THE INFLUENCE OF NEURAL REGULATION OF THE LIVER ON EXPERIMENTAL ATHEROSCLEROSIS

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It is well known that metabolic disorder, in particular disorders of lipid metabolism, form the basis of atherosclerosis. In this metabolism the liver plays an important role. The formation and destruction of phospholipids, the formation of fatty acids and also apparently of lipoproteins, take place for the most part in the liver [14].

The immediate causes of disorders of lipid metabolism in atherosclerosis are still obscure. There are clinical and experimental data [2, 4, 9, 10, 11, 17] pointing to the influence of the higher nervous system on the genesis of changes in lipid metabolism and atherosclerosis, but the mechanism of these influences has not yet been discovered.

Observations have been made [1] on the markedly increased secretion of cholesterol in the bile after liver desympathization in dogs, and on the effect of desympathization on adiposis of the liver caused by phlorizin. In the available literature we could not find data on the relative course of atherosclerosis after nerve section.

Thus it appeared desirable to us to study the role of nervous regulation of the liver on lipid metabolism and on the course of experimental atherosclerosis; these were the problems of the present work.

METHOD

The investigation was carried out on 27 male rabbits of the same breed, 2.5-3 kg in weight; 15 of these were used in the experiments and 12 served as controls. The liver nerve sections were performed according to the method of I. M. Ishchenko [5], with minor variations.

The operation took place under morphine narcosis (1 ml of 1% solution for each kg of weight), with continuous administration of oxygen. After opening the abdominal cavity the tissues of the hepato-gastric ligament were laid back and the adventitia removed from the hepatic artery, portal vein and common bile duct; a 5% solution of phenol was applied to these. The adventitia was taken off the lower area of the vein for a distance of 2-3 cm and then (after cutting the falciform ligament) also from the anterior surface between the liver and diaphragm.

Judging by the data in the literature [3, 7, 8], such an operation assures a functional effect, i.e., it causes corresponding changes in carbohydrate metabolism and in the composition of the blood.

The control operation consisted of laparatomy, and was done under the same conditions as the partial denervation of the liver.

Fourteen days after the operation the animals began to receive cholesterol in powder form, mixed with vegetables or in a solution of sunflower-seed oil administered through a tube. Before the operation, 14 days after,

and then once a month, blood was taken from the ear and the cholesterol content determined by Sackets's method, lecithin by Samson's method and the lipoproteins by paper electrophoresis. After the end of the feeding period the animals were killed by air embolism, and the aorta fixed in formalin and then stained with sudan III.

RESULTS

Two series of experiments were conducted. In the first series, four contol and seven experimental animals received cholesterol for 110 days, which they ate with shredded carrots (for the first two month, 0.5 and thereafter 1.0 unit per day). In the second series the animals (eight control and eight experimental) received 0.5 cholesterol in 10 ml of sunflower seed oil for 100-81 days.

The blood lipid levels showed practically no effect of the partial section of the hepatic nerves. In the group of control rabbits the average cholesterol level 14 days after the operation was 77 mg % ± 8.3, with variations from 144 to 150 mg%; in those with denervated liver, it was 76 mg% ± 4.5, with variations from 64 to 110 mg%.

The average lecithin level in the control group was 96.3 mg% ±11, varing from 52 to 195 mg%; in the group of experimental animals the average was 88mg% ±9, varing from 57 to 114 mg%. The percentage of β-lipoproteins in the control animals varied from 52 to 80, averaging 62 ± 2.82, and in the experimental group varied from 51 to 85, averaging 63 ± 2.82. Statistical analysis confirmed the absence of difference between the

TABLE 1

Changes in Blood Lipids during the Development of Experimental Atherosclerosis

Interval (in months) after beginning cholesterol feeding	Series of experiments	Group of rabbits: Control — Variation	Control — Average level	Experimental — Variation	Experimental — Average level	P (%)
Cholesterol (in mg%)						
1	First	192—664	355±38	167—450	308±37	55
	Second	190—760	406±68	187—920	507±79	61
2	First	150—964	442±155	192—668	360±61	37
	Second	250—1 100	488±113	300—1 400	685±87	81
3	First	342—940	720±111	267—990	543±78	74
	Second	300—496	403±33	520—800	631±43	97
Lecithin (in mg%)						
1	First	95—267	161±32	119—224	157±13	0
	Second	76—310	197±28	162—282	193±15	0
2	First	90—279	163±33	110—200	141±14	55
	Second	114—466	239±47	70—577	317±70	96
3	First	138—375	273±46	133—353	257±26	22
	Second	60—428	199±55	129—411	254±30	43
Ratio $\frac{\text{lecithin}}{\text{cholesterol}}$						
1	First	0,40—0,72	0,53±0,037	0,26—0,85	0,55±0,06	19
	Second	0,36—0,72	0,51±0,05	0,25—0,94	0,44±0,08	40
2	First	0,28—0,60	0,44±0,066	0,24—0,68	0,44±0,056	0
	Second	0,34—0,81	0,52±0,065	0,11—0,62	0,45±0,07	50
3	First	0,29—0,42	0,37±0,093	0,26—0,68	0,47±0,13	9
	Second	0,12—1,42	0,56±0,16	0,20—0,55	0,40±0,04	66
β-lipoproteins (in %)						
1	First	57—84	73±3,7	60—85	69±3,5	37
	Second	63—89	78±5,1	84—100	89±3,1	88
2	First	72—89	78±4,4	56—99	71±4,5	55
	Second	73—92	80±3	81—91	88±1,2	99
3	First	57—87	85±8	60—184	77±10	73
	Second	56—100	84±6	84—100	89±22	93

blood lipid levels in the control and experimental groups 14 days after the operation (P for cholesterol was 23% for lecithin 37%, for beta-lipoproteins 0). After beginning administration of cholesterol there were in all animals as described in the literature [6, 12, 16], heightened levels of cholesterol, lecithin and beta-lipoproteins, with a lowering of the lecithin/cholesterol ratio. The data of all experiments are summarized in Table 1.

