SPECIES DIFFERENCES IN THE DISTURBANCES OF CARBOHYDRATE AND ASCORBIC ACID METABOLISM OF RODENTS FOLLOWING ADMINISTRATION OF PLAGUE TOXIN

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We have shown [1, 2] that administration of toxins extracted from strain EV plague microbes leads to a fall in the ascorbic acid content of various organs of rodents. For these experiments we used white mice and rats, which are very sensitive to the plague toxin, and the less sensitive gerbils; we found that certain species differences could be discerned in the effect of the toxin on the vitamin C content of the organs examined. We found that 7-18 times as great a dose of strain EV Pasteurella pestis autolyzate was required to bring about the same degree of depletion of ascorbic acid in the resistant rodents as in rats or mice.

The connection between species differences in resistance of rodents to plague toxin and the intensity of the metabolic disturbances caused by the toxin was even more clearly shown by the results of our investigations of carbohydrate metabolism in plague intoxication. The fall in liver glycogen content of white mice and rats following administration of P. pestis EV autolyzate or fraction II of virulent plague microbes was greater than in guinea pigs, which are highly resistant to plague toxin.

These findings may be of interest not only for the better understanding of the pathogenesis of plague intoxication, but also for the elucidation of the nature of the species differences in sensitivity of animals to plague toxin. For this reason we repeated our earlier experiments on white mice, guinea pigs, and gerbils, including also two more species of rodent, viz., emuranchiks and dwarf gophers, which are more resistant to the action of plague toxin than are white mice.

In view of published reports [7] that ascorbic acid metabolism is closely bound up with that of carbohydrates, our analysis of the effect of plague toxin on the experimental animals included determination of the ascorbic acid contents of the suprarenal glands, spleen, liver, and lungs, as well as of liver glycogen and blood sugar.

EXPERIMENTAL METHODS

The wild rodents were trapped in the steppes of the Stavropol and Astrakhan Regions, as follows: gerbils (Meriones meridianus nogaiorum Heptn.) and dwarf gophers (Citellus pygmaeus Pall.) in the Levokum Province, and emuranchiks (Scirtopoda telum Licht.) in the Volga Province.

The animal material used in the experiments was as follows: 30 white mice (20 males and 10 females, weighing from 19 to 30 g), 20 gerbils (10 males and 10 females, weighing from 38 to 63 g), 20 emuranchiks (10 males and 10 females, weighing from 52 to 72 g), 29 dwarf gophers (14 males and 15 females, weighing from 140 to 300 g), and 21 guinea pigs (17 males and 4 females, weighing from 370 to 800 g).

TABLE 1

Changes in the Contents of Liver Glycogen, Blood Sugar, and Ascorbic Acid in Organs of Rodents 30 Minutes After Intraperitoneal Injection of Fraction II of Pasteurella pestis

Species of rodent	Dose of fraction II (mg/100 g)	Number of animals	Liver glycogen content (%)	Blood sugar content (mg, %)	Ascorbic acid content (mg, %)			
					of suprarenal glands	of spleen	of liver	of lungs
White mice	Control	15	3,51±0,9	127,2±6,8	209,4±56,4	35,8±7,6	32,0±5,3	17,6±7,5
	0.036	15	1,83±0,16	158,8±2,4	124,0±15,8	27,3±3,6	29,3±1,8	11,3±3,6
Gerbils	Control	10	3,71±0,6	119,4±3,9	119,0±28,3	46,3±11,1	27,0±4,4	9,6±1,4
	0.05	10	2,86±0,5	141,5±8,4	71,9±17,1	33,9±7,5	28,1±7,2	8,9±0,6
Emuranchiks	Control	10	5,32±0,97	147,9±12,4	169,0±50,0	99,0±21,5	18,1±4,2	20,1±5,1
	0.05	10	3,75±1,74	167,5±8,7	102,5±13,0	42,5±8,5	29,5±11,0	9,3±2,1
Guinea pigs	Control	12	3,62±0,42	104,7±0,9	74,8±27,6	25,5±5,9	12,5±2,1	9,3±1,0
	0.04-0.06	9	3,8±0,78	118,2±7,8	55,0±15,3	16,7±4,4	7,1±1,5	4,9±2,8
Dwarf gophers (experiment No. 1, October)	Control	10	5,48±1,1	150,3±24,5	114,8±2,5	17,3±4,8	18,5±3,1	9,1±3,8
	0.05-0.06	10	7,83±1,45	154,4±21,8	114,8±24,4	17,6±4,4	21,3±4,9	7,5±3,0
Dwarf gophers (experiment No. 2, April)	Control	4	2,44 (0,95—3,15)	103 (92—118)	105,2 (70,7—144,4)	16,6 (10,3—25,0)	20,5 (13,5—25,6)	10,0 (5,1—14,3)
	0.05	5	1,66 (0,56—3,0)	125,4 (65—145)	44,6 (28,0—77,0)	12,9 (5,1—22,2)	11,1 (6,1—14,9)	5,4 (3,6—6,6)

* The figures in parentheses indicate the range of the values found.

