THE INFLUENCE OF PLAGUE TOXIN ON DIAMINEOXIDASE

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According to A. G. Kratinov and M. A. Maksimenko [3, 4] the plague bacterium and its toxic metabolic products enhance the sensitivity of various species of animals to histamine, the cardiovascular and respiratory systems of dogs and rabbits being especially susceptible. According to Kratinov and Maksimenko, one of the reasons for the enhanced sensitivity may be a change of the amount of histaminase in the blood and tissues.

In the present work we have made a study of the influence of plague toxin on diamineoxidase (DO) both in vitro and in vivo.

METHOD

Most of our observations were made on the DO of white rats. These animals were selected on account of their high sensitivity to plague toxin and the relatively high activity of the enzyme in pulmonary tissue.* In all cases we used animals weighing between 200 and 250 g. For each experiment we used lungs from 2-3 animals. They were finely divided in the cold, and triturated with 5 times their volume of a 2.5% solution of sodium chloride [1].

Because DOs of various origins differ with respect to their action on inhibitors [9], as well as rat pulmonary DO we also studied the DO of the serum from one horse and from two rams.

TABLE 1. Influence of Plague Toxin on Rat Lung DO Activity in Experiments in vitro

Number of experiments	DO activity (M ± m) in tests		
	With 8 mg of toxin	With 0.8 mg of toxin	Without toxin
5	27.7 ± 6.8	55.7 ± 9.6	62.3 ± 8.9

TABLE 2. Influence of Plague Toxin on Rat Lung DO Activity in Experiments in vivo

Time after injection of the toxin (in hours)	Number of experiments	DO activity (M ± m)
No injection	24	59.9 ± 2.5
4	15	51.7 ± 3.5
24	16	57.5 ± 4.3
48	15	55.4 ± 2.8

Both the lung extract and the sera were dialysed in the cold for 17-20 hours against a phosphate buffer at pH 7.2. After dialysis and centrifugation the sera were freeze-dried and kept at − 20° ** until required, while the pulmonary extracts were examined on the day that they were made.

The toxin of the plague bacterium (strain 1) was obtained by Baker's method [7], and the lipopolysaccharide toxin of the pseudotuberculosis bacterium (strain 1) by Davies' method [8]. The Brucella toxin (type melitensis, strain 56) was extracted with trichloracetic acid [5]. The endotoxin of the cholera vibrio was prepared in the industrial section of the Institute from a collection of strain type ogava and inaba from instructions supplied by the L. A. Tarasevich State Control Institute.

*In other rat tissue (kidneys, liver, and blood serum) DO activity was much lower; we did not examine the intestinal mucosa.

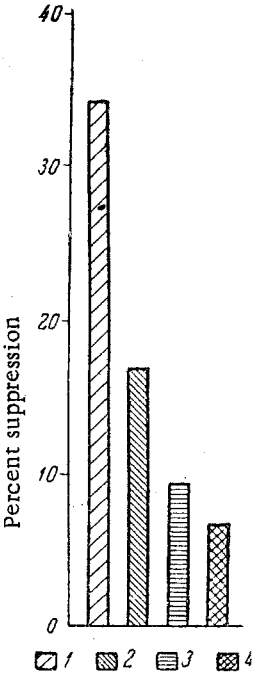
**As our experiments showed freeze-drying does not affect the DO activity of the sera. Before the experiment the freeze-dried sera were dissolved in physiological saline (50 mg of protein to 1 ml).

For white rats weighing 200-250 g, the LD_{50} of the plague toxin was 92 μg for an intraperitoneal injection.

In experiments in vitro we used 0.8 and 8 μg of toxin for the test; for the comparison of its action with that of other toxins, 4 mg of each was taken. In the experiments in vivo, 1 LD_{50} of plague toxin was injected intraperitoneally into white rats, and the surviving animals were investigated. Control animals received no toxin.

DO activity was measured by the indigodisulfonate method, described by I. L. Vaisfel'd [1]. For determination of the activity of the DO of sera, incubation was continued at 37° in oxygen for 2-2½ hours, and for lung extracts for 3-4 hours. Usually incubation was stopped when it could be seen that the contents of the samples had become decolorized. Protein was precipitated in 2 ml of 10% trichloracetic acid.

The intensity of the stain was measured in a horizontal photometer (model FMS) against water, and a M_{61} color filter was used. The activity of the enzyme was determined from the decolorization of the experimental samples as a percentage of the decolorization of the control portions containing no histamine. All the results were treated statistically by a method for a small number of samples.



