THE DEPENDENCY OF THE DIFFUSING CAPACITY ON THE HbO₂ SATURATION OF THE CAPILLARY BLOOD AND ON ANEMIA

MASAJI MOCHIZUKI, TAKEO ANSO, HIROSHI GOTO,
AKIRA HAMAMOTO AND YUKIO MAKIGUCHI*

Research Institute of Applied Electricity, Hokkaido University, Sapporo

The pulmonary diffusing capacity D_L can be explained by using two capacity components, *i.e.*, the membrane component D_M and the red cell component D_R as follows, as reported by Gibson *et al.* (7) and Roughton and Forster (23).

$$\frac{1}{D_L} = \frac{1}{D_M} + \frac{1}{D_R}. \quad (1)$$

In the previous paper theoretical studies on D_R were presented. It was concluded that D_R is expressed as

$$D_R = Q \cdot F_2 \cdot t, \quad (2)$$

where Q is a product of both the cardiac output and the hematocrit value, F_2 , a quantity called "the reacting capacity" and t , the contact time. F_2 is the rate of combination of oxygen and hemoglobin inside the red cell, when there is a difference of 1 mmHg between the O₂-tension in the plasma and the O₂-tension corresponding to HbO₂ saturation in the red cell; the latter tension is read from the dissociation curve of the blood.

Since D_R is equal to the product of three variables as expressed in Eq. (2), it is now possible to compare the magnitudes of D_M and D_R , because D_R can be changed quantitatively by varying Q and F_2 , though D_M is independent of them. For instance, F_2 is a function of HbO₂ saturation in the red cell which is controlled by the inspired oxygen tension and, on the other hand, the hematocrit value in Q can also be changed by experimental anemia in animals.

One of the authors was a coadjutor in experiments reported by Bartels *et al.* (11) which confirmed the dependency of D_L upon the inspired O₂-tension and found that D_L became greater in hypoxia than in air-respiration. However, the distribution of D_L values was not narrow enough to enable comparison of D_L and F_2 with each other. The dependency of D_L upon experimental anemia has not hitherto been investigated.

Gibson *et al.* (7) reported that D_M was c. 1/8 of D_R in case of O₂-diffusion and c. 1/4 in case of CO-diffusion. Their conclusion could be deduced from the assumption that the rate of combination of CO and HbO₂ in the red cell, namely the reacting capacity, was nearly equal to the rate of combination in

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望月政司, 安曾武夫, 後藤 宏, 浜本 昭, 牧口行雄

the laked hemoglobin solution. Such an assumption is not correct with regard both to the writers' theoretical consideration and to the recent experimental data presented by Roughton *et al.* (22), in which the rate of combination in the red cell became significantly smaller than the rate in a hemoglobin solution at a low oxygen tension of c. 100 mmHg, though both of the rates were nearly equal to each other at a higher O_2 -tension.

If the reacting capacity is applied to the estimation of D_M and D_B instead of the rate of combination in a hemoglobin solution, D_M becomes much greater than D_B . In the recent paper of Roughton and Forster (23) D_M 's were about 2-3 times as large as D_B . This numerical relation between D_B and D_M must be expected to occur in the measurements of D_L of oxygen.

This paper is concerned primarily with an investigation of the dependencies of D_L upon the HbO_2 saturation of the capillary blood and also upon the number of red cells in the circulating blood. Such dependencies are needed to be known in a comparison of D_M and D_B with each other.

METHOD

Dogs weighing 8 to 15 kg. were used throughout the experiments. They were anesthetized with morphine (2 mg/kg.) and urethane (1.2 g/kg.), ventilated by a pump, using a tightly fitting tracheal tube as a connection. The stroke volume and frequency were chosen individually for each dog, so that all spontaneous respiratory movements ceased. Three kinds of air were administered from a spirometer of 100 l., two of them (10-15% O_2) being used for hypoxic levels and the other for the hyperoxic level.

Anemia in the second experiment was brought about by draining a certain amount of blood and by injecting physiological NaCl solution with 6% dextran by the same amount as the depleted volume.

Analysis. The expired air was collected in a spirometer of 50 l. and analysed with Scholander's apparatus (24). The arterial and venous blood were sampled from aorta abdominalis and vena cave inferior respectively through catheters. The arterial and the venous oxygen tensions P_{aO_2} and P_{vO_2} , were polarographically measured by using oxigraph (12, 13). This is an instrument made by the Shimazu Seisakusho, Kyoto, for the purpose of measuring the oxygen tension in liquid as well as in tissue with a platinum electrode.

