

CONCERNING OXYGEN DEFICIENCY DURING EXPERIMENTAL PLAGUE INFECTION

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## CONCERNING OXYGEN DEFICIENCY DURING EXPERIMENTAL PLAGUE INFECTION

[Following is the translation of an article by K. M. Mokhin, published in the Russian-language periodical Trudy Rostovskogo-na-Donu Gosudarstvennogo Nauchno-Issledovatel'skogo Protivochumnogo Instituta (Trudy of the Rostov/Don State Scientific-Research Antiplague Institute), Vol XV, installment 1, pages 61-70, 1959. Translation performed by Sp/7 Charles T. Ostertag, Jr.]

The earlier established fact of the sharp reduction in the content of glycogen in the cardiac muscle of plague infected guinea pigs for 1-2 days prior to their death (K. M. Mokhin, L. N. Makarovskaya, A. M. Konno, 1957) is circumstantial proof of an oxygen deficiency setting in during this period.

The degree of oxygen deficiency during experimental plague of guinea pigs should be very significant, since it is known that a decrease of glycogen in the myocardium sets in only in the event of profound hypoxia. Thus, Boque, Chang and Gregory (1937) in tests on a cardiac-pulmonary preparation showed that the content of glycogen in the cardiac muscle begins to decrease with an extremely low saturation of the blood with oxygen. Only with a lowering of the pressure of the oxygen in the blood to 30 mm mercury column the aerobic processes of metabolism are exchanged by anaerobic, taking place with the splitting of glycogen. With a higher partial pressure of oxygen in the blood the content of glycogen in the myocardium is not reduced. On the basis of experimental investigations conducted on rats, Schuman (1942) makes the conclusion that with the exhaustion of air under the conditions of a pressure chamber, dissimilation of the glycogen of the cardiac muscle begins only with an altitude of 7200 meters.

Consequently, the lowering of the glycogen reserves of the cardiac muscle, which is observed during experimental plague of guinea pigs, may be evaluated as the result of a significantly expressed oxygen deficiency.

Under conditions of hypoxia oxygen deficiency is experienced not only by the cardiac muscle, but by all the organs and systems of the organism, which is doubtlessly reflected on all their functions. The nervous system is particularly sensitive to a shortage of oxygen. It is known that already during a brief anoxia irreversible changes set in in the nervous system, while other organs suffer less significantly. Therefore the first and most profound affections in response to an oxygen deficiency will be manifested on the part of the nervous system, which will become apparent in a disruption of its correlating role.

An hypoxic condition may be the result of various factors. Therefore, depending on the etiological factors hypoxia may be broken down into: 1) Hypoxic, 2) anemic, 3) circulatory 4) histotoxic.

Hypoxic or respiratory hypoxia is caused by an insufficient saturation of the blood with oxygen. A similar condition may take place during a disruption of respiratory movements, affection of pulmonary tissue, contraction of the lumen of the air passages, deficiency of oxygen in the inhaled air. All of these causes hinder the sufficient saturation of the blood with oxygen, as a result of which the content of oxygen in the arterial blood will be reduced.

During anemic hypoxia, which sets in as a result of the lowering of the content of hemoglobin in the blood, there is a decrease in the content of oxygen in the arterial and venous blood and the oxygen capacity is reduced.

Circulatory or stagnant hypoxia is characterized by a disturbance of blood circulation, which leads to an insufficient supply of oxygen to the tissues. During such a form of hypoxia the content of oxygen in the arterial blood is normal, but the tissues experience an oxygen deficiency, since in an unit of time a smaller amount of blood flows to them, and consequently a smaller amount of oxygen. In view of the slowed down flowing of blood the tissues absorb more oxygen. Therefore during circulatory hypoxia the content of oxygen in the venous blood will be significantly reduced and the arteriovenous difference will be great.

During histotoxic (tissue) hypoxia an affection of the oxidation-reduction ferment systems sets in, as a result of which the tissues cannot utilize the oxygen. During this form of hypoxia the content of oxygen in the arterial and venous blood is high and arteriovenous difference is insignificant.

