The “Tissue” Tension of Oxygen and Its Relation to Hematocrit and Erythropoiesis

By Eivind B. Thorling and Allan J. Erslev

An adequate supply of oxygen to the individual cell in a multicellular organism is dependent on a circulatory system by means of which oxygen, reversibly bound to various pigments, is transported from an uptake organ to the capillaries of the tissues. From these capillaries, oxygen diffuses to the cell via the capillary wall, the extracellular water, and the cellular membranes. An adequate supply of oxygen is thus dependent on a multitude of systems; the most obvious of these are: the ambient oxygen pressure, the effectiveness of the uptake organ, the blood flow through the consuming organ, the number and size of the capillaries per cubic unit, the concentration and chemical properties of the transport pigment and the oxygen consumption by the cells.

This complex of systems is integrated by a number of humoral and nervous mechanisms which enable the organism to adjust toward optimal working conditions and to counteract disturbances in one system by changing the function of others. Under physiologic conditions the oxygen supply to an organ can be adjusted through changes in the rate of blood perfusion and in the oxygen-carrying capacity. These two factors cannot be changed independently however, since the flow is dependent upon the blood viscosity which varies with changes in the hematocrit value. From determinations of the viscosity hematocrit relationship, it is possible to calculate the correlation between flow and hematocrit.

According to Castle and Jandl, this correlation is linear, based on in vitro determinations of the blood viscosity, Pirofsky. This is in good agreement with in vivo flow studies by Murphy et al. who studied the flow through the kidney in normovolemic dogs and found a linear dependency of the flow to the hematocrit. In studies on cardiac output, T. Q. Richardson and Guyton, Murray et al., and R. Replogle also found a linear dependency in normovolemic dogs.

Provided unchanged oxygen saturation of the hemoglobin at various hematocrit levels, the oxygen flow can be expressed as blood flow times hematocrit.
"Tissue" tension of oxygen and the curves for oxygen transport can be derived from the blood flow curves. Since the blood flow hematocrit relationship is linear, it follows that the oxygen transport-hematocrit relationship is represented by a parabola (Fig. 7).

Based on in vitro data, Erslev, Castle and Jandl found that the maximum for the oxygen transport curves were very close to the normal hematocrit value. This may be a coincidence, since the viscosity, as determined in vitro, is highly dependent upon the shear rate at which it is determined. However, Richardson and Guyton, Murray et al. and Weisse et al. also found a maximum for the oxygen transport close to the normal hematocrit value in normovolemic dogs. The data given by Murphy on the flow through the kidney allow calculation of the oxygen flow and here again, in the normovolemic dog the maximal oxygen flow is found to take place at close to normal hematocrit levels.

This means that one would expect an insufficient oxygen supply at low hematocrit levels which is in good agreement with clinical observations. Also one should find a similar decrease in oxygen transport in erythrocytosis. This actually contradicts the fact that erythrocytosis appears to be compensatory to low ambient oxygen pressure and to deficient oxygenation of the arterial blood.

Based on the assumption that the essential stimulus for the erythropoiesis is hypoxia, a study was performed previously in an attempt to evaluate whether normovolemic erythrocytosis might be associated with tissue hypoxia. No evidence was found for this, but the data were inconclusive as it was impossible to maintain the normovolemic erythrocytosis for a sufficiently long time. It was therefore decided to evaluate the relationship between tissue tension of oxygen and the hematocrit in a more direct fashion by measuring the partial oxygen pressure in subcutaneous and peritoneal air pockets in mice and rats with induced anemia and erythrocytosis.

**Materials and Methods**

**Mice:** Young female, Swiss Webster. Weight 20–25 g.

**Rats:** Young female, Sprague Dawley. Weight 130–150 g.

Donors of red cells were usually older animals of both sexes and the same strain.

