The Effect of Transfusions of Erythrocytes on Untreated Pernicious Anemia

By James D. Mason, Jr. and Byrd S. Leavell

Examination of the bone marrow in untreated pernicious anemia reveals that erythropoiesis is megaloblastic in type. Either spontaneous remission, or the administration of liver extract, folic acid, or vitamin B₁₂ causes a disappearance of the characteristic megaloblasts and the appearance of normoblastic maturation. This change begins a few hours after specific therapy is started and is complete within a few days. During the first week after treatment is started reticulocytosis occurs and reaches a maximum value in 5 to 10 days. These changes occur as a result of the correction of a deficiency of a specific substance that is necessary for normoblastic maturation.

In 1946, Davidson, Murphy, Watson, and Castle reported the effects of giving massive transfusions of whole blood and packed erythrocytes to 5 patients with pernicious anemia in relapse. The authors reported that transfusions had no effect on the low leukocyte and platelet counts and did not produce striking clinical improvement in the patients even when the erythrocyte level was brought to normal. They also reported that the “megaloblasts characteristic of pernicious anemia” disappeared from the bone marrow within 4 to 12 days after transfusions were started. In 2 patients this occurred when the erythrocyte count was raised to levels of only 3.2 and 4 million. Their data did not indicate that any further change in the morphology of the erythrocyte precursors in the marrow was produced by the administration of liver extract after transfusions were given, although a small reticulocyte response occurred in all but one case. These observations suggested to them that the nutritional deficiency state that was manifested by a megaloblastic marrow was relative rather than absolute and that it could be affected by an artificial decrease in the bone marrow anoxia.

In the conclusions of the paper of Davidson et al., it was stated that “Following the blood transfusions, but prior to liver extract therapy, the bone marrow megaloblasts characteristic of pernicious anemia disappeared.” This important observation has been quoted widely but it would appear that the statement is open to two interpretations that do not have the same significance. One possible interpretation would mean that multiple blood transfusions produce a change in the erythrocyte precursors in the marrow of patients with pernicious anemia in relapse that is similar to that seen after specific therapy with vitamin B₁₂ or liver extract. Since the transfusions did not supply an effective amount of vitamin B₁₂, this interpretation would mean that with the same amount of available vitamin B₁₂, erythropoiesis may be normoblastic in type during reduced erythrocyte production and become megaloblastic (vitamin B₁₂ deficient) when erythrocyte production is rapid enough to deplete the available supply of vitamin B₁₂. A different conclusion is suggested if the term “normoblast” as
Table 1.—Data on Four Patients with Pernicious Anemia

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>Color</th>
<th>Ht. (°C)</th>
<th>R.B.C. (mill.)</th>
<th>Hebc. (Gm.%)</th>
<th>W.B.C. (cu.mm.)</th>
<th>Retic. (cu.mm.)</th>
<th>Platelets (cu.mm.)</th>
<th>Amount of erythrocytes given, (cc)</th>
<th>Length of transfusion period, (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1</td>
<td>69</td>
<td>M</td>
<td>W</td>
<td>16</td>
<td>1.2</td>
<td>5.0</td>
<td>4,700</td>
<td>21,400</td>
<td>150,000</td>
<td>1750*</td>
<td>4</td>
</tr>
<tr>
<td>#2</td>
<td>67</td>
<td>M</td>
<td>C</td>
<td>10</td>
<td>0.8</td>
<td>3.5</td>
<td>4,100</td>
<td>16,000</td>
<td>50,000</td>
<td>1750*</td>
<td>5</td>
</tr>
<tr>
<td>#3</td>
<td>65</td>
<td>M</td>
<td>W</td>
<td>25</td>
<td>1.6</td>
<td>7.6</td>
<td>7,800</td>
<td>17,000</td>
<td>190,000</td>
<td>1500</td>
<td>8</td>
</tr>
<tr>
<td>#4</td>
<td>60</td>
<td>F</td>
<td>C</td>
<td>16</td>
<td>1.2</td>
<td>4.0</td>
<td>2,300</td>
<td>21,600</td>
<td>40,000</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

