Diffusive insights: on the disagreement of Christian Bohr and August Krogh at the Centennial of the Seven Little Devils

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The year 2010 is the centennial of the publication of the “Seven Little Devils” in the predecessor of Acta Physiologica. In these seven papers, August and Marie Krogh sought to refute Christian Bohr’s theory that oxygen diffusion from the lungs to the circulation is not entirely passive but rather facilitated by a specific cellular activity substitute to secretion. The controversy is concerned with the transport of oxygen from the lungs into the bloodstream: are passive transport and diffusion capacity together sufficient to secure the oxygen supply in all circumstances or is there an additional specific (“energy consuming” or “active”) mechanism responsible for the transport of oxygen from the alveoli into the bloodstream? The present discussion purports to show that the contestants’ views were closer than the parties themselves and posterity recognized. Posterity has judged the dispute unilaterally from the Nobel laureate’s point of view, but it is evident that August Krogh’s Nobel Prize was awarded for the discovery of a cellular activity (Christian Bohr’s expression), represented by Krogh’s discovery of capillary recruitment. Christian Bohr appears to have been correct in the narrower sense that the diffusion capacity at rest is not great enough to explain the transport during work; a special mechanism intervenes and optimizes the conditions under which diffusion acts. August Krogh, of course, was right in the wider sense that the transport mechanism itself is always entirely passive.

oxygen; diffusion; pulmonary circulation

The first part (1880–1908) is enacted in German and takes place first in Leipzig, Germany, at Leipzig University’s famous Institute of Physiology, whose head is Prof. Carl Ludwig, and later in Copenhagen, Denmark, at the Physiology Institute founded by P. L. Panum and located in the courtyard behind the College of Surgeons in Bredgade in Copenhagen.

The second part (1906–1910) is enacted in English and takes place at Panum’s Institute of Physiology and in the 23rd volume of Skandinavisches Archiv für Physiologie, whose publisher is another student of Carl Ludwig, Robert Tigerstedt, a Professor of Physiology at Helsinki University in Helsinki, Finland.

The third part (1909) is enacted in German and takes place at Panum’s Institute of Physiology and in the 22nd volume of Skandinavisches Archiv für Physiologie.

The fourth part (1910–1920) is enacted in English and takes place at Panum’s Institute of Physiology and the Zoophysiological Laboratory in Ny Vestergade in Copenhagen and later at the Nobel Committee in Stockholm, Sweden, whose Chairman is J. E. Johansson, a Professor of Physiology at the Karolinska Institutet, who himself spent time with Carl Ludwig and who, as a personal friend, conducted physiological experimentation in Alfred Nobel’s villa in Paris, France. He is Tigerstedt’s pupil and successor.

The controversy concerns the transport of oxygen from the lungs into the bloodstream: are passive transport and diffusion capacity together sufficient to explain the oxygen supply in all circumstances or is there an additional specific (“energy consuming” or “active”) mechanism responsible for the transport of oxygen from the alveoli into the bloodstream through the barriers (as shown in Fig. 1)? The barriers include the alveolar epithelium, basement membrane, capillary endothelium, a layer of plasma in the capillaries, erythrocyte membranes, and the binding of oxygen to hemoglobin.

There are two objectives to the reenactment of the debate. The main purpose is to show that the contestants’ views were closer than the parties themselves and posterity recognized. Posterity has judged the dispute unilaterally from the Nobel laureate’s point of view, but it is evident that August Krogh’s Nobel Prize was awarded for the discovery of a cellular activity (Christian Bohr’s expression), represented by Krogh’s discovery of capillary recruitment. Christian Bohr appears to have been correct in the narrower sense that the diffusion capacity at rest is not great enough to explain the transport during work; a special mechanism intervenes and optimizes the conditions under which diffusion acts. August Krogh, of course, was right in the wider sense that the transport mechanism of oxygen itself is always entirely passive.

