EFFECT OF PHENYLHYDRAZINE ANEMIA ON THE SUSCEPTIBILITY OF WHITE MICE TO PLAGUE INFECTION

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[Following is a translation of an article by G. G. Korobkov in the Russian-language periodical Izvestiya Irkutskogo gosudarstvennogo nauchnoissledovatel'skogo protivochumnogo instituta Sibiri i Dal'nego Vostoka (News of the Irkutsk State Scientific Research Anti-Plague Institute of Siberia and the Far East), Vol 25, 1963, pages 144-147.]

The study of the effect of oxygen starvation on the sensitivity of animals to plague is of certain theoretical and practical interest. ~~First of all,~~ such investigation can help in the study of the pathogenesis of plague infection. In addition, in the case of various ailments of infectious and non-infectious origin, as a rule, hypoxia occurs. Considering the great frequency of varied ailments in animals, ~~it is necessary to consider that~~ it is fully realistic under natural conditions to have a combination of oxygen starvation caused by some pathologic process with plague.

On the basis of these considerations ~~we conducted~~ a series of tests, in which we established the relation between oxygen starvation of the organism and the sensitivity of the animals to plague infection.

The tests were conducted according to the following method: white mice were given subcutaneous injections of 0.2 ml of a 1% solution of phenylhydrazine for a total of 3-5 injections. During the process of the development of anemia from 10-25% of the mice died at various times.

In the test animals periodic counts were made of erythrocytes; after their number had decreased by not less than 20% the animals were infected with a virulent strain (1435) of the plague microbe. The sensitivity of the anemic test mice and the control (intact) mice to infection with plague was established through the determination of DL_{50}. With this purpose the mice were infected with 10, 100, 1000, and 10,000 microbe

cells. The test was conducted with 75 animals; 35 were test animals and 40 were control animals.

The DL_{50} as calculated according to the Kerber formula was equal for the anemic white mice to 144 microbe cells; for the healthy mice it was 40 microbe cells. The fluctuation of the DL_{50} according to the Pizzi formula amounts to 77-202 microbe cells for the test animals and 25-63 for the control animals. The increase for the test animals is statistically reliable.

These data indicate that the anemic animals are more resistant to infection with plague than are healthy animals.

The second series of tests was conducted by a similar method but with a larger number of animals (160 white mice). In these tests the DL_{50} for the anemic mice was equal to 20 and for the control mice it was 12 microbe cells. The fluctuation of the DL_{50} according to the Pizzi formula is 14-28 microbe cells for the test animals and 8-15 for the control animals.

In these tests, as in the preceding ones, the anemic mice were more resistant to infection by plague than were the control mice. However, the data of these tests are not statistically reliable. This was a consequence of the fact that the sub-culture of the strain of plague which was used for infecting was highly virulent and the animals survived only with the administration of doses of 10 and 100 microbe cells.

Upon the infection of the animals with 10 microbe cells 8 of the 20 animals in the test group survived while 3 of 20 in the control group survived.

The third series of tests differed from the preceding ones in that the test animals, depending on the degree of lowering of the number of erythrocytes (under the influence of the administration of phenylhydrazine) were divided into three groups and for infecting all the animals one dose was used -- 75 microbe cells (Dlm).

These data indicate the reliability of the presence of anemia in the test mice. In addition, upon examining smears of blood of the anemic mice a characteristic picture was found for hemolytic anemia. This was indicated by the lymphocytosis, neutropenia, the presence in the blood of aniso and poikilocytosis (degenerative changes of the erythrocytes) and also considerable regnerative changes -- 40-50% polychromatophils, individual normoblasts, and in some preparations even megaloblasts.

Table 1

Number of erythrocytes in the blood of test animals before infection

Severity of anemia	M	m	σ	V%
Light anemia	6,560,000	71,430	200,000	3
Anemia of average severity	4,700,000	88,400	460,000	10
Severe anemia	3,450,000	88,000	370,000	8
Control	8,920,000	25,550	115,000	1

All the mice, including both the test and the control (healthy) animals, were infected with 75 microbe cells of strain 1435 Bact. pestis (Dlm).

Table 2

Severity of anemia	Number of animals being tested	Died by the 20th day after infection	Died of plague	Survived	
				Number	%
Light anemia	7	5	4	2	28.5
Anemia of average severity	36	30	21	6	20.8
Severe anemia	14	14	10		
Control	45	44	38	1	2.2

The data which were obtained are given in Table 2.

