Search for Diffusion Limitation in Pulmonary Gas Exchange

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I dedicate this chapter to the memory of those who introduced me to physiology and guided and promoted me through the years: Wolfgang Schoedel, long-time head of the Department of Physiology and my predecessor; Kurt Kramer, head of the Department of Physiology at Göttingen University (later at the University of Munich) and my academic promoter, whose enthusiasm for physiology inspired me; and Hermann Rahn, of Buffalo, New York, who was unsurpassed as a questor of things physiological.

From Estonia to Germany, Through War and Peace

My father was born in a poor family in Tallinn, capital city of the Government of Estonia in the Russian Empire. With the support of well-off relatives, he was able to complete secondary school in Tallinn and study biological sciences at the University of St. Petersburg. He became professor of zoology at the University of Tartu when it reopened as an Estonian university in 1919, after Estonia had won its independence from czarist Russia after fighting both the Germans and the Red Army in the years following World War I.

I was born in 1924 and grew up in a free country during a peaceful time of economic growth and cultural development. All this came to an end with the establishment of Soviet military bases in Estonia in the fall of 1939 and the annexation of Estonia by the Soviet Union in the summer of 1940. After the onset of the
German-Soviet war in June 1941, many Estonians were arrested and deported to northern Russia, and young Estonians were drafted into the Red Army. Being one year too young, I escaped the draft.

The German forces advanced quickly, but when they reached Tartu, they were halted for several weeks by the river that flowed through the city. During that time a large part of the city was destroyed by the fires resulting from artillery shelling and arson by Red partisans. In 1942 I graduated from secondary school in Tartu, after which I had to join the Luftwaffe, first as an auxiliary serviceman, but soon thereafter as a regular soldier stationed in Estonian territory.

As the German army retreated from the Baltic countries in September 1944, my unit was shipped across the Baltic to East Prussia. I was wounded on the outskirts of Königsberg, escaped from the encircled city on horse cart, and was taken on board a hospital ship to the port of Sassnitz on Rügen Island. There the less seriously wounded were immediately disembarked and taken on a hospital train. The severely wounded stayed on board the ship, which left the port and headed to the roadstead for safety, but it was hit by bombs dropped by Allied aircraft the following night and sank with all the severely wounded. My remarkable luck was that, though graded as severely wounded, I had been helped by lightly wounded German soldiers to the train, thereby escaping the shipwreck. During the following four days the hospital train traveled across the whole of war-torn Germany, from north to south. Finally we stopped near Garmisch-Partenkirchen in Bavaria and were put into an auxiliary hospital.

Soon after American forces occupied Bavaria, my wound had healed sufficiently, and I was taken to an American prisoner-of-war camp in Garmisch-Partenkirchen. Being Estonian, I could not be released due to lack of regulations, whereas Germans were continually discharged. But a fellow professor of my father had come as refugee to Göttingen and searched for me by advertising in a refugee newspaper, which was smuggled into the camp. Finally, by the end of 1946, I was discharged along with a number of Estonians, Latvians, and Lithuanians. The reason I went to Göttingen, where a large camp for Baltic refugees was located, was that I wanted to enter the university's medical school. This had been my parents' wish, and I never hesitated to comply with it, although I was attracted more by biology than medicine.

I had lost all my documents including school certificates, and it was utterly impossible to obtain substitutes from Estonia. All I could do was procure written declarations from two of my former teachers who had come as refugees to Germany. But the main reason I was accepted was the extraordinary considerateness of Göttingen University's counselor for foreign students, Dr. Wienert, who, after having heard my story, arranged my admission pending my passing an oral admission examination. Because of my long absence from school and my poor mastery of scientific German, this was the most difficult exam I have ever taken. My examiner in mathematics and physics was Professor Hans Loeschcke, the well-known respiratory physiologist. I remember one of the problems he gave me was determining the height of Egyptian pyramids using a yardstick but without climbing the structures (Luckily I had always been good in geometry.)

With much luck I had survived the war and was admitted to medical school, but I was alone. My parents and my sister remained in Estonia. I received some support from Estonian refugees, and I lodged clandestinely in a Displaced Persons' camp supported by refugee organizations. I could not obtain official permission to stay there, because of my military past. Later I was admitted to a student home supported by a Lutheran church organiza-
tion. After finishing two and a half years of my preclinical studies, I applied for immi-
grant to the United States but was de-
nied because of my military involvement
on the wrong side. About one year later I
was requested to come to an emigration
camp for a new interview, but then I was
the one who declined, intending to finish
medical school in Germany.

After passing the state medical exami-
nation I looked for a place to work and to
prepare my medical doctor thesis. After
some unsuccessful applications, I was in-
troduced by Professor Loeschcke to Pro-
fessor Schoedel, the director of the physi-
ology laboratory of the research institute
(later named the Max Planck Institute),
where I was accepted in January 1953. An
important asset was that I could get a
fellowship, worth about 100 German
marks a month, which at that time and in
my circumstances was a good income. I
never felt as rich since then. Today I am
still working in that institute, and since
1973 I have served as director of the De-
partment of Physiology.

