Presence of a Nonsplenic Platelet Pool

By M. Freedman, N. AltSzUler, and S. Karpatkin

A nonsplenic platelet pool was noted in humans subjected to exercise and in rabbits and dogs subjected to epinephrine infusion. Following vigorous exercise, 10 normal subjects raised their platelet count 1.43-fold (p < 0.01) and megathrombocyte number 2.17-fold (p < 0.01) at a peak interval of 8 min. Two healthy splenectomized subjects raised their platelet count 1.46- and 1.37-fold and megathrombocyte number 1.77- and 1.57-fold at peak intervals of 8 and 3 min, respectively. Nine rabbits subjected to a bolus of epinephrine intravenously (15μg/kg) raised their platelet count 1.32-fold (p < 0.05) and megathrombocyte number 2.91-fold (p < 0.05). Eight splenectomized rabbits treated similarly raised their platelet count 1.15-fold (p < 0.05) and megathrombocyte number 1.41-fold (p < 0.05). Six dogs subjected to a bolus of epinephrine intravenously (1 μg/kg) raised their platelet count 1.50-fold (p < 0.01) and megathrombocyte number 2.37-fold (p < 0.05).

Seven experiments performed in dogs following splenectomy resulted in an average peak rise in platelet count of 1.21-fold (p < 0.01) and megathrombocyte number of 1.33-fold (p < 0.05). The rise in platelet count and megathrombocyte number was transient in both intact and splenectomized animals; peaking at 3 min and returning to baseline at 5–7 min. The nonsplenic platelet pool in rabbits and dogs represented approximately 45% of the total rapidly mobilized platelet pool and was not appreciably enriched with megathrombocytes.

The spleen is the source of a rapidly mobilizable pool of platelets\(^1\) representing 30%−40% of the total population in humans,\(^2\) dogs,\(^7\) and rabbits.\(^6\)\(^,\)\(^7\) The spleen also preferentially sequesters megathrombocytes (large platelets).\(^6\)\(^,\)\(^7\) We have examined this platelet pool in intact and splenectomized humans, dogs, and rabbits following epinephrine administration (dogs and rabbits) and following exercise (humans) and provide evidence for an additional rapidly mobilizable nonsplenic platelet pool. The present report provides the experimental details and kinetics of exercise-induced thrombocytosis in humans and epinephrine-induced thrombocytosis in dogs and rabbits.

**MATERIALS AND METHODS**

Ten intact and two splenectomized healthy adults (splenectomized for trauma) gave informed consent and were subjected to vigorous exercise—bicycle pedaling on an ergometer with weights adjusted to give heart rates of greater than 150 beats/min for 11 min. Blood samples were collected at 2−3-min intervals from a vein in the antecubital fossa via a 23-gauge needle.

Nine intact rabbits and eight splenectomized rabbits were subjected to a bolus of intravenous adrenaline hydrochloride (15μg/kg) obtained from Parke Davis & Co., Detroit, Mich. The rabbits were premedicated with 50 units of heparin 2−3 min prior to the intravenous (marginal
ear vein) infusion of adrenaline in 10 ml of saline. Blood samples were collected from the ear artery at 1-min intervals. White New Zealand rabbits of both sexes, weighing 3-4 kg, were used. Splenectomies were performed under light intravenous sodium pentobarbital anesthesia. The rabbits were given nothing by mouth for 24 hr postsurgery, and received an intravenous injection of 500,000 units potassium penicillin G immediately following surgery. The animals were allowed to recover for 3-4 wk before they were employed for kinetic studies.

Six intact mongrel dogs and one splenectomized trained dog received a bolus of intravenous adrenaline hydrochloride (1μg/kg). Two dogs received a constant infusion of adrenaline hydrochloride (1μg/kg) for 12 min. Premedication with heparin was not employed. The dog's jugular vein was cannulated percutaneously and a polyethylene catheter was kept patent by a slow intravenous drip of saline. Blood samples were collected and drug injected via the indwelling catheter. Splectomy was kindly performed by the laboratory of Dr. Alan Dumont, Professor of Surgery, New York University Medical Center.

