The Erythropoietic Effect of Hematocrit Variations in Normovolemic Rabbits

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In 1960 Jandl and coworkers1 presented a graph which related blood flow of oxygen through a rigid capillary tube (Ostwald's Viscosimeter) to the hematocrit of the blood. The bell-shaped curve with its maximal oxygen flow at "normal" hematocrit values suggested that the evolutionary choice of a 45 per cent hematocrit as normal in most mammals was related to the flow properties of whole blood. Since then, physiologic support for the concept of an optimal supply of oxygen to the tissues at normal hematocrit has been provided by several groups who calculated systemic oxygen transport on the basis of cardiac output and arterial oxygen content.2-4 However, the acceptance of this concept must mean that the oxygen transport to the tissues is suboptimal both at anemic and at polycythemic hematocrit values. In anemia, a suboptimal oxygen transport is supposed to lead to tissue hypoxia and in turn to a compensatory increase in the rate of red cell production.5 In transfusion polycythemia, on the other hand, the presumed suboptimal oxygen transport does not lead to an increase in red cell production. Since transfusion polycythemia is associated with an increase in blood volume, it is possible that the resulting increase in tissue vascularity may influence significantly the perfusion rates and tissue tension of oxygen. Consequently, it was decided to study the effect of changes in hematocrit and viscosity on red cell production under conditions of a constant blood volume.

METHODS AND MATERIALS

Adult, male, white New Zealand rabbits weighing between 2 and 3 Kg. were used throughout.

1. Viscosity Determinations: Whole rabbit blood anticoagulated with balanced oxalate (6 mg. ammonium oxalate + 4 mg. potassium oxalate per 5 ml. whole blood) was adjusted to hematocrits ranging from 0 per cent to 70 per cent. An Ostwald Viscosimeter was used to measure viscosity at high shear rates (>200 inverse seconds) and a Brookfield Viscosimeter was used to measure viscosity at shear rates ranging from 230 inverse seconds to 11.5 inverse seconds. All determinations were made at 37 C.

2. Induction of Normovolemic Anemia and Polycythemia: Normal rabbits were bled by cardiac puncture with immediate isovolumetric replacement via the marginal ear vein by either normal rabbit serum or packed normal rabbit red cells. The volumes replaced were 10, 20 or 30 ml./Kg. in a single bleeding or 40 ml./Kg. in two bleedings 2 hours apart.

3. Serum Iron Turnover and Blood Volume: Exactly 48 hours after the induction of normovolemic anemia or polycythemia the rabbits were given one-half μC of Fe⁵⁹ preincubated with 4 ml. normal rabbit serum intravenously. After 10, 20, 30 and 40 minutes,
3 ml of blood was removed by cardiac puncture. Serum radioactivity was determined and the disappearance rate of radioactive iron calculated. The iron content was determined on the serum samples by the method of Peterson and the serum iron turnover calculated from the equation given by Bothwell et al.:

\[ \text{Serum iron turnover} = \frac{\text{Serum iron concentration} \times (100 - \text{hematocrit})}{\text{T/2} \times 100} = \text{mg. Fe/100 ml.} \]

blood/24 hr. Plasma volume was determined by extrapolation of the Fe\(^{59}\) disappearance curve to 0 hour and using the isotope dilution technic. Venous hematocrit was used to calculate blood volume.

4. **Determination of Erythropoietin Titer**: Male Sprague-Dawley rats were rendered polycythemic by means of a single red cell transfusion (2 ml packed red cells per 100 Gm. rat). On the next 2 days they received serum extract intravenously (1 ml extract per 100 Gm. rat X 2). On the following day 0.5 uc Fe\(^{59}\) was given i.v. and the 24-hour red cell utilization was determined. The serum extract was prepared by heating acidified serum (pH 5.5) to 100 C. for 6 minutes. Figure 1 gives the dose response curve of sheep erythropoietin (NIH Step IV) in this assay system.

**RESULTS**

1. Viscosity of whole oxalated blood measured in an Ostwald Viscosimeter is recorded in Figure 2. When estimating oxygen flow, using the equation:

\[ O_2 \text{ Flow} = Hct \times \frac{1}{\text{Viscosity}} \]

an optimal oxygen flow occurred at hematocrits in the normal range. Deviations from this range caused a decrease in oxygen
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Fig. 2.—Viscosity of whole oxalated rabbit blood measured by means of an Ostwald Viscosimeter at 37 C. and related to hematocrit. Oxygen flow is computed from the formula $O_2 \text{ Flow} = \text{Hematocrit} \times \frac{1}{Viscosity}$ and is recorded in arbitrary units.

When $O_2$ flow was calculated from viscosity studies at lower shear rates (Brookfield Viscosimeter) the high hematocrit blood displayed an even more striking reduction in oxygen flow (Fig. 3).

2. The blood volume in normal rabbits, determined by means of isotope dilution and venous hematocrit, was found to be 6.5 per cent of body weight with a standard deviation of 1.4 per cent. It was possible to induce and maintain a normovolemic anemia for 48 hours but not a normovolemic polycythe-
Fig. 3.—Oxygen flow computed from the viscosity of whole oxalated blood measured at various shear rates by means of a Brookfield Viscosimeter.

When intensive replacement with red cells was undertaken, an expansion of the blood volume took place "absorbing" additional red cells and preventing the hematocrit from reaching levels much above 60 per cent. The mortality of rabbits replaced with more than 30 ml./Kg. of red blood cells was also exceedingly high and impeded attempts to achieve high hematocrits. Figure 4 charts the mean blood volume of rabbits with 10, 20 or 30 ml. Kg. serum replacement or 10, 20, 30 and 40 ml./Kg. red cell replacement.