* Additional 250 cc. given after vitamin B₂ started.

used by Davidson et al. is synonymous with the term “late megaloblast,” (Type III and IV). In this circumstance the disappearance of the “megaloblasts” would mean that the most immature cells have disappeared, while the remaining more mature erythrocyte precursors, “normoblasts,” may still show morphologic evidence of a specific deficiency. We have conducted a study similar to that of Davidson, Murphy, Watson and Castle. The results of this study are reported in this paper.

**Material and Methods**

The four patients selected for study were diagnosed as pernicious anemia by the presence of macrocytic anemia, gastric achlorhydria after histamine, megaloblastic bone marrow, and a clinical picture consistent with pernicious anemia. The pertinent data on these patients are shown in Table 1. As well as could be determined none of these patients had ever received previous therapy with vitamin B₁₂, folic acid, or liver extract.

Two patients were transfused with washed erythrocytes and one was transfused with unwashed erythrocytes until the hematocrit value became normal. The fourth patient was given 250 cc. of freshly frozen type AB plasma. The quantity of plasma given was limited by the fact that this patient showed early signs of heart failure following the administration of 250 cc. Subsequently the first 3 patients were treated with 30 micrograms of vitamin B₂ administered intramuscularly daily for 12 days. The patient who received plasma was treated with a potent oral preparation of vitamin B₁₂ and desiccated hog stomach. Blood counts were done at frequent intervals before, during, and after the institution of vitamin B₁₂ therapy. Bone marrow aspirations were performed at appropriate times before and after transfusions and after treatment with vitamin B₁₂.

The blood cell counts were made on heparinized venous blood with U.S. Bureau of Standards pipets and counting chambers. The hemoglobins were determined by the use of the Evelyn colorimeter using 15.6 Gm. of hemoglobin per 100 cc. of blood as the standard. The red cell indices were obtained by the methods of Wintrobe. Blood platelets and reticulocytes were determined by the indirect method from blood smears. The reticulocyte smears were stained with brilliant cresyl blue and counterstained with Wright's stain. All bone marrow aspirations were obtained by one of the authors from the iliac crest and counts were made from direct smears stained with Wright's stain. Differential counts of marrow specimens were done by both authors, a combined total of 1,000 nucleated red blood cells being counted on each marrow specimen. The myeloid erythroid ratio was computed on the basis of the number of myeloid cells counted per 1,000 nucleated red blood cells. Wintrobe's terminology of nucleated red blood cells has been used. Stage I represents the promegakoblast or pronormoblast; Stage II represents the basophilic megaloblast or normoblast; Stage III represents the polychromatic megaloblast or normoblast; and Stage IV represents the orthochromatic megaloblast or normoblast.

Whole blood for transfusion purposes was collected by the hospital blood bank. One hundred and twenty cc. of acid citrate dextrose solution was used as an anticoagulant for
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each 500 cc. of blood obtained. The red cell concentrates were prepared by centrifuging each unit of blood and then withdrawing the plasma and dextrose solution. Cold normal saline was then added to the red cells in an amount equal to that of the plasma and dextrose withdrawn and the blood was then centrifuged for 30 minutes after which the saline was withdrawn. The blood used was always less than 21 days old and in most instances was freshly drawn on the day of transfusion. Each unit of red cells represents the cellular component of 500 cc. of whole blood. Sterilon* recipient sets were utilized in giving the transfusions, and a limit of 2 units of red cells per day was observed.

