FUNCTIONAL STATE OF THE ANTICLOTTING SYSTEM IN SOME FORMS OF EXPERIMENTAL DISTURBANCE OF LIPID METABOLISM

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In rats with "hypothalamic" obesity developing after injury to the ventro-medial nuclei of the hypothalamus, the function of the anticlotting system is undisturbed. If these animals are transferred to an atherogenic diet, depression of the anticlotting system arises, leading to the development of prethrombosis, just as in intact rats receiving the same diet.

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Previous investigations [1, 4-6] showed that Wilgram's experimental diet [11] causes profound depression of the anticlotting system and leads to a state of prethrombosis. This state is characterized by spontaneous and massive transition into thrombosis, mainly in the chambers and blood vessels of the heart, and also in the vessels of the kidneys.

In the present investigation the function of the anticlotting system was studied in other forms of disturbance of lipid metabolism, notably in alimentary obesity caused by destruction of the ventro-medial nuclei of the hypothalamus (the "satiety center" [3, 10]).

EXPERIMENTAL METHOD

Destruction in the region of the ventro-medial nuclei of the hypothalamus was produced in rats with an average weight of 200 g by means of the microelectrodes of Belenev's [2] stereotaxic apparatus, with a

TABLE 1. Indices of Functional State of Anticlotting System of Rats with Hypothalamic Obesity Kept on Laboratory Diet

Animals	No. of animals	Blood fibrinogen concentration (in mg%)	Blood fibrinolytic activity (in %)	Plasma heparin tolerance (in min)	Total blood cholesterol concentration (in mg%)
Intact rats aged 2 years (control I)	50	587.7 ± 38.2	35.0 ± 4.3	9.86 ± 0.48	78.6 ± 1.94
Rats with hypothalamic obesity, operation 2 years earlier	15	604.7 ± 11.9	49.04 ± 0.06	8.0 ± 1.01	122 ± 8.0
Intact rats in prethrombotic state kept on atherogenic diet for 7-8 months (control II)	50	814 ± 31	9.1 ± 0.95	3.5 ± 0.21	612 ± 15.7
Intact rats aged one year two months (control I)	36	429.15 ± 17.1	52.8 ± 3.2	13.9 ± 0.89	72.5 ± 2.31
Rats with hypothalamic obesity, operation 5 months earlier	36	414 ± 11.3	43.0 ± 4.42	11.6 ± 0.85	81.7 ± 2.33
Intact rats in prethrombotic state kept on atherogenic diet for 5 months (control II)	30	847.0 ± 9.9	14.3 ± 1.7	4.04 ± 0.21	427 ± 14

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TABLE 2. Indices of Functional State of Anticlotting System of Rats with Hypothalamic Obesity Kept on Atherogenic Diet

Animals	Time kept on atherogenic diet (days)	No. of animals	Blood fibrinogen concentration (in mg%)	Blood fibrinolytic activity (in %)	Plasma heparin tolerance (in min)	Total blood cholesterol concentration (in mg%)
Intact rats aged one year two months (control I) . .	–	36	431 ± 16.6	37.2 ± 3.17	15.3 ± 0.89	183.6 ± 2.2
Rats with hypothalamic obesity, operation 5 months earlier	40	28	568 ± 18.1	30.4 ± 6.15	9.75 ± 0.74	163.6 ± 1.6
Intact rats in prethrombotic state (control II)	40	16	593.4 ± 28.1	24.3 ± 0.34	5.54 ± 0.29	304.7 ± 2.29
Intact rats aged one year two months (control I) . .	–	36	475 ± 14.3	55.6 ± 5.2	14.7 ± 0.71	76.1 ± 1.9
Rats with hypothalamic obesity, operation 5 months earlier	120	22	630 ± 37.2	17.4 ± 5.2	5.28 ± 0.34	534 ± 635
Intact rats in prethrombotic state (control II)	120	16	703 ± 39.1	18.0 ± 0.99	4.0 ± 0.32	410 ± 21.4