Steady-State Gas Exchange:
Diffusion Limitation versus
Ventilation/Perfusion
Inequality and Shunt

In September 1958 I came to the
United States to work for one year with
Hermann Rahn in the Department of Phys-
iology at the University of Buffalo, later
called the State University of New York at
Buffalo. The timing was awkward because
my wife was expecting our first child, and
I could not afford to take her to the United
States. In Buffalo, I was introduced to the
American way of life and to the analysis of
pulmonary gas exchange by Pierre Haab,
who had come to Rahn from the laboratory
of Alfred Fleisch at Lausanne a year ear-
lier. (An interesting coincidence is that
Fleisch had invented the pneumo-
tachograph as professor of physiology at
the Estonian University of Tartu). I was
fortunate to find in Pierre Haab not only
an indispensable coworker in Buffalo, but
also a scientific companion and a friend
for life.

I learned that the alveolar-arterial $P_{O_2}$
difference ($AaD_{O_2}$) was conventionally
attributed to three mechanisms: ventilation-
perfusion ($V_A/Q$) inequality, shunt, and
diffusion limitation, which were not eas-
ily differentiated (Fig. 1). Hermann Rahn’s
idea was to demonstrate the effect of venti-
lation-perfusion inequality by an experi-
ment that had a simple and straight-
forward design but could not be easily
performed. According to Farhi and Rahn
the $AaD_{O_2}$ due to $V_A/Q$ inequality should
be much reduced when $N_2$ is eliminated
from inspired gas. This could be achieved
by breathing 100% $O_2$ in a hypobaric
chamber at a total pressure reduced to
such a level that alveolar and arterial $P_{O_2}$
stayed normoxic, i.e., at a total pressure
of about 197 Torr, equivalent to 10 km of
altitude. The result of the exciting experi-
ments we carried out, with all three of us
(the experimental dog, Pierre Haab, and
myself) confined in the hypobaric cham-
ber for many hours, was unexpected and
disappointing: the $AaD_{O_2}$ in anesthetized,
artificially ventilated dogs remained un-
changed after elimination of $N_2$ from in-
spired gas. We considered several possi-
bable “loopholes” such as incomplete elimi-
nation of $N_2$, accelerated development of
atelectatic shunt in the absence of $N_2$, and
$V_A/Q$ inequality of a mainly alveolar dead
space-like character (the $AaD_{O_2}$ due to al-
veolar dead space would be little affected
by elimination of $N_2$). We also considered
the possibility that the $AaD_{O_2}$ in dogs in
our experimental conditions was not due
to $V_A/Q$ inequality but to another kind of
maldistribution, unequal distribution of
diffusing capacity to blood flow ($D/Q$ in-
equality) (Fig. 2).

I had developed the concept of $D/Q$
ratio to explain the experimentally deter-
mined absorption rates of inert gases of
varied solubility and diffusivity from subcutaneous gas pockets in rats that Canfield and Rahn observed prior to my arrival in Buffalo.\textsuperscript{50} When allowing for variance of the $D/Q$ ratio in lungs, the dependence of $\text{AaD}_O_2$ on alveolar $P_{O_2}$ found in our experiment could indeed be explained. In normoxia the $\text{AaD}_O_2$ was mainly attributable to a lung compartment with low $D/Q$. In hyperoxia there would be no diffusion limitation in this compartment (no $\text{AaD}_O_2$), and in deep hypoxia the blood flow of this compartment would be functionally close to a complete shunt.\textsuperscript{53}
As the CO₂ electrode became available (after 1960), we and others showed that there existed an arterial-to-end-expired P co₂ difference in anesthetized dogs that explained part of the total AaD O₂ not attributable to shunt or (evenly distributed) diffusion. The larger part of AaD O₂ measured in normoxia could not, however, be explained by this factor (e.g., reference 2). Yet by the elegant multiple inert gas elimination technique (MIGET), 66,67 AaD O₂ in man and dog has been shown to be mainly explainable by VA/Q variance. From my perspective today, our results with elimination of N₂ may have been mistaken, but they did stimulate us to look for other solutions and thus led to the concept of D/Q and its variance. On the other hand, it would be strange if the sizable variance of pulmonary capillary transit time, as measured by videofluorescence microscopy in subpleural, pulmonary vessels by Wiltz Wagner, 4,69,70 had no effect on O₂ exchange. Moreover, the pulsatile pulmonary capillary blood flow as demonstrated by Grant de Jong Lee and associates 29 is expected to have effects analogous to those of local blood flow variance.

The combination of D/Q inequality with that of VA/Q inequality in a “V/Q=D/Q field” 45 remained theory for a long time. Only recently have Yamaguchi and coworkers attempted to apply this concept. Combining experimental results of MIGET with CO₂, O₂, and CO exchange data obtained from pulmonary patients, Yamaguchi and coworkers 74 have been able to estimate quantitatively both VA/Q and D/Q inequality.

Back in Europe from Buffalo, Pierre Haab and I returned to the conventional analysis of alveolar gas exchange in anesthetized dogs. To increase the relative diffusion limitation effects on AaD O₂, we measured gas exchange variables in deep hypoxia (Fig. 1). This time we used the recently available blood P co₂ electrodes and mass spectrometry for continuous gas analysis. 16 The results showed only a very small AaD O₂ to be attributable to diffusion limitation (Fig. 3). But this was a maximal effect based on the assumption that alveolar dead space was the only effective mode of VA/Q heterogeneity. When other modes of VA/Q inequality were assumed, the diffusion limitation component of AaD O₂ approached zero.

