Platelet Size in Man

By Jean-Michel Paulus

The shape and parameters of platelet size distributions were studied in 50 normal persons and 97 patients in order to test the proposed thesis that platelet size heterogeneity results mainly from aging in the circulation. This thesis was contradicted (1) by size distributions of age-homogeneous, newly-born cell populations which were lognormal with increased (instead of decreased) dispersion of volumes and (2) by the macrothrombocytosis found in some populations with normal age distribution. For these reasons, thrombocytopoiesis appeared to play the major role in determining platelet size. A model was built in which the volume variation of platelet territories due to megakaryocyte growth and membrane demarcation at each step of maturation was a random proportion of the previous value of the volume. This model explains the lognormal shape of both newborn and circulating platelet size distributions. It also implies that (1) the mean and standard deviation of platelet logvolumes depend on the rates of volume change of the individual platelet territories (growth rate minus demarcation rate) as well as on megakaryocyte maturation time; (2) platelet hyperdestruction causes an increase in the mean and dispersion of the rates of territory volume change; (3) Mediterranean macrothrombocytosis and some hereditary macrothrombocytopenias or dysthrombocytopeieses reflect a diminished rate of territory demarcation, and (4) platelet size heterogeneity is caused mainly by the variations in territory growth and demarcation and not by aging in the circulation.

Studies of circulating platelets indicate that these cells are markedly heterogeneous in size, density, and metabolic, functional, and biochemical properties. It has been suggested that this heterogeneity results from aging in the circulation and that young platelets are larger, denser, and more active than the older ones. Thus, disorders involving an increased proportion of young platelets, because of hyperdestruction or a sudden release of newly produced cells, would normally be accompanied by macrothrombocytosis. In fact, such is the case in idiopathic thrombocytopenic purpura (ITP), although opposite opinions have been expressed, in systemic lupus erythematosus, certain drug-induced thrombocytopenias, disseminated intravascular coagulation, the hereditary macrothrombocytopenia of the Bernard-Soulier syndrome, and the regeneration phase following experimentally induced thrombocytopenia.

However, in the disorders listed above, changes in thrombocytopoiesis also occur, and it is therefore possible that altered platelet production plays a role in the genesis of macrothrombocytosis. Macrothrombocytosis has also been
associated with normal platelet survival in certain hereditary disorders, indicating that factors other than the percentage of young platelets determine platelet size distributions. Similar conclusions have been drawn from animal experiments and from the microthrombocytosis associated with the Wiskott-Aldrich syndrome, in which platelet life span is reduced. It is the purpose of this paper to investigate the role and nature of the megakaryocytic mechanisms involved in the determination of platelet size. The shape and parameters of size distribution in normal subjects were therefore compared with those of patients with macrothrombocytosis having reduced or normal platelet survival.

MATERIALS AND METHODS

Blood samples (3.3 ml) for determination of platelet volume distribution (PVD) were collected in 4-ml, internal diameter 10 mm, Vacutainer tubes (Becton-Dickinson, Rutherford, N.J.) containing 0.7 ml acid-citrate dextrose solution (ACD, USP, formula A, Fenwall Laboratories, Morton Grove, Ill.) from 50 normal persons and 97 patients. The tubes were kept at 37°C, and studies were performed within 2 hr. Preliminary studies showed that this procedure maintained constant platelet shape, PVD, and count. Specimens kept at room temperature for up to 1 hr could also be used provided they were incubated for 1 hr at 37°C before size measurement. Incubation at 4°C was not satisfactory, since cold temperature caused the amplitude of platelet pulses to increase because of volume and/or shape changes.

Microscopic Determinations of Size Distribution

Two techniques for determination of size distribution were used. (1) Platelets in ACD blood samples were sized after smearing on glass slides and staining with the May-Grünwald-Giemsa method. Diameters were measured with a 100 x objective and a calibrated screw-micrometer fixed on a 8 x eyepiece (Zeiss, Oberkochen, Germany, catalog No. 46 3972; one division in the micrometer represented 0.1 \( \mu \text{m} \)). The surface was calculated from the average of two diameter measurements. (2) Platelets in platelet-rich plasma (PRP) prepared from EDTA blood specimens were measured as a vital preparation on a glass slide by use of a phase-contrast microscope and the same screw-micrometer as above. Under these conditions, platelets were spherical, and their volumes could be calculated from the diameters. The optical system comprises a 40 x objective and a 2 x magnifying glass so that each division corresponded to 0.117 \( \mu \text{m} \). All measurements were made within 15 min after blood collection.

