STUDIES ON THE EFFECT OF SHOCK ON RED CELL SURFACE CHARGE IN PRIMATES

D. L. HORWITZ

Red cell electrophoretic mobilities have been determined on cells from 6 baboons subjected to hemorrhagic shock and 2 baboons in E. coli shock. Studies of cells suspended both in saline and in their own plasma showed electrophoretic mobility in hemorrhagic shock to be unchanged from control values. Power function analysis showed the maximum undetectable change for cells measured in saline would be 6.7%, and in plasma 12.2%. Use of either heparin or EDTA as anticoagulant, or absence of anticoagulant, did not affect the mobilities of either control or shock cells. It is concluded that loss of red cell electronegativity is not an important factor in producing red cell aggregation in hemorrhagic shock, but that this may be of significance in the septic shock.
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Studies on the Effect of Shock on Red Cell Surface Charge in Primates

D. L. Horwitz

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Introduction

The phenomenon of erythrocyte aggregation or sludging has been observed in a number of types of shock, including septic shock and traumatic shock, as reviewed by Kniely [1965]. Although Kniely [1965] has stated that "simple hemorrhage" does not give rise to intravascular red cell aggregation, recent microscopic observations by vanek et al. [1969] have revealed progressive erythrocyte aggregation in Rhesus monkeys subjected to hemorrhagic shock with arterial pressures of 20-60 mm Hg.

In vivo red cells are known to repel one another [see Kniely et al., 1960] and numerous investigators have asked why this property is lost in shock. Billis and snow [1950] have suggested that agglutination of erythrocytes is due to lowered electrical surface charge on individual cells, thus enabling adsorption of cells onto each other. It is the purpose of this report to look at the possible loss of electrostatic repulsion as a factor in producing the red cell aggregation observed in shock.

Methods

Healthy male baboons (Papio anubis) were used after a 30-day quarantine period. On the day of study, they were sedated with phencyclidine hydrochloride (Sernylan) in a dose of 1 mg/kg, and catheters were placed in the femoral artery and vein. Samples of blood were collected in plain glass test tubes, and in tubes containing sodium heparin (143 USP units/10 ml blood) or disodium EDTA (12 mg/10 ml blood). The blood was promptly diluted 1:100 in isotonic phosphate-buffered saline at pH 7.4 and electrophoretic mobility determined within 15 min of the time of blood collection. Another set of blood samples was centrifuged to separate cells from plasma (or serum) and the cells were resuspended in their own plasma (serum), again in a 1:100 dilution. The animals were then bled to a mean arterial pressure of 60 mm Hg and maintained at that level for 1 h. Ringer's lactate solution was given as needed to maintain the pressure at that level. After 1 h, the pressure was lowered to 40 mm Hg by further bleeding and maintained at this level for an additional hour. Blood samples were obtained as before.

Two animals were also studied in septic shock, induced by i.v. injection of live E. coli in a dose of 10¹¹ organisms per kilogram. The E. coli were grown from a strain of known serotype under constant conditions, and this dose was found to uniformly bring the animal's mean arterial pressure below 60 mm Hg within 1 h. Samples were collected before and 1 h after injection of E. coli.

Electrophoretic mobility was determined in a

1 From the Bureau of Medicine and Surgery, Navy Department, Research Task No. MR005.20-0277B.
2 The experiments were conducted according to the principles enunciated in "Guide for Laboratory Animal Facilities and Care".
3 The opinions or assertions contained herein are those of the author and are not to be construed as official or reflecting the views of the Navy Department or the Naval Service at large.
commercially available Northrup-Kunzit cata-
phoresis apparatus (A. N. Thomas Co.) similar
to that described by Abrahamson et al. ([42]). The
time for red cells to migrate 0.04 mm in an elec-
trical potential gradient of 2.86 V/cm was deter-
mined for 10 cells from each sample. All measure-
ments were made in a room maintained at
25°C ± 1. Plasma and serum viscosities were
measured in a cone-plate microviscometer (Brook-
field Laboratories, Inc.) at shear rates of 10-100
sec⁻¹, on serum and on plasma collected in
heparin and EDTA at concentrations similar to
those described above.
Erythrocyte surface charge is related to electro-
phoretic mobility by the equation
\[ Q = \frac{6\pi r v E \Delta V}{r} \]
where \( Q \) is the charge in C, \( 6\pi r \) is a pro-
portionality factor from Stokes' Law, \( r \) is the
radius of the red cell, \( v \) is the viscosity of plasma,
and \( E \Delta V \) is the electrophoretic mobility (velocity
potential gradient). \( 10^{-5} \) is a factor to convert the
final result to C. The formula must be considered
an approximation as the factor \( 6\pi r \) is exact only for
a sphere.

**Results**

Table I shows the electrophoretic mobilities
of red cells obtained from 6 separate ani-

Table II compares data from the 2 animals
subjected to E. coli septicemia with the
hemorrhagic shock animals. We find that
while red cells in buffered saline show the
same mobilities in both types of shock,
there is a substantial decrease in mobility
in the E. coli shock cells when measured
in plasma, as compared to hemorrhagic
shock. Although this difference is statisti-
cally quite significant, with \( P < 0.005 \), it
must be interpreted cautiously because of
the very small number of animals studied,
especially in view of substantial animal-to-
animal variability.

