ATHEROSCLEROSIS: A HYPOTHESIS CONCERNING THE INITIATING STEPS IN PATHOGENESIS

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The rate of progression of atherosclerosis as manifested by coronary artery disease is correlated with the serum levels of low density lipoproteins (11). What may be the basis for this relationship? It is suggested that this correlation exists because the concentration of the material involved in the initiation steps in atherosclerosis parallels that of the low density lipoprotein. It is further suggested that this material is produced in the lipoprotein through oxidative-polymerization of some of its lipid constituents.

From the general character of oxidation reactions involving molecular oxygen and the ready oxidation of fats by oxygen (17) it might be expected that some of the lipids in the serum lipoproteins would undergo oxidative polymerization to higher molecular weight materials containing one or more groups such as hydroxyl, peroxy, carbonyl, or carboxyl. The type of product produced and the rate of formation would depend on such factors as the following: the concentration and types of cholesterol esters, detergents (such as the phospholipids and the globulins), oxidation catalysts (iron, copper, cobalt, for example), oxidation inhibitors (such as mercaptans, vitamin K, and vitamin E), fats liberated into the blood stream from the fat depots, and ingested fat. In addition, hydrolysis of ester groups of oxidized-polymerized material would yield compounds containing hydroxy and carboxyl groups. Some of the data which support this concept of the in vivo oxidation of fats to form higher molecular weight compounds follow: Fats have been shown to be oxidized by molecular oxygen in vitro at body temperature and the reaction is catalyzed by such common metallo-organics as cytochrome-C, catalase, and hemoglobin (32). Hemin catalyses the autoxidation of linoleic acid in heterogeneous emulsion (16); the catalysis is stopped by the addition of acetic acid, dioxane, pyridine, alkali or bile which bring about homogenization of the system—that is, this oxidation reaction occurs at a measurable rate only in the dispersed linoleic acid phase. It is well known that some fats can be oxidized to high molecular weight compounds at ambient temperatures; the drying of paints is an example.

It has been shown that atherosclerotic aortae contain lipoperoxides and that the content of the peroxides parallels the degree of severity of the atherosclerosis while normal aortae are free of lipoperoxides (8). Such peroxides are produced in the oxidation of fats. The following is an excerpt from the paper which reported this study:

"It may be that the peroxidation is secondary to the deposition of lipids, but the irreversible nature of the formation of lipoperoxides and the ability of such compounds to form a variety of secondary products suggests the possibility that an active part is played by these compounds in the pathogenesis of atherosclerosis."

Compounds that can act as oxidation inhibitors, such as ascorbic acid, thiamine, iodide (22), and thyroxine (10), in the diet of rabbits being fed cholesterol, decreases the severity of the cholesterol-induced atherosclerosis. Futher, a mixture of vitamin A palmitate and α-tocopherol acetate, fed orally, very significantly reduced plaque formation, aortic total fat, and free and esterified cholesterol in chickens on a regular diet (34).

Pyridoxine deficiency favors development of atherosclerosis (14, 28, 31). Is this because pyridoxine, a good heavy metal chelating agent, decreases the effective concentration in the serum of metal oxidation catalysts?

Atherosclerosis is a prominent feature of chronic disease states associated with an elevated cholesterol blood level (10). This is not surprising from the point of view of the present theory, since cholesterol is easily oxidized by molecular oxygen (26) and about two-thirds of the cholesterol in the serum lipoproteins occurs as esters of fatty acids (10, 12) (these are chiefly unsaturated fatty acids whose ready oxidation is well known).

