FLUCTUATIONS IN THE BLOOD CLOTTING SYSTEM AFTER EXPERIMENTAL ADMININSTRATION OF FIBRINOLYSIN

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UDC 612.115.014.46:615.273.55

A dynamic investigation of the blood of animals receiving fibrinolysin and calcium chloride revealed fluctuating changes in clotting activity.

The same substances have different actions on blood coagulation in vitro and in vivo. This indicates that experiments in vitro do not fully reflect processes taking place in vivo, for the blood clotting system is an open system [3] and does not obey the rules of a closed system such as blood contained in a test tube.

According to Von Bertalanffy's theory [5, 6] any open system reaches a state of dynamic equilibrium (a stationary state) which, unlike the ordinary equilibrium in closed system, is maintained in the course of the continuous exchange and movement of the substances composing the system. By stationary state of the blood clotting system must be implied dynamic equilibrium between the processes of formation of the clotting factors (procoagulants and anticoagulants) in the organs and their utilization during metabolism. In this case the conversion from one stationary state into another must take place through a series of alternating phases, i.e., through extreme states of a maximum or minimum of concentration (or activity) of the clotting factors or of the clotting activity of the blood as a whole. The behavior of certain biochemical systems has been shown to be of this nature [4, 7].

On the basis of these views an explanation can be obtained for the writers' earlier observations [1, 2] to the effect that in fibrinolysis the processes of formation and lysis of fibrin are joined together by a period of alternation of opposite phase states of blood clotting activity.

To verify the existence of these fluctuations, a series of dynamic tests of the blood clotting activity during experimental fibrinolysis was undertaken.

EXPERIMENTAL METHOD

Clotting activity was judged from the change in electrical resistance of blood samples during retraction of blood clots in the ERM-1 apparatus.

Experiments were carried out on 50 albino rats and 6 rabbits. Fibrinolysin in a dose of 30,000 units was dissolved in 10 ml 10% calcium chloride solution and in a dose of 20,000 units in 10 ml physiological saline. Altogether 3 series of tests were conducted. In series I and II rats were anesthetized with ether, the jugular vein dissected, and fibrinolysin solution in calcium chloride injected quickly into it in doses of 300 units (series I) and 900 units (series II) per 100 g body weight. In the experiments of series III fibrinolysin in physiological saline was injected in a dose of 1000 units/kg body weight into the marginal vein of the ear over a period of 1 min. Blood samples were taken at various times after injection of the preparation (from the heart of those rats which died, from the opposite jugular vein of surviving rats, and from the marginal vein of the opposite ear of the rabbits). In the experiments of series I 4 rats died from

Department of Pathological Anatomy, S. M. Kirov Military Medical Academy, Leningrad. (Presented by Academician V. N. Chernigovskii.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 73, No. 3, pp. 15–18, March, 1972. Original article submitted April 2, 1971.

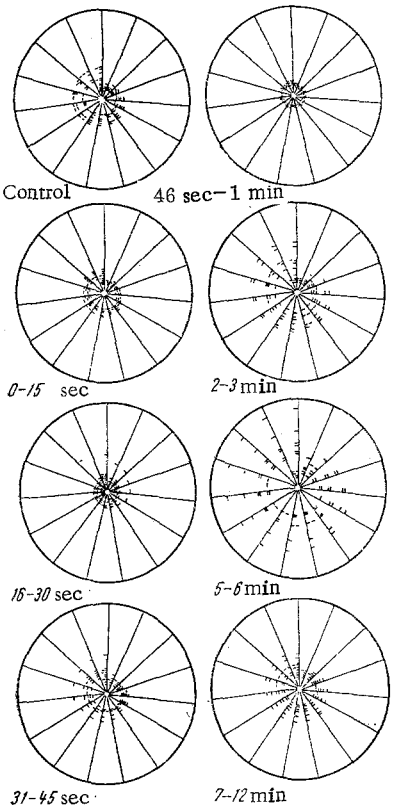

Fig. 1

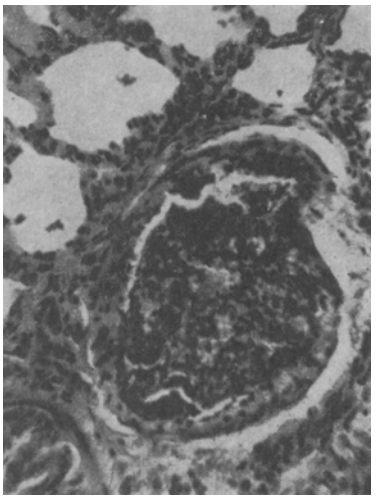

Fig. 2

Fig. 1. Dynamics of changes in electrical resistance of blood samples during clotting and retraction after injection of 300 units/100 g body weight fibrinolysin in calcium chloride solution into rats. Marks in conventional units made clockwise every 4 min during testing for 1 h. Explanation in text.