current of 3 mA acting for 20 sec. On the average 50% of the animals undergoing the operation developed obesity. Experiments were carried out on animals of two groups: on 15 old female rats aged 3 years with "hypothalamic" obesity, used in the experiment 2.5 years after the operation and with a body weight 2-3 times greater than the controls, and 36 rats of both sexes aged one year two months, used in the experiment 5 months after the operation, when their body weight on the average was three times greater than initially. The rats of the second group with "hypothalamic" obesity were kept for 5 months on an ordinary laboratory diet, and then transferred to Wilgram's atherogenic diet, which they received for 4 months. Normal intact rats of the same age served as controls. Some of these animals were kept on a normal laboratory diet, while others received an atherogenic high-fat diet for the same period.

Blood for analysis was taken with a syringe from the jugular vein. At different times of the experiment the following determinations were made on the animals: the blood fibrinogen concentration and the degree of its fibrinolytic activity by Bidwell's method [7], the plasma heparin tolerance by Gormsen's method [8], the total blood cholesterol concentration by Grigaut's method [9]. To investigate the lipid content in the aorta and liver, sections were cut on a freezing microtome. The sections were stained with Sudan III or Sudan black. The functional state of the anticlotting system of the experimental animals was determined from diagnostic signs of prethrombosis, i.e., from changes in the blood fibrinogen concentration, the fibrinolytic activity of the blood, and its tolerance to heparin.

EXPERIMENTAL RESULTS

As Table 1 shows, the rats undergoing the operation with hypothalamic obesity and kept for a long time on an ordinary laboratory diet showed no appreciable tendency toward the development of a prethrombotic state in contrast to the intact animals kept on the atherogenic diet.

It will be clear from Table 2 that depression of the anticlotting system appeared in the rats with hypothalamic obesity following the operation when transferred from the ordinary laboratory diet to the atherogenic diet, and a prethrombotic state developed after 30-40 days on this diet. In its degree and the time taken for its development, this depression was similar to the corresponding phenomena observed in intact animals kept on the same high-fat diet. The only difference was a slightly higher blood cholesterol concentration in the rats with the hypothalamic obesity, presumably on account of the increased intake of atherogenic food by these animals containing an excess of cholesterol.

The results of histological investigation of the lipid content in the aorta and liver of the experimental animals showed that in rats with hypothalamic obesity kept for 4 months on a natural diet, no accumulation of lipids in the endothelium of the aorta was observed, as in normal, intact animals. In the liver of normal

rats the lipid content was low: in occasional parenchymatous cells and in the cytoplasm of the Kupffer cells, lipids were found in the form of small inclusions. However, in rats with hypothalamic obesity and kept on the normal laboratory diet, the lipid content in the liver was considerably higher than normal, and deposition was observed mainly in cells concentrated in the center of individual liver lobules. In rats with hypothalamic obesity and kept on an atherogenic diet for 4 months, the histological picture of lipidosis both of the aorta and of the liver showed the same degree of development as in intact animals kept on the same diet. Considerable accumulations of lipids were observed in the endothelium of the arch of the aorta in the form of droplets or inclusions. In some places the endothelium was swollen and protruded into the lumen of the aorta. With an increase in the time during which the animals received the atherogenic diet, progressive penetration of fat into the intima of the aorta was observed. The lipid content in the liver of both groups of animals kept on the atherogenic diet was very great: fat in the form of small, and sometimes large, inclusions was found in the cytoplasm of nearly every cell of the liver lobules. The Kupffer cells were swollen and their cytoplasm contained an appreciably increased fat content.

It follows from these results that the state of prethrombosis arising as a result of depression of the anticlotting system in animals kept on an atherogenic high-fat diet is associated with a disturbance of metabolism in the body caused by the composition of the diet. Accumulation of lipids taking place in the body after excessive consumption of natural food in the experiment with hypothalamic obesity had no appreciable depressant effect on function of the anticlotting system. Consequently, thrombotic complications arising in experimental atherosclerosis are not the result of the direct accumulation of fat in the body but are the result of a disturbance of metabolism caused by an excess of cholesterol, saturated fats, and methylthiouracil in the diet, causing depression of function of the anticlotting system.

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