

## THE EFFECT OF CHOLINE ON THE CHOLESTEROL METABOLISM OF STARVING RABBITS

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As shown by N. N. Anichkov and his co-workers [1, 2], the development of atherosclerosis is based on disturbance of the cholesterol metabolism. For this reason it must be regarded as an important task to discover substances which could restore to normal a disturbed cholesterol metabolism. In this respect great importance is attached to lipotropic factors and, in particular, to choline. However the reports in the literature on the role of choline in cholesterol metabolism are conflicting.

Morrison and his co-workers [15], Yu. T. Pushkar' [5] and T. A. Sinitsyna [6] pointed out that the administration of choline has a restraining action on the development of experimental atherosclerosis in rabbits fed on cholesterol. Morrison and Rossi [14] observed absorption of atheromatous plaques in rabbits as a result of the action of choline. T. A. Sinitsyna [7] showed the accelerating effect of choline on the regression of atherosclerotic lesions. Other workers, however, deny the importance of choline in preventing the development of experimental atherosclerosis in rabbits [8, 10], dogs [9] and fowls [16].

For this reason we carried out investigations in order to study the effect of choline on the development of hypercholesteremia in starving rabbits, and we also attempted to explain the mechanism of action of choline on this process.

The effect of choline on the development of hypercholesteremia in starving rabbits. For the study of disturbances of the cholesterol metabolism, a convenient experimental model is hypercholesterolemia in starving rabbits, since it develops rapidly and equally rapidly disappears when the rabbit is put back on a normal diet. The cholesterol level in the blood of starving rabbits varies considerably, and so in investigating the effect of choline on the development of hypercholesteremia in these conditions we carried out the experiments in such a way that the effect of choline could be compared in the same animal.

The experiments were performed on young rabbits of both sexes, weighing from 2200–2500 g. Blood was taken from the rabbit's heart, after which it was kept without food but was given water for 5 days. On the 3rd and 6th days of starvation blood was also taken and the cholesterol concentration in the plasma was estimated by Bloor's method and the lipoid phosphorus by the method of Fiske and Suffarow. After about 2 weeks, in the course of which the rabbit was kept on an ordinary diet, an analogous experiment was carried out again on the same animal, differing only in that the rabbit received choline daily in the form of a 10% solution of choline chloride in distilled water in a dose of 0.5 g per kg body weight (the choline was introduced into the stomach through a tube). In some animals the starvation experiments were carried out in the opposite order; the experiment giving choline was carried out first and was followed after the interval of time mentioned by an analogous experiment without choline.

In 18 of 25 experiments a well marked inhibition of hypercholesteremia was observed, in 4 experiments choline had no effect, and in three there was even a slight increase in the hypercholesteremia. The most characteristic results of these 18 experiments are shown in Table 1.

TABLE 1

Changes in the Blood Cholesterol Concentration in Rabbits on a Starvation Diet under the Influence of Choline

Experiment No.	Time of taking blood	Conditions of experiment					
		Starvation			Starvation + choline		
		Cholesterol (in mg%)	Lipoid phosphorus (in mg%)	Coef-ficient $\frac{1}{c}$	Cholesterol (in mg%)	Lipoid phosphorus (in mg%)	Coef-ficient $\frac{1}{c}$
1	Before starvation	20	3.5	4.3	23.5	4.0	4.1
	After 3 days of starvation	52.6	3.5	1.6	44.4	3.3	1.9
	After 4 days of starvation	71.1	3.8	1.3	50.0	4.1	2.0
2	Before starvation	44.4	4.0	2.2	40	3.9	2.4
	After 3 days of starvation	72.7	4.5	1.7	40	4.1	3.1
	After 5 days of starvation	100	4.0	1.0	46.6	4.1	2.2
3	Before starvation	40	3.5	2.1	40	4.1	2.5
	After 3 days of starvation	64	4.1	1.5	34.7	4.5	3.3
	After 5 days of starvation	88.8	5.0	1.4	36.7	3.8	2.6