Preparation of Platelet Suspensions for Electronic Counting and Sizing

Platelet-rich plasma (PRP) was prepared by centrifugation of the Vacutainer tube in a table centrifuge for 25 sec at 40 g (800 rpm) according to the method of Bull. A plastic head holding the tubes vertically and with a capacity of 20 tubes was build in order to keep the acceleration applied to the sample independent of the height of the blood column, i.e., of the sample volume; this device made it possible to prepare a reproducible PRP whether or not blood had been drawn previously from the tube for red and white cell measurements with the Coulter Counter Model S. The final platelet suspension was prepared from PRP by diluting 6.6 \( \mu \text{l} \) of PRP in 20 ml of Isoton (Coulter Electronics, Hialeah, Fla.). In order to verify that size distributions were not affected by centrifugation at 40 g, platelet surface distributions on smears (see below) were compared in the original uncentrifuged blood sample and in the PRP. No significant change in shape or parameters of the distribution was observed in four experiments using samples from normal subjects or patients with macrothrombocytosis.

In one experiment, platelets were rounded by addition to the PRP of 0.04 g/100 ml promethazine hydrochloride. The cells were then incubated from 3 to 30 min at 37°C and diluted in Isoton as above.

Platelet Counting.

Platelet counting was performed from Isoton suspensions prepared from EDTA or ACD samples using the Coulter Counter method of Bull, Schneiderman, and Brecher. The hematocrit
Platelet size was measured with the Coulter Model S. For patients with platelet volumes higher than ~15 cu µm, the usual discriminator settings were invalid, and the count was verified by phase-contrast microscopy.

Electronic Determination of Platelet Size Distribution

A model Coulter Counter, model F (using a 70-µm aperture; attenuation and current intensity settings were ½), was connected to a 400-channel analyzer (Model SA40B, modified to analyze pulses with maximum rising time of 50 µsec; Intertechnique, Plaisir, France) according to Le Go, Le Go, and Crouillère.33 In conditions where particle shape, electrolyte resistance, elec-

Fig. 1. Frequency (A) and log-frequency (B) distributions of volumes, as well as frequency distribution of logvolumes (C) in platelets from a normal subject. The abscissa covers a range of 0–22 cu µm. Platelet logvolumes have a bell-shape, symmetric distribution, suggesting normal distribution. On a logarithmic ordinate, the descending slope of the volume distribution is approximately linear.
tronic amplification and capillary aperture diameter were kept constant, the Coulter Counter pro-
duced electrical impulses whose amplitude was proportional to the volume of the individual
particles passing through the aperture. The channel analyzer recorded a 100-200 class histogram
of the pulse amplitudes produced by platelet particles. Determinations made with calibrated
latex particles (3.423 cu μm) showed that corrected mean channel number (mean channel num-
ber x amplification factor) was linearly related to particle volume. No correction of PVD for
particle concentration was made, since nearly identical curves were recorded from suspensions con-
taining from 59,000 to 680,000 platelets per ml. The counting was discontinued when the channel
corresponding to the modal (peak) volume contained 4000 counts (the total number of pulses
counted was about 100,000).

Probit Plots of Platelet Size

In order to study the shape of platelet size distributions, probit plots of cumulative frequency
versus size parameters were made from the microscopic or electronic determinations. The latter
often had to be truncated at the upper end of the spectrum because of red cell contamination.
It was then necessary to correct the experimentally obtained total platelet count. This was done
by using the empirical finding that the frequency count in the second half of the volume distribu-
tion decreased approximately exponentially with the channel number (Fig. 1). The corrected,
absolute count in the sample was

\[ Z = \sum_{0}^{100} X_j + [X_{100} \cdot h/0.693] \]

where \( X_j \) was the count in channel \( j \) and \( h \) the number of channels associated with halving of the
count per channel. The probit plots were then drawn for the range 0 to 22-27 cu μm, excluding
the truncated fraction.

Computation of Size Parameters

As will be shown later, nearly all platelet volume distributions so far studied were compatible
with the lognormal model, i.e., platelet logvolumes were normally distributed. Since logarithmic
transformation of the Coulter pulses was not available until recently, the following formula was
used to convert the original volume distribution into a logvolume curve with constant class width:

\[ f_{\ln x} = f_x \cdot \frac{L1}{L2} \]

where \( f_{\ln x} \) and \( f_x \) represent the relative frequency in the classes \( \ln x \) with width \( L1 \) and \( x \) with
width \( L2 \), respectively. \( L1 \) and \( L2 \) were calibrated daily and varied by about 5% during a 1-yr
period. Typical values were 0.027 (logvolume units) and 0.21 cu μm, respectively.*

The logvolume distribution of the Coulter pulse amplitudes was influenced by both electronic
and particle contamination at the lower range of the curve and by small erythrocytes at the higher
end. These artifacts prevented the calculation of absolute platelet size values from the usual
formulae relating the mean and standard deviation to particle frequency in the various classes.
This difficulty was overcome by using Bhattacharya’s technique (Fig. 2). A normal distribution,
such as that of logvolumes, can be described by an equation of the form \( F = w \cdot \exp(hz^2) \). Hence,
\( \ln F_{\logvol} = \ln w + hz^2 \) is a parabola, and the derivative of the latter equation is \( d(\ln F_{\logvol})/dz = \)2hz, indicating that the differences of the logfrequencies in the lognormal distribution can be
fitted by a straight line (Fig. 2). It can further be shown that this line crosses the logvolume
abcissa at the mean logvolume estimate plus one-half the channel width and has a slope very

