Effects of Degree of Thrombocytopenia on Thrombocytopoietic Response

By T. T. Odell, Jr., and J. R. Murphy

Rats were exchange transfused with platelet-poor blood to reduce the number of circulating platelets. When thrombocytopenia was moderate, the rate of platelet increase toward normal levels was relatively slow, whereas after severe thrombocytopenia an early slow rate of increase was followed by a rapid rate. It seems likely that the rapid platelet increase that begins about 36–48 hr after acute, severe thrombocytopenia is engendered by a ploidy shift among megakaryocytes and by an increased input of cells into the maturing population. The basis of the moderate increase in circulating platelets before 48 hr in severely thrombocytopenic rats and for about 5 days in moderately thrombocytopenic rats is less clear. Both peripheral changes in the age distribution of the platelet population and in platelet depots, as well as hemopoietic changes in the marrow, probably contribute.

It has been shown that the rate of platelet production is markedly increased after induction of severe thrombocytopenia by exchange transfusion with platelet-poor blood. Responses to moderate thrombocytopenia have, however, received little attention. Knowledge of such responses is important to an understanding of the homeostatic mechanisms that regulate the megakaryocyte–platelet system. The experiments reported here were undertaken to explore and compare the effects on platelet production of various degrees of platelet depression.

MATERIALS AND METHODS

Sprague-Dawley-derived rats, averaging 382 g in body weight, were exchange transfused with platelet-poor blood, and their platelet counts were taken at intervals for several days. The volume exchanged varied from 5 to 50 ml, or about 0.25–2.5 times a rat’s blood volume. This procedure reduced the platelet count to about 72%–10% of the pretreatment count.
Platelet-poor blood was prepared by collecting blood from normal rats and subjecting it to centrifugation procedures to remove platelets. Blood was collected from the abdominal aorta of normal rats into syringes containing 1.5–2 ml of 1% EDTA in 0.7% saline. The volume withdrawn from each donor was about 15–20 ml. Twelve-milliliter conical plastic centrifuge tubes were about half filled with this blood and centrifuged at about 100 g for 40 min (650 rpm in an IEC refrigerated centrifuge using head No. 253). The platelet-rich plasma was removed to a 50-ml centrifuge tube and spun at about 150 g (2400 rpm, head No. 253) for 2 hr. The platelet-poor plasma was saved for subsequent remixture with the red cells, and the platelet pellet was discarded. The red cells were washed four times with an equal volume of saline in 50-ml centrifuge tubes (250 g for 15 min). Red cells and plasma were then mixed in suitable volumes to approximate a normal hematocrit. The platelet count of the platelet-poor blood was reduced to a few per cent of normal platelet levels (often <1%). The platelet-poor blood was usually collected one day, held overnight in a refrigerator, and used for exchange transfusion the next day.

For exchange transfusion, rats were anesthetized with ether and maintained with a Metofane-dampened head cone. After shaving, the jugular veins were exposed through two cuts in the skin, and the overlying tissues were spread away from them. Blood was removed with a 10-ml syringe containing 2 ml of 1% EDTA. Usually 7–10 ml was removed at a time and was replaced by injecting an equal amount of platelet-poor blood with another 10-ml syringe. This procedure was repeated until the desired amount of blood had been exchanged. The time from beginning to completion of transfusion varied from about 5 min in the 5-ml cases to 45–60 min for 50 ml. After exchange the skin was closed with wound clips. Usually 0.1 ml of a 0.027 M solution of CaCl₂ was injected intraperitoneally to bind to any residual EDTA in the injected blood. The first post-transfusion platelet count was taken approximately 15 min after completion of the exchange.

Other rats were subjected to a sham procedure in which the animals were anesthetized, the jugulars exposed, and the vessels entered with a needle but without any removal or transfusion of blood.