Studies of the degradation of isolated lipoproteins (both human and rabbit) indicated that the observed changes were due to oxidation (29):

The changes noted on dialysis against a large volume of buffered NaCl solution or ordinary water, as observed by ultracentrifugal analysis were: 1) The heterogeneity of the lipoprotein material increased, as evidenced by the spreading of the peak; 2) Low density material of lipid nature appeared—this rose rapidly to the meniscus and tended to form a compact layer; 3) The concentration under the lipoprotein peak diminished and there was an increased amount of material which sedimented under the experimental condition; 4) The rate of flotation of the lipoprotein peak progressively decreased. In addition there frequently developed a visible turbidity due to aggregation of lipid-like material mentioned above in second instance. If all traces of cupric ion (less than circa 0.1 ppm) were removed from the environment the lipoprotein was found to be stable upon dialysis; numerous other metal ions were without effect. Several substances were found which would stabilize the lipoprotein—these were antioxidants or else complexing agents for copper. It was concluded that the data supported the view that the degradative process was oxidative in nature and was catalyzed by copper.

In addition, changes were produced in lipoprotein concentration similar to the changes resulting from dialysis by the addition of 1 per cent hydrogen peroxide and a trace of cupric ion (cupric ion decomposes hydrogen peroxide to water and oxygen via a free radical reaction involving hydroxy radicals) (33). When cupric ion was not present or when it was complexed with Versene, the peroxide did not seem to affect the lipoprotein.

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Thus, oxidation of some of the constituents of the lipoproteins may contribute to the formation of lower groups from the higher S_f groups. Hydrolysis of glyceryl esters of lipoproteins also results in formation of lower S_f molecules from higher ones (13).

The foregoing data on oxidation taken as a whole suggest that oxidative-polymerization proceeds in the lipoprotein in vivo and that this process is analogous to that occurring in simple emulsion polymerization systems (5).

There is a good correlation between the steady state concentration of low density lipoproteins of the S_f 0-400 group and the extent of atherosclerosis as manifested by coronary artery disease. Hence, if oxidized-polymerized lipids are the initiating agents in atherosclerosis, their steady state concentration must parallel that of these low density lipoproteins. This does not mean that lipid oxidation-polymerization does not occur elsewhere than in members of the S_f 0-400 group, but implies that if it does, the oxidized material is formed in relatively small amounts or eventually comes to be carried by lipoproteins of the S_f 0-400 range—for example, oxidation of lipid might occur in a lipoprotein of $S_f > 400$, but as indicated above, this tends to convert it into one of lower S_f.

Tissue response to foreign material in the arterial wall probably plays a part in the development of human atherosclerosis as shown by experiments in which lesions were produced in arteries of dogs by injecting into the media human fat alone or mixed with fat acids, calcium soaps or cholesterol (4). The severity and chronicity of the lesions varied with the acidity and speed of dispersal of the fat mixture. Human fat and fatty acids produced marked inflammatory lesions, which healed rapidly because the lipids were absorbed readily. Human fat mixed with calcium soaps or cholesterol was absorbed slowly and caused chronic lesions. Repeated intravenous injections of solutions of large molecules such as polyvinyl alcohol or sodium cellulose glycolate produced atheromatosis in the arteries of dogs (18, 19).

Thus, it would seem that both the type of molecule entering the arterial wall and the length of time it stays there are involved in the development of atherosclerosis. In the case of human atherosclerosis, the types of molecules involved in pathogenesis are apparently primarily some lipids associated with the lipoproteins of the S_f 0-400 class. The time that these lipids—presumably those which have undergone oxidative-polymerization—remain in the arterial wall is a function of both the size of the molecules (and their chemical nature) and the degree of interaction between the lipids and the constituents of the arterial wall. Interaction would range from relatively slight (as between molecules of the arterial wall and lipids containing comparatively non-polar groups such as carbonyl, hydroxyl, and esters) to quite strong, as in the formation of mixed calcium salts between carboxyl groups of the lipid and those on arterial wall constituents.