RESULTS OF TRANSFUSIONS

The bone marrows of Cases 1, 2, and 3 who received packed erythrocytes were examined 3, 7, and 8 days respectively after transfusions were started. The bone marrow of Case 4 who received plasma was examined 8 days after the plasma was given. An example of the results obtained with packed erythrocytes is shown in figures 1, 2, and 3. These marrow samples were obtained from the same patient (Case 2) before transfusion with packed erythrocytes (fig. 1), 7 days after transfusions were started when the hematocrit was 45 per cent (fig. 2), and after 12 days of vitamin B₁₂ therapy (fig. 3). Erythropoiesis in figure 1 and 2 is thought to be megaloblastic and that shown in figure 3 is considered normoblastic. In all 3 patients who received erythrocyte transfusions, there was a decrease in the percentage of immature megaloblasts (Stages I and II) and

* Produced by Sterilon Corp., Buffalo, N. Y.
an increase in the percentage of more mature forms (Stages III and IV). There was a small and probably insignificant increase in percentage of early forms in the marrow of the patient who received plasma. These results are shown in table 2. In general the cellularity of the post-transfusion marrows appeared diminished in those patients who were transfused with erythrocytes, and the ME ratios of these marrows were increased over those of the pre-transfusion marrows in every patient (table 2). The cause of the increase in ME ratio observed in the patient who received plasma is not obvious. At this time she had a urinary tract infection which may have influenced the cellular composition of the marrow.

The absolute reticulocyte counts decreased in those patients who received erythrocyte transfusions and remained essentially unchanged in the patient who received plasma (table 2, figs. 4, 5, 6). The morphologic appearance of the leukocytes was not altered by transfusions, and the peripheral leukocyte and platelet counts were essentially unchanged.

Except for the relief of angina pectoris in one patient, there was no significant subjective improvement in the patients after transfusion.
Response to Specific Therapy

Erythrocytic maturation was considered to be normoblastic in all 4 patients when the bone marrows were examined 5, 12, 8 and 16 days respectively after vitamin B₁₂ was started (fig. 3). In all of the patients a reticulocytosis followed therapy with vitamin B₁₂ (figs. 4, 5, 6). The magnitude of response was small in the patients who were previously transfused to a normal hematocrit. However, a definite increase in reticulocytes total was observed in each case on the 7th to 9th day after vitamin B₁₂ was started. The patient who received plasma prior to specific therapy and whose hematocrit was only 14 when vitamin B₁₂ was started exhibited a much greater reticulocyte response which reached a total of 434,000 cu.mm. on the 9th day of treatment.

Discussion

Our interpretation of the changes observed in the three patients of the present study is that the erythrocyte transfusions diminished erythropoiesis but did not
Table 2.—Hematologic Changes After Administration of Red Cells or Plasma

<table>
<thead>
<tr>
<th>Patient</th>
<th>Type Transfusion</th>
<th>% Nucl. R.B.C. By Stages</th>
<th>M/E Ratio</th>
<th>Hct.</th>
<th>Hb. (Gm.)</th>
<th>R.B.C. (Million)</th>
<th>Absolute Retics.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
<td>II</td>
<td>III</td>
<td>IV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case #1 H. R.</td>
<td>7 Days Before Transfusion</td>
<td>10</td>
<td>17</td>
<td>65</td>
<td>8</td>
<td>1.4/1</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>3 Days After Transfusion</td>
<td>Unwashed red cells</td>
<td>3</td>
<td>14</td>
<td>65</td>
<td>18</td>
<td>2.3/1</td>
</tr>
<tr>
<td>Case #2 J. T.</td>
<td>7 Days Before Transfusion</td>
<td>9</td>
<td>6</td>
<td>55</td>
<td>30</td>
<td>.9/1</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>7 Days After Transfusion</td>
<td>Washed red cells</td>
<td>3</td>
<td>4</td>
<td>61</td>
<td>32</td>
<td>1.6/1</td>
</tr>
<tr>
<td>Case #3 J. M.</td>
<td>7 Days Before Transfusion</td>
<td>2</td>
<td>0</td>
<td>67</td>
<td>47</td>
<td>2.2/1</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>8 Days After Transfusion</td>
<td>Washed red cells</td>
<td>0.5</td>
<td>4</td>
<td>54</td>
<td>41.5</td>
<td>5.2/1</td>
</tr>
<tr>
<td>Case #4 B. B.</td>
<td>5 Days Before Transfusion</td>
<td>3</td>
<td>8</td>
<td>72</td>
<td>17</td>
<td>1.5/1</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>8 Days After Transfusion</td>
<td>Fresh frozen plasma</td>
<td>2</td>
<td>11</td>
<td>79</td>
<td>8</td>
<td>2.3/1</td>
</tr>
</tbody>
</table>

