CHANGES IN THE MORPHOLOGICAL COMPOSITION OF BLOOD IN EXPERIMENTAL PLAGUE

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U. S. ARMY
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Laboratory investigations of blood during the process of disease, and also during the period of treatment and recovery has occupied a stable place in the complex of clinical examinations of a sick organism. Up until the present time, Soviet and foreign investigators have accumulated a vast amount of material on the changes of the morphological composition of the blood during various illnesses, infections and intoxications.

Up until now the blood picture during plague infection has been studied least of all. On the one hand this may be explained by the absence of plague morbidity in a number of countries, and secondly by the difficulties which inevitably confront the investigator in getting blood from plague striken humans or infected animals.

The data cited in literature relative to the change of the morphological composition of blood in plague stricken persons are quite diverse and contradictory and deal mainly with changes on the part of leukocytes.

Thus, V. K. Vysokovich (1901) points to the minor leukocytosis in patients with the bubonic form of plague, while H. Mueller and R. Poch, 1900, detected a fluctuation in the number of leukocytes during plague from 8,000 to 45,000 in 1 mm^3 of blood.

More than once S. I. Zlatogorov and L. V. Padlevskiy (1915) observed the clearly expressed increase in the number of leukocytes during pestilential pneumonia, and consider that the absence of leukocytosis may in certain cases serve as an auxiliary index for differential diagnosis with croupous pneumonia.

On the contrary, D. K. Zabolotnyy (1956) observed hyperleukocytosis in the blood during all the forms of plague, and the number of leukocytes reached 20--30 thousand.

During primary pulmonary plague, G. P. Rudnev (1940) encountered, along with minor leukocytosis, a sharply expressed hyperleukocytosis (up to 20,000 and more).

M. Schar and K. Meyer, 1956, while studying the effect of the toxic fraction of P. pestis on the blood and the hemopoietic system of experimental animals, invariably found leukopenia and eosinopenia in white mice and rats already in 30 minutes following the introduction of both the toxic and the atoxic fraction of the plague microbe. These investigators note the increase in the number of erythrocytes up to 22--30% in white mice and rats in the premortal condition -- in two hours following the administration of a lethal dose of toxin.

In the literature available to us, we have not encountered a description of the changes in the blood picture of laboratory animals -- guinea pigs and white mice -- during the process of development of plague infection in them following their infection with a virulent strain of the plague microbe.

Changes in the leukocytic formula of susliks during their infection with the plague microbe have been characterized in the work by R. F. Akulova and G. P. Rudnev, 1930. In the case of sepsis the authors, on the basis of smears, ascertained the increase in the number of leukocytes by approximately three times in comparison with noninfected animals.

N. Ehrenkranz and L. White, 1954, refer to the changes in the number of eosinophils during experimental pulmonary plague in monkeys.

Significant leukocytosis (up to 50,000 in 1 mm^3) during experimental bubonic plague in monkeys and the absence of specific changes in the number of erythrocytes are noted by G. Hoessly, D. Walker, A. Larson and K. Meyer, 1955.

In spite of the fact that the third formed element of blood -- the blood platelets -- have served as the subject of investigation for more than 80 years, and changes in their numbers have been studied during many acute infectious diseases, under the influence of ionizing radiation, cancerous tumors, etc., we were not able to find a description of the change in blood platelets during the course of plague infection.

Investigation of the change in the number and morphology of formed elements of the blood in the dynamics of plague infection provides the basis for the differential appraisal of the condition of the hemopoietic apparatus in various periods of illness.

Besides this, knowledge of the peculiarities of changes in the formed elements of the blood during plague is necessary for delimiting the action of medicinal preparations from the toxic factors of the plague microbe, and also when studying the effect of vaccine strains of P. pestis on the organism.

The present work is devoted to the investigation of the above mentioned problem.

The tests were set up on 33 guinea pigs, weighing from 300 to 500 grams, and 38 white mice.

The animals were infected with various doses (from 100 to 10,000 microbes) of a virulent strain of P. pestis No 772 and 773, the minimum lethal dose of which comprised 100 microbes for guinea pigs and 10 for white mice. For infection we used a suspension of a 24 hour and a 48 hour agar culture of the plague microbe, incubated at 28°.

Preliminarily calculations were made of the number of erythrocytes, leukocytes and blood platelets in all the animals. These were made over a period of a month, in various times of the day, and on an empty stomach and after eating.

