Effects of Short-Term Hypoxia on Platelet Counts of Mice
By T. P. McDonald, Marilyn Cottrell, and Rose Clift

Recent studies have shown that long-term hypoxia causes decreased platelet counts in mice and short-term hypoxia increased platelet counts. In an attempt to explain the mechanism that increases platelet counts of mice after exposure to short-term hypoxia, we measured platelet counts, total circulating platelet counts (TCPC), total circulating platelet masses (TCPM), percentages of $^{35}$S incorporation, and platelet sizes. Platelet counts, as well as TCPC and TCPM of mice, increased after 1–3 days of hypoxia, but these values were decreased after 6–7 days of hypoxia. Although platelet counts were increased in hypoxic mice, the percentage $^{35}$S incorporation into platelets and platelet sizes did not show a concurrent increase. After 6 days of hypoxia, average platelet diameters began to increase as platelet counts decreased. Splenic release did not account for the increase in platelet counts of mice after short-term hypoxia. It seems possible, therefore, that megakaryocytes “shed” platelets into the circulation in response to hypoxia. The platelets that enter the circulation in response to short-term hypoxia are smaller and incorporate less $^{35}$S than platelets that are produced in response to acute thrombocytopenia.

EXPOSURE OF MICE TO LONG-TERM HYPOXIA has been shown$^{1,4}$ to decrease platelet counts. Our studies$^3$ showed that hypoxia decreases platelet counts by decreasing platelet production. It was postulated that platelet depletion was caused by stem cell competition between the erythroid and megakaryocytic cell lines$^1$ and/or inhibition of production of a thrombopoiesis-stimulating factor (thrombopoietin).$^4$ However, hypoxia of short duration (1–3 days) was shown in several experiments$^{1,5,6}$ to cause increased platelet counts. Increased incorporation of isotopes into platelets of mice$^1,5$ and rats$^6$ after short-term hypoxia was also reported; this result was interpreted to indicate increased platelet production. Since 3–4 days would be required for thrombopoietin to stimulate immature megakaryocytes to produce a measurable change in platelets, an increase in platelet counts within 1–2 days was too short a time for the phenomenon to be attributed to humoral stimulation. Increased platelet counts in mice exposed to hypoxia would have to be caused by factors other than known humoral agents. In an effort to explain this early increase in platelet counts, we measured the effects of short-term hypoxia on platelet counts, total circulating platelet counts (TCPC) and masses (TCPM), percentages of $^{35}$S incorporation into platelets, and platelet sizes.

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MATERIALS AND METHODS

Male C3H mice, 10–11 wk of age and weighing approximately 23 g, were used. Mice were enclosed 1–7 days in cages covered with dimethyl-silicone rubber membranes. The membrane cages were opened three times during the week for cleaning, feeding, and watering the mice. After equilibration (about 8 hr), the oxygen levels in the cages were 5.5%–6.5%. Control mice were kept at ambient air levels in latex mouse cages that were placed on shelves next to the membrane cages.

Peripheral platelet counts were determined by use of phase microscopy; packed cell volumes (PCV) were measured by use of a standard technique on fresh whole blood obtained without anticoagulants from the retroorbital sinus.

Blood volumes were estimated using the $^{59}$Fe-labeled erythrocyte dilution technique. Normal mice, which served as erythrocyte donors, were given a single intraperitoneal (i.p.) injection of 0.5 μCi of $^{59}$Fe (ferric citrate); 48 hr later, the donor mice were sacrificed and approximately 6 ml of blood were collected into a tube containing 1 ml of 1% sodium citrate. The blood was centrifuged, the citrate–plasma portion was removed, and the red cells were mixed with saline to the original blood volume. Normal mice, mice injected with rabbit anti-mouse platelet serum (RAMPS), and mice that had been exposed to hypoxia for 1–7 days were injected intravenously with 0.1 ml of $^{59}$Fe-labeled red cells. After 15–20 min, the mice were bled and 100 μl of blood, diluted into 2 ml of water, was counted for radioactivity in a gamma scintillation spectrometer. Blood volume (BV) was expressed as milliliters of blood per 100 g of body weight.

Since platelet counts of mice were increased after 1–3 days of hypoxia (at a time when PCV and blood volumes of mice were rapidly increasing and the body weight was decreasing), the TCPC and the TCPM were calculated. The TCPC was calculated by multiplying the peripheral platelet count per milliliter of blood by the total blood volume. Total blood volume for each mouse was estimated by multiplying the BV (milliliters of blood/100 g) by the body weight of the mouse in grams. The TCPM was calculated by multiplying the TCPC by the average platelet volume (μm$^3$). Platelet volume was calculated with a standard formula as previously described.

