long as 10 years when they adhered to the appropriate diet. Approximately 60% of type IIA patients also maintained the lower serum cholesterol levels initially achieved by diet;

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the other 40% showed decreasing response after a year or more on the diet.[34]

The diets originally described have served as a guide, and many interesting and nutritionally excellent menus utilizing these concepts have subsequently been developed. The interdependence of various factors in the composition of the diets must always be remembered.[37] These include composition of dietary fat, i.e., total fat, saturated and polyunsaturated fatty acid content, cholesterol, and other dietary factors including carbohydrate. These diet patterns served as a basis for subsequent studies including the National Diet-Heart Study.[38, 39] The Multiple Risk Factor Intervention Trial that is being conducted through the National Heart, Lung, and Blood Institute also uses them as the basis for their dietary recommendations.[40]

It is frequently asked when a hypolipidemic-type diet should be started. Since we observed that children of hypercholesteremic mothers may have significantly elevated serum cholesterol levels by 4 days of age, and extremely high levels by 1 year,[41] it appears certain that desirable food patterns should be developed and adhered to from the time that these children are young infants. A modified diet low in cholesterol and high in polyunsaturated fat is usually recommended.

In an early study on excessively obese subjects who were unable to be treated effectively by diet, a short-circuiting (jejunocolic bypass) procedure was carried out.[42, 43] It was observed that following this operation the serum cholesterol, LDL, and VLDL levels of the patients decreased approximately 50%, and that the lower level was maintained even after the patients' weights stabilized near desirable levels. These observations have been extended and modified by Buckwald et al,[44] and an ileal bypass operation developed which effectively reduced serum cholesterol and lipoprotein levels without affecting body weight. It may be especially useful in treatment of patients with extreme hyperlipidemias who have failed to achieve desirable serum lipoprotein and lipid levels with diet or diet plus drugs.

Lipoprotein levels in patients with hypertension. The correlation of blood pressure with serum lipid levels was very low in a cooperative study among "normal" persons.[20] It was only when individuals with lowest lipid levels were compared with those with highest that differences in blood pressure levels were demonstrated. The serum lipid levels were significantly higher in the high blood pressure group, but it was pointed out that although the differences were significant in the large population studied, they had little meaning for individual cases.

Corcoran et al[45] reported in 1956 that atherosclerotic complications were common in patients who did not respond well to antihypertensive treatment. The lipoprotein levels of patients with essential hypertension varied little from the "normal"; patients with malignant hypertension showed high levels of LDL and VLDL.

In St. Kittitian women, the serum cholesterol and lipoprotein patterns of normotensives and hypertensives were similar, and as noted under "normal patterns," they showed no increase in concentration with increase in age. The incidence of ischemic heart disease was very low despite the prevalence of hypertension. The low incidence of hyperlipidemia and heavy work may have been factors contributing to their protection.[23]

Serum lipids and lipoproteins of 54 hypertensive patients, 28 of whom had

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atherosclerosis of the abdominal aorta as demonstrated by arteriography, were investigated by Tarazi and Lewis.[46] There was little difference in the incidence of abnormal serum lipoprotein patterns and elevated lipids between those with (54% abnormal) or without (46% abnormal) aortic atherosclerosis. The incidence of aortic atherosclerosis was similar in those with essential and in those with renal arterial disease. The lipoprotein abnormalities included those typical of type IIA, IIB, and IV phenotypes; no one phenotype was characteristic of the hypertensive patients. The results suggest that in the hypertensive-atherosclerotic prone patient, factors other than hyperlipidemia probably play a dominant role in accelerating the disease process.

Lipoprotein patterns in patients with coronary artery disease. It is generally agreed that high serum cholesterol, triglyceride, and LDL levels are associated with an increased incidence of atherosclerosis. Results of serum lipid and lipoprotein determinations on 355 men 30 to 50 years of age who had cinecoronary arteriography at the Cleveland Clinic by Dr. F. Mason Sones lend further support to this concept (unpublished data). As seen in *Figure 1,* the concentrations of serum cholesterol, triglyceride, and β- and pre-β-lipoproteins were significantly greater in the 285 men who showed atherosclerotic plaques in the coronary arteries when studied by arteriography than those found in men who were free of atherosclerotic lesions. In contrast with the changes in serum β- and pre-β-lipoprotein levels in patients with lesions, their α-lipoprotein concentrations decreased (*Fig. 2*). Thus, only half as many subjects with lesions had