The O_2 and the CO_2 concentrations in the arterial and the venous blood were measured by Van Slyke's apparatus. The measurements of the arterial CO_2 tension P_{aCO_2} was made in part by the bubble method of Riley *et al.* (18) by using Roughton-Scholander's syringe and in part by Van Slyke's apparatus. In the latter case the arterial CO_2 tension was calculated with reference to the alkali reserve by the use of Henderson's nomogram and the diagram of Dill *et al.* (16).

Calculation. The alveolar oxygen tension P_{AO_2} was calculated from the arterial CO_2 tension and O_2 and CO_2 fractions in in- and expired air, F_{IO_2} , F_{ICO_2} , F_{EO_2} and F_{ECO_2} , according to Bohr's formula (4) as

$$P_{A_{O_2}} = P_{I_{O_2}} - P_{a_{CO_2}} \frac{F_{I_{O_2}} - F_{E_{O_2}}}{F_{E_{CO_2}} - F_{I_{CO_2}}} . \quad (3)$$

The oxygen uptake in STPD $\dot{V}_{O_2}$ was obtained from the ventilation volume and from the fractions of oxygen and carbon dioxide in in- and expired air using the following equation (24).

$$\dot{V}_{O_2} = V_E \frac{F_{I_{O_2}}(1 - F_{E_{CO_2}}) - F_{E_{O_2}}(1 - F_{I_{CO_2}})}{1 - F_{I_{O_2}} - F_{I_{CO_2}}} . \quad (4)$$

The venous admixture Q_{sh} (%) was calculated from $(P_{A_{O_2}} - P_{a_{O_2}})$ and the arterial venous O_2 -difference (Vol. %) both measured in hyperoxia according to

$$Q_{sh} (\%) = \alpha (P_{A_{O_2}} - P_{a_{O_2}}) \times 10^{-4} / (C_{a_{O_2}} - C_{v_{O_2}}), \quad (5)$$

as given by Bartels and Rodewald (2), where α is the solubility of oxygen in water per 1 mmHg of P_{O_2} , and is equal to Bunsen's coefficient divided by 760 mmHg.

The oxygen tension of the capillary blood at the end terminal was obtained from the arterial O_2 -tension with reference to the venous admixture Q_{sh} and the HbO_2 dissociation curves.

The calculation of the diffusing capacity depended on the mathematical treatment reported by Bohr (5).

RESULTS

(1) The dependency of D_L upon the HbO_2 saturation of the red cell in the lung capillary

The diffusing capacities were measured in 10 dogs. Firstly it was found that their values depended on the size of the dogs, that is, the dogs with a greater cardiac output showed the greater D_L . In other words, D_L 's showed a positive correlation against the cardiac output as is graphed in fig. 1. The dotted line in this figure is a regression line of D_L 's obtained by the least mean square method; it can be reasonably comprehended from this line that D_L has a linear relation against the cardiac output, which will be discussed later in detail.

Consequently, D_L 's per 1 l. of the cardiac output were obtained by dividing D_L 's by the cardiac output in order to minimize the individual variance. In fig. 2, D_L 's/I. are illustrated versus the average HbO_2 saturation in the lung capillary. The latter values were read from the dissociation curve corresponding to the average oxygen tension in the red cell. The dotted lines in fig. 2 are the curves of D_B which are calculated according to Eq. (2) at three contact times, 0.1, 0.2 and 0.3 sec. by assuming hematocrit value = 0.48. The diffusion through membrane was absolutely disregarded in the derivation of these curves, but it is apparent that values of D_L are distributed between two curves of 0.1 and 0.3 sec. contact time, and that the distribution of D_L 's/I. fits quite well to the calculated curves of D_B .

FIG. 1. The diffusing capacity D_L plotted against the cardiac output. The dotted line is a regression line obtained by the least mean square method.

FIG. 2. The diffusing capacity D_L per 1 l. of cardiac output versus the average HbO_2 saturation of the capillary blood. The dotted lines are D_B -curves calculated from Eq. (33) at the contact time of 0.1, 0.2 and 0.3 sec., provided that $Q=480$ cc.

The magnitude of D_M can be theoretically obtained from Eq. (1) by illustrating both reciprocals of D_L and D_B , or D_L and F_2 in a rectangular coordinate as in fig. 3 and by reading the value of $1/D_L$ extrapolated at $1/D_B = 0$.