3. The serum iron turnover was determined in a total of 39 rabbits and the results are related to the hematocrit concentration in Figure 5.

The correlation coefficient is −0.86, a highly significant value. The solid line charts the mean turnover of animals with 10, 20 or 30 ml./Kg. serum replacement or 10, 20, 30 or 40 ml./Kg. red cell replacement.

4. Erythropoietin titers on serum obtained from rabbits with serum or red cell replacements are given in Figure 5. The mean Fe³⁺ uptake of polycythemic rats receiving serum from normal or polycythemic rabbits lie within the standard deviation of rats receiving saline. This suggests that the amount of erythropoietin present in the blood of normal or polycythemic rabbits is too small to
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Fig. 4.—Mean blood volume of rabbits 48 hours after the induction of normovolemic anemia or polycythemia. The determinations were made on 8 normal rabbits with a mean hematocrit of 42 per cent and on rabbits with 10, 20 or 30 ml./Kg. serum replacement and 10, 20, 30 or 40 ml./Kg. red cell replacement.

be recognized in this assay. Despite the theoretic impairment of oxygen flow at hematocrits around 60 per cent, there was no evidence of a release of erythropoietin.

Discussion

It is generally accepted that the rate of red cell production is influenced, if not actually controlled, by the tissue tension of oxygen in a specific target organ. Although many studies strongly suggest the kidney as this target organ, the persistence of erythroid activity in nephrectomized rabbits in anephric man indicate that extrarenal areas also are involved. Wherever the target organ is, its tissue tension of oxygen must be determined by its supply and its consumption of oxygen. The supply of oxygen to the tissues appears to be dependent on blood viscosity with an optimal supply at normal hematocrit values. Richardson and Guyton showed in normovolemic dogs that the cell flow defined as hematocrit times cardiac output is optimal at a 40 per cent hematocrit. Murray, Gold and Johnson showed that systemic oxygen transport defined as cardiac output times arterial oxygen content also is optimal at this value both in normovolemic and hypervolemic dogs. Weisse and co-workers
Fig. 5.—Upper chart shows erythropoietic titer expressed in 24-hour Fe\textsuperscript{59} utilization of recipient polycythemic rats. The numbers in parenthesis above the standard deviation brackets give the number of donor rabbits while the numbers below the brackets give the number of recipient polycythemic rats used for the assays. Double circle denotes the normal donor animals. The lower chart gives the individual and mean (double circle) serum iron turnover values of rabbits rendered anemic and polycythemic.

confirmed this data and stated that the bell-shaped curve with its optimum at normal hematocrit values most likely is related to viscosity of blood in peripheral vessels (capillaries and veins).

In addition to suggesting why normal hematocrits are adjusted to 40-45 per cent, these studies also indicate that the oxygen transport to the tissues is reduced both at anemic and at polycythemic hematocrit values. Since the oxygen consumption in the tissues is fairly constant regardless of hematocrit, a deviation of hematocrit from normal should result in a low oxygen saturation of venous blood and consequently a tissue hypoxia. Weisse and co-workers'
state that a reduction in oxygen transport associated with a polycythemia does not necessarily imply a disadvantage in terms of oxygen supply to tissues. However, the data they use to support this conclusion (their Table 2) is derived from a study in which the oxygen transport happened to be the same at 30, 48 and 65 per cent hematocrits. If data are used from studies showing the characteristic decrease in oxygen transport at low and high hematocrits, the mixed venous oxygen saturation will be correspondingly low.

The studies presented here show that in regard to release of erythropoietin and rate of red cell production, normovolemic anemic rabbits with low "oxygen flow" behave very differently from normovolemic polycythemic rabbits with equally low "oxygen flow." Since the bone marrow in a polycythemic animal is not suppressed but actually hypersensitive to exogenous erythropoietin, it appears that the polycythemia with its associated high viscosity in some manner prevents the hypoxia from generating erythropoietin. Although the blood volume was higher in the polycythemic rabbits than in the anemic rabbits, the increase was only slight and it does not seem likely that the associated increase in vascularity could abolish tissue hypoxia. If the kidneys actually produce and release erythropoietin, it is possible that the intricate microcirculation here permits a differentiation of tissue hypoxia with low viscosity from tissue hypoxia with high viscosity. However, it appears that the simple hypothesis that tissue hypoxia in a target area directly leads to the generation of erythropoietin needs considerable modification.

**Summary**

Measurements of erythropoietin titer and serum iron turnover were carried out in rabbits 48 hours after the induction of normovolemic anemias and polycythemias. Although viscosity studies of blood at various hematocrits indicate that the oxygen flow to the tissues is impaired both in anemia and polycythemia, only anemias were found to be associated with production of erythropoietin and with accelerated serum iron turnover. It was concluded that if polycythemia with its associated high viscosity leads to tissue hypoxia, this tissue hypoxia will not cause production of erythropoietin.

**Summary in Interlingua**

Mesurationes del titro de erythropoietina e del metabolismo seral de ferro esseva effectuate in conilios 48 horas post le induction de anemia e polycytihemia normovolemic. Ben que studios del viscositate de sanguine a varie hematocrites indica que le fluxo de oxygeno verso le tissu es imperfeecte tanto in anemia como etiam in polycytihemia, il esseva trovate que solmente anemia esseva associate con le production de erythropoietina e accelerate metabolismo seral de ferro. Esseva concludite que, si polycytihemia con su associate alte viscositate duce a hypoxia tissular, iste hypoxia tissular non causa ulle production de erythropoietina.

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REFERENCES


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