*Because these standards were inert spherical particles which differed in shape and membrane
properties from platelets, the size values do not represent absolute estimates. The mean and stan-
dard deviation of volumes determined after calibration with red cells were 37% greater, i.e., log-
volumes were increased by 0.31 unit.
The subscripts \(L\) and \(V\) will be used in the symbols of size parameters to designate the log-volume \((L)\) and volume \((V)\) distributions. For instance, \(\mu_L\) and \(\delta_L\) represent the mean of the log-volume and volume distributions and \(\mu_V\) and \(\delta_V\), the corresponding standard deviations. Other symbols are explained in Table 1. All logarithms used are Naperian.

Fig. 2. Determination of mean and standard deviation of logvolumes by the computer method described in the text. The top panel has a linear volume abcissa, while the lower panel has a geometric scale for the abcissa. In both graphs, the total range covers 26.47 cu mm. The vertical lines indicate the limits from 3.5 to 15 cu mm between which background and red cell pulses do not influence the curves. Because of the change in scale, these limits correspond to channels 16 and 69 in the top panel and 45 and 99 in the lower one. The volume distribution recorded in the channel analyzer, which has a constant volume class interval, is converted into a straight line using the conversion of the ordinate explained in the text. The regression line reaches the zero ordinate at the median volume, whose logarithm is the mean logvolume \(\mu_L\), and the slope is inversely proportional to the variance of logvolumes \(\delta^2_L\), close to \(-1/\delta^2_L\), where \(\delta_L\) is the logvolume standard deviation.† In this analysis, distinct pulse populations may appear as separate lines in the graph of logfrequency differences. Using channel limits corresponding to 3.5 and 15 cu mm, it was possible to delineate a region where platelet pulse distribution was not influenced by electronic or particle contamination. The differences of the logfrequencies for about 50 classes in the above interval were therefore entered into a linear regression subroutine. From the estimates of \(\mu_L\) and \(\delta_L\) obtained, the parameters of the original volume distribution were then computed (Table 1). The thrombocytocrit (ml of platelets per 100 ml of total blood) was calculated as the product of platelet count and volume.

The validity of this mathematical analysis was demonstrated in the mean volume range 4.5-20 cu mm as follows: (1) In seven cases where contamination at both ends of the curve was minimal, \(\mu_V\) and \(\delta_V\) were calculated by both the present and standard methods. The results differed on the average by 0.9\% \((\mu_V)\) or 6.4\% \((\delta_V)\). (2) Forty different lognormal distributions with \(\mu_L = 1.5\) to 2.4 and \(\delta_L = 0.5\) to 1.2 were simulated on a 1800 IBM computer according to Aitchison and Brown. To each simulated volume distribution, a background distribution measured for 1 min on a day of usual laboratory activity was added. The simulated values were then used as data for the size parameter calculations described above. It was found that the calculated values were closely correlated with those expected \((r\) varying between 0.96 and 0.99), although there was a small systematic error, reflected by the value of the ordinate at the origin of the regression equation between expected and recorded values (Table 1). (3) The influence of red cell contamination was assessed by adding various volumes of Coulter Counter Cell Control (4C, Coulter Electronics) to a platelet suspension in Isoton. Red cell contaminations of 3.5\%, 15.6\%, 23.5\%, and 40.1\% caused no significant variation of \(\mu_V\) and \(\delta_V\) (coefficient of variation of the four determinations was 2.5\% for \(\mu_V\) and 2.7 for \(\delta_V\)). (4) The reproducibility of the method in normal subjects is also given in Table 1 and shows that a reproducible estimate can be obtained for most parameters. The greatest variability was observed for \(\delta_V\).
Determination of Platelet Survival

Samples of the circulating platelet populations were labeled with $^{51}$Cr by use of the acid-citrate method\textsuperscript{16} and injected into ten normal persons and 28 patients in order to correlate the survival and size parameters. Autologous platelets were used in all cases. In all patients with macrothrombocytosis, whole-blood samples were used as an index of platelet survival in order to avoid the loss of large platelets during platelet isolation procedures. In all the cases reported here, red cell-bound $^{51}$Cr activity injected was less than 2.5% of the total platelet activity injected. The survival data $S(t)$ (i.e., the proportion of injected activity remaining in the circulation at times varying from 30 min to a time corresponding to zero activity) were fitted to a polynomial regression using a program written for a 360/50 IBM computer. From the best fit survival equation $S(t) = c(0) + c(1)t + c(2)t^2 + \ldots + c(n)t^n$, platelet mean life span* could be computed as $q = -c(0)/c(1)$, and the mean age of circulating platelets was calculated as the area under $S(t)/S(0)$. Previous studies using simulated survival data with known mean life span and age showed that this method gave an accurate estimate (error less than 10%–15%) of the survival parameters.\textsuperscript{39} Furthermore, in all the cases reported here, repeated platelets counting in the days preceding platelet separation and transfusion showed no significant change which could prejudice the use of the above formulae.\textsuperscript{35} For short survivals ($q$ less than a day), however, the best results were obtained when the logarithms of survival data, rather than the survival data themselves, were entered into the polynomial regression program. In all the cases of short survival studied here the logarithms of activity could be fitted by a first-degree polynomial, and both the mean life span and age were equal to $-1/c(1)$.

