Distribution of Size and Shape in Populations of Normal Human Red Cells

By P. B. Canham and Alan C. Burton

ABSTRACT

The diameter, area, and volume of individual human erythrocytes (of 8 subjects, newborn to age 71) were determined by photographing the cells hanging on edge. Measurements from high magnification prints were processed by computer. The distributions of diameter, area, and volume are described statistically, with the unexpectedly linear regression equations for their interrelations. The plot of area vs. volume for the 1016 normal cells from seven subjects (newborn excluded) was remarkably linear with a "straight-line" boundary restricting the distribution. Shape was characterized by a dimensionless "sphericity index" (4.84 × volume $^{2/3}$/area). Cells of larger volume tended to be thinner than the smaller cells.

The red cell can easily be deformed at constant volume, but an increase in membrane area results in hemolysis. A theoretical geometric parameter, the "minimum cylindrical diameter" (MCDiam), in microns, the thinnest cylindrical channel through which each individual cell could pass, predicts the linear boundary of the plot of area vs. volume. The MCDiam value of 3.66 μ ± 0.04 SEM accurately represents the thinnest channel through which 95% of the cells can pass.

In two splenectomized patients with hereditary spherocytosis the MCDiam was increased to approximately 4.0 μ, suggesting that the severest restriction is located in the spleen.

ADDITIONAL KEY WORDS

normal RBC area

sphericity index

RBC volume

minimum cylindrical diameter

spleenic restriction

hereditary spherocytosis

minimum cylindrical diameter splenectomy

The membrane of the human red cell is very flexible, as reported by Bränemark and Lindström (1), whose motion pictures show the rapidly changing shapes of red cells in the microcirculation. In contrast, a small degree of stretching of the membrane (increase in area) results in hemolysis (2). It follows that if red cells were spherical, any deformation at all would increase the area, and therefore "nonsphericity" is essential to the tolerance of erythrocytes to deformation in the circulation. It follows also that the relation of volume to shape or area of each cell determines the degree of tolerance of that cell to deformation, as for example, in passing through a narrow cylindrical channel. Therefore, a statistical study of the distribution of volume, area, and shape and the correlations between these, might give evidence of the existence of such restrictions in the microcirculation. This would bear upon the general questions of the life span of red cells, their mechanical fragility, and the role of the spleen in removing cells that no longer could tolerate some specific geometric restriction imposed by the vascular bed. On this view, the distribution of cellular parameters in circulating blood may differ from that of cells put out from the bone marrow, since those in the circulation are being filtered continuously (about 0.8% of the total red cell mass being removed every day).

We recognize the importance of the chemical and osmotic processes in the spleen which Prankerd (3) and others have suggested play a role in the eventual destruction of old red cells.

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cells. These processes require time for their completion. Therefore the immobilization or trapping of a red cell seems to be the essential first step in its destruction. You must first catch your hare before you can cook it.

Within a single sample of human blood, red cells vary in shape, in volume, and in surface area, as noted by Ponder (4). In a preliminary investigation in this laboratory (2) on a group of normal cells we estimated this variation not only in surface area but also in volume. Furthermore, the more spherocytic cells in a normal sample had a significantly smaller volume than the less spherocytic (thinner) cells. In a small sample of cells from a subject with hereditary spherocytosis, the mean volume was still less. Consequently, there was strong reason to expect the variation in shape, or area, to be correlated to the variation in volume.

We are considering the deformability of red cells but are not, in this paper, concerned about the dynamics of deformation. Many investigators are also concerned about red-cell deformation but are interested in the forces required, and the filtration rate of cells through filters. As long as the red cell can be considered flexible and not rigid, changes in membrane stiffness (e.g., changes due to pH fluctuations) do not concern us. Such changes will alter the force required to deform the cells, but will not necessarily change the consequences of excessive deformation.

Materials and Methods

BLOOD SAMPLES

Venous blood taken from healthy adults and children was put in sodium heparin vacuum containers and refrigerated. The blood was examined microscopically at room temperature within a few hours after being obtained. Cells were suspended in modified 1 isotonic Tris-buffered Ringer’s solution and observed under an oil immersion objective and a 25X photo eyepiece. A Nikon 35-mm camera attachment was used in conjunction with a Wild electronic flash illuminator to obtain micrographs of cells on edge. The measurements were made on high magnification prints (0.7 inches/μ).

MEASUREMENTS OF CELLS

A membrane outline was drawn on the prints according to rules established by Ponder (5). To translate inches measured on the prints into microns, each series of photographic negatives contained one photomicrograph of two etched lines of known separation on a stage micrometer. This separation was determined by comparing its measured value, with a Leitz micrometer eyepiece, with the total 1-mm length of the etched stage micrometer. This calibration was essential because it was found that the nominal 10-μ spacing between etched lines varied as much as 10%. Two different stage micrometers, one made by Leitz and another by Zeiss, were used for this calibration.

CALCULATIONS

The numerous cellular dimensions were punched on IBM cards. Fortran IV programs were written for the IBM 7040 to determine areas and volumes, plot graphs, histograms, and other derived quantities.

We introduced the term sphericity index to provide a comparison between the shape of a cell and a sphere. It is defined by

\[
\text{Sphericity Index} = \frac{4.84 \cdot V^{2/3}}{A},
\]

where \( V \) is volume and \( A \) is area.

The factor 4.84 makes the sphericity index unity for a sphere. The sphericity index is a dimensionless constant with values ranging from zero for a laminar disc to unity for a sphere. The outlines included on Figure 1 show the relation between sphericity index and cross-sectional appearance of the cells. The sphericity index is inversely proportional to the shape factor previously used by Rand and Burton (2).