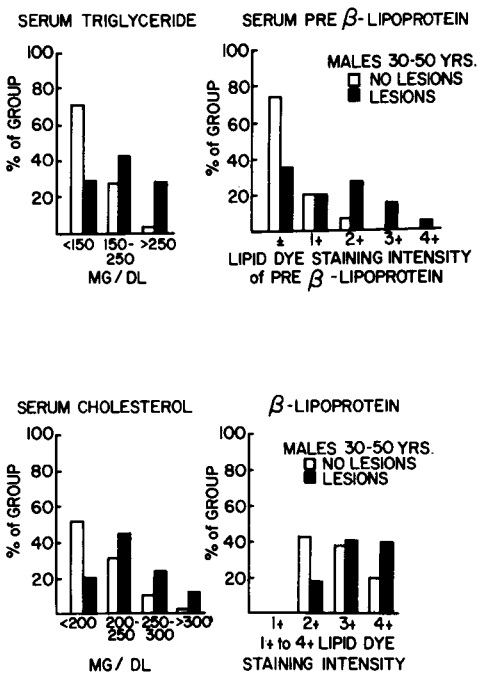

Fig. 1. Concentration of serum cholesterol, triglycerides and β- and pre-β-lipoproteins in men who showed demonstrable atherosclerotic lesions by arteriography and in those who had no demonstrable atherosclerotic lesions by arteriography.

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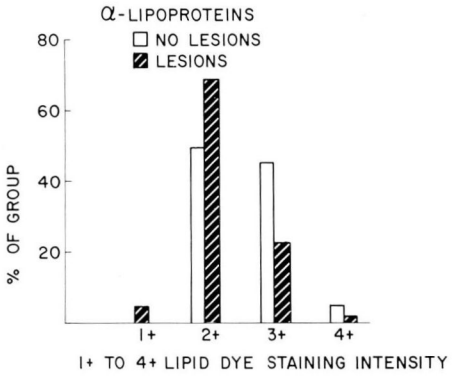

Fig. 2. Concentration of serum α-lipoproteins in men who showed demonstrable atherosclerotic lesions and in those who had no demonstrable atherosclerotic lesions by arteriography.

3+ α-lipoprotein as those without lesions. The lipoprotein and lipid distribution of the patients free of lesions was similar to that found when sera of 50 healthy Fellows of the Cleveland Clinic and 33 healthy company executives of similar ages were evaluated at the time of their physical examinations. The type of lipoprotein abnormalities did not fall into any one group.

Hyperlipoproteinemias associated with other abnormalities. *Renal disease.* Extreme hyperlipoproteinemia occurs in the nephrotic phase of nephritis or in nephrosis. Elevation of chylomicron, VLDL, and LDL is observed and HDL levels are usually low.[47] In patients with renal failure being maintained by hemodialysis, serum lipid and lipoprotein levels show relatively small variations from normal in comparison with those of the nephrotic. Serum VLDL and triglyceride levels are somewhat elevated, but cholesterol and LDL and HDL levels may be low, due probably at least in part to the very low protein intake of the subjects.[48]

Following renal transplantation, serum triglyceride levels tend to decrease during the first month or two, but later increase to an average level above 250 mg/dl; much of the triglyceride is in the VLDL fraction. Serum cholesterol and LDL may increase to above normal levels. This is especially true in the younger patient. HDL levels following transplantation tend to be maintained near normal.[49] It is likely that the increased triglyceride and VLDL lipoprotein concentrations may be an effect of the prednisone the patient is receiving, as the levels tend to decrease when the drug dose can be decreased. A higher incidence of cardiovascular disease as a cause of death occurs in the patient undergoing chronic dialysis and following renal transplantation than in the population as a whole. This difference is especially marked in the younger age group. It is possible that the lipid-lipoprotein abnormalities may play a part.[48]