\*In this study, mean life span refers, as usual, to mean life expectancy of newly born cells whereas mean age refers to the circulating population.

### Table 1. Size Parameters in Normal Subjects

<table>
<thead>
<tr>
<th>Size Parameter</th>
<th>Symbol</th>
<th>Normal Values*</th>
<th>Accuracy of Mathematical Analysis</th>
<th>CV of Repeated Determinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Logvolume distribution ($L$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>$\mu_L$</td>
<td>1.68 1.38–1.86</td>
<td>$\gamma = -0.08 \pm 1.02 X$</td>
<td>0.99</td>
</tr>
<tr>
<td>SD</td>
<td>$\delta_L$</td>
<td>0.61 0.50–0.74</td>
<td>$\gamma = 0.04 \pm 0.98 X$</td>
<td>0.98</td>
</tr>
<tr>
<td>CV</td>
<td>$K_L = 100 \mu_L / \delta_L$</td>
<td>36.0 29.8–44.5</td>
<td>$\gamma = 1.32 \pm 1.03 X$</td>
<td>0.97</td>
</tr>
<tr>
<td>Volume distribution ($V$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>$\mu_V = \exp(\mu_L + 0.5 \delta_L^2)$</td>
<td>6.6 4.8–8.5</td>
<td>$\gamma = -0.16 \pm 1.02 X$</td>
<td>0.98</td>
</tr>
<tr>
<td>SD</td>
<td>$\delta_V = (\mu_V - \mu_L)/100$</td>
<td>4.3 3.1–7.2</td>
<td>$\gamma = -0.34 \pm 1.15 X$</td>
<td>0.96</td>
</tr>
<tr>
<td>CV</td>
<td>$K_V = 100 \exp(\delta_V^2 - 1)$</td>
<td>66.0 53.8–86.8</td>
<td>$\gamma = 3.22 \pm 1.02 X$</td>
<td>0.96</td>
</tr>
<tr>
<td>Median</td>
<td>$Med_V = \exp(\mu_L)$</td>
<td>5.4 4.0–6.4</td>
<td>$\gamma = -0.19 \pm 0.99 X$</td>
<td>0.99</td>
</tr>
<tr>
<td>Mode</td>
<td>$Mode_V = \exp(\mu_L - \delta_L^2)$</td>
<td>3.7 2.8–4.6</td>
<td>$\gamma = -0.05 \pm 0.93 X$</td>
<td>0.99</td>
</tr>
</tbody>
</table>

*Results of determinations made in 50 laboratory personnel. 
1 Accuracy of mathematical estimation was determined by calculating the regression equation and correlation coefficient of recorded ($\gamma$) versus expected ($x$) value of the parameter, using simulated volume distributions as described in text. 
2 CV, coefficient of variation (100 times SD of experimental results divided by mean). 
3 Eight determinations made from one platelet suspension in Isoton. 
4 Eight determinations made in one normal subject. 
5 Normal values, and errors of determination of size parameters measured in suspensions of normal platelets using ACD blood. 
6 Equations relating the parameters in the volume distribution to the mean $\mu_V$ and SD $\delta_V$ of the log-volume distribution are shown in the second column. All volumes are expressed in cubic micrometers. Naperian logarithms are used in all cases. The calibration was made by means of latex particles measuring 3.42 cu pm. 
7 All column.

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*In this study, mean life span refers, as usual, to mean life expectancy of newly born cells whereas mean age refers to the circulating population.
RESULTS

Distribution of Platelet Sizes in Normal Subjects

When sized by the electronic method, circulating platelets of 50 control laboratory personnel had a unimodal, positively skewed distribution of volumes (Fig. 1). However a bell-shape, symmetrical curve was obtained when the frequency distribution of logvolumes was recorded either by interfacing a logarithmic amplifier between the Coulter Counter and the analyzer (Fig. 1) or by converting the original volume curve as described above. Furthermore, a linear relationship could be demonstrated between the differences of the logfrequencies and the corresponding logvolumes (Fig. 2) or between the probits corresponding to the cumulative frequencies and the logvolumes (Fig. 3). These results suggested that platelet logvolumes were normally distributed, i.e., that platelet volumes had a lognormal distribution.