Skewness and kurtosis are standard statistical parameters used to give information about the shape of frequency distributions.

\[
\text{Skewness} = \frac{1}{N} \sum_{i=1}^{N} (x_i - \bar{x})^3
\]

\[
\text{Kurtosis} = \frac{1}{N} \sum_{i=1}^{N} (x_i - \bar{x})^4
\]

10.5 mEq/liter calcium; 4.2 mEq/liter potassium; 1.0 g/liter glucose; 0.04 N tris-hydroxymethylamino methane and HCl buffer for pH 7.4; sodium chloride was the osmotic adjuster; sodium approx. 100 mEq/liter.
SIZE AND SHAPE OF HUMAN RED CELLS

Possible Bias in the Samples

Only those cells in a blood sample that satisfactorily remained hanging on edge from the cover slip could be photographed. It is important to the interpretation of the results to know whether this introduces a factor of selection in the sample, which might bias the results. For example, thicker cells might remain hanging more readily than thinner cells.

We decided that an independent study of the average diameter of each cell population would provide a measure of the degree of sampling bias when compared to the average population diameter of the cells on edge. For this, lower power photomicrographs were obtained with a Nikon model M inverted microscope. Cells were suspended in the Ringer's solution and allowed to settle. Micrographs of cells on their side (flat) provided completely unbiased samples, in which every cell could be used to determine an unbiased distribution of the cellular diameters. Table 1 contains data for the two independent diameters studied for eight cell populations. The means are the same within 0.5% for each subject, and the grand means are almost identical. (The t-test showed no significant difference.) However, in each case the standard deviation is greater for the cells on edge. This probably resulted from the method of measurement. Only one diameter (the horizontal) can be measured for cells on edge, whereas for the cells lying flat the diameter recorded was the average of a maximum and a minimum diameter. The explanation seemed perfectly adequate when examined statistically. In other respects, such as skewness and kurtosis, the distributions, for a given subject, are remarkably the same (Table 1). The correspondence of the results by the independent methods suggests that the bias in selecting only the cells on edge cannot be significant.

Results

The basic data are summarized in Table 1 for diameter, and Table 2 for area, volume, and sphericity index. The distributions of these parameters were remarkably Gaussian. The sex, age, and size of sample are given in Table 2 for each subject, arranged in order of age. In the general averages (last column) the data for the newborn were excluded, as the cells here are obviously very different in...
### TABLE I

**Comparison of Independent Measurements of Cell Diameter as Test for Bias**

<table>
<thead>
<tr>
<th>Subject, sex, age</th>
<th>On edge, E flat, F</th>
<th>No. in sample</th>
<th>Mean diam (μm)</th>
<th>Significance of Diff., E and F</th>
<th>SD (μm)</th>
<th>Skewness significance</th>
<th>Kurtosis*</th>
<th>Kurtosis significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>P.S., ♂, newborn</td>
<td>E</td>
<td>131</td>
<td>8.848</td>
<td>P &gt; 0.4</td>
<td>±0.561</td>
<td>+2%</td>
<td>L</td>
<td>1%</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>178</td>
<td>8.797</td>
<td></td>
<td>±0.532</td>
<td>-10%</td>
<td>L</td>
<td>5%</td>
</tr>
<tr>
<td>D.J., ♂, 5</td>
<td>E</td>
<td>154</td>
<td>7.827</td>
<td>P &gt; 0.7</td>
<td>±0.450</td>
<td>†</td>
<td>†</td>
<td>5%</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>195</td>
<td>7.812</td>
<td></td>
<td>±0.398</td>
<td>†</td>
<td>P</td>
<td>5%</td>
</tr>
<tr>
<td>S.D., ♂, 9</td>
<td>E</td>
<td>128</td>
<td>7.929</td>
<td>P &gt; 0.2</td>
<td>±0.522</td>
<td>†</td>
<td>P</td>
<td>5%</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>170</td>
<td>7.997</td>
<td></td>
<td>±0.411</td>
<td>†</td>
<td>†</td>
<td>5%</td>
</tr>
<tr>
<td>R.S., ♂, 14</td>
<td>E</td>
<td>157</td>
<td>8.083</td>
<td>P &gt; 0.7</td>
<td>±0.558</td>
<td>†</td>
<td>†</td>
<td>5%</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>137</td>
<td>8.069</td>
<td></td>
<td>±0.417</td>
<td>†</td>
<td>†</td>
<td>5%</td>
</tr>
<tr>
<td>T.O., ♂, 24</td>
<td>E</td>
<td>150</td>
<td>8.194</td>
<td>P &gt; 0.6</td>
<td>±0.502</td>
<td>†</td>
<td>†</td>
<td>5%</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>167</td>
<td>8.212</td>
<td></td>
<td>±0.379</td>
<td>†</td>
<td>†</td>
<td>5%</td>
</tr>
<tr>
<td>P.C., ♂, 25</td>
<td>E</td>
<td>103</td>
<td>8.116</td>
<td>P &gt; 0.6</td>
<td>±0.631</td>
<td>†</td>
<td>†</td>
<td>5%</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>253</td>
<td>8.077</td>
<td></td>
<td>±0.495</td>
<td>†</td>
<td>†</td>
<td>5%</td>
</tr>
<tr>
<td>D.E., ♀, 25</td>
<td>E</td>
<td>181</td>
<td>8.153</td>
<td>P &gt; 0.4</td>
<td>±0.607</td>
<td>†</td>
<td>†</td>
<td>5%</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>185</td>
<td>8.108</td>
<td></td>
<td>±0.444</td>
<td>+2%</td>
<td>†</td>
<td>5%</td>
</tr>
<tr>
<td>B.L., ♂, 71</td>
<td>E</td>
<td>143</td>
<td>8.182</td>
<td>P &gt; 0.8</td>
<td>±0.561</td>
<td>†</td>
<td>†</td>
<td>5%</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>140</td>
<td>8.109</td>
<td></td>
<td>±0.460</td>
<td>-10%</td>
<td>L</td>
<td>1%</td>
</tr>
</tbody>
</table>

