Stimulation of Megakaryocytopoiesis by Acute Thrombocytopenia in Rats

By T. T. Odell, J. R. Murphy, and C. W. Jackson

Rats were made acutely thrombocytopenic by injection of antiplatelet serum. Marrow sections and squash preparations were made at intervals during 120 hr. Determinations were made of mitotic index, stage of maturation, ploidy level, and cell size of megakaryocytes; number and size of platelets were measured. Increased endomitosis among megakaryocytes was followed by an increase in the proportion of immature megakaryocytes, a greater average ploidy level of recognized megakaryocytes, and larger megakaryocytes. Maximum changes in these several parameters occurred between 32 and 72 hr after induction of thrombocytopenia. By 120 hr all megakaryocyte parameters were near normal. For about 3 days, beginning at about 36 hr, platelet numbers increased rapidly. Average platelet size rose and returned to normal within about 60 hr. Changes in ploidy and size of megakaryocytes were measured in the immature and mature maturation stages. The results suggest that the initial stimulus in response to acute thrombocytopenia acts primarily on diploid precursors, programming them to mature into a population of megakaryocytes with an average ploidy approximately one level greater than in normal rats and a proportionate increase in cell size. The larger megakaryocytes presumably produce more platelets, accounting for a major part of the increased rate of platelet production. Since the changes in megakaryocytes begin to reverse before circulating platelet numbers have reached the normal level, reversal of the stimulus appears to be initiated by some change other than platelet mass.

It has been shown that megakaryocytes have a characteristic frequency distribution among several ploidy classes in normal animals of several species.1,2 Morphological studies suggested that megakaryocytes of thrombocytopenic rats were larger than those of normal animals.4 Indeed, measurements confirmed that rat megakaryocytes increased in size after thrombocytopenia induced by injection of antiplatelet serum, younger megakaryocytes being the first members of the population to be affected, followed by the more mature megakaryocytes.5,6 An increase in the number of endomitotic figures among megakaryocytes of experimentally thrombocytopenic rats was also observed,7 and an increase in megakaryocyte ploidy was then demonstrated.8 The study reported here was undertaken to investigate effects of acute thrombocytopenia on megakaryocytopoiesis and platelet production. We measured changes in mitotic index, ploidy, size, and proportions of immature and mature megakaryocytes in rats, as well as platelet numbers and size. In many cases, various measurements were made on the same cells, so that it was possible to relate changes in ploidy, size, and maturity of individual cells.
Samples were taken at intervals during the 120-hr period after induction of acute thrombocytopenia.

MATERIALS AND METHODS

General

Male albino rats (Charles River Breeding Laboratory) with an average body weight of 376 g were used. They were given a single intravenous injection of antplatelet serum (APS) to produce thrombocytopenia and were killed at intervals during a 120-hr period. Marrow smears, squash preparations, and sections were prepared and examined for progressive changes. Marrow smears were made from tibial marrow as previously described.9 Marrow squash preparations were also prepared from tibial marrow as follows. The marrow plug was blown onto a slide with a needle and syringe, and the marrow was mixed with a plasma expander, polyvinylpyrrolidone, into a single cell suspension. A small drop of the suspension was placed on a slide, and a cover slip was placed on the drop, adding slight finger pressure if necessary to spread the drop. Then the slide was placed horizontally on dry ice until well frosted (10-15 min). The cover slip was popped off with a razor blade, and the slide was fixed in cold Carnoy’s fixative for 25-30 min and then air dried. Staining was as described for smears in the foregoing reference. Antiserum to rat platelets was made in rabbits as previously described.7

Platelets

Platelet counts were made by the phase microscope method on all rats before treatment. Blood was collected from the saphenous vein. The average pretreatment count of 58 rats was 1 x 10^6 platelets/cu mm of blood (1 SD = ±0.09). Counts were also taken during the experiment and at time of killing. Relative sizes of platelets were measured by taking 35-mm photomicrographs in hemocytometer counting chambers, placing the photomicrographs in an enlarger and tracing the platelet perimeter on paper, cutting out the tracing, and weighing it. Electrostatic-copier paper was chosen from several kinds of paper tried because it was found to be uniform in weight and relatively heavy. Each sheet was preweighed; any sheets not falling within ±1% of an average weight (4.5 g ± 0.05) were discarded. In most cases 50 platelets per rat were measured.

