**Decreased Deformability of Erythrocytes From Smokers**

By James M. Norton and Peter W. Rand

The deformability of erythrocytes from smoking and non-smoking human subjects was examined by filtration through 3-μm pores and capillary viscometry of cell suspensions. In both cases, small but significant differences were found between the two groups that are consistent with a reduction in the surface area-to-volume ratio and/or a diminished membrane flexibility in erythrocytes from smokers. Additional evidence suggests that these findings represent a chronic rather than an acute effect of smoking on erythrocyte deformability.

**Materials and Methods**

Blood samples were obtained by venipuncture from the antecubital vein of human volunteers* using heparin (10 U/ml) as the anticoagulant in all cases. All measurements described were made within 2 hr. Hematocrit was determined using a microhematocrit centrifuge with no correction applied for trapped plasma. Hemoglobin concentration was determined using the cyanmethemoglobin method and commercial standards (Hycel, Inc., Houston, Texas). Red cell concentration (RBC) was measured using a Coulter Counter Model F calibrated with a standard blood preparation (4 C, Coulter Electronics, Hialeah, Fla.). MCV, MCH, and MCHC were calculated from the preceding measurements.

Subject were placed in the smoking group on the basis of history of cigarette smoking was associated with much larger alterations in red cell filterability and cell suspension viscosity than the original target variables. In consideration of the growing body of literature on the effects of smoking on erythrocyte function,1 we wish to document here our observations of reduced deformability of erythrocytes from smokers and present evidence suggesting the reasons for this finding.

**Measurement of Relative Filtration Time**

Erythrocyte deformability was assessed by passing dilute (Hct 0.5%) erythrocyte suspensions through polycarbonate sieve filters (13-mm diameter, 3 μm nominal pore size, Nuclepore Corporation, San Francisco, Calif.). All filters used in these experiments were from a single batch. The erythrocyte suspensions were prepared by direct addition of an aliquot of whole blood to a standard phosphate buffer (300 mosmole/kg, pH 7.40, prepared fresh from a stock solution of the following composition in g/liter: NaCl, 90.0; Na₂HPO₄, 13.66; NaH₂PO₄, 24.33). The relative filtration time (RFT) for each sample was determined as the ratio of the passage time through the filter, under a 10-cm H₂O hydrostatic pressure head, of 1 ml of cell suspension compared to 1 ml of the buffer alone. Values for RFT were expressed as the mean of triplicate determinations on each sample using three different filters.

Determinations of RFT performed in the above fashion have been shown, for a given blood sample, to be relatively insensitive to minor (+10%) variations in hematocrit, cell number, and reticulocyte percentage (Rand and Norton, unpublished observations).

**Statistical Analysis**

Analysis of the data in this report was performed using statistical programs supplied with or written for the HP-67 programmable calculator (Hewlett-Packard, Corvallis, Ore.); formulae for the comparison of two regression lines were taken from statistical texts.5,6

*Informed consent was obtained from all subjects, and all procedures were performed according to the Declaration of Helsinki and with the approval of the Committee for the Protection of Human Subjects of the Maine Medical Center.

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Table 1. Erythrocyte Indices and Relative Filtration Times for Controls and Smokers*

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 20)</th>
<th>Smokers (n = 9)</th>
</tr>
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<tbody>
<tr>
<td>Hct (%)</td>
<td>43.1 ± 2.2</td>
<td>46.8 ± 3.6†</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>16.07 ± 1.08</td>
<td>16.07 ± 1.45†</td>
</tr>
<tr>
<td>RBC (10^6/cu mm)</td>
<td>4.77 ± 0.38</td>
<td>4.96 ± 0.47</td>
</tr>
<tr>
<td>MCV (cu µ)</td>
<td>90.81 ± 6.19</td>
<td>95.19 ± 3.14†</td>
</tr>
<tr>
<td>MCH (pg/cell)</td>
<td>31.32 ± 2.51</td>
<td>32.45 ± 1.09</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>34.45 ± 1.58</td>
<td>34.09 ± 1.05</td>
</tr>
<tr>
<td>Carboxyhemoglobin (%)</td>
<td>1.64 ± 0.63</td>
<td>5.63 ± 1.62†</td>
</tr>
<tr>
<td>RFT</td>
<td>2.28 ± 0.18</td>
<td>2.67 ± 0.23†</td>
</tr>
</tbody>
</table>

*Values expressed as means ± standard deviation.
†Significantly different from control (p < 0.05 using the Student's t test for unpaired data).