TABLE 2

Species Differences in the Liver Glycogen Content, the Blood Sugar Content, and the Ascorbic Acid Content of Different Organs of Rodents Injected with Plague Toxin

Species of rodent	Degree of change (as % of the value found for the controls)					
	content		ascorbic acid content			
	of glycogen in the liver	of blood sugar	of the adrenals	of the spleen	of the liver	of the lungs
White mice	−47.9	+24.8	−40.8	−23.8	− 8.5	−35.8
Gerbils	−22.9	+18.5	−39.6	−26.8	+ 4.1	− 7.3
Emuranchiks	−29.5	+13.2	−39.4	−57.1	+16.3	−53.8
Guinea pigs	+ 5.0	+12.8	−26.5	−34.5	−43.2	−47.4
Dwarf gophers (experiment No. 1, October)	+42.9	+ 2.7	0	0	+15.1	−17.6
Dwarf gophers (experiment No. 2, April)	−32.0	+22.0	−58.0	−22.0	−46.0	−46.0

Note: − fall, + rise

The experiments on white mice, gerbils, and emuranchiks were performed in the second half of July, on guinea pigs towards the end of September, and on dwarf gophers during the hibernation period (the first half of October), and after hibernation (early April).

The animals of each species were divided into two groups, one of which served as the controls, while the other was given plague toxin. The same numbers of males and females were included in each group.

The toxic material used was fraction II of virulent plague microbes (strain No. 137), prepared in our Institute by M. I. Levi by the method of Baker et al. [5]. The LD_{50} of this fraction, when given by intraperitoneal injection to male white mice, amounted to 0.009 mg, or 0.032 mg/100 g. Freshly prepared solutions of fraction II in 0.85% NaCl were given by intraperitoneal injection into white mice, at a dosage rate of 0.036 mg/100 g, and into the other four species of rodents at the rate of 0.04, 0.05, or 0.06 mg/100 g, according to their weight.

Our earlier experiments showed that plague toxin [1, 3], similarly to other stress-provoking factors [8, 9], caused more or less pronounced response reactions during the first few minutes of their action on the organism. Bearing this in mind, in the present research we killed the animals by decapitation or by a heavy blow on the head 30 minutes after injection of toxin.

Liver glycogen was determined by Pflüger's method, as modified by Osterberg, blood sugar by the Hagedorn-Jensen method, and ascorbic acid by indophenol titration according to Harris and Ray.

EXPERIMENTAL RESULTS

As is shown by the mean values given in Tables 1 and 2, the effect of 30 minutes of action of the plague toxin was to cause a more or less considerable fall in liver glycogen content, a rise in blood sugar, and depletion of the ascorbic acid content of the organs examined.

Does there exist any connection between the intensity and nature of these changes and the species sensitivity of rodents to plague toxin?

The results obtained in our laboratory for the sensitivity of five species of rodents to plague toxin are shown in Table 3. These comparative data for species sensitivity to plague toxin are incomplete, and require further work for the determination of LD_{50} of fraction II for emuranchiks, guinea pigs, and dwarf gophers. Preliminary experiments showed that, as for autolyzates of plague microbes, guinea pigs and dwarf gophers were the most resistant to fraction II: guinea pigs survived doses of 0.5 mg/100 g, and dwarf gophers of 2.5 mg/100 g.

