

OBSERVATIONS ON THE MECHANISM OF THE HEMORRHAGES IN PLAGUE
REPORT I. STATE OF THE FACTORS OF BLOOD COAGULATION IN EXPERIMENTAL PLAGUE

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OBSERVATIONS ON THE MECHANISM OF THE HEMORRHAGES IN
PLAGUE. REPORT I. STATE OF THE FACTORS OF BLOOD
COAGULATION IN EXPERIMENTAL PLAGUE

[Following is the translation of an article by I. Ye. Kiseleva, appearing in the Russian-language periodical Trudy Rostovskogo-na-Donu Gosudarstvennogo Nauchno-Issledovatel'skogo Protivochumnogo Instituta (Transactions of the Rostov on the Don State Scientific-Research Antiplague Institute), Installment 1, Vol XV 1959, pages 97-105. Translation performed by Sp/7 Charles T. Ostertag Jr.]

In spite of the fact that plague actually represents a hemorrhagic septicemia and hemorrhages occupy one of the dominating places in the pathology of this infectious disease, the systematic study of the causes and mechanisms which determine the development of hemorrhagic symptoms has still not been carried out.

Hemorrhages on the skin in the form of large or small petechiae, coarse and fine cutaneous hemorrhages, hemorrhages under the mucous membrane of the intestines and stomach (hematemesis, melena), and hemorrhages under the mucous membrane of the bladder, detected due to the presence of erythrocytes in the urine, have been described in the clinical syndrome of all forms of plague beginning already with the middle ages (Black death") Rudnev, G. P., 1940; Wu Lien-teh, 1926; R. Pollitzer, 1954).

The pathoanatomical data from various investigators supplement the materials of the clinical picture, indicating numerous hemorrhages in the parenchymatous organs, the walls of veins in the region of the primary bubo, the serous and the mucous membranes. Thus, H. Albrecht and A. Ghon (1898) described hemorrhages in the skin, bubo, gastrointestinal tract, pancreas, skeletal muscles, in the pericardium and under the endocardium, and in the walls of vessels close to the primary bubo; D. P. Kishenskiy et al. (1911) described hemorrhages in cardiac muscles, and in large and medium vessels; M. M. Tizengauzen (1911) -- in the brain substance; Pollitzer (1954) -- in the kidneys and under the mucosa of the stomach and intestines; V. N. Labanov (1956) -- in the lymph nodes, in the folia of the pleura, and the visceral folium of the pericardium; A. N. Cherventsov (1904) -- in the adrenal glands of white rats and in the lungs of rabbits; V. V. Donskov (1939) - in the adrenal glands of guinea pigs.

There is no doubt that wide spread hemorrhages in the various tissues and organs, especially those that are vitally important, can exert a considerable influence on the course and outcome of the infectious process, aggravating the disturbances of the functions of the various organs and systems which ordinarily are progressing during plague.

Since for each nosological unit, accompanied by the syndrome of hemorrhage, peculiarities and its own pathogenetic moments are characteristic, the establishment of all the aspects of the mechanism of hemorrhag origination during plague helps to clear up the unclear aspects of the general pathogenesis of plague infection and thereby seek to find more rational means for the pathogenetic therapy of plague.

It is known that the capacity for hemorrhages depends on a number of causes which are assembled into two groups (Kh. Kh. Vlados, 1937; Ye. I. Freyfeld, 1947; I. A. Kassirskiy and G. A. Alekseyev, 1955 and others)

The first group includes the vascular factors, expressed in the disruption of the functional value of the vascular wall and, primarily, changes in its permeability, stability and resorptive ability.

The second group of reasons, which cause a tendency toward hemorrhages, is made up of factors connected with the disruption of the normal processes of blood coagulation and stopping of bleeding. Among the causes, influencing the process of coagulation of fibrinogen (factor I) is the amount of prothrombin (factor II) -- a glycoprotein which is produced by the liver and is a constituent factor of the globuli fraction of plasma proteins. Also taking direct part in the process of blood coagulation are the blood platelets, which due to their biological properties are also an important factor of hemostasis.