Mitotic Index

The percentage of recognized megakaryocytes in endomitosis was determined on sections of femoral marrow stained with hematoxylin and eosin. Mitoses among at least 800 megakaryocytes were scored on each section, with an average of 1242.

Classification of Megakaryocytes

Megakaryocytes on Giemsa-stained smears of tibial bone marrow were classified as belonging to one of three stages of maturation on the basis of morphological characteristics—immature cells, mature cells, and naked nuclei, types I, II, and III, respectively, as previously described.7 Samples for classification were taken at 2, 6, 9, 12, 18, 24, 30, 36, 42, 48, 60, 72, 84, 96, and 120 hr after injection of APS. Squash preparations of tibial marrow were also stained with Giemsa, and the cells were classified.

DNA Determination

After cell classification, the squash preparations were treated by the Feulgen procedure for quantitative staining of DNA; relative amounts of DNA in individual cells were determined by the two-wavelength microspectrophotometric method of Ornstein and Patau.3 DNA measurements were made on cells of rats killed 9, 12, 18, 24, 36, 48, 60, 72, and 120 hr after injection of APS. The number of megakaryocytes measured per rat ranged from 198 to 527 (total of 3482). Diploid marrow cells of each rat were also measured.

The DNA values were ordered according to increasing amounts of DNA within each of the maturation stages (I, II, and III) and were converted to log_{10}. The log_{10} values were placed in class intervals of 0.06 log_{10} units. The number of megakaryocytes in each class interval was plotted to provide histograms of the frequency of cell distribution according to amount of DNA.
MEGAKARYOCYTOPOIESIS IN RATS

The histograms of the distribution of mature megakaryocytes (Type II) were used to establish the boundaries of ploidy classes; it is believed that mature megakaryocytes do not synthesize DNA, and therefore the class boundaries should be more distinct than those of immature megakaryocytes, which spend a large part of their cell generation cycle time in DNA synthesis. Examination of the histograms of all the rats indicated that peak frequencies of megakaryocytes in ploidy classes occurred at intervals of approximately 0.31 log10 units. One exception was the 18-hr rat, in which the estimated best interval was about 0.33 log10 units. The dividing points between ploidy classes were taken as half the distance between the log10 values of the estimated peaks. These dividing points coincided with the regions of lowest frequency on the histograms. All of the DNA values were included in one ploidy class or another, none being omitted. The average DNA value of diploid cells was used to determine the actual ploidy level of the various polyploid classes. Frequencies of megakaryocytes in the several ploidy classes of the three maturation stages of both immature and mature megakaryocytes were tabulated.

Size of Megakaryocytes

The size of megakaryocytes was determined in some of the squash preparations on which megakaryocyte DNA was also measured, namely, the 18-, 24-, 36-, and 48-hr rats, and a control. Relative size was determined by weighing tracings of cells enlarged from photomicrographs of the cells, in a manner similar to that used to determine platelet size.

RESULTS

Platelets

Platelet counts were depressed to about 2% of the pretreatment count by injection of 0.2 ml of APS. By 24 hr they had begun to increase. The increase was most rapid between 48 and 96 hr, and a linear regression plot of platelet count against time during this period showed a rate of increase of approximately 333,000 platelets/cu mm of blood/day (Fig. 1).

Platelet size measurements were made on platelets of a control rat and of rats killed at 12, 18, 24, 30, 42, and 60 hr after injection of APS. By Student's t test, average platelet size was found to be larger statistically (p < 0.001) at 12, 18, 24, 30, and 42 hr than in controls (Fig. 2). The frequency distribution appeared to be bimodal at 18 hr, and average size was much greater than at any other time examined; by 60 hr, average platelet size and size distribution had returned to a pattern essentially like that of the control.

