confirmed by flow cytometric analysis of fresh unfixed cells. the megakaryocytes and nonmegakaryocytes were con-

very small percent of the other marrow cells. This size range and the existence of a visual threshold size between

megakaryocytes, to be able to compare experimental and clinical observations.

Normal guinea pig and human megakaryocytes in suspen-

EGAKARYOCYTES were first named in 1943.912 so that a criterion for the identification of megakaryocytes for

identifying large populations of megakaryocytes was larger than all but a just larger than the nonmegakaryocytes, and thus may fail to detect much of the megakaryocyte popula-

size due to degree of polyploidy and to matunational changes.3'4 The present article extends those observations.

As the Feulgen procedure for ploidy determination was introduced, but ploidization and maturation were corre-

sponding changes in nuclear morphology. Within each ploidy group, the sizes of megakaryocytes increased

doubled with each ploidy doubling. Within each maturation stage the mean cell volume of guinea pig

maturation appear to be linked; the data showed that 80% of

megakaryocytes doubled with each ploidy doubling. Within each maturation stage the mean cell volume of guinea pig

progressive changes in nuclear morphology. It thus became possible to determine size, ploidy, and matura-

from direct measurement of

quantitative relationship between any two of these parameters by holding the third constant. Size was

found to vary with ploidy or with maturation indepen-

ments, but ploidization and maturation were corre-

dently, but ploidization and maturation were corre-

Additionally,
required up to 1 hr to analyze 1000–3000 megakaryocytes. The flow rate was 2200 cells/sec, with a coincidence level of less than 1%.

**Human Marrow Smears; Ploidy Determination on Guinea Pig Cells**

Routine human marrow aspirates were anticoagulated with 0.1 ml 5% EDTA solution, smeared on clean glass slides and stained with Wright-Giemsa. Coverslip preparations were made of guinea pig cell suspensions by centrifugation onto cover slips and subsequent controlled air drying. Feulgen stains were carried out as described previously. Densitometric quantitation of relative DNA content in megakaryocytes was performed on an Artek 800 Image Analyzer (Artek Systems Corp., Farmingdale, N.Y.). The data were obtained in arbitrary units of relative DNA content. As before, all megakaryocytes were assigned to the ploidy level of the nearest modal value of DNA content.

**Criteria for Identification of Megakaryocytes**

By phase contrast, megakaryocytes were identified by nuclear morphology (bilobed or multilobed), small granule size, prominent light refraction, and occasionally, by surface bullae. Wright-Giemsa stained megakaryocytes were identified by nuclear configuration, granule size and color, and in the younger forms by an overall deep blue not found in monocytes or in any myeloid cells. Feulgen-stained megakaryocytes were recognized by nuclear configuration. Additionally, the younger megakaryocytes have a very high nuclear:cytoplasmic ratio. In normal marrow, no other cell type has a nucleus with multiple large lobes. In this difference, megakaryocytes are distinct from osteoclasts (Howell's polykaryocytes) and from binucleate plasma cells, both of which seem to have clearly separate nuclei, and from myeloid or erythroid precursors of any stage. Monocytes and macrophages often have folded or irregular nuclei but not distinct lobes. In suspension, they have diameters of only 9-10 µm and more cytoplasm than young megakaryocytes; thus, the small megakaryocytes have a higher nuclear volume than monocytes.

