EFFECT OF HYPERCHYLOMICRONEMIA AND HYPERPREBETALIPOPROTEINEMIA ON THE VASCULAR WALL IN RATS OF VARIOUS AGES

P. P. Chayalo and G. V. Kopylova

UDC 616.13-004.6-092:616.153.965.915-074-092.9

The effect of hyperchylomicronemia and hyperprebetalipoproteinemia on the vascular wall was studied in rats aged 6-10 and 26-30 months. The animals were given an intravenous injection of blood serum from rats which themselves had previously been injected intraperitoneally with Triton WR1339. Inclusions of chylomicrons and lipoproteins of very low density were found electron-microscopically in the endothelium of the old rats only. Swelling of the Golgi complex and mitochondria and dilatation of the cisternae of the endoplasmic reticulum in the endothelial cells of the young rats indicated activation of intracellular metabolism. The study of biosynthesis of certain classes of lipids in the aorta under these conditions showed inhibition of biosynthesis of free cholesterol, the intensity of which was the same in rats of different ages. Increased phospholipid biosynthesis was observed only in the young animals.

KEY WORDS: age; vascular wall; hyperchylomicronemia and hyperprebetalipoproteinemia; lipid biosynthesis

In the modern view the development of atherosclerotic lesions of the walls of blood vessels is due largely to the character of disturbances in lipid and lipoprotein metabolism in the blood, namely in the type of hyperlipoproteinemia. Types IIa, IIb, and IV, characterized by a high level of cholesterol and low-density lipoproteins (β-lipoproteins), are generally regarded as the most atherogenic [1, 4]. A problem not yet finally settled is the atherogenicity of other types of hyperlipoproteinemias, characterized by elevation of the blood level of very low density lipoproteins (VLDL) and chylomicrons (ChM). Several workers state that ChM cannot penetrate through the endothelium and are split up in the blood stream or on the surface of the endothelial cells [9, 10]. However, Foroglou-Kerameos [7] and Mjos et al. [8], using electron microscopy, found formations resembling VLDL and ChM in character in the endothelial cells and subendothelial space under the conditions of hyperlipidemia. When the role of these classes of lipoproteins in cardiovascular pathology is assessed it must be remembered that a high frequency of hypertriglyceridemia and disturbance of tolerance to exogenous glucose are regular phenomena which accompany the development of atherosclerotic lesions, especially in old age, and they may be reflected in particular by changes in VLDL and ChM metabolism.

The object of the present investigation was to study the character of the effect of hyperprebetalipoproteinemia and hyperchylomichronemia on the vascular wall in animals of different ages.

EXPERIMENTAL METHOD

Experiments were carried out on 68 rats. Hyperlipidemia was produced in young animals by intraperitoneal injection of Triton WR1339 in a dose of 500 mg/kg body weight. Analysis of changes in lipid and lipoprotein metabolism developing under these circumstances in the blood (from the standpoint of the type of hyperlipoproteinemia [4]) showed that they belonged to type V, i.e., hyperchylomicronemia and hyperprebetalipoproteinemia [3]. In the basic experiments, to rule out the possibility of the local effect of Triton on the vascular wall, lipemic blood serum from "Triton" animals was injected intravenously into young (6-10 months) and old (26-30 months) rats (1 ml/100 g body weight). Under these circumstances, biochemical determination of total cholesterol, triglycerides, total lipids, and the combined fraction of lipoproteins ($\alpha < 1.063$; β-lipoproteins, prebetalipoproteins, and chylomicrons) was carried out after animals of different ages had been injected with blood serum containing the same concentration of lipids and lipoproteins (the concentration of the latter was 1246 mg%).

Laboratory of Pathological Physiology, Institute of Gerontology, Academy of Medical Sciences of the USSR, Kiev. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Gorev.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 84, No. 12, pp. 664-667, December, 1977. Original article submitted April 20, 1977.

 0007-4888/77/8412-1696$07.50

Fig. 1. Area of cytoplasm of aortic endothelial cell from old rats after injection of lipemic blood serum. V) Vacuoles, M) mitochondria, SE) subendothelium. Stained with uranyl acetate and lead, 21,900 ×. [Figures 1, 2, and 3 not labeled in Russian original – Consultants Bureau.]

The biosynthesis of the individual classes of lipids in the tissues of the aorta was determined in vitro by the method described in [3]. An electron-microscopic technique was used.

The significance of the results was assessed statistically [2].

EXPERIMENTAL RESULTS

Part of an endothelial cell from the aorta of an old rat is illustrated on the electron micrograph in Fig. 1. The large vacuoles 0.3-1.5 μ in diameter, filled with amorphous electron-dense material, appeared a few minutes after injection of the lipemic serum. They could also be found when the vessel wall was examined after 1 h. Similar inclusions have been found in the endothelium of the coronary arteries of animals. Casley-Smith [5], who compared the structure of isolated ChM and of ChM in the mammary gland, showed that these complexes acquire this appearance in tissue sections. Meanwhile, in the control old animals no such formations were observed in the endothelium (Fig. 2).

After injection of lipemic blood serum into the young animals no electron-dense inclusions of lipid–protein complexes could be found in the endothelial cells (Fig. 3). Nevertheless, swelling of the Golgi complex and mitochondria and dilatation of the cisternae of the endoplasmic reticulum, which were observed under these circumstances, are evidence of activation of intracellular metabolism, possibly in connection with the action of the injected lipoproteins on the vessel wall. An argument in support of this explanation is given by the results of a study of the biosynthesis of certain classes of lipids (phospholipids, triglycerides, cholesterol esters, and free cholesterol) in the aortic tissue of experimental rats of different ages, which was carried out 1 h before the experiments began.