To obtain platelets for size measurements, mice were injected i.p. with a heparin-nembutal solution and bled from the heart into plastic syringes containing heparin–saline. The blood from each mouse was expressed into a plastic tube and the platelet-rich plasma (PRP) was obtained by centrifugation at 360 g for 4.5 min at 22°C. Average platelet sizes (on a logarithmic scale) were determined by use of an Electrozone/Celloscope (Particle Data) equipped with a 128-channel analysis accessory and direct readouts to an X-Y oscilloscope and an X-Y plotter. The instrument settings were log,0 (logarithmic span of about ten doublings of the particle volume or 10:1 in diameter), current 1/v$^2$, and gain 17. The multichannel analyzer was set for acquisition to a count of 4000 in the peak channel. Calibration was maintained at all times via frequent verification with latex particles of known size (2.02 and 3.49 μm diameter; Dow Chemical). Size determinations were made by use of an X-Y plotter. Duplicate platelet samples were sized; platelet size (micrometers in diameter) was calculated as previously described.

Platelet production was measured by determining the amount of radiosulfate incorporated into the newly formed platelets: 30 μCi of Na$_2^{35}$SO$_4$ diluted into 0.5 ml of saline was injected i.p. into each mouse 24 hr before assay and the percentage $^{35}$S incorporation into platelets was measured using PRP that was prepared for platelet sizing. The PRP was centrifuged at 600 g for 15 min at 4°C to obtain platelet buttons. The platelets from each mouse were resuspended in 0.6 ml of 1% ammonium oxalate, mixed, centrifuged (15 min at 600 g), and washed two additional times with 0.5 ml of 1% ammonium oxalate and 1.5 ml of saline. A yield of about 35% platelets was obtained by this procedure. Platelet counts of suspensions were made and radioactivity was determined. The percentage $^{35}$S incorporation into platelets was calculated as previously described using the appropriate blood volume for the corresponding time after hypoxia (Fig. 2B) or rabbit anti-mouse platelet serum (RAMPS) treatment (Table 1).

For positive controls, some mice were injected with RAMPS for production of acute thrombocytopenia. In previous experiments, mice responded with altered platelet production rates and sizes. Platelet count, PCV, body weight, blood volume, TCPC, TCPM, percentage $^{35}$S incorporation, and platelet sizes were measured in blood of these mice on days 2, 3, and 7 after antisera injection.

To test the possibility that splenic release of platelets caused the increase in platelet counts after 1–3 days of hypoxia, some mice were splenectomized and allowed to recover from surgery.
for 2 wk prior to placing them in hypoxia chambers. As before, platelet count, TCPC, TCPM, PCV, body weight, percentage $^{35}$S incorporation, and platelet sizes were measured.

In all cases, Student's $t$ test was used to test for statistical significance.

**RESULTS**

Figure 1 shows the platelet counts and PCV of mice after 1–7 days of hypoxia. As shown in Fig. 1A, mice had increased ($p < 0.0005$) platelet counts on days 1–3, but the counts returned to near normal on days 4 and 5; the counts decreased ($p < 0.0005$) to below normal on days 6–7. PCV of mice (Fig. 1B) showed a linear increase ($p < 0.0005$) with time in hypoxia, reaching 65% by day 7.

Figure 2 shows the body weights and blood volumes of normal and hypoxic mice. Mice enclosed in hypoxia chambers lost weight ($p < 0.0005$) after 1–7 days when compared with mice kept at normal ambient atmospheres (Fig. 2A). BV increased ($p < 0.0005$) with time in hypoxia (Fig. 2B).

As shown in Fig. 3, the TCPC essentially paralleled the peripheral platelet counts (Fig. 1A versus Fig. 3). Significantly increased ($p < 0.0005$) TCPC were found in mice after 1–3 days of hypoxia; normal values were observed on days 4 and 5; and significantly depressed ($p < 0.0005$) TCPC were observed on days 6–7 of hypoxia.

