In Vitro Measurements of the Effects of Anticoagulants on the Flow Properties of Blood: The Relationship of these Effects to Red Cell Shrinkage

BY WILLIAM I. ROSENBLUM

The apparent relative viscosity (ARV) of blood usually decreases as the hematocrit decreases. However, the data described in the following report indicate that the ARV will increase if hematocrit is reduced by exposure of blood to anticoagulants, like citrate, which induce erythrocyte shrinkage. A similar phenomenon has been reported by other workers, but appears to have attracted little notice.

MATERIAL AND METHODS

Animals

Blood was obtained by cardiac puncture from anesthetized male mice, and by venipuncture from unanesthetized rabbits and monkeys.

Anticoagulation

Mouse blood was drawn into syringes containing 0.02 ml. of anticoagulant. Rabbit and monkey bloods were ejected from syringes into clean tubes containing dry sodium citrate, sodium oxalate or disodium ethylene diamine tetracetic acid (EDTA), or containing an aqueous solution of heparin (final concentrations of all anticoagulants are indicated in the text). When ACD solution (NIH formula B) was used, each ml. of blood was diluted with 0.25 ml. of ACD so that it contained 9 mg. sodium citrate, 3 mg. citric acid and 9 mg. dextrose. For comparison with ACD-blood, 4 parts of heparinized blood (1 U heparin per ml. blood) were diluted with 1 part normal saline. In three additional experiments, controls were prepared by adding 9 mg. dextrose to each ml. of diluted heparinized blood.

Measurements

Microhematocrit determinations were made on all samples. The apparent relative viscosity (ARV) was measured in a capillary viscosimeter of the type designed by Swank and Roth. This instrument resembles, in principle, the viscosimeters used by Fahreus and Lindquist and others. Blood was drawn by a negative pressure of -100 mm. Hg. into a horizontal steel capillary 36 mm. long and approximately 306µ in diameter. A hand-activated electric stopwatch, accurate to 0.01 seconds, was used to measure the time taken by 0.1 ml. of blood to pass through the capillary into a calibrated reservoir. The blood and apparatus were kept at 38 C. The time taken by blood was compared with that taken by an
Fig. 1.—Heparin dosage was 2–4 U per ml. The concentration of sodium citrate was 5–10 mg per ml. The relative viscosity was higher in 6 of the 7 citrated bloods than in any of the controls, while the hematocrits were lower.

equal volume of distilled water under the same conditions (0.1 ml. water took 1 sec. to pass through the capillary in these experiments).

\[
\text{ARV} = \frac{\text{Time taken by blood}}{\text{Time taken by water}}
\]

In some experiments the viscosity index was also determined.  

\[
\text{Viscosity index} = \frac{\text{ARV} \times 10}{\text{Hematocrit}}
\]

This parameter enables one to more easily compare the viscosities of two samples with widely disparate hematocrits by providing a correction for the effect of hematocrit itself. This is made possible by two facts: (a) the relationship of ARV to hematocrit is essentially linear between hematocrits of 30 and 501-4 and (b) the slope of the line representing this portion of the relationship is relatively constant.

RESULTS

Effects of Citrate

Figures 1 and 2 summarize the results obtained when citrated and heparinized mouse bloods were compared. Citrate reduced the hematocrit but elevated
EFFECTS OF CITRATE ON VISCOSITY INDEX OF MOUSE BLOOD

Fig. 2.—The data from figure 1 is replotted to show viscosity indices in the 2 groups of animals. All the citrated samples have viscosity indices greater than any of the heparinized samples. Viscosity index = relative viscosity \times 10.

The differences between citrated and heparinized samples with respect to the distribution of values representing ARV, viscosity index and hematocrit are each significant at the .002 level (Mann-Whitney Test). When a citrated aliquot from a given rabbit, monkey or mouse was compared with a heparinized aliquot from the same animal, the hematocrit was reduced and the ARV elevated (Figure 3, p = .002 Sign Test). Citrate had no effect on the viscosity of plasma.

Effects of Citrate on Heparinized Cells

Five mg. of Na Citrate were added to each ml. of pooled, heparinized mouse blood. Two experiments were performed. In both, the addition of citrate elevated the ARV and viscosity index (Table 1).

Effects of Citrate in ACD Solution

Monkey blood was diluted with ACD solution (Methods) and compared with heparinized (1U/ml.) blood from the same animal to which saline or saline plus dextrose (Methods) had been added. In only one out of six experiments (Table 2) was there a significant elevation in both the ARV and viscosity index, while in four experiments, a slight to moderate fall in these parameters was noted.

