

Accumulation of lipids in the leukocytes of rats fed atherogenic diets

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SUMMARY Wistar rats were fed various atherogenic diets containing 5% cholesterol, 0.3% thiouracil, 2% bile salt, and 40% fat (butter, corn oil, peanut oil, or medium-chain triglycerides (MCT)). Blood specimens were obtained weekly for counting lipid-laden leukocytes (lipophages) and serum cholesterol levels were determined. The lipophages in circulating blood were defined as leukocytes, usually monocytes and lymphocytes, containing more than one vacuole stainable with oil red O in the cytoplasm.

Rats fed the butter diet showed significantly higher numbers of lipophages and higher levels of serum total cholesterol than any other group, and grossly recognizable atherosclerosis of the thoracic and abdominal aorta was present only in these rats. The number of lipophages in circulating blood did not necessarily correspond to levels of blood cholesterol; the group of rats fed MCT had a relatively high level of blood cholesterol and low count in lipophages. Lipidosis on the aortic valve of the heart was noted on rats of all groups after two months on the diet. Postprandial hyperlipemia did not affect the frequency of circulating lipophages in these hypercholesterolemic rats compared with the rats fasted for 16 hr in the same group.

THERE HAVE BEEN numerous reports concerning studies of plasma or serum lipids in relation to experimental atherosclerosis, but only a few deal with lipids within the cells of the circulating blood (1). Morphologic studies (2, 3) have led us to consider that the circulating lipophage may well be important in atherogenesis. This paper is a preliminary report concerning studies on lipid-laden leukocytes in the peripheral blood of rats that were fed atherogenic diets containing various types of fat.

METHOD

One hundred Wistar albino male rats weighing 70–90 grams were divided into five groups and housed in individual wire-bottom cages. They were fed a variety

of atherogenic diets ad lib. as shown in Table 1. Blood smears were made by clipping the tail veins of two rats from each group at weekly intervals from the 4th to the 13th week of the experiment. The blood smears were immediately fixed in formalin vapor and then stained with oil red O by the technique described previously (1). Differential counts of 500 leukocytes were made on blood smears of each rat and the percentage of lipid-laden cells (lipophages) was obtained for each of the three major classes of leukocytes (neutrophil, lymphocyte, and monocyte). In order to define the lipophage microscopically, the following arbitrary criteria were adopted: (a) the lipophage should contain more than one vacuole in the cytoplasm; (b) the vacuoles should be stainable with oil red O; and (c) the vacuoles should be approximately 0.5–1 μ in diameter. Air-dried, Wright-stained smears were simultaneously prepared and examined for confirmation and comparison of the differential cell morphology. Blood specimens were also obtained weekly from half of the rats of each group and the levels of serum total cholesterol were determined by the method of Zak et al. (4). All rats that died during the experiment or survived until its termination were autopsied and the extent of gross intimal lipidosis of aorta was evaluated.

RESULTS

On blood smears stained with oil red O the lipophages were readily demonstrated in rats of all groups. On differential counts of leukocytes in the rats of all groups the lipophages were found most frequently to be monocytes (Table 2). Lipid vacuoles were only rarely encountered in band-form neutrophils. The number of eosinophils in rats of all groups was decreased, numbering from 0 to 7 cells per 500 leukocytes of the three major classes compared with the range of 2–10 cells in the chow-fed control rats. Lipophages among eosinophils were

TABLE 1 DIET, LIPHAGE RATIO, AND SERUM CHOLESTEROL OF RATS

Group	No. of Rats	Diet*	Lipophage Ratio†	Average Serum Total Cholesterol
			%	mg/100 ml
A	20	40% Butter	23.4	2338
B	20	40% Butter, no added thiouracil	9.6	960
C	20	40% Corn oil	9.4	1429
D	20	40% Peanut oil	10.3	1277
E	20	40% MCT‡	5.0	1928
Control	10	Chow	0.1	90

* Additional dietary ingredients fed to all groups except the control were: 5% cholesterol, 0.3% thiouracil (except Group B), 2% sodium cholate, 20.5% sucrose, 2% vitamin mix, 0.2% choline chloride, 4% salt mix, 20% casein, and 6% alphacel. Average dietary consumption per rat per day has been determined in previous experiments during the 5th week, and has been approximately 9 g in Group A, 11 g in Group B, and 8 g in the groups of animals consuming oily diets such as C, D, and E.

† Average percentage value of lipophages in 500 leukocytes, counted on blood smears stained with oil red O, of each rat during the 4th to 13th week of the experiment.