Autoimmune hyperlipidemias. When normal serum is subjected to ultracentrifugation, chylomicron, VLDL, and LDL are concentrated in the supernatant fraction if the density has been adjusted to d 1.063, and HDL is also floated if d is 1.21. Electrophoresis of the top fraction shows that it contains only lipoproteins. In 1952 a patient's serum showed high cholesterol and triglyceride content and unusual flotation properties and physical characteristics which could not be explained. With the development of starch-gel electrophoretic techniques the unusual supernatant lipoprotein concentrate was found to contain a band not normally present, which could not be identified at that time. A few years later when IgA was described and antisera to it were available, the previously unidentifiable band was found to be IgA globulin. It was firmly bound to VLDL and LDL and only small amounts of HDL were resolved as a separate band when the concentrate was studied by electrophoresis and immunoelectrophoretic techniques. Since

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then the patient has been followed and has consistently showed serum cholesterol levels greater than 800 mg/dl and triglyceride greater than 1000 mg/dl, despite his rigorous adherence to diet and use of various hypolipidemic drugs over the years.[50–53] The serum IgA levels have been consistently greater than 1500 mg/dl. There is no indication that the abnormality is genetically determined as all family members had normal lipid and lipoprotein levels: About 16 years after recognition of his hyperlipidemia, the patient had resection of an aortic aneurysm. Atherosclerotic areas showed presence of β-lipoprotein and IgA globulin. These two proteins were also demonstrated in plasma cells of his bone marrow.[53] The bone marrow showed 10% to 20% plasma cells, but there was no clinical evidence of myelomatosis. Despite the extremely high lipid and lipoprotein levels for 23 years, the patient's coronary arteriograms have showed only minimal changes.

The association of the lipoprotein with immunoglobulin has many characteristics of a soluble antigen-antibody complex. Beaumont et al[54, 55] have also reported on sera with these characteristics.

A second serum with association of lipoprotein with immunoglobulin showed the presence of IgM globulin bound with VLDL and LDL lipoproteins to form a complex with cryoglobulin properties.[56] All of the complex precipitated from the serum at room temperature, but after dissociation of IgM from lipoprotein by ultracentrifugation at high salt concentration neither the lipoprotein nor the IgM had the properties of a cryoglobulin. The 44-year-old woman had a lymphomatous type of disease and no evidence of atherosclerosis. Her serum lipid and lipoprotein concentrations were only slightly elevated. When the IgM and lipoproteins were mixed, cryoglobulin properties were restored.

A third instance of lipoprotein immunoglobulin association was found in a patient with multiple myeloma in whom the lipoproteins were combined with IgG globulin.[51] Using electrophoresis, all serum lipoproteins were found to migrate to the γ-3-globulin position with the IgG. The complex was readily broken by ultracentrifugation at high salt concentration, the IgG concentrating at the bottom of the tube and the lipoproteins floating to the top. When the IgG concentration decreased following treatment of the patient with melphalan (Alkeran), the concentration of serum lipids also decreased from high pretreatment levels to normal, where they remained for several years. When relapse occurred both IgG and serum lipoproteins increased. The isolated IgG and β-lipoproteins when mixed together interacted and migrated as a single band during electrophoresis to the γ-3-globulin position. Moderately advanced atherosclerosis was found at autopsy. Atherosclerotic areas showed positive immunofluorescence against both anti IgG and anti β-lipoprotein antisera. The plasma cells of her bone marrow showed a similar reaction.[52]

It thus is evident that an antigen-antibody type of complex of lipoproteins and immunoglobulins in the serum does not permanently protect against the development of atherosclerosis, although both of our patients who have had arterial studies were in their seventies; the first patient is 72 years old at present and the third was 72 years old at the time of death.

Conclusion

The results of our studies clearly indicate the validity of the multifaceted

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concept of the etiology of atherosclerosis. Hyperlipidemia, of genetic origin or acquired as in renal disease and in the "autoimmune" types, has been shown to be an important contributing factor associated with atherosclerosis. The effectiveness of diet, exercise, hormones, and drugs in regulation of serum lipid and lipoprotein patterns has been demonstrated in human beings and experimental animals. The significance of other facets including high blood pressure also have been reinforced. Future answers to the control of atherosclerosis will probably come only when the many blocks involved can be fitted together in correct, integrated order. Before this can be accomplished greater knowledge of the role of blood vessel injury and altered vessel metabolism in physiologic and pathologic conditions will be needed. The results we have obtained over the years should be helpful in new explorations to achieve the completion of the atherosclerotic mosaic.

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