EXPERIMENTAL ATHEROSCLEROSIS IN THE RAT *

JOSEPH H. BRAGDON, M.D., and OLAF MICKELSEN, Ph.D.

From the Laboratory of Metabolism, National Heart Institute (Dr. Bragdon), and Laboratory of Nutrition, National Institute of Arthritis and Metabolic Disease (Dr. Mickelsen), Bethesda 14, Md.

The rat has proved refractory to the development of experimental atherosclerosis. Page and Brown[1] were able to maintain very high serum-cholesterol levels for prolonged periods by feeding high-cholesterol, high-cholic-acid diets to hypothyroid rats. Although the aortas and coronary arteries of their animals showed lipid deposition, they found no foam cells or cellular proliferation and concluded that the vessels of the rat did not respond to the presence of lipid. Several other groups, however, have claimed success in producing lesions simulating atherosclerosis in this species. In 1952, Wissler *et al.*[2] reported moderate hypercholesterolemia and coronary atheromas in old rats fed a high-fat diet for many months. Increasing the choline content of the diet increased the incidence of lesions. In the same year Hartroft *et al.*[3] reported the production of lipid deposits in the coronary arteries of rats on choline-deficient diets. More recently Malinow *et al.*[4] have reported atheromatous lesions following the forced feeding of vegetable oil in the presence of unilateral cellophane perinephritis. Also in 1952 one of us[5] reported the production of early lesions in rats by the repeated intravenous injection of lipoproteins from the serum of cholesterol-fed rabbits. Lesions are readily produced in rabbits by this method,[6] but the occurrence of spontaneous lesions[7] is a complicating factor in acute experiments. The purpose of the present paper is to report in more detail the effects of injecting serum lipoproteins into rats and to illustrate the tissue response of rats to hypercholesterolemia.

METHODS

Young rabbits were rendered hypercholesterolemic by feeding a 1 per cent cholesterol diet. The cholesterol was dissolved in ether and the solution distributed over commercial rabbit pellets. After 6 to 10 weeks the animals were exsanguinated. Ten gm. of sodium chloride were added to each 100 ml. of serum. This increased the saline density to about 1.065. The serum was then centrifuged for 18 hours in the 30 rotor of the Spinco model L ultracentrifuge at 78,000 G. Under these

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conditions all the low-density lipoproteins (Gofman's Sf classes[8]) rose to the surface, where they appeared as a butter-like mass. This material re-emulsified readily in physiologic saline solution, against which it was then dialyzed to remove any excess salt. No attempt was made to keep the emulsions sterile. In the chronic experiments fresh emulsions were prepared weekly.

The experimental animals were male Sprague-Dawley rats weighing about 200 gm. Injections were made into a tail vein. The volume injected varied from 1 to 4 ml. At the termination of an experiment the animals were exsanguinated from the aorta under sodium pentobarbital anesthesia. The heart and thoracic aorta were removed en bloc and irrigated with physiologic saline solution. They were then fixed in formalin, embedded in gelatin, sectioned with the freezing microtome, and stained with oil red O and hematoxylin.

The following lipid analyses were made both of the emulsions and of the recipients' serum: free and total cholesterol,[9] phospholipid,[10] and total fat.[11] From these, neutral fat was determined by difference.

Other rats were offered the following diet: vitamin-test casein, 19 per cent; dextrin, 31 per cent; lard, 30 per cent; wheat bran, 6 per cent; salt mixture, 6 per cent; complete vitamin powder, 5 per cent; cholic acid, 2 per cent; oleic acid, 1 per cent; cholesterol, 0.3 per cent, and 6-propyl-2-thiouracil, 0.065 per cent. Fat-soluble vitamins were supplied as Natola,* 10 drops per kg. of diet. The animals were housed in individual cages and offered the diet and water ad libitum.

Results

Because the lipoprotein emulsions represented pools of several donor rabbits, they were relatively constant in chemical composition. The following analysis, expressed as mg. per 100 ml., is typical: total cholesterol, 6,860; free cholesterol, 1,740; phospholipid, 2,440, and neutral fat, 980. The average cholesterol-phospholipid ratio was 2.8. Some preparations were more concentrated.