Hence, for a given size and type of lipid, other conditions being constant, the rate of passage through the arterial wall, and hence the time spent in the wall, is a function both of the chemical character of the lipid and that of the arterial wall. What determines the contribution of the arterial wall to the anchoring of the lipid molecule? According to a recently proposed theory of aging (which relates aging to the deleterious side effects of free radicals normally produced in the body) a slow net oxidation of the constitutents of the body would be expected, including the connective tissues of the large vessels such as the aorta (15). Further, it would be anticipated that carboxyl groups would be among the oxidized groups formed (on the side chains of proteins, for example) and that these in turn would form insoluble calcium salts. Thus, an increase in the calcium content of the tissues with increasing age would be expected. It has been known for a long time that the calcium content of the tissues of the body increases with age. Further, it has been shown that the content of aspartic and glutamic acid (both dibasic acids) of the elastin of the aorta increases with age, and it has been suggested that the increasing calcium content of the aorta is related to this fact (21). Thus, the ability of the arterial wall to anchor material such as oxidized lipids would be expected to increase with age through the formation of polar groups on the constituents of the wall. That arterial age is a factor in the development of atherosclerosis was indicated by the experiment (27) in which, under analogous experimental conditions, rabbits one year of age responded to cholesterol feeding with atherosclerosis, whereas 5, 10, and 25 week old animals did not. All rabbits, however, regardless of age, reacted with hypercholesterolemia of almost equal degree.

In addition to the interaction of the oxidized-polymerized lipid with fixed tissues of the arterial wall, such as elastin, there may also be one involving the cells, which may contribute to the fixation of the lipid material.

The expected effect of age on the contribution of the arterial wall to the rate of progression of atherosclerosis is paralleled by an increase in the level of the low density lipoproteins. This increase with time (over the major portion of the life span) follows the same function as does the rate of aging, as measured by the mortality rate.* That is, the rate of increase at any given time is proportional to the lipoprotein level, i.e. $\frac{dS_f}{dt} = kS_f$ or $\log S_f = kt + c$, where c is a constant of integration. Thus, the lipoprotein levels appear to reflect the rate at which the body as a whole is aging. For females the equation is good from age 25 to 70 years, while for males it is valid between ages 15 and 35. The slope of the curve log S_f—age is essentially the same for both males and females below age 35; above age 35, when S_f 0-400 is circa 600 mg% (9), the curve for males begins to bend downwards. Recently it has been shown that the lipoprotein values also decrease, from a maximum S_f 0-400 of circa 700 mg%, with advancing age in women past 70 (6). Consideration of the data for abnormal lipoprotein states (10, 30) indicates that this phenomenon is due, at least in part, to saturation of the β-globulins, normally associated with the low density lipoprotein, with lipid.

The fact that a maximum level of S_f 0-400 is attained eventually with increasing age does not necessarily mean that the steady state concentration of the oxidized-polymerized lipid assumed to be responsible for initiating atherosclerosis does not continue to rise un-

* Calculated from data in the paper by Glazier, F. W., and Associates. Human Serum Lipoprotein Concentrations. *J. Gerontol.*, 9: 395-403, 1954.

abated, but rather that when the Sf 0-400 lipoproteins are saturated the excess oxidized-polymerized molecules are carried elsewhere—probably in the higher S_f groups (or in higher concentration in the S_f 0-400 or both).

Thus, atherosclerosis appears to be the result of three primary processes: 1) Oxidative-polymerization of constituents of serum lipoproteins; 2) Anchoring of the oxidized material in the arterial wall; 3) An inflammatory reaction induced in the arterial wall by these oxidation products. With increasing age the contributions of the first two processes increase while that of the latter decreases. Hence, it is conceivable that a point might be reached where the rate of progression of atherosclerosis might decrease with age in spite of increasing contribution of the first two primary processes. In addition to age, a number of other modifying factors are superimposed on the primary processes (1, 2, 3, 7, 20, 23, 24, 25, 35)—their influence seems to be best understood when considered from the standpoint of their effect on the suggested primary processes.

This concept of the pathogenesis of atherosclerosis is suggestive of means to slow down the process. One of the most obvious is to decrease the chain length of the oxidative-polymerization reactions by increasing the serum concentration of compounds capable of acting as oxidative inhibitors. A study of the oxidative characteristics of lipoproteins formed on different dietary regimens may be quite productive. This may be particularly helpful in understanding why increasing the unsaturation of dietary lipids tends to decrease cholesterol and lipoprotein levels.

