Humoral Regulation of Red Cell Production

By Allan Erslev, M.D.

It is generally accepted that the oxygen content of arterial blood regulates erythropoietic activity. However, whether this regulatory effect is exerted on the bone marrow directly or through an intermediary humoral factor is still unknown.

The oxygen content of arterial blood is dependent on the oxygen tension of alveolar air, on pulmonary and cardiac function and on the amount of normal hemoglobin in circulating blood. Increased red cell production is induced by a low oxygen content of arterial blood and decreased production follows a high oxygen content. The oldest and still most commonly held explanation for this regulation is that the oxygen in arterial blood influences the activity of nucleated red cells directly, either by controlling the oxygen tension and saturation of bone marrow blood or by determining the amount of oxygen delivered to the bone marrow tissue. Recent experimental evidence appears to weaken this hypothesis. Oxygen tension and saturation of bone marrow blood have been shown by Grant and Root to be normal in dogs in which increased erythropoiesis was induced by acute and chronic bleeding. Normal oxygen saturation of bone marrow blood was also found in patients with various chronic anemias and with polycythemia vera. Birkhill has emphasized that the amount of oxygen delivered to the bone marrow probably is more important than the oxygen tension of the bone marrow in regulating red cell production. However, as Tinsley et al. have stated, it is hard to believe that normoblasts which consume large amounts of oxygen should be stimulated to increased activity by decreasing their supply of oxygen. Furthermore, tissue culture studies have indicated that anoxia of bone marrow cultures depressed and arrested rather than increased normoblastic activity.

In 1906 Carnot and Deflandre suggested that blood oxygenation regulates red cell production by means of an intermediary factor. According to this theory the arterial blood oxygen regulates the production of a factor capable of stimulating erythropoiesis. This factor is carried to the bone marrow by the blood stream. To test the theory, Carnot and Deflandre injected 9 ml. of plasma obtained from slightly anemic rabbits into normal rabbits and noticed a small rise in the number of red cells in the peripheral blood. However, plasma from severely anemic rabbits did not have any effect on the red blood cell count.

During the next twenty years a number of investigators attempted to prove the existence of a plasma factor, the so-called erythropoietin, which would stimulate red cell production. From 0.5 to 10 ml. of plasma obtained from anemic
rabbits or rabbits kept at low barometric pressure were injected into test rabbits. The injections were followed, often within 12 hours, by moderate or marked increase in the red blood cell counts. These studies are conflicting and unconvincing but were interpreted as being in support of a humorai theory.

Gordon and Lubin (1934), and Feenders (1936) repeated most of these experiments and failed to find any red cell stimulating property of "anemic" or "low pressure" plasma.

In 1948 Bonsdorff and Jalavista revived the humoral theory. They reported that 3 ml. of plasma from patients with congestive failure or from rabbits exposed to low barometric pressure produced a slight increase in red blood cell count when injected into normal rabbits. In 1949 Oliva et al. injected 20 ml. of plasma obtained from patients with bleeding anemia or pernicious anemia into normal subjects. Within 12 hours there was a brief rise in reticulocyte count to 1.8 to 3.0 per cent. These reports are not very convincing.

In 1950 Reismann pointed out that the use of very small amounts of plasma to demonstrate the existence of a plasma factor capable of stimulating red cell production is almost certain to be unsuccessful. He proposed to use parabiotic rats as a more adequate approach. Parabiotic rats were exposed for five weeks in a special breathing chamber to defined gas mixtures in such a way that one rat was breathing an oxygen-nitrogen mixture whose oxygen content was 8 vol. per cent whereas its partner was breathing normal air. Both partners developed normoblastic hyperplasia of the bone marrow. This result led Reismann to conclude that a humoral factor stimulating the erythropoiesis had been elicited by the hypoxemia in one rat and transferred to its partner. Blood gas studies were performed in several pairs and revealed that in these pairs the arterial oxygen tension corresponded to the air each partner was breathing without being influenced by the blood mixing through the anastomosis. However, the experimental technic is so difficult to standardize that of 139 operated pairs only 15 could be used and in none of the latter were blood gas studies made. Reismann's excellent study has supported the theory of a humoral regulation of red cell production but has not proved it.

The study reported here was carried out in order to secure definite and reproducible information as to the presence or absence of a plasma factor capable of stimulating red cell production. Large amounts of plasma from rabbits made anemic by bleeding were injected into normal rabbits. As a control, the same amounts of plasma from normal rabbits were used. The resulting effects on red cell production in the injected rabbits were compared.

**Materials and Methods**

White New Zealand male rabbits weighing 2.8 to 3.2 Kg. and fed on Red Rose rabbit pellets and water were used as blood donors and plasma recipients.