**Subcutaneous air pockets.** Pockets were induced under the skin of the neck and back of the animals by injecting 6–7 ml. (mice) or 15–25 ml. (rats) of sulfur hexafluoride. This gas is absorbed slowly and causes a certain amount of fibrosis. The sulfur hexafluoride was removed after one week and replaced with atmospheric air. Assays of the oxygen pressure in the induced air pockets showed a gradual decrease until constant values were reached after about 8–10 hours (Fig. 1). During the experiments, a minimum of 20 hours were used to insure equilibration. The pockets opened three weeks after induction revealed a shiny, smooth, moist lining with many newly formed vessels.

**Pneumoperitoneum.** This technic was used in mice only and consisted in 6–7 ml. of air being insufflated into the peritoneum. Assays of the gas mixture showed that equilibrium was reached in four hours (Fig. 1). In most instances, a 20-hour period of equilibration was used in the experiment. The equilibrated air was removed with a disposable plastic syringe "Stylex" and a No. 25 hypodermic needle. The needle was sealed with a rubber stopper from the aspiration of gas until the assay.

**The Po2 determinations** were performed using a modified Clark electrode type E-5044 and an oxygen monitor type PHA928, supplied by Radiometer®, Copenhagen. A reference gas mixture was used as a standard reference in the daily calibration of the monitor.
Erythrocytosis induced by transfusions. Packed red cells were given i.v. to mice, i.p. to the rats, in doses of 2 ml. red cells per 100 g. body weight. The red cells had been washed in cold saline and packed to a hematocrit of 65-70 percent before infusion.

Erythrocytosis induced by exchange transfusions. The rats were anesthetized with ether and bled 20 ml. per kg. from the tail. The blood was replaced by the same volume of red cells packed to a hematocrit value of 80-85 percent given i.v. If the infusion of the packed cells was given immediately after the bleeding, all the rats would die, whereas most of them would survive if 15-20 min. were allowed to pass before the infusion was completed.

Anemia induced by bleeding. The very tip of the tail was cut off and the tail was submerged in lukewarm saline.

Anemia induced by phenylhydrazine. The rats were given 0.5 ml. of a 2 percent neutralized solution of phenylhydrazine (Baker) in saline s.c. on two consecutive days. The mice received 0.7-1.0 ml. of an 0.2 percent solution s.c. for two days.

Hematocrits were determined in oxalated capillary tubes (Aloe) using a “Drummond” micro hematocrit centrifuge and reader.

RESULTS

Transfused and Phenylhydrazine Anemic Rats

Normal rats with established skin pockets were either given three i.p. transfusions of 2 ml./100 gm. each or two s.c. injections of 0.5 ml. 2 percent phenylhydrazine solution. The Po2 of the pocket air was determined at various points during the induction of the erythrocytosis and anemia. A total of 150 rats were employed in ten separate studies.

The variations in the oxygen tension in one such study are shown on Fig. 2.
Fig. 2.—$P_0_2$ changes in the rat skin pockets induced by anemia and hypervolemic erythrocytosis. The bars indicate plus and minus the standard deviation.

Fig. 3.—Scatter plot of the individual values for $P_0_2$ and hematocrit. The "linear" part of the curve was determined according to the method of the "least squares." The rest of the curve represents the "best fit." The outer lines demonstrate plus and minus a mean value of two times the standard deviation calculated for various parts of the curve.

The first bar represents the values before randomization. The next bars show the changes in $P_0_2$ during the following days. It appears that the mean $P_0_2$ value increases in the transfused group and decreases in the anemic group. The correlation between the hematocrit and the $P_0_2$ is shown in Fig. 3. If linear dependency is assumed in the range of hematocrit from 20–60 percent,
Fig. 4.—Comparison of the effect of hypertransfusion and exchange transfusion on the $P_{O_2}$ in the skin pockets of rats. The heavy lines represent the changes in the mean value.

A coefficient of correlation of 0.9 is found. However, the relationship is more complex, probably curvilinear through all hematocrit ranges. There is a maximum of 60–65 percent hematocrit above which there is a slight decrease toward normal values. The highest hematocrit obtained in rats is in the seventies. Attempts to increase the hematocrit further were limited by a marked tendency to develop hemorrhagic infarctions of the stomach mucosa, usually leading to the death of the rats. Our normal values closely corresponded with those obtained by Bartlett and Tenney who used the Scholander microassay technique for the $P_{O_2}$ determinations.