Also, measurements of O₂ exchange in isolated, blood-perfused dog lung lobes in hypoxia led to results that were not easily explained by diffusion limitation. 46 Following an idea of Masaji Mochizuki (with whom I had interminable discussions as he worked with Heinz Bartels in the physiology department of the University of Göttingen 1953 and 1954), we performed measurements with varied hematocrit. The AaD O₂ was found to be independent of hematocrit, in agreement with Mochizuki and coworkers 40 and correspondingly the calculated D O₂ came out proportional to hematocrit. This could be explained by a model with all resistance to O₂ uptake in red blood cells (and no resistance in the blood-gas barrier or plasma). But the same effect would be produced by a shunt beside a major compartment exhibiting no diffusion limitation. The shunt model could also explain the independence of AaD O₂ of blood flow, which led to a directly proportional relationship between D O₂ and blood flow. 46

It followed from all the theoretical models and experimental results that an AaD O₂ due to diffusion limitation was not easily evaluated. Even its existence could not be convincingly demonstrated, apparently due to disturbing influences of functional inhomogeneities like ventilation-perfusion and other inequalities.

Rebreathing: Attempt to Isolate Diffusion Limitation

The stimulus came from Paolo Cerretelli, from the laboratory of Rodolfo Margaria in Milan (now in Geneva), with
whom I had collaborated since 1962 on studies of \textsuperscript{2}O\textsubscript{2} supply to stimulated isolated muscles and exercising animals. With Hermann Rahn and Leon Farhi, he had attempted to determine mixed venous \(P_{O_2}\) in man by rebreathing, using mass spectrometry.\textsuperscript{6} Be selecting a gas mixture of appropriate composition and volume, he and his coworkers attempted to obtain mixed venous \(P_{O_2}\) and \(P_{CO_2}\) from rebreathing plateau values of \(CO_2\) and \(O_2\). But in most cases this ideal condition of true plateau could not be achieved. Instead, the mixed venous \(P_{O_2}\) had to be extrapolated or interpolated from a plot of rate of change of \(P_{O_2}\) against \(P_{O_2}\); the true value was assumed where the rate of change of \(P_{O_2}\) became zero.\textsuperscript{7}

I became involved when Cerretelli asked me to analyze the factors that might determine the kinetics of the approach of lung gas \(P_{O_2}\) toward the equilibrium value, i.e., mixed venous \(P_{O_2}\). According to the theory, in an ideal system these kinetics were determined by the ratio of the overall \(O_2\) conductance, determined by pulmonary diffusing capacity for \(O_2\) (\(D_{O_2}\)), pulmonary capillary blood flow, and the slope of the blood \(O_2\) dissociation curve, to the \(O_2\) capacitance, given by the total gas volume of lungs and rebreathing bag.\textsuperscript{51} Because an infinitely high ventilation cannot be achieved, in practice the effective ventilation and the partitioning of the total gas to lungs and rebreathing bag also had to be taken into account (Fig. 4). The feasibility of the method was tested in experiments on anesthetized dogs\textsuperscript{1} and on man at rest and during exercise.\textsuperscript{64}

The method could be improved by introducing a stable isotope of \(O_2\), \textsuperscript{18}O\textsubscript{2}, in low concentration (0.07\% in the rebreathing mixture) as test gas.\textsuperscript{37} This had two important advantages. First, mixed venous partial pressure of the isotope could be neglected. Second, the effective blood dissociation curve of the isotopic \(O_2\) against its partial pressure could be re-

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**Figure 3.** Analysis of alveolar gas exchange in hypoxia. Left: model of lung with series dead space, alveolar dead space, and effective alveolar ventilation, capillary, and shunt blood flow. Mean values (±SE) of \(P_{O_2}\) and \(P_{CO_2}\) differences, obtained in 11 anesthetized, paralyzed, artificially ventilated dogs are indicated. After Haab et al.\textsuperscript{16} Right: \(P_{CO_2} - P_{O_2}\) diagram with blood and gas R lines underlying the analysis of alveolar gas exchange according to the model at left.
Figure 4. Determination of diffusing capacities (D) for O₂ and CO by rebreathing: model and recordings of partial pressures in rebreathing gas by mass spectrometry. Vᵦ, (average) volume of rebreathing bag; Vₑᵦ, (average) lung volume; Vₑff, effective alveolar ventilation; Q, pulmonary blood flow; β, slope of effective blood dissociation curve. For DCO, Q and β are not required because CO uptake may be regarded as not limited by blood flow. Time available for measurement is limited by onset of recirculation (about 12th s).

Figure 5. Simultaneous determinations of the diffusing capacities for O₂ and CO (D₀₂, D₉₀) by rebreathing in man at rest and during bicycle ergometer exercise. Mean values ± SE obtained in 6 normal males, 20–33 years of age. After Meyer et al.³⁷

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garded as a straight line through the mixed venous point of the abundant isotope (O₁₆₂). From simultaneous determination of CO diffusing capacity, a stable isotope of CO, ¹³CO, was used, again to be able to neglect CO in mixed venous blood and, more important, to be able to separate CO from N₂ when using continuous respiratory mass spectrometry with relatively low mass resolution.

The advantage of rebreathing is the homogenization of lung gas by rebreathing, thus reducing or even practically eliminating the effects of unequal distribution of ventilation to blood flow. Simultaneous determination of D₀₂ and D₉₀ in normal man showed that the resting values were higher than usually reported (using other methods), their increase in exercise was moderate, a plateau value was reached, and the ratio D₀₂/D₉₀ was about 1:2, i.e., close to the ratio of Krogh diffusion constants for tissue³⁷ (Fig. 5). The last finding was taken to indicate that simple diffusion was the main factor involved.