The donor rabbits were bled by heart puncture (150 ml. in 48 hours) reducing the hematocrits to less than 20 per cent. These anemic donors were bled 20 to 40 ml. daily; the blood was mixed with heparin (1.0 ml. of a heparin solution containing 1.0 mg. of heparin per 20 ml. of blood) and plasma obtained by centrifugation of the heparinized blood. Plasma from normal rabbits was obtained in the same way. The plasma was injected slowly into the marginal ear vein of the recipient rabbit within a few hours of its being drawn.

Blood counts were carried out on a sample of 1.5 ml. of oxalated blood obtained from the
ear vein or the heart. However, platelet counts were carried out directly on a drop of blood from a small ear vein.

Hematocrit determinations were made by centrifuging blood in Wintrobe hematocrit tubes for 30 minutes at 3000 r.p.m. Hematocrits in 20 normal rabbits varied between 36 per cent and 45 per cent.

Red blood cell counts, white blood cell counts and platelet counts were carried out in two pipets each. Red blood cell counts per cu. mm. in 20 normal rabbits varied between 5.02 million and 7.99 million. Reticulocyte counts were made on dry smears prestained with brilliant cresyl blue and counterstained with Wright’s stain. From 1000 to 2000 red cells were counted. The absolute reticulocyte count per cu. mm. was calculated by multiplying red blood cell count by the per cent of reticulocytes.

In 81 determinations on 20 normal rabbits the reticulocyte count varied between 80,000 per cu. mm. (1.2 per cent) and 310,000 per cu. mm. (5.1 per cent). Mean value ± 1 standard deviation was 155,000 ± 60,000 per cu. mm. Differential leukocyte counts were made on 200 cells. Bone marrow aspirations were carried out from the upper third of the tibia. A 20 gauge needle with adapter was introduced without any local or general anesthesia into the bone marrow cavity and 0.1 ml. of material was aspirated. Coveralip preparations were made and stained with Wright’s and Giemsa’s stain. Differential counts were made on 1000 nucleated cells and the percentage of nucleated red blood cells determined.

RESULTS

Twenty-five ml. of heparinized plasma from anemic rabbits (“anemic” plasma) was injected once a day for eight days into 2 normal rabbits. There was no significant change in the number of reticulocytes, the red blood cell count or the hematocrit.

Fifty ml. of heparinized plasma from anemic rabbits was injected once a day for two days into 2 normal rabbits and once a day for four days into 7 rabbits.
There was a significant rise in the number of reticulocytes per cu. mm. in both groups of recipients but much more marked in the group which received a total of 200 ml. of "anemic" plasma (fig. 1).

Fifty ml. of heparinized plasma from normal rabbits injected once a day for four days into 6 normal recipient rabbits failed to elicit any significant rise in the total number of reticulocytes (fig. 1).
Figure 2a and b records the reticulocyte counts in 2 rabbits. One received first a total of 200 ml. of “anemic” plasma followed three weeks later by a course of 200 ml. of normal plasma. The other rabbit received the normal plasma three weeks prior to a course of “anemic” plasma. In each recipient only “anemic” plasma elicited a reticulocyte response.

Figure 3 summarizes the reticulocyte counts in each of 7 rabbits receiving plasma from anemic donors, and in each of 6 rabbits receiving plasma from normal donors.
rabbids receiving 50 ml. of plasma from normal donors once a day for four days. The “anemic” plasma is seen consistently to produce a significant reticulocytosis.

Figure 4 illustrates the average changes in red blood cell counts and hematocrits in 7 rabbits receiving “anemic” plasma and in 6 rabbits receiving normal plasma. The changes are probably not statistically significant but they would seem to indicate that there is a more rapid and complete recovery from the dilution anemia in the group receiving “anemic” plasma than in the group receiving normal plasma. The plasma injections induced in all recipient rabbits an immediate drop in hematocrit of from 6 to 9 per cent, but the hematocrit values returned to near normal within 4 to 6 hours.

In 4 rabbits, 2 receiving “anemic” plasma and 2 receiving normal plasma, the bone marrow was examined before the first plasma infusion and after the fourth one. Table 1 indicates that in these rabbits the infusion of “anemic” plasma was

### Table 1—Nucleated Red Blood Cells in Per Cent of Total Nucleated Cells in the Bone Marrow of Four Rabbits Before and After Receiving 200 ml. of Normal or “Anemic” Plasma

<table>
<thead>
<tr>
<th>Rabbit</th>
<th>Plasma infused</th>
<th>Per cent nucleated RBC in B.M.</th>
<th>Before infusion of plasma</th>
<th>After a total of 200 ml. of plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>45</td>
<td>“Anemic”</td>
<td>30.4</td>
<td>49.0</td>
<td></td>
</tr>
<tr>
<td>48</td>
<td>“Anemic”</td>
<td>29.6</td>
<td>49.7</td>
<td></td>
</tr>
<tr>
<td>53</td>
<td>Normal</td>
<td>26.6</td>
<td>33.5</td>
<td></td>
</tr>
<tr>
<td>66</td>
<td>Normal</td>
<td>32.8</td>
<td>34.9</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 5.** Hematologic response in 2 rabbits to thirteen infusions of 50 ml. of plasma from anemic donors.
followed by a definite erythroblastic hyperplasia while normal plasma did not change the ratio between the cellular elements appreciably.