Effects of Sodium Oxalate and EDTA

Dry sodium oxalate, like citrate, will shrink erythrocytes when added to blood. Oxalated blood (5 mg Na oxalate per ml.) displayed an increased ARV and viscosity index in 5 out of 6 animals when compared with heparinized (1U heparin per ml. blood) blood from the same animal (Table 3). Additional studies revealed that oxalate had no effect on the ARV of plasma. EDTA, like
EFFECTS OF ANTICOAGULANTS ON BLOOD VISCOITY

Fig. 3—The lines in the figure connect the 2 points representing samples from a single animal. Citrate elevated the relative viscosity and decreased the hematocrit. Citrate dosage was 3–5 per mg per ml. Heparin dosage was 1 U per ml in monkey and rabbit blood and 4–5 U per ml in mouse blood.

citrate and oxalate, removes calcium from the blood, but unlike the latter anticoagulants, EDTA leaves cell size and shape intact. EDTA (1 mg./ml.) failed to significantly elevate ARV or viscosity index (Table 3).

Effects of Cell Shrinkage Induced by NaCl

Heparinized rabbit blood was centrifuged and the erythrocytes resuspended in phosphate-buffered NaCl solutions of increasing tonicity. As the concentration of NaCl increased and the cells shrank, there was a progressive rise in the viscosity index (Fig. 4).

DISCUSSION

The anticoagulants used in this study can be divided into two groups: (a) those which shrink erythrocytes—citrate and oxalate, and (b) those...
Table 1.—Effects of Na Citrate on Heparinized Pooled Mouse Blood

<table>
<thead>
<tr>
<th></th>
<th>Experiment 1</th>
<th>Experiment 2</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Heparin alone</td>
<td>Heparin plus</td>
<td>Heparin plus</td>
<td>Heparin plus</td>
</tr>
<tr>
<td>Heparin</td>
<td></td>
<td>Citrate</td>
<td>alone</td>
<td>Citrate</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>40</td>
<td>35</td>
<td>39</td>
<td>32</td>
</tr>
<tr>
<td>Relative Viscosity</td>
<td>2.940</td>
<td>3.100</td>
<td>3.360</td>
<td>3.670</td>
</tr>
<tr>
<td>Viscosity Index</td>
<td>0.740</td>
<td>0.890</td>
<td>0.740</td>
<td>1.130</td>
</tr>
</tbody>
</table>

*Heparin 4–5 U/ml blood.  
†Citrate 5 mg/ml of heparinized blood.

Table 2.—Effects of ACD Solution on the Relative Viscosity and Viscosity Index of Monkey Blood

<table>
<thead>
<tr>
<th>Animal Number</th>
<th>Heparin</th>
<th>ACD</th>
<th>Heparin + Dextrose</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.760†</td>
<td>3.230 (0.990)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>3.970</td>
<td>3.140 (0.920)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4.590</td>
<td>3.250 (0.910)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>3.100 (1.010)</td>
<td>2.920 (0.970)</td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>3.900 (1.340)</td>
<td>2.760 (1.000)</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>3.070 (0.850)</td>
<td>3.280 (0.910)</td>
</tr>
</tbody>
</table>

*Apparent relative viscosity.  
†Viscosity index.

Table 3.—Effects of Oxalate and EDTA on Blood Viscosity

<table>
<thead>
<tr>
<th>Species</th>
<th>Age</th>
<th>Treatment</th>
<th>Relative Viscosity</th>
<th>Viscosity Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit</td>
<td>6 mo.</td>
<td>Heparin</td>
<td>3.200</td>
<td>0.800</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oxalate</td>
<td>3.970</td>
<td>1.140</td>
</tr>
<tr>
<td>Rabbit</td>
<td>26 mo.</td>
<td>Heparin</td>
<td>3.070</td>
<td>0.780</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oxalate</td>
<td>4.380</td>
<td>1.330</td>
</tr>
<tr>
<td>Rabbit</td>
<td>36 mo.</td>
<td>Heparin</td>
<td>3.430</td>
<td>0.840</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oxalate</td>
<td>4.210</td>
<td>1.080</td>
</tr>
<tr>
<td>Monkey (M.irus)</td>
<td>Adult</td>
<td>Heparin</td>
<td>3.760</td>
<td>0.990</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oxalate</td>
<td>4.120</td>
<td>1.210</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EDTA</td>
<td>3.790</td>
<td>1.000</td>
</tr>
<tr>
<td>Monkey (M.speciosa)</td>
<td>Adult</td>
<td>Heparin</td>
<td>3.970</td>
<td>1.070</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oxalate</td>
<td>4.720</td>
<td>1.450</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EDTA</td>
<td>3.870</td>
<td>1.050</td>
</tr>
<tr>
<td>Monkey (M.speciosa)</td>
<td>Adult</td>
<td>Heparin</td>
<td>4.590</td>
<td>1.020</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Oxalate</td>
<td>4.230</td>
<td>0.810</td>
</tr>
<tr>
<td></td>
<td></td>
<td>EDTA</td>
<td>4.020</td>
<td>0.910</td>
</tr>
</tbody>
</table>

