

MECHANISM OF CHANGES IN ADHESIVENESS  
AND AGGREGATION OF PLATELETS  
IN ACUTE RADIATION SICKNESS

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Adhesiveness and aggregation of blood platelets are reduced in rats with acute radiation sickness. It is postulated that this may be due to a decrease in activity of plasma factor XIII.

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In the pathogenesis of the hemorrhagic diathesis following irradiation considerable importance is attached to changes in the megakaryocyte-platelet system.

Until recently the efforts of investigators were concentrated principally on studying the number, morphology, and thromboplastin activity of the blood platelets in acute radiation sickness [6-8, 11]. Only a few scattered references are found in the literature to changes in physiological properties of the platelets, and in particular, to their adhesiveness in acute radiation sickness [4, 14]. The ability of the platelets to aggregate and the role of plasma factors in the mechanism of adhesiveness and aggregation of the platelets have not been studied.

The object of the present investigation was to study the physiological properties of the platelets in acute radiation sickness and to examine some aspects of the mechanism of disturbance of their physiological function.

EXPERIMENTAL METHOD

Experiments were carried out on 330 sexually mature Wistar rats of both sexes, weighing 140-350 g. In the experiments of series I (229 rats) the number, morphology, adhesiveness, and aggregation of the platelets, the bleeding time and volume of blood lost, and the activity of factor XIII were determined for healthy and irradiated rats; in the experiments of series II (101 rats) the effects of serum and platelet-free plasma from healthy animals on adhesiveness and aggregation of rat platelets were investigated at the height of acute radiation sickness (7th and 15th days).

Acute radiation sickness was produced by irradiating the animals with  $\text{Co}^{60}$   $\gamma$ -rays on the Gammacell 220 apparatus in a dose of 600 R (dose rate 77 R/sec). The animals were irradiated in containers constructed from organic glass. Tests were carried out 6 h and 1, 2, 3, 7, 15, and 30 days after irradiation. The severity of the radiation sickness was judged from the clinical picture, changes in the leukocyte count in the circulating blood, and pathomorphological changes.

The platelet count [10] was determined using the diluting fluid of Fisher and Germer [5], and the differential platelet count [2], adhesiveness [13, 16] of the platelets (by a slightly modified method), and ag-

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TABLE 1. Changes in Number, Morphology, and Physiological Functions of Platelets of Rats with Acute Radiation Sickness ( $M \pm t$ )

Index	Healthy	After irradiation						
		6 h	day					30-th
			1-st	2-nd	3-rd	7-th	15-th	
Number of platelets (in thousands/mm <sup>3</sup> )	880±50	720±40 <sup>1</sup>	690±30 <sup>1</sup>	750±30 <sup>1</sup>	620±50 <sup>1</sup>	170±20 <sup>1</sup>	460±50 <sup>1</sup>	900±30
Differential platelet count (in %):	4,05±0,56	1,67±0,50 <sup>1</sup>	0,80±0,34 <sup>1</sup>	0,45±0,07 <sup>1</sup>	0,62±0,08 <sup>1</sup>	0,27±0,05 <sup>1</sup>	2,25±0,47 <sup>1</sup>	5,05±0,75
Young			78,0±2,3 <sup>1</sup>	75,5±1,8 <sup>1</sup>	70,9±2,6 <sup>1</sup>	41,4±4,1 <sup>1</sup>	53,0±3,3 <sup>1</sup>	79,9±1,8 <sup>1</sup>
Mature			11,1±1,6 <sup>1</sup>	10,4±1,0 <sup>1</sup>	12,8±1,3 <sup>1</sup>	24,1±3,4 <sup>1</sup>	17,5±2,5 <sup>1</sup>	10,5±1,1 <sup>1</sup>
Old			2,5±0,4 <sup>1</sup>	6,8±0,7 <sup>1</sup>	7,5±0,8 <sup>1</sup>	17,0±1,2 <sup>1</sup>	15,0±1,8 <sup>1</sup>	1,2±0,4 <sup>1</sup>
Degenerative			8,1±1,5 <sup>1</sup>	6,8±1,0 <sup>1</sup>	9,5±1,7 <sup>1</sup>	17,2±2,5 <sup>1</sup>	12,3±1,3 <sup>1</sup>	3,4±0,5
Irritation			—	—	254,0±18,6 <sup>1</sup>	270,0±12,9 <sup>1</sup>	300,0±75,6 <sup>1</sup>	—
Bleeding time (in sec)	3,8±0,5	—	—	—	208±61	333±45 <sup>1</sup>	328±76 <sup>1</sup>	—
Volume of blood lost ( $\times 10^{-4}$ ml)	200,0±5,5	—	—	—	10±2 <sup>1</sup>	3±1 <sup>1</sup>	13±3 <sup>1</sup>	20±1
Adhesiveness (in %)	116±25	5±2 <sup>1</sup>	10±2 <sup>1</sup>	13±2 <sup>1</sup>	—	88	91	118
Aggregation (in %):	22±1	—	—	—	—	57	68	72
Time of beginning of aggregation	100	—	176	—	55	65	69	—
Decrease in optical density of plasma	100	—	72	—	—	—	—	—
Fibrinase activity (in %)	100	—	—	—	—	—	—	—

\* Here and in Table 2, significance of the difference is taken relative to data obtained by investigation of healthy rats ( $P < 0.05$ ).

gregation of the platelets under the influence of ADP [9] were determined. ADP ("Reanal," Hungary) was used in a final concentration of 0.2 mg/ml. Observations on platelet aggregation were continued for 12 min. Plasma, rich in and free from platelets, was obtained by differential centrifugation of blood at 120 and 15,000 g respectively for 15 min. The bleeding time [2] was measured, the volume of blood lost was calculated from the hemoglobin concentration in blood escaping from an incision, and the activity of factor XIII [1] was determined. To obtain serum, whole blood was incubated at 37° for 3 h.