Measurements of plasma and serum
viscosity showed considerable animal-to-
animal variation, and the values can hence
not be determined with great precision.
The viscosities, which were essentially
independent of shear rate, were 1.3 ± 0.08
eP (±S.E. of mean) for control serum.
and 1.4 ± 0.08 eP for serum from
hemorrhagic shock animals, a statistically

<table>
<thead>
<tr>
<th>Anticoagulant</th>
<th>State</th>
<th>Mobility (μm sec⁻¹/Vcm)</th>
<th>S.E.</th>
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</thead>
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<tr>
<td>None</td>
<td>Control</td>
<td>1.33 ± 0.004</td>
<td>1.27 ± 0.004</td>
</tr>
<tr>
<td></td>
<td>Shock</td>
<td>1.35 ± 0.003</td>
<td>1.33 ± 0.006</td>
</tr>
<tr>
<td>Heparin</td>
<td>Control</td>
<td>1.29 ± 0.035</td>
<td>1.24 ± 0.009</td>
</tr>
<tr>
<td></td>
<td>Shock</td>
<td>1.34 ± 0.033</td>
<td>1.34 ± 0.060</td>
</tr>
<tr>
<td>EDTA</td>
<td>Control</td>
<td>1.35 ± 0.033</td>
<td>1.51 ± 0.087</td>
</tr>
<tr>
<td></td>
<td>Shock</td>
<td>1.38 ± 0.040</td>
<td>1.24 ± 0.072</td>
</tr>
</tbody>
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Overall 1.32 ± 0.019 1.34 ± 0.070

To detect any changes in erythrocyte
charge which might be related to loosely
adsorbed plasma components, mobilities
of cells in their own serum or plasma
were determined. These are also given in
Table I. Again, no significant differences
are observed, although EDTA measure-
ments border on significance at the \( P = 0.05 \)
level. A 3-way analysis of variance again
indicated that neither anticoagulant nor
shock contributed to net variation. 7.1
Table II. Red cell mobility in hemorrhagic and septic shock, as determined in saline and plasma

<table>
<thead>
<tr>
<th>Type of shock</th>
<th>Mobility, µm/sec/V cm</th>
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<tbody>
<tr>
<td></td>
<td>In saline</td>
</tr>
<tr>
<td>Hemorrhagic</td>
<td>1.30 ± 0.035</td>
</tr>
<tr>
<td>Septic</td>
<td>1.57 ± 0.049</td>
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<tr>
<td>P*</td>
<td>n.s.</td>
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* P values based on Student 't' test

effect was not observed, we must ask whether our methodology was sensitive enough to detect this effect. Statistically, this is done by means of a 'power function', which gives the probability of rejecting the null hypothesis (that is, the hypothesis that red cell mobility is unchanged in shock) when the actual change in red cell mobility is a given value. Such an analysis shows that a change in mobility of 0.088 µm/sec/V cm, or 6.7% of control, will be detected with 95% certainty. From this, we may conclude that red cell mobility in hemorrhagic shock does not change by more than 6.7%. A similar analysis for the erythrocytes in plasma indicates that a change in mobility of 0.163 µm/sec/V cm, or 12.2%, would be detected with 95% confidence.

We may also ask whether erythrocyte mobility, the quantity actually determined, is directly related to the cell net charge, the desired quantity. We note that, in addition to mobility, the viscosity of the suspending medium enters into the calculation of surface charge. Viscosity does not change at all in the case of red cells in buffered saline, which was of the same composition throughout the experiment. Also, our studies indicated no significant change in plasma viscosity during shock.

The well-known changes in whole blood viscosity in shock do not affect our studies, in which the red cell suspension is sufficiently dilute that only plasma viscosity need be considered. Thus, we may consider that electrophoretic mobility is a direct measure of cell charge, and we may set maximum limits of 6.7 and 12.2% for the change of erythrocyte surface charge in hemorrhagic shock as measured in saline and plasma, respectively.

We therefore conclude that erythrocyte aggregation observed in hemorrhagic shock is unlikely to be due to changes in cell surface charge. ONLEY [1965] reached a similar conclusion using a rather different approach based on iso-hemagglutin-
duced aggregation. However, Hissen et al. [1966] have reported that heparinized dogs subjected to hemorrhage show a net decrease of red cell surface charge averaging -25% for erythrocytes suspended in plasma, but not for those in saline. This difference from our results could possibly be attributed either to species difference or to the prior in vivo heparinization. Finally, the possibility of decreased surface charge promoting red cell aggregation in septic shock remains, and is especially interesting in view of electron microscopic observations by Mergenhagen et al. [1969] of physical defects in the red cell surface after treatment with endotoxin.

Summary

Red cell electrophoretic mobilities have been determined on cells from 6 baboons subjected to hemorrhage shock and 2 baboons in E. coli shock. Studies of cells suspended both in saline and in their own plasma showed electrophoretic mobility in hemorrhage shock to be unchanged from control values. Power function analysis showed the maximum detectable change for cells measured in saline would be 67 °, and in plasma 12.2 °. Use of either heparin or EDTA as anticoagulant, or absence of anticoagulant, did not affect the mobilities of either control or shock cells. It is concluded that loss of red cell electronegativity is not an important factor in producing red cell aggregation in hemorrhagic shock, but that this may be of significance in the septic shock animal.

References