The presented classification of hypoxic conditions points out a whole number of causes which are capable of causing the oxygen deficiency of an organism. It is understandable that depending on the form of hypoxia the appropriate therapeutic measures should be selected.

In this connection there is natural tendency to study the gaseous composition of the blood, and in particular the content of oxygen in the arterial and venous blood during various phases of plague infection.

The present report presents materials from an investigation of the content of oxygen in the blood of guinea pigs during their infection with *B. pestis*.

#### Methods

The tests were set up on 22 guinea pigs. The content of oxygen was determined in the arterial and venous blood after 1, 3, 4, and 5 days following

infection. The guinea pigs were infected in the right rear paw with 1,000 microbial bodies of *B. pestis* 773 (100 Dlm).

The investigations were carried out with a Barkroft apparatus (micro-model). The arterial blood was taken with a syringe from the left section of the heart. The venous blood was taken from an ear vein or from a surface vein of a rear extremity, for which a small cut of the skin was preliminarily made. In view of the fact that it is extremely difficult to use a syringe to take blood from the vein of a guinea pig, especially one infected with plague, we collected the blood in a graduated micropipette. For this, having pricked the vein with a needle, we applied to this site a micropipette with a small container attached to its end. The blood was easily raised along the capillary of the micropipette without coming into contact with the air.

#### Results of the tests and their discussion.

Initially we investigated the content of oxygen in the arterial and venous blood of healthy guinea pigs.

The tests showed that the content of oxygen in the arterial blood of the guinea pigs prior to infection comprised 18.1--20.2 percent by volume (an average of 19.2 per cent by volume), the percentage of saturation fluctuated from 89 to 95 (an average of 91%). The oxygen capacity of the blood corresponded to 20.4 per cent by volume. In the venous blood the content of oxygen was on the average of 15.1 per cent by volume. The saturation of venous blood with oxygen fluctuated from 59 to 72% (an average of 63%).

Following the infection of the guinea pigs with *B. pestis*, already in one day changes were noted in the gaseous composition of the blood. It can be seen from table 1 that after a day following infection the content of oxygen was reduced in comparison with the initial values, in the arterial blood by almost 2 per cent by volume, and in the venous blood by more than 4% by volume. The arteriovenous difference increased up to 6.4 per cent by volume and the coefficient of incomplete saturation of capillary blood comprised 5.6 per cent by volume. These data testify to the development of oxygen deficiency of the organism already by the end of the first days of experimental plague infection.

The profoundness of the hypoxic condition progressed with the course of the infectious process. Thus, after 3 days following infection the incomplete saturation of blood with oxygen had increased still more, and the content of oxygen in the venous blood decreased by almost 2 times. On the 4th day of the plague process the content of oxygen in the arterial blood was reduced to 14.6 per cent by volume. In the venous blood there was somewhat less oxygen than on the previous days. After 5 days following infection, by the time of death of the guinea pigs, there was a sharp reduction in the amount of oxygen in the arterial blood, while in the venous blood only a small reduction in the content of oxygen was noted in comparison with the previous determination.

It is interesting to note that on the 5th day of plague infection, even in animals which were in a state of agony, the content of oxygen in the venous blood did not drop lower than 6.2 per cent by volume and on the average

comprised 7.3 per cent by volume. The arteriovenous difference in this period of plague infection was less than in the initial condition prior to the infection of the animals.

A comparison of the figures of the oxygen capacity of the blood in various periods of plague infection shows a significant lowering of them. An analysis of the data presented in table 1 reflects the certain dependency between the values of oxygen capacity and the content of oxygen in the arterial blood. This dependency is especially visually brought out in a graphic depiction of the digital material (figure 1).

It is known that under normal conditions the oxygen capacity is dependent on the amount of blood hemoglobin -- the more hemoglobin the erythrocytes contain, then the more oxygen will be bound with 1 ml of blood, and consequently the greater will be the oxygen capacity.