Blood for platelet counts was taken from the saphenous vein with the Unopette system and counted by the phase microscope method. Counts were taken before exchange transfusion and at intervals thereafter. The average pretreatment platelet count was 0.984 x 10⁶ platelets/cu mm of blood.

Blood volume was assumed to be 5.5% of body weight, an estimate derived from our experiments involving blood cell transfusions and recovery in Sprague-Dawley rats, and from values in the literature.⁴

### RESULTS

**Platelet Reduction**

Reductions in platelet counts after exchange transfusion with platelet-poor blood approximated expectations (Table 1). Observed and calculated values were in good agreement after single exchanges of 5 and 10 ml. They tended to be more discrepant in rats subjected to successive withdrawal and reinfusion.

<table>
<thead>
<tr>
<th>Volume Exchanged (ml)</th>
<th>Platelet Count (% of pretreatment)</th>
<th>Number of Rats</th>
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<tr>
<td></td>
<td>Observed*</td>
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</tr>
<tr>
<td></td>
<td>Calculated</td>
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<td>Sham</td>
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<td>10</td>
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<td>8</td>
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</table>

*Count taken approximately 15 min after completion of blood exchange.
the observed reductions being less than the calculated ones. For calculation of the expected values, it was assumed that the remaining platelets were uniformly mixed in the circulating blood after each infusion of platelet-poor blood. It was also assumed that blood volume in these rats was 5.5% of body weight. Calculations were made individually for each rat and were based on the volume of each withdrawal. In ten rats exposed to sham exchange, the average platelet count was 97% of normal at 15 min and 104% at 3, 6, and 12 hr. It appears, therefore, that the sham operation did not alter the early platelet count. The standard deviation of 66 pretreatment platelet counts was 66,300 platelets/cu mm of blood or ±6.7%.

Posttransfusion platelet counts responded differently, depending on the degree of thrombocytopenia induced (Fig. 1). After exchanges of 5, 10, or 15 ml, the rates of platelet increase during the first 96 hr could be fitted to single regressions having slopes of 0.47–0.66 (Table 2). These exchanges reduced the platelet count, taken about 15 min after exchange, to 72%, 52%, and 44%, respectively, of the normal (pretreatment) counts. After exchanges of 30 and 50 ml, the data between 15 min and 96 hr were fitted to two regressions. In the 30 ml-group, the rate during the first 36 hr was similar to that in the 10 and 15 ml groups, and between 48 and 96 hr it was more than twice as great. The 50-ml

![Fig. 1. Average platelet counts at intervals after platelet depletion by exchange transfusion with platelet-poor blood, and regressions (Table 2). Platelet counts are presented as percentages of pretreatment counts. Symbols: ▼ denotes the average of ten sham-exchanged rats; ○ denotes rats exchanged with 5 ml, the number (N) of rats being ten when indicated or 14 otherwise; ◊, 15 ml, N = 6 or 4, 8 and 7 ml/exchange; ▲, 30 ml, N = 8 or 4, four exchanges of 7 or 8 ml each; ■, 50 ml, N = 4, seven exchanges.](image-url)
Table 2. Platelet Return After Depletion

<table>
<thead>
<tr>
<th>Volume* (ml)</th>
<th>Platelets of 15 min</th>
<th>Time Period (hr)</th>
<th>Population Increase (pLs/cu mm/day x 10^9)</th>
<th>Number of Counts</th>
<th>Number of Platelets</th>
<th>Standard Error</th>
<th>Standard Deviation</th>
<th>Standard Deviation of the Deviations from the Regression</th>
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<td>97</td>
<td>101</td>
<td>10</td>
<td>0.25-96</td>
<td>0.16</td>
<td>0.017</td>
<td>1.9</td>
<td>13</td>
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<td>74</td>
<td>14</td>
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<td>0.85</td>
<td>0.069</td>
<td>2.4</td>
<td>8</td>
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</tbody>
</table>