**Maturation Stages**

Representative photomicrographs for the different stages are presented in Fig. 1. The youngest (stage I) megakaryocytes had...
bi-, tri-, or multilobed nuclei that occupied almost the entire cell volume. The next stage (II) was comprised of cells with the nuclear lobes moving apart a bit, although still somewhat overlapped, to appear as a "C," "U," or cup-shaped configuration, with a nuclear:cytoplasmic ratio still greater than 1:1. Stage III were the more familiarly appearing megakaryocytes with multiple, separated nuclear lobes (not separate nuclei) and a large cytoplasmic volume. The final stage (IV) were the megakaryocytes preparing for platelet formation with the first parameter by phase contrast and the second parameter by optical micrometer and compared to the sizes of other cell types in the same samples (Fig. 2). The diameters of a large number of isolated megakaryocytes and of unseparated megakaryocytes in bone marrow cell suspensions were measured with an optical micrometer and compared to the sizes of other marrow cell types in the same samples (Fig. 2). The mean size of the marrow (unseparated) megakaryocytes was 25.94 ± 2.35 μm. Megakaryocytes ranged from 10 to 56 μm, while the nonmegakaryocytes were 3–17 μm. The distributions of the megakaryocytes and nonmegakaryocytes were substantially different, with minimal overlap. For unseparated marrow cell populations, only 1.16% of the megakaryocytes were smaller than 13.0 μm, while 2.05% of nonmegakaryocytes were larger than that cut-off. Thus, megakaryocytes are not only larger than the nonmegakaryocytes, but a distinct visual threshold value separated most of the megakaryocyte population from most of the other cells. The distinction between the megakaryocytes and the non-megakaryocytes can be seen in Fig. 1. The smallest megakaryocyte illustrated (row I, first panel) had a cell diameter of 14 μm; the largest nonmegakaryocyte shown (in row IV, third panel, at 12 o’clock) had a cell diameter of 13 μm. Megakaryocyte enrichment altered the distribution of megakaryocyte sizes slightly, so that some of the smaller megakaryocytes were lost. Although fewer megakaryocytes with diameters of 12–21 μm were counted, the threshold phenomenon at 13 μm was not altered.

Flow Cytometry

To confirm this distribution and the existence of a size threshold that discriminates megakaryocytes from the other marrow cells, we examined megakaryocytes in marrow suspensions and at different stages of purification by electronic volume determination as well as by optical measurements. Specimens from three guinea pigs were divided in half for direct comparison of results by the two methods. Data by both measurement techniques are shown for unseparated megakaryocytes, after a density gradient separation, and after a second velocity sedimentation (Fig. 3). A wide range of cell volumes was found, requiring the electronic analyses to be recorded originally on a logarithmic scale; the data were transposed to a linear scale and normalized to obtain peak heights approximating those of the optical data. Electronic trigger levels were set to accumulate data from the downslope of the nonmega-
SIGNIFICANCE OF MEGAKARYOCYTE SIZE

Fig. 3. Size histograms of megakaryocytes measured by an optical micrometer (heavy lines) and of cells in the same samples by electronic volume measurements (thin lines). The data are from a single guinea pig and are representative of two additional similar experiments. The upper histogram in each is of megakaryocytes from unseparated marrow, the middle after a density gradient centrifugation, and the bottom after two velocity sedimentations. The megakaryocyte frequencies were, respectively, 0.3%, 7%, and 88% of the cells in each preparation. Data from electronic volume measurements are shown only above certain trigger levels. In each histogram, the ordinate scale refers only to the optically measured megakaryocytes; the electronic data were normalized to obtain similar peak heights (see text). The upper data are of 533 megakaryocytes (N optical), mean diameter $\bar{x} = 26.4 \pm 7.4$ (SD) $\mu m$, and 287 cells with diameter $>17.0 \mu m$ (N flow); the middle N optical was 1026, $\bar{x} = 28.2 \pm 6.6 \mu m$, while N flow was 43,100; and the bottom N optical was 1026, with $\bar{x} = 27.2 \pm 6.4 \mu m$ and N flow 94,240.

In every case, a distinct inflection point was seen in the downward curve of the nonmegakaryocyte subpopulation, beyond which the electronic size distribution closely matched the megakaryocyte sizes determined optically. In these comparisons the location of these inflection points varied with the proportion of nonmegakaryocytes present. With a much greater frequency of nonmegakaryocytes in unseparated marrow (99.7%) and in partially enriched preparations (93%), the width of the nonmegakaryocyte size distribution was greater at the base than in Fig. 2, displacing to the right the threshold value between the two subpopulations. With increasing frequency of megakaryocytes in the suspensions (top to bottom in Fig. 3), these inflection points in the right tails of the nonmegakaryocyte distributions moved progressively from about 20 to 13 $\mu m$.

