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Oxygen Consumption and Tissue Oxygen Tension as a Function of the Hematocrit in Polycythemic Mice

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Partial pressures of oxygen in subcutaneous gas pockets and oxygen consumption of mice with hematocrit ratio from 25 to 75 and exposed to various degrees of hypoxic hypoxia were determined.

Higher than normal values for both parameters were found in plethoric ex-hypoxic mice even in mild hypoxia (from 25 to 30 % of normal). The implications of these findings on the oxygen transport capacity of plethoric mice and on the role of erythropoietin in the control of erythropoiesis are discussed.

Erythropoietic activity in mice rendered polycythemic by red cell transfusion or by chronic exposure to lowered barometric pressures becomes minimal. This effect is now widely admitted to be due to diminished erythropoietin (EP) formation caused by a plus in the oxygen supply to the tissues of the organs responsible for EP production (1, 2, 3). The significant changes of blood viscosity which take place as the hematocrit ratio increases raise the question whether these changes could deteriorate the blood flow, hence the effectiveness of polycythemia in maintaining the oxygen supply above normal would be impaired (4, 5, 6).

Observations made in other species indicate that oxygen transport is accomplished at its optimal, around the normal values of the hematocrit (7, 8). On the other hand studies on the relationship between the hematocrit ratio and tissue oxygen tension made in rats and mice at sea level oxygen tension, show a linear relationship between the two parameters in the hematocrit range from 25 to 60, the optimal oxygen transport taking place at higher than normal hematocrits. Above 60 the relationship disappears and a slight tendency to fall was reported though never below normal values (9). Therefore it appears that small rodents are able to adjust the hemodynamics involved in blood flow so that no impairment in the oxygen transport occurs when hematocrit values are well above those capable to cause a substantial fall in other species. The results of those observations however cannot be directly extrapolated to conditions of airway hypoxia which happens to be of particular importance in the understanding of the physiological role of polycythemia as a compensatory mechanism for hypoxic hipoxia.

Here we report the results of studies on the influence of the hematocrit ratio on

the oxygen consumption (O_2c) and tissue oxygen tension (pO_2t) in mice exposed to various levels of hypoxia.

Materials and Methods

Female mice 7 to 9 weeks of age from the imbred of this Institute were used. Reduction of the hematocrit was produced by a single bleeding of volumes from 0.2 to 0.6 ml, made 24 hours before the O_2c and pO_2t determination were performed. Polycythemia was induced by exposure to hypoxia (0.4 atmosphere in a low pressure chamber) 12 hours daily for various periods of time from 5 to 21 days and the studies made 24 hours after returning the animals to normal ambient air. Another group of mice was rendered polycythemic by intraperitoneal transfusion of 0.2 to 1.2 ml of packed red cells made 5 days before the determinations. Using these procedures hematocrits ranging from 25 to 75 were attained.

Determinations of the O_2c were made using a modification of the technique described by GRAD (10). The animals after being placed in the respiratory chamber were allowed to adjust themselves to the new enviroment for 30 minutes and the O_2c measured over a 30 minute period. From that time on the feeding of the respiratory chamber was shifted from pure O_2 to normal air. In this way since each volume of O_2 consumed is replaced by an equal volume of a mixture containing only 20.9 % of oxygen a continuous drop in O_2 concentration inside the container takes place, resulting in a O_2 concentration of around 7.0 % in a 80 minutes period. Every 5 minutes the volumes of O_2 consumed were read in a scale adequately designed to show both volumes of O_2 consumed and the corresponding O_2 concentration. Simultaneously with this reading a microsample of the admixture was taken from the chamber and its O_2 concentration was measured in order to check on the accuracy of the O_2 concentration read from the scale. All gas analysis were performed using the Scholander analizer on a 0.5 ml sample (11). Determinations were made in the unanesthesized animal in front of an intense light source which reduces the spontaneous activity of this species of nocturnal habits to a minimun.

The pO_2t was calculated from the values of oxygen concentration in the gas pocket and expressed at 760 barometric pressure of dry air at 0°. Using a similar technique to that described by RAHN *et al.* (12) gas pockets were induced by injection of 3.0 ml of nitrogen under the skin of the cervicodorsal region. 24 hours later the animals were placed in groups of 6 in a cylindrical glass container (3 inches wide-25 inches long) provided with an air inlet at one end. A plastic bag with an air oulet was attached to the other end. Air or the corresponding gas admixture was allowed to flow through the container at a rate of 4 liters per minute over a 4 hour period. Soda lime was placed in the container to prevent CO_2 accumulation. Samples of the gas admixture were taken from the inlet and the outlet to check on the posible variations in O_2 an CO_2. The changes were always negligible for both gases (less than 1.5 mm Hg).

