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Increasing Stimulation of Megakaryocytopoiesis with Decreasing Platelet Count (40590)[1]

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It has been shown in rats that the rate and character of platelet recovery (platelet production) after induction of acute thrombocytopenia is dependent on the degree of thrombocytopenia induced (1). After relatively moderate thrombocytopenia, when platelet counts were 44–72% of pretreatment level, the rate of increase of platelets in the peripheral blood was approximately constant for 4 to 5 days but varied among groups, depending on the severity of induced thrombocytopenia. Platelets increased in the blood at a rate of about 6%/day when the count was reduced to 72% of pretreatment values and about 16%/day when it was reduced to 44%. In contrast, when thrombocytopenia was marked, with platelet counts of about 10–40% of pretreatment values, platelet recovery was biphasic, having an initial slow rate (<21%/day) for approximately the first 36 hr after induction of thrombocytopenia followed by a more rapid rate (up to 43%/day). These differences in rate of platelet production among animals exposed to different degrees of thrombocytopenia suggested differences in degree of stimulation of megakaryocytopoiesis.

Our experiments were undertaken to investigate and quantify alterations in megakaryocytopoiesis after induction of moderate to marked thrombocytopenia. Our results demonstrate a direct relationship between severity of acute thrombocytopenia and the percentage of the megakaryocyte population engaged in endomitosis, the latter being an established indicator of megakaryocytopoietic activity.

Materials and methods. Sprague–Dawley-derived rats, averaging 372 g in body wt, were exchanged transfused with platelet poor blood. Platelet counts were taken at 1- and 31-hr intervals following transfusion, and marrow samples were taken at 31 hr for determination of the endomitotic index (M_EI) of megakaryocytes. The volume of blood exchanged ranged from 3.5 to 35 ml, or about 0.17–1.7 times a rat's blood volume. This procedure reduced the platelet count to 72–32% of the pretreatment count. The platelet counts were determined by the phase microscopy method. The average pretreatment count of 62 recipients was 1.07×10^6 platelets/mm^3.

Platelet-poor blood was prepared by collecting blood from normal rats and centrifuging it to remove platelets. Approximately 15 ml of blood was collected from the dorsal aorta into 20-ml plastic syringes containing 1.5 ml of 1% EDTA in 0.7% saline. Four-milliliter aliquots were centrifuged in 12-ml plastic conical tubes at about 100*g* for 40 min (650 rpm, IEC refrigerated centrifuge, using No. 253 head). The platelet rich plasma was then pooled in 50-ml plastic tubes for further centrifugation at 2000*g* (3500 rpm, Servall RC-5 refrigerated centrifuge, Rotor SS-34) for 2 hr. The platelet-poor plasma was pipetted off and stored overnight in the refrigerator for mixing with red blood cells the next day. Red blood cells were washed two times with equal volumes of saline, pelleted for 30 min at about 360*g*, combined, and stored in a refrigerator overnight. The following day a hematocrit of the red blood cells was determined, and platelet-poor plasma was added in a volume necessary to reach a normal hematocrit. The platelet count of this platelet poor blood averaged approximately 7% of normal.

Exchange transfusions were performed on ether-anesthetized rats via surgically exposed jugular veins. Seven milliliters of blood was removed at one time with a 10-ml syringe containing 1 ml of 1% EDTA (except for the 3.5-ml exchange transfusions) and an equal

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amount of platelet-poor blood was replaced each time, again using a 10-ml syringe. This procedure was repeated until the desired amount of blood was exchanged. A 1-ml injection of a 0.027 *M* solution of $CaCl_2$ was given to each transfused rat i.p. in order to bind any residual EDTA from the injected platelet-poor blood.

Control rats were either sham injected or given a 0.25-ml injection of antiplatelet serum (APS) i.v. Sham-injected rats were anesthetized and their jugular veins surgically exposed and a needle was inserted, but without the removal or transfusion of blood. The rabbit antiserum against rat platelets was prepared as previously described (2).

Rats were killed ~31 hr following the time of exchange transfusion, their femurs collected, fixed in Zenkerformol, and 5-μm longitudinal sections cut and stained with hematoxylin and eosin. The 31-hr sacrifice time was chosen since the M_EI of megakaryocytes has been found to reach a peak value at that time (3). The M_EI (percentage of the megakaryocyte population in endomitosis) was determined by examining two longitudinal sections per rat. Only polyploid megakaryocytes that are readily recognized by the size of the cells and nuclei were included. Smaller diploid and tetraploid megakaryocytes were not included since they cannot readily be distinguished from the blast cells of other hemopoietic cell lines by usual hematologic staining methods. The number of megakaryocytes counted per rat was 1555 to 3720.

Results. The platelet counts at 1 and 31 hr after exchange transfusion with platelet-poor blood or after injection of 0.25 ml of APS are shown in Table I. The reductions in platelet counts approximate those observed in earlier experiments using the same method. Variation from a stepwise reduction in counts is likely due in large part to errors inherent in making platelet counts, but it may also be related to small differences in the residual platelet counts of different batches of the transfused, platelet-poor blood.

