A Form of Immunological Atherosclerosis

Louis Levy

Riker Laboratories
Northridge, California

During the last two decades, the literature on atherosclerosis has become overwhelming. Not only have there been many original scientific contributions, but the subject has been discussed extensively in review articles and books. The pathology observed in human coronary atherosclerosis appears to be unique but does bear some resemblance to certain phases of both induced and spontaneous atherosclerosis in animals. Factors that appear to contribute to both human and animal vascular derangement embrace a wide diversity of stimuli. Dietary components such as lipids have been, and still are, fashionable as pathogenic agents [1]. There is a strong school of thought that believes the early formation of thrombi is of paramount importance in the human disease [2]. The rheologists believe that the turbulence and flow through vessels of certain sizes contribute profoundly to the pathogenesis of atherosclerosis [3]. Other investigators believe that prospective coronary artery disease patients can be found using personality test interviews [4]. Correlations have been found relating coronary heart disease with hypertension, obesity, cigarette smoking, circulating catecholamines, and heredity [5]. It is obvious from the numerous studies by reputable investigators that the formation of the ultimate vascular change called "atheroma" is probably a common result of a variety of stimuli. Not only do these multiple etiologic factors have a similar pathologic manifestation, but also there must be a receptive vessel in an appropriately receptive individual for the formation of an atheromatous lesion.

The histologic morphology of the experimental and spontaneous atherosclerotic lesion has also been the subject of much controversy. The primary lesion has been thought to be in the intima, the elastic membrane, or the media by different workers. Variabilities in experimental design and observations such as the age of the lesion or the means used for inducing the lesion, as well as in the species under study, have contributed to these different opinions [6].

Our interest is in the relationship of vascular hypersensitivity to atherosclerosis. It had been shown that arterial lesions similar to those seen in humans could be induced in rabbits by immunologic means [7]. The unique localization of the vascular lesion in the arterial tree in experimentally induced serum sickness indicated that the large coronary arteries and the aorta are the prime sites for vascular damage [8, 9]. There have been few studies reported in which high blood cholesterol levels have been superimposed on immunologically induced vascular lesions. Serum sickness was induced in rabbits both on normal diets and cholesterol-supplemented diets, and observations were made on the hearts and aortas. The microscopic observations of these hearts and aortas are the subject of this report.

METHODS

Male New Zealand albino rabbits of approximately 2 kg body weight were obtained locally and used throughout these experiments. A total of 80 rabbits was studied using four different treatments. One group of rabbits was given a single intravenous injection of physiological saline solution and regular rabbit diet (Purina). Another group was given the same i.v. saline injection but also placed on regular feed containing 1% cholesterol. A third group received a single i.v. injection of 250 mg/kg bovine serum albumin (BSA)* plus regular feed. A fourth group received 250 mg/kg of BSA i.v. plus regular feed containing 1% cholesterol. Groups of rabbits were sacrificed at either 2 or 3 weeks from the date of i.v. injection and organs were removed. Gross examinations of the heart, aorta, and kidneys were made at the time of sacrifice and these tissues were fixed in Bouin's solution or 10% neutral formalin for subsequent histological study. Serial sections of either 6 or 20 μ were made of the hearts and aortas. Approximately 300 sections were made of each heart. Some sections were stained with hematoxylin and eosin (H and E), others with Sudan IV for fat, and another group with Weigert–Von Giesen stain for elastic tissue.

RESULTS

The main object of this study was to determine the existence of a phenomenon, and the data will be described and illustrated. Three general types of lesions were observed, depending on the treatment given the animals. Those animals that received the 1% cholesterol diet had a very low frequency of almost pure foam cell growths that occurred exclusively in the aorta. In the group that received the BSA without the cholesterol, about 20% of the animals had endothelial proliferation without discernible amounts

*Nutritional Biochemicals, 2× recrystallized.

Fig. 1. Foam cell plaque in coronary artery of rabbit No. 51. Hematoxylin and eosin preparation from Bouin's-fixed tissue (x 100). The animal received a single injection of 250 mg/kg of BSA and a 1% cholesterol diet for 2 weeks, after which it was sacrificed.