A decrease in the oxygen capacity, which is observed in plague infected pigs, suggests an assumption concerning a decrease in the content of hemoglobin in the blood. However the experimental check did not confirm these assumptions. It was established that the amount of hemoglobin in the dynamics of plague infection in guinea pigs increases, especially in the peripheral blood (table 2).

Statistical processing of the data obtained showed the authenticity of the differences in the content of hemoglobin in the peripheral blood and blood from the heart in plague infected animals ( $P < 0.001$ ), while a difference in the content of hemoglobin in normal guinea pigs is virtually little probable ( $P > 0.05$ ).

The unequal distribution of hemoglobin in the blood system during experimental plague of guinea pigs, in our opinion, may be explained by the clotting of blood due to a slowing down in the rate of blood flow, deposition of blood in the capillary vessels and the discharge of plasma through the damaged capillary membranes into the intertissuespaces. However, this proposal requires the appropriate verification.

A disturbance of the proportionality between the content of hemoglobin in the blood and its oxygen capacity is possible only in cases of a qualitative change of the hemoglobin. Consequently, a reduction of oxygen capacity with an increase in the content of hemoglobin, which is noted in plague infected guinea pigs, may be explained only by a qualitative change in the blood hemoglobin. The possibility of lowering of oxygen capacity with a normal and even an increased content of hemoglobin in the blood is an established fact and was observed with other pathological conditions -- tuberculosis, pneumonia, benzol poisoning (B. Ya. Sadogurskiy, 1941, 1947; V. L. Eynis, 1940; A. A. Tregubov, 1938, 1947; S. M. Genkin, 1940).

An analysis of the experimental material obtained testifies to the significant oxygen deficiency of an organism, setting in already in a day following infection. The reasons which provoke a hypoxic condition during

experimental plague in guinea pigs are diverse and interrelated. This is testified to by the presence in the indices of oxygen deficiency in plague guinea pigs of elements which are characteristic of hypoxia of a various etiology. Thus in the cited classification of hypoxic phenomena we pointed out that a significant lowering in the content of oxygen in the arterial blood is a characteristic of the hypoxic form of hypoxia. During this the oxygen capacity is changed little. In our tests we observed not only a lowering of the content of oxygen in the arterial blood, but also a significant decrease in oxygen capacity, which is characteristic for anemic hypoxia. Whereupon a decrease of oxygen capacity took place with an increase in the content of blood hemoglobin, which testifies to the change in the biological properties of hemoglobin as a carrier of oxygen.

It is little probable that the decrease in the content of oxygen in the arterial blood is connected just with the drop in oxygen capacity. Without a doubt the change in the nature of respiration which takes place during plague infection also exerts an influence on the saturation of arterial blood with oxygen. Besides this, a change in the capillary wall, which is observed in all the organs, including the lungs, during plague infection (V. N. Lobanov, 1956; Albrecht and Ghon, 1898 and 1900; and others) disturbs the process of gas diffusion in the pulmonary alveoli, as a result of which the incomplete saturation of the blood with oxygen takes place. It is necessary to also take into consideration the possibility of toxic effects on the respiratory center, which lead to a change in its sensitivity, and consequently is reflected in the nature of respiration. All of these enumerated moments take place to some measure in the disturbance of the external respiration in plague infected guinea pigs.

From the data obtained it is seen that during experimental plague infection one cannot deny the presence of the symptoms of circulatory hypoxia. Circulatory hypoxia is testified to by the increase in the arterio-venous difference. At the same time attention is drawn to the fact of the relatively small reduction in the content of oxygen in the venous blood, which may be observed even in animals which are in agony. At first appearance it seems that between the serious condition of plague animals (shortness of breath, cyanosis, etc.) and the mildly reduced oxygen pressure in the venous blood a disparity takes place, since it is difficult to imagine that the tissues experience oxygen starvation with a high oxygen pressure in the blood.