To measure platelet production, percentage $^{35}$S incorporation into platelets and platelet sizes were determined (Fig. 4). Figure 4A shows that the percentage

![Graph](image-url)
Fig. 2. (A) Body weights and (B) blood volumes of mice after exposure to hypoxia for 1–7 days. Vertical bars: ±SE. In A, body weights represent the average of 19–32 mice per point, except at time 0 (horizontal broken line), when 94 normal control mice were used. In B, blood volumes represent the average of 9–10 mice per point except at time 0 (horizontal broken line), when 20 normal control mice were used. In both A and B, all values of hypoxic mice were significantly different from normal control values (p < 0.0005).

Fig. 3. Total circulating platelet count (TCPC), of mice after exposure to hypoxia. Each point represents the average of 19–32 mice, except at time 0 (horizontal broken line), when 94 normal control mice were used. TCPC was calculated by multiplying the peripheral platelet count by the blood volume (see Materials and Methods). Vertical bars: ±SE. Values were significantly different from normal control mice: ** p < 0.0005.
Fig. 4. (A) Percentage $^{35}$S incorporation into platelets and (B) platelet sizes of mice after exposure to hypoxia. Each point represents the average of 10-24 mice, except at time 0 (horizontal broken line) when 43 untreated control mice were used. Vertical bars: ±SE. Values were significantly different from normal control mice: *, p < 0.005; ***, p < 0.0005.

Fig. 5. Total circulating platelet mass (TCPM) of mice after exposure to hypoxia. Each point represents the average of 19-32 mice, except at time 0 (horizontal broken line), when 94 normal control mice were used. TCPM was calculated by multiplying the total number of circulating platelets by the average platelet volume (see Materials and Methods). Vertical bars: ±SE. Values were significantly different from control values: *, p < 0.005; ***, p < 0.0005.
Table 1. Effects of Rabbit Anti-Mouse Platelet Serum (RAMPS) on Hematologic Values and Platelet Production in Mice

<table>
<thead>
<tr>
<th></th>
<th>Days After RAMPS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>Platelet count/cu mm</td>
<td>9.73 ± 0.19 (41)*</td>
</tr>
<tr>
<td>× 10^-5</td>
<td></td>
</tr>
<tr>
<td>Packed cell volume</td>
<td>46.14 ± 0.24 (40)</td>
</tr>
<tr>
<td>(%)</td>
<td></td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>24.31 ± 0.32 (41)</td>
</tr>
<tr>
<td>Blood volume (ml/100 g)</td>
<td>5.65 ± 0.05 (20)</td>
</tr>
<tr>
<td>Total circulating platelet count × 10^-8</td>
<td>13.37 ± 0.29 (41)</td>
</tr>
<tr>
<td>35S incorporation × 10^4 (%)</td>
<td>27.36 ± 1.23 (43)</td>
</tr>
<tr>
<td>Platelet size (μm in diameter)</td>
<td>1.73 ± 0.01 (41)</td>
</tr>
<tr>
<td>Total circulating platelet mass × 10^-8 (cu μm)</td>
<td>36.23 ± 0.78 (41)</td>
</tr>
</tbody>
</table>

Each value is the mean ± SE of at least two experiments.

*Number of mice.

†Significantly different from untreated control mice, p < 0.0005.

Table 2. Effects of Splenectomy on Platelet Count, Total Circulating Platelet Mass, and Platelet Production of Mice Enclosed in Hypoxia Chambers