With the aim of clearing up the reasons which cause hemorrhagic diathesis in plague, we studied the changes in the amount of blood platelets, the content of prothrombin and the time for blood coagulation, and also the resistance of the vascular wall in guinea pigs and white mice infected with plague.

The results of the first part of the investigations are presented in this article. Changes in the functional state of the vascular wall during experimental plague are the subject for the next communication.

The tests were set up on 56 white mice and 43 guinea pigs (males) infected subcutaneously with 500 and 1000 microbes of B. pestis 772 and 773 (50 and 100 Dlm for white mice and 10 -- for guinea pigs). In all the animals for a month prior to infection and on all the days after the infection with a virulent strain of the plague microbe, a determination was made of the number of blood platelets, the time for blood coagulation and the prothrombin time.

The blood coagulation time was determined according to the method of Byurker, prothrombin time -- by the method of Quick as modified by D. P. Borovskaya and S. D. Rovinskaya (1948), utilizing as a substitute for thromboplastin an emulsion of rabbit brain (antirabic vaccine according to Fermi).

The number of blood platelets was calculated according to the method of Foniyu. Control investigations were carried out on healthy animals which were studied under similar conditions with the infected ones.

Changes in the amount of blood platelets in guinea pigs and white mice in the dynamics of experimental plague were described in detail in our previous communication (See this same collection). They amount to an increase in the number of blood platelets in the first days following infection and a decrease in their number in the last days, before the death of the animal. Thus, after 24 hours following the infection of guinea pigs with a virulent strain of the plague microbe, the amount of blood platelets was increased on the average by 46%, and after 3 days -- by 6.5%. From the fourth day following the inoculation the number of blood platelets dropped sharply (on an average to 20%) only on the sixth day, several hours prior to the death of the animal. At the same time in cases when the plague process proceeded rapidly, when the guinea pigs and white mice died in early periods (3-4 days), the reduction in the amount of blood platelets did not exceed the physiological fluctuations of their number in this species of animals.

Changes in the blood coagulation time in plague infected guinea pigs and white mice are shown in table 1. For obtaining comparable results based on the stages of the illness, the animals were divided into groups corresponding to the time of their death and the average data are presented for each group.

As the tests showed, in the white mice in the first days following infection with plague an acceleration of blood coagulation was observed. Up to the outcome of the disease the blood coagulation time had built up insignificantly and before death it usually somewhat exceeded the initial indices, which was most clearly expressed in animals with a duration of the infectious process greater than four days. In mice which had died on the third day following infection, a slowing down of blood coagulation before death was not noted.

In guinea pigs in the overwhelming majority of the tests, in the first three days after infection, just as with the white mice, the process of blood coagulation was accelerated. In the following stage of plague infection and in animals which were in agony, no regular changes were observed in the blood coagulation time. In more than half the tests the blood coagulation time was lessened in comparison with the original time; under certain conditions, following an initial lessening it increased and reached the original value. We did not observe a dependency between the blood coagulation time in this period and the duration of the infectious process or the peculiarities of its course. Only in one test, in guinea pig No 3804, which died on the eighth day following infection, a slowing down was noted of the beginning and end of the second phase of blood coagulation. This was observed on the eve of its death.

As is seen from the table, the prothrombin time was increased in the plague infected white mice in all the days following infection. This increase, though it did not exceed 7 seconds (32%), was observed with great constancy in contrast to the control group of animals. The prothrombin time of the control animals in all the times of the investigation did not change by more than 1-2 seconds.

In guinea pigs the prothrombin time in all the days following infection also increased on comparison with the initial time. In animals which were sick for 4-5 days, following an initial increase the prothrombin time lessened correspondingly on the 3rd and 4th days, but the initial values were not reached. On the following days the prothrombin time in these animals again increased but more significantly than in the first days, and in individual cases it exceeded the normal time by almost two times. It is necessary to note that in the healthy guinea pigs a fluctuation was observed in the prothrombin time depending on the physiological state of the animals, the time of taking blood, and the frequency of repeated investigations, however in the majority of tests it did not exceed 5-7 seconds and only in individual cases did it reach 12-15 seconds.

