

THE ROLE OF THE CEREBRAL CORTEX AND SUBCORTICAL CENTERS
IN THE REGULATION OF BLOOD CHOLESTEROL LEVELS

COMMUNICATION I: CHANGES IN BLOOD CHOLESTEROL LEVEL ON FORMATION OF A CONDITIONED
REFLEX TO RESPIRATORY INHIBITION IN RABBITS

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Disorders of lipid metabolism and particularly that of cholesterol play an important role in a number of pathologic processes, such as atherosclerosis of blood vessels, lipid nephrosis, cutaneous xanthomatosis and others [1, 7, 8].

The work of S. S. Khalatov [7, 8], A. A. Znachkova [3], P. D. Gorizontov [2], N. T. Shutova [9] et al has shown that there is increased diminution of cerebral cholesterol and a rise in blood cholesterol level on emotional excitation, oxygen starvation, intoxication and destructive processes in the brain. On the basis of these facts S. S. Khalatov concluded that a number of hypercholesterinemic conditions were of cerebral etiology.

It was later shown that prolonged narcotic inhibition led to a decrease in blood serum cholesterol and its increase in the brain [4, 6].

Stimulation of the carotid sinus by bacterial filtrate of enteric bacillus [5] and electric stimulation of the brain [10, 11] led to a rise in blood cholesterol.

In the present work investigation of the blood cholesterol levels (using the Engelhardt-Smirnova micro-method) during changes in the functional state of the cerebral cortex and subcortical structures was undertaken.

EXPERIMENTAL

2 series of experiments were carried out on 10 rabbits. Following thrice-repeated estimation of blood serum cholesterol in 4 rabbits its level was determined on formation of a conditioned reflex to defensive reflex inhibition of respiration. The intervals between determinations in the course of the whole investigation did not exceed 5 days.

The sound of a metronome served as the conditioned stimulus. Unconditioned inhibition of respiration was produced by stimulation of the nasal mucosa by threshold doses of CO₂ admitted under a certain pressure onto a reflector held by means of a mask near the animal's nose. The distance between the reflector and the nose was 5 mm. The mask and reflector did not cover the animal's eyes, nose or ears and did not restrict its movements.

The sound of the metronome preceded the action of CO₂ by 3-4 seconds. 6 combinations of the stimuli with 5 minute intervals were used during the experiment; the pneumogram, the application of stimuli and the time were recorded on a kymograph (Fig. 1, C).

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Formation of the conditioned reflex passed through the stage of conditioned reflex increase in the rate of respiration and decrease in the amplitude of respiratory movements (Fig. 1, B). The typical form (cessation of respiration) of the conditioned reflex (Fig. 1, D) was established in rabbits (No. 2 and 4). In two other rabbits (No. 3 and 1) the reflex in most cases manifested itself by increased rate and decreased amplitude of respiration (Fig. 1, B) and only in 5-7% by cessation of respiration.

The first series included 217 experiments using a conditioned stimulus and 168 blood serum cholesterol estimations. The animals were kept under observation for 6 months.

RESULTS

During the formation of the conditioned reflex, as early as 10-15 days after the beginning of the experiments (60-80 combinations) all the rabbits showed a 25-30% decrease of blood serum cholesterol. On further prolonged work with the animals the average content of cholesterol decreased by 50% (Table 1) as compared with the average initial level. The maximal decrease noted towards the end of the procedure amounted to 80% in some cases.

A comparison of the number of positive responses to the conditioned stimulus (increase in respiratory rate with decrease in amplitude, cessation of respiration) and the degree of decrease of blood serum cholesterol shows that in the case of rabbit No. 3 the conditioned reaction appeared in a smaller percentage of cases than in other rabbits and the decrease in blood serum cholesterol in the same rabbit was insignificant (Table 2).

Therefore the decrease in cholesterol content is in direct ratio to the extent to which the conditioned reflex is pronounced.

On prolonged and continuous work with the animals disturbances of conditioned reflex activity were observed, which manifested themselves in a decrease of positive responses to the conditioned stimulus. These disturbances as a rule corresponded with minimal blood serum cholesterol levels. Towards the end of a two-months interval following termination of experiments using a conditioned stimulus the blood serum cholesterol remained decreased in 2 rabbits; in two others the cholesterol level exceeded the original value by 60-75%.

In the second series of experiments the blood serum cholesterol levels were studied in rabbits under conditions of dietary cholesterol "loading" against a background of conditioned reflex activity.