To study the mechanism of disturbance of the adhesiveness and aggregation of platelets, 1 ml blood or 1.5 ml platelet-rich plasma from irradiated animals was treated with 0.2 ml serum or platelet-free plasma of healthy animals. The effect of platelet-free plasma from healthy animals on the physiological functions of platelets from irradiated rats was also determined from changes in the adhesiveness of the platelets, the bleeding time, and the volume of blood lost by irradiated rats after intravenous injection of 1 ml platelet-free plasma from healthy rats stabilized with 3 units heparin. The investigations were carried out 40 min later. In control tests 1 ml 0.85% NaCl solution containing 3 units heparin was injected.

## EXPERIMENTAL RESULTS

All the experimental animals developed moderately severe radiation sickness ( $LD_{9/30}$ ). Post-mortem examination of the animals which died revealed congestion of the organs and hemorrhages of varied severity into the subcutaneous areolar tissue, muscles of the fore- and hind limbs, lungs, visceral and parietal pleura, mesentery, and mucous membranes of the gastro-intestinal tract.

Similar changes were found in rats sacrificed on the 7th day of radiation sickness. On the 15th day of radiation sickness the changes were less marked: hemorrhages were found principally in the lungs.

The results obtained show that ionizing radiation causes quantitative and qualitative changes and changes in function of the blood platelets during the first few days after irradiation (Table 1).

The most marked changes were observed at the height of acute radiation sickness. On the 7th day the number of platelets fell to 20% of the initial level and the differential count revealed a sixfold increase in the number of old and irritation forms the young forms had almost completely disappeared, and the number of mature forms was reduced by half; degenerative forms of platelets appeared (mean number 17%).

TABLE 2. Changes in Physiological Properties of Platelets from Irradiated Rats after Intravenous Injection of Platelet-Free Plasma from Healthy Animals ( $M \pm t$ )

Index	0.85% NaCl solution		Platelet-free plasma	
	before in- jection	after in- jection	before in- jection	after in- jection
Bleeding time (in min)	5,5	6,2	6,4	5,4
Volume of blood lost ( $\times 10^{-4}$ ml)	308,0	350,0	307,0	185,0 <sup>1</sup>
Adhesiveness (in %)	7,6 $\pm$ 6,9		36,5 $\pm$ 8,6 <sup>1</sup>	

\*See Table 1.

Besides quantitative and morphological changes in the irradiated animals, disturbances of platelet function were observed. The number of adhesive forms was reduced by 75% 6 h after irradiation compared with healthy animals. In the next 3 days a small increase in the number of adhesive forms of platelets was observed, but on the 7th day their number fell again to 13.5% of its initial value. The time of onset of platelet aggregation was lengthened 24 h after irradiation on the average by 19 sec, and the decrease in optical density of the plasma was reduced by 28%; at the height of acute radiation sickness (7th day) the time of onset of aggregation was shortened by 12% and the decrease in optical density of the plasma was 43% higher than in healthy animals. In 3 of 14 rats on the 7th day and 5 of 7 rats on the 15th day of radiation sickness, the two successive phases of aggregation and deaggregation were interchanged. In the healthy animals, very slight deaggregation of platelets aggregated under the influence of ADP was observed in only one case. The bleeding time on the 7th day of the sickness was increased by 35% and the volume of blood lost was increased by 2.5 times compared with healthy animals. Complete restoration of the platelet count was observed 30 days after irradiation, but changes in the differential platelet count still persisted: an increase in the proportion of old forms of platelets and the presence of degenerative forms; the physiological properties of the platelets were at a lower level than in healthy animals.

Besides changes in the platelets of the irradiated rats, a marked decrease in fibrinase activity was also observed.

To explain the mechanism of disturbance of the physiological functions of the platelets in rats with acute radiation sickness, the experiments of series II were carried out. These showed that after addition of platelet-free plasma or serum from healthy rats in vitro to blood or platelet-rich plasma from irradiated animals, the adhesiveness of the platelets was increased from 6.6 to 20.8%, and their ability to aggregate was improved. The addition of physiological saline produced no such changes. After intravenous injection of platelet-free plasma from healthy animals into irradiated rats, the adhesiveness of the platelets of the irradiated rats was increased by 5.5 times. Meanwhile the bleeding time was shortened by 1 min and the volume of blood lost reduced almost by half (Table 2). Intravenous injection of physiological saline, on the other hand, led to a slight depression of physiological function of the platelets.

The mechanism of disturbance of the physiological functions of the platelets, of their adhesiveness and ability to aggregate, in acute radiation sickness remains unexplained. It has been suggested [14] that the decreased adhesiveness of the platelets in acute radiation sickness is associated with the appearance of an inhibitor in the blood.

Various investigations have shown that the blood of healthy animals and man contains an adhesiveness and aggregation factor [12, 15]. The nature of this factor is not known. It was shown previously that there is a definite relationship between the changes in the activity of factor XIII and the adhesiveness and aggregating power of the platelets: the higher the activity of factor XIII the higher the adhesiveness and aggregation power of the platelets, and conversely, with a decrease in activity of factor XIII, the adhesiveness and aggregation power of the platelets falls. On the basis of these observations it was postulated that besides other factors, factor XIII is concerned in the adhesiveness and aggregation of platelets [3].

A direct relationship between changes in the activity of factor XIII and platelet function was also observed in the course of the present investigation.

The results obtained thus suggest that disturbance of the adhesive and aggregation properties of platelets in acute radiation sickness are due principally to a decrease in the activity of factor XIII, rather than to changes in the morphology of the platelets.

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