Mitotic Index (Endomitosis)

The mitotic index (MI) of megakaryocytes increased markedly after treatment with APS, reaching a peak value around 32–36 hr that was more than five times greater than the average of controls (Fig. 3). At 18 hr after APS, the MI was significantly greater than that of controls (p < 0.001), and it continued to differ at that probability level through 60 hr. The control rats were injected with saline at approximately the same time of day as the experimental animals (8:30 p.m.). The results did not suggest a difference in MI related to time of sample collection.

Ploidy

Marked progressive changes in the distribution of megakaryocytes among ploidy classes occurred after induction of acute thrombocytopenia by injection of APS. In general, there was a shift to the right; i.e., the average ploidy of the recognized megakaryocytes increased. The proportions of 8N and 16N mega-
Fig. 1. Platelet counts of rats at intervals after injection of APS. The numbers by the points indicate the number of rats whose platelets were counted; two rats were counted where no number appears. The line between 48 and 96 hr is a calculated straight-line regression (slope 1.375). Solid circles indicate platelet counts of controls at time of killing in relation to their preexperiment counts (100% line).

Fig. 2. Platelet size distribution at various times after injection of APS. A class interval was chosen that would include all platelets of rats having the biggest range of platelet size in nine intervals. Platelet size is expressed in terms of the number of the class interval. The values of average platelet size are shown for each time period; they have been expressed as the average weight of the platelet picture.

Fig. 3. Mitotic index. Percentage of megakaryocytes in sections of femoral marrow that were in mitosis at intervals after injection of APS. The average mitotic index of 13 control rats was 0.27% (SD, ±0.03). The total number of megakaryocytes examined per rat ranged from 805 to 2259. Two rats were examined at each sampling time after injection of APS except where noted otherwise by numbers. The t test indicated that the averages of experimental rats were different (p < 0.001) from that of controls at 18, 24, 36, 42, 49, and 60 hr after injection of APS (at 120 hr p < 0.025).
Fig. 4. Changes in frequencies of immature (type I) and mature (type II) megakaryocytes in ploidy classes at intervals after injection of APS. Type I megakaryocytes are denoted by an open symbol and type II by a solid symbol. Horizontal lines at left of graphs indicate control values. Numbers at the top of each graph indicate the total number of megakaryocytes in the ploidy class at the indicated time. In two controls (1127 megakaryocytes) from another experiment 9% of the population were 8N I, 10% were 8N II, 17% were 16N I, 45% were 16N II, 3% were 32N I, and 12% were 32N II.

Megakaryocytes declined, while the proportion of 32N megakaryocytes increased (Fig. 4). At 48 hr, 32N megakaryocytes comprised 53% of the total population, in comparison with about 14% in the control. Moreover, a new ploidy class of 64N megakaryocytes appeared, and comprised nearly one-fourth of the total megakaryocyte population at 48 hr.

The frequencies of megakaryocytes in ploidy classes within the immature and mature stages are plotted in Fig. 4. Both immature and mature megakaryocytes of the 8N and 16N ploidy classes declined during the 48 hr after APS and then returned toward normal. The decline of immature 8N and 16N megakaryocytes was moderate, while that of mature cells was marked and rapid. The 32N and 64N megakaryocytes increased after thrombocytopenia, the proportion of immature megakaryocytes rising first, followed by mature megakaryocytes. The maximum frequency of the immature 32N cells occurred at approximately 40 hr and of the mature 32N cells at about 65 hr. The immature 64N megakaryocytes...
Table 1. Percentages of Megakaryocytes in Maturation Stages at Intervals After Injection of Antiplatelet Serum