After infection the investigations of the blood for determining all the formed elements were conducted daily right up until the death of the animal.

All told 893 determinations were made.

With the white mice the batches of blood for investigation were obtained from the blood vessels of the tail by cutting the tip of it each time. With the guinea pigs it was obtained from the ear vein by pricking it with a thin needle. In some cases, in the last days before the death of the animal, it was sometimes necessary to make an incision of the concha auriculae with the edge of a safety razor.

The diluting liquid for calculating erythrocytes was Gayem's liquid, prepared on mercuric chloride; for calculating leukocytes -- Turk's fluid in G. P. Rudnev's modification (1940). Calculation of formed elements was made in a Goryayev chamber.

In the first series of tests the blood was drawn up into the mixer with the help of a syringe, connected by a thin rubber tube with the end of a blood count pipette. Taking blood in such a manner, which guaranteed the safety of the investigator, is not entirely convenient for carrying out the technique, and also for transporting the blood count pipettes. Therefore in subsequent series we somewhat modified the method proposed by Nikolayev (1954), which is relative to plague, and switched to taking blood with a graduated pipette and diluting it in test tubes. For this, in one row of small numbered test tubes (the so-called agglutination test tubes are the most convenient) we preliminarily poured 2 ml of Gayem's liquid into each one, and in the other row -- 0.2 ml of Turk's fluid into each one. On the distal end of a Pasteur pipette which is graduated for 10 mm^3 (such a graduation for one division is easily made in the laboratory with the help of a chemical micropipette), a short rubber tube in the form of a balloon is attached, the pipette is led up to a drop of blood which is emerging following pricking, and by preliminarily pressing on the balloon, or sometimes by simply inclining the pipette, the blood is collected up to the mark which has been made. Then the pipette with the blood is immersed into a test tube with the diluting liquid, and by the pressure of the fingers on the rubber ball the blood is discharged into the test tube. It is washed several times with diluting liquid, withdrawn from the test tube and immersed

into a beaker with a disinfectant solution. The test tubes are closed with rubber corks, vibrated and transferred to the laboratory.

Such a method is convenient because it makes it possible to obtain the required solution (1:200 and 1:20) of blood in the test tube and guarantees safety when taking blood, and also when transporting it and disinfecting the pipettes and test tubes.

The bactericidal action of Gayem's and Turk's fluids on the plague microbe was noted by investigators (G. P. Rudnev, 1940; I. S. Tinker and G. P. Rudnev, 1930) and verified by us in respect to the strain of the plague microbe which we used in the experiment.

As regards the preservation of erythrocytes in Gayem's liquid, then in the test tubes which were closed with rubber corks the number of erythrocytes during repeated determinations did not change for us over a period of two months (limiting period of observation). This is testified to by the data of E. L. Volfson (1936), who cites the case of the preservation of erythrocytes in Gayem's liquid, nonhemolyzed for a period of three years.

The number of blood platelets was calculated according to the method of A. Fonio (1912), who used a 14-percent solution of magnesium sulfate as the anticoagulant, and also by the method proposed by A. P. Yegorov (1939, 1954) with a 4-percent solution of sodium citrate. Staining of smears was carried out with the Giemsa-Romanovskiy stain according to the method of Pappenheym.

As the repeated investigations of the blood in healthy animals showed, the number of erythrocytes in 1 mm^3 of blood in a guinea pig was subject to considerable fluctuation in the course of a day.

Depending on the time of the investigation and the physiological state of the animal, the number of erythrocytes in the same animal changed by 0.30 $\pm$ 0.24 million in 1 mm^3.

Following the infection of animals with a virulent strain of the plague microbe, an increase was noted in the number of erythrocytes in all the days after inoculation (table 1).

As is apparent from the table, the greatest increase in the number of erythrocytes is reached on the second day following infection, exceeding the initial value by 9.1%. In addition to the quantitative change, on the part of the blood corpuscles the presence was noted of normoblasts, anisocytosis and poikilocytosis. It must be stipulated that the uneven form and size of the erythrocytes, and also the presence of normoblasts and polychromatophilia are observed to a somewhat lesser degree even in healthy guinea pigs.