Exchange Transfusions in Rats

Exchange transfusions were carried out in rats in order to study the effect of normovolemic erythrocytosis on the “tissue” $P_{O_2}$. It became evident that the normovolemic conditions were unstable and could not be maintained for the period necessary for equilibration of the air in the skin pockets. The hematocrit which right after the exchange was close to what could be predicted from the amount of blood and packed cells exchanged, fell during the following 20 hours. We were therefore unable to get any specific information on the strictly normovolemic state. However, the $P_{O_2}$ found in the pockets after 20 hours would represent an integrated value of the changing conditions during the transformation of the normovolemic erythrocytosis to the hypervolemic state. Fig. 4 shows the results obtained after exchange transfusions compared to simple hypertransfusion to the same hematocrit level. It appears that while hypervolemic erythrocytosis improves the oxygen transport to the tissues, normovolemic erythrocytosis does not.
"TISSUE" TENSION OF OXYGEN

Fig. 5.—Po$_2$ changes in the mice pneumoperitoneum induced by anemia and hypervolemic erythrocytosis. The bars indicate plus and minus the standard deviation.

Fig. 6.—Analogous to Fig. 3 showing the correlation between the oxygen tension and the hematocrit in the mouse pneumoperitoneum.

Transfused, Phenylhydrazine-anemic and Bled Mice

Earlier in this study, assays were carried out in a few groups of mice using the skin pocket technic. The changes found in Po$_2$ in the pockets in response to transfusions were consistent with the results obtained in the rat series. However, due to the small amount of obtainable air from the air pockets in mice, it was decided to use the pneumoperitoneum technic in mice.

Fig. 5 shows the results obtained in one such experiment with three groups of mice: normal, transfused and bled mice. The procedure and the results are
similar to the rat studies, although the level of Po2 is higher in the mouse peritoneum than in the skin pockets of rats. Anemia induced by bleeding or phenylhydrazine showed no significant difference in the effect on the Po2 in the pneumoperitoneum, although mice given phenylhydrazine have probably a moderate degree of methemoglobinemia. If all observations are combined and linear dependency is assumed up to a hematocrit value of 60 percent, a coefficient of correlation of 0.7 was found (Fig. 6). Again, as found in the rat skin pockets, the correlation is more complex and shows a Po2 maximum in the hematocrit range 60–65 percent above which the oxygen tension approaches the normal values. In the mice, the hematocrit could be increased to nearly 90 percent at which point a few mice developed hemorrhagic stomach infarction as found in the rats.

Mice with hematocrits above 85 percent are not included to avoid inclusion of sick mice. Up to this point they appeared healthy.

**DISCUSSION**

In 1928, Campbell\(^2\) first attempted to correlate oxygen carrying capacity of the blood with tissue tension of oxygen by introducing air under the skin and in the peritoneum of rabbits. A similar method with few modifications as used in the present study on rats and mice, has been described in detail by Rahn,\(^15\) by van Liev,\(^9\) and again by Bartlett and Tenney.\(^1\) According to Rahn, the Po2 found in an equilibrated air pocket is within ± 1 mm./Hg. of the Po2 in the “tissue.” This is based on a comparison of the results obtained in equilibrated pockets and the values obtained by an interpolation technic in which various gas mixtures were induced into the pocket. The Po2 at which no net in- or e-flux of oxygen takes place, can be calculated from serial determination of the Po2 during equilibration. This Po2 is considered to be the tissue tension of oxygen. According to van Liev, this tension is probably very close to the oxygen tension in the venous capillaries draining the area. It should be stressed, that the skin pockets represent an artificial “organ” and that the oxygen tension in this pocket does not necessarily equal the Po2 of undamaged tissue.