<table>
<thead>
<tr>
<th>Time after APS (hr)</th>
<th>No. of Rats</th>
<th>Average Percentage (Range)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Type I</td>
</tr>
<tr>
<td>Controls 10</td>
<td>2</td>
<td>32 (24-38)</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
<td>32 (30-33)</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>34 (34-35)</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>38 (36-40)</td>
</tr>
<tr>
<td>12</td>
<td>2</td>
<td>26 (24-28)</td>
</tr>
<tr>
<td>18</td>
<td>2</td>
<td>38 (34-42)</td>
</tr>
<tr>
<td>24</td>
<td>2</td>
<td>38 (31-45)</td>
</tr>
<tr>
<td>30</td>
<td>2</td>
<td>40 (38-41)</td>
</tr>
<tr>
<td>36</td>
<td>2</td>
<td>58 (56-59)</td>
</tr>
<tr>
<td>42</td>
<td>2</td>
<td>58 (56-60)</td>
</tr>
<tr>
<td>48</td>
<td>2</td>
<td>60 (54-65)</td>
</tr>
<tr>
<td>60</td>
<td>2</td>
<td>38 (36-41)</td>
</tr>
<tr>
<td>72</td>
<td>2</td>
<td>40 (31-48)</td>
</tr>
<tr>
<td>84</td>
<td>2</td>
<td>32 (30-33)</td>
</tr>
<tr>
<td>96</td>
<td>2</td>
<td>30 (29-31)</td>
</tr>
<tr>
<td>120</td>
<td>2</td>
<td>27 (24-30)</td>
</tr>
</tbody>
</table>

peaked around 48 hr, the mature ones later. All then declined and were close to control levels by 120 hr. It was striking that 64-ploid megakaryocytes were absent in control rats, but came to comprise more than 20% of the total megakaryocyte population 48 hr after induction of thrombocytopenia.

Maturation Stages

In a group of ten control rats, immature megakaryocytes averaged 32% of the population (1 SE = ±1.4%), mature megakaryocytes 65% (1 SE = ±1.4%), and naked nuclei 3% (1 SE = ±0.4%). The number of megakaryocytes classified for each rat ranged from 275 to 837 (total of 5202). The control rats were killed 6, 12, 24, and 48 hr after intravenous injection of 0.2 ml of saline. The time of day of sample collection had no obvious effect on the composition of the population. (Samples of control rats were taken at approximately 8:30 a.m., 2:30 p.m., and 8:30 p.m.)

The proportion of immature megakaryocytes began to increase during the first day after induction of thrombocytopenia, reached a peak at around 40 hr, and then declined (Table 1). At maximum, the proportion of immature megakaryocytes was about 185% of that seen in controls. The proportion of immature cells present in the population was statistically different from controls at 30 (p < 0.05), 36, 42, and 48 hr (p < 0.001). The proportion of mature cells in the population declined while immature cells were increasing. No change was discerned among naked nuclei. The number of megakaryocytes classified per experimental rat ranged from 174 to 1239; the average was 449.

Megakaryocyte Size

Size determinations were made on some of the megakaryocytes in squash preparations whose stage of maturity had been classified and whose DNA was measured. Average sizes of megakaryocytes in ploidy classes within maturation stages are presented in Table 2. It is apparent that immature megakaryocytes
**MEGAKARYOCYTOPOIESIS IN RATS**

Table 2. Average Sizes* of Megakaryocytes in Ploidy Classes Within Maturation Stages at Intervals After Injection of Antiplatelet Serum