Recently a group in Oxford³ and a group in Bordeaux¹⁴ have reported measurements of pulmonary diffusing capacity in patients using a new test gas, nitric
oxide (NO). The advantage of NO is that it reacts much faster with hemoglobin than does CO. But NO is a highly reactive and toxic gas, and it must be used in very low concentrations. It could be measured in gas samples using highly sensitive chemiluminescence detectors developed for monitoring atmospheric pollution. Using mass spectrometry, we had to go to considerably higher concentrations than used in man by the other groups (600 ppm vs. 10 to 40 ppm), and therefore we preferred to work on dogs. Because of the reactivity and instability of NO, particular measures had to be taken for its continuous recording by the mass spectrometer during rebreathing.

Simultaneous rebreathing of NO and CO yielded average $D_{NO}$ in anesthetized dogs about four times higher than $D_{CO}$ (Fig. 6). According to literature data, the Krogh diffusion constant ratio NO/CO is close to 2. The higher figure for the $D_{NO}/D_{CO}$ ratio probably is due to the slow reaction of CO with hemoglobin. Even in hypoxia, where substantial free Hb is also present in arterialized blood, the $D_{NO}/D_{CO}$ ratio averaged 3.2. The inference that CO uptake is strongly reaction limited is evidently in disagreement with our conclusions from simultaneous measurements of $D_{CO}$ and $D_{O_2}$ in humans (see above). Because the ratio $D_{O_2}/D_{CO}$ was found close to the Krogh diffusion constant ratio $O_2/CO$, it was concluded that both $O_2$ and CO were limited by simple diffusion. The high $D_{NO}/D_{CO}$ ratio would mean that true $D_{CO}$ was higher than measured and the estimated $D_{O_2}$ was an underestimation, possibly due to remaining inhomogeneity effects ($D/Q$ inhomogeneity?).

Of particular interest was a small but significant increase of $D_{NO}$ in hypoxia and decrease in hyperoxia. Because the reaction of Hb with NO is known to be very fast, we would like to attribute these changes to real changes in $D$ (increase in size or number of perfused pulmonary capillaries).

![Figure 6](https://example.com/figure6.png)

**Figure 6.** Simultaneous determinations of the diffusing capacities (D) for NO and CO by rebreathing in 8 anesthetized, artificially ventilated dogs. Mean values relative to $D_{NO}$ in normoxia. The differences between normoxia and hypoxia and between normoxia and hyperoxia are statistically significant for both $D_{NO}$ and $D_{CO}$.

The problem of $CO_2$ equilibration kinetics of blood in pulmonary capillaries has been experimentally investigated by Hyde and coworkers in humans. They applied a breath-holding method in which the kinetics of $^{13}CO_2$ equilibration was compared with that of acetylene. But the equilibration limitation effect on $^{13}CO_2$ uptake was too small to be measurable. The effective diffusing capacity, $D_{CO_2}$, was estimated to be higher than 200 ml/(min·Torr). Using the same $CO_2$ isotope and acetylene but applying rebreathing and continuous recording of $^{13}CO_2$ during rebreathing by mass spectrometry, we could estimate the $CO_2$ diffusing capacity at 180 ml/(min·Torr) at rest and at 300 ml/(min·Torr) during moderate exercise.

Our main interest was comparing $D_{CO_2}$ with $D_{O_2}$, both measured by rebreathing in similar experimental conditions. The mean ratios $D_{CO_2}/D_{O_2}$ were 3.3 at rest and 4.7 during exercise, thus averaging 4. But the Krogh diffusion constant ratio $K_{CO_2}/K_{O_2}$ is about 20–25. Thus the capillary-alveolar equilibration of $CO_2$ was found to be much slower than expected on the basis of $CO_2$ diffusion. It is well known that the blood-gas $CO_2$ transfer in lungs is a complex process consisting of many compo-
nent processes. The bicarbonate-chloride exchange between red blood cells and plasma, the dehydration of carbonic acid to CO$_2$ in plasma, and the same reaction in the red cells (in spite of the presence of carbonic anhydrase) are known or suspected to be relatively slow. Thus the diffusion of CO$_2$ may lead to a rapid equilibration of CO$_2$ in blood with alveolar gas, but the subsequent slow reequilibration between CO$_2$, HCO$_3^-$, and H$^+$ in red cells and plasma would lead to an elevation of P$_{CO_2}$ in arterialized blood after it has left the lungs (to be eventually completed as the blood is in a CO$_2$ electrode) (Fig. 7).

**Diffusion/Perfusion Model: Extent and Site of Diffusion Limitation**

In the analysis of gas exchange, a basic question is to what extent gas transfer is limited by perfusion and by diffusion. The simplest model, applicable to many situations (such as absorption from tissue gas pockets, gas transfer through skin, gas transfer in lungs), is a gas phase maintained at constant composition having gas exchange with flowing blood, of a certain effective solubility for the gas considered, across a barrier constituting a resistance to diffusion.$^{55}$ The calculated partial pressure profiles are shown in Figure 8. They are determined by the ratio of diffusing capacity (D) and the product of blood flow (Q) and the effective solubility ($\beta$), which is the slope of blood dissociation curves in terms of concentration (content) versus partial pressure ($\beta = dC/dP$). The ratio, $D/(Q\beta)$, may be termed the equilibration index. For the relative equilibration deficit of arterial blood $(PA - Pa)/(PA - P\nu)$, the following equation holds:

$$\frac{PA - Pa}{PA - P\nu} = e^{-\frac{D}{Q\beta}}$$

(1)

Clearly for $D/Q \to \infty$, $PA - Pa \to 0$, meaning absence of diffusion limitation. With decreasing $D/Q$, $PA - Pa$ increases, denoting increasing diffusion limitation. The relationship of Equation (1) can be applied to any gas including O$_2$.