**Mean, all subjects:***

| E | 8.068 | †     | ±0.547 | †     |
| F | 8.065 | †     | ±0.429 | †     |

*L = leptokurtic; P = platykurtic. †Not significant. ‡Excluding the newborn in column 1.
### TABLE 2

**Data on Distribution of Area, Volume, and Sphericity Index for Eight Subjects**

<table>
<thead>
<tr>
<th>Subject</th>
<th>P.S.</th>
<th>D.J.</th>
<th>S.D.</th>
<th>R.S.</th>
<th>T.O.</th>
<th>P.C.</th>
<th>D.E.</th>
<th>B.L.</th>
<th>Avg., all subjects*</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Area (µ²)</td>
<td>170.2</td>
<td>132.6</td>
<td>133.5</td>
<td>139.4</td>
<td>140.7</td>
<td>136.9</td>
<td>139.8</td>
<td>143.5</td>
<td>138.1</td>
</tr>
<tr>
<td>sd</td>
<td>±20.6</td>
<td>±13.8</td>
<td>±15.6</td>
<td>±18.0</td>
<td>±16.1</td>
<td>±19.4</td>
<td>±19.7</td>
<td>±18.0</td>
<td>±17.4</td>
</tr>
<tr>
<td>Coeff. of variation</td>
<td>±12.1%</td>
<td>±10.4%</td>
<td>±12.0%</td>
<td>±12.9%</td>
<td>±11.5%</td>
<td>±14.2%</td>
<td>±14.1%</td>
<td>±12.6%</td>
<td>±12.5%</td>
</tr>
<tr>
<td>Skewness significance†</td>
<td>+2%</td>
<td>§</td>
<td>§</td>
<td>§</td>
<td>§</td>
<td>§</td>
<td>+5%</td>
<td>+2%</td>
<td>§</td>
</tr>
<tr>
<td>Kurtosis, significance‡</td>
<td>L 1%</td>
<td>§</td>
<td>§</td>
<td>§</td>
<td>§</td>
<td>§</td>
<td>§</td>
<td>L 5%</td>
<td>L 5%</td>
</tr>
<tr>
<td><strong>Volume (µ³)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>136.8</td>
<td>99.2</td>
<td>101.0</td>
<td>105.3</td>
<td>111.2</td>
<td>104.8</td>
<td>109.2</td>
<td>108.2</td>
<td>107.5</td>
</tr>
<tr>
<td>sd</td>
<td>±20.9</td>
<td>±13.1</td>
<td>±15.4</td>
<td>±17.2</td>
<td>±16.1</td>
<td>±18.5</td>
<td>±20.1</td>
<td>±17.2</td>
<td>±16.8</td>
</tr>
<tr>
<td>Coeff. of variation</td>
<td>±15.3%</td>
<td>±13.3%</td>
<td>±15.2%</td>
<td>±16.3%</td>
<td>±14.5%</td>
<td>±17.7%</td>
<td>±18.4%</td>
<td>±15.8%</td>
<td>±15.9%</td>
</tr>
<tr>
<td>Skewness significance†</td>
<td>+2%</td>
<td>§</td>
<td>§</td>
<td>§</td>
<td>§</td>
<td>§</td>
<td>+10%</td>
<td>+2%</td>
<td>§</td>
</tr>
<tr>
<td>Kurtosis, significance‡</td>
<td>L 1%</td>
<td>§</td>
<td>§</td>
<td>§</td>
<td>§</td>
<td>§</td>
<td>§</td>
<td>L 5%</td>
<td>L 1%</td>
</tr>
<tr>
<td><strong>Sphericity Index</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>0.755</td>
<td>0.781</td>
<td>0.786</td>
<td>0.774</td>
<td>0.795</td>
<td>0.786</td>
<td>0.790</td>
<td>0.766</td>
<td>0.783</td>
</tr>
<tr>
<td>sd</td>
<td>±0.023</td>
<td>±0.021</td>
<td>±0.025</td>
<td>±0.025</td>
<td>±0.026</td>
<td>±0.032</td>
<td>±0.028</td>
<td>±0.029</td>
<td>±0.026</td>
</tr>
<tr>
<td>Coeff. of variation</td>
<td>±3.4%</td>
<td>±2.7%</td>
<td>±3.2%</td>
<td>±3.2%</td>
<td>±3.2%</td>
<td>±4.0%</td>
<td>±3.5%</td>
<td>±3.8%</td>
<td>±3.4%</td>
</tr>
<tr>
<td>Skewness significance†</td>
<td>§</td>
<td>+2%</td>
<td>+2%</td>
<td>+2%</td>
<td>+10%</td>
<td>+10%</td>
<td>+2%</td>
<td>§</td>
<td></td>
</tr>
<tr>
<td>Kurtosis, significance‡</td>
<td>§</td>
<td>L 1%</td>
<td>L 1%</td>
<td>L 1%</td>
<td>§</td>
<td>§</td>
<td>L 1%</td>
<td>§</td>
<td></td>
</tr>
</tbody>
</table>

*Averages of the table entries, excluding the newborn, in column 1.
†Positive or negative skewness considered significant at below the 5% level.
‡Considered significant at below the 10% level. L = leptokurtic.
§Not significant.
size. The large size of the red cells of the newborn is well known (6).

