Erythropoietic Function in Dilution Anemia

By ALLAN J. ERSLEV

DILUTION ANEMIA OR HYDREMIA is a condition characterized by an increase in total plasma volume without corresponding change in total red cell volume. Certain anemias complicating pregnancy,\(^1\) malnutrition,\(^2\) neurasthenia,\(^3\) and the therapeutic use of phenylbutazone\(^4\) and steroids\(^5\) have been considered to be caused by such a dilution of the red cell mass. The erythropoietic response to these anemias has not been studied adequately, but they are usually not associated with any significant reticulocytosis despite the decrease in oxygen-carrying capacity of the arterial blood. However, the reduction in hemoglobin concentration takes place gradually and is often so moderate that it is unlikely that occasional reticulocyte counts would give accurate information in regard to erythropoietic activity.

In order to determine the response of erythropoietic tissue to dilution of blood, an acute and pronounced dilution anemia was induced in rabbits. Dextran was used as a plasma expander because of its lack of side reactions\(^6\) and its ability to exert an effective expansion lasting 6 to 24 hours.\(^7\) Erythropoietic activity was evaluated from daily reticulocyte counts and from examination of bone marrow smears.

MATERIAL AND METHODS

White New Zealand rabbits weighing 2-3 Kg. and fed rabbit pellets and water were used. Hemoglobin concentrations were determined spectrophotometrically on oxyhemoglobin from venous blood.

Reticulocyte counts were made on smears of venous blood mixed with new methylene blue.\(^8\) Small pieces of polyethylene tubing were used for mixing blood and stain rather than white blood cell counting pipets in order to prevent clotting and to reduce trauma to the friable reticulocytes. Duplicate counts were made by having two observers count 1000 red blood cells each. The results were reported in per cent.

Bone marrow aspirations were made from the tibia and smears prepared and stained with Wright's stain. 1000 nucleated cells were examined and the number of normoblasts was expressed in per cent.

RESULTS

(a) Erythropoietic Response to Acute Blood Loss

Four rabbits were bled 20 ml./Kg. by cardiac puncture. In order to minimize the effect of shock the blood volume was immediately restored to normal by injecting 20 ml./Kg. of a 6 per cent solution of dextran into the marginal ear vein.

Daily reticulocyte counts and hemoglobin concentrations are charted in figure 1. Three and four days after the hemorrhage, the mean reticulocyte counts reached a high of 13.7 per cent and 12 per cent. In order to evaluate the absolute...
Fig. 1.—Hemoglobin concentrations, reticulocyte counts and per cent normoblasts in bone marrow in four rabbits after a hemorrhage of 20 ml./Kg. with immediate replacement by 20 ml./Kg. of a 6 per cent solution of dextran. Stippled line records the mean reticulocyte counts corrected for the reduced number of red cells found after the initial hemorrhage.

increase in reticulocyte production the percentage values were corrected for the drop in the number of red cells produced by the initial bleeding. The stippled line in figure 1 records the mean reticulocyte counts as percentages of the number of red blood cells in blood with a hemoglobin concentration equal to the average of the baseline determinations. It can be seen from this curve that the absolute number of reticulocytes in the peripheral blood starts to increase 48 hours after the hemorrhage and reaches a maximum of about three times the baseline count on the next two days.

Bone marrow examinations four days after the hemorrhage revealed that the number of normoblasts increased from a mean of 28 per cent of nucleated hematopoietic cells to a mean of 49 per cent.

(b) Erythropoietic Response to Sustained Dilutions of Blood with Dextran

Six rabbits received 25 ml./Kg. of a 6 per cent solution of dextran intravenously every twelve hours for four days. Two animals died shortly after the first injections but the remaining four tolerated the injections well. Hemostasis was moderately impaired but there was no evidence of a generalized bleeding tendency. Hemoglobin concentrations and reticulocyte counts were determined
daily before the morning injection and hemoglobin concentrations were again determined immediately after this injection. The results are charted in figure 2. The mean drop in hemoglobin concentrations in this dilution anemia compares very closely to the drop found after a single hemorrhage of 20 ml./Kg. (fig. 1). The reticulocyte counts in per cent of red cells did not change significantly during the period of anemia, and the bone marrow did not reveal any signs of increased erythropoiesis.

(c) Erythropoietic Response to Acute Blood Loss followed by Sustained Dilution of Blood with Dextran

The effect of hypervolemia and dextran per se on erythropoietic function was studied in a separate experiment. Five rabbits were first bled 20 ml./Kg. and then at once given 20 ml./Kg. of a 6 per cent solution of dextran as in experiment (a). Immediately following this procedure they received an additional 25 ml./Kg. of dextran intravenously after which they received the same amount of dextran every twelve hours for four days as in experiment (b). Two rabbits died but the remaining three tolerated the ordeal very well. Figure 3 shows the acute drop in hemoglobin concentration, the rise in normoblastic activity of the
Fig. 3.—Mean hemoglobin concentration, reticulocyte counts and per cent normoblasts in bone marrow of three rabbits bled 20 ml./Kg. with immediate replacement by 20 ml./Kg. of Dextran and subsequently receiving 25 ml./Kg. of dextran every 12 hours for four days.

bone marrow, and the resulting reticulocytosis. The degree of erythropoietic activity in this experiment resembles closely that found in experiment (a) (fig. 1), indicating that neither hypervolemia nor dextran have an inhibiting effect on erythropoieses.