![Figure 7. Blood-gas CO$_2$ equilibration in lungs. Qualitative model to explain the experimental finding that the CO$_2$/O$_2$ ratio of diffusing capacities was much smaller than the corresponding Krogh diffusion constant ratio for tissue. The equilibration of CO$_2$ proper is rapid and practically complete during the pulmonary capillary transit time, but the equilibrium for total CO$_2$ content is not reached.](image)
Figure 8. Diffusion-perfusion limitation in alveolar-capillary gas transfer. Model, partial pressure profiles for various values of $D/(Q \cdot \beta)$ and a dissociation curve (concentration, $C$, as function of partial pressure, $P$) showing definition of the capacitance coefficient $\beta = \Delta C/\Delta P$. After Pitzer and Scheid.\textsuperscript{55}

Whereas in the normoxic and slightly hypoxic regions the assumption of a constant $\beta$ is problematic because of the evident curvature of the $O_2$ dissociation curve, and a procedure like Bohr integration should be applied, in deep hypoxia the $O_2$ dissociation is sufficiently linear. The effects of the curvature of blood $O_2$ dissociation curves have been recently investigated.\textsuperscript{27,28} With the value of $D_{O_2}$ determined in our laboratory in deep hypoxia and $Q$ and $\beta$ calculated after the values estimated by Cerretelli\textsuperscript{5} in man in the base camp of Mt. Everest (altitude 5,350 m), a strong diffusion limitation results for rest (12%) and an even stronger one for maximum $O_2$ uptake exercise (64%). The $AaD_{O_2}$ estimated therefrom (2 Torr at rest, 27 Torr at maximum $O_2$ uptake exercise) is in rough agreement with measurements performed by Wagner and coworkers in normobaric or hypobaric simulated altitude.\textsuperscript{19,63,65,68}

There is also growing evidence for diffusion limitation of pulmonary $O_2$ uptake in very strenuous exercise in human athletes in normoxia.\textsuperscript{9} In these cases the $D/Q$ inhomogeneity may play a role, as the transit times in some pulmonary capillaries may be reduced so much as to turn their flow to functional shunt flow.

Let us make a short excursion to lower vertebrates (indeed, the comparative physiology of gas exchange in vertebrates has been in the center of my research interest for a long time). In amphibians, cutaneous gas exchange is important, particularly for $CO_2$ elimination, but also for $O_2$ uptake. Because skin must have a certain thickness to provide mechanical protection, diffusion limitation in cutaneous gas exchange is expected to be much more prominent than in lungs. Remarkably, in various groups of salamanders lungs have been reduced so that all exchange occurs through skin (and oral and pharyngeal mucosa).

Randall Gatz and Eugene Crawford, zoologists from Lexington, Kentucky, took lungless salamanders captured in the Alleghenies to Göttingen to study their cutaneous gas exchange. We could measure $P_{O_2}$ and $P_{CO_2}$ in blood sampled from the single ventricle and roughly estimate the
blood flow and diffusing capacity of the skin using soluble inert gases. Calculations with the values thus obtained revealed a strong degree of diffusion limitation for both \( O_2 \) uptake and \( CO_2 \) output.\(^{52}\) Remarkably, animals are highly tolerant of hypoxia\(^ {13} \) and can substantially increase their cutaneous \( O_2 \) uptake during exercise. Evidently the diffusing capacity can be adaptively increased.\(^ {11} \)

Estimation of the pulmonary equilibration deficit of \( CO_2 \) in human lungs by introducing values of \( D, Q, \) and \( \beta \) into Equation (1) yielded 2% for rest but 18% for maximum \( O_2 \) uptake exercise.\(^ {54} \) The corresponding arterial-to-alveolar \( P_{CO_2} \) differences were 0.2 Torr and 7 Torr, respectively. Thus, against what is usually believed, blood-gas \( CO_2 \) equilibration in lungs may play an important role in limiting the efficiency of \( CO_2 \) elimination.

Site of Resistance to Gas Transfer: Red Blood Cells versus “Membrane”

The role of \( O_2 \) transfer resistance of the red blood cells in alveolar \( O_2 \) uptake (and \( O_2 \) delivery in tissues) has been discussed for many years. It was fortunate for us that in 1981 Robert Holland from Sydney, Australia, came to us for a sabbatical and initiated a number of studies on \( O_2 \) kinetics of red cells using an improved stopped-flow technique. Our attention was directed toward determining the extracellular resistance to \( O_2 \) transfer in stopped-flow experiments and to obtaining, by subtraction of the extracellular resistance, a better estimate of the true \( O_2 \) transfer resistance of red blood cells or its reciprocal, the specific \( O_2 \) transfer conductance, \( \Theta. \)\(^ {25} \) As expected the \( \Theta_0 \) values corrected for pericyrbohydrate \( O_2 \) transfer resistances were considerably larger than previously published values.\(^ {75} \)

