CHANGES IN THE STRUCTURE AND NUMBER OF PLATELETS DURING INTRAVASCULAR ACTIVATION OF THE BLOOD CLOTTING SYSTEM

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Injection of thrombin into the blood stream was followed at all times of observation by a decrease in the number of platelets and by marked disturbances of their structure.

There are differences of opinion in the literature regarding changes in the clotting power of the blood after injection of thrombogenic substances (thrombin, thromboplastin, etc.) into the blood stream. Some investigators [1, 2, 6, 7, 9] consider that intravenous injection of thrombin or other thrombogenic agents into healthy unanesthetized animals causes massive intravascular blood clotting and death of the animals. The size of the blood clot depends on the rate of injection of the thrombogenic substances: if they are injected rapidly, massive blood clots are formed, but if they are injected slowly clots are small and quickly undergo lysis. Other workers [4, 5] consider that intravenous injection of moderate doses of thromboplastic or thrombin into unanesthetized animals does not cause intravascular blood clotting, by contrast to what takes place in anesthetized animals.

An essential piece of evidence in support of intravascular blood clotting after injection of thrombin would be a disturbance of the structure of the platelets and a change in their number.

This paper describes changes in the structure of the platelets and in their number after intravenous injection of thrombin.

EXPERIMENTAL METHOD

Experiments were carried out on 74 August and Wistar rats of both sexes weighing 150-200 g.

In the experiments of series I the process of thrombosis was studied after injection of thrombin. Thrombin (Leningrad Institute of Blood Transfusion; activity: 0.1 ml thrombin coagulates 0.2 ml oxalated plasma in 25 sec) was injected in a dose of 1 ml into the jugular vein of unanesthetized rats. In control experiments physiological saline was injected in the same volume.

In series II the effect of intravenously injected thrombin on the structure and number of the platelets was studied. Tests were carried out 3, 15, and 60 min after injection of the thrombin.

The number of platelets in venous blood was estimated by the method of Brecher and Cronkite [8], using the diluting fluid of Fisher and Germer [10]. The structure of the platelets was studied by electron microscopic examination of total preparations of platelets [11] in blood taken from the jugular vein of the rats into silicone-treated syringes; the blood was mixed with anticoagulant (1.34% solution of sodium oxalate) in the ratio of 9 : 1.

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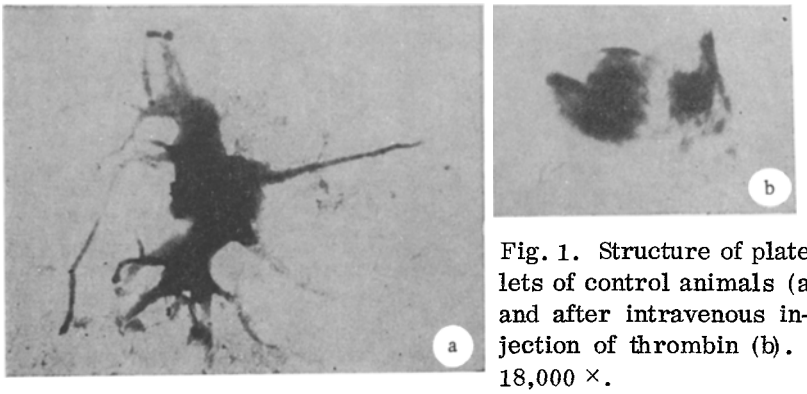

Fig. 1. Structure of platelets of control animals (a) and after intravenous injection of thrombin (b). 18,000 ×.

EXPERIMENTAL RESULTS

Injection of 1 ml thrombin led to intravascular clotting. In the animals killed 4 min after injection of thrombin, clots were found in the blood vessels of the lungs, intestines, and other organs. The blood clots were friable and easily broken up. Division of the vessels at the location of the clot was followed by hemorrhage. If the animals did not die and were sacrificed 15 or 60 min after injection of thrombin, no visible fibrin clots were found.

The platelet count in the circulating blood was reduced on the average by 20% when tested 3, 15, and 60 min after injection of thrombin, whereas injection of physiological saline caused no changes in the platelet count.

Intravenous injection of thrombin caused significant changes in the structure of the platelets.

For instance, the platelets from the control animals appeared on the electron micrographs as circular, ellipsoidal, or stellate structures measuring from 1 to 4 μ in diameter, with clear outlines of their outer membrane (Fig. 1a). An optically dense granulomere was present in the center of the structure, and was surrounded by a paler hyalomere. The differences in shape of the platelets were due to the presence of optically translucent pseudopodia, arising directly from the hyalomere. On the average, in every 12 platelets 1 was fragmented.

After injection of thrombin the number of fragmented platelets was increased by more than three times at all periods of investigation. Their structure was ill-defined and their outer membrane was destroyed. The granulomere and hyalomere were ill-distinguishable optically, and the pseudopodia were fewer in number (Fig. 1b). These changes were further evidence of intravascular blood clotting, which was observed in all cases where the animals died during the first 4-7 min after injection of thrombin.

These observations are in agreement with results obtained by other workers [3, 7, 12-14]. After intravenous injection of amniotic fluid possessing marked thrombogenic activity, Chernaya [7] found a marked decrease in the number of circulating platelets, activation of the blood clotting system, and inhibition of fibrinolysis.

Consequently, after injection of thrombin into the blood stream there is considerable utilization of platelets, and changes take place in their structure. These results are further evidence of the intravascular blood clotting which occurs after injection of thrombogenic agents. Otherwise the structure of the platelets and their number would remain unchanged.

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