The mean volume of the erythrocyte, based upon hematocrit and cell count (6), has long been considered to be about 90 μ³. The data of Table 2 indicate that the mean volumes determined by us are higher by 10 to 15%. Although we believe that the membrane has been traced correctly, we acknowledge that very little adjustment (in the form of a consistent error) would significantly reduce the volume. The change in volume, ΔV, if the line were shifted inward by a distance Δt, is given by ΔV = A • Δt. Assuming a Δt of 0.1 μ and an area of 130 μ² one would get a ΔV of 13 μ³. There are two other possibilities: (a) that the difference is due to a different cellular environment and some swelling has occurred in our experiments; (b) that a hitherto unrecognized error exists in the established method of obtaining the hematocrit, in that centrifugation can drive water from the packed cell column. We favor possibility (b) on theoretical grounds which we have not yet tested experimentally.

Houchin et al. (7) have reported a value of 84 μ³ for the volume, using studies on rouleaux. However, their calculation is based on the assumption that the individual cells in rouleaux are elliptical in cross section. We have recalculated the volume from their data on the assumption that the cells are thick discs (which seems more reasonable from the pictures in their article) and arrived at an average group volume of 106 μ³. This agrees with our value.

Lushbaugh et al. (8), using an electronic cell counter, have reported bimodal distributions of the “volume” of cells. The quantity that is actually measured by the counter is of course not directly the volume, but the amplitude of the voltage response, which may be also affected by cellular orientation. None
of our histograms of diameter, area, or volume, showed any hint of being bimodal.

The mean cellular area of a red cell population has been reported to be 134 μ² (7), which is supported by our findings. (Area is less sensitive than volume to a change in assumed configuration.)

**Correlations Between the Parameters of the Basic Data**

The distributions of the single parameters, as reported above, do not reveal the operation of any geometric restrictions, which must be sought in correlations between the parameters.

**Area and Volume vs. Diameter**

From geometry one would expect that, if the sphericity index remained constant (randomly variable about the mean), the area and volume would be proportional to the diameter squared and cubed, respectively. However, as illustrated in Figures 2 and 3, the area and volume have a remarkably linear dependence on diameter. This is only partly due to the relatively small percent variation of these parameters. When we tried tests of least squares, using the expected nonlinear relations from geometry, we found that the sum of squared deviations, M, was substantially higher than for the linear regression line. The constants of proportionality, K, for area (A = K₁ • D²), where D is diameter, and similarly for volume (V = K₂ • D³) along with M are recorded in Tables 3 and 4. This means that for both area and volume plotted against diameter, the data are better represented by a straight line than by the relation expected from geometry (f(x) = K • xⁿ). The linear correlation coefficient was in every case greater than 0.93, and highly significant.

The above finding that the correlations between diameter and area, and between diameter and volume are linear instead of the expected power laws was the first clue that the shape of cells cannot be varying independently of the volume. Other plots (e.g., sphericity index vs. volume) given later confirm this. The statistical data and linear

\[ M = \sum [y_i - f(x_i)]^2 \]
### TABLE 3

**Area vs. Diameter—Comparison of Linear Regression $A = a + b \cdot D$ with Geometrical Relation $A = K \cdot D^2$**

<table>
<thead>
<tr>
<th>Subject</th>
<th>P.S.</th>
<th>D.J.</th>
<th>S.D.</th>
<th>R.S.</th>
<th>T.O.</th>
<th>P.C.</th>
<th>D.E.</th>
<th>B.L.</th>
<th>All subjects*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept $a \pm se$</td>
<td>$-149.5 \pm 5.4$</td>
<td>$-103.9 \pm 3.3$</td>
<td>$-107.1 \pm 3.1$</td>
<td>$-119.0 \pm 3.1$</td>
<td>$-118.7 \pm 3.9$</td>
<td>$-110.0 \pm 3.6$</td>
<td>$-120.7 \pm 3.6$</td>
<td>$-115.4 \pm 3.8$</td>
<td>$-112.0$</td>
</tr>
<tr>
<td>Slope $b \pm se$</td>
<td>$36.1 \pm 0.61$</td>
<td>$30.2 \pm 0.42$</td>
<td>$30.3 \pm 0.39$</td>
<td>$32.0 \pm 0.38$</td>
<td>$31.7 \pm 0.48$</td>
<td>$30.4 \pm 0.44$</td>
<td>$32.0 \pm 0.44$</td>
<td>$31.7 \pm 0.47$</td>
<td>$31.0$</td>
</tr>
<tr>
<td>Corr. coeff.</td>
<td>$0.982$</td>
<td>$0.986$</td>
<td>$0.990$</td>
<td>$0.989$</td>
<td>$0.984$</td>
<td>$0.989$</td>
<td>$0.984$</td>
<td>$0.985$</td>
<td>$0.983$</td>
</tr>
<tr>
<td>$K$ ($A = K \cdot D^2$)</td>
<td>$2.16$</td>
<td>$2.16$</td>
<td>$2.15$</td>
<td>$2.14$</td>
<td>$2.13$</td>
<td>$2.12$</td>
<td>$2.12$</td>
<td>$2.12$</td>
<td>$2.12$</td>
</tr>
<tr>
<td>Sum of squares of deviations</td>
<td>$1991$</td>
<td>$815$</td>
<td>$673$</td>
<td>$1079$</td>
<td>$1269$</td>
<td>$817$</td>
<td>$2273$</td>
<td>$1383$</td>
<td>$1383$</td>
</tr>
</tbody>
</table>

*Excluding the newborn, in column 1.