The second purpose is to examine the nature of a scientific controversy. The disagreement on oxygen diffusion is exemplary because it possesses several elements that commonly...
hinder a comprehensive settlement. Here is the junior scientist working opposite the senior celebrity; here is the patrician sharing the laboratory with the austere provincial; here is the scientific paragon of Copenhagen University, the country’s oldest (and at that time only) university, in close contact with German scientists, mentoring a second-generation refugee from Schleswig-Holstein with a distrust of things German; here is the theoretician interacting with the genius of experimentation. Finally, there is the exchange of physiological reasoning with direct investigation in the dialectical dance around nature’s secrets, where hypothesis and experiment go hand in hand.

First Act (1880 –1908)

In which Christian Bohr mistakenly concludes that the oxygen tension in arterial blood is higher than in the alveolar air, such that transport must be active and subject to a special “specific activity.”

A new physiological science arose in the second half of the 19th century. It was based on the experimental exploration of bodily functions, and part of the responsibility for this development rested with the eminent French physiologist Claude Bernard. Bernard had visits in the years of 1852–1853 with the Danish physiologist Peter Ludwig Panum, who brought the principles of this new science, first to Kiel, Germany, and later, after 1864, to Copenhagen, Denmark. It was there, in the courtyard behind the Surgical College in Bredgade, that Panum built a new physiology laboratory with facilities specifically designed for animal experiments and that Christian Bohr (Fig. 2) began his physiological experiments in the late 1870s (10).

Panum was Christian Bohr’s mentor. Bohr’s earliest work focused on nutrients and their properties. The results were presented in a MD Thesis on the topic of milk (3). Bohr then went to Leipzig, Germany, where he spent several periods in the years of 1880–1885 working with the patriarch of German physiology, Carl Ludwig, at Leipzig University’s new Physiological Institute in Talstrasse (29). There, Bohr immersed himself in attempts to solve a novel scientific problem, inspired by Carl Ludwig himself, focused on the issues of the transport of oxygen from inhaled air into the bloodstream (6):

It was my intention by means of these tests to resolve whether the continuous migration of the gases through the tissue of the lungs can be explained by a simple diffusion process, or whether the tissue of the lung is involved in a manner that in a peculiar way resembles a glandular secretion process.

In this issue, Carl Ludwig held an unfortunate minority position, which would prove to be detrimental to the conclusion of Bohr’s scientific work: unlike the majority of physiologists, Ludwig believed that the passive diffusion of oxygen is inadequate in the lungs, and he therefore claimed that the transport of oxygen in whole or in part must be subject to the same process that is responsible for the known secretion of oxygen from a lower to a higher pressure of oxygen in the swim bladder of fish.

In the discussion of this process, two key elements must be taken into account, including the oxygen tensions on the two sides of the transport barrier and the barrier’s transport capacity (Fig. 1). If transport occurs against a pressure gradient, it cannot take place without energy expenditure. On the other hand, even in the presence of a gradient in the right direction, the transport may still depend on a supply of energy if the capacity of the passive transport does not match the total transport of oxygen. A higher oxygen tension in the bloodstream than in the air of the lungs certainly remains the most direct evidence of an active transport, but the hypothesis of an
active transport of oxygen in the lungs cannot be rejected outright only because the oxygen tensions are higher in alveolar air than in the bloodstream. In that case, the accuracy of the claim of active contributions to the oxygen transport must rest on different and more complex considerations.

In short, the hypothesis is supported by evidence that oxygen tensions are higher in the bloodstream, but it is not rejected automatically by evidence of the presence of higher oxygen tensions in alveolar air. In the latter case, the conclusion depends on the magnitude of the diffusion capacity under the applicable conditions.

The alternatives raise several experimental and physiological questions: Where in the bloodstream must the oxygen tension be measured to test the hypothesis, when the oxygen tension increases during the passage of the blood through the pulmonary capillaries? How are oxygen tensions determined simultaneously in the bloodstream and alveolar air, and how is the pulmonary diffusion capacity determined under different circumstances? In this context, what is meant by the active transport of oxygen? As a highly diffusible gas, oxygen is probably not transported in the classic sense of a transport protein giving oxygen molecules access to a channel or corridor through the cell membrane, where the molecule typically accompanies or is exchanged with another substance with a positive concentration gradient that is maintained by ATP-dissolving enzymatic processes. Bohr had this comment in his most famous and most quoted work (6):

The influence exerted by the lung tissue on the gas pressures in the blood and lung air can be understood either as direct uptake of oxygen and release of carbon dioxide by the epithelial cells of the alveolar wall, or as an influence on the blood that changes the tensions of the gas at the moment in which it leaves the lungs, keeping in mind of course that neither of these two modes of action excludes the other.