Cell Sizes on Marrow Smears

Human megakaryocytes were examined to see if their size distribution in marrow smears was similar to that in suspension and if a size threshold was present. Comparisons were made between the megakaryocyte sizes on traditional marrow aspirate smears and the sizes of cells from the same three samples examined in suspension by phase contrast (Fig. 4). The latter data were similar to that found in the guinea pig. In suspension (above), human megakaryocytes (solid lines) ranged from 12 to 63 $\mu m$ and formed a subpopulation larger than and distinct from the other marrow cells (dashed lines), which ranged from 5 to 20 $\mu m$. A definite separation between the two populations was observed at 14 $\mu m$ when equal numbers of megakaryocytes and nonmegakaryocytes were measured in a wet mount preparation. On marrow smears (below), the same size relationships were found between the megakaryocytes (solid lines) and the other cells (dashed lines), but all cell types appeared significantly larger on the smears than in suspension. The flattening of megakaryocytes on smears was associated with an apparent increase in mean diameter from 27 to 36 $\mu m$ ($p < 0.001$ by Student’s $t$ test). Thus, the point of overlap between the two marrow subpopulations also shifted to the right, from 14 $\mu m$ to 19–23 $\mu m$. While some megakaryocytes on marrow smears remained below this threshold, only 1.3%–2.0% of the nonmegakaryocytes were larger than this. In this study almost all cells with diameters greater than about 20 $\mu m$ were megakaryocytes. Morphological evaluation of all cells $>14 \mu m$ could simply and rapidly identify most (87%–97%) of the megakaryocytes on human marrow aspirate smears.

Relationship of Size to Ploidy

Because the large size of megakaryocytes has been related to their unusual DNA content, we examined on dried smears the sizes of guinea pig megakaryocytes...
Fig. 4. Histograms of megakaryocyte diameters from unseparated human samples (upper histograms) and on clinical smears (lower). Each panel is from a different patient. As in Fig. 1, the megakaryocytes (solid lines) comprised a broad peak of large cells and the nonmegakaryocytes (dashed lines) formed a narrow peak of smaller cells. The sizes of megakaryocytes and nonmegakaryocytes appeared larger on smears compared to their three-dimensional (rounded) diameters in suspension. The point of overlap between the two populations shifted to the right and provided better discrimination between them in each comparison. Left panel: the number of megakaryocytes counted in suspension was 279, on smears, 279, and nonmegakaryocytes, 279 and 279, respectively. The corresponding numbers for the middle panel were 268 and 278, and 250 and 270. For the third patient, 247 and 158, and 186 and 232, respectively.

Fig. 5. Histograms of megakaryocyte sizes by ploidy levels. A slightly enriched preparation (3% megakaryocytes) of guinea pig marrow was examined. Right panel is a composite histogram of all megakaryocyte sizes (n = 1105). Middle panel shows separate 8N, 16N, and 32N subpopulations of megakaryocytes. The size distributions of 4N megakaryocytes and 2N and 4N nonmegakaryocytes are plotted separately, in the left panel. Representative data from 15 different analyses. Some variation was found in the relative proportions, but not size distributions of the different ploidy groups, according to the degree of megakaryocyte purification.
Fig. 6. Three-dimensional display of 2052 megakaryocytes plus 210 nonmegakaryocytes (130 2N, 90 4N) evaluated for size and ploidy. Numbers of cells at each size/ploidy level are represented by topographic lines. The outer, heavy line encompasses all cells measured; succeeding contour lines mark increments of 5 cells/size-ploidy units. The peak is at 40 cells having 100-104 ploidy units and size 34-36 μm. In a histogram of cell number versus arbitrary ploidy units (not shown), the modal values were at 10, 21, 46, 94, and 198 ploidy units.

