Influence of Repeated Acute Thrombocytopenias on the Reappearance of Circulating Blood Platelets

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Repeated experimental thrombocytopenia has been performed in three groups of dogs, at intervals of 2 days, 5-7 days, and 10 days. In the first two of these groups, the circulating thrombocytes reappear significantly more rapidly and the maximal platelet counts average respectively 138 per cent \((p < 0.02)\) and 174 per cent \((p < 0.001)\) of the peak observed after a single platelet depletion. If the thrombocytopenic stimulus is repeated at a 10-day interval, this modification in the rate of reappearance of platelets and in the platelet counts does not occur. It is concluded that platelet production induced by a single acute depletion does not represent the maximal medullary response and that thrombocytopoiesis can be further increased if the stimulus is repeated 2-7 days after a first one. Recovery curves after platelet depletion alone or followed by bleeding are similar, suggesting that thrombocytopoiesis is not influenced by a factor stimulating erythropoiesis.

It has been shown that acute experimental thrombocytopenia stimulates thrombocytopoiesis in dogs\(^1,2\) and rats\(^3-5\) but the potential capacity of thrombocyte production in response to sustained or repeated stimuli has not been thoroughly studied. Craddock et al.,\(^1\) studying the regeneration of thrombocytes in dogs subjected to thrombocytophoresis, have observed that the experimental thrombocytopenia that persisted for 2-3 days was followed by a progressive rise in platelet counts reaching normal or supranormal levels on the sixth or seventh day. In the same study, the authors have also reported a single experiment in which, following their statement, “one dog made thrombocytopenic 6 days after a first thrombocytophoresis failed to develop thrombocytopenia.” Matter et al.\(^3\) produced in the rat an acute thrombocytopenia by replacing the animal’s blood with blood from a donor rat deprived of its platelets by differential centrifugation. Following this procedure, the authors observed a period of thrombocytopoiesis of 2 days and a subsequent gradual increase of platelets in the circulating blood reaching a maximum on the 4th or 5th day. In this study, one rat was subjected after 3 days to a second exchange transfusion with platelet-poor blood. The second recovery period was shorter and the response somewhat greater than that observed in singly depleted animals. These results suggest that thrombocytopoiesis induced by acute thrombocytopenia can be further increased if the stimulus is repeated. As the number of these experiments was very limited, we attempted in the present study to investigate systematically the effects of
repeated experimental thrombocytopenia at different times on the production of platelets in the dog. Additionally, as a decrease of the hematocrit was observed in our experiments, we also investigated the influence of bleeding on the regeneration of platelets after a single acute depletion.

**MATERIALS AND METHODS**

Mongrel dogs of both sexes, weighing between 14 and 22 Kg. were used. Enumeration of blood platelets, prior to any experimental procedure, averaged 169,000/mm$^3$ (SEM ± 5600).

Acute thrombocytopenia was produced by the technique previously described. This method utilizes the continuous extracorporeal circulation of blood through a column containing glass beads 1 mm. in diameter to which the platelets adhere. The platelets decrease to about 5 per cent of the initial count at the completion of the experiment, which lasts 3 hours. This procedure was applied to five experimental groups:

**Group I, controls.** Acute platelet depletion was performed 50 times in 34 dogs, submitted from one to four times to this procedure, at intervals of 8–20 weeks, when the effect of the previous stimulation had disappeared.

**Group II.** In seven dogs, acute thrombocytopenia was carried out twice at 2-day intervals.

**Group III.** In ten dogs, platelet depletion was repeated 5 to 7 days after the first depletion (5 days in four dogs and 7 days in six dogs), when reappearance of the platelets had reached a presumed maximal level.

**Group IV.** Seven dogs, randomly chosen in group I, were submitted to a second platelet depletion 10 days after a first thrombocytopenia.

**Group V.** In the last group, the effect of bleeding on platelet regeneration was investigated. Acute thrombocytopenia was carried out once in seven dogs. Immediately after the completion of the extracorporeal circulation, anemia was induced by bleeding from the femoral artery removing 200 to 300 ml. of blood (approximately 1.5 per cent of body weight), which was replaced by saline.

In order to detect an eventual intravascular coagulation due to breakdown material contained in the effluent from the column of glass beads, one-stage prothrombin time, kaolin-activated partial thromboplastin time, as well as factor I, V, VIII, IX, and XI were determined before and on the 4 days following the experimental procedure in four dogs.

To exclude a possible splenic sequestration of newly formed platelets, splenectomy was carried out in three dogs under nembutal anesthesia. Six to 12 weeks later, these animals were subjected to acute platelet depletion.

In four dogs, a sham operation was performed: The femoral artery and vein were catheterized with polyethylene tubing under local procaine anesthesia and connected to each other for 3 hours.