However it must be taken into consideration that the magnitude of the content of oxygen in the capillary vessels still is not a conclusive criterion of the intensity of supplying the tissue elements. Why with the presence of conditions which hamper the diffusion of gas through the capillary membrane, a significantly expressed oxygen deficiency may set in even under conditions of a high oxygen pressure in the capillary vessels.

The works of Eppinger et al. (1927, 1938) already showed the important role of the condition of the capillary wall in the course of diffusion processes. It has been established by numerous experiments that during various pathological conditions in the event of an affection of the capillaries, the serous fluid coming out into the precapillary spaces

inhibits the diffusion of gasses, and especially of oxygen from the blood to the tissues. As a result of this, conditions are created for the oxygen starvation of the tissues.

The investigations by O. Klein (1936, 1937) on the impairment of capillary membranes with histamine, uroselectan and other chemical substances supplement the conclusions of Eppinger. Thus, Klein established a reduction in the transition of oxygen to the tissues during the impairment of the vessels of greater circulation, while the impairment of the vessels of lesser (pulmonary) circulation causes an incomplete saturation of the arterial blood with oxygen.

Clinical observations also point to the possibility of oxygen starvation of the tissues with a relatively high content of oxygen in the blood as a result of a change in the vascular wall.

Thus, A. A. Tregubov (1947), having investigated the gaseous composition of the blood in patients with a cardiovascular deficiency, assumes an increased oxygen pressure in the capillary and venous blood with a simultaneous incomplete oxygen saturation of all the tissues of the organism, as a result of the disruption of the diffusion capability of the capillary membranes.

In persons with septic diseases V. M. Belonozhko (1949) noted a dependency between the severity of hypoxic symptoms and the disruption of the capillary wall. The author makes the conclusion that the oxygen deficit, experienced by the tissues, is the result of destructive changes of the hematoparenchymatous barrier under the influence of bacterial toxins.

Hypoxic symptoms, which are characteristic of the first stage of hypertonic illness, find their explanation in a change of the filterable membranes of the capillaries and a disruption of tissue permeability (A. I. Smirnova-Zamkova, 1949).

Taking into consideration the frequency of conditions, under which the gas exchange between the blood and the tissues is disrupted as a result of a change in the capillary walls, corresponding member of the USSR Academy of Sciences N. N. Sirotinin (1949), at a conference devoted to problems of hypoxia, proposed to distinguish a similar condition as a particular form of hypoxia.

As we already mentioned, during plague infection there is significant suffering on the part of the walls of the small vessels, especially the capillaries, venules, and arterioles. Thus, V. N. Lobanov (1956) found in all organs during experimental plague and plague in man profound destructive changes in the vascular wall, going as far as necrosis. Naturally the exchange of oxygen and nutrient substances through such a sharply changed vascular membrane will be disrupted. Therefore, it is necessary to think that oxygen is retained in the venous blood more than would be assumed, not because the tissues do not experience an oxygen deficiency,

but because through the changed wall the oxygen cannot diffuse into the tissues sufficiently rapidly.

Thus, an analysis of the experimental data obtained by us makes it possible to consider that during experimental plague in guinea pigs a significant hypoxia develops and its causes are very complex and cannot be contained in the framework of any one type of hypoxia and include elements of hypoxic, anemic, and circulatory hypoxia.

#### Conclusions

1. During experimental plague infection in guinea pigs, already after one day following infection an oxygen deficiency is observed.
2. In the arterial and venous blood of guinea pigs infected with *B. pestis* the content of oxygen lowers significantly with the course of the experimental plague process.
3. During experimental infection of guinea pigs the oxygen capacity of the blood is reduced along with a simultaneous increase in the content of hemoglobin, which indicates a change in the biological properties of hemoglobin as an oxygen carrier.
4. The oxygen deficiency which develops in guinea pigs during the process of plague infection cannot be confined to the framework of one type of hypoxia and includes elements of hypoxic, anemic and circulatory hypoxia.

