THROMBOPLASTIC ACTIVITY OF THE DIFFERENT LAYERS OF THE VESSEL WALL IN RADIATION SICKNESS

I. A. Andrushko

UDC 617-001.28-07:[616.151.5-02:616.1-07

The hemorrhagic syndrome in acute radiation sickness is one of the principal causes of death of animals injured by ionizing radiation.

The development of this syndrome depends on changes in the permeability of the blood vessels and disturbances of the blood clotting system. Many investigations have been made of the changes in the clotting power of the blood in radiation injuries. Slowing of blood clotting has been shown to be caused by moderate hypoprothombinemia and thrombocytopenia and a considerable fall in the thromboplastic activity of the blood [1-5, 7]. The last of these may also be detected in some cases before the development of an appreciable thrombocytopenia [1, 5]. Meanwhile, the hemostatic function of the vessel wall itself in radiation sickness has not been studied. The vessel wall is known to contain several tissue compounds influencing the clotting of the blood. One of these substances is the tissue thromboplastic factor [6, 9, 12, 14, 15].

The author's previous investigations [1] showed that in radiation sickness the increase in the clotting power of the blood, occurring as an adaptive response to acute blood loss, does not take place. In normal conditions the development of hypercoagulemia after acute blood loss, resulting in arrest of the bleeding, is due to the tissue thromboplastin of the vessel wall [4]. The development of the hemorrhagic syndrome in radiation sickness may possibly be due not only to changes in the clotting system of the blood itself, but also to disturbances in the tissue factors of hemocoagulation.

The object of the present investigation was to study the thromboplastic activity of the various layers of the vessel wall and also the relationship between the tissue and blood factors of blood clotting in the radiation hemorrhagic syndrome.

EXPERIMENTAL METHOD

Experiments were carried out on 40 rabbits of both sexes weighing from 2500 to 4500 g in a fasting state and without anesthesia. Whole-body irradiation was given with the RUM-11 apparatus, working with a voltage of 190 kV, current 10 mA, dose rate 9.5 R/min, filters 0.5 mm copper and 1 mm aluminum, and a skin-focus distance of 60 cm. The exposure dose of x-ray irradiation was 1200 R. After irradiation the animals developed a severe degree of acute radiation sickness. About 60% of the rabbits taken in the experiment died during the period of two weeks before the experiment began.

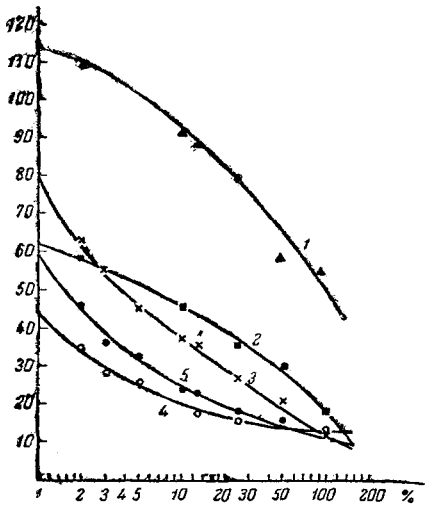
The blood clotting time of the animals was measured in Bazaron's apparatus [3] with automatic thermoregulation [4], the platelets and erythrocytes were counted directly in the phase-contrast microscope, the thromboplastic activity of the blood was determined by the method of Ulitina and Kudryashov [8], the capillary resistance by Borbeli's method, as described by G. G. Bazaz'yan [2], and the prothrombin time was estimated by Tugolukov's method. The thromboplastic activity of the vessel wall (the intima and the media with the adventitia), the brain, spleen, and bone marrow was investigated by Perlick's method [15].

The tissues for investigation were removed and immediately ground in a porcelain mortar with an equal of quartz sand and 0.85% sodium chloride solution (1 ml solution for each 0.2 g tissue) to give a homogeneous mince. This mixture was allowed to stand in the refrigerator at -24°. The tissue extracts from all the rabbits, both control and experimental, were collected in the refrigerator for the simultaneous determination of their thromboplastic activity. After a few days the thawed tissue mixture was centrifuged

Department of Biochemistry and Physiological Division, Central Research Laboratory, Kazan' Medical Institute (Presented by Active Member of the Academy of Medical Sciences of the USSR, S. R. Mardashev).
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 63, No. 5, pp. 29-32, May, 1967. Original article submitted March 24, 1965.

Effect of Irradiation of Rabbits on Thromboplastic Activity

Index	Mean		±S		±$S_{\bar{x}}$		P
	control	Irradiated	control	Irradiated	control	Irradiated	
Clotting time (in sec)	1330,7	3411,2	431,5	745,5	130,1	224,7	<0,001
Thromboplastic activity of blood (in sec)	44,52	78,83	7,114	12,98	2,145	3,913	<0,001
Platelets (per mm^3)	305 000	182 000	44 741	30 166	13 484	9 095	<0,001
Erythrocytes (per mm^3)	8 153 636	6 857 727	759 031	727 639	228 856	219 399	<0,001
Capillary resistance (in sec)	4 110,5	2345.8	545,9	738,7	164,6	222,7	<0,001
Prothrombin (in sec)	16,50	18,74	2,465	3,114	0,22	0,93	>0,1
Thromboplastic activity (in sec)							
Of intima	10,79	15,17	2,352	4,550	0,726	1,372	<0,02
Of media with adventitia	12,45	13,85	2,612	2,756	0,787	0,831	>0,5
Of brain	11,50	12,40	1,233	1,429	0,371	0,430	>0,1
Of bone marrow	65,47	100,10	15,11	40,60	4,554	12,23	<0,02
Of spleen	20,21	13,52	9,03	3,961	2,722	1,194	<0,05



