THE EFFECT OF *Pasteurella pestis* TOXIN ON THE HISTAMINE-BINDING POWER OF THE BLOOD OF ALBINO RATS AND GUINEA PIGS

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Previous research [1] has shown that the histamine sensitization of animals caused by Pasteurella pestis and its toxin is not attributable to depression of diamine oxidase activity, nor to the effect of the toxin on the process of histamine formation. To continue research in this direction, studies have therefore been made of the effect of plague toxin on the histamine-binding activity of the blood serum in relation to exogenous histamine.

The histamine-binding effect (HBE) was first discovered in normal human serum. It was later shown that an HBE occurs in the serum of guinea pigs, rats, mice, dogs, and cats; little or no such activity is found in the serum of rabbits, cattle, pigs, horses, and eels [9, 11]. Besides the blood serum, various tissue homogenates from rats, guinea pigs, and rabbits may also show the presence of HBE [4].

There is reason to suppose that the HBE plays an important part in the transfer and detoxication of histamine. This view is supported by the fact that the HBE is weak or absent in persons with allergic diseases [10, 12], in guinea pigs sensitized with egg albumen [13],and in rats after removal of the hypophysis, ovaries, and adrenals [11, 14, 15].

EXPERIMENTAL METHOD

Instead of 5% serum as recommended elsewhere [9, 11], we used citrated whole blood. The reason for this change was, firstly, the more marked HBE in citrated whole blood than in 5% serum, and secondly, the fact that the effect is manifested in the same way in serum and blood [5].

In experiments in vivo, one batch of albino rats weighing 160-180 g received an intraperitoneal injection of P. pestis toxin in a dose of 3 LD_{50} (fraction II from the EV strain); these animals were decapitated 4 h after injection of the toxin. A second batch of albino rats received a similar injection of 1 LD_{50} of toxin and were sacrificed after 18 h. The guinea pigs received corresponding doses of toxin, bearing in mind their greater body weight (300-350 g); they were sacrificed at the same intervals.

Blood from the sacrificed animals was collected in test tubes with citrate (1 mg/ml blood). At the same time blood was collected from the guinea pigs and albino rats not receiving injections of toxin (control group). To 1 ml of citrated whole blood of the experimental and control animals was added 10 μg of histamine dichloride in a volume of 1 ml. The mixture was rapidly agitated and placed on ice to stand. Histamine was estimated after 15 min.

In the experiments in vitro, histamine alone was added to one blood sample from healthy animals in a dose of 10 μg, and histamine plus plague toxin, in the doses indicated in the table, were added to the second sample.

The histamine was estimated by a biological method at 38° in an atropinized loop of guinea pig's ileum, suspended in Ringer-Locke solution, through which oxygen was bubbled. The error was ±3.5%. The histamine binding was calculated from the fall in its concentration in the samples with citrated blood and expressed as a percentage of the standard dose of histamine, namely 1 μg. All the conclusions are based on statistical analysis of the numerical results, using a small sample method [3].

Histamine-Binding Effect of Blood (M ± m)*

Species of animal	Without toxin (control)	With toxin in vivo: 3 LD_{50} 4 h after injection	With toxin in vivo: 1 LD_{50} 18 h after injection	With toxin in vitro: "small" dose †	With toxin in vitro: "large" dose †
Albino rats	56,9±3,1	6,5±1,0	30,7±3,4	63,0±2,5	71,1±1,3
Guinea pigs	53,9±3,9	4,9±1,6	24,4±1,2	61,7±2,8	69,9±0,9

* In the control series, mean results of 10 tests, in the other experiments – 5 tests.
† In the experiments with rats' blood the "small" doses were 90 μg (3 LD_{50}), and with guinea pigs' blood, 200 μg (3 LD_{50} for rats adjusted for the body weight of the guinea pigs); the "large" doses in the experiments with the blood of both species of animals were 1 mg.

EXPERIMENTAL RESULTS

It is clear from the table that citrated whole blood from albino rats and guinea pigs gave an HBE‡, and that no significant difference was found between the ability of the blood of the two species of animals to bind histamine ($P \ll 0.05$).

The same table shows that after injection of plague toxin the HBE of the animals' blood fell sharply. This was especially obvious 4 h after injection of 3 LD_{50} of toxin, although 18 h after injection of 1 LD_{50} of toxin the blood continued to show feeble HBE.