The most expressed change was on the part of the leukocytes -- the increase in their numbers in certain cases was by 4--5 times in comparison

with the initial number (table 2) already in 24 hours following infection and their was a subsequent sharp decrease in the number of leukocytes in the days preceding the death of the animal.

Only in two animals (No 1230 and 1476), the death of which in both cases set in on the fourth day following infection, the number of leukocytes which were measured 24 hours prior to death was increased by 17% in the first case and 120% in the second case in comparison with the initial.

Along with the change in the number of leukocytes, an increase was also observed in their size by approximately 1,5--2 times both during investigation in a native state and during investigations in stained preparations. Among the leukocytes, cells were sometimes detected which had a vacuolized protoplasm and sometimes cells in a condition of decay.

Changes on the part of the blood platelets, which are presented in table 3, amounted mainly to an increase in their number in the first 3 days after infection, and a decrease in the number of blood platelets beginning with the fourth day after inoculation.

The number of blood platelets in 4--5 days following infection turned out to be sharply reduced immediately before the death of the animal. Thus in guinea pig No. 1237, which died on the sixth day following inoculation, the number of blood platelets in the blood, taken two minutes before the death of the animal, comprised 5 per 1,000 erythrocytes. At the same time, during the rapid course of the plague process, when the animals died in early periods, the decrease in the number of blood platelets did not surpass the physiological fluctuations of their number in this species of animal.

In considering the role of blood platelets in the process of formation of agglutinates with bacteria as the first response of the organism to microbial invasion even before the onset of phagocytosis (Roskin, G. I., 1954; A. Copley, T. Balea, O. Chryssostomidon, 1955; L. Duchon, 1955), we made a calculation of the blood platelets and simultaneously of the erythrocytes and leukocytes in guinea pigs 5 minutes prior to infection and in 3--10 minutes after inoculation. No difference was established in the number of formed elements in comparison with the initial values.

The absence of thrombocytopenia in the first minutes following infection, as the above named investigators consider, points to the considerable virulence of the microbe which was introduced.

In our tests a highly virulent strain of P. pestis was used, and this in all probability explains the absence of a decrease in the number of blood platelets in the first five minutes after inoculation.

The change of formed elements of blood in white mice in the process of plague infection did not differ basically from the changes in the blood picture in plague infected guinea pigs. The number of erythrocytes, measured

in noninfected white mice, comprised an average of 9,141,000 in 1 mm^3 of blood with a minimum content of 5,940,000 and a maximum of 10,970,000. After infection of the white mice with 1,000 microbes (100 Dlm) of the 772 virulent strain of P. pestis a calculation of erythrocytes showed an increase of their numbers in all days following the infection. The greatest increase in the number of erythrocytes was reached on the 3rd day after infection in white mouse No. 2674, which died on the 4th day after inoculation. The amount of erythrocytes in this animal, which was measured twice a day for two days prior to infection, comprised 7,200,000 $\pm$ 150,000 in 1 mm^3. In two days following the microbial invasion, the number of erythrocytes in 1 mm^3 of blood equaled 9,120,000, that is, an increase of 26% in comparison with the original. In two white mice the number of erythrocytes following infection turned out to be decreased by 18 and 14.4% in comparison with the original.

In the remaining 35 white mice the number of erythrocytes in all days following infection was increased on an average by 6--10% in comparison with initial values.

For taking into consideration the influence of the repeated taking of blood and, possibly, the conditions for housing the infected animals in jars covered with gauze moistened in a solution of Lysol, we made a calculation of the number of formed elements of blood not only in infected but in healthy white mice which were housed in the infectious-experimental division under the same conditions as the infected animals. The number of erythrocytes in these control animals during repeated investigations fluctuated insignificantly both in respect to an increase and to a decrease, but did not deviate by more than 3--4% from the initial value.

The number of blood platelets in the white mice in the first 4 days after infection increased. Thus, the average number of blood platelets in 13 white mice which had died in 4 days following inoculation comprised 194,000 prior to infection, 202,400 in 24 hours after infection, and after 3 days -- 273,000 in 1 mm^3 of blood.

Thus, as these investigations showed, during experimental plague changes are observed in the number of all formed elements of blood. Erythrocytosis in plague is not great (up to 10% from the initial value), however it is encountered with great constancy. It must be assumed that the increase in the number of erythrocytes in 1 mm^3 of blood which was observed by us during plague does not reflect the true change in the number of formed elements of blood, but is the result of a decrease in the volume of the plasma, that is, blood coagulation. As was shown in the investigations by K. M. Mokhin (1958), the disruption of the permeability of the vascular endothelium, which is developing in a plague afflicted organism, and the discharge of plasma into the tissue causes blood coagulation, and by this, probably, an increase in the number of erythrocytes.

