THE PARTICIPATION OF INSULIN AND GLUCOCORTICOIDS IN THE REGULATION OF THE CHOLESTEROL METABOLISM

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It is known that during the state of starvation, changes in the hormone metabolism are observed in the organism: the insulin production is reduced [4, 6] and the production of glucocorticoids is increased [9, 13]. In addition, and apparently as a result of this, an inhibition of the glucokinase reaction is noted [2, 11], which leads to an inhibition of the carbohydrate metabolism as a whole, a deficiency of plastic (TPN-H) and energy (ATP) products [1, 14]. An organism with a "carbohydrate" type of metabolism changes to a "fat" type. Mobilization of fat from the fat depots occurs [10], along with breakdown of fatty acids in the liver to ketone bodies [8] and the accumulation of fatty inclusions in the tissues [7]. In humans [5] and certain species of animals, for example, rabbits and guinea pigs [12], hypercholesterinemia develops during the state of starvation.

In this work we undertook to determine the possible pathways of the influence of insulin and cortisone on certain indices of the lipid metabolism in animals.

EXPERIMENTAL PROCEDURE

Three series of experiments were conducted on rabbits 2.5-3 kg in weight. In one series all the animals were left without food, but they received water for five days. Before starvation, and on the third and fifth days of starvation, blood was removed from their hearts, after which the content of lipid phosphorus was investigated in the blood serum according to Fiske-Subbarow, and free cholesterol and its esters by the method of chromatography on Al_2O_3. The cholesterol esters were washed out with benzene, and free cholesterol with ethyl ether. The total cholesterol was determined according to the sum of the two preceding indices.

After restoration of the original weight of the rabbits (after 10-12 days), they were again subjected to starvation according to this same scheme, receiving daily intramuscular injections of 20 mg cortisone and 10 units of insulin during the entire experiment. The hormone doses were selected so that the dominant role in the organism would be played by insulin. To prevent the development of hypoglycemic coma, leading to death of the animals, cortisone was injected 2-2.5 h before insulin. This is due to the fact that the compensating effect of cortisone on glucokinase is manifested more slowly than that of insulin. During the same periods, blood was taken from the heart, and the same indices were determined in the blood serum.

At the end of the experiment we studied the dynamics of the change in the blood sugar after the injection of cortisone and insulin in the indicated ratio. For this purpose, blood was withdrawn from the ear of the rabbit in the morning, and it immediately received an injection of cortisone (20 mg), then blood was taken again after two hours, and insulin was immediately injected (10 units). Subsequently the blood was taken at two-hour intervals until restoration of the initial results. After the end of the experiment, the animals were killed, and the total cholesterol content was determined in the liver, lungs, kidneys, and muscles.

The second series of experiment was conducted according to the same scheme as the preceding, only the ratio of hormones introduced was changed—the dose of cortisone now comprised 30 mg, while the dose of insulin was five units per day.

TABLE 1. Joint Action of Cholesterol (20 mg) and Insulin (10 Units) on the Development of Hypercholesterinemia in Starving Rabbits (Average Data of Eight Experiments)

Conditions of experiments	Time of taking of blood	Free cholesterol	Cholesterol esters	Total cholesterol	Lipid phosphorus
		In mg %			
Starvation five days	Before starvation	16.3 ± 0.7	41.3 ± 2.6	57.6 ± 2.3	9 ± 0.2
	On third day of starvation .	30.2 ± 3.4	61.8 ± 5.2	92 ± 8	11 ± 0.7
	On fifth day of starvation .	42.5 ± 5	86 ± 7	128.5 ± 10	14.8 ± 0.5
	Rest for 12 days				
Starvation five days Cortisone (20 μg/day) Insulin (10 units/day)	Before starvation	15.8 ± 0.9	41.8 ± 2.9	57.6 ± 3.3	8.1 ± 0.3
	On third day of starvation .	12.7 ± 1.3	44.8 ± 4.4	57.5 ± 5	7 ± 0.3
	On fifth day of starvation .	12.2 ± 2.5	47.7 ± 7.8	59.9 ± 8.4	7.1 ± 0.3

The third series of experiments was conducted on rabbits with dithizon diabetes. For this purpose, the sugar content in the blood of the animals was preliminarily determined according to Hagedorn-Jensen, along with the free cholesterol content, cholesterol esters, total cholesterol, and lipid phosphorus in the blood serum by the methods indicated above. On the following day, all the rabbits received intravenous injections of dithizon in a dose of 80 mg/kg. Beginning with the 11th day, the animals received intramuscular injections of hydrocortisone in a dose of 8 mg/kg. Blood was taken on the fourth, sixth, ninth, 11th, 14th, and 16th days (from the moment of its first removal) and investigated according to the same indices.

RESULTS OF THE EXPERIMENTS

During five-day starvation, the rabbits developed substantial hypercholesterinemia and less substantial hyperphosphatidemia (Table 1). However, in the case of repeated starvation, when the animals received injections of cortisone (20 mg) and insulin (10 units), the development of hypercholesterinemia and hyperphosphatidemia was almost entirely prevented. This pertains especially to free cholesterol, the content of which frequently dropped below the initial level. The same may also be stated concerning the lipid phosphorus content. As for cholesterol esters, their content in the blood serum either did not change at all, or increased negligibly.