**DISCUSSION**

It is well established that a decrease in the oxygen content of arterial blood caused by decreased oxygen tension or by decreased oxygen-carrying capacity is associated with an increase in red cell production. The study on dilution anemia reported here shows that a reduction in the content of oxygen per unit arterial blood will not invariably result in an increased red cell production. Dilution of blood with large amounts of dextran resulted in a reduction in hemoglobin concentration and oxygen content per unit of blood but did not induce a reticulocytosis or a normoblastic hyperplasia of the bone marrow. In a control experiment it was shown that neither dextran nor hypervolemia interfered with erythropoietic activity. In interpreting this lack of compensatory erythropoiesis in dilution anemia it must be recalled that the cardiac output is increased in hypervolemia. With an increase in cardiac output, tissues can be supplied...
with a normal amount of oxygen without decrease in the venous oxygen tension. Consequently, the oxygen tension in the capillaries and tissues will be maintained near normal.

These observations indicate that it is the tissue tension of oxygen and not the oxygen content per unit of blood which regulates red cell production. The tissue responsible for erythropoietic regulation is still unknown. Until recently it was assumed to be the bone marrow. However, in vivo studies have failed to demonstrate a measurable fall in marrow oxygen tension in the anemic animal, or man, and in vitro studies of bone marrow have shown that erythropoietic activity is depressed rather than stimulated by a reduction in oxygen tension. Furthermore, a number of studies have directly or indirectly demonstrated the existence in serum from anemic animals of a factor (the erythropoietic serum factor), capable of stimulating red cell production. In view of these findings it seems more likely that the oxygen tension of an extramedullary organ or cellular system controls erythropoietic activity. As a working hypothesis it is proposed that this “erythropoietic center” responds to a decrease in the tissue tension of oxygen by releasing the erythropoietic factor into the circulation, consequently causing an increased red cell production.

The tissue tension of oxygen in the hypothetical erythropoietic center probably depends, as the tissue tension in other organs, on the hemoglobin concentration, the oxygen tension of inspired air, the cardiac output, the respiratory function and on local vasomotor activities. The present experiments suggest that when an anemia is associated with a reticulocytosis, as after blood loss, it must mean that the cardiovascular and pulmonary mechanisms for increasing the tissue tension of oxygen do not compensate adequately for the reduction in the oxygen-carrying capacity of blood. One reason for this lack of compensation seems to be that a measurable increase in cardiac output and pulmonary function does not take place before the hemoglobin concentration is less than 7 to 8 Gm. per cent. Another possibility is that the erythropoietic center is located in one of the organs, i.e., skin or kidney, from which, in anemias, blood is shunted to more oxygen-sensitive tissues. This local diversion of blood flow away from the center would counteract any tendency to improved oxygenation due to increase in cardiac output or respiratory minute volume.

In conclusion it can be stated that the lack of response of the erythropoietic tissue to dilution anemia supports the old theory that red cell production is controlled by the tissue tension of oxygen.

**Summary**

For a period of four days, four rabbits received intravenously at 12-hour intervals large amounts (25 ml./Kg.) of a 6 per cent solution of dextran. This caused a pronounced dilution anemia. However, no increase in erythropoietic activity was observed despite the reduced oxygen content of each unit of arterial blood.

Since hypervolemia and dextran per se were found not to interfere with red cell production it was concluded that the increased cardiac output in dilution anemia compensates for the reduction in the oxygen content of each unit of arterial blood.
Consequently, the stimulus to erythropoietic function must depend on the tissue tension of oxygen rather than on the oxygen content of arterial blood.

**Summario in Interlingua**

Iuratste (luatro dies, quatro coisilios recipeva a intervalllos de 12 horas large dosages (25 ml. Kg.) de un solution de 6 pro ceisto de dext.rano. Ist.o resultava its protsutsciate hydremia. Nonobstante, nulle augmetsto del activitate erythropoietic esseva observate in despecto del reducit.e cotsteisto de oxygetso its cata utsitate de sanguilse arterial.

Proque il esseva constatate que hypervolemia e dextraiso per se non disturba le production de erythrocyt.os, le conclusion se impo.ssa que in hydreissia le augmentate rendimento cardiac compensa le reduction del conten.to de oxygetso in cata unitate de sanguine arterial.

Consequentemente le stimulo del activitate erythropoietic debe depetider del tension oxygenic in le texito plus tosto que del coisteisto de oxygetso its le sanguine arterial.

**References**

14. **Thomas, E. D.**: Hemin synthesis in marrow culture. (In press.) (See also pp. 600-615, this issue.)
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