In the case of oxygen, Bohr had several options: 1) an energy-consuming release of oxygen on the proximal side of the diffusion barrier, 2) an energy-consuming disposal of oxygen on the distal side of the diffusion barrier, or 3) an energy-consuming maintenance of a sufficiently high permeability of the barrier by shortened diffusion distances or expansion of diffusion areas. The complicated nature of the proof contributed to the subsequent complication of the dispute, because the numerous possibilities for an active intervention in the diffusion of oxygen made it very difficult in practice to deny that an active intervention ever occurs, even when the tension is higher in the alveolar air, but this was Krogh’s basic claim.

Bohr was appointed to a lectureship in Copenhagen in 1886 and to Panum’s Chair of Physiology a few years later. Experiments made first in Leipzig and later in Copenhagen suggested that the oxygen tension is higher in arterial blood than in a sample of air that Bohr knew as “bifurcation air,” which is air sampled at the bifurcation of the trachea. To make these measurements, Bohr used an analytic appliance originally invented by Carl Ludwig, but the measured values had considerable variability, as shown in Fig. 3. The measured difference was so small that the significance yielded by a Wilcoxon test gave a probability of barely less than 0.05. However, with these measurements, Bohr held it to be confirmed that the lung tissue plays an active role in the transport of gases, but he acknowledged that this conclusion differed from the position of the majority of physiologists (6):

Fig. 3. Average oxygen tensions in bifurcation air and arterial blood, as measured in dogs with Ludwig’s gas analyzer. The difference is barely significant ($P = 0.0494$) (6).

Overall, my studies now show that the lung tissue plays an active role in the transport of gases, such that the function of the lung is similar to that of a gland. That conclusion, at least with respect to oxygen, in no way is consistent with the view which has hitherto been regarded as predominant in the field of physiology. On the other hand, it appears to me not to fall into conflict with the experimental results that have hitherto been published.

Bohr’s conclusions were nonetheless supported by Haldane and Smith (13) for human beings, but with highly error-prone measurements.

Second Act (1906–1910)

In which August Krogh shows that the oxygen tension is always higher in the lungs than in the arterial blood and in which, in the first five of seven “Little Devils,” he finds that oxygen transport is always passive.

In 1885, Bohr showed that oxygen binds to hemoglobin solutions (4), and, in 1890, he further showed that carbon dioxide inhibits this binding (5). August Krogh began his work with Christian Bohr in 1897, and he became Bohr’s fellow and teaching assistant in 1899. Their work together primarily focused on the gas exchange between the environment and the organism. In 1904, Bohr, Hasselbalch, and Krogh published the definitive work on the effect of carbon dioxide tensions on the binding of oxygen to hemoglobin, known as the Bohr effect (7, 12, 32). It is evident that the success of the work largely rested on the use of a new precision instrument for measuring gas tensions, the microtonometer, which was designed by Krogh.

This precision instrument became the method of choice for the retesting of the hypothesis of an active factor in the transport of oxygen from the cavities of the lungs to the bloodstream. In 1906, Krogh began a series of attempts at replication in collaboration with the physician Marie Jørgensen, whom he had married in 1905 (Fig. 4), but to the researchers’ surprise the results pointed to purely passive transport (24):

The experiments described in the present paper were undertaken in the summer of 1906 with a view to investigate in greater detail than had hitherto been possible the gas-secretion...
supposed to take place in the lungs. The results were contrary to our expectations, and the publication has been put off until now, in order that the problem might be approached in some other ways and conclusions, representing, if possible, some sort of finality in the protracted discussion of this most complicated subject, arrived at.

