ON BLOOD PLASMA COAGULATION BY PLAGUE AND PSEUDOTUBERCULOSIS MICROBES

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It is known that the plague microbe lyses human and animal fibrin [1, 2, 3, 6]. Along with this, there are reports that the plague microbe is capable of coagulating the plasma of rabbits [5] and man [4].

Inasmuch as fibrinolytic and plasma-coagulating activity are directed toward the very same substrate*, it was of interest to investigate the interdependence between these two properties in various strains of the plague microbe. Attention was also merited the question of whether the pseudotuberculosis microbe, which does not possess fibrinolytic activity, was capable of coagulating plasma.

EXPERIMENTAL METHOD

Since there are no comparative data characterizing plasma coagulation by the plague microbe in different species of animals, to investigate the coagulative properties of the strains we used human, rabbit and guinea pig plasma.

Human blood was obtained from donors by means of venapuncture. In the animals, the blood was taken from the heart, observing the principles of asepsis, quickly mixed with an anticoagulant solution, centrifuged, and the plasma siphoned off. As the anticoagulant, we used a 5% solution of sodium citrate, the final concentration of which was 0.5% in the human and guinea pig whole blood, and 0.7% in the rabbit blood. In individual experiments, in order to stabilize the human blood, in addition to the sodium citrate we used a 2.5% solution of potassium oxalate, producing a final concentration of 0.25% in the whole blood. The rabbit and guinea pig plasma were used only in the fresh state, while the human plasma was either fresh or lyophilized. For the plasma coagulation reaction, the plasma was diluted with physiological saline to a ratio of 1:5, since, according to our preliminary data, in the presence of rabbit or human whole plasma the reaction is depressed.

The results of preliminary experiments, in which we compared the activity of bouillon and agar cultures of different ages (24 and 48 hours), showed that only the number of microbes in the sample determines the outcome of the reaction. In view of this, as the source of coagulase activity we used a suspension of microbes cultured on Hottinger's agar for 48 hours. The strains of plague microbes were grown at 28°, and the pseudotuberculosis – at 28 and 37°.

For the reaction of plasma coagulation, 0.1 ml of the microbe suspension, with a density of 1 and 5 billion microbes per ml, was introduced into sterile test tubes, and then 0.5 ml of plasma was added. The test tubes were placed in an incubator at 37°, and, over a period of 48 hours, were periodically observed for the formation of clots. The reaction was accompanied by controls for the capacity of the plasma to coagulate spontaneously, and for coagulation in the presence of microbes of the coagulase – positive strain of staphyllococcus aureus.

*In purified systems, fibrinogen, as well as fibrin, may be the substrate for the action of the blood's fibrinolytic enzyme, independent of the means by which the latter is activated.

Simultaneously with the plasma coagulation test, we set up the reaction of fibrinogenolysis, using the same suspensions of microbes. The substrate in the fibrinogenolysis reaction was a preparation of lyophilized ox fibrinogen.

EXPERIMENTAL RESULTS

For the investigation, we used 116 fibrinolytically active, and 57 inactive, strains of the plague microbe, as well as 68 strains of the pseudotuberculosis pathogen.

The group of fibrinolytically active strains of the plague microbe included strains of differing origin and of different durations of laboratory custody. Their virulence varied within wide limits. In addition to highly virulent strains, whose Dcl for guinea pigs and white mice was 50-100 microbes, we used strains with lower virulence, down to avirulent types. The latter were represented by the vaccine strains No. 1, 17, and EB. Along with the strains that were virulent for both guinea pigs and white mice, there were also strains virulent primarily for one of these two species of laboratory animals.

The group of fibrinolytically inactive strains consisted of 57 types, isolated from various subjects in the peri-Caspian, middle-Asiatic and Ciscaucasus pools, chiefly from 1912 to 1939. Their virulence was low: Dcl for guinea pigs did not exceed 1 million microbes. Chief among the culture-biochemical properties of these strains was their ability to ferment rhamnose in 1-5 days (fermentation of rhamnose was noted in 51 of the 57 strains). A second characteristic was related to the morphology of their growth on agar plates: all of them formed plately pigmented colonies with a smooth surface.

The strains of the pseudotuberculosis microbe (68) were represented by both smooth and rough forms. They differed in virulence. The greater portion of the strains consisted of freshly isolated cultures, and the minority – museum specimens.

As a result of the investigations, it was shown that the plague microbe regularly coagulated rabbit plasma, and that this ability of the strains was correlated with their fibrinolytic activity. All 116 fibrinolytically active strains yielded a positive reaction for plasma coagulation with rabbit plasma, and all the fibrinolytically inactive strains produced a negative reaction.