†95% confidence limits of correlation coefficients in no case exceeded ±0.02.

‡$K$ was calculated on the least squares basis.

### TABLE 4

**Volume vs. Diameter—Comparison of Linear Regression Equation $V = a + b \cdot D$ with $V = K \cdot D^3$**

<table>
<thead>
<tr>
<th>Subject</th>
<th>P.S.</th>
<th>D.J.</th>
<th>S.D.</th>
<th>R.S.</th>
<th>T.O.</th>
<th>P.C.</th>
<th>D.E.</th>
<th>B.L.</th>
<th>All subjects*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercept $a \pm se$</td>
<td>$-172.9 \pm 10.0$</td>
<td>$-118.1 \pm 5.8$</td>
<td>$-122.4 \pm 6.0$</td>
<td>$-134.6 \pm 5.4$</td>
<td>$-133.8 \pm 7.9$</td>
<td>$-120.5 \pm 7.8$</td>
<td>$-146.6 \pm 6.3$</td>
<td>$-126.6 \pm 7.2$</td>
<td>$-131.0$</td>
</tr>
<tr>
<td>Slope $b \pm se$</td>
<td>$35.0 \pm 1.13$</td>
<td>$27.8 \pm 0.74$</td>
<td>$28.2 \pm 0.76$</td>
<td>$29.7 \pm 0.66$</td>
<td>$29.9 \pm 0.96$</td>
<td>$27.8 \pm 0.96$</td>
<td>$31.4 \pm 0.78$</td>
<td>$28.7 \pm 0.88$</td>
<td>$29.3$</td>
</tr>
<tr>
<td>Corr. coeff.</td>
<td>$0.939$</td>
<td>$0.950$</td>
<td>$0.957$</td>
<td>$0.964$</td>
<td>$0.932$</td>
<td>$0.945$</td>
<td>$0.950$</td>
<td>$0.940$</td>
<td>$0.948$</td>
</tr>
<tr>
<td>$K$ ($V = K \cdot D^3$)</td>
<td>$1.93$</td>
<td>$1.97$</td>
<td>$1.97$</td>
<td>$1.97$</td>
<td>$1.97$</td>
<td>$1.96$</td>
<td>$1.96$</td>
<td>$1.96$</td>
<td>$1.96$</td>
</tr>
<tr>
<td>Sum of squares of deviations</td>
<td>$6738$</td>
<td>$2604$</td>
<td>$2516$</td>
<td>$3305$</td>
<td>$5109$</td>
<td>$3790$</td>
<td>$7165$</td>
<td>$4923$</td>
<td>$4923$</td>
</tr>
</tbody>
</table>

*Excluding the newborn, in column 1.

†95% confidence limits of correlation coefficients in no case exceeded ±0.02.
SIZE AND SHAPE OF HUMAN RED CELLS

FIGURE 4

Sphericity index vs. volume. The plot is quartered by the centroid. The chi-square test indicates the shift to thinner cells with increase in cellular volume.

FIGURE 5

Area vs. volume plot. The dashed line is the least squares fit for a relation of constant sphericity index. The data fit the linear regression line much better.

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regression equations may have some practical empirical usefulness to further research where area and volume are relevant, but where only the diameter is easily measured. Computer programs were written to calculate regression equations for the combined data of seven normal subjects (excluding the newborn), whose ages range from 5 to 71, with values for 1016 cells in all. The results are:

\[ A = 31.0D - 112.0/x^2 \pm 3.3 \text{ SEE}; \]

\[ V = 29.3D - 131.0/x^3 \pm 5.6 \text{ SEE}. \]

The standard errors of estimate (SEE) mean that 95% of the predictions of area or volume from these equations will be within \( 2 \times \text{SEE} \) of the actual values. The use of such empirical equations is of course limited to normal blood samples. We do not have a sufficient number of subjects of different ages to study age dependence, which has been reported as slight (9), except for the newborn.

Sphericity vs. Volume

Figure 4 shows the relation between sphericity index and volume of each cell for one subject. The corresponding graphs for all subjects showed the same feature. There is obviously a significant negative correlation, but the facts are better expressed by noting that for the larger cells there is a much more severe upper limit to the sphericity index than for those cells which have a volume less than the mean volume. The statistical test for this statement is the chi-squared test applied to the number of cells whose points fall in the four quadrants. For all subjects except the newborn the \( P \) value was less than 0.02; for the newborn it was <0.1.

Area vs. Volume

The plot of area vs. volume is unexpectedly linear over its entire range (Fig. 5)—unexpected because the sphericity index has such a small percent variation (coefficient of variation approximately ± 3.5%). If a constant sphericity index was the link between area and volume, then area would be proportional to \( \text{volume}^{2/3} \) and the area-volume curve would be concave downward. In every case (Table 5) the linear regression was a better fit. We devised a test for curvature by cal-
Area = 4πR² + 2πRL
Volume = 4/3πR³ + πR²L

eliminating L;

Vol = Area · R² - 4/3πR³ · · · (1)

MCDiam = 2R

FIGURE 6

The calculation necessary to obtain a value for a cellular minimum cylindrical diameter (MCDiam).

Calculating a regression of the slope (ΔA/ΔV) vs. the volume V. Here the correlation coefficient was not significantly different from zero, meaning that no tendency to a curvilinear relation was detectable.