<table>
<thead>
<tr>
<th>Time after APS (hr)</th>
<th>8N (μm²)</th>
<th>16N (μm²)</th>
<th>32N (μm²)</th>
<th>64N (μm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>36 ± 20† (31)†</td>
<td>66 ± 28 (73)</td>
<td>129 ± 52 (10)</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>47 ± 16 (16)</td>
<td>91 ± 36 (44)</td>
<td>184 ± 44 (44)</td>
<td>(2)</td>
</tr>
<tr>
<td>36</td>
<td>59 ± 33 (42)</td>
<td>99 ± 42 (73)</td>
<td>147 ± 58 (67)</td>
<td>(3)</td>
</tr>
<tr>
<td>48</td>
<td>38 ± 18 (13)</td>
<td>101 ± 43 (30)</td>
<td>176 ± 75 (50)</td>
<td>260 ± 99 (10)</td>
</tr>
<tr>
<td><strong>Type I</strong></td>
<td>37 ± 18 (19)</td>
<td>73 ± 22 (25)</td>
<td>164 ± 56 (69)</td>
<td>386 ± 119 (47)</td>
</tr>
<tr>
<td>18</td>
<td>112 ± 54 (27)</td>
<td>179 ± 83 (183)</td>
<td>286 ± 118 (41)</td>
<td>0</td>
</tr>
<tr>
<td>24</td>
<td>139 ± 64 (12)</td>
<td>190 ± 62 (113)</td>
<td>308 ± 107 (63)</td>
<td>0</td>
</tr>
<tr>
<td>36</td>
<td>126 ± 78 (17)</td>
<td>206 ± 66 (116)</td>
<td>299 ± 112 (71)</td>
<td>(1)</td>
</tr>
<tr>
<td>48</td>
<td>174 ± 93 (7)</td>
<td>286 ± 87 (47)</td>
<td>416 ± 156 (48)</td>
<td>(1)</td>
</tr>
<tr>
<td><strong>Type II</strong></td>
<td>0</td>
<td>179 ± 82 (23)</td>
<td>370 ± 105 (84)</td>
<td>657 ± 208 (24)</td>
</tr>
<tr>
<td>18</td>
<td>23 ± 6 (14)</td>
<td></td>
<td></td>
<td>(2)</td>
</tr>
<tr>
<td>24</td>
<td>27 ± 9 (8)</td>
<td>49 ± 9 (4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>26 ± 6 (22)</td>
<td>49 ± 10 (16)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>28 ± 8 (11)</td>
<td>48 ± 5 (7)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Type III</strong></td>
<td>28 ± 5 (7)</td>
<td>44 ± 8 (10)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Weight in grams × 100 of paper image.
†1 SD.
†The numbers in parentheses represent the number of megakaryocytes averaged. Averages are included for groups having four or more cells.

(Type I) become larger as they attain a higher ploidy level. In addition, they become larger as they move from the immature to the mature stage at any given ploidy level. Similar size-distribution patterns were seen in control rats and those that received APS, except that large 64N cells not seen in normal animals were present in some of the treated rats. The results in Table 2 suggest that megakaryocytes may increase in size within groups (within a ploidy class of a maturity stage) in the stimulated rats. However, it does not presently seem justified to draw any definite conclusions in this regard because of uncertainties related to the methods. These size determinations were made on squash preparations, which were used in these experiments because of potential advantages for the DNA measurements. In general, the results indicate relative uniformity among preparations; notice that the size determinations on the naked nuclei were very consistent among rats, with the exception that the control value (16N III) is about 85% of the average of the others. Nevertheless, some of the apparent differences between animals may be the result of differences in degree of flattening of the cells during preparation. In addition, the standard deviations demonstrate the large variation in measured sizes of cells within individual groups. Moreover, only one animal was examined per treatment group. Therefore resolution of this question awaits additional investigation.

**DISCUSSION**

The nature of the response of the megakaryocyte-platelet system to acute thrombocytopenia is only partly understood and is the subject of this report.
It is well known that platelet antiserum can produce severe acute thrombocytopenia. It has also been shown by fluorescent antibody techniques on smears of bone marrow that antiserum made against platelets will also react with megakaryocytes, raising the question whether in vivo administration of platelet antiserum affects megakaryocytopoiesis directly in addition to its thrombocytopenic effect. Presently available evidence is inconclusive about effects of injected antiserum on megakaryocytopoiesis, but indicates that judicious choice of dose can produce acute thrombocytopenia without additional effects. Studies of the return of the peripheral platelet count after induction of thrombocytopenia with antiserum or by exchange transfusion with platelet-poor blood have demonstrated that the platelet count increase during 96 hr after treatment is essentially identical when the platelet count is reduced to about 2%-10% of normal levels by either method. If, however, a larger dose of antiserum is given, the platelet count remains depressed for a longer time and eventually returns at a very rapid rate. This response after larger doses can be interpreted either as the result of damage to megakaryocytes or as continued removal of newly formed platelets by persisting antiserum. In experiments reported here, care has been taken to inject antiserum in a dose just sufficient to reduce the platelet count to about 2% of normal circulating levels, and therefore we believe that the platelet count was markedly reduced without any important effect of antiserum directly on megakaryocytopoiesis.