As Table 1 shows, under normal conditions differences were observed only in the biosynthesis of free cholesterol in the vessel wall between the rats aged 6-10 and 26-30 months. The significance of the small decrease in the specific activity of the remaining classes of lipids studied during aging could not, however, be confirmed by statistical analysis.

TABLE 1. Specific Activity of Aortic Lipids of Young and Old Rats under Normal Conditions and after Injection of Lipemic Serum (cpm/mg lipids)

Index studied and age of animals, months	Control (normal)	Injection of lipemic serum
Free cholesterol		
6—10	1250±189[a,6]	527±114
26—30	695±117[6]	312±56
Cholesterol esters		
6—10	2055±302	3201±539
26—30	1610±293	2285±431
Phospholipids		
6—10	3828±594[6]	5926±747
26—30	3146±458	3637±543
Triglycerides		
6—10	5488±638	4635±574
26—30	4683±692	4482±780

Legend. a) Statistically significant differences between age groups of rats; b) the same, between experimental and control rats. Differences between arithmetic mean values of groups were considered to be statistically significant at the level of probability $P < 0.05$. The groups included at least seven animals.

Fig. 2

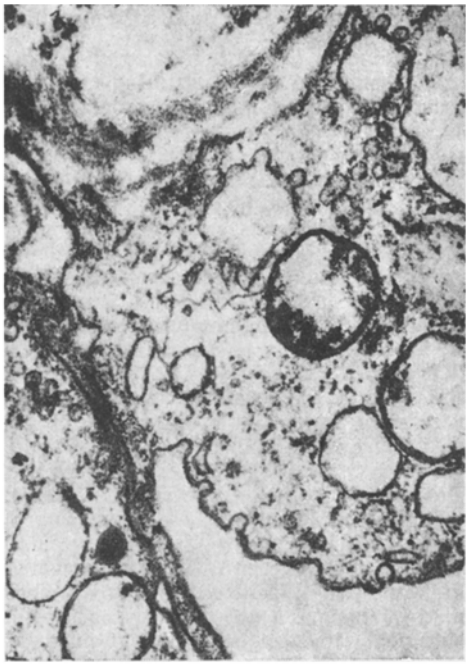

Fig. 3

Fig. 2. Area of aortic endothelial cell of normal old rat. R) Ribosomes, M) mitochondria. Stained with uranyl acetate and lead, 36,240 ×.

Fig. 3. Area of boundary between two endothelial cells in coronary artery of young rats after injection of lipemic blood serum. V) Vacuoles, M) mitochondria. Stained with uranyl acetate and lead, 36,240 ×.

After intravenous injection of lipemic serum into the animals differences were observed in the biosynthesis of individual classes of lipids in the animals. The differences related not so much to the character of the response as to the degree of change in the value of the specific activity. For instance, a decrease in the biosynthesis of free cholesterol, some increase in the biosynthesis of cholesterol esters and phospholipids, and

insignificant deviations in triglyceride biosynthesis were common features in rats of the two age groups. Inhibition of free cholesterol biosynthesis in the experimental rats was considerable and was significant in both the young and the old animals. The increase in phospholipid biosynthesis in the young animals also was statistically significant and may have been facilitated by expulsion of the hydrophobic cholesterol molecule formed during metabolic conversion of the lipoproteins [6, 11]. Meanwhile all the other changes relating to triglycerides and cholesterol esters in rats of different ages and to phospholipids in the old animals were not statistically significant.

The results obtained thus indicate that the character of the effect of hyperlipoproteinemia, due to an increased concentration of ChM and prebetalipoproteins, on the vascular wall is largely determined by the age factor. In particular, the less marked activation of biosynthesis in the vascular wall of the old animals after injection of lipemic serum, reflecting depression of catabolism of the lipoprotein complexes, could be the reason why ChM and VLDL were found electron-microscopically in the endothelium of the old animals under these experimental conditions.

LITERATURE CITED

1. A. N. Klimov, N. G. Nikul'cheva, and I. V. Krivoruchenko, Kardiologiya, No. 12, 103 (1974).
2. E. V. Montsevichyute-Éringene, Patol. Fiziol., No. 4, 71 (1964).
3. P. P. Chayalov, in: Working Capacity, Health, and Longevity (Annual Report of the Institute of Gerontology, Academy of Medical Sciences of the USSR) [in Russian], Kiev (1977), p. 177.
4. J. L. Beaumont, L. A. Carlson, G. R. Cooper, et al., Circulation, 45, 501 (1972).
5. J. R. Casley-Smith, J. Cell Biol., 15, 259 (1962).
6. S. Dayton and S. Hashimoto, Atherosclerosis, 12, 371 (1970).
7. C. Foroglou-Kerameos, Arch. Anat. (Strasbourg), 52, 391 (1969).
8. O. D. Mjos, O. Faergeman, and R. L. Hamilton, et al., J. Clin. Invest., 56, 603 (1975).
9. J. Scheuer and R. E. Olson, Am. J. Physiol., 212, 301 (1967).
10. A. Vost, J. Lipid. Res., 13, 695 (1972).
11. D. B. Zilversmit, M. L. Shore, and R. F. Ackerman, Circulation, 9, 581 (1954).