having no effect on cell size or shape—heparin,11-13 EDTA14 and ACD.15,16 It is important to note that citrate is the anticoagulant agent in ACD solution, but red cell shrinkage is prevented when erythrocytes are exposed to citrate in this milieu. Heparinized blood was employed as the standard because heparin, in the dosages used here, does not alter blood viscosity.17-19 Citrate or oxalate elevated the apparent relative viscosity (ARV) and viscosity index.
EFFECTS OF ANTICOAGULANTS ON BLOOD VISCOSITY

EFFECTS OF INCREASING CONCENTRATIONS OF NaCl ON THE VISCOSITY INDEX

Fig. 4.—The viscosity index of cell suspensions increased as the tonicity of the suspending medium increased. The hematocrit was 30 in the suspension with the highest viscosity and 43 in the suspension with the lowest viscosity.

Data in the literature, pertinent to the present investigation, are sparse. Observations during the current study indicated that blood flowed 3 to 4 times more slowly than water, depending upon the species. Others have obtained similar results with dog blood and human blood, examined in viscosimeters with dimensions comparable to those employed herein. Present observations on the effects of citrate confirm those of other workers utilizing a capillary viscosimeter of different dimension, while the observations concerning oxalate and EDTA confirm the results of studies in which an entirely different type of viscosimeter was employed. It would appear that the present data were not the product of a particular technic of measurement. This impression is further supported by the rare reports relating blood viscosity to erythrocyte size and to plasma tonicity. In the latter investigations, viscosimeters were used which differed greatly in design or dimensions from the instrument employed in the present study. Trevan observed an inverse relationship between blood viscosity and the size of erythrocytes altered by manipulating the osmotic pressure of their environment, while an investigation published...
shortly after preparation of the present report demonstrates a striking relationship between plasma tonicity and blood viscosity. The latter results support our conclusion that citrate and oxalate increased blood viscosity because these anticoagulants produced erythrocyte shrinkage.

The reason for an association between erythrocyte shrinkage and increased blood viscosity are not clear. Increased aggregation of red blood cells results in increased blood viscosity\(^2\) and may be caused by alterations of the cell surface.\(^3\) The cell membrane is certainly altered by erythrocyte shrinkage and these changes may result in cellular aggregation; however, the high shear rate at which the present observations were made would tend to disrupt aggregates.\(^2\) Consequently, it may be that some other mechanism, such as increased cellular rigidity,\(^2\) is responsible for the altered flow properties of shrunken cells.

**SUMMARY**

Citrate and oxalate increased the viscosity of blood as measured in a capillary viscosimeter. The elevated viscosity was accompanied by erythrocyte shrinkage, manifest by the decreased hematocrit of blood anticoagulated with either of these agents. Plasma viscosity was not affected. EDTA, which does not alter cell size or shape, also failed to alter blood viscosity, while citrate no longer affected viscosity, if utilized in ACD solution, a milieu which prevents cell shrinkage. When erythrocytes were suspended in hypertonic NaCl, “blood” viscosity was also elevated in comparison to that of suspensions in lower concentrations of NaCl. The data indicate that blood viscosity will be elevated by anticoagulants which are permitted to shrink erythrocytes.

**SUMMARIO IN INTERLINGUA**

Citrato e oxalato augmentava le viscositate de sanguine secundo mesurationes in un viscosimetro capillari. Le elevate viscositate esseva accompagniate per retraction erythrocytic, manifeste in le deprimite hematocrite de sanguine tractate con le citrato o le oxalato del mentionate agentes como anticoagulantes. Le viscositate del plasma non esseva afficite. EDTA, que non altera le dimensiones o le conformation del celular, etiam esseva sin influentia super le viscositate del sanguine, durante que citrato cessava afficer le viscositate quando illo esseva utilisate in un solution in ACD (le qual preveni le retraction cellular). Quando erythrocytos esseva suspendite in hypertonic solution salin, le viscositate del “sanguine” esseva etiam augmentate in comparation con illo de suspensiones in plus basse concentrationes de NaCl. Le datos indica que le viscositate del sanguine es augmentate per anticoagulantes que resulta in un retraction del erythrocytos.

**ACKNOWLEDGMENTS**

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**REFERENCES**

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