The Po2 in the skin pockets will depend on the vascularity of the lining walls, the blood flow through the vessel, and the degree of fibrosis. Since the vascularity and fibrosis change with time, the Po2 could not be expected to be the same on consecutive observations. It was, therefore, attempted to compare gas pockets of the same “age.” Although the Po2 in these artificial air pockets and pneumoperitoneum may not reflect the “true” tissue oxygen tension, the pockets are useful for the purpose of registering the changes in Po2 induced by variations in the hematocrit and the blood volume.

The data in this report confirm and amplify those published by Campbell\(^2\) and by Bartlett and Tenney.\(^1\) It was demonstrated that anemia is associated with a decrease in the Po2 in the skin pockets and that the decrease is proportional to the severity of the anemia. This we found also to be the case of the pneumoperitoneum of mice.

In animals with erythrocytosis, induced by transfusion, the Po2 was either normal or increased. When the Po2 was measured before and after hyper-
transfusion, an increase was found in every animal (Fig. 4). This must mean that the high viscosity in hypervolemic erythrocytosis does not interfere with the oxygen supply to the cells.

It has been theorized that normovolemic erythrocytosis would lead to hypoxia as the cardiac output is disproportionately decreased.\textsuperscript{5} We, therefore, attempted to induce normovolemic erythrocytosis in rats. Immediately after the exchange transfusion, the rats were in a rather poor condition with slight cyanosis. However, most of them recovered in the following 20-hr. period during which the hematocrit fell towards normal values. Thus, it appears that the normovolemic condition was converted into a hypervolemic state. The blood volume was not measured in these animals, but experiments in dogs,\textsuperscript{7,11} rabbits,\textsuperscript{5} and splenectomized dogs\textsuperscript{18} indicate that an increase in the plasma volume is responsible for the decrease in hematocrit. Since the \( P_{\text{O}_2} \) was found not to be increased in the pockets after 20 hours following the exchange transfusion, it was concluded that normovolemic erythrocytosis is an unfavorable condition, in respect to oxygen transport to the tissues. The inability to detect an increase in erythropoietin release 48 hours after the induction of a normovolemic erythrocytosis in rabbits\textsuperscript{5} is undoubtedly due to the fact that a rapid influx of extravascular fluid made it impossible to maintain the normovolemic state for this long period.

Studies in dogs by Murray et al.\textsuperscript{11} and by Smith and Crowell\textsuperscript{17} seem to provide the answers to why hypervolemic erythrocytosis enhances the transport of oxygen while normovolemic erythrocytosis impairs it. These authors found the peripheral vascular resistance markedly decreased in hypervolemia as compared to normovolemia at all hematocrit levels. Murray\textsuperscript{11} found a linear correlation between hematocrit and cardiac output in both hypervolemia and in normovolemia, but in hypervolemia it was at a higher level, evidently due to decreased peripheral vascular resistance. As the lines are close to parallel, it follows that the derived parabolic curves demonstrating the oxygen transport will have different maxima, the hypervolemic condition giving a maximal oxygen transport at the higher hematocrit (Fig. 7).

The cardiac output in hypervolemic erythrocytosis is not necessarily increased as compared to normal conditions. The cardiac output is higher than it would be at the same hematocrit level with no increases in blood-volume, but not necessarily larger than normal cardiac output.

If the blood pressure is not increased, this means that the cardiac workload may still be normal allowing an increased oxygen transport. In the same strain of rats, and in New-Zealand white rabbits, we found no increase in the heart size after sustained transfusion induced erythrocytosis (Table 1).

In late polycythemia vera, the blood volume will often remain elevated when the erythrocyte volume declines, due to a concomitant increase in plasma-volume, (Polycove 1966). In these cases, the cardiac output may very well be increased above the normal and the above-mentioned considerations do not apply.

It seems permissible to outline the following concept for the oxygen transport in different conditions.
Fig. 7.—Graff of working hypothesis.

The correlation cardiac output—hematocrit, and the derived correlation oxygen transport—hematocrit is shown for normovolemia and hypervolemia. (Modified from Murray.)