All blood samples were collected into EDTA Vacutainer test tubes (Becton-Dickinson & Co., Rutherford, N.J.). All determinations were performed in duplicate at room temperature. Platelet counts were performed manually under phase contrast microscopy, employing 3% procaine hydrochloride as diluent. On rare occasions platelet aggregates were noted. When such samples (which also gave identifiable patterns with the Coulter Counter) were encountered, the experiment was discarded. The average difference between 107 duplicate counts was 9.7%. Hematocrits were performed in microhematocrit tubes. Basal values represented the average of 2-3 measurements taken 1-5 min prior to exercise or injection of drug.

The megathrombocyte number was obtained by multiplying the percentage megathrombocytes by the platelet count. The percentage megathrombocytes was measured with a Coulter Counter for platelet-rich plasma, obtained by centrifugation in plastic 2-mm-diameter tubes at 600 g for 30 sec. The specimens were diluted in Isoton (Coulter Electronics, Hialeah, Fla.), with the aid of a 3-μl pipette, to a platelet count of 5000-10,000/100-μl volume in order to reduce coincidence counting to less than 1%. A P-64 Channel Analyzer with an automatic electronic recording device was attached to a model B Coulter Counter. A 70-μm aperture tube was employed and the equipment was calibrated with latex particles of 3.35 μm so that each window was equal to 0.25 fl. Windows 4-100 therefore represented 1-25 fl volumes.

The percentage megathrombocytes was arbitrarily defined as the upper 10% volume distribution of the total platelet volume distribution. For example, in rabbits the mean upper 10% window setting obtained was 42. Thus, all megathrombocyte measurements were made from windows 42-100. This value was divided by measurements made from the total platelet population, windows 4-100, and then multiplied by 100 to obtain the percentage. Windows 1-3 were excluded because of electronic noise interference. In order to standardize conditions, the diluting fluid, time interval between blood removal and volume measurement (less than 2 hr), and concentration of platelets were held constant at all times. All measurements were made in duplicate. The aperture tube was kept clear by continual monitoring of the oscilloscope screen for interference patterns. Background counts, as determined on the model B, were kept at below 50 prior to use of the P-64 Channel Analyzer.

Statistical analysis was performed by matched Student's t test.

RESULTS

Studies in Humans Subjected to Vigorous Exercise

Twelve experiments were performed on 10 healthy adults (2 repeat experiments), ages 27-45 (3 females, 7 males), who were subjected to vigorous bicycle pedaling (heart rate >150/min) for 11 min. This procedure resulted in a rise in platelet count as well as megathrombocyte number. The average rise in platelet count was 1.40-fold over the basal count at 11 min (p < 0.01). All patients did not peak at 11 min; the average peak occurred at 7.9 min, at which time the platelet count was 1.43-fold (p < 0.01) over the basal count (data not shown). The average rise in megathrombocyte number at 11 min was 1.75-fold (p < 0.01). The average peak rise at an average peak time interval of 7.6 min was
NONSPLenic PLATELET POOL

Table 1. Effect of Vigorous Exercise* on Platelet Count and Megathrombocyte Number in Healthy Subjects