The shape characteristics of platelet size distribution did not depend on the procedure used. The probit graph was not influenced by the dimensions of the aperture between 30 and 70 μm (Fig. 3), by particle concentration (from 50,000

![Graph showing distribution of platelet sizes](image)

**Fig. 3.** Lognormal distribution of platelet sizes in normal subjects. The cumulative frequencies are plotted on a Gaussian probability scale against the logarithms of volume (logvolumes). The curves have been displaced relatively to each other to facilitate comparison of shape. Each interval in the abscissa corresponds to an increase of logvolume or logsurface of 1.0 U, i.e., to an increase in volume or surface by a factor of 2.781. In experiments (A–C) sizes were determined by the Coulter Counter channel analyzer method. (A) One single suspension was measured with Coulter aperture tubes having diameters and lengths (μm) equal to 70 × 84; 70 × 56, 50 × 50, and 30 × 45, respectively. (B) Comparison between control discoid platelets and platelets sphered with promethazine. (C) Curvilinear graph obtained with mixed suspensions of normal (mean volume, 8.0 cu μm) and macrocytic (13.8 cu μm) platelet populations. (D) and (E) Distributions obtained from microscopic measurements of diameters on suspensions (D) or smears (E) (see Materials and Methods). (D) represents logvolume distributions from ten normal subjects. (E) are the logsurface distributions of normal platelets measured in smeared plasma or blood. The lognormal distribution of sizes is demonstrated by the linear curve obtained in all experiments except in the suspension mixture shown in (C).
to 400,000/ml), or by platelet shape (discoid in ACD–PRP or spherical after incubation of PRP with 0.04 g/100 ml of promethazine hydrochloride for 3–30 min) (Fig. 3). However, in contrast to the linear shape recorded in cases where a single platelet population was analyzed, a curvilinear probability plot was obtained with suspensions containing approximately equal numbers of control (mean volume, 8.0 cu μm) and macrocytic (mean volume, 13.8 cu μm) platelets (Fig. 3).* In order to verify that the distribution properties were not caused by electronic artifacts, probability plots were drawn from cumulative frequency curves obtained by the microscopic method. Linear plots were recorded for logdiameters or logvolumes of platelets in suspension and for logdiameters and logsurfaces of cells spread on smears (Fig. 3).

Finally, it was also demonstrated that the unimodal shape of the distribution was not due to a selective loss of cells during preparation of PRP, since in experiments made with four different specimens, including one with macrothrombocytosis, a linear plot was obtained microscopically both from the PRP and the initial blood sample (Fig. 3). Although slight differences in mean and standard deviation existed between PRP and blood samples, these did not show any constant trend and appeared to depend essentially on differences in spreading on glass.

The finding that PVDs are lognormal justifies the mathematical analysis described above. Lognormal distributions can be characterized by the eight parameters defined in Table 1, which include the mean μ, standard deviation δ, and coefficient of variation K in both the logvolume (subscript L) and volume (subscript V) distributions, plus the median and mode in the PVD. The values obtained in 50 normal laboratory personnel are given in Table 1, together with the 95% limits that were used as reference standards for patient studies.

Macrothrombocytosis in Cohorts of Young Platelets

Macrothrombocytosis in cohorts of young platelets could be studied in two clinical situations: (1) in 20 patients with stable platelet counts whose platelet life spans were less than 3 days and whose mean platelet age was generally well under 3 days (stationary hyperdestruction). The platelet count varied from 10,300 to 310,000/cu mm. This group included 11 cases of ITP, six cases of systemic lupus erythematosus, two cases of malignant lymphoma, and one case of uncertain etiology; (2) in five patients with rapidly increasing platelet counts, including three patients whose platelet counts rose from under 10,000/cu mm to 189,200, 153,400, and 184,000, respectively, in less than 24 hr following splenectomy for ITP; two patients whose counts had fallen from normal values to 3000 and 14,500/cu mm, respectively, after treatment with mechloretamine (for esophageal carcinoma) and cyclophosphamide plus amethopterine (for bronchial carcinoma) and rose again to 835,000 and 600,000/cu mm in 7 and 10 days, respectively.

*The addition of the macrocytic sample, which contained 35.5% megathrombocytes (platelets with volume greater than 13 cu μm) increased the megathrombocyte index of the control sample from 12.1% to 23.8%. It appeared that such a small increase in the percentage of large platelets was detectable by the probit plots (Fig. 3).
Because of red cell contamination, the upper end of the curve was truncated, and the total count was corrected as described in Materials and Methods. (A) refers to regenerating platelets from three subjects splenectomized for ITP whose platelet count rose from less than 10,000/cu mm to 189,000, 153,400, and 184,000/cu mm in less than 24 hr. Mean platelet age was 10–12 hr. (B) refers to young platelets from patients with platelet hyperdestruction whose platelet mean age was less than 1 day and whose platelet count ranged from 10,000 to 75,000/cu mm. All distributions were compatible with lognormality except the first one, which showed slight deviations from linearity on the logarithmic probability scale.