Relationship between clotting time of control plasma and concentration of tissue extract. Ordinate—clotting time (in sec); abscissa—concentration of extracts (in %, logarithmic scale); 1) bone marrow; 2) spleen; 3) brain; 4) media with adventitia; 5) intima.

for 15 min at 3000 rpm. The tissue extract was withdrawn and its pH adjusted to 7.4-7.5 by the addition of 0.014N NaOH solutuon or 0.5% acetic acetic acid. The tissue thromboplastic activity was determined on a water bath at 37°. The test sample contained 0.1 ml of tissue extract, 0.1 ml of normal plasma, and 0.1 ml of 0.025 M calcium chloride solution.

All the investigations on the control and experimental animals were carried out before and two weeks after irradiation. Blood for investigation was taken before irradiation from the marginal vein of the ear, and after irradiation from the femoral artery with silicone-treated cannulas.

The experimental results were analyzed by statistical methods [11].

EXPERIMENTAL RESULTS

The normal values of the indices investigated, as determined in unirradiated rabbits, are given in the Table.

In the 11 surviving rabbits 14 days after irradiation (altogether 28 animals were used in the experiment) the thromboplastic activity of the blood and the platelet count showed a marked dedrease (see Table). By comparison with the normal animals, their blood clotting was slowed and their capillary resistance was reduced by 57%. The prothrombin activity was only slightly lowered in agreement with observations by other authors [5].

Determination of the thromboplastic activity of the different tissues showed that in all cases the thromboplastic activity of the intima of the aorta and of the bone marrow was lowered. The thromboplastic activity of the media and adventitia of the aorta, like that of the brain, showed only very slight changes. Only in the spleen was the thromboplastic activity increased after irradiation.

To express the thromboplastic activity of the samples of the vessel wall and the other tissues of the irradiated animals as a percentage of the thromboplastic activity of the tissues from normal animals, standard dilution curves of the extracts were plotted (see figure).

It was thus shown that during development of the hemorrhagic syndrome the thromboplastic activity of the intima fell by approximately 59% and that of the bone marrow by approximately 94%. Since the increase in thromboplastic activity of the spleen exceeded the limits of normal maximal activity of its tissue, it was expressed as a percentage of the activity of the marrow thromoboplastin, as has been done by other workers [9, 10, 14]. An increase from 46 to 88%, i.e., approximately twofold, was observed.

A noteworthy feature was the marked decrease in the thromboplastic activity of the intima and bone marrow. Whereas the decrease in the thromboplastic activity of the marrow was most likely to be the result of disturbance of platelet formation in the marrow, its decrease in the intima may be ascribed both to the disturbance of thromboplastin formation in the endothelial cells and to a decrease in the supply of thromboplastin to its cells from the blood. The decrease in the thromboplastic activity of the blood may thus be either the cause or the effect of the decrease in the thromboplastic activity of the endothelial cells. Following the investigations of Johnson and co-workers [13], the first suggestion seems more probable. Evidently the platelets are assimilated by the vessel wall and maintain the physiological clevel of permeability.

Regardless of its mechanism, the observed decrease in the thromboplastic activity of the intima is an important factor contributing to the development of the hemorrhagic syndrome, for when the blood vessel is injured, the local factors of hemostasis are not put into operation as they should be.

In the author's opinion, the increase in the thromboplastic activity of the spleen extract is the result of the intensified destruction of the blood cells in this organ after irradiation.

LITERATURE CITED

1. I. A. Andrushko, and D. M. Zubairov, in the book: Scientific Publications of Kazan' Medical Institute [in Russian], Vol. 14, p. 75, Kazan' (1964).
2. G. G. Bazaz'yan, Vestn. Moskovsk. Univ. Seriya 6. Biologiya, Pochvovedenie, No. 1, 3 (1960).
3. S. Ts. Bazaron, Sov. Med., No. 3, 36 (1954).
4. D. M. Zubairov, Physiological and Pathophysiological Mechanisms of Regulation of the Clotting Power of the Blood, Author's abstract of doctorate dissertation,Kazan' (1964).
5. B. A. Kudryashov, G. V. Andreenko, P. D. Ulitina, et al., Probl. Gematol., No. 6, 3 (1957).
6. B. I. Kuznik and V. V. Al'fonsov, Probl. Gematol., No. 8, 8 (1964).
7. A. S. Petrova, Probl. Gematol., No. 3, 14 (1959).
8. P. D. Ulitina and B. A. Kudryashov, Lab. Delo, No. 6, 7 (1958).
9. T. Astrup and K. Buluk, Circulat. Res., 13, 253 (1963).
10. L. Donner, J. Atheroscler. Res., 2, 88 (1962).
11. R. A. Fisher, Statistical Methods for Research Workers [Russian translation], Moscow (1958).
12. C. E. Huggins, Surgery, 48, 724 (1960).
13. S. A. Johnson, R. S. Balboa, B. H. Dessel, et al., Exp. Molec. Path., 3, 115 (1964).
14. J. E. Kirk, Soc. Exp. Biol. (N. Y.), 109, 890 (1962).
15. E. B. Perlick, in the book: R. Emmrich, E. Perlick (Hrsg.), Gefässwand und Blutplasma, Jena, (1961), p. 211.