After assignment of all megakaryocytes, the size range of each ploidy group was as follows: 2N, 10 ± 6; 4N, 21 ± 5; 8N, 46 ± 19; 16N, 96 ± 30; and 32N, 198 ± 60 μm. Thus, in the three-dimensional data, discrete peaks show progressively increasing size with ploidy doublings between 8, 16, and 32N.

SIGNIFICANCE OF MEGAKARYOCYTE SIZE

Isolated megakaryocytes were grouped by ploidy (Fig. 5). The megakaryocytes at each ploidy level comprised different size groups. The cell diameters increased progressively with ploidy, from 4N to 32N. The 4N megakaryocytes were often not distinguishable from the great numbers of 4N nonmegakaryocytes; however, the diameters of 4N cells recognizable as megakaryocytes were the same as those of other 4N cells. The composite histogram of megakaryocyte diameters shows some features of its component ploidy groups (right panel). There is a prominent peak to the left, largely comprised of the 8N subpopulation, and another smaller one on the right, reflecting part of the 32N contribution. Such peaks were usually seen with optical measurements; with electronic data, a small shoulder was frequently seen in these two regions (see Fig. 3).

Another way of presenting this kind of data is in a three-dimensional histogram of megakaryocyte size and ploidy measurements (Fig. 6). Each ploidy group was clearly distinct. The individual peaks or hills, as indicated by the topographic lines, were the 2N non-megakaryocyte reference cells and the 8N, 16N, and 32N megakaryocyte groups.

Maturation, Size, and Ploidy

It had seemed that within each ploidy group, size might also be related to maturation. A large sample of 6N megakaryocytes, slightly enriched from the marrow of a single guinea pig, was examined to simultaneously determine the maturation, size, and ploidy of each cell (Fig. 7 and Tables 1-3). Megakaryocyte mean size progressively increased with maturation. Although definite overlaps were present in each comparison (Fig. 7), the ranges and mean sizes of megakaryocytes in maturation stages I, II, and III were clearly different from each other (Table 1).

Stage IV was only slightly, though significantly, greater in its mean size than that of stage III. A stepwise progression of sizes was also apparent from each ploidy group to the next higher one (Table I).

The same data were broken down into subgroups to examine the nature and significance of the relationships of size to maturation and of size to ploidy with the other component held constant (Table 2). Within each of the four maturation stages, mean diameter increased progressively with ploidy. Exact doublings (2.0 ± 0.1-fold) of mean cell volume were found in stage I from 4N to 8N and from 8N to 16N, in stage II from 8N to 16N, and in stage III from 8N to 16N and from 16N to 32N.* The groups in Table 2 can also be
Fig. 7. Histograms of megakaryocyte size by maturation stage (upper left) and by ploidy level (lower left). Composite histogram of megakaryocyte size frequencies at right (n = 1014). Each cell was examined for all three parameters. Megakaryocyte size increased progressively with both maturation and ploidy level. Quantitative data are given in Tables 1–3.

Table 1. Megakaryocyte Sizes by Maturation Stage and by Ploidy Group

<table>
<thead>
<tr>
<th></th>
<th>Maturation Stage</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Range</td>
<td></td>
</tr>
<tr>
<td>Maturation stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>14.4</td>
<td>3.5</td>
<td>7–28</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>22.8</td>
<td>5.7</td>
<td>10–40</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>33.0</td>
<td>8.1</td>
<td>16–61</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>37.5</td>
<td>6.0</td>
<td>20–49</td>
<td></td>
</tr>
<tr>
<td>Ploidy Group</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4N</td>
<td>11.8</td>
<td>2.8</td>
<td>7–22</td>
<td></td>
</tr>
<tr>
<td>8N</td>
<td>18.4</td>
<td>6.0</td>
<td>10–39</td>
<td></td>
</tr>
<tr>
<td>16N</td>
<td>28.8</td>
<td>7.8</td>
<td>12–52</td>
<td></td>
</tr>
<tr>
<td>32N</td>
<td>38.1</td>
<td>8.4</td>
<td>18–39</td>
<td></td>
</tr>
</tbody>
</table>