*Volume of platelet-poor blood exchanged.
†Slope equals the percentage of change of platelet count per hour.
§Standard deviation of the deviations from the regression.
¶The number of serial platelet counts on individual rats that determined the regression slopes.
| Population increase is the number of platelets added to the circulating population in 24 hr and can be obtained by multiplying the slope by 24 x 10^9.
|| Sham operation. |

The number of platelets added to the population beyond those needed to replace exiting platelets can be calculated from the rate slopes and can be used as one sort of comparison among animals with different degrees of thrombocytopenia (Table 2). Platelets added varied from 113,000 platelets/cu mm of blood per day after an exchange of 5 ml of platelet-poor blood to 430,000/cu mm per day when 50 ml were exchanged.

Peak Platelet Response

The time of maximum platelet count was estimated from the platelet curves (Figs. 2 and 3). The peak occurred between 120 and 130 hr after exchange transfusion, regardless of the degree of platelet depression.

The height of the platelet curve at its peak was related to the initial degree of platelet depression. In the 5-, 10-, and 15-ml groups (reduction of platelet count to 72%–44% of pretreatment count), the estimated peak was about 125%–130% of normal counts. In the 18–50-ml groups (reduction to 40%–10%), group behaved similarly except that both early and late rates were greater than in the 30-ml group.

Results of 18-ml exchange experiments suggested that the change from a single rate to two successive rates of platelet increase during the 96 hr after thrombocytopenia occurs when platelets are depleted to about 40% of the normal pretreatment count. Slopes resulting from fitting the data both to a single rate and to two differing successive rates are presented in Table 2.

Sham operation apparently induced a small increase above normal in the size of the platelet population, possibly beginning at about 48 hr (Fig. 1). There is a probability of less than 0.001 that the sham group belongs to a population that has a zero slope.

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Fig. 2. Average platelet counts at intervals after exchange transfusion with platelet-poor blood. Symbols: ○ denotes rats exchanged with 5 ml, the number (N) of rats being ten when indicated or 14 otherwise; ●, 10 ml, N = 6 or 4; ▲, 15 ml, N = 6 or 4. Lines from 15 min to 96 hr are the calculated regression lines; thereafter the lines are freehand.

Fig. 3. Average platelet counts at intervals after exchange transfusion with platelet-poor blood. Symbols: ▲ denotes rats exchanged with 18 ml, N = 13, 9, or 5; ● denotes rats exchanged with 30 ml, N = 8 or 4; △, 50 ml, N = 9 or 4. Lines from 15 min to 96 hr are calculated regression lines; thereafter the lines are freehand.
the highest point on the estimated curve was about 150%-180% of the pretreatment count. After sham exchange, platelets reached a peak of 118% of pretreatment counts at 5 days.

After reaching peak values the platelet counts declined toward normal levels by 9-10 days, although stabilizing possibly slightly above the pretreatment count. The elapsed time of 4-5 days is approximately one platelet life span.

DISCUSSION

The closeness of the observed (15 min) and calculated platelet counts after single exchange transfusions in the 5- and 10-ml groups supports the choice of 5.5% of body weight as a valid approximation of blood volume. These platelet count results suggest a blood volume between 5% and 5.5%. The higher observed than calculated platelet values after repeated exchanges may result from a lack of uniform mixing of the remaining platelets with the transfused platelet-poor blood between successive exchanges. It is also possible that severe or extended thrombocytopenia induces a release of platelets from a reserve pool. It has been reported that rats have a splenic platelet pool of about 12% of the total platelet mass.6

The results reported here suggest that the thrombopoietic system can respond in at least two ways to a stimulus of acute thrombocytopenia and that the degree of platelet need, as reflected by the circulating platelet mass, determines which type of response will be evoked. When thrombocytopenia was moderate (platelet count no lower than about 44% of normal), the rate of addition of platelets to the circulating population beyond replacement was moderate and appeared to be fairly constant during the 5-day period of replenishment. The response to more severe thrombocytopenia was biphasic, with the first phase being similar to the response observed after moderate thrombocytopenia. The second phase appeared about 2 days after acute thrombocytopenia and was characterized by a more rapid rate of platelet increase, around two times the rate seen in the first phase. In addition, the rates within both the first and second phases were greater in relation to the severity of the initial thrombocytopenia. A biphasic response to severe, acute thrombocytopenia, with a change to a more rapid rate of increase of circulating platelets at about 48 hr, has been reported previously.2,3