In fig. 3 the relation between the reciprocals of D_L and F_2 is plotted. Though the width of the distribution is too broad to show D_M exactly, $1/D_M$ can practically be assumed to be equal to zero as shown in the figure. Hence it is considered that the diffusion through the membrane occurs much more rapidly than that inside the red cell.

These experimental facts lead to the estimation of the contact time. It was obtained from Eq. (2) by assuming hematocrit value = 0.48 and, therefore, $Q = 480$ cc., and by dividing D_L by $Q \cdot F_2$.

The mean value of the contact time was 0.18 sec. and the standard deviation, 0.056 sec. Accordingly, the mean of the population of the contact time falls in the region of $t = 0.18 \pm 0.03$ sec.

(2) The diffusing capacity and experimental anemia

The relation between D_L and the red cell number was studied in 6 dogs; results are shown in fig. 4. The linearity of that relationship can be clearly observed in this figure.

To use $Q \cdot t$ is probably better than to use D_L/l for the purpose of investigating the dependency of D_L upon the red cell number, though the character of the distribution, that is, the linearity remains unchanged, even if $Q \cdot t$ was used instead of D_L/l in securing results shown in fig. 4.

FIG. 3. Graphical illustration of the relation of $1/D_L$ and $1/F_2$.

FIG. 4. A graph of the diffusing capacities D_L per 1 l. of the cardiac output versus the red cell number.

DISCUSSION

The dependencies of D_L both upon the HbO_2 saturation of the red cell in the lung capillary and upon the red cell number were primarily investigated. It was clarified from the experimental results with reference to the previous

paper that the diffusion in the lung is determined mainly by the one occurring within the red cell membrane.

The dependency of D_L upon the cardiac output, as shown by fig. 1, was obtained by measuring D_L under such a condition that there was no intentional correlation between the cardiac output and the contact time. If there is a correlation between them, for example, if D_L is measured at both rest and exercise, as in the experiments made by Riley *et al.* (20) and Filley *et al.* (6), the linearity of the relation between D_L and the cardiac output will not be observed.

$Q \cdot t$ in Eq. (2) is 30 times as large as the blood volume contained in the lung capillary. In the works of Lochner and Schoedel (9, 10), Ochsner (15) and Piper (17) it was clearly shown that the vascular volume in the lung increased with the increase of exercise and finally reached a maximum value. Although the whole blood volume in the lung was measured in their experiments, the capillary would also change its volume similarly to other parts of the vascular system. Therefore, it is expected from the relation of Eq. (2) that D_B also reaches a maximum value at a time of a heavy exercise.

The relation between D_L and the average HbO_2 saturation bore a resemblance quite similar to the one between the reacting capacity F_2 and HbO_2 saturation. This resemblance is deduced with certainty from the fact that $1/D_M$ in Eq. (1) can practically be equal to zero as in fig. 3; that leads to the conclusion that D_M is very great compared with D_F , that is, $D_L \doteq D_F$.

Roughton and Forster (23) insisted from the measurements of D_L of carbon monoxide in men that D_M is about 46 cc. by the breath-holding method and 55 cc. by the steady-state method. They used in their report the reaction rate θ , which represented the rate of combination of HbO_2 and CO within the red cell obtained from their preceding paper (22).

No matter whether θ may be theoretically correct or not, θ must reasonably be about 30 times as large as present writers' reacting capacity F_2 ; however, the product of solubility and diffusion coefficient of carbon monoxide, $\alpha D(\text{CO})$ in θ , was estimated too great and hence the relation between θ and O_2 -tension in the red cell differed from the relation between F_2 of carbon monoxide and O_2 -tension inside the red cell; the latter relation was represented in the previous paper. Being different from $1/\theta$, the reciprocal of $F_2(\text{CO})$ is not linear, but upwards convex against O_2 -tension. In the calculations of the previous report $\alpha D(\text{CO})$ was 0.8 of $\alpha D(\text{O}_2)$, namely, 0.6×10^{-10} cc/cm².mm Hg.cm.sec.; it was nearly 1/3 of $\alpha D(\text{CO})$ used by Roughton *et al.* (22). In the writers' derivation neither the transfer coefficient nor the red cell membrane was regarded, but $\alpha D(\text{O}_2)$ was determined from the data of Gibson *et al.* (8); furthermore the obtained reacting capacity of CO agreed quite well with the one obtained from the rate of combination of CO and HbO_2 inside the red cell reported by Roughton *et al.* (22). Consequently, so far as the writers' equation was used, the value of $\alpha D(\text{CO})$ must be correct.