Observations were carried out successively on 3 groups of animals. In each group there were 2 experimental and 2 control rabbits which received cholesterol in equal amounts. In the first and second groups, experiments using a conditioned stimulus were begun on the experimental animals after 4-6 feeds of cholesterol when its level had risen 3-4-fold as compared with the initial content.

Figure 1. Respiration in rabbit during various periods of the formation of conditioned reflex. Records (from above down): respiration, beginning of stimulation, time marker in seconds; the figures refer to the serial number of combination of stimuli. M - metronome in A, B, C, D.

First group (Table 3). Experimental rabbits No. 1 and 4 and control rabbits No. 5 and 6 were given 0.5 g crystalline cholesterol in 40 ml milk. The increment of cholesterol in the blood serum towards the end of feeding found in the experimental rabbits was 5 and 8 mg % respectively. In the course of the same period of feeding the cholesterol increment in control rabbits was 164 and 166 mg %.

TABLE 1

Changes in Cholesterol Levels in Rabbits During Formation of Conditioned Reflexes

No. of rabbit	Blood serum cholesterol							Number of experiments using conditioned stimulus
	Average of 3 determinations	During conditioned reflex activity				After 60 days		
		Average of 17 determinations		Minimal in mg %	Minimal in % of initial	Average of 3 determinations		
		In mg %	In % of Initial			In mg %	In % of Initial	
1	37	21	56	10	27	22	59	57
2	25	19	76	10	40	44	176	54
3	50	33	66	20	40	80	160	52
4	24	13	54	5	20	14	58	54

TABLE 2

Manifestation of Conditioned Reflex in % of Total Number of Stimulations

No. of rabbits	Respiratory changes	Cessation of respiration	Presence of conditioned reaction	Absence of response
1	77.8	5.5	83.3	16.7
2	36	60	96	4
3	70.6	4.1	74.7	25.3
4	40.6	54.7	95.3	4.7

The average blood serum cholesterol content in rabbits No. 1 and 4 during feeding was 62 and 32 mg%, the average content in control rabbits No. 5 and 6 was 217 and 141 mg%. Details of conditioned reflex activity in the experimental rabbits are given in Table 4.

Second group. Experimental rabbits No. 2 and 3 and control rabbits No. 7 and 8 received 38 doses (0.5 g) of cholesterol over a period of 70 days. By the end of feeding (Table 3) the increment of blood serum cholesterol in experimental rabbits No. 2 and 3 and 85 and 431 mg% respectively. In control rabbits No. 7 and 8 the increment was equal to 246 and 573 mg%. The average blood serum cholesterol content during feeding was 64 and 258 mg% in experimental rabbits. In control animals it was 162 and 393 mg%.

Thus, in 3 out of 4 experimental rabbits (from the first and second groups) ingestion of cholesterol against the background of conditioned reflex activity did not result in hypercholesterinemia, and the cholesterol increment towards the end of feeding was insignificant. In one experimental rabbit (No. 3), as well as in the control rabbits, hypercholesterinemia was clearly evident. It must be remarked that the degree of manifestation of the conditioned reflex was much less in rabbit No. 3 than in the other rabbits (Table 4).

TABLE 3

Blood Serum Cholesterol Content

No. of rabbit	Blood cholesterol content in mg %					Number of cholesterol feeds	Duration of observation (days)
	Initial	At the end of feeding	Increment towards end of feeds	Average (of 11) during feeds	Average increment during feeding		
1	28	36	8	62	34	34	64
4	15	20	5	32	12	34	64
5	39	203	164	217	178	28*	53
6	24	190	166	141	117	28*	53
1 ₂	14	30	16	75	61	19	36
4 ₂	20	53	33	77	57	19	36
9	21	206	185	241	220	19	36
10	86	396	310	302	218	13*	26
2	21	106	85	64	43	38	70
3	35	466	431	258	223	38	70
7	40	286	246	162	122	38	70
8	60	633	573	393	333	38	70

TABLE 4

Manifestation of Conditioned Reflex in % of Total Number of Stimulations

No. of rabbit	Respiratory changes	Cessation of respiration	Presence of conditioned reaction	Absence of response	Number of experiments using a conditioned stimulus
1	92	3	95	5	18
4	34	66	100	0	19
1 ₂	68.0	9.3	77.3	22.7	24
4 ₂	68.0	29.3	97.3	0.3	24
2	56	40	96	4	28
3	76.1	0.7	76.8	23.2	27