Fig. 2. Intravascular blood clotting with thrombus formation. Lung of rat after injection of fibrinolysin with calcium chloride. Chromotrope 2B aqueous blue, 200 times.

spreading thrombosis (one died after 20 sec and three after 1 min). Blood samples were tested in the following time intervals: 0-15 sec, 16-30 sec, 31-45 sec, 46sec -1min, 2-3 min, 5-6 min, and 7-12 min. Six samples were tested at each time. The control group contained 6 rats. After blood samples had been taken the surviving rats were killed and pieces of their viscera were taken for histological investigation. In the experiments of series II 4 of the 10 animals died from thrombosis (2 after 30 sec and 2 after 1 min); the remainder were sacrificed at intervals of 20-30 sec and 45 sec - 1 min after injection of the preparation. In series III all the rabbits survived. Blood samples were tested 30 sec, 1, 2, 3, 5, 9, 18, 27, 54, and 108 min, and 6 h and 24 h after injection of the fibrinolysin; the blood loss sustained during sample taking did not exceed 6-12 ml.

EXPERIMENTAL RESULTS

The retraction curves are shown as vector diagrams in Fig. 1. These curves for the control animals for blood tested within the first hour consisted of helices which unwound in a clockwise direction.

In the experiments of series I the character of the retraction curves remained the same for blood samples taken within the interval 0-15 sec after injection of the fibrinolysin; on the whole, however, the helices were more compressed and the fluctuations in electrical conductivity of the individual blood samples were less marked. The retraction curves were more compressed in the interval from 16 to 30

sec, and in their shape they were almost closed circles. In 3 of the 6 animals the helices actually coiled in the clockwise direction, indicating a reduction of retraction and fibrinolysis. In the interval 31-45 sec the general appearance of the retraction curves again was indistinguishable from the controls, and only in 1 experiment did the helix coil clockwise. Between 46 sec and 1 min the retraction curves in all tests were virtually closed circles with very slight fluctuations. In 4 of the 6 experiments the curves coiled clockwise. From 2 to 3 min their character was restored, but 5-6 min after the beginning of the experiment there was a marked increase in the electrical resistance of the blood samples after the first few minutes and, in particular, at the end of the first hour of investigation, indicating increased clotting activity of the blood and increased retraction. Finally, between 7 and 12 min the normal changes in electrical resistance of the blood samples during retraction began to be restored.

Histological investigation of animals killed or dying during the first minute after the beginning of the experiment and autopsied during the subsequent 3 min showed granular thrombotic masses (Fig. 2) similar to those observed previously in experiments on rabbits in the capillaries of the lungs. The extent and severity of the vascular thrombosis varied, being most marked in the animals killed or dying between the intervals of 16-30 sec and 46 sec - 1 min when, as the retraction curves showed, the clotting activity of the blood was reduced. This discrepancy can be explained by the fact that the time elapsing from collection of the blood for testing and sacrifice and autopsy of the animal was measured in minutes; consequently, intravascular blood clotting had already developed in association with the wave of hypercoagulation.

The experiments of series II showed that an increase in the dose of fibrinolysin and calcium chloride injected caused death of the animals from thrombosis more frequently than in the experiments of series I. The retraction curves were of the same character as in the experiments of series I.

In the experiments of series III weakening of retraction was observed 30 sec after the beginning of fibrinolysin injection and the retraction curves came to resemble closed circles just as was observed in the rats. A slight increase in retraction took place after 2 min, with normalization at subsequent periods of testing.

Injection of fibrinolysin thus caused fluctuating changes in the coagulating activity of the blood, with alternation of states of hypocoagulation and hypercoagulation.

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