In view of the fact that insulin and cortisone exert opposite influences on the rate of the glucokinase reaction, we determined the dynamics of the blood sugar content. It was found that cortisone in a dose of 20 mg does not change the blood sugar level two hours after its injection, while subsequent injection of insulin in a dose of 10 units leads to a sharp drop in the sugar content in the blood (from 120 to 35-40 mg %), which is restored to normal only after six hours. Since we injected the hormone daily over a five-day period, it may be assumed that the comparatively rapid restoration of the sugar content in the blood is evidence of active supplementation of the reserves of glycogen in the liver under the influence of cortisone, and, consequently, of an intensification of the processes of glycogenogenesis. In the absence of cortisone, the animals died even from the injection of 1 unit of insulin. We know of cases in the literature of unsuccessful study of the effect of insulin on the cholesterol metabolism in starving animals precisely as a result of the development of hypoglycemic coma [3].

In order to resolve the question of whether the inhibition of hypercholesterinemia in starving rabbits after the injection of cortisone (20 mg) and insulin (10 units) is related to the deposition of cholesterol in the tissues, we determined it in the muscles, liver, lungs, and kidneys—the most active organs with respect to cholesterol metabolism. The results of the determinations are presented in Table 2, from which it is evident that the cholesterol content in the abovementioned organs became greater during the process of starvation. However, during starvation with the injection of cortisone (20 mg) and insulin (10 units), the cholesterol content in the investigated tissues was substantially below that in the usual starvation and practically did not differ from the norm. This indicates that cortisone with insulin in this ratio not only does not promote the deposition of cholesterol in the tissues, but even prevents this. Thus, the impression is created that the basic factor in the joint action of insulin and cortisone on cholesterol metabolism during starvation is the stimulation of carbohydrate metabolism. This is in good agreement with the

TABLE 2. Effect of Cortisone (20 mg) and Insulin (10 units) on the Total Cholesterol Content (in mg %) in the Tissues

Number of experiments	Conditions of experiments	Liver	Lungs	Kidneys	Muscles
10	Norm	235 ± 25	420 ± 30	298 ± 12	56 ± 10
10	Five-day starvation	345 ± 32	508 ± 25	395 ± 16	57 ± 13
8	Five-day starvation + cortisone + insulin	241 ± 25	436 ± 24	315 ± 12	57 ± 10

TABLE 3. Joint Action of Cortisone (30 mg) and Insulin (5 units) on the Development of Hypercholesterinemia in Starving Rabbits

Number of experiments	Conditions of experiments	Time of taking of blood	Free cholesterol	Cholesterol esters	Total cholesterol
			in mg %		
6	Five-day starvation	Before starvation	13.7 ± 0.7	32.5 ± 1.8	46.2 ± 2
		On third day of starvation	24.3 ± 3.2	54.8 ± 6	79.1 ± 9.4
		On fifth day of starvation	40.5 ± 5	80.2 ± 8	120.7 ± 16
		12-day rest			
6	Five-day starvation + cortisone + insulin	Before starvation	13.9 ± 0.6	32 ± 1.8	45.9 ± 2.2
		On third day of starvation	23.7 ± 2.9	47.8 ± 4.4	71.5 ± 8
		On fifth day of starvation	36.1 ± 4.6	68.6 ± 7.8	104.7 ± 15

fact that the most powerful factor in the inhibition of hypercholesterinemia during starvation is the injection of carbohydrates. Proteins and fats, on the other hand, intensify hypercholesterinemia.

It should be mentioned that the injection of cortisone together with insulin is expedient only when the effect of insulin in the organism predominates over the action of cortisone. This can be seen from Table 3, which presents the results of experiments in which the dose of cortisone was increased to 30 mg, while the dose of insulin was reduced to 5 units. In this case, stimulation of the processes of glycogenesis evidently did not occur, since cortisone, intensifying the new formation of carbohydrates from protein and fats, simultaneously inhibited the glucokinase reaction. In the experimental animals that received an increased dose of cortisone and a reduced dose of insulin, as before, a relatively high hypercholesterinemia developed.

The sugar content in the blood of such animals underwent less of a change than in the preceding case: it was reduced two hours after the injection of insulin (from 120 to 70 mg %) and was already restored to normal after four hours.

Consequently, the ratio of insulin to cortisone in the starving rabbit organism plays an important role in the determination of the degree of development of hypercholesterinemia.

In conclusion, we attempted to show that reduction of the insulin production and the increased glucocorticoid production is a quite sufficient condition for the development of hypercholesterinemia. For this purpose, rabbits which dithizon diabetes received injections of hydrocortisone in a dose of 8 mg/kg from the moment when the sugar content in the blood was still above normal, while the content of cholesterol fractions was approaching the norm. The injection of hydrocortisone under these conditions led to a substantial increase in all the investigated blood indices: sugar—from 175 to 453 mg %, total cholesterol—from 35 to 90 mg %, and lipid phosphorus—from 8 to 19 mg %.

A detailed determination of the effects of the indicated hormones on cholesterol metabolism will require further study.

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