Fig. 2. Marked proliferation in coronary artery of rabbit No. 101. Hematoxylin and eosin preparation from formalin-fixed tissue and cut by frozen section (x 100). The animal received a single injection of 250 mg/kg BSA and a 1% cholesterol diet for 2 weeks, after which it was sacrificed.

Fig. 3. Sudanophilic material below the proliferative area in a coronary artery from rabbit No.101. Sudan IV preparation from formalin-fixed tissue and cut by frozen section (x 100). The animal received a single injection of 250 mg/kg BSA and a 1% cholesterol diet for 2 weeks, after which it was sacrificed.

Fig. 4. Mitotic figure observed in aortic valvular area of rabbit No. 1. Hematoxylin and eosin preparation from Bouin's-fixed tissue (oil immersion, x 1000). The animal received a single injection of 250 mg/kg BSA and a 1% cholesterol diet for 2 weeks, after which it was sacrificed.

Fig. 5. Foam cell accumulation at the point of branching off the aorta of rabbit No. 77. Hematoxylin and eosin preparation from Bouin's-fixed tissue (x 40). The animal received a single injection of 250 mg/kg BSA and a 1% cholesterol diet for 2 weeks, after which it was sacrificed.

Fig. 6. Foam cells in the artery of the aorta of rabbit No. 77 beyond the branching as seen in Fig.5. Hematoxylin and eosin preparation from Bouin's-fixed tissue (x 40).

Fig. 7. Greater magnification of foam cells seen in Fig. 6 of the vessel beyond the branch in the aorta. Hematoxylin and eosin preparation from Bouin's-fixed tissue (x 100).

Fig. 8. Sudan stain seen at a branch in the aorta of rabbit No. 92. Sudan IV preparation from formalin-fixed tissue and cut by frozen section (x 100). The animal received a single injection of 250 mg/kg BSA and a 1% cholesterol diet for 2 weeks, after which it was sacrificed.

Fig. 9. Small breaks in the internal elastica of a coronary artery directly beneath small foam cell plaques in rabbit No. 4. Weigert–Von Giesen stain from Bouin's-fixed tissue (x 450). The animal received a single injection of 250 mg/kg BSA and a 1% cholesterol diet for 2 weeks, after which it was sacrificed.

and eosin preparation
250 mg/kg of BSA and

ylin and eosin prepara-
al received a single
ch it was sacrificed.

ery from rabbit No.101.
100). The animal
2 weeks, after which

natoxylin and eosin
eceived a single injec-
was sacrificed.

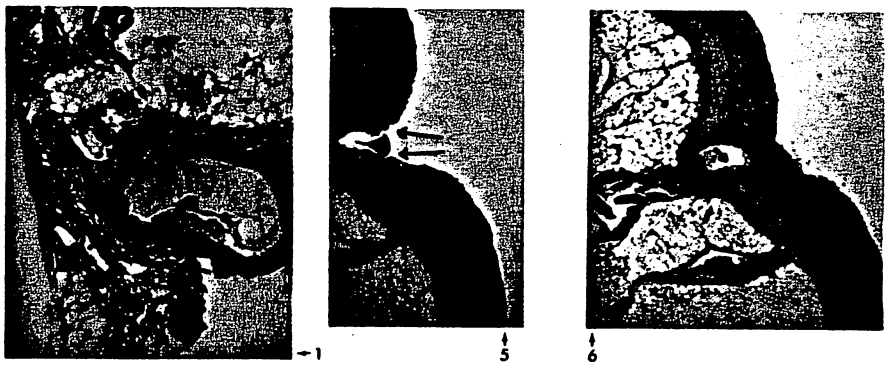
bbit No. 77. Hema-
eceived a single injec-
was sacrificed.

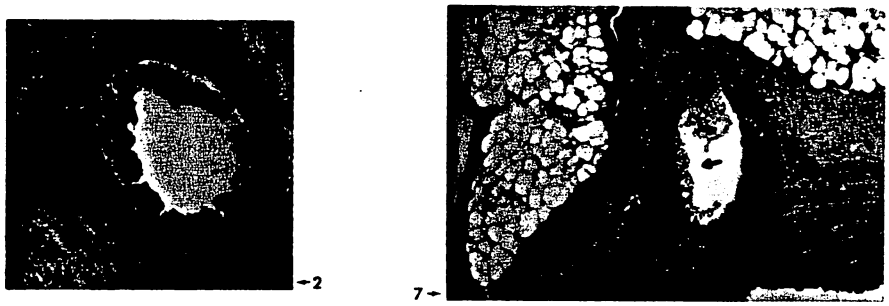
nching as seen in Fig.5.

