Stimulation of Platelet Production by Serum of Platelet-Depleted Rats. (26958)

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Investigations into the effects of platelet depletion on peripheral platelet counts and on megakaryocytes of the bone marrow (1-4) have demonstrated that such thrombopenic measures bring about, after a delay of a day or two, an increased rate of entry of platelets into the peripheral circulation, platelet numbers rising to values more than 1½ times normal levels. Both the delay in response and the changes seen in the megakaryocytic series have indicated that the response is caused by an increased production of platelets rather than by a quick release from a pool. Thus, it appears that a subnormal level of platelets in the circulation triggers an increased production of these blood elements. It has also been reported that normal human serum injected into a splenectomized thrombopenic patient (5), and extracts of normal human serum injected serially into rabbits (6) produced an increase in number of circulating platelets, presumably by stimulating platelet production. An increase in production might result either by the action of a stimulating agent or by removal of an inhibitor. If a stimulating agent were responsible, it should be present in greater-than-normal amounts in the blood of platelet-depleted individuals.

Therefore, we have assayed in untreated and in splenectomized rats serum obtained from platelet-depleted rats, using peripheral platelet counts as the measure of effect. We observed increases in platelet counts to about 150% of control values 5 days after serum injection.

Materials and methods. We used male Sprague-Dawley rats for serum donors and for assay animals; the latter weighed 350 to 450 g. Reduction in number of platelets of serum donors was accomplished in either of 2 ways. One method was to remove by cardiac puncture 40% of the blood volume (taken as 5% of body weight) at about 9 a.m. and another 25% at about 2 p.m. After each withdrawal, a volume of Tyrode's solution equal to half the amount of blood removed was injected intraperitoneally. By this method, as much as 50% of the circulating platelet mass was removed (4). A second method of platelet depletion was to inject rat-platelet antiserum that was made in rabbits. Usually 2.5 ml of a 5% suspension of rat platelets were injected intravenously into rabbits twice a week for 3 weeks, and serum was collected about 10 days after the last injection. In some instances, platelets were mixed with Freund's reagent and injected into footpads and axillary and inguinal regions. Similar titers against rat platelets were obtained by both methods. Serum collected from the platelet-injected rabbits was heated at 56° C for 45 minutes and then absorbed 3 times with an equal volume of homologous rat erythrocytes to remove anti-rat antibodies not specific to rat platelets. Red cells were prepared for this purpose by defibrinating normal rat blood to remove platelets (4). Intraperitoneal injection of 0.25 ml of this antiserum reduced platelet counts in most rats to less than 10% of normal values.

Approximately 24 hours after the initial bleeding or after injection of rat-platelet antiserum, the rats were anesthetized with ether and bled from the abdominal aorta into siliconized syringes. The yield was 3 to 4% of body weight collected in about 2 minutes. This blood was expressed into 15-ml centrifuge tubes, allowed to clot, then centrifuged. The serum was pipetted to

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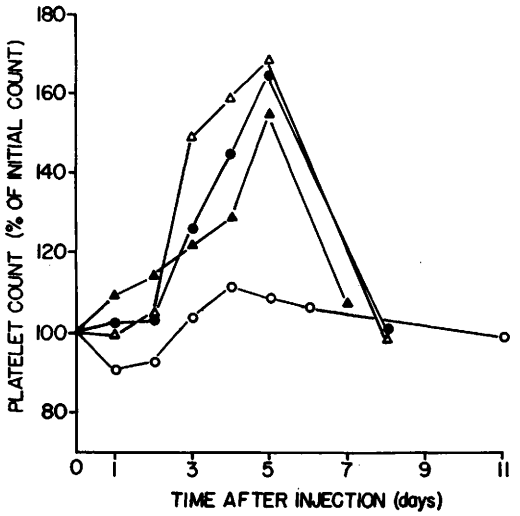

FIG. 1. Peripheral platelet counts of rats inj. with serum of bled donors. Counts were plotted as a percent of the initial count of each rat (Initial Count = 100%). *Active serum*—△, 4 × 12 ml (N = 2); ●, 4 × 6 ml (N = 2); ▲, 4 × 3 ml (N = 4). *Control serum*—○, 4 × 6 ml (N = 5).

other tubes and frozen for storage. Serum recipients were injected twice daily, morning and afternoon, for 2 days. Serum was injected subcutaneously in amounts of 1 to 12 ml per injection, or a total of 4 to 48 ml per recipient.