Platelet counts were made on arterial blood in duplicate by the method of Piette with a phase microscope using 200-times magnification. Platelets and hematocrit were determined before and immediately after the experimental procedure, as well as on the subsequent days.

**RESULTS**

**Group I.** The reappearance of circulating platelets in the control group is shown in Fig. 1. Platelet depression persisted for approximately 3 days and was followed by a gradual, sometimes sharp, increase reaching generally the initial control value on the fifth day (mean 4.7 day) and frequently a supranormal value (mean: 173.3 per cent of the initial mean value) between the fifth and seventh day (Fig. 1). The maximal peak lasted 1 day and was followed by a more or less rapid decrease of the platelet count toward the initial level, the average value still being above normal on the 10th day. After
the experimental procedure of depletion, the hematocrit decreased usually in this group from an initial mean value of 42 per cent (range: 39–44%) to a mean value of 38 per cent (range: 36–40%), which corresponded partly to the quantity of blood contained in the extracorporal circuit and in a lesser degree to a slight hemolysis caused by the passage of blood through the glass beads.

Factors V, VIII and X, as well as the one-stage prothrombin time, the kaolin-activated partial thromboplastin time and the thrombin time, did not vary significantly during the first 4 days following the acute platelet depletion, whereas factor I showed a gradual rise from a mean initial value of 186 mg. per cent on day 0 to 413 mg. per cent on day 4.

**Group II.** The mean value on the day of the second acute experimental thrombocytopenia carried out 2 days after the first one, averaged 15,000 platelets/cu. mm. Platelet depression persisted for the first two days thereafter. The recovery was more rapid than in the control group, the mean initial value of group I being reached on day 3.0 (p < 0.01). After the sixth day, the platelet counts rised to approximately 200 per cent or more of the mean initial value of the control group, amounting to 239 per cent 10 days after the second experimental depletion.

**Group III.** Reappearance of platelets after the second acute thrombocytopenia performed 5 to 7 days after the first one was different from the control group I as for time relationship and magnitude of the response (Fig. 1). The platelets started to increase earlier after the second experimental depletion; platelet depression lasted only 1 day and reached higher levels than in the control group. The mean initial value of the control group I was reached on day 2.6 (p < 0.001), while the mean maximal value was observed on the 7th day, averaging 302.3 per cent of the initial mean value in the control group I. Levels as high as 829,000 and 761,000 platelets/cu. mm. were occasionally found on the seventh or eighth day in group III. No significant difference was observed in the pattern seen in dogs who received their second challenge on day 5 as compared to day 7.
Group IV. When the second depletion was performed 10 days after the first, the regeneration curve observed had the same shape as in the control group, the rebound thrombocytosis reaching 207.3 per cent of the initial mean control value on the ninth day.

In groups II, III and IV, the hematocrit decreased after the second platelet depletion from a mean initial value of 44 per cent (range: 39–51 per cent) to a mean value of 35 per cent (range: 31–37 per cent).

Group V. The curve of platelet reappearance in these dogs subjected to a single thrombocytopenia followed by bleeding was similar to the curve observed in the control group (Fig. 1). The hematocrit was lowered in this group from a mean value of 41 per cent (range: 38–47 per cent) to a mean value of 32 per cent (range: 27–35 per cent).

In the splenectomized dogs submitted to an acute platelet depletion, the pattern of response from day 1 to day 9 was the same as in the control group. The mean initial value before the procedure was 414,000/cu. mm. The platelet counts on days 1, 2, 3, and 4 averaged, respectively, 9.9, 24.1, 58.9, and 86.5 per cent of this initial value.

In the sham-operated dogs, the platelet counts did not show significant variations until the seventh postoperative day, when they averaged 126 per cent of the initial value.