Using the well-known Roughton-Forster relationship, which is based on the additivity of intraerythocyte (RBC) and extraerythocyte (“membrane”) transfer resistances,

\[
\frac{1}{DL} = \frac{1}{Dm} + \frac{1}{Vc \cdot \Theta}
\]

where \( DL \) is pulmonary \( D; \) \( DM, \) “membrane” \( D; \) and \( Vc, \) pulmonary capillary blood volume. We calculated for the ratio \( DL/DM, \) equivalent to the fraction of extraerythrocyte \( O_2 \) uptake resistance in total \( O_2 \) uptake resistance, 0.88 (assuming \( D_{O_2} = 54 \text{ ml/(min \cdot Torr)} \) and \( Vc = 100 \text{ ml).} \) With a previously determined value of \( \Theta, \) 1.5\(^ {20} \) the figure would be 0.67. Thus a major part of the \( O_2 \) uptake resistance in lungs appears to be located outside the red cells, in the plasma and tissue barrier. This conclusion is not in good agreement with morphometric data, which show that diffusion distances within red cells are larger than those across the air-blood tissue barrier.\(^ {71} \) But in red cells \( O_2 \) transport appears to be facilitated by hemoglobin (diffusion of \( O_2 \)-hemoglobin). Moreover, intracapillary hematocrit is generally lower than large vessel hematocrit. Because \( \Theta \) is defined for normal hematocrit (0.45), a lower hematocrit (lower \( \Theta \)) would lead to an increased \( DM \) for a given (measured) \( DL \). Another factor that is difficult to assess is the convective mixing of red cell interior and plasma during blood flow through pulmonary microvessels in vivo.

In other studies using the stopped-flow apparatus we attempted to further analyze the factors determining the \( O_2 \) transport within the red blood cells. Using a particular model for evaluation of measurements, we concluded that the diffusion coefficients of \( O_2 \) and hemoglobin were the most important variables, but \( O_2 \) reaction kinetics of hemoglobin also had a limiting effect.\(^ {23} \) In contrast, measurements of \( O_2 \) uptake kinetics at varied tem-
perature left very little space for a limiting role of the chemical reaction. Both studies may be considered as evidence for a minor role of the kinetics of the chemical reaction of hemoglobin with $O_2$ in limiting $O_2$ uptake.

Many years ago, I had tried to measure the red cell–plasma equilibration of $CO_2$-bicarbonate in vitro using the rapid mixing–continuous flow technique combined with filtration for plasma sampling. At that time I looked for a possible explanation of the relatively large arterial-alveolar $P_{CO_2}$ differences found in anesthetized dogs. But the results showed the kinetics of bicarbonate to be very rapid, leading to 90% equilibration in 0.11 s on the average. Because the usually assumed transit time of pulmonary capillaries, 0.3 to 1 s, is longer, a discrepancy appears to exist: the in vitro results suggest no measurable equilibration limitation in contrast to the in vivo $^{13}$CO$_2$ equilibration results. But both in vivo and in vitro techniques are difficult and fraught with potential errors. A reevaluation using improved methods would be welcome.

**Blood-Gas Equilibrium of $CO_2$: “Anomalous” versus Conventional**

In the Symposium on $CO_2$: Chemical, Biochemical and Physiological Aspects, which took place in Philadelphia in August 1968 (as a satellite to the International Congress of Physiological Sciences in Washington, D.C.) a remarkable finding was presented by Gail Gurtner (at that time in Buffalo). He showed experimental data obtained in anesthetized dogs in rebreathing equilibrium, exhibiting large negative $P_{CO_2}$ differences between alveolar gas and mixed venous blood, averaging about 10 Torr but reaching up to 28 Torr. Moreover, he presented an elegant physiochemical theory, which came to be known as the charged membrane hypothesis, to explain the experimental data. This remarkable paper gave rise to a lively debate in which two discussants, Gabriel Laszlo (London) and Jack Hackney (Downey, California) reported that they had not been able to reproduce the negative blood-gas $P_{CO_2}$ differences in alveolar gas-blood $CO_2$ equilibrium.

In the wake of the Gurtner paper, a number of studies have been performed, most of them confirming negative mixed venous-to-alveolar or arterial-to-alveolar $P_{CO_2}$ differences in rebreathing equilibrium in varied experimental conditions (reviewed in references 49 and 57). The excitement about this finding was understandable. There was every reason to believe that this phenomenon of a negative blood-gas $P_{CO_2}$ difference would not be confined to rebreathing equilibrium but would be operative also in steady-state gas exchange (Fig. 9). But this would invalidate the classical approach to the analysis of alveolar gas exchange in which the equality of $P_{CO_2}$ between alveolar gas and end-capillary blood in equilibrium was a central, seemingly obvious, assumption.

Indeed, soon Donald Jennings (Kingston, Ontario) claimed that negative blood-gas $P_{CO_2}$ differences occurred in anesthetized dogs during steady-state gas exchange in hypercapnia. Having spotted this paper, my coworkers and I immediately set out to repeat the experiments, essentially duplicating the experimental conditions. But we could not reproduce the results: there were no negative arterial-to-alveolar $P_{CO_2}$ differences. Soon after the completion of these experiments, I had the opportunity to present the results at the 1978 fall meeting of the American Physiological Society in St. Louis. Donald Jennings was present, and our discordant experimental results were discussed. After the session, we continued the discussion with some beer. The outcome was a visit by Peter Scheid and myself to Jennings’s laboratory at Kingston, followed by joint experiments in Göttingen.
The experiments were performed on awake dogs prepared with a chronic tracheostomy and exteriorized carotid loop (exactly as done in Kingston) but using our mass spectrometer for alveolar-expired $P_{CO_2}$ and our blood electrodes for $P_{CO_2}$. The result was that in hypercapnia no single measurement in any dog had a negative value of the arterial-to-end-expired $P_{CO_2}$ difference: with 5% inspired $CO_2$ the mean value was +0.9 Torr (significantly different from zero), with 10% $CO_2$, +0.1 Torr (not significantly different from zero). The results were published in a joint communication (26), although Jennings was not quite convinced. We planned a later continuation of the research in Kingston, but it did not occur.