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Platelets x 10^9/liter</th>
<th>p &lt; t</th>
<th>Megathrombocytes x 10^9/liter</th>
<th>p &lt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>338 ± 221</td>
<td>—</td>
<td>32.3 ± 3.0</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>396 ± 22</td>
<td>0.01</td>
<td>43.2 ± 5.3</td>
<td>0.10</td>
</tr>
<tr>
<td>5</td>
<td>431 ± 21</td>
<td>0.01</td>
<td>50.4 ± 4.8</td>
<td>0.01</td>
</tr>
<tr>
<td>8</td>
<td>419 ± 19</td>
<td>0.05</td>
<td>56.4 ± 3.9</td>
<td>0.01</td>
</tr>
<tr>
<td>11</td>
<td>473 ± 30</td>
<td>0.01</td>
<td>56.2 ± 6.5</td>
<td>0.01</td>
</tr>
<tr>
<td>22</td>
<td>336 ± 14</td>
<td>NS</td>
<td>42.1 ± 5.6</td>
<td>NS</td>
</tr>
<tr>
<td>31</td>
<td>356 ± 30</td>
<td>NS</td>
<td>38.5 ± 5.1</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Ten normal subjects underwent vigorous exercise (bicycle pedaling, heart rate > 150/min) for 11 min, followed by a rest period of 20 min.
†Probability of being significantly different from 0 time value; NS, not significant, p > 0.10.
 Mean ± SEM.

2.17-fold (p < 0.01) (data not shown), which represented 26% of the total platelet increment. The peak rise in megathrombocyte number was significantly greater than the peak rise in platelet count (p < 0.01). The average platelet count and megathrombocyte number for each time interval are shown in Table 1. The shift in platelet volume distribution for three different experiments is shown in Fig. 1.

Two healthy male splenectomized adults (S1, age 40, and S2, age 45) were also subjected to the same vigorous exercise. There was a rise in platelet count in both subjects to 1.46- and 1.37-fold, respectively, at 8 and at 3 min, respectively (Table 2). This increase was associated with a peak rise in megathrombocyte number of 1.77-fold in S1 and 1.57-fold in S2. These data strongly suggest the presence of a rapidly mobilizable nonsplenic pool. Because of the difficulty in obtaining splenectomized healthy volunteers further experiments were performed in animals.

Studies in Rabbits Subjected to Adrenaline Infusion

Nine intact rabbits were subjected to a bolus of adrenaline intravenously, which resulted in an average rise in platelet count of 1.32-fold (p < 0.05) and in megathrombocyte number of 2.91-fold (p < 0.05) (Table 3). The peak rise oc-

![Fig. 1. Effect of exercise on platelet volume distribution in three different subjects, A, B, and C. One window is equivalent to 0.25 ft. Solid line, preexercise values; broken line, values after 8 min of exercise for A, 11 min of exercise for B, and 4 min of exercise for C; dotted line, postexercise values following 11 min of rest for A and B.](image)
Table 2. Effect of Vigorous Exercise on Platelet Count and Megathrombocyte Number in Two Healthy Splenectomized Subjects*

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Platelets x 10^9/liter</th>
<th>Megathrombocytes x 10^9/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S1</td>
<td>S2</td>
</tr>
<tr>
<td>0</td>
<td>380</td>
<td>315</td>
</tr>
<tr>
<td>3</td>
<td>510</td>
<td>430</td>
</tr>
<tr>
<td>5</td>
<td>470</td>
<td>410</td>
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<td>8</td>
<td>555</td>
<td>395</td>
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<td>11</td>
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<td>400</td>
</tr>
<tr>
<td>15</td>
<td>370</td>
<td>380</td>
</tr>
<tr>
<td>21</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Two healthy splenectomized subjects (S1, age 40, and S2, age 45) underwent vigorous exercise for 8-11 min, followed by a rest period of 7-10 min.

The peak rise in megathrombocyte number was significantly greater than the peak rise in platelet count (p < 0.01) and represented 35% of the total platelet increment. Control experiments in which EDTA-anticoagulated rabbit blood was incubated with heparin and adrenaline, simulating concentrations achieved in vivo, revealed no effect on the platelet volume distribution curve; platelet aggregation was not noted under phase microscopy.