Under the influence of anemia of average severity there was an increase in the resistance of the mice to infection by plague (the difference in the percentages of test and control animals which survived after infection was 18.8%; reliability with such a number of animals is achieved with a difference of 12%).

In the case of slight anemia the number of surviving animals is even greater percentagewise and it is possible that this degree of oxygen insufficiency causes an increase in the resistance of the animals to plague. However, the small number of animals used in the tests of this group does not provide a basis for such a conclusion.

Somewhat different data were obtained when the anemia was severe; All the animals which were tested perished; in one third of the cases a plague culture was not isolated from the victims. This was a consequence

of the fact that anemia itself in such a degree is an extremely severe ailment. During the process of the development of anemia from the introduction of phenylhydrazine 13 white mice died out of the 70 in the test (before infection).

Considering the great significance of intoxication in the pathogenesis of plague, we conducted tests in which we studied the sensitivity of the anemic animals to the introduction of plague toxin.

Anemia in these tests was caused by a 1% solution of phenylhydrazine which was introduced every other day in subcutaneous doses of 0.2 ml. After the third administration of phenylhydrazine anemia developed in the mice. The number of erythrocytes in the test animals was equal to (M) 3,820,000± ±25,550; in the healthy animals it was 9,770,000±180,000.

For the determination of DL_{50} the test and control animals were given subcutaneous injections of an autolysate of the plague microbe in doses of 0.05, 0.072, 0.086, and 0.103 ml -- a ratio between doses of 12:10. For each dose nine animals were used, a total of 36 test animals and 36 healthy (control) animals. The DL_{50} for the anemic mice 24 hours after the administration of the toxin was equal to 0.051 ml; for the control animals it was 0.069. The fluctuation of the DL_{50} according to the Pizzi formula was 0.048-0.052 ml for the test animals and 0.056-0.060 ml for the control animals. This difference of the DL_{50} was reliable since upon statistical processing of the data obtained with respect to the percentage of surviving animals the coefficient of reliability was (t)> 2.

The data which were obtained on the action of the plague microbe toxin on animals in which hemic hypoxia developed show an increase in the sensitivity of the organism to plague intoxication. This fact is somewhat contradictory in comparison with the results obtained by us in the case of the combination of anemia with plague infection.

We do not have sufficient factual material in order to support the results which were obtained; it is possible only to express hypotheses. The degree of severity of the course of various infectious ailments involving hypoxia can depend on the interrelationship between the defense mechanism with respect to the given infectious process and the defense mechanism in the case of hypoxia.

There is no doubt that in this case the range of adaptability of the organism to changed conditions of existence is of great importance. The greater the simultaneous load on any physiological system or organ, the greater are the possibilities for a more severe course for the infection.

In the pathogenesis of plague intoxication the resulting circulatory hypoxia is of great importance. It must be considered that the introduction of plague toxin in animals which are already experiencing oxygen starvation should cause a very severe pathological process.

In the case of infection with plague during the initial period of the development of the infection intoxication as a pathogenetic factor is not significant; during this period of the disease the main role in the protection of the organism belongs to the physiological systems of the latter which can retard the spread and multiplication of the microbes. As is known in this process the functional state of the reticulo-endothelial system is of great importance.

Most plague specialists -- M. P. Pokrovskaya, L. S. Kaganova, N. N. Zhukov-Verezhnikov, Ye. I. Korobkova, Pollittser, Meyer, etc. -- attach decisive importance to the reticulo-endothelial system in the process of receptivity and recovery from plague.

An increase in the functioning of the cells of the reticulo-endothelial system during the period of sanogenesis from hypoxia is noted by N. Ye. Kavetskiy et al. and N. N. Sirotinin. In these investigations the activity of the reticulo-endothelial system was determined from the growth of the titer of antibodies and the cancerolytic index and by skin tests with red congo and trypan blue and also by other methods.

The considerable regenerative changes of the red blood which were observed in our tests also point to an increase in the functioning of the reticulo-endothelial system.

Consequently, the increase in the resistance of anemic animals to plague can be explained by the fact that under the influence of hypoxia in the organism of these mice there is an increase in the functioning of the reticulo-endothelial system.

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