Peripheral platelet counts were taken before the initial injection and at intervals thereafter. In some experiments counts were taken daily and in others only at 5 days after initial injection. Blood samples, taken in a red cell pipette from a puncture in the lateral vein of the hind limb, were diluted with ammonium oxalate. Counts were made by the direct phase microscope method of Brecher and Cronkite (7). In some experiments, total white blood cell counts were also made before the initial injection of serum and again 5 days later.

Results. Injection of serum from platelet-depleted rats caused increases in peripheral platelet counts as high as 167% of the initial counts (Fig. 1). No appreciable change took place during the first 48 hours after the injections (Table I). The increase was rapid, however, between 48 and 120 hours. The peak count occurred at 5 days, and by the 7th or 8th day platelet numbers had returned to normal levels. Equal amounts of serum from untreated donors did not produce such an effect.

The results (Fig. 1) suggest that the 4 × 6-ml dose (4 injections of 6 ml each) produced a greater response than the 4 × 3-ml dose. Additional groups of rats were injected with these 2 doses of active serum, obtained either from bled donors or from donors injected with rat-platelet antiserum, and the 5-day gains in numbers of platelets were determined (Table II). The data were examined using the covariance analysis method, with gain as the variable and initial platelet count as the covariable; the level of rejection was taken as 5%. Responses to the 2 dose levels were significantly different. However, at each dose level it made no difference whether the active serum was obtained from bled or from antiserum-injected donors.

TABLE I. Early and 5-Day Platelet Response to Serum of Bled Rats (4 × 6 ml).*

Hour	No. of rats	% of initial count	Platelet count range (× 10^{-3}/mm^3)
0	6	100	730-1085
1	6	92	620-1120
8	6	105	745-1240
18	3	102	990-1055
24	3	89	705- 895
36	3	94	870- 970
48	3	97	780- 945
120	6	158	1230-1770

* Platelet counts were taken on all recipients twice before treatment (at −24 and 0 hr) and at 1, 8, and 120 hr after treatment. At other intervals, counts were taken on alternate halves of the total group, as indicated.

TABLE II. Gain in Platelet Count at 5 Days; 2 Doses and 2 Sources of Active Serum.

	Recipients		
Treatment of donors	Dose (ml)	No. of rats	Mean platelet gain (× 10^{-3}/mm^3)
Bleeding	4 × 3	16	348
"	4 × 6	31	473
Antiserum	4 × 3	7	209
"	4 × 6	11	427

TABLE III. Comparison of Platelet Response of 3 Untreated and of 3 Splenectomized Rats to Serum of Bled Rats (4 × 3 ml).*

	Splenectomized			Untreated		
	Platelet count ($\times 10^{-3}$/mm^3)			Platelet count ($\times 10^{-3}$/mm^3)		
Day	Mean	Range	% of initial count	Mean	Range	% of initial count
0	915	735-1050	100	1008	885-1105	100
5	1330	1255-1435	146	1343	1235-1475	133

* Platelet counts were made twice on both days, 0 and 5, and the 2 counts from each rat were averaged. Range includes all counts.

Splenectomized rats were also used as assay animals. The response to active serum may have been somewhat greater in splenectomized than in normal rats (Table III), but the data are not conclusive.

We also found that serum of bled rats that was kept frozen for 50 days or was lyophilized retained its platelet-stimulating activity.