The rabbit plasma began to coagulate after only 2-4 hours. At this time, some of the strains caused complete coagulation of the plasma, terminating in the formation of a dense clot that stuck firmly to the walls of the test tube. The principal portion of the strains coagulated the rabbit plasma at later intervals. After 20-25 hours, all the strains that were capable of clotting rabbit plasma had yielded a positive reaction when 100 million microbes were present in the sample. A positive reaction was characterized by complete or partial coagulation of the plasma.

Out of 116 strains that yielded a positive reaction for coagulated human plasma. Coagulation of the human plasma was observed primarily when 500 million microbes were present in the sample. Similar results were obtained with both the fresh and lyophilized human plasma. There were also no differences noted in the coagulation of the oxalated and citrated plasma, using the indicated concentrations of the anticoagulants.

The data in the literature pertaining to coagulation of human plasma by the plague microbe are contradictory. Zawetz and Meyer [5] did not observe human plasma coagulation by the plague microbe, while Eisler [4] observed human plasma coagulation in 23 of 32 strains that he investigated. In our experiments, the human plasma was coagulated by a relatively small number of strains. This can be explained partially by the fact that the concentration of sodium citrate in our experiments significantly exceeded the optimal figures established by Eisler (2 mg/ml). In addition, as the source of activity Eisler used a whole bouillon culture, in which the content of living microbes was as high as 640 million, and the total number of microbial bodies possibly exceeded even that figure.

Under the standard conditions of the experiment, the guinea pig plasma was coagulated by 27 of the 116 strains. One of these occurred with 100 million microbes in the sample, and the rest, with 500 million microbes. Partial, but not complete, coagulation was noted after one day; after this we observed lysis of the clots that formed, and after 48 hours they had completely disappeared.

It must be noted that both strains of staphyllococcus, used as the control, regularly coagulated the human and rabbit plasma, and did not coagulate the guinea pig plasma. The opinion exists that guinea pig plasma contains very small amounts of the factor needed for plasma coagulation by staphyllocoagulase, and that at 37° the small amounts of this factor are destroyed, due to its thermolability. At 25° this factor is not destroyed, and in this case, one observes slow coagulation of guinea pig plasma by staphyllococci [7].

In order to verify this hypothesis, we observed the coagulation of guinea pig plasma by the plague microbe simultaneously at 37 and 28°. At 37°, 9 out of the 17 investigated strains produced partial coagulation; at 28°, all 17 strains produced predominantly complete coagulation after one day. Under these conditions, both strains of staphyllococci coagulated the plasma only partially, after 48 and 96 hours.

On the basis of preliminary data, we cannot conclude whether or not the coagulase factor in the guinea pig plasma is actually destroyed at 37°. It is also too early to judge the reasons behind the differences in coagulation of the plasma from the different species of animals by the plague microbe.

It was indicated above that the plague microbe regularly coagulates rabbit plasma. The capacity to coagulate rabbit plasma is inherent to strains of the plague microbe, independent of the intervals of their laboratory custody, which shows the stability of the characteristic in this species of microbes. Moreover, the data presented, characterizing the virulence of the coagulase – positive strains, show that the capacity to coagulate plasma is manifested by strains of the most divergent virulence. This latter fact testifies to the absence of a correlation between the ability to coagulate plasma and virulence in the plague microbe. It should be noted that a similar opinion was expressed by Jawetz and Meyer [5] and Eisler [4]. The direct dependence which we observed between the capacity for plasma coagulation and fibrinolytic activity, in turn, confirms the earlier drawn conclusion that there is no correlation in the plague microbe between fibrinolytic activity and virulence.

The data of this investigation showed that the capacity to coagulate plasma is a characteristic feature of the plague microbe, but not of the pseudotuberculosis microbe, since all investigated strains of the pseudotuberculosis pathogen failed to demonstrate the ability to coagulate plasma, despite numerous testings. The constant absence of plasma-coagulating and fibrinolytic activity in the pseudotuberculosis microbe justifies postulating that, in this case, there is a qualitative distinction between the plague and pseudotuberculosis microbes. Since the capacity to coagulate plasma, as well as the presence of fibrinolytic activity, are features characterizing the qualitative difference between the plague and pseudotuberculosis microbe, the plasma coagulation test may offer considerable interest in the differential diagnostics of the plague and pseudotuberculosis microbe.

SUMMARY

The authors investigated the coagulative capacity of human, rabbit and guinea pig plasma in various strains of Past. pestis and found that the latter coagulated rabbit plasma regularly. There was a direct relationship between the capacity of rabbit plasma coagulation and the fibrinolytic activity. No correlation between the capacity to coagulate plasma and the virulence of Past. pestis was detected. Capacity to coagulate plasma is a characteristic sign of Past. Pestis but not of pseudotuberculosis microbes.

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