Within 12 hr after APS injection, large platelets began to appear in the circulation, and at 18 hr nearly half of the population was composed of such platelets. Because the new large platelets were bigger than any present in controls, they apparently did not correspond to normally occurring, large, young platelets, but, rather, represented a specific response to the acute thrombocytopenic condition. Moreover, they apparently did not represent the principal response to platelet need, because the greatest influx of platelets occurred later, between 48 and 96 hr after acute platelet depletion. The early appearance of the large platelets suggested that they were derived from Type II megakaryocytes that were already undergoing the later stages of their maturation process at the time of APS injection. Therefore, the message to produce these large platelets was received by postreplication megakaryocytes, and the stimulus was platelet deprivation. By 24 hr, the frequency distribution of platelet size had begun to shift back toward the control condition, suggesting that the period of delivery of these large platelets to the circulation was quite short.

These experiments demonstrated a marked shift toward a higher average ploidy level in megakaryocytes of rats subjected to acute thrombocytopenia, and were in agreement with an earlier study covering fewer sampling times. We found that changes in ploidy had begun to occur by 18 hr, and were most marked around 48 hr. By 120 hr values had returned to near normal. The appearance of a new ploidy class with 64 complements of DNA made a substantial contribution to the increased average ploidy. In addition, the ploidy class having the highest frequency of cells shifted from 16N in controls to 32N in rats that were responding to platelet depletion. Our results have shown directly, by measuring DNA and size of the same cells, that the size of megakaryocytes varied according to their amount of DNA. Moreover, difference in the size of cells between ploidy classes was due not only to added DNA, but also to a
major growth of the cytoplasm of megakaryocytes after DNA replication had ceased. Presumably the number of platelets produced by each megakaryocyte was proportional to its amount of cytoplasm, thus accounting for a major part of the increased platelet production in these stimulated rats. Calculations based on the changes observed in ploidy distribution of the mature megakaryocytes at 48 hr, and assuming a doubling of platelets produced with a doubling of DNA, indicated that about twice as many platelets could be produced by the population existing at 48 hr after induction of thrombocytopenia as in controls. The amount of cytoplasm may actually have increased by more than a doubling. Two other changes may also have contributed to the increase in platelet production. It has been reported that the number of megakaryocytes increases after acute thrombocytopenia, and that the rate of maturation of megakaryocytes accelerates.

In addition to extending our understanding of the overall increase in ploidy level and size of responding megakaryocytes, these experiments have demonstrated the changes that occur in relative frequencies of cells within the several ploidy classes of both the immature and mature compartments after induction of acute thrombocytopenia. The latter changes indicated modifications of the flow of cells through the maturation process. The proportions of immature cells in the 8N and 16N classes declined only moderately, probably because all cells pass through these compartments during their maturation to classes of higher ploidy. In contrast, the proportions of mature 8N and particularly 16N cells declined markedly, accounting to a large extent for the reductions observed in these classes as a whole, and specifically demonstrating the relative failure of megakaryocytes to mature at the 16N ploidy level in stimulated subjects. These lower proportions of 8N and 16N cells were balanced by high proportions of both immature and mature megakaryocytes of the 32N and 64N classes. The marked increase in proportions of immature 32N and 64N cells accounted for the increase in overall proportion of immature megakaryocytes; immature 32N megakaryocytes increased from about 2.5% to about 24%, and immature 64N megakaryocytes from zero to about 15%. As expected, the time of maximum frequency of immature 32N and 64N megakaryocytes preceded the time of maximum frequency of the mature cells of those same ploidy classes.

The earliest change in megakaryocytes after acute thrombocytopenia in these experiments was an increase in the endomitotic index, occurring sometime between 12 and 18 hr, with its peak at about 32 hr. Succeeding changes were a peak frequency of immature megakaryocytes at about 40 hr, and time of highest average ploidy of the total population at about 48 hr.