In both groups, linear plots were recorded on probability graphs relating the cumulative frequency to the corresponding logvolume (Fig. 4). However, the upper tail of these curves could not always be analyzed because of truncation due to red cell pulses. For this reason, the cumulative percentages from 1% to 93%–99% were plotted, omitting the values above. In these conditions a slight deviation from linearity was recorded in only one postsplenectomy experiment, (second curve in A, Fig. 4), presumably because the suspension contained a mixture of changing populations.

In both groups, the size parameters were clearly related to platelet count, the greatest changes being found in the most thrombocytopenic patients. In these patients, the size profile of the circulating platelet population consisted of increased mean, median, and modal logvolumes and volumes, together with increased dispersion of volumes. Results obtained in the 20 patients with stationary hyperdestruction are shown in Fig. 5. There was a highly significant negative correlation between platelet count and volume ($r = -0.51$, $p < 0.001$) or standard deviation of volumes ($r = -0.52$, $p < 0.001$).

**Macrothrombocytosis With Normal Platelet Age Distribution**

Macrothrombocytosis with normal platelet age distribution was found in the seven subjects described in Table 2 whose size parameters are shown in Fig. 5. Subjects 1–5 presented the recently described Mediterranean macrothrombocytosis together with various nonhematologic diseases, except for case 5 who had liver cirrhosis with palpable splenomegaly. No patient had erythrocyte macrocytosis or marrow megaloblastosis. All had moderate thrombocytopenia (86,400–138,300 platelets/cu mm), moderately to greatly increased platelet volume (11.5 to more than 20 cu μm), normal thrombocytocrit (0.10–0.23 ml/100 ml), and near-normal or normal platelet life span (5.5–9.4 days) and age (3.6–5.1 days) (Fig. 6). When their mean volumes and standard deviation of volumes were plotted against platelet count, these five subjects formed a group quite distinct from those with hyperdestruction. The son of patient 3, who had no survival study, presented a slightly different picture, including normal platelet count (216,000/cu mm), macrothrombocytosis (13.18 cu μm), and borderline thrombocytocrit (0.29 ml/100 ml).
Fig. 5. Relationship between platelet mean volume $\mu_V$ (left) or standard deviation of volume $\delta_V$ (right) and platelet count. Each point represents one determination. Values higher than 20 cu $\mu$m are represented by a triangle. The 50 subjects included in the normal group are indicated as white dots. The hyperdestruction group, which includes 20 patients with platelet mean life span and age less than 3 days, demonstrated a significant negative correlation between size parameters and count. In Mediterranean macrothrombocytosis (five cases) and dystrombocytopenia (two cases), the usual relationship was disturbed.
PLATELET SIZE

Table 2. Platelet Kinetic and Size Parameters

<table>
<thead>
<tr>
<th>Count (per μl)</th>
<th>Thrombocytocrit (ml/100 ml)</th>
<th>Logvolume Distribution</th>
<th>Volume Distribution</th>
<th>Survival Parameters</th>
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<tr>
<td></td>
<td></td>
<td>Mean μ4</td>
<td>SD δ4</td>
<td>CV</td>
</tr>
<tr>
<td>Mediterranean macrothrombocytosis</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>CUSU 90,800 0.23</td>
<td>2.72 0.99 36.3</td>
<td>25.0 32.2 129.0 15.3 5.7</td>
<td>5.5 3.6</td>
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<tr>
<td>MICE 125,000 0.22</td>
<td>2.51 0.84 33.5</td>
<td>17.7 18.1 102.1 12.4 6.1</td>
<td>7.4 4.3</td>
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<tr>
<td>FARI 109,300 0.14</td>
<td>2.45 0.82 33.7</td>
<td>16.3 16.2 99.1 11.6 5.9</td>
<td>6.4 4.3</td>
<td></td>
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<tr>
<td>PACF 138,300 0.23</td>
<td>2.47 0.81 32.8</td>
<td>16.5 16.0 96.7 11.9 6.1</td>
<td>9.4 5.1</td>
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<tr>
<td>RUSS 86,400 0.10</td>
<td>2.19 0.70 32.2</td>
<td>11.5 9.2 80.4 9.0 5.4</td>
<td>6.0 4.0</td>
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<tr>
<td>Dysthrombocytoysis</td>
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<tr>
<td>TASl 388,800 0.38</td>
<td>1.96 0.79 40.6</td>
<td>9.8 9.3 94.5 7.1 3.8</td>
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<tr>
<td>PROV 555,800* 0.90</td>
<td>2.37 0.90 38.1</td>
<td>16.2 18.2 112.5 10.7 4.7</td>
<td>7.9 4.2</td>
<td></td>
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<tr>
<td>Normals 189,500</td>
<td>0.116</td>
<td>1.38 0.50 29.8</td>
<td>4.8 3.1 53.8 4.0 2.8</td>
<td>6.1 3.8</td>
</tr>
<tr>
<td>395,300 0.300</td>
<td>1.86 0.74 44.5</td>
<td>8.5 7.2 86.8 6.4 4.6</td>
<td>9.3 4.8</td>
<td></td>
</tr>
</tbody>
</table>

*Value corresponding to size determination. Average count during survival study was 294,100.