Eight splenectomized rabbits were similarly treated, resulting in a rise in platelet count of 1.15-fold (p < 0.05) and in megathrombocyte number of 1.41-fold (p < 0.05). The peak rise occurred at 3 min, with a return to basal levels at 4-6 min. The peak rise in megathrombocyte number was just significantly greater than the peak rise in platelet count (p = 0.05), and considerably less than the rise in megathrombocyte number in intact animals.

Table 3. Effect of Intravenous Adrenaline on Platelet Count and Megathrombocyte Number in Intact Versus Splenectomized Rabbits*

<table>
<thead>
<tr>
<th></th>
<th>Platelets x 10^9/liter</th>
<th>Megathrombocytes x 10^9/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Peak</td>
</tr>
<tr>
<td>Intact (9)</td>
<td>449 ± 461</td>
<td>594 ± 79</td>
</tr>
<tr>
<td>Splx (8)</td>
<td>510 ± 31</td>
<td>585 ± 39</td>
</tr>
</tbody>
</table>

*Rabbits were injected intravenously with adrenaline (15 µg/kg) and their platelet counts and megathrombocyte numbers were followed at 1-min intervals for 10 min. The peak response was at 3–4 min. Number of experiments is given in parenthesis.
†Probability of being significantly different from basal value.
Mean ± SEM.
Table 4. Effect of Intravenous Adrenaline on Platelet Count and Megathrombocyte Number in Intact Versus Splenectomized Dogs*

<table>
<thead>
<tr>
<th></th>
<th>Platelets x 10^9/liter</th>
<th>Megathrombocytes x 10^9/liter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Basal</td>
<td>Peak</td>
</tr>
<tr>
<td>Intact (6)</td>
<td>378 ± 481</td>
<td>565 ± 76</td>
</tr>
<tr>
<td>Splx (7)</td>
<td>504 ± 104</td>
<td>609 ± 91</td>
</tr>
</tbody>
</table>

*Dogs were injected intravenously with adrenaline (1 μg/kg) and their platelet counts and megathrombocyte numbers were followed at 2-min intervals for 12 min. The peak response was at 3-4 min. Number of experiments is given in parentheses.

†Probability of being significantly different from basal value.

‡Mean ± SEM.

Seven experiments were performed in a splenectomized dog who was subjected to adrenaline intravenously. There was an average rise in platelet count of 1.21-fold (p < 0.01) and in megathrombocyte number of 1.33-fold (p < 0.05). The peak rise occurred at 4 min, with a return to basal levels at 6-8 min. The peak rise in megathrombocyte number was not significantly greater than the peak rise in platelet count (p > 0.1). No appreciable rise in peripheral blood hematocrit was noted in six of seven experiments.

DISCUSSION

These data clearly indicate the presence of a rapidly mobilizable pool of platelets in humans, rabbits, and dogs during exercise or epinephrine administration, representing at least 29% [(0.4/1.4) × 100] of the total platelet pool in humans, 24% in rabbits [(0.32/1.32) × 100], and 33% in dogs [(0.5/1.5) × 100]. This phenomenon was first quantified by Aster in humans, employing epinephrine infusion experiments in splenectomized and nonsplenectomized subjects, and it was confirmed by others. However, these workers attributed this rapidly mobilizable platelet pool to splenic sequestration.

From our data, it is now evident that another rapidly mobilizable pool of platelets also exists which cannot be attributed to splenic sequestration and which can be mobilized by exercise in humans and by epinephrine in rabbits and dogs. It is of interest in this regard that Dawson and Ogston have also reported an increase in platelet count in three of six splenectomized humans, following exercise, which averaged 25%. The nonsplenic pool represents a significant portion of the total rapidly mobilizable pool, approximately (0.15/0.32) × 100 (47%) in rabbits and (0.21/0.50) × 100 (42%) in dogs. This value represents approximately 13% of the total platelet pool in rabbits, dogs, and humans (the splenic pool represents approximately 16%). In contrast to the splenic pool, the nonsplenic pool is not enriched with megathrombocytes in dogs and is only slightly enriched in rabbits. The number of observations obtained with humans is insufficient to draw any definitive conclusions regarding megathrombocyte enrichment of the nonsplenic pool.