All values are in μm. The sizes of stages I, II, III, and IV are different from each other with \( p < 0.001 \) in each case. The sizes of 4N, 8N, 16N, and 32N megakaryocytes are also different from each other, with \( p < 0.001 \) for each comparison. (Student's t test). The population mean diameter is 27.6 ± 10.4 (SD) μm.

Rearranged to show the relationship of size to maturation independent of the ploidy levels. Thus, megakaryocyte size was related independently to these two parameters. Two-way analysis of variance was performed with log-transformed data and demonstrated highly significant and independent influences of maturation stage and ploidy level on megakaryocyte size.

That ploidy and maturation stage are not independent of each other, however, was suggested by the similarities in peak height and configuration in Fig. 7 between the 8N and stage I megakaryocytes and between 16N and stage III. In fact, almost half of the 8N cells were stage I megakaryocytes and almost two-thirds of the 16N cells were stage III. In Table 3 it can be seen that low ploidy was strongly associated with immaturity, and vice versa. In the rows, of 4N megakaryocytes, 90.2% were in stage I, 9.8% in stage II, and none in stages III–IV. Of 32N cells, 0.5% were stage I, and 85.4% were in stages III–IV. In the
columns, 70.9% of stage I cells were 4N or 8N and 0.5% were 32N. Of stage IV cells, 95.3% were 16N or 32N, while 4.6% were 8N and none were 4N. Chi-square analysis of this distribution indicated that it was very unlikely to have arisen by chance \( (p < 0.001) \). Even excluding the 4N megakaryocytes, which are difficult to detect, the maturation pattern of ploidy groups 8N–32N does not have a random distribution \( (p < 0.001) \).

### DISCUSSION

In the present study the size distribution of megakaryocytes was different from many estimates.\(^\text{3-9}\) Several investigators of megakaryocyte sizes have used photognavimetnic techniques and reported only relative enal investigators of megakaryocyte sizes have used distribution (ploid level

| Table 2. Megakaryocyte Sizes by Ploidy Groups, Independent of Maturation Stages |
|----------------------------------|------------------|------------------|------------------|------------------|------------------|
| Maturation Stage | Ploidy | Mean \( \mu m \) | SD \( \mu m \) | \( n \) |
|------------------|--------|------------------|------------------|------------------|------------------|
| I                | 4N     | 11.4             | 2.3              | 46               |
|                  | 8N     | 14.3             | 2.5              | 83               |
|                  | 16N    | 17.4             | 2.8              | 51 NS            |
|                  | 32N    | 18.2             | 1               | 1 NS             |
| II               | 4N     | 16.7             | 3.6              | 5               |
|                  | 8N     | 19.2             | 5.8              | 60 NS            |
|                  | 16N    | 22.8             | 5.6              | 122 NS           |
|                  | 32N    | 27.5             | 5.2              | 27 NS            |
| III              | 4N     | 0               | 0               | 1 NS             |
|                  | 8N     | 24.4             | 4.7              | 44 NS            |
|                  | 16N    | 31.3             | 6.6              | 376 NS           |
|                  | 32N    | 39.8             | 7.6              | 155 NS           |
| IV               | 4N     | 0               | 0               | 1 NS             |
|                  | 8N     | 24.1             | 5.2              | 2 NS             |
|                  | 16N    | 36.9             | 4.7              | 32 NS            |
|                  | 32N    | 42.5             | 5.0              | 9 NS             |