Note: The coefficient  $\frac{1}{c} = \frac{\text{lecithin}}{\text{cholesterol}}$

TABLE 2

The Effect of Choline on the Stability of the Blood Cholesterol of Rabbits Kept on a Starvation Diet

Experiment No.	Time of taking blood	Conditions of experiment									
		Starvation					Starvation + Choline				
		Before precipitation of proteins		After precipitation of proteins			Before precipitation of proteins		After precipitation of proteins		
		Cholesterol (in mg%)	lipoid phosphorus (in mg %)	Cholesterol (in mg%)	lipoid phosphorus (in mg %)	% of initial cholesterol concentration	Cholesterol (in mg%)	lipoid phosphorus (in mg %)	Cholesterol (in mg%)	lipoid phosphorus (in mg %)	% of initial cholesterol concentration
1	After 5 days of starvation	71.1	3.8	14.7	8.5	20.1	50.0	4.1	17.7	3.6	32
2	After 5 days of starvation	58.8	5	12.5	4.0	22	32	3.7	14.8	3.6	46.5
3	After 5 days of starvation	64	4.5	11.4	3.7	17.8	34.7	3.8	12.1	3.5	35.2
4	After 5 days of starvation	94.4	5.0	22.8	4.1	24.4	66.6	3.5	31.3	3.1	46.9
5	After 5 days of starvation	64	4.1	13.3	3.3	20.7	32	2.6	11	2.1	34.3

Table 3

The Effect of Choline on the Excretion of Cholesterol in the Bile

Experimental conditions	Number of experiments	Number of animals with a concentration of cholesterol in the liver of (in mg%)			
		38-50	51-100	101 and over	Average concentration
Starvation + choline	11	—	6	5	97.8
Starvation	9	4	3	2	68.1

TABLE 4

Results of Experiments to Study the Effect of Choline on the Cholesterol Metabolism in the Liver

Experimental conditions	Number of experiments	Number of animals with a concentration of cholesterol in the liver of (in mg%)				
		250-350	351-400	401-450	451 and above	Average concentration
Starvation + choline	19	4	3	6	6	416
Starvation	9	6	2	1	—	359

As may be seen from Table 1, the administration of choline to starving rabbits considerably retards the hypercholesteremia which develops under these circumstances. The concentration of lipid phosphorus remained unchanged, although the coefficient  $l/c$  was considerably higher in the rabbits receiving choline than in those which were starved and did not receive choline, since the level of the cholesterol was lower in the experiments in which choline was given.

The effect of choline on the stability of the blood cholesterol in starving rabbits. There is no unanimity on the causes of the stability of the blood cholesterol level. Some authors [12, 13] account for the stability of the blood cholesterol by its combination with proteins, others [4, 11] by its combination with phosphatides. Since the coefficient  $l/c$  in all the experiments with starving rabbits which received choline was significantly higher than in the experiments on the starving rabbits not receiving choline, it appeared that this must be the feature which led to the greater stability of the blood cholesterol of these animals.

Experiments were carried out by a method described in a paper by S. V. Nedzvedskii and S. S. Ratnitskaya [4]. The results of some typical experiments are shown in Table 2.

The results in Table 2 show that the administration of choline to starving rabbits increased the stability of the blood cholesterol: after precipitation of the plasma proteins, 24-69% of the total cholesterol remained in the protein-free filtrate whereas in the experiments in which no choline was given, this figure was 15-27%.

The effect of choline on the excretion of cholesterol in the bile of starving rabbits. Many authors have pointed out that the liver plays an essential part in the excretion of cholesterol with the bile. We therefore considered it of interest to study the effect of choline on the cholesterol concentration in the bile.

Two groups of rabbits were used in these experiments. Animals were given choline daily (0.5 g per kg body weight), and after starvation for 5 days, were killed by air embolism; their bile was collected in a tube and the cholesterol concentration of the bile estimated accurately by the method of Sperry and Brand. Similar experiments were carried out on rabbits which received no choline during the period of starvation.