Discussion

The procedure of experimental thrombocytopenia utilized in this study induces platelet depression persisting for 2 or 3 days. This temporary depression could be due to conditions inherent to the process of thrombocyte regeneration, but other mechanisms could be evoked, such as intravascular coagulation, with platelet consumption, or splenic sequestration of newly formed platelets. Activation of the blood coagulation mechanism could be achieved by breakdown products contained in the effluent from the column of glass beads and released from red cells or aggregated platelets. However, disseminated intravascular coagulation seems unlikely since we have found normal value of the parameters generally depressed in this condition. Furthermore, Craddock et al. induced acute experimental thrombocytopenia in the dog by a different method and observed the same pattern of response. Significant splenic sequestration of newly formed platelets seems likewise to be excluded, since the same pattern of response as in the control group was observed in splenectomized dogs after acute platelet depletion. Therefore, the delay in reappearance of circulating platelets could be ascribed to a function of the megakaryocyte system. This platelet depletion is followed by a rebound thrombocytosis beginning toward the fifth day. This thrombocytosis can be attributed to the experimental thrombocytopenia and not to a nonspecific thrombocytosis induced by tissue trauma, since no significant variations of the platelet counts occurred in the sham-operated animals until the seventh postoperative day. When a second experimental thrombocytopenia is performed 2–7 days after a similar procedure, reappearance of platelets occurs more rapidly than after a single platelet depletion. The accelerated thrombocyte reappearance is not observed if the second stimulus is applied 10 days after the first one. It is of
interest to consider how this acceleration of platelet recovery could be accomplished. Regulation of thrombocytopoiesis meets some extent the demand for circulating platelets. Accordingly, the studies of de Gabriele and Penington suggest that platelet production is regulated by the number of circulating platelets themselves. Thrombocytopenia in experimental animals has been shown to induce an increase in the number of megakaryocytes, as well as an increased production of platelets. Preceding these modifications, maturation of megakaryocytes is accelerated and some macropoiesis is observed in megakaryocytes. The more rapid reappearance of circulating platelets after the second depletion in groups II and III could then be imputable to several factors: (1) the increased megakaryocytic mass induced by the first thrombocytopenia; (2) a stimulation of the rate of maturation of megakaryocyte precursors into macromegakaryocytes, perhaps induced more precociously than after a single platelet depletion; (3) the possibility that the second thrombocytopenia accelerates the formation and/or the release of more platelets by preexisting megakaryocytes; (4) the possibility that the first thrombocytopenia has increased the number of “committed stem cells” serving, in accordance with Stohlman’s scheme, as the immediate precursors for the identifiable megakaryocytes. Neither of these possible mechanisms satisfactorily explain the lag period of 1 day preceding the rapid rise in platelet counts after the second thrombocytopenia in group III. It is interesting to note that Witte observed in thrombocytopenic rats that the maturation time of thrombocytes was about 1 day, beginning at the stage of pseudopodial megakaryocytes containing well-defined areas of granulations and ending with the appearance of platelets in the circulating blood. If this observation is transposable to the dog, the lag period of 24 hours could represent the time necessary for a mature megakaryocyte to release its platelets in the circulation.

In groups II and III, the platelets not only reappear more rapidly, but reach significantly higher values than after a single platelet depletion. The mean maximal values in these groups are respectively 138 (p < 0.02) and 174 percent (p < 0.001) of the mean maximal value in the control group. The days on which the maximal counts are reached are different in groups II, III, and IV, occurring later in group II and IV (days 10 and 9, respectively) than in the control group and group III (day 7). The reason for these temporal differences is not apparent. It could be objected that the higher platelet counts observed after the second depletion in group III is related to a higher starting value (mean 234,000 platelets/cu. mm.) than in group I. However, if we compare a subgroup of nine control dogs from group I (with an initial starting value of ± 250,000 platelets per cu. mm.) with group III, the same difference as between control group I and group III is observed.

The higher peak of the second regeneration curve in groups II and III indicates that the thrombocytopoiesis induced by a single acute platelet depletion does not represent the maximal medullary response and that thrombocytopoiesis can be increased further by successive stimuli. Recent studies suggest that the increased differentiation of precursor cells into recognizable megakaryocytes evoked by thrombocytopenia is perhaps mediated by a humoral substance present in the plasma of thrombocytopenic animals. One might
conceive that the second depletion in group II and III induced a supplementary production of the humoral factor. The short lag period existing in these two groups between the second thrombocytopenia and subsequent reappearance of circulating platelets is in accordance with the little delay that Odell et al.23 observed between the stimulus and the production of the active principle. Several authors28,29 studying the effect of fractionated doses of erythropoietin on erythropoiesis in mice have demonstrated an increased dose response up to 4 days after a previous erythropoietic stimulation. By analogy with these studies, the increased thrombocytopenia observed in groups II and III could represent an increased dose response in dogs previously stimulated by a thrombopoietic humoral factor. The similarity of platelet regeneration curves in groups I and IV indicates that the additive effects of repeated stimuli is not seen after 10 days.

The moderate decrease of the hematocrit to about 35 per cent observed after the experimental procedures does not influence the regeneration curve of the platelets. The reappearance of thrombocytes in dogs made simultaneously thrombocytopenic and anemic by bleeding is not significantly different than in animals subjected to only one experimental thrombocytopenia. This seems to agree with the observations of de Gabriele and Penington,37 who showed that, in the rat, platelet production is not influenced by the factors that stimulate or suppress erythropoiesis and that it may vary independently regardless of the state of red cell production.

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REFERENCES


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