Thereafter another North American physiologist who had found indirect evidence for negative blood-gas $P_{CO_2}$ differences in dogs in steady-state gas exchange came to us, initially with the intention of restudying the phenomenon, but then he drifted to other experiments. Other actions and reactions in this campaign followed. Steinbrook and coworkers reported large negative blood-gas $P_{CO_2}$ differences, averaging −12 Torr in 178 measurements during long-lasting rebreathing in awake goats. We repeated the experiments on awake dogs in similar conditions but found no significant blood-gas $P_{CO_2}$ difference (average −0.4 Torr in 266 measurements). Because previously larger anomalous blood-gas $P_{CO_2}$ differences had been reported to occur in exercise, we repeated measurements in dogs rebreathing while running on a treadmill. The result was the same: no negative blood-gas $P_{CO_2}$ differences were observed.

During these years I frequently asked colleagues their opinions on the controversial matter of negative blood-gas $P_{CO_2}$ differences, or they approached me about it. The opinions fell into two opposite camps. Many encouraged us to continue our efforts, with improved methodology, to reproduce this interesting phenomenon.
non, which we evidently had failed to find. Others wondered why we wasted time and effort trying to reproduce something that was nonexistent because it evidently contradicted physicochemical laws. In the last few years some reports of small negative $P_{CO_2}$ differences have appeared, but studies explicitly aimed at checking the phenomenon, with negative results, have also been published.$^8,^{12}$

In a detailed analysis of potential sources of error it seemed that more factors led to falsely negative than to falsely positive blood-gas $P_{CO_2}$ differences, the simplest and possibly most important one being loss of $CO_2$ from blood during sampling or transfer into the electrode.$^5^7$ But it was practically impossible to explain the large differences amounting to tens of Torr $P_{CO_2}$. It seems wise to consider this interesting issue to be open, and I encourage all students of $CO_2$/acid-base balance to look out for evidence pro or con.

It is important to point out that the equilibration kinetics of labeled $CO_2$, discussed in a preceding section, concerns a fundamentally different aspect: the kinetics of approach to an equilibrium that, in principle, is independent of the position of the equilibrium. Thus the kinetic data and their interpretation are compatible with any constellation of blood-gas $P_{CO_2}$ differences in the equilibrium state.

**Diffusion Resistance in Gas Phase: Conflicting Evidence**

There has been considerable controversy, revived recently about what part of the overall diffusion resistance to movement of $O_2$ and $CO_2$ between inspired gas and pulmonary capillary blood may be located in the alveolar gas. The consequence of such a transfer resistance would be a partial pressure gradient in the terminal airways, often referred to as stratification. This gradient could be detected during a prolonged expiration as a rising (falling) alveolar plateau of $CO_2$ ($O_2$) or of inert foreign gases previously inspired.

These problems were investigated half a century ago by, among others, my teacher and predecessor, Wolf Schoedel, then at the Department of Physiology, University of Göttingen, headed by Hermann Rein.$^{41,5^9}$ It was realized that not only stratification proper, but also continuing gas transfer between blood and alveolar gas and sequential emptying of lung areas with varied gas concentrations might be involved in producing sloping alveolar plateaus (Fig. 10). With the advent of rapid gas concentration recording techniques like infrared absorption and mass spectrometry, the shape of expirograms of various gases could be recorded more accurately.

The simultaneous measurement of several gas species of different diffusivities is of particular interest for the detection of diffusion limitations. Because of its great contrast in diffusivity, the pair He/$SF_6$ has been frequently used, the ratio of their diffusivities amounting to 6.0 from Graham’s Law (diffusivity inversely proportional to the square root of molecular weight). After measurements in our laboratory, the ratio of diffusion coefficients of He and $SF_6$ in a gas mixture of 14% $O_2$, 6% $CO_2$, and 80% $N_2$ was 7.2.$^{7^2}$ For a simple diffusion resistance model, one expects the gradient per transfer rate to be inversely proportional to diffusivity. Because the transfer rate is proportional to the end-expired-to-inspired (or mixed-expired-to-inspired) partial pressure difference, the relative slope of the alveolar plateau (i.e., standardized to the abovementioned partial pressure differences) should be inversely proportional to diffusivity, i.e., the $SF_6$/He ratio of standardized alveolar slopes should be about 7.

Scherer’s group in Philadelphia has studied the expirograms of $CO_2$ and intravenously infused He and $SF_6$ in man. The reported $SF_6$/He ratios of the standardized slopes were 3:13$^{5^8}$ and later, with im-
proved technique, 1.85. As pointed out by the same group, due to the trumpetlike shape of the lung airways (when considered as total airways cross section as function of length), the expected ratio is considerably smaller than the diffusivity ratio and may reach the values that were experimentally found. The authors concluded that diffusion resistance in the airways is the main factor in producing the alveolar slope.