RESULTS

Erythrocyte indices and relative filtration times for controls and smokers are summarized in Table 1. Significantly higher hematocrit, hemoglobin concentration, and MCV were seen in the smokers, similar to previous reports. These findings were accompanied by a significant increase in RFT.

A logical assumption would be that the increases in MCV and RFT seen in the smokers are directly related. To investigate this possibility in more detail (and to determine in general the relationship between erythrocyte size and RFT in our system), relative filtration times were measured for erythrocyte suspensions prepared from blood of rats and rabbits as well as humans. The use of these three mammalian species with red cells of differing volume (60–65 cu µ, 70–75 cu µ and 90 cu µ, respectively) but of the same basic biconcave shape provided a natural model for studying the effect of cell size alone on RFT using our method. The results of these studies are shown in Fig. 1. A highly significant (t = 5.0911 for 40 degrees of freedom, p < 0.001) relationship does indeed exist between MCV and RFT over the range of 60–100 cu µ, as indicated by the solid line. The point indicating the mean MCV and RFT for the human smokers lies well outside the 95% confidence limits, suggesting that the smokers' RFT is higher than can be expected on the basis of increased size alone.

An important determinant of RFT in our system is the surface area to volume ratio (SA/V). Osmotically produced increases in cell volume (decreases in SA/V) 5%–10% above the original MCV dramatically increase RFT (Fig. 2). For example, rabbit erythrocytes swollen osmotically from a normal MCV of 73 cu µ to an MCV of 80 cu µ demonstrate RFTs of 6–8, whereas normal human erythrocytes of a much larger volume but a more favorable SA/V show RFTs of about 2.3. A possible explanation for the increased RFT seen in smokers may, therefore, be a decreased SA/V, scarcely discernible morphologically but apparent as a decrease in filterability. Such a condi-

Fig. 1. Relationship between MCV and RFT for normal biconcave erythrocytes from rats, rabbits and humans, and erythrocytes from smokers. The solid line represents the curve: RFT = 1 + (0.000187)4(MCV)2.0121 (R2 = 0.5133, n = 42) obtained from the data points indicated by the filled circles. The dashed lines indicate the 95% confidence limits. The rat and rabbit erythrocytes were handled using the methods outlined in the text for human cells; the only difference lay in the osmolality of the PBS, which was increased to 310 mosmole/kg, a level more nearly isotonic to the plasma of these animals. The open symbol indicates the location of the mean values (± standard deviation) of MCV and RFT for the smokers taken from Table 1.

Fig. 2. Relationship of relative filtration time (RFT) to osmotically induced changes in MCV. Filled circles represent RFTs determined on dilute erythrocyte suspensions from a single donor containing the same number of cells but with osmolalities ranging from 250 to 400 mosmole/kg. The asterisk (*) represents the MCV determined on the whole blood sample (MCVwb). The vertical and horizontal dashed lines indicate the location of the minimum RFT obtainable through osmotic manipulation of MCV (RFTmin) and the approximate MCV at which the minimum RFT occurs (MCVmin). Small increases in MCV above the MCVmin produces large increases in RFT, presumably due to an alteration in the surface area to volume ratio of the cells.
tion could arise if the well documented increase in MCV in smokers is not accompanied by a proportional increase in membrane surface area during the course of erythrocyte development.

Smokers also differed significantly from controls with respect to the relationship between MCV and red cell concentration (RBC). This relationship was recently proposed to be a valuable tool in exploring the control of hematopoiesis,8 has been confirmed and extended in our laboratory,9 and has been used to analyze the effects of smoking on erythrocytes.7,10 Linear regression analyses of MCV on RBC were performed for our control and smoking subjects using the least squares method, and the results are shown in Fig. 3. Clearly different relationships exist in the two groups, and the regression coefficients were found to differ significantly ($t = 2.7677$ for 25 degrees of freedom, $p < 0.01$). This finding, although not in full agreement with a previous report,7 nevertheless suggests that the differences in MCV between controls and smokers may represent an effect of smoking on erythrocyte development.

The capillary viscometry results, shown in Fig. 4, substantiate the findings of reduced deformability of erythrocytes from smokers apparent in the filtration studies. Suspensions of washed erythrocytes from smoking subjects have significantly higher apparent viscosities at all shear rates below 20 sec$^{-1}$. Since MCHC was not different for the two groups, and in the absence of fibrinogen and serum globulins that might serve to increase aggregation at low shear rates, the most likely explanation for this increase in low shear rate viscosity is a reduction in erythrocyte deformability, consistent with an alteration in surface area to volume ratio but not ruling out a decrease in intrinsic membrane flexibility.11

**DISCUSSION**

The differences in the erythrocyte indices reported here for whole blood do not appear to be acutely reversible changes dependent on some factor(s) present in smokers' plasma. Indices measured on the thrice-washed suspensions used in the viscometry studies are not different from the whole blood indices and confirm the pattern shown in Table I.