Several explanations for the moderate rate of increase of peripheral platelets after moderate thrombocytopenia can be hypothesized. It might result in part from peripheral changes, such as a decreased use or decreased senescent loss of platelets. No direct evidence pertaining to change in use or loss of platelets under these circumstances is presently available. However, young platelets presumably comprise a larger proportion of the population in animals recovering from thrombocytopenia than in controls, and, therefore, platelet loss from senescence might be less than in untreated animals. If so, an increase in the size of the circulating population would be expected from this source even without an increased rate of production, and presumably it would be proportional to the percentage of the population made up by new platelets. Indeed, the early curves (to 36 hr) of platelet population increase among the more thrombocytopenic groups approximate those calculated from the life span model of
platelet survival (Fig. 4), assuming no change in the production rate of platelets and an exit change in accordance with the age composition of the circulating population. Such calculated curves do not coincide with experience in mildly thrombocytopenic groups where the new young platelets comprise a smaller proportion of the total population. Among the latter, the observed rates of increase were greater than expected if population increase were due only to a moderate shift toward a younger population. In summary, the population may realize some accretion by changes in age composition, especially during the first day or two when the age distribution of platelets is the youngest.

In addition, it has been reported that the proportion of the megakaryocyte population in DNA synthesis is increased following moderate thrombocytopenia. The 24-hr labeling index of megakaryocytes of rats injected with tritiated thymidine was greater at 48 and 60 hr after moderate thrombocytopenia brought about by exchange transfusion with platelet-poor blood than it was in...
untreated controls. Moreover, the overproduction indicated by the excursion of the peripheral platelet count above 100% in the experiments reported here also suggests a modification of megakaryocytopoiesis in mildly thrombocytopenic rats. Therefore, the modest increase in rate of platelet production after moderate thrombocytopenia is probably due partly to a stimulation of megakaryocytopoiesis, especially in the period beyond 2 days.

The platelet response to sham operation was about one-fourth of that observed after an exchange transfusion (5 ml) that resulted in a 28% reduction in platelet count. Whether the response to sham operation depends on mechanisms similar to those operating after exchange transfusion is not presently known. It is conceivable that platelet utilization in wound repair stimulates thrombocytopoiesis. This lability of the system suggests cautions in assessment of thrombopoietin assay experiments that utilize peripheral platelet counts as the endpoint.

The fact that the rise in peripheral platelet count begins soon after thrombocytopenia, both moderate and severe, suggests that mature megakaryocytes rather than very early cells are the source of platelets, because maturation from an early megakaryocyte precursor to a platelet-producing cell normally requires about 3 days. One possible explanation of an early increase, associated with a change in age composition of the circulating platelet population, has already been discussed. In addition, the observation by electron microscopy of a sparse amount of cytoplasm and an absence of demarcation membranes in megakaryocytes taken from rats shortly after completion of exchange transfusion with platelet-poor blood suggests the possibility that the more mature megakaryocytes may produce platelets prematurely in response to thrombocytopenia, leaving a population composed of younger megakaryocytes. Perhaps the large platelets seen in the circulating population after thrombocytopenia are produced in this manner. Moreover, some platelets may be contributed from a reserve pool soon after induction of thrombocytopenia. The platelet pool in rats is relatively small, however, probably less than 15% of the circulating population, so that this source is limited.