White blood cell counts were not altered 5 days after injection of active serum that produced increases in platelet numbers.

Discussion. The results demonstrate that subcutaneous injection of large amounts of serum from platelet-depleted rats into normal or splenectomized assay animals produced a substantial increase in number of platelets in the recipients' peripheral circulation. The increase seems not to be the result of a release of platelets from a platelet pool, since it did not commence until about 2 days after we began the injections of active serum. Experiments on the effects of bleeding on megakaryocyte and platelet response in rats also indicated the absence of a rapidly mobilizable pool of platelets (3,4).

The increase in number of platelets in circulation to levels higher than normal between the 2nd and 5th days indicates that platelet input, and thus platelet production, was greater than normal during this period (or that survival time of platelets was temporarily lengthened). Thereafter, the rate of platelet production apparently declined since platelet numbers returned to normal levels on the 8th day. Since we have reported that platelet life span in Sprague-Dawley rats is about 4.5 days (8), the rapid drop suggests either that some of the platelets produced between the 2nd and 5th days had a shorter than normal survival time in the circulatory system, or that the rate of platelet production was temporarily reduced below normal between the 5th and 8th days. Cronkite (9) showed that transfusion of large numbers of platelets into rats caused peripheral platelet counts to fall to subnormal levels over a period of several days, presumably by inhibiting production.

The mechanism responsible for increased platelet production is not yet known, but we assume that one or more chemical agents play a mediating part. The increased production that occurs after removal of platelets from the circulation (3, 4) could result from removal of an inhibitor to production, possibly residing in or emanating from platelets (9). Theoretically, such a substance could either directly inhibit megakaryocytic cells from producing platelets or could inhibit elaboration of an agent that is stimulatory to megakaryocytic cells. The platelet increase seen after injection of active serum seems more readily attributable to the presence of a stimulatory agent in the donor serum. Thus there may be a balance in normal individuals between an inhibiting agent that derives from platelets and a stimulatory agent, the source of which is not presently known. This type of humoral balance occurs commonly, as, for example, between thyroid hormone and thyroid-stimulating hormone.

An alternative mechanism can be imag-

ined, namely that a diminution in number of platelets, rather than in concentration of an inhibitor deriving from platelets, brings about some functional imbalance that in turn is responsible for initiating elaboration of a stimulatory agent.

We believe that serum of platelet-depleted rats contains an agent that stimulates production of blood platelets. This agent might act on the megakaryocyte compartment of the bone marrow and spleen either by increasing the rate of maturation of megakaryocytes, or by increasing the rate of cell division among the earlier cells in the megakaryocytic series, or both. Examination of the effects of platelet depletion by bleeding on the total and differential counts of cells of the megakaryocytic series suggests that both processes may play a part (4).

The lack of response to similar quantities of serum from untreated donors shows that the observed platelet response is not due simply to injection of relatively large amounts of serum.

It is interesting that an increase to a level of about 150% of normal platelet numbers usually constituted the maximum response to injection of active serum. Perhaps the marrow is not capable of producing any greater short-term response, or an opposite regulatory action (platelet-derived inhibitor?) may come into play by the time these platelet levels have been reached.

Spector has been engaged in similar experiments with rabbits. The results (10), presented concurrently with ours on rats (11), demonstrated that serum from donor rabbits that had been depleted of platelets by specific antiserum or by treatment with Myleran produced an increase in platelets when injected intravenously into untreated or splenectomized rabbits.

Summary. Four subcutaneous injections, given during a 2-day period, of serum from platelet-depleted rats into untreated or splenectomized rats caused an increase in the number of circulating platelets to as much as 167% of the initial count. The rise in peripheral platelet count began 2 days after the first injection, reached a peak at 5 days, then declined to control values at 8 days. Equal amounts of serum from untreated donors did not produce this response. The results indicate that the active serum contains an agent (thrombopoietin) that stimulates production of blood platelets.

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