The greater the injected dose, expressed as mg. of total cholesterol per kg. of body weight, the higher was the serum cholesterol level 24 hours later. Thus, rats receiving a dose of 2,650 mg. had serum levels of about 2,650 mg. per 100 ml.; animals receiving 2,000 mg. had serum levels of about 1,250, and those receiving 650 mg. had levels of 180. The serum of normal rats contains about 50 mg. of cholesterol and 100 mg. of phospholipid per 100 ml. After subtracting these values, the cholesterol-phospholipid ratio in several rats receiving the same

* Parke, Davis & Co., Detroit, Michigan.

emulsion dropped from the immediate post-injection value of 2.9 to about 2.5 at 7 hours and 1.6 at 24 hours.

Control rats have never shown stainable lipid within the intima or media of the aorta or coronary arteries, or in the endocardium. Animals receiving lipoproteins in doses of 1,000 mg. total cholesterol per kg. uniformly show focal deposits of sudanophilic material at 24 hours. These areas are characterized by small, discrete granules of lipid within the cytoplasm of endothelial cells and, in the endocardium, in cells immediately beneath the endothelium. In the aorta there may also be very fine sudanophilic particles, apparently extracellular, along the inner surface of the innermost elastic lamella. In the aorta these deposits appear to have no sites of predilection, such as about the mouths of the great vessels. In the heart they appear on the aortic cusps and mitral leaflets. The endothelium of the coronary arteries is only rarely involved. With larger doses these changes have been observed as early as 4 hours after injection.

More advanced lesions may be produced either by continuous intravenous infusion or by repeated daily injections. Two rats received a constant infusion at a rate slightly in excess of 1 ml. per hour for 24 hours, at which time their serum cholesterol levels were 11,700 and 10,700 mg. per 100 ml., respectively. Endothelial and subendothelial cells of the endocardium had assumed the appearance of foam cells and there was evidence of their focal proliferation.

Figure 1 shows a section of aorta of a rat which had received five daily injections. At one end of the photomicrograph the endothelial cells contain lipid granules; at the other end there is a collection of sudanophilic material which appears to have accumulated along the inner surface of the most internal elastic lamella. There is no evidence of tissue reaction.

Sixteen animals received daily intravenous injections for periods varying from 22 to 30 days. All animals gained weight during that time. Figures 2 and 4 illustrate lesions in a rat killed 24 hours after receiving 28 daily injections. Figure 2 shows a lesion on the endocardial surface of the left ventricle. The endocardium is thickened by masses of foam cells. Figure 4 shows a coronary artery at its origin from the aorta. Two focal accumulations of foam cells are protruding into the lumen of the vessel. Schultz stains revealed cholesterol in these lesions. Neither necrosis nor inflammatory response was seen. The sections originally showed no anisotropism, but doubly refractile crystals became abundant with the passage of time. Other coronary lesions have been limited to infiltration of the intima and media with-

out cellular reaction. In the aorta this infiltration has been limited to the intima.

Some of the rats receiving repeated injections were permitted to survive for periods up to 3 months following the last injection. The amount of sudanophilic material in the endocardium gradually diminished although even at 3 months some was present both intracellularly and extracellularly. Anisotropic crystals became a prominent feature of the lesions. Eventually they were embedded in fibrous tissue, although at no time was there evidence of necrosis or active inflammation.

Examination of other organs of rats killed 24 hours after 28 daily injections showed slight enlargement of the adrenal glands, and microscopic, but not gross, fatty infiltration of the liver. Sudanophilic material was present within a few cells of the splenic corpuscles and within the renal glomeruli. The tubules, however, were practically free of fat. The pulmonary arteries occasionally showed some sudanophilia. Other vessels were not examined.

No quantitative or qualitative differences were observed in the tissue response of 2 female and 2 male rats receiving repeated injections of the same material. Old males were compared with young males, the dose being constant on a weight basis. The older animals appeared to have more extensive lesions than the younger. Old animals were not used routinely in this study because greater difficulty was encountered in injecting their tail veins.

Twenty-four hours following intraperitoneal injection the serum cholesterol level was higher than following an intravenous injection of the same dose. Foam-cellular lesions were produced following repeated intraperitoneal injections, but peritonitis was a complicating factor.