Cases 6 and 7 had severe dysthrombocytoysis associated with leukemia or marrow abnormalities. Case 6 suffered from refractory anemia requiring monthly transfusions, and case 7 had chronic myelogenous leukemia. In both patients, normal to elevated platelet counts and increased thrombocytocrit were associated with macrothrombocytosis and normal life span and age (Table 2).

DISCUSSION

The tests for lognormality used in this study included analyses of platelet size distribution by two graphic methods, relating either the differences in log-frequencies (Fig. 2) or the cumulative frequencies (Fig. 3) to the logarithms of platelet size. Combined with electronic measurements made on approximately 100,000 elements, the latter method was the most sensitive for the subpopulation smaller than 22–27 cu μm, since it could detect an increase in the percentage of large platelets (more than 13 cu μm) from 12.1% to 23.8%. Furthermore,
microscopic measurements made on whole-blood smears, although less sensitive, demonstrated that no element was omitted from the distributions and that the whole-blood curves were similar to those recorded from the corresponding PRP.

Both microscopic determinations of suspended and smeared platelets and electronic measurements performed with various Coulter aperture tubes and cell concentrations and shapes indicated that the diameter (after sphering), surface (in smeared cells), and volume of normal platelets are lognormally distributed. In fact, lognormality is characteristic of all platelet size parameters so far studied, including volume,24,29,41,42 dry weight,43 computed cross-sectional area or diameter, and diameter or surface determined on smears.23,42 These results conform to the theorem stating that lognormality is preserved when the variable is multiplied by a constant factor or elevated at a constant, even fractional power.35

Because the curves obtained in thrombocytopenic patients were often contaminated by red cell or background pulses and since the extrapolation used to calculate the total count was only an approximation, the results of probit plots in hyperdestruction could not be evaluated with as much confidence as those from normal subjects. Nearly all curves, however, were compatible with a lognormal distribution, as were the graphs relating the differences in logfrequencies to the logvolumes. Lognormality of platelet volumes was also suggested by measurements made on the young cohort released during the regeneration phase after platelet depletion.2 These results made it possible to apply the same general analysis to most platelet curves, using the mean and standard deviation of logvolumes as primary size indices, from which all other values could be calculated. The advantages of this mathematical treatment were the absence of background or red cell interference and the satisfactory accuracy and reproducibility of the estimated parameters (Table 1).

The fact that platelet populations less than 3–10 hr old already have a lognormal distribution of volumes indicates that the shape of the PVD is determined either during thrombocytopoiesis or shortly after platelet release, and suggests that aging in the circulation does not alter the shape properties of the PVD. In addition, macrothrombocytosis does exist in cases where the age distribution of circulating platelets is normal. Examples of this situation are Mediterranean macrothrombocytosis and various dystrombocytopoieses associated with leukemia or refractory anemia (Table 2). Similar results have previously been obtained in some hereditary macrothrombocytopenias15,24–26 including the May-Hegglin anomaly.4,27 Finally, if macrothrombocytosis in hyperdestruction is due only to the young age of the circulating population, a decreased dispersion of volumes should be obtained, since young platelets have a much smaller age heterogeneity than do normal populations containing cells of all ages. The results shown in Fig. 5 indicate that, on the contrary, platelet size is not only increased but is more heterogeneous in hyperdestruction. In the latter situation, small platelets, as well as large ones, are present. Although this conclusion does not exclude the possibility that some decrease in size occurs during aging in the circulation, it demonstrates that such a phenomenon cannot alone provide an adequate explanation for the genesis of macrothrombocytosis and that thrombocytopoiesis must play a major role.
The genesis of lognormal distributions has been extensively studied in the field of small particles.\textsuperscript{35} Such distributions are produced if the variation in size at each step of the formation of the particle is a random proportion of the size reached at the preceding step. This process explains the lognormal distribution of small particles resulting from a multistep fragmentation\textsuperscript{44} or growth\textsuperscript{45} of another particle. Considering that platelets are released as a result of progressive growth and fragmentation of megakaryocytes, one may explain the genesis of platelet size curves by postulating that the mean and standard deviation of platelet volumes are determined essentially by the combined rates of growth and demarcation of the platelet territories. This model, which is presented in mathematical terms in the appendix, might conceivably explain the increased mean and standard deviation of platelet volumes occurring in hyperdestruction as a result of stimulated synthesis of platelet constituents per platelet territory or of depressed membrane demarcation or both. For this reason, the increase in size,\textsuperscript{2,9,21,23} density,\textsuperscript{7,9} protein, phospholipid, and cholesterol\textsuperscript{22} found in young platelets released after platelet depletion may depend more on thrombopoietic alterations than on the young age of the regenerating cells; such cells may be different from physiologic young platelets. Also in accordance with the model is the syndrome of increased platelet size, generally reduced platelet count, and normal thrombocytocrit recorded in Mediterranean macrothrombocytosis\textsuperscript{42} (Table 2) and recently reported in the May-Hegglin anomaly.\textsuperscript{4,27} This syndrome is best explained by diminished membrane demarcation in megakaryocytes.\textsuperscript{4,27} Finally, ultrastructural studies demonstrate that, in leukemia or refractory anemia, micromegakaryocytes with heterogeneous or deficient demarcation can produce highly heterogeneous platelets including macrothrombocytes.\textsuperscript{46}

