ACTIVITY OF α-AMYLASE AND GLUCOSE-6-PHOSPHATASE OF THE LIVER AND SUGAR LEVEL IN THE BLOOD OF ANIMALS DURING PLAGUE INTOXICATION

(UDC 616.981.452-07:[616.36-008.931+616.153.45]-092.9)

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 60, No. 8,
pp. 61-64, August, 1965
Original article submitted February 5, 1964

During plague intoxication, as in other bacterial intoxications, an exhaustion of the reserves of liver glycogen and a change in the blood sugar level are noted, which, in the opinion of certain authors [4, 5], are due to a disturbance of the activity of the adrenals; however, the state of the enzyme systems that cleave glycogen during plague intoxication has practically not been studied. It is known only that the activity of the liver phosphorylase of white mice is increased in this case [3].

In this work we investigated the influence of the toxin of the plague microbe on the activity of α-amylase and glucose-6-phosphatase in the livers of animals. In addition, we determined the sugar level in the blood of white rats at various periods of plague intoxication, since the data pertaining to glycemia in various species of animals, in particular, in white rats, are contradictory. In addition, we studied the dynamics of the exhaustion of the glycogen reserves in the livers of white rats during plague intoxication by an adrenal test.

EXPERIMENTAL PROCEDURE

White mice 18-20 g in weight and white rats 180-200 g in weight were used in the experiments. A preparation of fraction II, produced from the plague microbe according to the Baker method, was used as the plague toxin (LD_{50} for white mice was equal to 1.8 μg, for white rats—8 μg). The toxin was administered to the animals intraperitoneally. The white mice received one LD_{50} of the toxin. After 17 h they were decapitated. The white rats received doses of 10 LD_{50} of the plague toxin and were killed after 2 h. The livers of the animals were pulverized in a glass homogenizer with twice the volume of physiological saline cooled on ice.

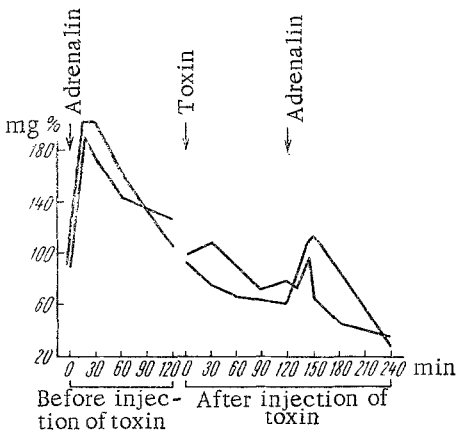

Fig. 1. Influence of adrenalin on the sugar level in the blood of white rats before and after injection of plague toxin (10 LD_{50}).

The amylase activity of the livers of the white mice was determined according to the method of Rutter and Brosemer [12]. The amylase activity was expressed in milligrams of decomposed starch in 30 min of incubation at 37°, converted to 100 mg of dry weight of the liver.

The activity of glucose-6-phosphatase was investigated in the blood serum and livers of the animals according to the method of Dosta and Ostrovskii [2]. The enzyme activity was expressed in millimoles of phosphorus, liberated in 1 h at 37°, converted to 1 ml of serum or to 100 mg of dry weight of the liver.

The experimental results were subjected to statistical treatment [1].

Activity of Glucose-6-Phosphatase in the Liver and Blood Serum of Healthy Animals and Animals Poisoned by Plague Toxin

Species of animal	Source of enzyme	Time after injection of toxin	Dose of toxin (in LD_{50})	Enzyme activity	Number of experiments
White mice	Liver	Control	–	11.5±0.9	7
		After 17 hours	1	11.5±0.9	
	Blood serum	Control	–	3.1±0.5	5
		After 17 hours	1	2.9±0.6	
White rats	Blood serum	Control	–	6.6±0.7	11
		After 2 hours	10	8.2±0.6	

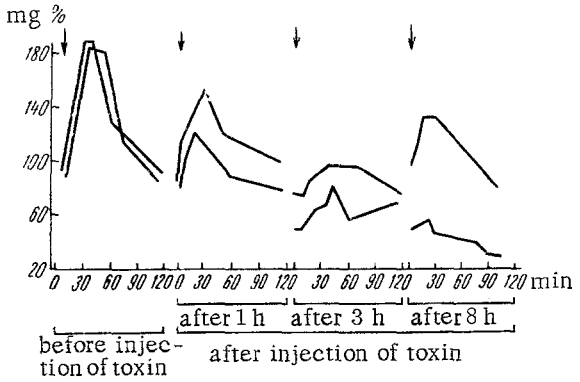

Fig. 2. Influence of adrenalin on the blood sugar level of white rats before and after injection of plague toxin (3 LD_{50}). Arrows –moment of injection of adrenalin.