Data on changes in the prothrombin time in healthy and infected animals make it possible to calculate in the latter the relative content of prothrombin in the circulating blood. The content of prothrombin in the blood of the healthy mice and guinea pigs is taken as 100%. The percentage content of prothrombin in animals infected with *B. pestis* is the ratio of the prothrombin time of healthy animals to the prothrombin time of infected animals, multiplied by 100.

The results of the calculations are presented in table 3.

It is seen from the table that in white mice following infection with plague the amount of prothrombin in the circulating blood is lessened on the average by 13% on the 2nd day and by 26% on the 4th day.

In guinea pigs in the first two days following infection the amount of prothrombin is decreased insignificantly, averaging 17%. On the 3rd day this reduction already comprises 29%, and on the 4th day the amount of prothrombin even increases somewhat in comparison with the previous day, after which it drops sharply. The content of prothrombin reaches its greatest decrease (up to 36-42%) on the 5th and 6th day after infection. In all cases with the duration of the plague process greater than four days there is a marked lowering in the content of prothrombin in the circulating blood in the days before the death of the animal.

The decrease in the amount of prothrombin in the blood of plague infected animals may be caused by a disruption of the prothrombin-forming capability of the liver during plague. Thus, Ehrenkranz and L. White (1954) found a worsening of the liver function during a pneumonic form of plague in monkeys already on the second day following infection. and M. Schar and K. Meyer (1956) found the same in white mice two days after

they had received a toxic fraction of the plague microbe. It is very possible that during plague, as well as during other infections, in the beginning of the illness there is an increased detoxication capability in the liver which causes a disruption of its other functions, in particular prothrombin formation. In this period we observed an insignificant lessening (up to 17%) in the content of prothrombin in the blood. In the last phase of the plague process the depression of the activity of the liver cells under the influence of the toxic products of bacteria metabolism causes a more significant reduction in the amount of prothrombin.

In the last days of life of an organism infected with plague we observed also a significant reduction in the amount of blood platelets, which in connection with the leukopenia noted in this same period may be explained by the toxic injury to megakaryocytes of the bone marrow.

The speeding up of blood coagulation, observed by us in the process of development of plague, is apparently caused both by the stimulation of the sympathetic nervous system and the strong interoceptive irritations, leading to the mobilization of the defensive mechanisms of the blood.

Thus, as the investigations demonstrated, in guinea pigs and white mice following infection with a virulent strain of the plague microbe, changes are observed in the amount of blood platelets and prothrombin and the time for blood coagulation.

As is known, changes of these factors are observed in many diseases, accompanied by a tendency for bleeding, and in many cases they are the etiological moment of the hemorrhagic nature of the disease.

However, our noted decrease in the number of blood platelets on the 4th to 5th days following infection did not in the majority of cases exceed a half of their original amount, and only several hours prior to the death of guinea pigs, on the sixth day following infection, the amount of blood platelets in them decreased by 5 times. It is considered that hemorrhages, connected with thrombocytopenia, set in only when the amount of blood platelets is reduced by 10-12 times (Kh. Kh. Vladimirov, 1937).

The content of prothrombin in the blood of plague infected white mice did not drop lower than 74%, and in guinea pigs, even before death, lower than 58% of the content of it in healthy animals. Taking into consideration the fluctuation of the prothrombin time for these rodents, it cannot be considered as significant. Usually hemorrhagic diathesis, depending on a deficiency of prothrombin, is lower than 35% of the standard (I. A. Kassirskiy and G. A. Alekseyev, 1955).

It must also be stressed that all the disruptions noted by us during experimental plague of the factors of blood coagulation, which would be the etiological cause of hemorrhages, are observed constantly only in the last days of life of the plague infected organism. At the

same time hemorrhages in the internal organs during experimental plague are noted not only during the highest point of illness but even in the incubation period (A. N. Cherventsov, 1904; V. V. Donskov, 1944).