The increase in mitotic index is a prerequisite for the increase in average ploidy level of the megakaryocyte population. Its initiation between 12 and 18 hr suggests either that the target cell must pass through a differentiation and maturation process for 12–18 hr before it is recognized, or that it takes a relatively long time for the stimulator to reach or act on the target cell. The presence of measurable amounts of thrombopoietin in plasma within 2 hr after induction of thrombocytopenia indicates the possibility of early action on the target cells, and, therefore, that an early cell might be the target. The time of increase of the mitotic index is consistent with stimulation of a diploid precur-
sor, because passage of a cell from G1 of the 2N state to mid-S between 4N and 8N, when megakaryocytes are probably first recognized, would require about 14 hr, assuming a cell generation cycle time of 9.3 hr.6 The mitotic index continues to be elevated for another 1–1.5 days as the megakaryocytes undergo additional replication of DNA, some reaching a ploidy level of 64N.

The increase in the proportion of immature megakaryocytes, as well as the increased mitotic index and the time of appearance of additional mitoses and immature megakaryocytes, in a population whose total size equals or is greater than normal,6,8 suggests an influx of cells from an unrecognized precursor compartment. Theoretically, the increased proportion of cells in the immature compartment could be attributed to increased time spent in the immature compartment as a result of the added mitoses (cell generation cycles) necessary to provide additional 32N megakaryocytes and a new class of 64N megakaryocytes. However, the evidence from 3H-thymidine-uptake studies shows that the time spent in the immature compartment is no longer, and may even be shorter, than in untreated rats.7,14 The latter results imply a shortening of the cell generation cycle time of maturing megakaryocytes in subjects with stimulated thrombocytopoiesis. The increase in mitotic index of recognized megakaryocytes would not be expected to produce an increase in the number of immature megakaryocytes, because (according to present evidence) such cells do not undergo cell division. The maximum change in cell size occurs last, as the added 32N megakaryocytes and the new 64N cells undergo the final stages of maturation.

The time of maximum frequency of the increased 32N and 64N megakaryocytes agrees with the interpretation that diploid cells are the original source of these new cells. However, the appearance of a few 64N megakaryocytes at 18 and 24 hr (two and four cells, respectively) and increased numbers of 32N megakaryocytes at these times would require a major shortening of the cell generation cycle time. Perhaps a few polyploid cells retain their sensitivity to a regulator, while most are insensitive beyond their programming as diploid cells.

In conclusion, the nature of the waves of change among cells of the megakaryocytic system in response to platelet depletion suggests a temporally short stimulus to early cells, which then proceed through the maturation process, ultimately producing platelets. The return toward normal in most megakaryocyte parameters by 60 hr or earlier, even before platelets have reached normal levels in the peripheral circulation, indicates that the size of the circulating platelet mass does not constitute the signal to turn off stimulated megakaryocytopoiesis. The inhibitory effect may rather arise from some activity of an earlier cell in the maturation sequence, or from a preponderance in the circulating platelet population of young highly functional platelets.13 Thus a decreased platelet mass appears to constitute the mechanism for stimulating megakaryocytopoiesis, while some other change acts to reverse or shut off that stimulus.

REFERENCES
3. Odell TT, Jackson CW, Gosslee DG: Maturation of rat megakaryocytes studied by
MEGAKARYOCYTOPOIESIS IN RATS


Stimulation of megakaryocytogenesis by acute thrombocytopenia in rats

TT Odell, JR Murphy and CW Jackson

Updated information and services can be found at:
http://www.bloodjournal.org/content/48/5/765.full.html
Articles on similar topics can be found in the following Blood collections

Information about reproducing this article in parts or in its entirety may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#repub_requests

Information about ordering reprints may be found online at:
http://www.bloodjournal.org/site/misc/rights.xhtml#reprints

Information about subscriptions and ASH membership may be found online at:
http://www.bloodjournal.org/site/subscriptions/index.xhtml