The curve through the maxima for the oxygen transport curves represents the optimal oxygen transport at the corresponding hematocrit level.

Point 1: Normal conditions at rest. Normal hematocrit, normal oxygen transport.

Point 2 and the curve through the maxima: Moderate erythrocytosis with compensatory changes in plasma volume. Increased hematocrit and increased oxygen transport.

Point 3: Normovolemic erythrocytosis, induced by exchange transfusion. Increased hematocrit, decreased oxygen transport. Point 4: "Dilution anemia." Disproportionate increase in plasma volume and red cell mass. Hypervolemia, decreased hematocrit, increased oxygen transport. Point 5: Excessive erythrocytosis. The possibility for plasma volume increase limited. Very high hematocrit and decreased plasma volume, normal or slightly increased oxygen transport.

1. In hypervolemia, the optimum oxygen transport will take place at higher than normal hematocrits explaining the beneficial effect of a compensatory hypervolemic erythrocytosis in conditions of hypoxemia.

2. In the moderately hypertransfused animal, the oxygen transport is enhanced, as demonstrated in Fig. 7 by the curve point 1 → point 2, in agreement with the findings in the present study. The fact that the oxygen pressure in the skin pockets and pneumoperitonea in our study approaches the normal value as infusion of red cells is continued, is believed to be the result of a lim-
Table 1.—Heart Size in Percent of Body Weight in Rats and Rabbits

<table>
<thead>
<tr>
<th>Weeks treated</th>
<th>Weight range g.</th>
<th>Number of animals</th>
<th>Heart Weight per cent of body weight</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control rats</td>
<td>0 200-250</td>
<td>10</td>
<td>0.355</td>
<td>0.033</td>
</tr>
<tr>
<td>0 300-400</td>
<td>13</td>
<td></td>
<td>0.344</td>
<td>0.027</td>
</tr>
<tr>
<td>Transfused rats</td>
<td>1 250-300</td>
<td>9</td>
<td>0.367</td>
<td>0.026</td>
</tr>
<tr>
<td>3 300-350</td>
<td>12</td>
<td></td>
<td>0.358</td>
<td>0.041</td>
</tr>
<tr>
<td>6 375-425</td>
<td>8</td>
<td></td>
<td>0.343</td>
<td>0.037</td>
</tr>
<tr>
<td>9 375-425</td>
<td>9</td>
<td></td>
<td>0.331</td>
<td>0.031</td>
</tr>
<tr>
<td>Control rabbits</td>
<td>0 2500-4500</td>
<td>6</td>
<td>0.197</td>
<td>0.008</td>
</tr>
<tr>
<td>Transfused rabbits</td>
<td>10 2500-4500</td>
<td>6</td>
<td>0.195</td>
<td>0.017</td>
</tr>
</tbody>
</table>

No statistical significant change in this relation could be demonstrated after sustained transfusion induced erythrocytosis. The hematocrit level in the transfused groups was maintained at 15-20 percent above the normal.

3. The normovolemic erythrocytosis, as demonstrated by point 3, Fig. 7, is evidently unfavorable for the oxygen transport, as confirmed in the present study. The broken line (3 → 3′, Fig. 7) demonstrates the compensatory changes taking place after the transfusion, transforming the condition into a hypervolemic erythrocytosis with a lower hematocrit value, but an improved oxygen transport capability.

4. The disproportionate increase in plasma volume and red cell mass in last trimester of normal pregnancies results in a hypervolemic condition with a slightly decreased hematocrit value. As exemplified by point 4, Fig. 7, one would expect to find this condition beneficial for the oxygen transport in spite of the decreased hematocrit value. However, as opposed to the improved oxygen transport of hypervolemic erythrocytosis, this increased oxygen transport does take place at the expense of an increased cardiac output. Consequently, the capacity of further increasing the oxygen output is decreased which may explain that these women even if they may be better off at rest, may still experience "anemic symptoms" at exercise. In this connection, it should be mentioned that we could not demonstrate any stimulation of the erythropoiesis by decreasing the hematocrit value in rabbits by infusion of large doses of dextran and plasma.