It is not clear why others working with splenectomized human subjects have been unable to demonstrate this nonsplenic pool of platelets. These workers have employed epinephrine rather than vigorous exercise as the inducer. It is therefore possible that some "exercise-induced factor," rather than epinephrine, is required for mobilization of this nonsplenic pool. This possibil-
ity is supported by the observations of Dawson and Ogston,8 who could not block exercise-induced thrombocytosis with 20 mg of propranolol, a β-adrenergic blocker known to inhibit adrenaline-induced thrombocytosis.9 Longer and fewer sampling intervals following epinephrine administration may also have contributed to the lack of recognition of this nonsplenic pool. The epinephrine effect was noted to be transient in our animal studies, peaking at 3 min in rabbits and 4 min in dogs and returning to baseline levels in 4-6 min in rabbits and 6-8 min in dogs. Indeed, preliminary observations in dogs suggested a refractory period of approximately 12 min following a peak epinephrine response.

As demonstrated previously by our group,6-7 and confirmed by the present data, enhanced megathrombocytes in the rapidly mobilizable pool(s) are released by exercise in humans and epinephrine infusion in rabbits and dogs. Megathrombocytes appeared to be mostly confined to the splenic pool in rabbits, dogs, and humans since appreciable increases were not noted in splenectomized animals and humans following epinephrine and/or exercise. This fraction represented approximately 46% of the total splenic platelet pool in rabbits and dogs. Although the increment in megathrombocyte release into the peripheral circulation was only 26%-35% of the total platelet increment (i.e., splenic and nonsplenic) this could represent a sizable platelet functional pool. Indeed, it has been proposed that only some platelets are functional.10 Some authors have reported enhanced platelet function following exercise.11-14 We have made similar observations in some of our exercise subjects15 and attribute this mechanism, in part, to the release of megathrombocytes from the spleen into the peripheral circulation. Megathrombocytes have been shown to be more functional than other platelets by relatively crude techniques.16 More recent studies have revealed a direct correlation between platelet size (mean platelet volume and/or megathrombocyte number) and ADP-, collagen-, and epinephrine-induced platelet aggregation.17

It should be recognized that the splenic and nonsplenic pools of platelets may be part of a more generalized organization of the microcirculation including mature erythrocytes, reticulocytes, and granulocytes, which are rapidly mobilizable with stress and or exercise. For example, the peripheral hematocrit is known to rise following epinephrine infusion in intact animals (according to our own data and that of others18,19). The red blood cell mass has been shown to increase in splenectomized dogs following epinephrine infusion as well as conditions which lead to increased peripheral arterial resistance.18 Epinephrine infusion in intact sheep19 and splenectomy in dogs20 as well as humans21 are associated with a reticulocytosis which persists following splenectomy, suggesting preferential sequestration of reticulocytes. Epinephrine infusion is also associated with leukocytosis,22 presumably secondary to the rapid mobilization of a marginal pool of leukocytes.

The source of the nonsplenic platelet pool is not yet well documented. One might speculate that other reticuloendothelial organs such as the lungs, bone marrow, liver, etc. may be the sequestering organ. Indeed, Bierman et al.23 have claimed a significant release of platelets from the pulmonary circulation during epinephrine administration. An alternative explanation would be platelet margination to the endothelial vascular surface with entry into the axial flow following exercise and/or epinephrine, as has been suggested for granulocytes.24
Whatever the source, it is clear that a nonspenic platelet pool exists. It is rapidly mobilizable with exercise in humans and epinephrine infusion in rabbits and dogs. It is not enriched with megathrombocytes in dogs and only slightly enriched with megathrombocytes in rabbits. The nonspenic pool represents approximately 45% of the total rapidly mobilizable platelet pool.

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REFERENCES

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