Two normal rabbits received 50 ml. of plasma from anemic donors thirteen times in seventeen days. The effect on the blood picture is recorded in figure 5. There was a definite rise in red blood cell count, hematocrit and per cent nucleated red cells in the bone marrow but no significant change in platelet count or granulocyte count.

**DISCUSSION**

A decreased oxygen content of the arterial blood is known to stimulate red cell production. This stimulation is generally thought to be induced by anoxemia of the nucleated red blood cells in the bone marrow. However, it has been suggested that the anoxemia stimulates the production of a humoral factor which in turn increases red cell formation.

It has never been possible convincingly to demonstrate or isolate this postulated humoral factor and only Reismann's experiment\textsuperscript{19} can be said to support the theory that such a factor exists. Unfortunately the complicated parabiotic technic employed in his investigation leaves it open to some criticism.\textsuperscript{1} Other investigators interested in this theory have attempted to bioassay small amounts of plasma (from 0.5 to 10 ml.) obtained from animals rendered anoxemic by bleeding or by breathing air with a low oxygen content. It seems unlikely that these amounts constituting so small a fraction of the donors' and the recipients' plasma volumes would contain enough of the hypothetical factor to elicit a demonstrable increase in the red cell production of the recipient.

Larger amounts of plasma have heretofore not been used because the dilution effect of the injected plasma, causing a relative anemia, was thought to obscure the results. However, this relative anemia is of short duration (from 4 to 6 hours) and it is doubtful that it will cause any appreciable stimulation of red cell production. Intermittent anoxemia of like duration, as experienced by flyers, has not been shown to cause a significant reticulocytosis or polycythemia\textsuperscript{19}. In the present study 50 ml. of normal rabbit plasma was injected once a day for four days into rabbits with a plasma volume of approximately 100 to 150 ml. without evidence of significant stimulation of red cell production.

This control experiment made it possible to evaluate the effect on erythropoiesis of similar amounts of plasma obtained from anemic animals. The results indicate that plasma from rabbits made anemic by bleeding stimulates red cell production. This strongly supports the theory that red cell production is controlled at least to some extent through an intermediary humoral factor.

The physiology of the bone marrow in rabbits is so similar to that of man that it seems probable that human erythropoiesis is controlled by a similar humoral factor. Conceivably isolation and purification of this factor would provide an agent useful in the treatment of conditions associated with erythropoietic depression, such as chronic infection and chronic renal disease.

It has been suggested that the bone marrow functions as a unit.\textsuperscript{22} If true, this would explain the fact that increased or decreased erythropoiesis is frequently associated with similar changes in myelopoiesis and thrombocytopoiesis. Polycythemia vera and early myelocytic leukemia are often characterized by an
increased activity of all three bone marrow elements. Prolonged and intense stimulation of red cell production as seen in chronic hemolytic anemia and chronic bleeding are often associated with an increase in white blood cell count and platelet count. Conversely, chronic aplastic anemia frequently begins with a depression of erythropoiesis to be followed at a later time by depression of white cell and platelet production. Transfusion polycytemia maintained for twenty-five days in a small number of rabbits has been shown not only to depress endogenous red cell production but eventually to produce leukopenia and fatty replacement of the red active marrow.

In the study reported here there was no change in the white blood cell count or platelet count in 2 rabbits in which thirteen injections of plasma from anemic donors had produced a significant increase in red cell production. However, it is possible that the employment of larger amounts of the stimulating factor contained in plasma from anemic donors over longer periods of time may reveal an effect on granulocyte and platelet production.

SUMMARY AND CONCLUSION

Large amounts of plasma from rabbits, rendered anemic by bleeding, were injected into normal rabbits. A significant rise in the number of reticulocytes was observed in these rabbits. Control rabbits receiving the same amount of plasma from normal donor rabbits failed to show any significant change in the reticulocyte count. A definite increase in red blood cell count, hematocrit and per cent nucleated red cells of the bone marrow was noticed in 2 rabbits receiving repeated injections of plasma from anemic rabbits. It is concluded that plasma from rabbits rendered anemic by bleeding contains a factor capable of stimulating red cell production.

REFERENCES

23. Chetwick, A.: Transfusion plethora: Effects on bone marrow and blood cells. Thesis for the degree of Doctor of Medicine at Yale University School of Medicine, 1953.
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