As shown in Fig. 5, the tension difference between bifurcation and alveolar air samples, on one side, and the arterial blood, on the other side, was both positive and highly significant. However, the findings were not published until 1910. This happened in the first of the articles nicknamed the “Little Devils” by August Krogh himself, as an expression of the weight of this evidence against active transport of oxygen that he and his wife presented. The seven papers filled a total of 100 consecutive pages in the 23rd volume of *Skandinavisches Archiv für Physiologie* (p. 179–278) in 1910. In the first five Little Devil papers, the authors criticized and discussed studies conducted before 1909, whereas the last two Little Devil papers more directly served as rebuttals to the defense of the theory that Bohr published in 1909.

The delayed publication of the first five Little Devil papers reflected Krogh’s desire to reach an understanding in the thorny matter of a fundamental disagreement with the laboratory’s director, who was also his mentor, but, for more or less obscure reasons, such an understanding was never reached. It is unlikely that Bohr directly would have prohibited Krogh’s publication of the new results, but Krogh’s daughter, Bodil Schmidt-Nielsen, believes that the mood in the laboratory became increasingly dark, as Bohr apparently avoided debate (31). Although Bohr was known to be “sensitive, kind, and helpful,” it was also said that he “shied away from conflict.” This reluctance, however, does not fully explain the widespread perception at the time that Bohr “hardly ever engaged in scientific controversy” (2). Ignorance of the dispute with Krogh was expressed by a contemporary Professor of Pharmacology, Johannes Bock, and by others who held similarly idyllic views of Bohr’s personality. In contrast, readers of the seven Little Devil papers would not have failed to notice how completely the Kroghs dismantled the foundations of Bohr’s major scientific claim and how effectively the parties had avoided a more direct exchange of opinions.

Bohr’s relations with Carl Ludwig, the father of the physiological revolution of the 19th century (2), whom Bohr called “my beloved teacher,” probably played a role as well. Carl Ludwig and his generation had distanced themselves from Johannes Müller’s vitalism, and it is a major paradox that Bohr, Ludwig’s most highly praised pupil, now stood accused of cryptovitalistic sympathies wrapped in concepts such as “specific cell activity.” Vitalism is the claim that life depends on mechanisms that are fundamentally different from those that explain the properties of nonliving matter,
and Bohr’s notion of a “specific property” of pulmonary oxygen transport was seen by some observers as suggestive of vitalism. Another factor may have played role: did the parties carefully read their respective papers? Bohr wrote in French and German, whereas Krogh wrote most of his works in English. Krogh’s parents had moved north from the former Danish dukies of Slesvig and Holsten South of Jutland to avoid the Prussian occupation in 1864. Schmidt-Nielsen speculates that August Krogh developed a distrust of things German (31), to the extent that he, on some occasions, resisted writing papers in German, although at least a 10th of his publications used this language. His favored medium was certainly English.

By means of the microtonometer invented by August Krogh, in the first of the Little Devil papers, Marie and August Krogh showed that the oxygen tension in the bloodstream of rabbits is always lower than in the alveoli (Fig. 5) and that the magnitude of the difference is always a few percent or more of the atmospheric pressure, equivalent to 10–30 mmHg (24):

The tension of carbon dioxide in the arterial blood of the rabbit always follows closely the corresponding alveolar tension. The oxygen-tension of the blood is always lower than the alveolar oxygen tension, and the difference is generally 1–2 or in some cases even 3–4 per cent of the atmospheric pressure.

In the second Little Devil paper, Krogh (18) ruled out the possibility that the oxygen measurements could be affected by any conversion of oxygen to carbon dioxide in the bloodstream:

Metabolic processes, involving the consumption of from 10 to 50 ccm of oxygen, per kg and hour, take place in the arterial blood of rabbits at body-temperature. Their intensity is not sufficient to affect appreciably the determinations of CO\textsubscript{2} and O\textsubscript{2}-tensions by means of my tonometers.