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of Mice</th>
<th>Platelet Count/cu mm × 10^-5</th>
<th>TCPC × 10^-8</th>
<th>Packed Cell Volume (%)</th>
<th>35S Incorporation × 10^4 (%)</th>
<th>Platelet Size (μm diameter)</th>
<th>TCPM × 10^-8 (cu μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No hypoxia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>41</td>
<td>9.52 ± 0.18</td>
<td>13.37 ± 0.29</td>
<td>46.18 ± 0.21</td>
<td>27.79 ± 1.25</td>
<td>1.73 ± 0.00</td>
<td>36.23 ± 0.78</td>
</tr>
<tr>
<td>Splenectomized</td>
<td>11</td>
<td>10.65 ± 0.60</td>
<td>14.63 ± 0.81</td>
<td>43.96 ± 0.61†</td>
<td>34.82 ± 3.17</td>
<td>1.70 ± 0.00†</td>
<td>37.61 ± 2.09</td>
</tr>
<tr>
<td>1 day of hypoxia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>29</td>
<td>11.09 ± 0.34*</td>
<td>15.12 ± 0.39*</td>
<td>46.98 ± 0.34*</td>
<td>30.64 ± 2.39</td>
<td>1.72 ± 0.01†</td>
<td>40.72 ± 1.06†</td>
</tr>
<tr>
<td>Splenectomized</td>
<td>11</td>
<td>13.48 ± 0.58†</td>
<td>19.70 ± 0.91§</td>
<td>46.27 ± 0.65†</td>
<td>51.53 ± 9.06†</td>
<td>1.68 ± 0.03§</td>
<td>48.85 ± 2.26§</td>
</tr>
<tr>
<td>2 days of hypoxia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>29</td>
<td>11.24 ± 0.26**</td>
<td>14.56 ± 0.33†</td>
<td>49.31 ± 0.27*</td>
<td>32.60 ± 2.99</td>
<td>1.71 ± 0.01†</td>
<td>37.81 ± 0.86</td>
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<tr>
<td>Splenectomized</td>
<td>11</td>
<td>13.32 ± 0.52†</td>
<td>19.53 ± 1.05§</td>
<td>48.91 ± 0.57†</td>
<td>36.16 ± 3.81</td>
<td>1.66 ± 0.01§</td>
<td>46.68 ± 2.51§</td>
</tr>
<tr>
<td>3 days of hypoxia</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>29</td>
<td>11.72 ± 0.24*</td>
<td>15.67 ± 0.48*</td>
<td>51.41 ± 0.22*</td>
<td>37.08 ± 2.75†</td>
<td>1.73 ± 0.01†</td>
<td>43.04 ± 1.32*</td>
</tr>
<tr>
<td>Splenectomized</td>
<td>11</td>
<td>11.20 ± 0.52</td>
<td>15.06 ± 0.78</td>
<td>49.41 ± 0.69†</td>
<td>31.18 ± 3.02</td>
<td>1.68 ± 0.02†</td>
<td>37.35 ± 1.93</td>
</tr>
</tbody>
</table>

*Significantly different from nonhypoxic control mice, p < 0.0005.

†Significantly different from nonsplenectomized control mice, p < 0.005.

§Significantly different from nonhypoxic control mice, p < 0.005.
\(^{35}\)S incorporation into platelets of hypoxic mice was not different from normal, except for an increase \((p < 0.005)\) on day 3 of hypoxia. Platelet diameters were unchanged after 1–5 days of hypoxia (Fig. 4B), except that the platelets were slightly smaller \((p < 0.01)\) than normal on day 2. Platelet sizes were significantly \((p < 0.0005)\) increased on days 6 and 7 of hypoxia.

Since platelet sizes were increased after 6–7 days of hypoxia, the TCPM was calculated (Fig. 5). As shown, significant increases in TCPM were found after 1–3 days of hypoxia. However, at day 2 the increase in TCPM was not as large as the TCPC (Fig. 3), probably due to the smaller platelet size (Fig. 4B). As before, the platelet values returned to near normal on days 4–5 and were decreased \((p < 0.0005)\) by days 6–7. The larger platelets on days 6–7 of hypoxia (Fig. 4B) resulted in TCPM that were not as depressed as platelet counts (Fig. 1) or TCPC (Fig. 3).

In order to compare the platelet production of hypoxic mice with that of mice that were producing platelets at a greater than normal rate, other mice were injected with RAMPS and various blood determinations were made after 2, 3, and 7 days. As shown in Table 1, platelet counts of mice injected with RAMPS were decreased \((p < 0.0005)\) 2 and 3 days later, but the counts were elevated on day 7 \((p < 0.005)\). PCV, body weight, and blood volume were unaltered by RAMPS. As in the hypoxia experiments, TCPC paralleled the platelet count. The percentage \(^{35}\)S incorporation was elevated at 3 days after RAMPS \((p < 0.0005)\), but the production of new platelets was not different from control values on days 2 and 7. Platelets were larger than normal on days 2 and 3 after RAMPS treatment, but returned to normal size by day 7. TCPM paralleled the platelet count and TCPC except at 3 days after RAMPS, when large platelets appeared to offset the decreased platelet counts.