Within each stage, the ploidy subgroups progressively increased in size. If the converse was examined similarly, that is with the ploidy level held constant, megakaryocyte sizes increased with maturation stage

#### Table 3. Number of Megakaryocytes of Particular Maturation Stages and Ploidy Groups

<table>
<thead>
<tr>
<th>Stages</th>
<th>Ploidy</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>4N</td>
<td>46</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>8N</td>
<td>83</td>
<td>18.6</td>
</tr>
<tr>
<td></td>
<td>16N</td>
<td>51</td>
<td>57.3</td>
</tr>
<tr>
<td></td>
<td>32N</td>
<td>1</td>
<td>18.9</td>
</tr>
<tr>
<td>II</td>
<td>4N</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>8N</td>
<td>60</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>16N</td>
<td>44</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>32N</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td>III</td>
<td>4N</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>8N</td>
<td>122</td>
<td>37</td>
</tr>
<tr>
<td></td>
<td>16N</td>
<td>376</td>
<td>57</td>
</tr>
<tr>
<td></td>
<td>32N</td>
<td>155</td>
<td>25</td>
</tr>
<tr>
<td>IV</td>
<td>4N</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>8N</td>
<td>2</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>16N</td>
<td>32</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>32N</td>
<td>9</td>
<td>2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total n</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>181</td>
<td>17.9</td>
</tr>
<tr>
<td>214</td>
<td>21.1</td>
</tr>
<tr>
<td>575</td>
<td>56.7</td>
</tr>
<tr>
<td>1,014</td>
<td>4.2</td>
</tr>
</tbody>
</table>

DISCUSSION

The discovery of a relative size threshold between the megakaryocytes and all other marrow cell types should facilitate reliable detection of essentially all the megakaryocytes in clinical and research studies. The threshold phenomenon was found in each of several different kinds of specimens and methods of measurements, although the exact size as an essentially single criterion of cell identification varied with the specific technique. A size criterion of 20 \( \mu m \) seems sufficient to detect only megakaryocytes. Recognition of the smaller megakaryocytes on marrow aspirate smears has usually been more tenuous than identification of the larger megakaryocytes. In the past, during rapid scanning of marrow, one generally sought only the notably large megakaryocytes, of at least 25–30 \( \mu m \) in diameter, unaware that a significant proportion were not so large and that subtle differences in size from the background nonmegakaryocytes (i.e., >14 \( \mu m \)) could be readily detected. The nonmegakaryocytes in this in-between range (14–20 \( \mu m \)) can be readily distinguished from the smaller megakaryocytes by the application of a few morphological criteria. The use of nuclear lobulation for identification of megakaryocytes of any size was based on correlated ultrastructural phase contrast,\textsuperscript{13,14,16,28} and Wright-Giesma studies. All cells with multiple nuclear lobes
had ploidy levels of ≥4N, thus excluding the diploid
great majority of marrow cells and providing further
support for the specificity of the nuclear configuration
criterion.

The larger nonmegakaryocytes were generally
premitotic myeloid precursors, particularly myelocytes,
which were easily identified by their particular grana-
nulation and nuclear configuration. Since the
nuclei of myeloblasts, promyelocytes, and myelocytes
are mostly round or oval and did not appear to flatten
out on smears, the smallest megakaryocyte nuclei (10
μm in diameter) generally appeared bigger than the
nuclei of these other cell types. Conversely, the young-
est megakaryocytes have, besides a very high nuclear:cytoplasmic ratio, deeply basophilic cyto-
plasm, which sometimes obscures the nucleus. There-
fore, whole fields of marrow cells can be scanned
rapidly for the small number of the total that are larger
than the others; of those cells just slightly larger, the
megakaryocytes can be distinguished on the basis of
their cytologic features. With this approach, almost all
normal megakaryocytes can be detected simply, with-
out histochemical or immunochemical manipulations.
Our previous report showed that these phase contrast
criteria can be used to identify the whole population of
megakaryocytes detected by acetylcholinesterase his-
tochemistry in rats, down to diameters of 10 μm.28 The
exceptions are the relatively rare, even smaller (6–10
μm)29,30 newly differentiated megakaryocytes that
have not yet developed sufficiently to reach this size
threshold and these morphological criteria. The abso-
lute data on the ranges of megakaryocyte sizes and
mean diameters in different ploidy groups can provide
a simple perspective on the normalcy or aberrancy of
megakaryocytes in clinical specimens, without the
need for expensive and time-consuming quantitation of
ploidy levels. On the other hand, studies are underway
to determine what absolute size can be used as a single
criterion in flow cytometry to count and describe
population size characteristics in megakaryocytes.