Influence of toxins of different origins on white rat pulmonary DO. 1) Plague; 2) cholera; 3) pseudotuberculosis; 4) Brucellosis.

RESULTS

As can be seen from Table 1, even a few experiments were sufficient to demonstrate at a level of probability of 0.99 that DO activity is suppressed by a plague toxin dose of 8 mg. We were unable to demonstrate any effect on DO of a smaller dose of toxin (P is less than 0.95).

A similar influence was exerted by plague toxin on the DO of horse and sheep serum. However, unlike the DO from rat lungs, these sera were more sensitive to plague toxin. For example, 0.8 mg of toxin suppressed the activity of horse serum DO by 44.3% (one sample of serum in the tests with the toxin, and 6 tests without it), while the DO of sheep serum reduced the activity by 67.5% (2 samples of serum in the tests with toxin, and 3 measurements without it).

The effect of large doses of plague toxin on rat pulmonary DO was fairly specific (Fig. 1). Suppression of rat pulmonary DO by 4 mg of plague toxin at a probability higher than 0.99, which may be taken as highly significant and not due to chance. In the same experiments, the probability that cholera toxin suppresses rat pulmonary DO lay between 0.90 and 0.95, so that the significance was not demonstrated. The same thing was true to a greater extent of the Brucella and pseudotuberculosis toxins.

A somewhat different relationship was observed in the experiments with the DO of sheep serum. Here, not only plague toxin but also the toxin of the cholera vibrio had a marked effect on the activity of the enzyme ($P < 0.99$). However, inhibition of DO by the cholera vibrio toxin was five times weaker than that produced by plague toxin. No influence of the toxins of two other species of bacteria on sheep serum was observed.

Experiments in vivo were carried out on rats only with plague toxin. The results of the experiments are shown in Table 2.

In white rats studied 24 and 48 hours after injection of the toxin no consistent changes in the activity of pulmonary DO was observed: in some of the rats it was somewhat greater and in others less than in healthy animals. In general however the difference between the mean values of the activity of the DO of healthy and poisoned animals was not significant, and was apparently due to chance. Rather more definite changes in the activity of pulmonary DO occurred in rats killed 4 hours after injection of the toxin. However in this case despite the relatively large number of experiments, the degree of significance was not high ($P > 0.05$).

In evaluating the results, the first impression is of a lack of correspondence between the results of the experiments in vitro and in vivo. However, it must be remembered that in the in vivo experiments the white rats received one LD_{50} of toxin, and it was only the surviving animals which were investigated. However, in the in vitro experiments we used doses of toxin far greater than the LD_{50} used in the experiments in vivo; but then, as the dose of toxin was reduced to 0.8 mg, i.e., approximately to 9 LD_{50}, the ability to suppress the activity of the enzyme in this species of animal was lost.

It is therefore not difficult to deduce that in neither case was there any marked effect of plague toxin on rat pulmonary DO, and that it was essential to continue the investigations with other species of animal. In particular, it would be interesting to study the changes in the DO of various organs during plague infection, when the toxic condition develops gradually, and all the changes in the body are very clearly marked.

The explanation for the sensitization of many animal species to histamine [3, 4] must be sought in the action of the plague toxin not only on DO but also on other enzyme systems. The point is that the function of the amine-oxidase, and of DO also is still by no means clear [9]. A great deal remains controversial concerning the metabolic pathways of histamine. In any case, in certain animals and in man methylation is the starting point from which inactivation of free histamine starts; DO appears to play a subsidiary part [6]. Further, the splitting off of DO in vivo by means of aminoguanidine appears not to affect vital activities [9].

SUMMARY

In experiments in vitro, large doses of plague toxin depressed the activity of diamineoxidase (DO) of the lungs of albino rats. The action was relatively specific, as could be judged by the absence of any significant effect on it of toxins of Brucella, cholera vibrio, or the pseudotuberculosis bacterium. The effect of a small dose of the P. pestis toxin on the DO of rat lungs was quite insignificant ($P > 0.05$). The DO of the horse and sheep sera was more sensitive to P. pestis, but the action was less specific. From experiments in vivo on rats which were investigated 4-48 hours after receiving an intraperitoneal injection of 1 LD_{50} of plague toxin showed that no definite changes in the activity of the DO of the lungs was caused ($P > 0.05$).

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