A different picture was seen in the experiments in vitro; the addition of "small" doses of toxin to the blood samples taken from the healthy animals did not repress the HBE. When the dose of toxin was 1 mg per sample, the histamine-binding power of the blood was actually slightly increased (probability of error < 0.01).

For this reason it is interesting to note that after estimation of histamine in the blood samples with "large" doses of toxin, in order to relax the loop of bowel completely the Ringer-Locke solution in which it was immersed had to be changed twice. When histamine was estimated in the blood samples without the addition of toxin from outside (1 mg), it was sufficient to change the solution once. By itself this dose of toxin did not cause the intestine to contract, but together with histamine and without blood it gave the same reaction as histamine without toxin. This aspect of the action of the toxin in the blood sample with histamine was not studied in greater detail.

Hence, one of the manifestations of the action of P. pestis toxin on the animal organism is the depression of the HBE of the blood. This is observed not only in albino rats, sensitive to the toxin, but also in guinea pigs which are relatively resistant to it. So far as we know, it is rare for P. pestis toxin to have an action of similar intensity on animals differing in their sensitivity to the toxin.

The fact that the toxin had no action on the HBE of the blood in the experiments in vitro suggests that the depression of the HBE of the blood in the experiments in vivo did not result from the direct action of the toxin on the mechanism of histamine binding, but from other causes. One such cause could be a lesion of the adrenals which, according to V. V. Donskov [2], is an important factor in determining death from plague. We have already mentioned that the serum of adrenalectomized rats possesses no HBE.

The other factor on which depression of the HBE depends, namely allergization of the animal [10, 12, 13], is not important in regard to the action of plague toxin.

It is difficult at the moment to say whether the depression of the HBE of the blood in plague toxemia is the reason for the increased sensitivity of the animals to histamine, for it has not yet been finally agreed that the metabolism of exogenous and endogenous histamine follows on identical lines [5, 7], and the effect of the toxin on the removal of histamine from the tissues has not been studied**.

‡ Its intensity was largely dependent on the dose of histamine added: with 5 μg histamine the binding was on the average of 18% higher. However, for technical reasons, we used a larger dose of histamine (10 μg).
** In certain other infections the mechanism of the increased sensitivity of the animals to histamine may be different. For example, after administration of whooping cough vaccine to animals it is due mainly to activation of histidine-decarboxylase [16], whereas in brucellosis it is due to liberation of histamine from the depots [8].

SUMMARY

Whole citrate blood of albino rats and guinea pigs produced a histaminopexic effect: there was no difference between the capacity of the blood of both species of animals to bind histamine. The histaminopexic effect of the blood showed a marked reduction after administration of Past. pestis toxin to these animals. This effect was established 4 h and even 18 h after administration of the toxin. In experiments in vitro no depression of the histaminopexic effect of the blood by the toxin was noted.

LITERATURE CITED

1. I. V. Domaradskii and I. M. Klimova. Byull. éksper. biol., 5, 69, (1962).
2. V. V. Donskov. Med. byull. Irkutsk, 1, 23 (1939).
3. L. S. Kaminskii. Analysis of Clinical and Laboratory Data [in Russian]. Leningrad, 1959.
4. E. I. Krichevskaya and G. V. Kapitonova. Doklady Akad. Nauk SSSR 123, 1, 68 (1958).
5. K. M. Larionova. Radiobiologiya 1, 2, 247 (1961).
6. M. D. Kyrstay. Uspekhi sovr. biol. 51, 1, 21 (1961).
7. B. N. Halpern and Th. Neveu, J. Physiol. (Paris), 1961, Vol. 53, p. 358.
8. S. Malkiel and B. Hargis, J. Allergy, 1958, Vol. 29, p. 524.
9. C. Laborde, J. L. Parrot, and D. A. Urquia, Presse méd. 1953, Vol. 61, p. 1151.
10. J. L. Parrot and C. Laborde, Presse méd. 1953, Vol. 61, p. 1267.
11. J. L. Parrot and C. Laborde, C. R. Soc. Biol., 1953, Vol. 147, p. 1203.
12. J. L. Parrot and C. Laborde, J. Physiol. (Paris), 1956, Vol. 48, p. 677.
13. J. L. Parrot and C. Laborde, J. Physiol. (Paris), 1960, Vol. 52, p. 183.
14. J. L. Parrot, M. Tuchmann-Duplessis, and C. Laborde, J. Physiol. (Paris), 1957, Vol. 49, p. 333.
15. R. W. Schayer and O. H. Ganley, J. Allergy, 1961, Vol. 32, p. 204.

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