The role of other factors in determining platelet size cannot be excluded. Microthrombocytosis has been found in iron deficiency\textsuperscript{13} and the Wiskott-Aldrich syndrome.\textsuperscript{15,28} Megaloblastosis without thrombocytopenia is not accompanied by macrothrombocytosis.\textsuperscript{39} The role of the spleen is still unclear, although splenectomized human subjects\textsuperscript{39} or dogs\textsuperscript{23} have normal platelet size distributions. Finally, as stated above, this study cannot completely exclude that a distinct population of large, young platelets has not been detected because of imperfect electronic and/or mathematical separation of erythrocytes or insufficient sensitivity of graphic analyses. However, the evidence for such a population is minimal. The large elements ranging from 13 to 27 cu \textmu m are well accounted for by the tail of a continuous, unimodal, lognormal distribution. The recently demonstrated loss of membrane labeling with aging\textsuperscript{47} does not prove net loss of membrane, since label elution and/or concomitant membrane synthesis have not been disproved. Furthermore, loss of membrane would not necessarily indicate decrease in volume, as is well known from the example of erythrocyte spherocytes. Finally, contradictory results have been obtained when platelet specific activity in various density fractions is measured sequentially after injection of \textsuperscript{75}Se-selenomethionine\textsuperscript{48,49} or \textsuperscript{51}Cr-chromate-labeled platelets.\textsuperscript{49}

The model presented provides a new interpretation of the origin of platelet heterogeneity. In a given subject, large platelets may be produced by those megakaryocyte territories which, by chance, have had the highest combined rate of growth and demarcation. If the growth rate is a significant fraction of the
combined rate, large platelets should be expected to show signs of increased growth other than their large size. Their greater metabolic and functional properties per unit volume could also be explained on this basis. In other words, the experimental results and the model presented are compatible with the thesis of one single platelet population, showing, for probabilistic reasons, a high correlation between apparently distinct properties. Platelets may in fact have little other heterogeneity than that inherent in the concept of population, wherein the properties of each single element result from the multiplicative effects of a large number of random variations.

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APPENDIX

Megakaryocyte maturation is conceived as a sequence of growth and demarcation steps. Starting with a potential volume \( X_0 \), each future platelet territory has a volume \( X_j \) at step \( j \) and a volume \( X_n \) at platelet release. It is postulated that the volume variation due to growth is, at each step, a random proportion of the volume reached at the preceding step so that

\[
\frac{X_j - X_{j-1}}{X_{j-1}} = \epsilon_j
\]

where the \( \epsilon_j \) are random and mutually independent; moreover in the simplest cases they are also independent from the \( X_j \).

It is similarly postulated that the volume variation due to territory demarcation is, at each step, a random proportion \( \eta_j \) of the previous value of the volume. The \( \eta_j \) are random and mutually independent, independent from the \( \epsilon_j \) and, in the simplest case, from the \( X_j \).

The net relative volume variation at each step is then \( \omega_j = \epsilon_j + \eta_j \), where the \( \omega_j \) have the same properties of independence as the \( \epsilon_j \). The mean \( (\omega_j) \) and standard deviation \( (\sigma_{\omega_j}) \) of the \( \omega_j \) can be expressed as per cent volume increase during the step considered.

If the volume variation at each step is small, it follows that

\[
\sum_{j=1}^{n} \frac{X_j - X_{j-1}}{X_{j-1}} = \sum_{j=1}^{n} \omega_j \sim \int_{X_0}^{X_n} \frac{dX}{X} = \ln X_n - \ln X_0
\]

and

\[
\ln X_n = \ln X_0 + \omega_1 + \omega_2 + \omega_3 + \cdots + \omega_n.
\]

From the central limit theorem, \( \ln X_n \) is asymptotically normally distributed with mean \( \ln X_0 + \sum \omega_j \) and standard deviation \( (\sum \omega_j^2)^{1/2} \).

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