If $F_2(\text{CO})$ is used for the estimation of D_M instead of θ , the expected value for D_M becomes greater than the ones reported by Roughton *et al.* (23). As values of D_M , 55 and 70 cc. were obtained from the data derived from a breath-

holding and a steady-state method respectively, though there was a difficult problem concerning the way of estimating the average O_2 -tension inside the red cell.

Apart from the above troublesome discussion, the magnitude of D_B and D_M can be practically compared with each other in another experiment in which the relation between D_L and anemia is investigated. The hematocrit value, that is, Q in Eq. (2) becomes smaller by diluting the blood with a dextran Ringer solution. Therefore, D_B must become smaller linearly as the anemia proceeds.

Now, as D_L is expressed in Eq. (1), if D_M is smaller than D_B , or D_M and D_B are of comparative magnitude, D_L must not reduce linearly, but hyperbolically as the number of red cells becomes smaller. Thus, the fact that D_L linearly reduced in proportion to the red cell number explains at least that the diffusion of oxygen in the lung is controlled in greater part by the diffusion inside the red cell.

In the previous paper as the contact time in men, 0.23 sec. was estimated from the difference between the alveolar and arterial O_2 -tensions measured by Bartels and Rodewald (1, 2). However, if the time is calculated from D_L measured also by Bartels *et al.* (3) at hypoxia by using Eq. (2) in which hematocrit value is assumed to be 0.48, it becomes 0.15 sec. in case $D_M = \infty$, where $D_L = 24.4$, $F_2(O_2) = 0.038$ and $Q = 9,040 \times 0.48$ cc. Provided that $D_M = 70$, the contact time becomes 0.23 sec.

There have, up to the present time, been two sets of data as to the contact time, the one was measured by Roughton (21) and the other by Vogel (26). The former value was c. 0.7 sec. and the latter was 0.05 to 0.15 sec. The values of Vogel coincide quite well with those of the present workers, but Roughton's data are about three times as large. When Roughton calculated the contact time, he did not regard the diffusion rate inside the red cell and assumed that the reaction rate of combination of CO and HbO₂ in the red cell was almost equal to the one in a hemoglobin solution. The incorrectness of this assumption was pointed out recently by Roughton *et al.* (22). If the reacting capacity F_2 was used for this estimation, the contact time was to become smaller.

The capillary volume, on the other hand, is evaluated from both the contact time and the cardiac output; it is given as 23 and 35 cc. in cases of $D_M = \infty$ and $D_M = 70$ respectively. These values are 1/2 to 1/3 of the ones obtained by Roughton and Forster (23).

Concerning the practical calculation of D_L , Riley and co-workers (8, 19) assumed that D_L of oxygen was independent of O_2 -tension in the alveolar air. However, this assumption is not correct, because D_L as well as F_2 depends upon the average HbO₂ saturation of the red cells in the lung capillary. Hence, D_L must be calculated through the procedure employed by Bartels and Rodewald as follows. 1) At first, the venous admixture is calculated by the use of Eq. (5) at hyperoxia, 2) the terminal O_2 -tension of the capillary blood P_{CO_2} is determined with reference to the venous admixture and the arterial O_2 -tension at hypoxia and 3) at last, D_L is calculated by means of the above value as to P_{CO_2} .

Throughout the calculation of D_L the ununiformity of the perfusion and ventilation ratio was disregarded; however, it was sure that the differences

between the alveolar and the arterial O_2 -tension, AaD , caused by this effect could be neglected as shown by another experiment (14) in which AaD 's were measured in hyperoxia at over 300 mmHg in normal men and patients with pulmonary diseases.

SUMMARY

The diffusing capacities D_L 's of oxygen were measured in dogs at various inspired O_2 -tensions and at various red cell numbers in the circulating blood in order to compare two capacity components, that is, the red cell component D_B and the membrane component D_M .

D_B is related to the average HbO_2 saturation of the capillary blood, the red cell concentration, the cardiac output and the contact time as theoretically investigated in the previous paper. Hence, the authors compared D_M and D_F , or D_L and D_B with each other by using the relations both between D_B and HbO_2 saturation, and between D_B and the red cell number, because D_L measured can be expressed as

$$1/D_L = 1/D_M + 1/D_B.$$

From the results it was shown to be highly possible that D_M is at least three to four times as great as D_B , so that one could practically put $D_L = D_B$. It was further made clear that the contact time became c. 0.18 sec. on an average in dogs in case $D_M = \infty$.

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