Another response of megakaryocytes to thrombocytopenia is an increase in their average ploidy. Measurement of the relative amounts of DNA in individual megakaryocytes disclosed an appreciable change in the ploidy distribution of the population within 24 hr. We have seen the beginnings of a ploidy shift by 18 hr. Since it has been shown that the amount of cytoplasm per megakaryocyte in untreated rats is related to the ploidy level of the nucleus, the increase in ploidy in thrombocytopenic rats may be accompanied by an increased synthesis of cytoplasm and thereby an increase in the number of platelets produced per megakaryocyte. Indeed, an increase in megakaryocyte size in acutely thrombocytopenic rats has been reported. It is not yet clear how soon platelets produced by this mechanism contribute to the circulating platelet mass. It seems that at least the length of one cell-generation cycle, which takes about 9.5 hr in immature megakaryocytes of normal rats, plus a postreplication maturation period of from several hours to more than a day, would be required. Postreplication maturation time of megakaryocytes probably extends for 10-40 hr after the last replication, the length of time depending on terminal ploidy
level and on shortening of maturation time under pressure of platelet demand. Although the first indications of ploidy shift appear about 18 hr after induction of thrombocytopenia,\textsuperscript{11} the maximum shift in ploidy\textsuperscript{10,11} and in size of megakaryocytes\textsuperscript{3} occurs about 48 hr after acute thrombocytopenia. It seems likely that an increased platelet production resulting from an increase in ploidy of megakaryocytes makes its major contribution to the circulating population during the period of rapid platelet increase between about 48 and 96 hr.

It has also been reported that the megakaryocyte population increases in size, as well as ploidy level, in response to thrombocytopenia, and this could provide another source of additional platelets. New platelets by this avenue would not be expected to appear in the circulation for 2 or 3 days because of the length of the maturation period. The average maturation time in normal rats is 2.5–3 days from diploid precursor to mature platelet-producing megakaryocyte, but it has been reported that recognizable megakaryocytes mature more rapidly in thrombocytopenic animals, perhaps by half a day.\textsuperscript{14}

The apparent paradox of shortened maturation time and a concomitant increase in average ploidy (more generation cycles) has not been fully explained but may result in part from a shortened generation cycle time and in part from a shortening of the "cytoplasmic maturation period" that follows completion of all DNA synthesis.

The foregoing interpretations suggest that both the ploidy shift of megakaryocytes and an increased input of precursor cells may make major contributions to the peripheral platelet population during the time of its maximum increase between about 48 and 96 hr. The ploidy level seems to increase on the average by one DNA complement in severely thrombocytopenic rats, the class with the greatest frequency of cells shifting from 16N to 32 N.\textsuperscript{10} This doubling of DNA might result in a doubling of the amount of cytoplasm of the megakaryocyte population and thereby a doubling of the number of platelets produced, provided the platelets are normal in size. Additional increase in the rate of platelet production beyond a doubling from ploidy changes might be accounted for by the increase in size of the megakaryocyte population.

Perhaps it should be emphasized that measurements of changing population size in these experiments do not provide estimates of rates of platelet production. Population size depends on rates of both entry and exit. If the exit rate and time spent by individuals in the compartment remain constant while population size increases, the change in population can be attributed to new production. However, we do not have a measurement of exit rate and indeed suspect that it may temporarily decline in this population recovering from decimation. The reason for a decline in exit would be a temporary shift toward a younger age composition of the population, as previously discussed.

The results of these experiments also raise questions about regulatory processes. Does an acute stimulus (thrombocytopenia) initiate the progress of a cohort of cells through the megakaryocyte maturation process, the size of which is related to the strength of the stimulus (degree of thrombocytopenia)? Such a hypothesis is supported by the common time of peak height among all groups, and the variable rates of platelet production and the variations in maximum platelet mass, which could result from different cohort sizes. On the other hand,
one would expect the system to include a mechanism for continued stimulation during variance from homeostatic conditions.

REFERENCES

11. Odell TT Jr, Jackson CW, Murphy JR: Unpublished data
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