To the Editor:

A recent study of membrane transport in red cells lacking the rhesus antigenic locus (Rhnull) showed a 40%–60% increase in both active and passive influxes of K+. Moreover, the number of active cation pumps measured by the 3H-ouabain binding technique was 30% higher in Rhnull red cells than in Rhnull (D) control cells. Another study of Rhnull red cells reported in abstract showed increased Na+ efflux and K+ influx, and both the ouabain-sensitive and -insensitive components were raised. Lauf and Joiner concluded that the membrane defect in Rhnull cells was pleiotropic and directly affected both active and passive cation transport as well as expression of the rhesus antigen.

An alternative explanation is that these cation transport changes are associated with an immature red cell membrane and that cation fluxes might be altered on that basis alone. Evidence against this possibility is the normal content of water, Na+, and K+ in Rhnull cells, normal mean corpuscular volume (MCV) (94 fl), and slightly raised reticulocyte count (2.1%). However, neither the size of a cell nor its content of water, Na+, or K+ would be expected always to reveal the degree of maturation of the cell membrane. Evidence for an immature membrane in Rhnull disease is the persistence of i as well as I antigen on the cell surface, a finding characteristic of “marrow stress” and associated with premature release of red cells from the marrow. This change might indicate a persistent membrane immaturity of the entire red cell population, even in reticulocyte-depleted populations.

Cation fluxes were studied in Rhnull red cells drawn from an Aboriginal woman (E.N.) who was the first person described with this condition. Blood from the subject and controls was drawn into acid citrate dextrose (ACD) anticoagulant, placed on ice, and promptly carried by plane from the Western Desert region of Australia to Melbourne. Approximately 24 hr elapsed between the times of venesection and cation flux measurements. Red cell 22Na and 42K influx were measured by standard techniques. Reticulocytes were enumerated after supravital staining with new methylene blue and were 6.2% for the Rhnull blood. This elevated value was consistent with previous reports of hemolytic anemia in Rhnull subjects, although it did not accord with the normal reticulocyte value (0.6%) previously reported for this woman. Na+ influx (active) was increased by 50%, while ouabain-sensitive K+ influx (active) was raised by 60% in the Rhnull cells (Table 1). In contrast, the ouabain-insensitive K+ influx (passive) was normal in these Rhnull cells. This permeability pattern found for patient E.N. closely corresponded with that expected for immaturity of the red cell membrane, since reticulocyte-rich red cell suspensions have an increased Na+ influx and active K+ influx but a normal passive K+ influx.

Thus the permeability changes in E.N. may simply relate to the reticulocytosis, possibly combined with immaturity of the red cell membrane, both of which are constant features of this disease. The difference in ouabain-insensitive K+ influx between cells from E.N. and the other two patients studied to date might reflect a genetic heterogeneity in this disease. Other changes reported for Rhnull red cells, such as weaker MNSsU, the unique S+U- blood group combination, or the increase in membrane fluidity, could also reflect a persisting and generalized immaturity of the red cell membrane, rather than a specific effect of the Rh gene deletion. Future studies on Rhnull disease would be helpful.

Table 1: Influx of Na+ and K+ Into Rhnull Red Cells

<table>
<thead>
<tr>
<th>Reticulocytes</th>
<th>Na+ Influx*</th>
<th>K+ Influx*</th>
<th>Glucose + Ouabain</th>
<th>Active Flux*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhnull patient</td>
<td>6.2%</td>
<td>3.2</td>
<td>3.2</td>
<td>0.11</td>
</tr>
<tr>
<td>Aboriginal control</td>
<td>0.8%</td>
<td>2.1</td>
<td>2.2</td>
<td>0.12</td>
</tr>
<tr>
<td>White control</td>
<td>1.4%</td>
<td>1.9</td>
<td>1.9</td>
<td>1.7</td>
</tr>
</tbody>
</table>

*All fluxes are in μequiv/ml cells/hr measured with either 22Na or 42K in a medium of composition of 145 mM NaCl and 5 mM KCl plus 20 mM imidazole Cl, pH 7.5. Red cells were washed prior to these measurements, and the removal of theuffy coat during this procedure reduced reticulocytes in the Rhnull cells to 4.8%. Active flux is the difference between K+ influx with glucose and K+ influx with glucose plus ouabain.
should include control red cells with comparable immaturity of the cell membrane.

The assistance of Dr. Martin Davey and Sister Scorer is greatly appreciated.

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REFERENCES

Cation Fluxes in Rhnull Red Cells: Reply

To the Editor:

In his Letter to the Editor, Wiley reported on Na+ and K+ influxes into Rhnull red cells of the Aboriginal Australian woman (E.N.) whose red cells lacking the entire Rh-Hr antigen complex were the very first reported Rhnull red cells. Observing both increased Na+ leak and K+ pump fluxes but a normal passive K+ influx, Wiley concluded that “the permeability changes in E.N. may simply relate to the reticulocytosis, possibly combined with immaturity of the red cell membrane.” Since similar transport changes were found in reticulocyte-rich red cells of Rh-Hr individuals.

Wiley's observation was different from that of Lauf and Joiner because the Rhnull red cells of their case showed significantly higher passive K+ influxes than Rh-Hr cells. Studying yet a third case, Lee and Stevenson also found an elevated passive K+ influx. Wiley's explanation that the cation transport changes reflected immaturity of the Rhnull red cell membrane is an alternative explanation to that of Lauf and Joiner, who suggested that absence of the Rh-Hr antigens, changes of antigenic activities unrelated to the Rh system, and altered cation permeability may be expressions of a pleiotropic membrane defect.

The question of immaturity of the red cell membrane was explicitly discussed and ruled out by Lauf and Joiner. Hematologically, the reticulocyte counts were 2.1% and 1.3% for the patient of Lauf and Joiner and hence very close to that of Wiley's white control. This fact eliminates marrow stress. The physiologic parameters did not indicate the presence of young cells, since cell size, cell water, cation composition, and osmotic fragility were found to be in the range of Rh-Hr cells.

A subsequent analysis of the cation transport parameters of separated young Rh-Hr red cells revealed that young red cells were larger and had more cell water and a higher cellular K+ content than unseparated red cells. Particularly, the leak K+ influx was lower than in unseparated cells, while K+ pump flux was higher and attributable to an increased number of pumps per cell. However, the Rhnull red cells studied by Lauf and Joiner showed considerably higher K+ leak and K+ pump fluxes than the young cells from Rh-Hr individuals. Furthermore, Lee and Stevenson reported that K+ leak flux was increased in both young and old Rhnull erythrocytes. In light of these experimental findings, the presence of the i antigen seems rather weak evidence for “immaturity” of the Rhnull cells, particularly since the unrelated MNUS system is also affected. Hence there is good reason to assume that the Rhnull red cell has a pleiotropic...
Cation fluxes in Rhnull red cells [letter]

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