Following a three-months interval experiments were repeated on rabbits No. 1 and 4. A new pair of control rabbits (No. 9 and 10) was taken, and the animals were fed egg yolk. Four egg yolks were mixed with milk and the volume made up to 160 ml. A single feed consisted of 40 ml of mixture of milk with one egg yolk (0.3 g cholesterol). Each rabbit received 19 yolks in the course of 36 days. Results of the experiments are presented in Tables 3 and 4. By the end of feeding the blood cholesterol increment in the experimental rabbits No. 1₂ and 4₂ was 16 and 33 mg%. During the same period the control rabbit No. 9 showed a cholesterol increment of 185 mg% and rabbit No. 10 showed an increment of 310 mg% over a considerably shorter period (13 feeds). The average blood serum cholesterol content in experimental rabbits No. 1₂ and 4₂ during feeding was 75 and 77 mg%, the average cholesterol content in control rabbits No. 9 and 10 was equal to 241 and 302 mg%. It is thus seen that ingestion of egg yolks against the background of conditioned reflex activity did not lead to the development of hypercholesterinemia in rabbits. No changes in the conditioned reflex activity of rabbits could be observed during administration of cholesterol. A more or less constant level of such activity was maintained.

In summary, the results of the present series of experiments indicate that during conditioned reflex activity the blood serum cholesterol increment was insignificant in 5 out of 6 cases by the end of the feeding and there was no development of hypercholesterinemia in rabbits (Fig. 2). During the same period in 8-10-fold rise in blood serum cholesterol was observed in control rabbits (Fig. 2). It must also be noted that the level of blood serum cholesterol during feeding was in inverse relation to the extent of conditioned reflex manifestation.

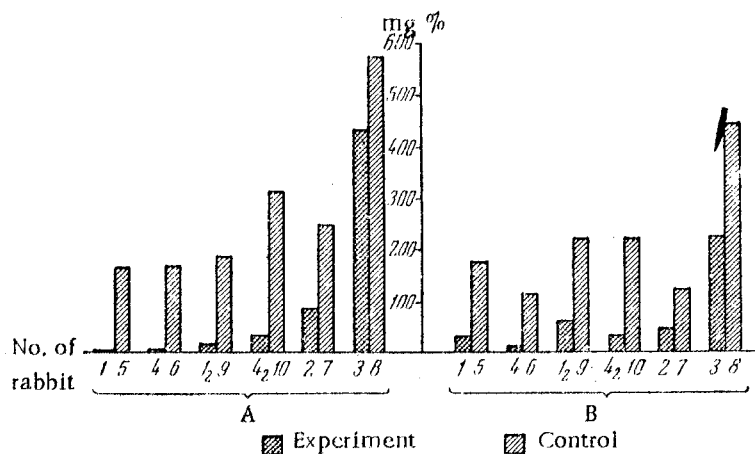


Figure 2. Cholesterol increment in blood serum of rabbits during ingestion of cholesterol. A) Increment towards end of feeding (in mg%); B) average increment during feeding (in mg%).

Thus, rabbit No. 3 showed development of hypercholesterinemia on feeding, and exhibited a weak type of nervous system with the lowest number of positive responses to the conditioned stimulus.

Upon cessation of observations the experimental and control rabbits were sacrificed and a histologic examination of the blood vessels with respect to lipids was carried out. It was found that both the experimental and control animals showed primary lipoidosis of the vessels despite the low blood serum cholesterol in the former group. The data obtained, with their evidence of primary deposition of lipids within the blood vessel wall in rabbits, pose the question whether it is possible for vascular lipoidosis to develop at normal blood cholesterol levels in the presence of altered nervous regulation.

SUMMARY

Development of a conditioned reflex to inhibition of respiration in the rabbit is accompanied by a decline of blood cholesterol. Feeding cholesterol to animals in a state of conditioned reflex activity did not result in hypercholesterinemia, while in control animals cholesterol feeding caused a rise of blood serum cholesterol. The cerebral cortex and subcortical formations are thus participating in the regulation of cholesterol metabolism.

Preliminary data demonstrating deposition of lipoids in vascular walls of experimental rabbits, point to the possibility of vascular lipoidosis being caused by altered nervous regulation, the cholesterol blood level being normal.

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