Table 5

The Effect of Choline on the Cholesterol Content of Liver Tissue Incubated under Various Conditions

Experiment No.	Experimental conditions	Cholesterol (in mg%)		
		Control	Experiment	
		Incubation of liver tissue		
		with NaOH, 15%	with CH <sub>3</sub> COOH, 5%	with KH <sub>2</sub> PO <sub>4</sub> , 1%
1	Starvation + administration of choline	500	454	384
2	Starvation + administration of choline	395	416	326
3	Starvation + administration of choline	470	470	390
4	Starvation + administration of choline	400	363	333
5	Starvation + administration of choline	454	454	400
6	Starvation without choline	333.3	327	327
7	Starvation without choline	347.8	347	347
8	Starvation without choline	307	307	301
9	Starvation without choline	250	250	250
10	Starvation without choline	400	400	400

The results of these experiments are shown in Table 3.

It can be seen from Table 3 that the cholesterol content of the gall bladder was higher in the majority of the starving rabbits receiving choline than in the starving rabbits not receiving choline. Administration of choline therefore increased the cholesterol content of the gall bladder of starving rabbits, thereby facilitating its excretion.

The effect of choline on the cholesterol metabolism in the liver of starving rabbits. Recent work has shown the important role of the liver in cholesterol metabolism. Since choline reduces hypercholesteremia in starving rabbits, the question arose whether this phenomenon depended on the cholesterol metabolism in the liver of these rabbits. This appeared more likely since it had been established in a recently published paper by V. N. Kolmakov [3] that there was a relationship between the fall in the hypercholesteremia and the cholesterol metabolism in the liver of starving rabbits under the influence of vitamin C. We therefore studied the effect of choline on the cholesterol content of the liver in starving rabbits. These experiments were carried out as described in the paper cited [3]. In all 28 experiments were performed, in 19 of which choline was given to starving rabbits, and in 9 no choline was given (the number of the latter experiments was smaller because the results were the same as those obtained on this subject in earlier experiments in our laboratory). In Table 4 are shown the results of these experiments.

As can be seen from Table 4, the cholesterol content of the liver of starving rabbits which were given choline was higher in the majority of cases than in those which did not receive choline. It could therefore be considered that under the influence of choline the liver had a greater restraining action on the blood cholesterol, which led to a fall in the hypercholesteremia. The higher cholesterol content of the liver could possibly be due,

however, to the fact that in these conditions the synthesis of this substance is stimulated or its breakdown is inhibited in the liver. In order to clarify this point we carried out experiments in which the liver tissue of experimental animals was incubated: 1) with acetate, where synthesis of cholesterol was postulated, and 2) with potassium phosphate, where breakdown of cholesterol was postulated, as described by V. N. Kolmakov [3]. The results of some typical experiments are given in Table 5.

It follows from the results given in Table 5 that both the synthesis and breakdown of cholesterol are inhibited in the liver of starving rabbits. The administration of choline to starving rabbits restored the breakdown of cholesterol in the liver tissue but had no essential effect on its synthesis.

It may thus be considered that under the influence of choline the power of the liver to store and break down the cholesterol of the blood is increased, which leads to a reduction of the hypercholesteremia.

It may be concluded from these experiments that the administration of choline to starving animals delays the development of hypercholesteremia, increases the stability of the blood cholesterol of these animals, promotes storage by the liver of the cholesterol of the blood and the excretion of cholesterol in the bile, and stimulates the breakdown of cholesterol in the liver.

#### SUMMARY

The effect of choline on the cholesterol metabolism was studied in fasting rabbits. Administration of choline (0.5 gm per kg of body weight) delays the development of hypercholesteremia, increases the resistance of cholesterol in the plasma of these animals, promotes the retention of blood cholesterol by the liver and cholesterol excretion in the bile and intensifies the processes of cholesterol destruction in the liver.

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