The effect on the M_EI of megakaryocytes produced by acute reduction of the platelet count by exchange transfusion with platelet-poor blood is shown in Fig. 1. Depression of the platelet count to 72% of normal circulating level had no discernible effect on the index. However, as the platelet count was further reduced, the index gradually increased until it approached three times that of controls in animals having platelet counts about one-third of normal. In rats whose platelet counts were reduced to a few per-

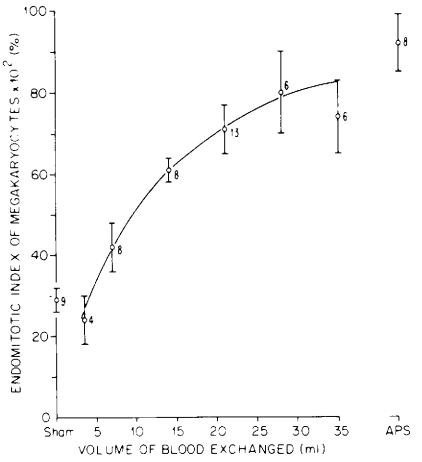

FIG. 1. Effect of exchange transfusion of platelet-poor blood on the M_EI of megakaryocytes. Bars represent the standard errors. Numbers indicate number of rats in each treatment group.

TABLE I. PLATELET COUNTS AFTER EXCHANGE TRANSFUSION WITH PLATELET-POOR BLOOD OR INJECTION OF ANTIPLATELET SERUM[a]

	Percentage of pretreatment counts							
		Platelet-poor blood						
Time (hr)	Sham (9)[a]	3.5 ml (4)	7 ml (8)	14 ml (8)	21 ml (12)	28 ml (6)	35 ml (6)	APS (8)
1	104 ± 3	72 ± 6	69 ± 3	42 ± 7	45 ± 3	32 ± 2	32 ± 2	6 ± 1
31	97 ± 3	82 ± 15	80 ± 3	60 ± 6	62 ± 5	49 ± 2	46 ± 5	20 ± 4
1[b]	99	74	65	51	39	31	24	

[a] Values are means ± standard error. The numbers in parentheses are number of rats in each treatment group, except last row.

[b] Approximate expected 1-hr count based on combined results of this and earlier experiments.

TABLE II. COMPARISONS OF MEANS OF ENDOMITOTIC INDICES (PERCENTAGE) USING STUDENT'S *t* TEST

		Platelet-poor blood						
	Sham	3.5 ml	7 ml	14 ml	21 ml	28 ml	35 ml	APS
Mean	0.29	0.24	0.42	0.61	0.71	0.80	0.74	0.92
		Comparisons with sham						
df[a]		11	15	15	20	13	13	15
P		<0.50	<0.10	<0.001	<0.001	<0.001	<0.001	<0.001
		Comparisons with APS						
df		10	14	14	19	12	12	
P		<0.001	<0.001	<0.005	<0.05	<0.40	<0.20	

[a] Degrees of freedom.

centage of normal by injection of APS, the index was somewhat greater than in the rats exchange transfused with the larger volumes of platelet-poor blood. Table II compares the means of the various exchange-transfused groups with the sham-control group and with the APS group, using Student's *t* test. The results show that the index was significantly ($P < 0.001$) greater than in shams after exchange of 14 ml or more of platelet-poor blood, which lowered the platelet count to less than 50% of the pretreatment count. Conversely, groups with exchange transfusions of 21 ml or less differed significantly from the APS group.

Discussion. Our results show that the severity of induced, acute thrombocytopenia determined the magnitude of stimulation of megakaryocytopoiesis, as indicated by the progressive changes in the M_EI of megakaryocytes. Although the M_EI was not discernibly changed after an approximate 25% reduction in peripheral platelet count, it appeared to be greater than normal when the platelet count was reduced by 30–35% and was significantly ($P > 0.001$) when the count was reduced to 40–50% of pretreatment value or less. The increasing M_EI of megakaryocytes with decreasing peripheral platelet count corresponds to the increasing rates of platelet production observed when platelet counts were similarly reduced (1).

These findings are consistent with the concept of a humoral regulator of thrombocytopoiesis (4), and they satisfy the general principle that the amount of regulator produced and/or released for action on the target tissue is directly related to the level of product, in this case the circulating platelet population.

The results indicate that a dose–response relationship between the amount or potency of humoral agent and the magnitude of stimulation of megakaryocytopoiesis and platelet production can be expected when assaying materials thought to have thrombocytopoiesis-stimulating activity. They also indicate the limits of change of the M_EI in its response to acute stimulation. These findings will be useful in the development of methods for assaying humoral agents that regulate megakaryocytopoiesis and in understanding dose–response relationships of such regulators.

Summary. Rats were exchange transfused with platelet-poor blood to produce varying degrees of thrombocytopenia. Marrow samples were taken 31 hr later and the megakaryocyte population was scored for the percentage in endomitosis. The M_EI progressively increased as the circulating platelet count was reduced. These findings are consistent with the concept of a humoral regulator of thrombocytopoiesis, the activity of which is related to the severity of depression of circulating platelets.

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