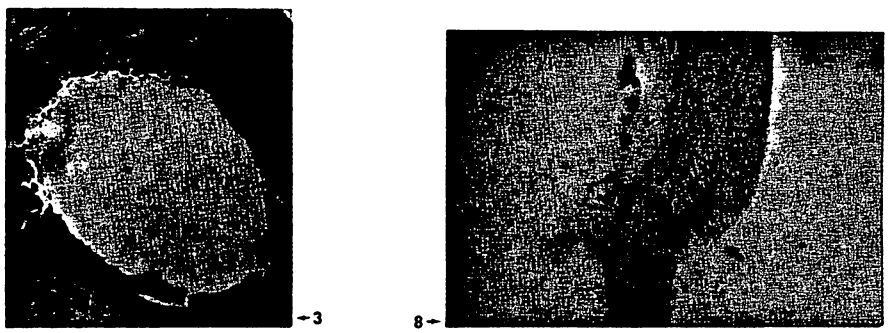
nd the branch in the

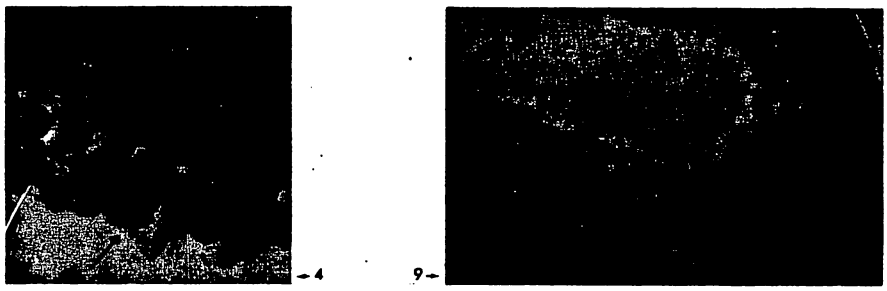
preparation from form-
single injection of 250
ed.

neath small foam cell
(x 450). The animal
2 weeks, after which









of foam cells in the aorta, valvular areas, or coronary arteries. When both BSA and cholesterol were administered, about 50% of the animals had endothelial proliferation with foam cells also present. None of the saline control animals showed any changes. No gross pathology was observed at autopsy in the heart, aorta, or kidneys in any of the four groups of animals.

General Locations of Lesions

In all cases where lesions were observed in the heart, they were in areas of the aortic valves and the coronary artery outflows from the aorta. Those parts of the aorta that showed changes were primarily at branches coming off the aorta.

Histological Description

Three general types of lesions were observed. Some showed only a proliferation, whereas others appeared to be comprised of foam cells. A third group contained a mixture of foam cells and proliferation. In Fig. 1, a coronary vessel can be seen with an intimal growth identified as foam cells. Figure 2 illustrates a coronary artery with marked endothelial or intimal proliferation. The tissue in Fig. 2 was also stained for fat, and Fig. 3 illustrates sudanophilic material in the proliferative area. Figures 2 and 3 were made from thick (20 μ) frozen sections. Figure 4 illustrates the process of mitosis in the proliferative area. It can be seen from these illustrations that the alterations observed are in the endothelium with no apparent involvement of the media.

In the aorta, foam cells appeared at the branches, as can be seen in Fig. 5. The proliferation continued in the branch beyond the aortic orifice, as can be seen in Figs. 6 and 7. Areas of aortic branches stained for fat contained sudanophilic material, as seen in Fig. 8. Figure 9 is a vessel examined with Weigert stain, showing the internal elastic membrane. Small breaks are noticeable in the membrane directly under the growth points.