‡ A hydrogenated synthetic fat (medium-chain triglycerides) containing 1.9% C₆, 77.7% C₈, 19.6% C₁₀, and 0.8% C₁₂ fatty acids (8).

rare. Nucleated red cells were frequently found in rats of all groups, ranging from 1 to 33 per 500 leukocytes compared with 0-7 in the control group. The monocytes not only demonstrated the highest frequency of lipophages but also contained a larger amount of intracytoplasmic lipids than other leukocytes. The cytoplasm of some lipophages, which appeared to be monocytes and lymphocytes, was distended and filled by lipid vacuoles and in these cells the nuclei were frequently compressed (Fig. 1). Sometimes the differentiation between monocyte and lymphocyte was difficult but these two cell types could usually be distinguished by considering the over-all cell size, the nuclear shape, and the staining intensity of chromatin (Fig. 2 and 3). Azurophilic granules in the lymphocytes were not readily visible on oil red O preparations, but on Wright-stained smears they were distinct and often coexistent with lipid vacuoles. Bilobed lymphocytes were occasionally encountered and some of them contained large amounts of lipid (Fig. 4).

The numbers of lipophages in blood increased sharply during the first few weeks of the experiment. Rats of Group A that were fed an atherogenic diet containing 40% butter had a significantly higher percentage of lipophages than other groups ($P < 0.001$) and the rats of Group E that were given the medium-chain triglycerides (MCT) had a significantly lower percentage of lipophages than any other group ($P < 0.01$) (Table 1). Levels of serum total cholesterol were highest in the rats of Group A and lowest in Group B. Although at

autopsy lipidosis was noted on the aortic valve of rats of all groups after 3 months of the experiment, grossly recognizable lesions of atherosclerosis were present in the thoracic and abdominal aorta of only Group A rats (Fig. 5).

The effect of fasting on the frequency of circulating lipophages was studied in the rats fed an atherogenic diet containing 40% butter (Group A). The percentage of lipophages in 500 leukocytes of 10 rats that were fasted for 16 hr averaged 13.4% compared with 11.9% in another 10 rats that were fed ad lib., a difference that was not statistically significant.

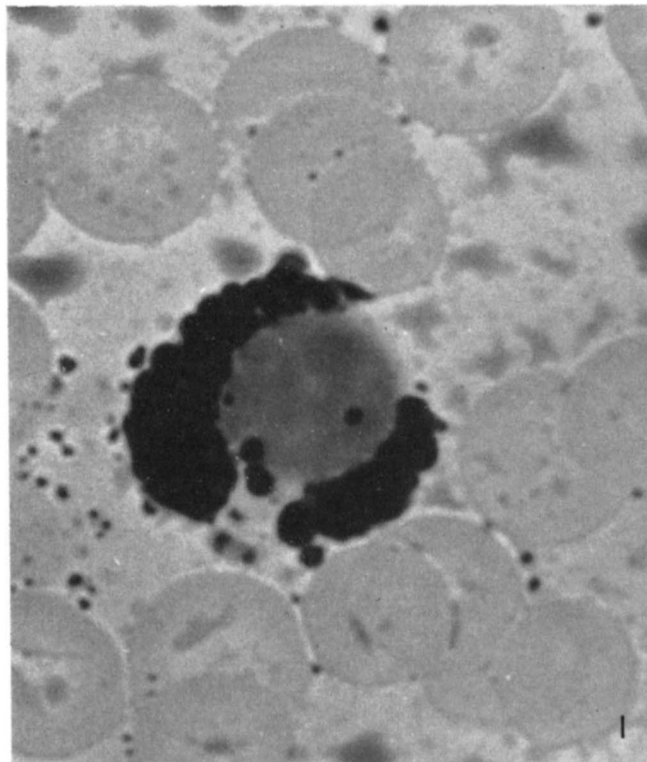
DISCUSSION

The lipophages of peripheral blood are readily recognizable in rats fed atherogenic diets and also can frequently be found in control chow-fed rats. The frequency and lipid content of lipophages vary considerably, depending on the kind of added fat in the diet. In rats of all experimental groups the percentage of monocytes containing lipid was proportionally very high, usually over 50% and sometimes exceeding 80-90% within its cell type, but the actual number of lymphocytic lipophages occasionally outnumbered monocytic lipophages because in rats lymphocytes normally comprise over 70% of the blood leukocytes (5). The large lymphocytes of the rats revealed abundant cytoplasm and constituted the majority of lymphocytic lipophages, whereas small lymphocytes with scanty cytoplasm were usually devoid of lipid vacuoles. Bilobed lymphocytes which are normally present in rat blood were frequently found to contain lipid vacuoles in the cytoplasm. Lipophages among polymorphonuclear neutrophils were few and none contained a large number of vacuoles. Postprandial hyperlipemia appeared to have no effect on the frequency of lipophages, as judged by lipophages present in blood of rats fed an atherogenic diet containing 40%

TABLE 2 DIFFERENTIAL COUNTS OF LEUKOCYTES AND THEIR LIPHAGES ON RAT BLOOD SMEARS*

Group	Neutrophil		Lymphocyte		Monocyte	
	Total	Lipophage	Total	Lipophage	Total	Lipophage
	%	%	%	%	%	%
A	37.7	4.3	43.8	6.6	18.5	12.5
B	41.4	1.9	46.8	2.0	11.8	5.7
C	26.1	1.5	62.9	3.0	11.0	5.0
D	24.3	1.6	64.7	3.8	11.0	4.9
E	36.3	0.5	55.2	1.9	8.5	2.6
Control	16.0	0.06	76.9	0	7.1	0

* Five hundred leukocytes were counted on all smears stained with oil red O during the 4th to 13th week of the experiment, and average percentage values obtained.