The three tests of the presence of hidden correlations, i.e. area and volume, therefore all agree. The last of these (area vs. volume) has a special usefulness in verifying the theory that follows on the nature of the restriction. It seems possible that the constant linking area with volume is connected with a new parameter, the “minimum cylindrical diameter.” The minimum cylindrical diameter is expressed in microns, and represents the diameter of the thinnest long cylindrical channel through which each red cell could theoretically pass, without increase in area, the volume remaining constant. The osmotic equilibrium ensures that the volume is constant, and Rand and Burton (2) have reported that the area will not tolerate more than about 20% increase before hemolysis. Hoffman (10) considers that much less stretching than this will produce hemolysis. Considering that they expressed caution about this value, it would seem reasonable for the calculation to consider the area as well as the volume to be unchanging.

The Minimum Cylindrical Diameter

The most successful shape that a cell could have to pass through a narrow tube would be that of a cylinder, with spherical end-caps (hot dog) (Fig. 6). The soup-can shape (cylinder with flat ends) is not as successful in conserving area and would also require a discontinuity in the curvature at the edges. Rand (11) has shown that erythrocytes will fold like crêpe suzettes when passing into a pipette of about 5 μ. However, if the cell is forced through a smaller channel it must adopt a more conservative configuration. The bullet shape of cells observed in the motion picture studies of Brånenmark and Lindström (1) is very similar to the ideal shape (hot dog) for going through thin channels. Any shape involving involution will mean that the minimum diameter of a channel through which the cell could pass is increased. Our calculation represents the “best that a given cell could do.”

Equation 1 of Figure 6 is a cubic equation for the variable R, with volume and area

### Table 6

<table>
<thead>
<tr>
<th>P.R.</th>
<th>D.J.</th>
<th>S.D.</th>
<th>R.S.</th>
<th>T.D.</th>
<th>P.C.</th>
<th>T.L.</th>
<th>Average+</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean μ</td>
<td>3.47</td>
<td>3.26</td>
<td>3.30</td>
<td>3.38</td>
<td>3.47</td>
<td>3.24</td>
<td>3.41</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>±0.17</td>
<td>±0.14</td>
<td>±0.16</td>
<td>±0.14</td>
<td>±0.17</td>
<td>±0.19</td>
<td>±0.19</td>
</tr>
<tr>
<td>Coeff. of variation</td>
<td>±4.9%</td>
<td>±4.3%</td>
<td>±4.7%</td>
<td>±4.3%</td>
<td>±4.9%</td>
<td>±5.6%</td>
<td>±5.5%</td>
</tr>
<tr>
<td>5% cut-off point</td>
<td>3.20</td>
<td>3.03</td>
<td>3.02</td>
<td>3.05</td>
<td>3.19</td>
<td>3.02</td>
<td>3.09</td>
</tr>
<tr>
<td>95% cut-off point</td>
<td>3.80</td>
<td>3.52</td>
<td>3.59</td>
<td>3.57</td>
<td>3.77</td>
<td>3.70</td>
<td>3.70</td>
</tr>
</tbody>
</table>

*Excluding data for newborn, in column 1. None of the populations showed significant skewness or kurtosis.

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as parameters. The value of $2 \cdot (V/A)$ is always slightly smaller and serves as a very good first approximation. Newton's method (found in most first-year calculus texts) was used to solve the cubic equation for each cell. Results are given for all the samples in Table 6 and Figure 7 gives the histogram for one subject. Even for newborn, whose cells are much larger, the minimum cylindrical diameter is only slightly larger than the very closely grouped values for the 7 normals. The diameter of the thinnest cylinder through which 95% of the cells can pass lies between 3.5 and $3.8 \mu$ for all the eight subjects.

**Discussion**

**The Nature of the Restriction**

The idea of long cylindrical channels is in harmony with the anatomy of the vascular system. The walls of the small blood vessels are remarkably nondistensible (12). The possibility is that somewhere in the vascular bed through which red cells must pass, either every time in the circuit or eventually after many circuits, there are channels that require deformation of the cell sufficient to increase the area of the membrane of cells that do not have an appropriate relation between their shape and volume. Those that do not have this appropriate relation would be hemolyzed and eliminated from the circulation. This might explain the restriction, and the dependence of the variation of sphericity on volume that is seen in the statistical data.

A much more exacting test of the minimum cylinder theory is to see if it predicts the type of restriction on the distribution that is evident in the graphs already shown. If in equation 1 of Figure 6 the radius, $R$, of the minimum cylinder is constant, there is a linear relation between the area and volume. The loci of the combination of areas and volumes necessary for cells to have the same given
minimum cylindrical diameter are therefore straight lines, as shown in Figure 8. It is evident that, for the data of Figure 8, the line for $R = 1.8 \mu$ (diameter = 3.6 $\mu$) is a very good "boundary" limiting the distribution. Note that the linear regression line for the data obviously would have a slope different from the minimum cylindrical diameter line passing through the centroid (say for $R = 1.64 \mu$). The line thus fits as a boundary to the data rather than as a regression line. Since the value of $R$ fixes not only the slope, but also the intercept of these lines, the test of fitting the limit of the scatter is indeed severe. The fit was good in the graphs of area vs. volume for all subjects, Figure 8 being neither the best nor the worst example. We conclude that the cylindrical-channel theory of the restriction is entirely consistent with the results, if the diameter of the cylindrical channels is about 3.7 $\mu$. The 95% cut-off point at the right-hand end of each histogram of minimum cylindrical diameter was determined by interpolation on the actual data and found to have a mean of $3.66 \pm 0.04 \mu$ SEM.