In other experiments, mice were splenectomized before enclosure in hypoxia chambers. The results (Table 2) showed that intact mice responded to hypoxia with essentially the same results as before (Figs. 1, 3–5). Mice that had been splenectomized prior to enclosure in hypoxia chambers showed similar increases in platelet counts and TCPC, as did intact mice. PCV of splenectomized mice were statistically \((p < 0.005)\) lower than those of intact mice after 0 and 3 days of hypoxia, but not after 1 and 2 days of hypoxia. The percentages of \(^{35}\)S incorporation of hypoxic splenectomized mice were similar to those of intact mice, except sulfate uptake was greater in hypoxic splenectomized mice at 1 day of hypoxia. Platelets were smaller in splenectomized mice than in intact mice, regardless of hypoxia treatment. TCPM were similar to the platelet counts and TCPC, except at 2 days after hypoxia for both the intact and splenectomized mice; apparently, decreased platelet sizes influenced the values.

**DISCUSSION**

The present work (Fig. 1A) confirms the previous findings of several workers\(^ {1,5,6}\) of increased peripheral platelet counts in animals after 1–3 days of hypoxia. In the present study, the platelet counts returned to near normal on days 4 and 5, and thereafter dropped to below normal counts after 6 and 7 days of hypoxia. This result is in agreement with previous findings\(^ {1,6}\) that mice exposed to hypoxia for 6–7 days had mean platelet counts that were lower than
normal controls. In agreement with the results of the present study, Shreiner and Levin found no change in incorporation of $^{75}$Se-selenomethionine ($^{75}$SeM) into platelets after 6-7 days of hypoxia. However, other studies have shown that after 14 days of hypoxia a significant decrease in isotopic incorporation, along with severe thrombocytopenia, occurred in hypoxic mice. These data on long-term hypoxia were interpreted as evidence in favor of hypoxia causing decreased platelet production. The present work showed that the observed thrombocytopenia after 6-7 days of hypoxia was not due to expanding blood volumes, and previous data ruled out the possibility that excess sequestration of platelets by the hypertrophic, erythropoietic spleen causes reduced platelet counts. Decreased counts with normal production of platelets (isotopic uptake) might indicate the production of platelets that have structural or metabolic defects leading to shortened survival times. This explanation would account for reduced platelet counts after 6-7 days of hypoxia. Other studies are obviously required to clarify the mechanisms.

It has been hypothesized that the early increase (1-3 days) in platelet counts might be due to nonspecific factors released in response to stress of hypoxia or to dehydration of mice since a rapid decrease in body weight has also been observed. In order to measure more accurately the thrombocytosis, we determined platelet counts and TCPC of mice. TCPC of mice essentially paralleled the peripheral platelet counts. Therefore, hypoxia not only increased the platelet count, but also increased the total number of circulating platelets in mice. Moreover, the increase was apparently not due to changes in blood volumes, because TCPC of hypoxic mice were greater than counts of control mice.

Other workers found an increase in the incorporation of $^{35}$S or $^{75}$SeM into platelets of mice after 1 day of hypoxia. These studies were confirmed in rats, in which increased $^{35}$S incorporation into platelets after 2-4 days of hypoxia was found. In disagreement with these studies, the present data (Fig. 4) show that $^{35}$S incorporation into platelets was unchanged in mice after 1-2 days of hypoxia, while platelet counts were being elevated. For some unexplained reason, we found increased percentages of $^{35}$S incorporation into platelets only after 3 days of hypoxia, at the same time that platelet sizes were increasing and platelet counts were elevated. Evatt and co-workers found no increase in $^{75}$SeM of platelets from mice after 1 or 3 days of hypoxia and normal platelet counts were observed after 40 and 88 hr of hypoxia. The reason for this discrepancy between our data and those of others is not known, but it may be related to different mouse strains, different platelet labels, the use of inappropriate blood volumes in calculating the results, or different degrees and methods of producing hypoxia.

Platelets produced in response to thrombocytopenia are larger and heavier and incorporate more radiosulfate or $^{75}$SeM than platelets from normal populations. However, since the isotopic uptake and sizes of platelets entering the circulation after hypoxia were not elevated in our mice, the mechanism by which platelets are increased in response to hypoxia does not appear to be analogous to the platelet production that occurs in response to antiserum-produced thrombocytopenia (Table 1). In fact, platelets were slightly smaller than normal (Fig. 4B) after 2 days of hypoxia, in spite of rapidly increasing platelet counts (Fig. 1).
The presence of larger than normal platelets in mice after 6–7 days of hypoxia suggests stimulation of platelet production by the thrombocytopenia caused by hypoxia. In dogs with cyclic hematopoiesis, platelet sizes increase as platelet counts decrease, suggesting that when platelet counts are low, megakaryocytes release large platelets, and when platelet counts are high, smaller than normal platelets are found in the circulation. Therefore, the present finding agrees with these previous results and indicates that thrombocytopenia (caused by hypoxia of 6–7 days or antiserum injection) causes the release of large platelets from megakaryocytes into the circulation. It is not known whether large platelets that are produced in response to hypoxia are “stress” platelets or platelets that are produced “normally” in response to thrombocytopenia.