In our laboratory we found in anesthetized dogs well-established alveolar slopes, but SF₆/He slope ratios were much closer to unity, and in many conditions the ratios were even below unity. This finding indicated that the slopes were due to other factors besides incomplete diffusional equilibration in gas phase. Because the effects of continuing gas exchange were compensated for by a special technique, only sequential expiration of lung areas of differing gas composition, due to $\dot{V}_A/Q$ or $\dot{V}_A/V_A$ inequalities, was a possible explanation. The simplest model to explain the experimental data consisted of two peripheral compartments connected to a central common compartment. The gas exchange between the central and each of the peripheral compartments was implemented by both diffusion and ventilation, characterized by a diffusive and a ventilatory conductance. In subsequent experiments on dogs, the sloping alveolar plateaus of CO₂, O₂, and intravenously infused acetylene and Freon-22 were determined. In another series, the alveolar slopes of He and SF₆ administered through inspired gas and through venous blood were comparatively studied (Fig. 11). In all cases only a minor dependence of the test gas alveolar slopes on diffusivity was found.

The picture emerging from these experiments performed in Göttingen is that the alveolar slope is mainly due to continuous gas exchange (particularly for well-soluble gases, but also for little-soluble gases like He and SF₆ when intravenously infused), secondarily to sequential expiration from lung areas with differing test gas composition (due to ventilation-perfusion or ventilation-volume inequalities). Finally, but to a lesser extent, the slope is
due to diffusion resistance in gas phase (as indicated by dependence on the diffusion coefficient).

The differing results from Philadelphia and Göttingen have led to discussions, reciprocal laboratory visits, and a joint symposium at a meeting of the Federation of American Societies for Experimental Biology (FASEB, Washington, D.C., April 1990). What we could agree on was that the source of the discrepancies might be sought in the species (humans in Philadelphia vs. dog in Göttingen) and in the experimental conditions (spontaneous breathing in Philadelphia vs. artificial ventilation with prolonged, linear expiration in Göttingen). An interesting aspect of this story should be mentioned: both the Philadelphia group and the Göttingen group had difficulty getting their papers accepted for publication by a leading journal of respiratory physiology, apparently because the concepts, models, and interpretations of both groups were in disagreement with the views of influential referees. Indeed, other attractive lung models producing sloping alveolar plateaus are models with asymmetrically branching “trumpets” of different lengths leading to locally varying diffusion resistances.31,43

We have not tried to apply such models because of their complexity and a lack of...
information about the anatomy, preferring simpler models composed of a few compartments as functional analogs.

According to model calculations, the relative alveolar slopes due to incomplete diffusive mixing are expected to increase with increased breathing frequency and reduced total volume and have been found to do so during rapid shallow breathing in humans. An extreme form of rapid shallow breathing is panting in the dog, with breathing frequencies of about 5/s and tidal volumes close to anatomical dead space. Using a special technique, expirograms during panting could be obtained. They showed a very steeply rising $P_{CO_2}$ (falling $P_{O_2}$) during expiration, immediately followed by a rapid fall of $P_{CO_2}$ (rise of $P_{O_2}$) during inspiration (Fig. 12). This could be described in two ways: absence of an alveolar plateau or an extremely steeply sloping alveolar plateau, not differentiable from phase II of the normal expirogram. Moreover, the arterial $P_{CO_2}$ was much higher than the peak expired $P_{CO_2}$.

This looked very much like a well-developed stratification. But the expirograms of two intravenously infused gases of equal solubility but different diffusivity, acetylene and Freon 22, were close to identical. (Fig. 12). Moreover, the multiple breath washout kinetics of helium and SF$_6$ were very similar. This points to a relatively small role for diffusion limitation, and the prevalent role of convection, probably a “stratified ventilation” in a serial system. But unequal parallel ventilation with relatively hypoventilated areas expiring last could not be excluded. Similar results and conclusions were reached from experiments with high-frequency ventilation in anesthetized dogs, with respiratory frequencies varied from 10 to 40/s.

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**Figure 12.** Gas concentration profiles of intravenously infused acetylene ($C_2H_2$), Freon 22 (F22), and of $O_2$ and $CO_2$ during a single respiratory cycle in dogs during panting (= 5 breaths/s), plotted against respired volume (abscissa). Note reversed scale for $O_2$ as compared to other gases. After Sipinková *et al.*, 61
It is rather difficult to separate airway diffusion gradients proper (stratification) from unequal distribution effects due to inhomogeneity of ventilation and diffusion among parallel and serial airway elements.

**Epilogue**

This is a fleeting report of the main features of my meandering, erratic journey through diffusion problems in lungs. No aspect has been completely clarified, but my coworkers and I had in many cases the pleasure of experimentally verifying what we expected and, what is at first less gratifying but more fruitful in the long run, finding unexpected results. Of fundamental importance were personal contacts with scientists from other laboratories. Most of our contributions have arisen from such contacts, which gave rise to reciprocal visits and joint projects.

**Acknowledgment:** The work reported here could not have been performed without the collaboration of many coworkers whose names appear as coauthors of the publications in the reference list. I am particularly grateful to Dr. Peter Scheid (now in the Department of Physiology, University Bochum) and Dr. Michael Meyer, who participated, in many cases as principal investigators in most of the recent studies on which this report is based.

**References**


16. Haab, P., G. Duc, R. Stucki, and J. Piiper. Les échanges gazeux en hypoxie et la capacité de diffusion pour l’oxygène chez le...