SUMMARY

A hypothesis has been developed concerning the initiating steps in atherosclerosis. These postulated steps are: 1) Oxidative-polymerization of constituents of serum lipoproteins; 2) Anchoring of the oxidized materials in the arterial wall; and 3) An inflammatory reaction induced in the arterial wall by these condensed products. With increasing age the contribution of the first two processes increases while that of the latter decreases. This hypothesis is suggestive of means whereby the rate of progression of atherosclerosis may be inhibited.

REFERENCES

1. Ahrens, E. H., Tralter, T. T., Hirsch, J., and Insull, W., Jr.: Effects of Dietary Fats on the Serum Lipids of Human Subjects. *J. Clin. Investigation, 34:* 918, 1955.
2. Becker, G. H., Meyers, I., and Necheles, H.: Fat Absorption and Atherosclerosis. *Science, 110:* 529-530, 1949.
3. Byer, S. O., Friedman, M., and Rosenman, R. H.: Review: On the Regulation of Blood Cholesterol. *Metabolism, 1:* 479-503, 1952.
4. Christianson, O. O.: Observation on Lesions Produced in the Arteries of Dogs by Injection of Lipids. *Arch. Path., 27:* 1011-1020, 1939.
5. D'Adelio, G. F.: Fundamental Principles of Polymerization. John Wiley and Sons, Inc., New York, 1952.
6. Eiber, H. B., Goldbloom, A. A., Boyd, L. J., Chapman, I., and Deutschberger, O.: Newer Clinical and Laboratory Studies in the Aged: II. Correlated Serum Lipid Partitions and Lipoprotein Molecules (S_f 0-400) in Patients 80-100 Years of Age: Preliminary Report. *Bull. N. Y. Acad. Med., 30:* 719-720, 1954.
7. Engelberg, H., Kuhn, R., and Steinman, M.: A Controlled Study of the Effect of Intermittent Heparin Therapy on the Course of Human Coronary Atherosclerosis. *Circulation, 13:* 489-498, 1956.
8. Glavind, J., Hartmann, S., Clemmensen, J., Jessen, K. E., and Dam, H.: Role of Lipoperoxides in Human Pathology II. The Presence of Peroxidized Lipids in the Atherosclerotic Aorta. *Acta path. microbiol. Scandinav., 30:* 1-6, 1952.
9. Glazier, F. W., Tamplin, A. R., Strisower, B., de Lalla, O. F., Gofman, J. W., Dawber, T. R., and Phillips, E.: Human Serum Lipoproteins. *J. Gerontol., 9:* 395-402, 1954.
10. Gofman, J. W., de Lalla, O. F., Glazier, F., Freeman, N. K., Lindgren, F. T., Nichols, A. V., Strisower, B., and Tamplin, A. W.: Serum Lipoprotein Transport System in Health, Metabolic Disorders, Atherosclerosis and Coronary Heart Disease. *Plasma, 2:* 413-484, 1954.
11. Gofman, J., Glazier, F., Tamplin, A., Strisower, B., and de Lalla, O.: Lipoproteins, Coronary Heart Disease, and Atherosclerosis. *Physiol. Rev., 34:* 589-607, 1954.
12. Gould, R. G.: Lipid Metabolism and Atherosclerosis. *Am. J. Med., 11:* 209-227, 1951.
13. Graham, D., Lyon, T., Gofman, J., Jones, H., Yankley, A., Simonton, J., and White, S.: Blood Lipids and Human Atherosclerosis. II. The Influence of Heparin Upon Lipoprotein Metabolism. *Circulation, 4:* 666-673, 1951.
14. Greenberg, L. D., and Rinehart, J. F.: Plasma Cholesterol Levels and Cholesterol Fed Controls and Pyridoxine Deficient Monkeys. *Proc. Soc. Exper. Biol. & Med., 76:* 580-583, 1951.
15. Harman, D.: Aging: A Theory Based on Free Radical and Radiation Chemistry. *J. Gerontol., 11:* 298-299, 1956.
16. Haurowitz, F., and Schwerin, P.: Hemin Catalysis in the Interfacial Film between Water and Oil Phases. *Enzymologia, 9:* 193-197, 1941.
17. Holman, R. T.: In *Progress in the Chemistry of Fats and Other Lipids.* Vol. 2, page 51. Pergamon Press Ltd. London, 1954.
18. Hueper, W. C.: Experimental Studies in Cardiovascular Pathology. XI. Thesaurosis and Atheromatosis Produced in Dogs by the Repeated Intravenous Injections of Solutions of Sodium Cellulose Glycolate. *Am. J. Path., 21:* 1021-1029, 1945.
19. Hueper, W. C.: Experimental Studies in Cardiovascular Pathology. III. Polyvinyl Alcohol Atheromatosis in the Arteries of Dogs. *Arch. Path. 31:* 11-24, 1941.
20. Kritchevsky, D., Moyer, A. W., Tesar, W. C., Logan, J. B., Brown, R. A., Davies, M. C., and Cox, H. R.: Effect of Cholesterol Vehicle in Experimental Atherosclerosis. *Am. J. Physiol., 78:* 30-32, 1954.
21. Lansing, A. I.: editor, *Cowdry's Problems of Aging,* 3rd Ed. Williams and Wilkins Co., Baltimore, 1952.