At the end of the 4 hour period gas samples from the gas pockets were taken for O_2 concentration measurement. To avoid variations in the inhaled atmosphere used in each particular case while the gas samples were taken from the gas pocket, the animals were gently made to move into the plastic bag being thus possible to hold the animal by the tail to obtain a gas sample in a sealed syringe from the gas pocket by puncturing the plastic wall and the skin. Very little manipulation is required and the whole operation takes less than 10 seconds for each animal. From the syringe the gas was transferred to the chamber of the Scholander microanalizer using for this purpose the modification described by VAN LIEW (13). Hematocrits

were determined on blood taken from the retroorbital sinus at the begining of all determinations using the microtechnique. The animals were kept at air ambient temperature of 24 to 26° C.

Results

Figure 1 shows the O_2c values in ml/minute/100 g of body weight reduced to 0° and 760 mm Hg barometric pressure of dry air. For these determinations anima's with hematocrit ratio on the neighbourhood of 28, 45 and 67 were selected. Transfused and ex-hypoxia polycythemic mice were studied separately. In normoxic airway conditions the anemic group shows the lowest O_2c. The transfused polycythemic as well as the ex-hypoxic polycythemic group instead exhibit higher values than the normal group. As the partial pressure in the inhaled air (pO_2a) decreases a fall in O_2c ocurres in all groups. As it can be seen in figure 1 the fall in O_2c is iniciated in the anemic group at a pO_2a of 119.0 mm Hg, followed by normal, transfused polycythemic and ex-hypoxic polycythemic animals in which the O_2c, falls below the range found in normal mice in normoxia, at pO_2a of 110.0, 96.5 and 94.0 respectively.

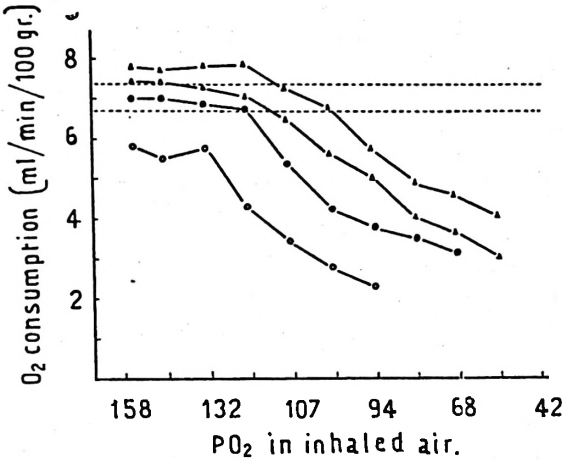

Fig. 1. *Oxygen consumption in mice with hematocrits of 28-45 and 67 exposed to progressive decrease in partial oxygen tension in the ambient air.*

The area between the dashed lines indicates the range of variations of oxygen consumption found in normal animals breathing normal ambient air (760 mm Hg). (—O—) Anemic; (—●—) Normal; (—△—) Transfusion polycythemia; (—▲—) Ex-hipoxia polycythemia. Each point represents the average of 20 individuals determinations.

Table I. *Coefficient of correlation between hematocrit and tissue partial oxygen tension in mice breathing various ambient partial oxygen pressure from normal to 68.9 mm Hg.* p values were obtained from the *Values of the coefficient of correlation for different levels of significance.* Statistics Tables. Fisher, R. A., and Yates, F. Eds. Aguilar, S. A. Ediciones Madrid, p. 64, 1954.

Air pO_2	Hematocrit			
	25 to 60		60 to 75	
	r	N	r	N
158.8	+ 0.845	24*	- 0.726	19**
137.1	+ 0.814	77*	+ 0.024	22***
121.3	+ 0.836	43*	+ 0.030	19***
108.2	+ 0.661	41*	+ 0.042	18***
95.1	+ 0.820	32*	+ 0.029	20***
68.9	+ 0.815	30*	+ 0.050	20***

Levels of significance (P): * >0.001; ** >0.01; *** No significative.