Figure 4
CASE #2 J.T. 67 C.M.

CASE #3 J.M. 65 W.M.
basically alter the manifestations of the deficiency state as exhibited by elements of the bone marrow and peripheral blood. Evidences for diminished erythropoiesis are the decrease in the absolute reticulocyte counts, the decrease in cellularity of the bone marrow together with an increase in the ME ratio, and a reduction in number of the more immature megaloblasts (Stages I and II) to less than 50 per cent of pretransfusion levels in two patients and to 63 per cent in the third patient. The interpretation of the post-transfusion marrows as megaloblastic appears to be supported by the marked changes in morphology of the marrow and the reticulocytosis that followed the administration of vitamin B$_{12}$.

Summary and Conclusions

1. Three patients with untreated pernicious anemia were transfused with packed erythrocytes to produce a normal hematocrit before they were treated with vitamin B$_{12}$. One patient with untreated pernicious anemia was transfused with 250 cc. plasma prior to therapy with vitamin B$_{12}$. No significant changes were noted in this patient who received plasma.

2. Following erythrocyte transfusions the bone marrows of these patients were considered to show the megaloblastic type of maturation with a reduction in percentage of the early megaloblasts (Stages I and II). The ME ratio of the marrows increased. A decrease in the absolute reticulocyte count occurred. No change occurred in the peripheral leukocyte and platelet counts and there was no significant change in the clinical condition of the patients.

3. After therapy with vitamin B$_{12}$, at a time when the hematocrits were normal, the bone marrows became normoblastic, a small but definite reticulocytosis occurred, the leukocyte and platelet counts returned to normal, and there was subjective improvement in the condition of the patient.

4. It is concluded that in these three patients with untreated pernicious anemia multiple transfusions of packed erythrocytes decreased erythropoiesis but did not cause any basic change in the type of erythrocyte maturation.

Summario in Interlingua

1. Tros patientes con nontractate anemia pernicioso recipeva transfusiones de paccate erythrocytos, con le objectivo de establir un normal hematocrite ante le initiatio del tractamento a vitamina B$_{12}$. Un patiente con nontractate anemia pernicioso recipeva un transfusion de 250 cm$^3$ de plasma ante le initiatio del therapia a vitamina B$_{12}$. Nulle significative alterationes esseva notate in iste ultime patiente.

2. Post transfusiones de erythrocytos le medullas ossee de iste patientes pareva exhibir le typo megaloblastic de maturation con un reduction del procentage de precoce megaloblastos (stadios I e II). Le proportion M:E (myeloide: erythroide) del medulla accresceva. Occurreva un reduction del absolute conto reticulocytic. Nulle alteration occurreva in le peripheric contos leucocytic e plahetto, e il habeva nulle significative alteration in le condition clinic del patientes.

3. Post le therapia a vitamina B$_{12}$, a un tempore quando le hematocrites esseva normal, le medullas ossee deveniva normoblastic, un leve sed definite
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reticulocytosis ocurrieva, le contos leucocytic e plachettal retornava a valores normal, e le stato subjective del paciente se meliorava.

4. Nos conclude que in le tres patientes con nontractate anemia perniciose le multiple transfusiones de paccate erythrocytos reduceva le erythropoiese sed non causava un alteration fundamental in le typo del maturation erythrocytic.

REFERENCES

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