The present study showed a strong association
between maturation stage and ploidy level in unperturbed
animals. In vitro, development of both parameters
appears to depend on thrombopoietin-like materi-
als.31,32 The in vivo response to thrombocytopenia
includes increases in ploidy and size of the megakaryo-
cytes and has also been thought to include an accelera-
tion in the rate of maturation.33,34 The observations
supporting the latter idea were based on movement
of tritiated thymidine-labeled cells through the different
maturation steps. This idea had been considered with
caution, because some degree of thymidine reutiliza-
tion occurs even within 24 hr.35 Nonetheless, there may
be an acceleration of maturation as a consequence of
higher ploidy levels,37 perhaps because of increased
RNA synthesis per DNA unit.38 Such an increase in
protein synthesis may be the basis of present assays for
thrombopoietin that measure isotope incorporation
into proteins or glycoproteins of newly formed plate-
lets.39–41

The present demonstration of a link between
maturation stage and ploidy level was more marked than in
past studies, probably because of the simultaneous
measurements, the quadruplicate scheme of maturation
stages, and the inclusion of 4N megakaryocytes. Future
studies will examine the simultaneous kinetics of these parameters in thrombocytopenia and
thrombocytosis.

REFERENCES

1. Howell WA: Observations on the occurrence, nature, and
function of the giant cells of the bone marrow. J Morphol 4:117,
1890
2. Japa J: A study of the morphology and development of the
3. Dameshek W, Miller EB: The megakaryocytes in idiopathic
thrombocytopenic purpura, a form of hypersplenism. Blood 1:27,
1946
X. Morphologic characteristics of megakaryocytes by phase contrast
microscopy in normals and in patients with idiopathic thrombocy-
topenic purpura. Blood 8:703, 1953
Thrombopoiesis in vitro: Experiments with animal and normal
York, Springer-Verlag, 1973
8. Wintrobe MM, Lee GR, Boggs DR, Bithell TC, Foerster J,
Athens JW, Lukens JN: Clinical Hematology (ed 8). Philadelphia,
Lea & Febiger, 1981
1968
10. Cowan D, Hines JD: Thrombokinetics in dietary-induced
12. MacPherson GG: Changes in megakaryocyte development
13. Levine RF, Fedorko ME: Isolation of intact megakaryocytes
14. Levine RF: Isolation and characterization of normal human
15. Jolly J: Traite Technique d’Hematologie. Paris, Maloine,
1923
16. Levine RF: Culture in vitro of isolated guinea pig mega-
karyocytes: Recovery, survival, morphologic changes, and matura-
17. Behnke O: An electron microscope study of the megakaryo-
cyte of the rat bone marrow. I. The development of the demarcation
membrane system and the platelet surface coat. J Ultrastruct Res
24:412, 1968
18. MacPherson GG: Development of megakaryocytes in bone
22. Levine RF, Bunn PA Jr, Hazzard KC, Schlam ML: Flow cytometric analysis of megakaryocyte ploidy. Comparison with Feulgen microdensitometry and discovery that 8N is the predominant ploidy class in guinea pig and monkey marrow. Blood 56:210, 1980
The significance of megakaryocyte size

RF Levine, KC Hazzard and JD Lamberg