Thus, on the basis of experimental data presented in this and the previous communications it can be assumed that the disruption of factors of blood coagulation, in particular a decrease in the number of blood platelets and the amount of prothrombin, do not lie at the basis of hemorrhages in plague. These facts may have significance in the pathogenesis of hemorrhages only in the last days and even hours of a delayed plague process, when the balance is disrupted in the system of blood coagulation, which depends not only on humoral influences, but also on the functional condition of the entire organism.

Conclusions

1. A speeding up of blood coagulation is observed in white mice and guinea pigs in the first days following infection with a virulent strain of the plague microbe.
2. The blood coagulation time in white mice during the last stage of plague is increased. In the majority of guinea pigs no characteristic changes are detected in the blood coagulation time during this period.
3. In plague infected laboratory animals during the process of development of the infection, the content of prothrombin in the circulating blood is decreased.
4. Disruption of the factors of blood coagulation during experimental plague is not so great as to be a specific cause of hemorrhages.

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Table 1

Changes in the time for blood coagulation in white mice and guinea pigs in the dynamics of plague infection. *

Group of animals	Period of life in days	Coagulation time in minutes and seconds								
		Before infection	Days after infection							
			1	2	3	4	5	6	7	
White mice	2	1'50"	1'40"	1'40"						
1		4'	3'20"	3'35"						
2	3	2'35"	1'45"	1'40"	2'45"					
3	4	3'50"	3'45"	3'20"	4'10"					
		2'15"	2'10"	2'	2'50"	3'20"				
		4'45"	4'35"	4'20"	5'20"	6'40"				
Guinea pigs	3	1'30"	1'	1'	1'					
1		3'15"	2'30"	2'30"	3'					
2	4	2'	1'10"	1'20"	1'20"	1'20"				
		3'30"	2'50"	2'50"	3'	2'30"				
3	5	1'40"	1'15"	1'20"	1'30"	1'20"	1'20"	1'20"		
		3'10"	2'30"	3'	3'10"	3'30"	3'30"	3'10"		
4	6	1'40"	1'	1'45"	1'15"	1'15"	1'15"	1'15"	1'15"	
		3'15"	2'15"	3'	3'15"	3'30"	3'30"	3'15"	2'15"	
5	7	1'30"	2'	1'	1'	1'	1'30"	1'	1'30"	2'30"
		3'30"	4'	3'	3'	2'	4'30"	2'30"	3'30"	4'

* The table indicates the beginning of the 2nd phase of blood coagulation -- the appearance of the first threads of fibrin --- and the end of the 2nd phase -- the formation of a dense blood clot.

Table ?

Changes of prothrombin time in white mice and guinea pigs in the dynamics of plague infection (average data).

Group of animals	Period of life in days	Prothrombin time in seconds							
		Before infection	Days after infection						
			1	2	3	4	5	6	7
White mice									
1	2	23	24	27					
2	3	22	25	28	30				
3	4	23	24	25	28	31			
Guinea pigs									
1	3	29	36	42	47				
2	4	29	34	44	38	46			
3	5	32	39	44	51	45	55		
4	6	31	33	39	39	35	40	44	
5	7	33	34	34	46	46	51	50	51

Table 3

Changes in the amount of prothrombin in white mice and guinea pigs in the dynamics of plague infection (average data).

Group of animals	Period of life in days	Content of prothrombin in percentages							
		Before infection	Days after infection						
			1	2	3	4	5	6	7
White mice									
1	2	100	96	85					
2	3	100	88	88	78				
3	4	100	96	88	82	74			
Guinea pigs									
1	3	100	80.6	69	62				
2	4	100	85	66	76	63			
3	5	100	82	73	63	71	58		
4	6	100	94	79	79	88	77	70.5	
5	7	100	97	97	72	72	64	66	64