The TCPM of mice exposed to hypoxia in the present study correlate with the TCPC findings. The data of the present work also indicate that when platelet counts are low, large platelets are being produced (Table 1; Figs. 4, 5). Thus, there may be compensating effects of the production of large platelets to offset the effects of thrombocytopenia.

Previously, Minter and Ingram proposed that large heavy platelets obtained from acutely bled dogs are “stress” platelets and that these platelets do not decrease in size as they age. However, other workers believed that platelets produced in response to thrombocytopenia decrease in size as they age. In the present work, platelet sizes were larger in mice after RAMPS treatment and 6–7 days after initiation of hypoxia than in normal mice. The data of the present study are also consistent with the hypothesis that large platelets produced in response to thrombocytopenia decrease in size as they age (Table 1).

Previous work has shown that about 35% of the total number of platelets are sequestered in the spleen and that large platelets are preferentially stored. Moreover, Freedman and Karpatkin have demonstrated a rapidly mobilizable nonsplenic platelet pool that is not enriched with large platelets and is responsive to exercise and/or epinephrine. In agreement with these findings, the present study indicates that splenic release of platelets does not account for the increase in the platelet counts of mice after 1–3 days of hypoxia. Mice that were splenectomized 2 wk prior to enclosure in hypoxia chambers showed similar increases in platelet counts to those of nonsplenectomized mice after exposure to hypoxia (Table 2). Since these data illustrate that hypoxia must cause an increase in circulating platelets by mechanisms other than splenic release, it is tempting to speculate that hypoxia causes the release of platelets from the same platelet pool described by Freedman and Karpatkin.

Although Kraytman found that splenectomy does not alter the average platelet size of dogs, platelets from splenectomized mice in the present study were smaller than platelets from normal mice. This interesting finding may support a previous hypothesis that splenectomy causes increased platelet counts in animals by removing the site of destruction of the blood cells, i.e., the “graveyard” hypothesis. An increase in platelet counts and a decrease in the average platelet size of mice following splenectomy seems to indicate that splenectomy causes mice to retain the small old platelets in the circulation for longer periods of time than normal. Decreased platelet sizes in splenectomized mice, therefore, may be a key in explaining postsplenectomy thrombocytosis.

As shown here, short-term hypoxia caused increased platelet counts, TCPC,
TCPM, PCV, and blood volumes of mice while body weights were decreasing. The percentage $^{35}$S incorporation into platelets and platelet size measurements did not agree with an influx of new large platelets into the circulation. Moreover, splenectomy did not alter the platelet production rates nor the release of platelets into the circulation after hypoxia. It seems possible from these findings that hypoxia, a stressful situation, mobilizes a nonsplenic platelet pool that is composed of small platelets that do not incorporate $^{35}$S-sulfate. The thrombocytosis could be the result of hypoxia acting directly and/or indirectly to cause megakaryocytes to release these platelets by a mechanism other than that observed after thrombocytopenia. In this regard, Penington and Streatfield believe that $^{32}$N megakaryocytes may serve as a platelet reserve for stress situations.

Jackson and Edwards found increased megakaryocyte size and turnover in rats after hypoxia. Interestingly, they also found a decrease in the number of megakaryocytes in the marrow and an increase in the number of megakaryocytic naked nuclei after short-term hypoxia. These findings may indicate a rapid release of platelets from mature megakaryocytes into the circulation. Since an increase in megakaryocyte labeling index is possible by decreasing the proportion of mature megakaryocytes (producing naked nuclei), as well as increasing the number of immature cells, platelet “shedding” from mature megakaryocytes in response to hypoxia seems possible. This hypothesis would explain the early increase in platelet counts of mice and at the same time would explain the apparent increase in the proportion of immature megakaryocytes that has been previously observed. Alternate hypotheses are also possible, e.g., another organ, other than the spleen, might release large quantities of small-sized, poorly labeled platelets into the circulation in response to hypoxia. Further work is necessary to clarify the mechanism of hypoxia-induced thrombocytosis.

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