The influence of the toxin of the plague microbe and adrenalin on the blood sugar level was tested on white rats. The animals were divided into 3 groups–7 rats in each. The rats of the 1st and 2nd groups received 10 LD_{50} of the plague toxin (the animals died from this dose in 4-5 h), and blood was taken from the rats at 30 min intervals over a period of 2 h for sugar determination. After this time had elapsed, the 2nd group of animals (rats poisoned with toxin) received injections of adrenalin in a dose of 50 μg intraperitoneally. The same dose of adrenalin was administered to healthy white rats (3rd group). The blood sugar was determined 10, 20, 30, 60, and 120 min after the injection of adrenalin.

The dynamics of the influence of adrenalin on the blood sugar level of animals poisoned by plague toxin was studied on a group consisting of 10 white rats. Eight of them received injections of toxin in a dose of 3 LD_{50}. One hour after introduction of the toxin, 2 rats received injections of adrenalin, and the blood sugar level was determined after definite time intervals. A rat was subjected to the same treatment 3 and 8 h after injection of the toxin. The blood sugar ("true glucose") was determined according to the Shomon'i-Nil'son method [6].

EXPERIMENTAL RESULTS

As can be seen from the table, plague toxin does not affect the activity of glucose-6-phosphatase in the livers of white mice. No effect of it upon the activity of glucose-6-phosphatase in the blood serum of the animals was established: 17 h after the injection of 1 LD_{50} of the toxin, the enzyme activity in the blood serum of white mice remains unchanged. Even in the case of a lethal intoxication, when the animals received 10 LD_{50} of the toxin, the glucose-6-phosphatase activity was as before. In spite of the fact that the enzyme activity was almost twice as high as normal in the blood serum of certain poisoned animals, the difference between the average values of the experimental and the control was not statistically reliable ($P > 0.05$). Hence, it is impossible to resolve the question of the state of metabolism of this compound during plague intoxication on the basis of the absence of an effect of plague toxin on the liver glucose-6-phosphatase activity alone.

Plague toxin also does not affect the activity of liver α-amylase of white mice: 17 h after the injection of 1 LD_{50} of the toxin, the content of decomposed starch was 23.8±3.1 mg, while in healthy animals it was 22.6±1 mg. The α-amylase activity was not determined in the livers of guinea pigs.* We had earlier established a decrease in

* According to the communication of Brosemer and Rutter [9], no α-amylase activity is detected in livers of guinea pigs. At the same time, its activity in the livers of white mice is higher than the activity of phosphorylase [3]. These facts are interesting in view of the fact that white mice and guinea pigs, as is well known, manifest different sensitivities to plague toxin.

the activity of acid phosphatase* and an increase in the activity of the liver phosphorylase of white mice during plague intoxication [3]. The latter facts evidently indicate an intensification of decomposition of glycogen in the liver to lactic acid and a disturbance of the sugar-forming function of the liver.

As can be seen from Fig. 1, 30 min after the injection of 10 LD_{50} of plague toxin in white rats, a decrease in the sugar concentration in the blood is observed, and only in certain animals is an inconsequential increase in it noted.

The dynamics of the decrease in the blood sugar level of poisoned white rats after the administration of the smaller dose of the toxin (3 LD_{50}) can be followed in Fig. 2: after 1 h, the sugar concentration was within normal limits (75 mg %); hypoglycemia set in after 3 h, and turned into lethal hypoglycemia by the 8th hour. The cause of the hypoglycemia evidently is exhaustion of the reserves of glycogen in the liver during plague intoxication [4]. This hypothesis is supported by our data on the influence of adrenalin on the blood sugar level in healthy and poisoned white rats. Although the administration of adrenalin to healthy white rats induced the characteristic hyperglycemia after only 10-20 min, in the case of plague intoxication, judging by the results of 7 experiments, the reaction of adrenalin was reduced (see Fig. 1) or did not appear at all. Since the response to adrenalin determines the level of glycogen reserve, the absence of a reaction to it is a confirmation of the data of the indicated authors on the exhaustion of the glycogen reserves in the liver. In addition, as can be seen from Fig. 2, the response to adrenalin changes gradually: 1 h after the injection of 3 LD_{50} of the toxin, hyperglycemia was less pronounced in comparison with the norm; after 3 h, the rise in the hyperglycemic curve continued to decrease, while by the 8th hour, there was no response to adrenalin† (see Fig. 2).

Thus, it has been established that the toxin of the plague microbe does not affect the α-amylase and glucose-6-phosphatase activity of the livers of white mice and rats. At the early periods of plague intoxication, the blood sugar level in the white rats is unchanged, while at the late periods and in the agonal period, hypoglycemia develops, evidently, as a result of exhaustion of the glycogen reserves in the liver. These results were confirmed by an adrenalin test.

SUMMARY

The present research shows that the plague toxin does not affect the activity of alpha-amylase and glucose-6-phosphatase in the liver of albino mice and rats. In the early stages of plague intoxication the sugar content of the blood in albino rats remains unchanged, and in the later stages and the agonal period hypoglycemia comes on the scene, apparently as a result of depletion of the glycogen supply in the liver.

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* An interrelationship has been established between the acid phosphatase activity of the liver and the blood sugar reaction [8, 11].

† The absence of a response to adrenalin 4.5 h after administration of Salmonella endotoxin to white mice is communicated by Berry and Mitchell [7].