#### Literature

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Table 1.

Indices of supplying the organism of guinea pigs with oxygen prior to infection and in various periods of plague infection (average values)

No.	Index	Prior to infection	After infection			
			1 day	3 days	4 days	5 days
1	Content of oxygen in arterial blood	19.2 %vol	17.4 %vol	16.2 %vol	14.6 %vol	11 %vol
2	In venous blood	15.1 %vol	11 %vol	8.5 %vol	8 %vol	7.3 %vol
3	Oxygen capacity	20.4 %vol	19.8 %vol	18.7 %vol	17.4 %vol	15.3 %vol
4	% oxygen saturation of arterial blood	91	88	87	84	76
5	% oxygen saturation of venous blood	68	55.5	45.4	45.9	47.7
6	Arterio-venous difference	5.3 %vol	6.4 %vol	7.7 %vol	6.1 %vol	4.3 %vol
7	Coefficient of oxygen utilization	0.28	0.36	0.47	0.42	0.3
8	Coefficient of incomplete saturation of capillary blood	3.2 %vol	5.6 %vol	6.3 %vol	6.1 %vol	5.9 %vol

Table 2

Content of hemoglobin (according to Sali) in the peripheral blood and in blood taken from the heart, before infection and in the dynamics of plague infection in guinea pigs

No	Content of hemoglobin (according to Sali)	Before infection	After infection			
			1 day	3 days	4 days	5 days
1	In the peripheral blood	78	84	93	98	96
2	In blood from the heart	76	77	79	81	82

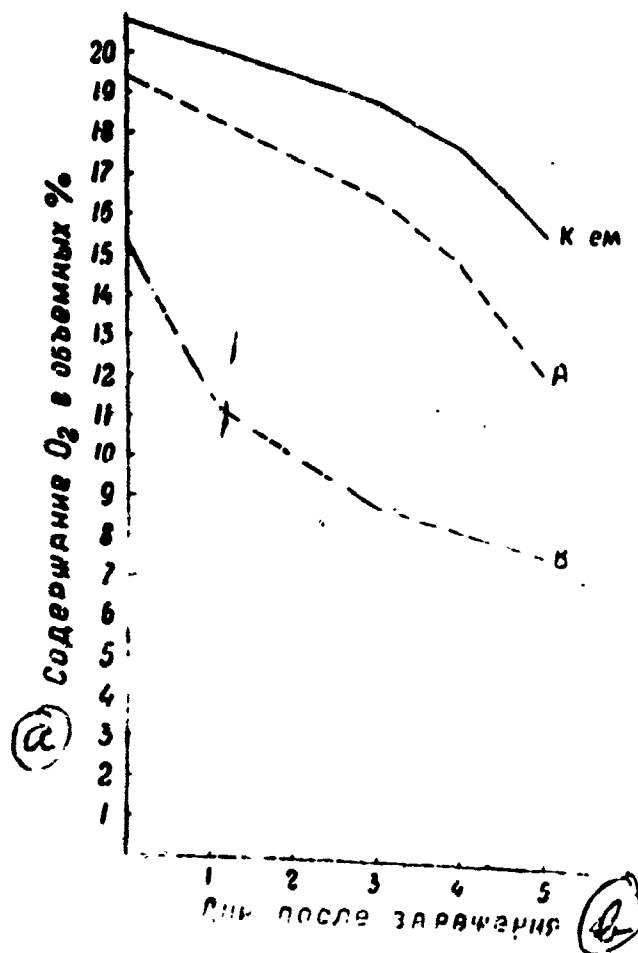


Figure 1. Dynamics of the content of oxygen in arterial and venous blood and the oxygen capacity during experimental plague of guinea pigs.

- K ем -- oxygen capacity  
 A -- content of oxygen in arterial blood  
 B -- content of oxygen in venous blood
- a -- oxygen content in per cent by volume  
 b -- days following infection