Three rats received 28 daily intravenous injections of a synthetic cholesterol emulsion in a dose of 200 mg. per kg. per day. Twenty-four hours after the last injection serum cholesterol levels were in the normal range and no sudanophilic material could be found in the endocardium or arterial intima. In this respect the rat differs from the rabbit, which converts injected cholesterol into lipoproteins. The emulsions could not be concentrated to permit larger doses.

In order to compare these lesions with those observed in hypothyroid rats fed cholesterol and cholic acid, 5 rats were placed on the diet described. All animals slowly lost weight over a period of 22 weeks, at the end of which time they were killed. Serum cholesterol levels at that time averaged 944 mg. (776 to 1,370) per 100 ml. The cholesterol-phospholipid ratio averaged 2.0 (1.7 to 2.2). All animals showed

vascular sudanophilic deposits. In 2 cases there were foci of foam cell proliferation in the endocardium. They appeared identical to those produced by injecting lipoproteins. Figure 3 illustrates such a lesion on a mitral leaflet. Lesions in the coronary arteries were characterized by foci of massive infiltration of the media with sudanophilic, anisotropic material but without tissue reaction. The infiltration occasionally was sufficient to reduce the lumen of the vessel substantially. Lesions in the aorta were, like those following lipoprotein injections, without cellular reaction.

Discussion

The evidence presented here demonstrates that parts of the vascular system of the rat are capable of responding to hypercholesterolemia in an atheromatous manner. Figures 2 and 3 from injected and dietary rats, respectively, show masses of foam cells thickening the endocardium. This has been a frequent finding. Figure 4 shows the same process in a coronary artery near its ostium. This remains a unique example in our material. Usually, in the smaller branches, there are seen only foci of massive infiltration of the wall with sudanophilic, anisotropic material, but without cellular reaction. An illustration in a recent paper by Wissler *et al.*[12] apparently shows foam-cellular proliferation in a coronary artery.

In our experience, with the one exception illustrated, tissue reaction to the presence of lipid has occurred only in the endocardium. These proliferating lesions may be lost when frozen sections are cut from unembedded hearts. This may explain the failure of Page and Brown[1] to observe them.

To date, apparently no one has succeeded in producing atheromatous lesions in the aorta of the rat. The lesions illustrated in the paper by Hartroft *et al.*[3] from choline-deficient rats represent primarily medial degeneration. It is possible that this immunity of the aorta of the rat has an anatomical basis. The intima consists of little more than endothelium. We have seen masses of lipid granules between the innermost elastic lamella and the endothelium, so that the latter protrudes into the lumen, and yet there has been no evidence of cellular reaction.

The data further suggest that the development of atheromatous lesions in the endocardium of the rat depends primarily on maintaining a sufficient concentration in the blood of cholesterol-bearing lipoproteins for a sufficient period. This does not deny that factors such as hypertension and local vascular injury may accentuate the atherosclerotic process.

Summary

Focal atheroma-like lesions have been produced in the rat by intravenous or intraperitoneal injection of lipoproteins from the serum of cholesterol-fed rabbits. The lesions are characterized by groups of foam cells in the endocardium and intima and later by masses of anisotropic crystals embedded in fibrous tissue. Similar lesions have appeared in hypothyroid rats fed a diet relatively high in cholesterol and cholic acid. The lesions occur most frequently on the aortic and mitral valves and in the endocardium of the left ventricle. One such lesion has been found in a coronary artery near its ostium. Coronary arteries usually show only massive infiltration of the wall without tissue reaction. The aortic intima may also show heavy deposits of lipid without tissue reaction. It is concluded that the arteries of the rat are relatively immune to those lipoproteins which in the rabbit are highly atherogenic.