Figure 9, an area-volume plot for 1016 cells from seven persons, demonstrates very strikingly the adherence to a straight boundary. The 98% cut-off line is calculated from the frequency distribution of minimum cylindrical diameter for the 1016 cells. This graph is almost a straight line over the threefold range in cellular size. The 95% cut-off point for the 1016 normal cells coincides with a minimum cylindrical diameter of 3.65 $\mu$.

For completeness, two other restrictions are considered: a system of closely spaced rigid parallel sheets, and a system of small holes (orifices) in the vascular walls through which the cells would pass as do white cells in "diapedesis." Would either of these geometric hypotheses fit the restriction seen in the data as nicely as does the cylindrical channel?

**THE MINIMUM SHEET SPACING**

This is defined as the minimum distance between two rigid parallel sheets through which a red cell could pass without area and volume changes. The most economical
Area vs. volume for 1016 normal cells from the seven normal subjects. Two densities in population are shown. (A unit rectangle on the graph has dimensions of area × volume, i.e., \( \mu^3 \).

MCDiam = minimum cylindrical diameter.

FIGURE 9

Vol = \( \frac{2}{\pi} \int_0^w y^2 dx \)
= \( 2\pi WH^2 + \frac{4}{3} \pi W^3 + \pi^2 W^2 H \)

Area (A) = \( 2\pi H^2 + 4\pi \int_0^w \sqrt{1 + [y']^2} dx \)
= \( 2\pi H^2 + 2\pi^2 WH + 4\pi W^2 \)

eliminating H;

Vol = \( \frac{\pi}{6} W^3(3H^2 - 16) + AW - \pi^2 W^2 \left( \frac{(H^2 - 8)W^2 + 2}{\pi^2} \right) \)

Equation (2)

Mini- Sheet Spacing = 2W

FIGURE 10

Calculations necessary to obtain the minimum sheet spacing for a single cell.

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FIGURE 11
Histogram of cellular minimum sheet spacings.

TABLE 7
Distribution of Minimum Sheet Spacing

<table>
<thead>
<tr>
<th>Subject</th>
<th>P.S.</th>
<th>D.J.</th>
<th>S.D.</th>
<th>R.S.</th>
<th>T.O.</th>
<th>P.C.</th>
<th>D.E.</th>
<th>B.L.</th>
<th>Average*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean μ</td>
<td>2.27</td>
<td>2.18</td>
<td>2.21</td>
<td>2.18</td>
<td>2.34</td>
<td>2.24</td>
<td>2.29</td>
<td>2.16</td>
<td>2.23 ± 0.026 SEM</td>
</tr>
<tr>
<td>Standard deviation</td>
<td>±0.14</td>
<td>±0.11</td>
<td>±0.13</td>
<td>±0.11</td>
<td>±0.14</td>
<td>±0.16</td>
<td>±0.15</td>
<td>±0.14</td>
<td>±0.13</td>
</tr>
<tr>
<td>Coeff. of variation</td>
<td>±5.9%</td>
<td>±5.1%</td>
<td>±5.8%</td>
<td>±5.0%</td>
<td>±6.2%</td>
<td>±7.2%</td>
<td>±6.8%</td>
<td>±6.8%</td>
<td>±6.3%</td>
</tr>
<tr>
<td>5% cut-off point</td>
<td>2.07</td>
<td>2.00</td>
<td>2.02</td>
<td>2.00</td>
<td>2.18</td>
<td>1.98</td>
<td>2.03</td>
<td>1.92</td>
<td>2.02 ± 0.030 SEM</td>
</tr>
<tr>
<td>95% cut-off point</td>
<td>2.50</td>
<td>2.37</td>
<td>2.43</td>
<td>2.36</td>
<td>2.57</td>
<td>2.51</td>
<td>2.35</td>
<td>2.38</td>
<td>2.45 ± 0.032 SEM</td>
</tr>
</tbody>
</table>

*Omitting the data for the newborn, in the first column. There was no significant skewness or kurtosis in the distributions.

geometry for this maneuver would be a pancake shape similar to a torus with a filled-in midsection (Fig. 10). Equation 2 was solved for each cell by Newton's method. Figure 11 gives an example of the results and Table 7 the details of the distribution for all subjects. The minimum spacing between sheets for red cells is about 2.2 μ, which is not much less than the maximum thickness of red cells. The loci of constant sheet spacing on the area-volume plot are almost straight lines in the region of interest, with slopes slightly lower than the minimum-cylinder diameter lines. These curves do not fit the data as well, as
shown by the fact that for each subject the percent variation is higher for the distributions of minimum sheet spacing (Table 7). Also a system of rigid parallel sheets does not seem to be as reasonable from a biological standpoint.

**DIAPEDESIS OF RED CELLS**

The possibility that red cells had to pass through orifices in a thin wall was also analyzed. The optimal case to conserve area would be for a cell to remain a segment of a sphere on each side of the orifice, and the maximum tendency to increase area would be with the cell equally divided on the two sides. This form of diapedesis of red cells is theoretically possible, on this assumption, for almost any hole, regardless of how small. A cell of volume 100 $\mu^3$ would need an area greater than 125 $\mu^2$ to go through a minute hole. Cells of this volume have a mean area of 135 $\mu^2$. However, the calculation is obviously unrealistic, since it ignores any rigidity of the membrane opposing the very acute curvature involved. Diapedesis has been observed through capillaries of inflamed tissue (13). Except in pathological states, we know of no reports of red cells passing through very small orifices by diapedesis. It may be that they could so pass, if the very high driving pressure required to make them do so existed in the circulation.