TABLE 3

Sensitivity of Different Species of Rodents to Plague Toxin Given by Intraperitoneal Injection

Species of rodent	Magnitude of lethal dose		
	MLD of Pasteurella pestis EV autolyzate (mg/100 g body wt.)	LD_{50} for fraction II of Pasteurella pestis No. 137	
		(mg per animal)	(mg/100 g body wt.)
White mice	0.2	0.009	0.032
Gerbils	0.3-2.2	0.078	0.18
Emuranchiks	0.4-0.5	–	–
Guinea pigs	1.8-2.4	–	–
Dwarf gophers.	6.0	–	–

A consideration of the data of Tables 1, 2, and 3 reveals the existence of a certain degree of correlation between the sensitivity of a given species of rodent to plague toxin and the extent of glycogenolysis in the liver of the given animals. The most pronounced fall in liver glycogen, with the correspondingly greatest rise in blood sugar content, was found in white mice, which are the most susceptible to the toxin. Much smaller changes were found in gerbils and emuranchiks, even when somewhat higher doses of toxin were injected. Liver glycogen did not fall in guinea pigs, although there was a slight rise in the blood glucose content. Injection of toxin into hibernating dwarf gerbils (experiment No. 1) caused a fairly considerable rise in liver glycogen content, without appreciable change in the blood sugar level. The same doses of toxin administered to the animals after their spring awakening (experiment No. 2) caused pronounced glycogenolysis and hyperglycemia. This seasonal difference in the response of gophers is evidently related to seasonal fluctuations in the state of their carbohydrate metabolism and reactivity [4, 6]. This conclusion is not contradicted by the results obtained with emuranchiks, which also belong to the class of hibernating rodents. They differ from dwarf gophers in entering into hibernation in the late fall, with the onset of the first frosts. The experiments on emuranchiks were conducted in July, when the animals were active.

Our second group of results concerned the effect of toxin on the ascorbic acid contents of different organs. The extent of lowering of ascorbic acid content after 30 minutes of toxemia showed no regular correlation with species sensitivity to toxin. However, the more susceptible species of rodents showed a greater fall in the ascorbic acid content of the adrenals than did the resistant species. This effect was particularly pronounced with gophers. Whereas during the hibernation period injection of toxin had no effect on the ascorbic acid content of the adrenals and the spleen, and caused only a slight fall in its content in the lungs, and a slight rise in the liver, after the spring wakening the same dose of toxin caused a fall in the ascorbic acid content of all the organs examined, most of all in the adrenals.

The fall in the vitamin C content of the adrenals found following exposure to extraordinary stressor agents is regarded as an expression of enhanced functional activity of the adrenal cortex, in response to the stimulatory action of adrenocorticotrophic hormone [7, 8, 9]. The species differences found by us in the extent of glycogenolysis in the liver and in the fall in ascorbic acid concentration of the adrenals of animals given plague toxin are evidence that the response of the organisms to plague toxin is determined not only by the nature of the agent used, but also by the reactivity of the given species of animal.

The nature of these species differences in the extent of disturbance of carbohydrate and ascorbic acid metabolism following administration of plague toxin is obscure. It has been found in our laboratory that plague toxin causes intensification of the medullary function of the adrenals, modifying the reactivity of various systems of the organism to acetylcholine, histamine, and adrenalin, as also the reactivity of the carbohydrate metabolism of the liver in response to adrenalin, histamine, and insulin. It hence appears probable that the differences in degree of disturbance of carbohydrate metabolism in different species of animals suffering from the effects of plague toxin are related to species peculiarities in disturbance of neurohumoral regulation. It may also be supposed that an important part is played in the way in which carbohydrate metabolism is disturbed in plague intoxi-

cation by differences in the degree of inactivation of various enzyme systems participating in carbohydrate metabolism. The elucidation of these questions may be of interest both for the study of the pathology of plague and for the understanding of the mechanisms responsible for differences shown in the sensitivity of different species to plague toxin.

SUMMARY

The effect of plague toxin (fraction II of Pasteurella pestis No.137) on the liver glycogen and blood sugar contents, and on the ascorbic acid contents of the adrenals, spleen, liver, and lungs has been examined for five species of rodents, which differed in their sensitivity to the toxin; these were: white mice (highly sensitive), gerbils (Meriones meridianus nogaiorum Heptn.) and emuranchiks (Scirtopoda telum Licht.) (less sensitive), and guinea pigs and dwarf gophers (Citellus pygmaeus Pall.) (highly resistant). Intraperitoneal injection of toxin was followed within 30 minutes by a fall in liver glycogen content, a rise in blood sugar level, and a fall in the ascorbic acid content of the organs examined. The magnitude of the changes observed showed some correlation with the sensitivity of the given species to the toxin. Injection of toxin into hibernating dwarf gophers had no effect on their liver glycogen or blood sugar contents, or on the ascorbic acid contents of the adrenals and spleen, whereas in the spring, after termination of hibernation, these animals were as sensitive to the toxin as were white mice.

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