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[*Illustrations follow*]

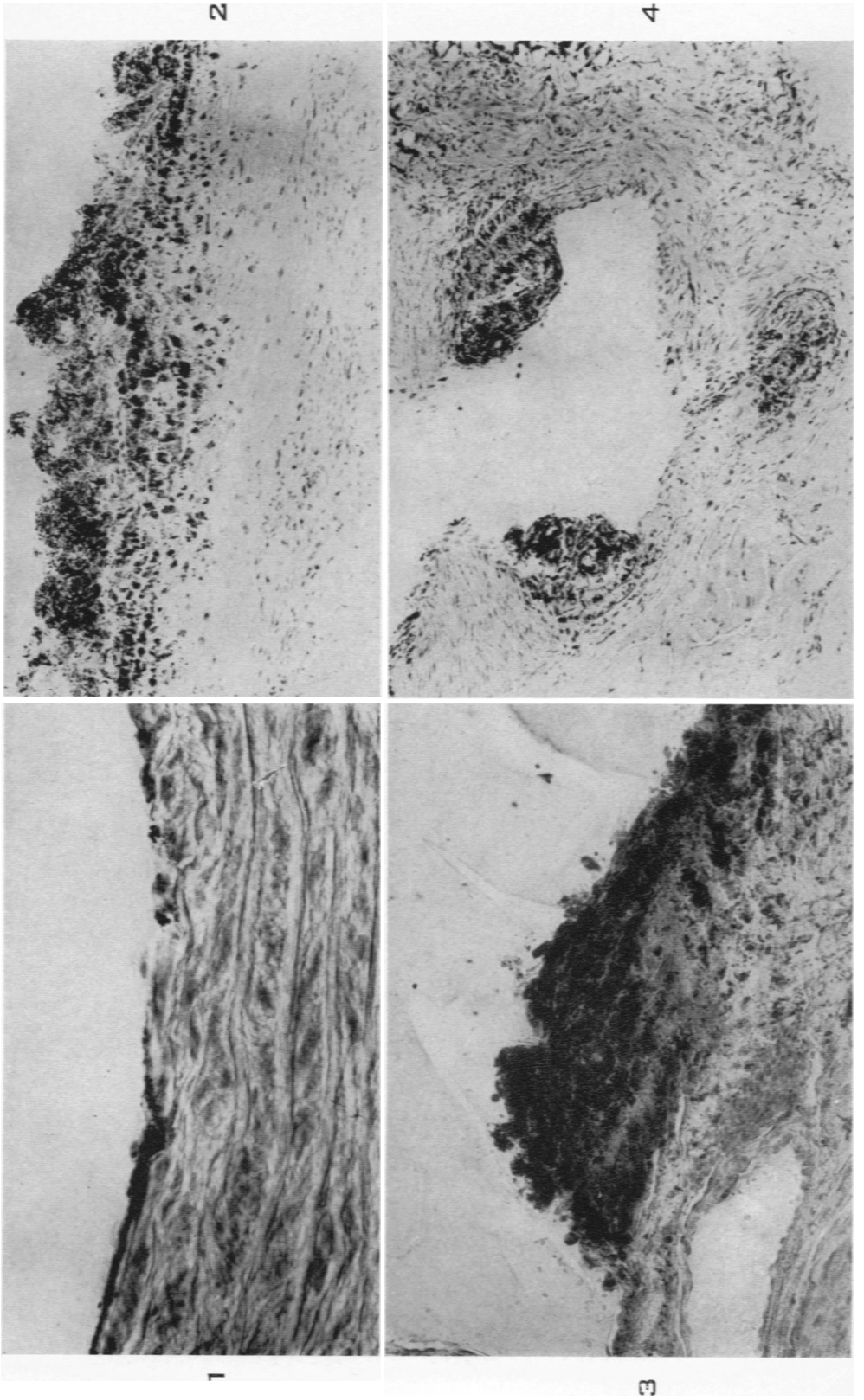
LEGENDS FOR FIGURES

FIG. 1. Aorta of a rat which received five daily intravenous injections of lipoproteins from the serum of cholesterol-fed rabbits. Sudanophilic material is apparent both within endothelial cells and extracellularly between endothelium and innermost elastic lamella. × 210.

FIG. 2. Endocardium of left ventricle of a rat which received twenty-eight daily injections of lipoprotein. The endocardium is thickened by masses of foam cells. × 140.

FIG. 3. Section of base of mitral leaflet from a rat with dietary hypercholesterolemia. The endocardium is greatly thickened by foam cells. Under polarized light this lesion contained doubly refractile crystals. × 170.

FIG. 4. Section through ostium of a coronary artery of the same rat from which Figure 2 was secured. Two masses of foam cells protrude into the lumen of the vessel. Sudanophilic material is seen also within the aortic intima and within the media of a small coronary branch, but without cellular reaction. × 140.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