DISCUSSION

Numerous theories have been put forth to explain the pathogenesis of atherosclerosis. From the morphological point of view, it is generally agreed that the areas of high blood pressure and great turbulence are the most susceptible to injury. This is consistent with our and other findings, that most lesions are found in the larger coronary arteries, directly off the aortic valves, and in the branches off the aorta, primarily near the arch. In addition to the physical aspects of pressure and turbulence, injury to the vessels and high blood lipid levels are the other members of the "troika" considered necessary parts of the optimal environment for the occurrence of the lesions. The mild injury induced by serum sickness in the presence of high lipid levels appeared to be capable of predisposing a vascular site for experimental atherosclerosis. The accompanying illustrations indicate that some lesions have been obtained superficially resembling early athero-

sclerosis. In the series of animals receiving both BSA and cholesterol, approximately 50% showed lesions. Those with BSA alone showed a 20% incidence, and in the cholesterol or saline control groups no lesions were seen in the heart.

Living systems are exposed to a hostile environment that includes immunological sensitization. This sensitization is at times acutely life-threatening, as in the case of anaphylactic shock, or life-sparing, as when there are circulating protective antibodies. It is reasonable to conceive of a condition which is not acute but can occur over many years, in which an individual episodically builds up circulating antibodies to circulatory antigens causing a mild, subclinical form of serum sickness. If these antigen–antibody complexes are formed in an environment of high blood lipids, the vascular damage can be self-perpetuating. Under these circumstances, the lipid incorporated in the intimal tissue can act as an injury (or irritant) to sustain the lesion. Under normal circumstances, serum thickness heals and leaves little or no residual damage. However, the inclusion of lipid may contribute to the preparation of a focal site for continued and future damage. Hypertension may be another synergistic factor in preparing the vascular bed for the ultimate atherosclerosis, since Wilens [10] showed that serum sickness is enhanced by elevated blood pressure. Small molecular substances acting as haptens can also cause immunological injury to the coronaries and the aorta [11].

If continued exposure to immunological insult leads to vascular damage, this offers some therapeutic opportunities. Since antigen–antibody complexes are localized in discrete areas, presumably by phagocytic mechanisms as some authors believe [9], we might alter the course of the reaction by pharmacologically influencing the reticuloendothelial system (RES) and phagocytic cells to remove these complexes. Indeed, some investigators are already looking into the problem of the role of the RES in systemic immunological reactions [12, 13].

Second, it is believed that leucocytes are instrumental in the phagocytic process, and may release chemical mediators that cause the inflammation or proliferation [14]. The use of antagonists to these chemical mediators might abort the pathological processes leading to atherosclerosis. Some evidence has already been obtained indicating that certain monoamine oxidase inhibitors [15], antihistaminics [16], antibradykinin agents [17], and cortisone [18] are capable of altering either serum sickness or experimental atherosclerosis.

In the light of the above argument, it is believed that ultimate decreased morbidity and mortality can be achieved. Pharmacologically, it is now possible to alter the blood pressure to decrease the risk from changes in cardiovascular dynamics. Either diet or drugs like heparin or heparinoids

[19, 20] can lower lipemia and thereby decrease added stress to the vessels. The probability of thrombus formation can be attacked from two major areas. If thrombus formation is believed to be independent of injury to the vessel, then anticoagulant agents or fibrinolytic agents can be used. However, if thrombus formation is part of the sequelae of injury, then certain types of anti-inflammatory agents should suppress the process. It is to this latter phenomenon that this investigation has been directed. Injury has been induced by mild immunological means and lipid has appeared to be incorporated into this inflamed area. When the mediator or mediators that are responsible for the injury have been found, then appropriate pharmacological depressants should be capable of reducing the morbidity and mortality from atherosclerotic heart lesions.

SUMMARY

Serum sickness was induced in hypercholesterolemic rabbits by a single i.v. injection of bovine serum albumin. After two or three weeks, the animals were sacrificed and serial sections were made of the heart and aorta. It was found that the animals on a cholesterol diet and with serum sickness had a high incidence of vascular damage at the aortic valves, coronary arteries, and the branches at the arch of the aorta.

ACKNOWLEDGMENTS

The author wishes to thank J.K. Smith, R. Gayek, L. Seifert, M. von Diepow, and P. Cohen for their help on this study.