Our present investigations support the current hypothesis of the regulation of the erythropoietin production by the oxygen tension in a sensitive target organ. The fact that the PO2 in the "tissue" does approach normal values as the hematocrit is increased in excess of 65 percent, and the fact that erythropoiesis is not reinitiated by transfusion to this high hematocrit level, point to the presence of possibly modifying mechanisms for the control of erythropoiesis. As opposed to Krzymowski and Krzymowska, and Whitcomb, we have so far...
been unable to detect an inhibitor for erythropoiesis in plasma from hyper-
transfused animals.\textsuperscript{8}

Whether "contact inhibition" at the cellular level is of any significance is not
known, but a high concentration of erythrocytes in the bone marrow might in-
fluence the erythropoiesis.

\section*{SUMMARY}

The variations in oxygen tension in relation to induced changes in the he-
matocrit, were investigated in mouse pneumoperitoneum and rat skin pockets.

A close to linear relationship was found in the range of hematocrit from 20–
60 percent. Above this hematocrit value, the correlation was lost and no further
increase in $P_o_2$ was noted with increases in the hematocrit. A tendency to a
minor fall in $P_o_2$ at very high hematocrit values was observed in both systems,
however, the fall was never below normal value.

Attempts to induce normovolemic erythrocytosis by exchange transfusions
in rat demonstrated that this condition is transient, transforming into a hyper-
volemic erythrocytosis within hours. In spite of the increasing viscosity of the
blood with increases in hematocrit, a hypervolemic erythrocytosis was found to
cause a slight increase in tissue $P_o_2$ while a normovolemic erythrocytosis did
not. Consequently, an accelerated rate of red cell production will have a dual
effect on oxygen transport: (1) it will increase the oxygen carrying capacity,
and (2) it will increase the blood volume and thereby decrease the peripheral
resistance, allowing the heart to transport more oxygen at no extra expense.

\section*{SUMMARIO IN INTERLINGUA}

Le variationes occurrente in le tension de oxygeno in relation con alterationes inducite
in le hematocrite eseva investigate in pneumoperitoneos murin e in tascas cutanee de rattos.

Un nette correlation lineari esseva constatate in le region del hematocrite ab 20 ad 60 pro
cento. Supra iste valor, le correlation se perdeva, e nulle augmento additional esseva notate
in $P_o_2$ con augmentos del hematocrite. Un tendentia del $P_o_2$ de declinar levemente a
altissime valores del hematocrite eseva observate in ambe systemas. Tamen, iste declino
nunquam descendeva ad infra le norma.

Effortios a inducer erythrocytosis normovolemic per transfusiones de excambio in rattos
demonstrava que le condition es transiente. Illo es reimplaciate intra pauc horas per eryth-
rocytosis hypervolemic. In despecto del accrescente viscositate del sanguine que occurre
con le augmento del hematocrite, il eseva trovate que un erythrocytosis hypervolemic
causa un leve augmento in le $P_o_2$ tissular, lo que non occurreva como effecto de erythro-
cytosis normovolemic. Per consequente, un accelerate erythropoiese exerce un duple effecto
super le transporto de oxygeno, i.e., (1) illo augmenta le capacitate vectori pro oxygeno e
(2) illo augmenta le volumine de sanguine e reduce assi le resistencia peripheric, con le
resultato que le corde pote transportar plus oxygeno sin expensa additional.

\section*{REFERENCES}


1928.

3. Castle, W. B., and Jandl, J. H.: Blood viscosity and blood volume: Opposing in-
fluences upon oxygen transport in polycy-
themia. Seminars in Hematology, Vol. 3:


5. Erslev, A. J.: The erythropoietic effect of hematocrit variations in normovolemic
The "Tissue" Tension of Oxygen and Its Relation to Hematocrit and Erythropoiesis

EIVIND B. THORLING and ALLAN J. ERSLEV