**ROLE OF THE SPLEEN IN THE RESTRICTION**

The spleen is considered to be the site of destruction of circulating red cells (3), and many think that this is where they are trapped prior to destruction. The evidence of the role

---

**FIGURE 12**

Distributions of minimum cylindrical diameter standardized according to area. The stippled zone indicates the variation about the mean. The two distributions for the splenectomized patients are well outside the normal zone.
of the spleen is convincing for injected "foreign" or artificially injected cells but not adequately documented for normal red cells. If so, the distribution of minimum cylindrical diameters after splenectomy might show the removal of critical cylindrical channels in the spleen. We have only preliminary results on this, which are consistent with this theory but not conclusive.

The stippled area of Figure 12 shows the distribution for the seven normal subjects, excluding the newborn, treated as a single population, with the standard deviation from the grand mean of the seven samples set off on each side of the mean values. The two other distributions are for postsplenectomy patients, who had hereditary spherocytosis. It is clear that the distributions are significantly shifted to higher minimum cylindrical values, and many cells are present which could not pass the restriction that applies to the data on normal subjects. This is reflected also in a deficit in the normalized distributions of cells that could pass the smaller cylindrical channels.

The weakness of this interpretation is, of course, that these two patients were not normal hematologically, and we have as yet no comparable data on healthy persons whose spleen has been removed, or on these patients before splenectomy. However, Rand and Burton (2) did report an average volume and shape factor for 16 cells from a patient with hereditary spherocytosis, prior to splenectomy. The calculated minimum cylindrical diameter for this small group of cells has the value of \( 3.32 \mu \). This value puts the group of 16 cells of a patient with spherocytosis before splenectomy very nicely with the normal group on Figure 12.

Recent electron microscopic evidence (14) suggests that the splenic circulation is not a system of simple tubes but rather a tortuous system of irregularly shaped channels and that the erythrocyte runs a "traumatic gauntlet" in the spleen. Our evidence is not necessarily contradictory. One might consider that the minimum cylindrical diameter model is equivalent to, but not anatomically the same as the limiting channels thought to be located in the spleen.

**IMPACT OF THIS RESEARCH ON THE EXPLANATION OF THE LIFE SPAN OF RED CELLS**

The analysis has shown that the distribution of size and shape of the population of circulating red cells is consistent with their eventual removal by a geometric restriction, by passing through a cylindrical channel, or its equivalent, of diameter less than about 3.7 \( \mu \) (probably in the spleen). If the aging of a red cell is accompanied by changes in volume and shape, e.g. becoming more spherocytic for the same volume, or increasing the volume with area constant, this could result in failure to pass the critical cylindrical channel; the trapping and elimination of a red cell after its life span would be by "mechanical hemolysis." While changes with age in the chemical composition of the red cell membrane have been reported, and increases in density with age are firmly established, changes in shape and volume, while they have been suggested, have not been sufficiently investigated. Our next aim will be statistical analysis of populations of old vs. young cells, separated by their known differences in density, to see if this next step in the logic of explaining the life span may be verified.

**Summary and Conclusions**

1. From microphotographs of red cells on edge, diameter, area, volume, and a dimensionless "sphericity index" have been computed for relatively large samples for eight normal subjects. While the distributions of these parameters are essentially normal (Gaussian), there are correlations between them that show they are not independently variable. For example, by several statistical tests, the shape of cells is not independent of their volume. Cells of less than average volume may have a wide range of sphericity, but those of greater than average volume are restricted to being thinner (lower sphericity index).

2. Because of these hidden correlations, the regressions between diameter, surface area,

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and volume are unexpectedly linear. Empirical regression equations are possible by which surface area and volume could be predicted from their diameters with good accuracy for normal blood.

3. If it is assumed that the red cell membrane can be easily deformed, but cannot increase its area without hemolysis, the minimum diameter of a cylindrical channel through which it could pass can be computed. This averaged 3.33 μ for the subjects, with a standard error of the mean ± 0.03 μ. In spite of considerable variations in volume and shape, the minimum-cylinder diameter has a narrow range in any blood sample. Ninety-five percent of the cells in a sample can pass a cylindrical channel of diameter about 3.7 μ, 5% a channel of about 2.9 μ.

4. The theory of the minimum cylindrical diameter predicts that in the plot of area vs. volume, the data should lie on one side of a limiting straight line, calculated for each diameter of the cylinder. The data fit this prediction well but do not fit restrictions imposed by other geometric restraints (such as passing between rigid sheets), as well.

5. In two patients after splenectomy (for spherocytosis), the distribution of the minimum cylindrical diameter differed from the normals, in that many cells were circulating that could not pass a cylindrical channel of 3.65 μ (through which 95% of the cells of normals can pass). This would be consistent with the theory that the spleen is the site of the geometric restriction. Since the blood was not normal, the data do not prove this.

It is concluded that a stretching of the membrane, leading to hemolysis, is required of cells of inappropriate size and shape, when they circulate through narrow cylindrical channels. Cylindrical channels of diameter about 3.7 μ (or equivalent), probably in the spleen, are consistent with the correlations between volume and shape found in blood samples. This is discussed in connection with the larger problem of what determines the life span of normal red cells. The present results represent only a small step toward the solution of this problem.

Acknowledgments

The authors wish to thank Dr. Carol Graham, Dr. W. B. Barton, and Dr. N. Jaco for their co-operation in obtaining suitable blood samples, and Mrs. Dorothy Elston for technical assistance.

References

Distribution of Size and Shape in Populations of Normal Human Red Cells
P. B. CANHAM and ALAN C. BURTON

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