Figure 2 shows the pO_2t values in mice with hematocrits between 25 to 75 breathing pO_2a within the range of 158.8 to 68.9 mm Hg. Decreasing the pO_2a from 158.8 to 137.1 mm Hg appears to cause no change in pO_2t (figure 2, curves 1 and 2). Below that point a decrease in pO_2t was caused by further reductions in pO_2a. At all levels of pO_2a the pO_2t increased steadily as a linear function of the hematocrit up to a value of 60. The linear dependency as shown by the straight lines drawn by the least squares method seems to be the most approximate function. To check on this assumption the coefficient

of correlation of the experiment in which mice breathed 121.3 pO_2a was calculated for the whole range from 25 to 60 and for three different fractions of this range comprising 12 points of the hematocrit each of them. Table II shows the values for r and p which compare well with those found for the whole range. When the hematocrit was higher than 60 no further increase in the pO_2t was found and the tendency to raise changes to a plateau, the best fitting free-hand line indicating a slight tendency to fall below the highest values which consistently was found around the 60 hematocrit ratio. In the range from 60 to 75 hematocrit ratio the scattering of individual values was greater. The average of individual values comprised in the plateau was never below that found at normal hematocrits.

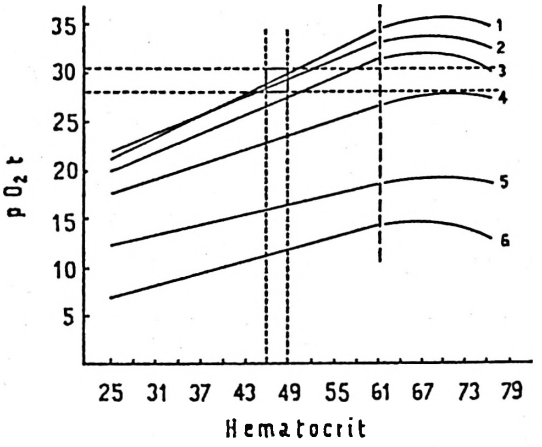

Fig. 2. *Relationship between hematocrit and tissue oxygen partial tensión (pO_2t) at different partial oxygen tension in the ambient air, which are indicated by the numbers at the right and of each coordinates as follows: 1 = 158.8; 2 = 137.1; 3 = 121.3; 4 = 108.2; 5 = 95.1, and 6 = 68.9 mm Hg respectively.* The area between the dashed horizontal lines indicates the range of variation for tissue partial oxygen tension in normal animals breathing normal ambient air (760 mm Hg). The area between the vertical dashed lines shows the range of variations of hematocrits in normal unmanipulated mice. The square of solid lines indicates the values of partial oxygen tension of normal animals breathing normal ambient air.

Table II. *Coefficient of correlation between hematocrits and tissue partial oxygen tension in mice breathing 121.0 mm Hg pO_2a calculated for three fractions and for the whole 25-60 hematocrit range.*
p obtained as stated in table I. $P > 0.001$.

Hematocrit	r	N
25-37	+ 0.802	17
35-47	+ 0.816	16
45-60	+ 0.800	17
25-60	+ 0.836	41

Figure 3 shows the relationship between the observed values for tissue pO_2 and the corresponding figure for O_2c in animals with hematocrits of 28, 45 and 67 as the pO_2a was increased. A consistent change in the steepness of O_2c curve to a plateau appears in all the groups when the pO_2a reaches the 108 mm Hg.

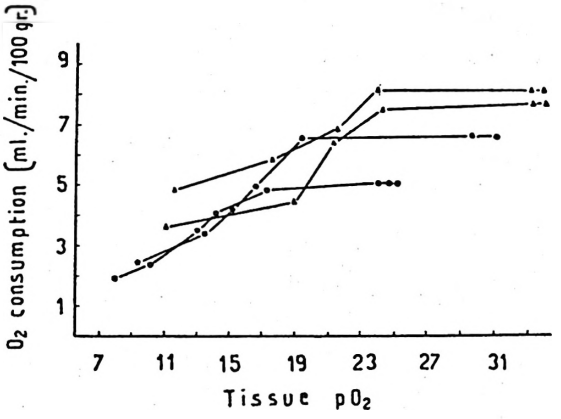

Fig. 3. *Relationship between oxygen consumption and tissue partial oxygen tension in normal (—●—); anemic (—○—); transfused polycythemic (—△—); and ex-hypoxic polycythemic (—▲—) mice as the partial pressure of oxygen in the inspired air increases from 68.9 to 158.8 mm Hg (left to right in the abssisa).*
Each point is the average found in 15 individual determinations.

Discussion

A fall in the oxygen consumption occurs at all levels of the hematocrit ratio as the pO_2a is reduced. A thereshold for this effect is found in all groups although the anemic mouse prove more sensitive to this effect. The greater efficiency for oxygen uptake was found in the ex-hypoxic polycythemic mouse. The larger difference between the O_2c in this groups and that found in animals with normal hematocrits takes place in the range of 108 to 88 mm Hg of pO_2a. This range of pO_2a includes most of the circumstances expected to set out the development of adaptatory mechanisms which are likely to evolve during evolution since they corresponds to the pO_2a existing at altitudes between 7.500 to 17.000 feet above sea level. The extra-efficiency provided by polycythemia in this range of pO_2a subsides as the ambient oxygen tension is further decreased.