1



3



2



4

FIG. 1. A typical lipophage, probably a lymphocyte, showing many intracytoplasmic lipid vacuoles (oil red O stain; $\times 2700$).

FIG. 2. A monocyte containing lipid vacuoles in the cytoplasm (Wright stain; $\times 2700$).

FIG. 3. A large lymphocyte showing abundant cytoplasm filled with numerous lipid vacuoles (Wright stain; $\times 2700$).

FIG. 4. A bilobed lymphocyte with many lipid vacuoles in the cytoplasm (Wright stain; $\times 2700$).

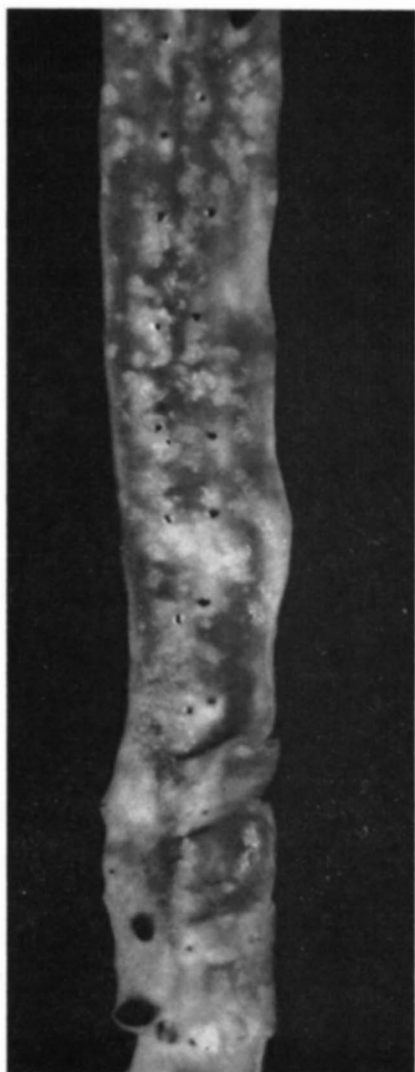


FIG. 5. Thoracic aorta of a Group A rat, showing numerous patchy areas of atherosclerosis.

butter ad lib. as compared with fasted rats in the same group.

Approximately 80% of the fatty acids in the medium-chain triglycerides (MCT) used in this experiment have fewer than 10 carbon atoms in the chain (6). Fatty acids with less than 10 carbon atoms are, after being absorbed from the intestine, transported through the portal venous system rather than by intestinal lymphatics (7), and therefore would not be expected to contribute directly to chylomicronemia. Hashim et al. (8) reported a serum cholesterol-lowering effect of MCT, as compared with butter, in the human. Similarly, in the present experiment rats fed a hypercholesterolemic diet developed a less pronounced hypercholesterolemia on MCT than on butter though more pronounced than on corn oil. After 2 months of the experiment the rats fed MCT showed fatty liver, lipophages in circulating blood and even lipidosis of the aortic valve of the heart, but athero-

sclerosis of the aorta was not detected grossly. The number of lipophages present in the circulating blood in rats fed MCT was less than would be expected in rats with such severe hypercholesterolemia induced by other fats; in a previous study (1), numbers of lipophages found were proportional to blood cholesterol levels. An attractive explanation for the results of the present study is that chylomicra form the principal source of the lipid found in circulating leukocytes, and that dietary MCT produce relatively few chylomicra.

The uptake and staining intensity of oil red O by various pure lipids were recently studied by Schjeide et al. (9), who concluded that oily triglycerides and unsaturated cholesterol esters took up large amounts of stain. Intracellular lipids of rat leukocytes in the present experiment are intensely stainable with oil red O and it is likely that a considerable portion of the lipids within those lipophages may be esterified cholesterol, since these rats were extremely hypercholesterolemic, as has been described in previous work (10). Further studies need to be carried out in order to elucidate the chemical nature of intracytoplasmic lipids of these circulating lipophages. In addition, it should be emphasized that the changes described in the results of this study probably represent only a small facet of the over-all response of the rats to these extreme dietary variations.

The authors are indebted to Mr. V. K. Babayan, Drew Chemical Co., Boonton, N. J., who supplied medium-chain triglycerides for this study.

This study was supported by PHS Research Grant HE-06964 from the National Heart Institute, National Institutes of Health, U. S. Public Health Service. The paper was presented in part at the Annual Meeting of the Federation of American Societies for Experimental Biology, April 13, 1964.

Manuscript received January 8, 1964; accepted June 25, 1964.

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