In the first series of experiments no substantial difference was detected in blood lipid levels between the control and experimental animals. In the second series, review of the data gave the impression of a higher blood lipid level in the experimental animals than in the controls, especially in blood cholesterol and β-lipoproteins. However, it was possible to confirm this statistically for cholesterol only 3 months after the beginning of feeding (blood cholesterol in the controls being 403 mg% ±33, and in the experimental animals 631 mg% ±4.3), and for β-lipoproteins 2-3 months after the beginning of feeding (the percentage of β-lipoproteins at two month was 80 ±3 in the controls and 88±1.2 in the experimental animals; at three months it was 84±6 and 89±2.2 respectively). The level of lecithin at two months also turned out to be higher in the experimental animals (317 mg% ±70) than in the controls (239 mg% ±47). However, the magnitude of the lecithin/cholesterol ratio did not differ substantially between the experimental and control animals during the entire course of observations.

TABLE 2

Indication of Aortic Atherosclerosis

Degree of indication, atherosclerosis	Series of experiments			
	first		second	
	group of rabbits			
	control	experimental	control	experimental
Marked	3	—	2	—
Moderate	1	1	5	1
Weak	—	3	1	4
Very weak	—	3		3
Total	4	7	8	8

Thus one can speak of some tendency to an intensification of the usual changes in lipid metabolism attendant upon cholesterol overfeeding in animals with disturbed neural regulation of the liver, in those cases where cholesterol is administered in sun flower-seed oil.

It was naturally expected that in this group of rabbits, anatomical changes in the aorta (particularly in the second series) would show a tendency to aggravation.

In Table 2 are shown the data on the relative indication of atherosclerotic changes in all animals. It is apparent that partial section of the liver nerves not only did not lead to greater anatomical changes in the aorta, but on the contrary distinctly inhibited the development of experimental atherosclerosis in the animals in both the first and second series of experiments. Thus if out of the 12 animals in the control group the indication of atherosclerosis could be described as marked in five, moderate in six and weak in only one, then out of the 15 experimental animals in not a single one were there marked indications of atherosclerosis; in two rabbits the indication was moderate, and in the remaining 13 weak or very weak. These facts indicate that the disturbance of neural regulation of the liver markedly influences the course of experimental atherosclerosis, in such a way as to hinder the deposition of lipids in the aortic wall. This action apparently is not connected with the influence of this liver operation on lipid metabolism.

The tendency to aggravated hypercholesterolemia, and increased percentage of β-lipoproteins in particular, in the group of rabbits receiving cholesterol in sunflower-seed oil should lead to the intensification of atherosclerotic changes in the aorta.

The mechanism of the described inhibition in the development of experimental atherosclerosis under conditions of disturbed neural regulation of the liver is still obscure. It should be noted, however, that some cases are known in which there is no parallel development of cholesterolemia and experimental atherosclerosis. Cortisone, for example, aggravates cholesterolemia and inhibits the development of atherosclerosis [13].

In conclusion we feel it imperative to report some facts received when the present work had already gone to press.

Histological investigations, kindly conducted by A. E. Seranova at various intervals (from 24 hours to 12 days) after partial section of the nerves of the liver, have indicated that there is destruction only of individual nerve fibers in the liver. Leukocytic infiltration is seen in the connective tissue of the porta hepatis, vascular adventitia and around the nerve stems; hypertrophy of the fibroblastic elements is noted in the epineurium of the nerve stems, with proliferation and hypertrophy of the cells of Schwann.

It may be suggested that in all likelihood the inhibition of experimental atherosclerosis in our experiments depended mainly on the irritation of the neural conductors of the liver.

SUMMARY

The author studied the effect of liver denervation on the lipid metabolism and the intensity of anatomical changes in rabbit aortas during cholesterol administration. Animals in which laparatomy was performed under the same conditions as denervation served as controls. Some animals received cholesterol in sunflower-seed oil, others – mixed with vegetables. Some tendency to intensification of the usual changes occurring in the lipid metabolism following alimentary cholesterol load was detected in animals after liver denervation when cholesterol was administered in sunflower-seed oil. Liver denervation considerably inhibited the development of anatomical changes in the aorta irrespective of the method of cholesterol administration. The mechanism of this inhibitory effect is still obscure. Additional histological investigations of A. E. Seranova have demonstrated destruction of only individual nervous fibers. Leucocytic infiltration is seen in the connective tissue of the porta hepatis, vascular adventitia and around the nerve stems; hypertophy of the fibroblastic elements is noted in the epineurium of the nerve stems with proliferation and hypertrophy of the Schwann cells.

It may be suggested that inhibition of experimental atherosclerosis in the aforementioned experiments depended mainly on the stimulation of the nervous conductors of the liver.

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