The observed difference in O_2c between the group in which plethora was induced by a sudden change in erythrocyte concentration by transfusion and the group with polycythemia developed by mice housed in the altitude chamber, might be explained through a better efficiency of pulmonary and systemic circulation evolved during the period of hypoxia exposure. Since the oxygen consumption is an approximation not only of oxygen transport but also of blood flow, an enlargement of the capillary bed enough to accomodate the expanded blood volume appears to be a beneficial adjustement for the cardiac work by reducing peripheral vascular resistance as pointed by THORLING *et al.* (9). In the series of experiments here reported no impairment of the O_2c, as reported for other species (7, 8) was found in spite of the high values of the hematocrit.

The use of the skin gas pocket as an *in vivo* tonometer, has been in use since CAMPBELL first described this technique (14). More detailed information on the significance of the pO_2 in the pocket as well as on the approximation of the true tissue pO_2 to the pO_2 measured in the pocket after equilibration, indicates that a gradient of no more than 1 mm Hg exists between the two mentioned values (12, 15).

The values for tissue pO_2 at various degrees of ambient, oxygent tension as shown in figure 2, indicate that in hypoxic conditions this parameter of the respiratory function is improved by polycythemia although it proved unable to maintain the tissue pO_2 whithin the normal range when pO_2a was reduced bayond a 35 % of the normal value. The correlation between the changes in O_2c and the pO_2t as the ambient oxygen tension is increased from 66.9 mm Hg upward at all levels of the hematocrit, shows an increase with an exponential tendency of the values of O_2c as the pO_2t increases linearly up to a level of 108 mm Hg in the inhaled air. Further increases in pO_2a only cause the tissues pO_2 to augment with a slight increase of O_2c which reaches a plateau at this point. This lack of correlation between the two parameters under study in animals breathing mixtures with pO_2 higher than 108 could be explained by the fact that arterial saturation, which is responsible for the larger amount of O_2 uptake, is almost completed at this level of pO_2a while the amount of the gas necessary for further increases of arterial pO_2 requires just a small fraction of the chemically combined O_2 .This would imply that up to an approximate value of pO_2a of 100 mm Hg, both saturation and arterial O_2 tension act as limiting factors for pO_2t. When the pO_2 is further increased toward normality the increases in pO_2t are mainly dependent on the increases in arterial pO_2 as expected. More points in the curve of pO_2a would be necessary to find a more accurate pO_2a value at which the plateau is iniciated.

Our data indicate that polycythemia in the mouse acts as a true compensatory

mechanism to help keep the tissue pO_2 close to normality which is achieved even after a 30 % reduction in pO_2a. The main physiological adjustment of the body in meeting the need for more oxygen is an increase in the volume of blood flow and oxygen carrying capacity of the blood. The striking changes in blood viscosity that accompany the raise of the hematocrit ratio above normal values pose a reasonable question on wether or not such changes would affect the hemodynamics involved in blood flow. These results do not fit well with the available experimental evidence found in other species (4, 7, 8) and the theoretical calculations (16, 17, 18) for the optimal hematocrit ratio in providing the best hemodynamic conditions to insure and adequate blood flow per cubic unit. Our data instead indicate that plethora in the mouse even at the highest range of the hematocrit is still able to maintain the oxygen consumption and the pO_2t above those found in the normal animal either in normal ambient air or in airway hypoxia.

It has been suggested that plethora in the mouse depresses erythropoiesis by a diminished erythropoietin formation due to a higher tissue pO_2. This interpretation although not shared by others (19, 20), finds further support in these results.

Resumen

La presión parcial de oxígeno en bolsillos subcutáneos de gas y los valores del consumo de oxígeno se estudió en ratones con distintos valores del hematocrito y sometido a varios grados de hipoxia ambiental.

En ratones con policitemia producida por exposición a bajas presiones barométricas, se encontró que ambos parámetros en estudio son más elevados que los encontrados en el animal normal aún cuando el grupo policitémico se expuso a ambientes con una reducción del 25 al 30 % en la presión parcial de oxígeno. Se discute las implicancias de estos hallazgos sobre la capacidad de transporte de oxígeno del ratón policitémico, como así también sobre la teoria del rol de la eritropoyetina en el control de la eritropoyesis.

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