In the plague infected organism the leukocytes are subjected to the gr atest changes. Their number increases by 3--4 times on the 2nd and 3rd

day following infection. After 2--3 days hyperleucocytosis is replaced by leukopenia and the animals usually die with a decreased number of leukocytes in the peripheral blood.

In contrast to the false increase in the number of erythrocytes, leukocytosis in plague is undoubtedly caused not only by blood coagulation, but also by hyperplasia of the leukopoietic part of the blood-producing system, which is testified to by the pathomorphological investigations of S. Damberg (1926), who studied the picture of the bone marrow of suslika in various periods following infection with plague.

The replacement of lukocytosis with leukopenia indicates the lowered reactivity of the organism in the last period of plague morbidity.

Based on the experimental data presented, it is possible to explain the contradictory, on first appearance, results obtained by various investigators when describing the blood picture in persons sick with plague. Thus, investigators who observed the patients for 6--20 hours prior to death speak of leukopenia during plague and consider it a distinguishing sign from croupous pneumonia (S. I. Zlatogorov and L. V. Padlevskiy, 1915). The same thing was noted by pathologoanatomists who were investigating the bone marrow of persons who died from plague (G. S. Kulesha, 1915). On the other hand, investigators who were studying the blood picture in the highest point of the disease, noted during plague a clearly expressed leukocytosis (Zabolotnyy, D. K., 1956; Mueller and Poch, 1900).

In our investigations we also observed in the same animals, following their infection with P. pestis, leukocytosis, hyperleukocytosis, and leukopenia, depending on during which period of plague infection the investigation of blood was carried out.

The increase in the number of blood platelets which we observed in the first days of plague infection in guinea pigs and white mice also points to the increased activity of the bone marrow in this period.

The lowering of the number of blood platelets in 4--5 days following infection speaks in its turn of the progressing depression of hematosis in this, the last, period of plague infection.

Conclusions

1. The method proposed by us for taking blood with a graduated pipette and diluting it in test tubes, according to the method of Nikolayev is safe for working with plague infected animals.

2. Following the infection of guinea pigs and white mice with a virulent strain of plague microbe the number of erythrocytes in 1 mm^3 of blood increased on all the days following infection.

3. In the first days of plague infection leukocytosis was observed in

the animals, sometimes hyperleukocytosis, which on the 4--5th day following infection is replaced by leukopenia.

4. The number of blood platelets is increased in the first 3 days after infection and is decreased in cases of a prolonged course of infection.

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Table 1

Change in the number of erythrocytes in guinea pigs in the dynamics of plague infection

Days	Number of erythrocytes in 1 cubic mm of blood		
	Minimum	Maximum	Average indices
Before infection	4,520,000	5,800,000	5,215,000
After infection			
1	4,290,000	6,210,000	5,417,000
2	4,770,000	6,750,000	5,693,000
3	4,550,000	6,500,000	5,629,000
4	4,390,000	6,470,000	5,430,000
5	4,650,000	6,900,000	5,475,000
6	4,900,000	6,080,000	5,480,000

Table 2

Change in the number of leukocytes in guinea pigs in the dynamics of plague infection

Days	Number of leukocytes in 1 cubic mm of blood		
	Minimum	Maximum	Average indices
Before infection	7,500	19,400	13,317
After infection			
1	6,600	30,500	13,853
2	15,700	59,600	26,745
3	15,300	47,000	30,833
4	12,850	33,900	21,462
5	4,930	12,000	7,523
6	4,400	8,300	6,350

Table 3

Change in the number of blood platelets in guinea pigs in the dynamics of plague infection

Days	Number of platelets in 1 cubic mm of blood		
	Minimum	Maximum	Average indices
Before infection	256,500	650,160	472,058
After infection			
1	560,490	793,650	689,834
2	414,990	992,250	623,022
3	383,400	628,320	502,728
4	232,670	401,140	318,732
5	117,300	399,230	255,207
6	25,000	197,600	98,493 [?]

? = Flaw in the original Russian version. This appears to be the number.