REFERENCES

1. H.F. Watts, "The mechanism of arterial lipid accumulation in human coronary artery atherosclerosis," in: W. Likoff and J.F. Moyer, Eds., Coronary Heart Disease. New York, Grune & Stratton, 1963.
2. G. Pickering, "Pathogenesis of myocardial and cerebral infarction: modular arteriosclerosis," Brit. Med. J., 1:517, 1964.
3. M. Texon, "The role of vascular dynamics in the development of atherosclerosis," in: M. Sander and C.H. Bourne, Eds., Atherosclerosis and Its Origin. New York, Academic Press, 1963.
4. R.H. Rosenman and M. Friedman, "Behavior patterns, blood lipids, and coronary heart disease," J. Am. Med. Ass., 184:934, 1963.
5. J.W. Gofman, Coronary Heart Disease. Springfield, Illinois, Charles C. Thomas, 1959.
6. J.C. Roberts and R. Strauss, Eds., Comparative Atherosclerosis. New York, Hoeber Medical Div. of Harper & Row, 1965.
7. O. Saphir, D. Stryzak, and L. Ohringer, "Hypersensitivity changes in coronary arteries of rabbits and their relationship to arteriosclerosis," Lab. Invest., 7:434, 1958.

8. F.G. Germuth, Jr., "A comparative histologic and immunologic study in rabbits of induced hypersensitivity of the serum sickness type," J. Exptl. Med., 97:257, 1953.
9. W.T. Kniker and C.G. Cochrane, "Pathogenic factors in vascular lesions of experimental serum sickness," J. Exptl. Med., 122:83, 1965.
10. S.L. Wilens, "Enhancement of serum sickness lesions in rabbits with pressor agents," Arch. Pathol., 80:590, 1965.
11. O. Saphir, M. Telischi, and L. Ohringer, "Rabbit sulfa drug hypersensitivity and lesions resembling arteriosclerosis," Arch. Pathol., 73:414, 1962.
12. D.A. Blickens and N.R. DiLuzio, "Induction of phagocytic inhibition and the generalized Shwartzman phenomena by the administration of plasma from reticuloendothelial hypofunctional mice," J. Reticuloendothelial Soc., 2:187, 1965.
13. P.D. Mott and S.M. Wolff, "The association of fever and antibody response in rabbits immunized with human serum albumin," J. Clin. Invest., 45:372, 1966.
14. W.T. Kniker and C.G. Cochrane, "Localization of circulating Ag–Ab complexes in serum sickness," Federation Proc., 25:474, 1966.
15. T. Shimamoto, "The relationship of edematous reaction in arteries to atherosclerosis and thrombosis," J. Atherosclerosis Res., 3:87, 1963.
16. D. Harman, "Inhibiting effect of an antihistaminic drug, chlorpheniramine," Circulation Res., 11:277, 1963.
17. T. Shimamoto, F. Numano, and T. Fujita, "Atherosclerosis-inhibiting effect of an antibradykinin agent, pyridanolcarbamate," Am. Heart J., 71:216, 1966.
18. M. Friedman, S. Byers, and S. St. George, "Cortisone and experimental atherosclerosis," Arch. Pathol., 77:56, 1964.
19. L. Levy and G. Cronheim, "Blood levels and clearing activity of a synthetic heparinoid," Biochem. Pharmacol., 7:27, 1961.
20. L. Levy and F.J. Petracek, "Chemical and pharmacological studies on N-resulfated heparin," Proc. Soc. Exptl. Biol. Med., 190:901, 1962.

The Reticuloendothelial System *and* Atherosclerosis

Proceedings of an International Symposium on Atherosclerosis and the Reticuloendothelial System, Held in Como, Italy, September 8–10, 1966

Edited by

N. R. Di Luzio

Department of Physiology and Biophysics
University of Tennessee Medical Units
Memphis, Tennessee

and

Rodolfo Paoletti

Institute of Pharmacology
University of Milan
Milan, Italy

PLENUM PRESS · NEW YORK · 1967

1006)Levy L.

A Form of Immunological Atherosclerosis.

Advances in Experimental Medicine and Biology. 1967;1:426-432.

MACROPHAGE /HEART /WOW

"Serum sickness was induced in hypercholesterolemic rabbits by a single i.v. injection of bovine serum albumin. After two of three weeks, the animals were sacrificed and serial sections were made of the heart and aorta. It was found that the animals on a cholesterol diet and with serum sickness had a high incidence of vascular damage at the aortic valves, coronary arteries, and the branches of the aorta."