Support for the contention that the decreased deformability noted in both the filtration and capillary viscometric studies is due to erythrocyte geometric or membrane properties and not to size or internal viscosity comes from several additional literature and experimental observations. First, the MCV alone has not been found to be an important rheologic factor in erythrocyte suspensions with a given MCHC and volume fraction of cells,12 erythrocyte geometry, especially the surface area to volume ratio, appears to play a greater role than absolute volume. Second, the high shear rate viscosity values for cell suspensions from controls and smokers shown in Fig. 2 are nearly identical, an indication that internal fluid viscosity is the same for both groups.12 This point is supported by the similarity in MCHC values in Table I. Third, in addition to MCHC, internal fluid viscosity may also be influenced by the physicochemical state of the hemoglobin, which may be altered in smokers by the presence of carboxyhemoglobin. The results of three capillary viscometry experiments designed to investi-
Fig. 5. Apparent viscosity of washed suspensions of erythrocytes from nonsmoking donors equilibrated with either oxygen or carbon monoxide. Symbols represent the mean value ± standard deviations. No differences could be demonstrated between the two groups at any shear rate, using the t test for paired samples.

gate this possibility are shown in Fig. 5. No differences in apparent viscosity were observed at any shear rate for washed cell suspensions divided into equal aliquots and equilibrated with either 100% oxygen (resulting in a carboxyhemoglobin concentration of less than 1%) or 100% carbon monoxide (greater than 98% carboxyhemoglobin). Acute effects of carbon monoxide on the physicochemical state of hemoglobin do not appear from these results to be responsible for the differences in apparent viscosity shown in Fig. 4. Fourth, additional filtration studies were performed in which the osmolality of the suspending buffer was varied over the range of 250–600 mosmole/kg. In these studies, the number of cells in each buffer suspension prepared from a given blood sample was constant and equal to the number of cells that would produce a 0.5% hematocrit in an equal volume of the isotonic (300 mosmole/kg) suspending buffer. The results of these studies are shown in Table 2. The proportional increases in MCV as suspending medium osmolality is reduced were essentially identical for the control and smoking subjects; the increases in RFT were not. The RFT appeared to increase more in the smokers than in the controls and, although the observed differences were not statistically significant, this observation supports the concept of a reduced SA/V in erythrocytes from chronic smokers. However, in osmotic fragility studies performed on blood samples from the control and smoking subjects, no differences were observed.

In conclusion, the balance of the experimental results and relationships described in this article suggest that the decreased deformability of the large erythrocytes of smokers is due to an alteration in cell geometry and/or membrane properties secondary to a chronic effect of cigarette smoking on cell development and not to changes in MCHC or in the properties of hemoglobin in these cells.

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REFERENCES

5. Edwards AL: An Introduction to Linear Regression and Correlation. San Francisco, Freeman, 1976

<p>| Table 2. Relationship Between Osmotically Induced Changes in RFT and MCV for Controls and Smokers* |
|----------------------------------------|--------------------|---------------------|-------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Osmolality (mOms/kg)</th>
<th>Controls (n = 4)</th>
<th>Smokers (n = 5)</th>
<th>Controls (n = 4)</th>
<th>Smokers (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>250</td>
<td>1.45 ± 1.33</td>
<td>1.11 ± 1.11</td>
<td>1.09 ± 1.01</td>
<td>1.11 ± 1.11</td>
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<td>275</td>
<td>0.99 ± 0.99</td>
<td>1.05 ± 1.05</td>
<td>1.04 ± 1.04</td>
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<tr>
<td>300</td>
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<tr>
<td>350</td>
<td>1.11 ± 1.11</td>
<td>1.10 ± 1.10</td>
<td>0.91 ± 0.91</td>
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<tr>
<td>400</td>
<td>1.24 ± 1.24</td>
<td>1.25 ± 1.25</td>
<td>0.87 ± 0.87</td>
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<tr>
<td>600</td>
<td>2.00 ± 2.00</td>
<td>1.85 ± 1.85</td>
<td>0.75 ± 0.75</td>
<td>0.74 ± 0.74</td>
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</table>

*Values given are means ± standard error.
†Values for RFT at each osmolality for each sample were divided by the RFT at an osmolality of 300 mosmole/kg for that sample.
‡Values for MCV at each osmolality for each sample were divided by the MCV in 300 mosmole/kg for that sample.
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