22. Loibman, G. E.: The Influence of Thiamine and Nicotinic Acid on the Cholesterol in the Blood of Rabbits. *Ukrain Biokhim. Zhur., 24:* 19-24, 1952.

23. Marder, L., Becker, G. H., Maizel B., and Necheles, H.: Fat Absorption and Chylomicronemia. *Gastroenterology, 20:* 43-59, 1952.

24. Markowitz, H., Gugler, C. J., Mahoney, J. P., Cartwright, G. E., and Wintrobe, M. M.: Studies on Copper Metabolism. XIV. Copper, Ceruloplasmin, and Oxidase Activity in Sera of Normal Human Subjects, Pregnant Women, and Patients with Infection, Hepatolenticular Degeneration and the Nephrotic Syndrome. *J. Clin. Investigation, 34:* 1498-1508, 1955.

25. Menz, H. C., Davis, W. S.: Effects of Heparin on Development of Atherosclerosis and Fatty Livers. *Arch. Path., 60:* 276-280, 1955.

26. Mosback, E. H., Nierenberg, M., and Kendall, F. F.: Separation of the Air-Oxidation Products of Cholesterol by Column Partition Chromatography. *J. Am. Chem. Soc., 75:* 2358-2360, 1953.

27. Pollak, O. J.: Age and Weight as Factors in the Development of Experimental Cholesterol Atherosclerosis in Rabbits. *Arch. Path., 43:* 387-392, 1947.

28. Rinehart, J. F., and Greenberg, L. D.: Arteriosclerotic Lesions in Pyridoxine-Deficient Monkeys. *Am. J. Path. 25:* 481-491, 1949.

29. Roy, B. R., Davisson, E. O., and Crespi, H. L.: Experiments on the Degradation of Lipoproteins from Serum. *J. Physical Chem., 58:* 841-846, 1954.

30. Rubin, L.: Serum Lipoproteins in Infectious Mononucleosis. *Am. J. Med., 17:* 521-527, 1954.

31. Schroeder, H. A.: Is Atherosclerosis a Conditioned Pyridoxal Deficiency? *J. Chron. Dis., 2:* 28-41, 1955.

32. Tappel, A. L.: The Mechanism of the Oxidation of Unsaturated Fatty Acids Catalyzed by Hematin Compounds. *Arch. Biochem. & Biophysics, 44:* 378-395, 1953.

33. Uri, N.: Inorganic Free Radicals in Solution. *Chem. Rev., 50:* 375-454, 1952.

34. Weitzel, G., Schon, H., and Gey, F.: Anti-Atherosclerotic Effect of the Fat Soluble Vitamins. *Klin. Wchnschr., 33:* 772-773, 1955.

35. Woldow, A., Chapman, J. E., and Evans, J. M.: Fat Tolerance in Subjects with Atherosclerosis: Heparin Effects Upon Lipemia, Lipoproteins and γ-Globulin. *Am. Heart J., 47:* 568-579, 1954.