Experiments by Vilhelm Maar, later Professor of History of Medicine at the University of Copenhagen, and Valdemar Henriques, Bohr’s successor as Professor of Physiology, suggested that vagal activity could promote secretion in the lungs. The results implied that oxygen transport in the lungs could be characterized as a form of secretion (19):

Maar has published experiments on the influence of the nervous system upon the gas-secretion in the lungs. When the vagus on one side was divided, the absorption of oxygen and to a less extent also the elimination of carbon dioxide in the corresponding lung was found to rise considerably, while a compensating fall generally occurred in the other. Maar concluded…that the nerves were truly secretory in character…

However, in the third Little Devil paper, Krogh’s experiment in turtles, performed with the help of Prof. Holger Møllgaard (who elected not to be a coauthor), showed that the vagus on the contrary acts as a vasopressor. Its severance lowered the air exchange by increasing blood flow and not by inhibiting active oxygen transport (19):

1. In the tortoise the vagus is a vasoconstrictor and not a gas-secretory nerve for the lung on the same side. Its section causes augmentation of the blood-flow through the lung. 2. The experiments detailed lend no support to the theory of gas-secretion in the lungs, but prove that the gas-exchange may be brought about solely by diffusion.

The oxygen tensions reported by Krogh in the first Little Devil paper also differed from previous calculations for humans, published by Haldane and Smith (13). In the fourth Little Devil paper, Krogh showed that oxygen tensions cannot be accurately calculated by means of carbon monoxide, because the relationship between the tensions of oxygen and carbon monoxide fails to observe the constant relation to oxygen’s binding to hemoglobin that Haldane and Smith had assumed (20):

The CO-tension of the blood is assumed by Haldane and Smith to be equal to the percentage of CO in the air breathed, provided the time has been sufficiently long for equilibrium to be established.

In the fifth Little Devil paper, Krogh (21) showed that the solubilities of oxygen and carbon monoxide in water are greater than those measured by Bohr and thus could be no impediment to the passive diffusion of oxygen. Krogh’s microtonometric measurements confirmed that the rates of “invasion” were much faster than those measured by Bohr (21):

As I had found by occasional microtonometric experiments that the invasion of oxygen must be much more rapid than was indicated by Bohr’s figure, I resolved to utilize my microtonometric methods for a definite series of experiments with the redetermination of this constant in view. It must be mentioned that Bohr has now repudiated the invasion coefficients given in Nagel’s Handbuch and the conclusions based upon them.

The coefficient of invasion must have a minimum value because it represents the theoretical upper limit of oxygen diffusion. According to Krogh, the magnitude of the coefficient of 90 ml·min\textsuperscript{-1}·mmHg\textsuperscript{-1} (given in the same units as the diffusion of oxygen) is more than adequate for the diffusion measured in actual practice (21):

The magnitude of the invasion-coefficient does not…put any difficulty in the way of the hypothesis that the gas-exchange in the lungs takes place by diffusion.

It was no longer possible to maintain the hypothesis of an active transport of oxygen in the lungs by reference to measurements of the tension difference between the lungs and the bloodstream. The reasoning now had to focus on the adequacy of the diffusion capacity. But the parties did not communicate, and only later did Krogh elaborate on the realization that the dispute had two themes: when Krogh focused on the diffusion mechanism, Bohr in reality but unknowingly addressed the question of the mechanism responsible for the adjustment of diffusion capacity.

Third Act (1909)

In which Christian Bohr publishes the carbon monoxide method of measuring the oxygen diffusion capacity of the lungs and finds that the diffusion capacity at rest is insufficient to explain the oxygen transport in the lungs during exercise, thus identifying a part of the transport as indicative of “specific cell activity.”

August and Marie Krogh postponed the publication of the results until 1910, but August Krogh’s hope in this way of persuading his teacher to participate in discussion was not fulfilled. The new and unexpected results apparently did not convince Bohr of the passive diffusion of oxygen. One explanation may be that the term “specific activity” was poorly defined. The phenomenon of capillary recruitment played no
role in the thinking of either Bohr or Krogh, who at this time had no idea of the importance of capillary density for the oxygen diffusion capacity. Capillary recruitment can mimic an active process, particularly when the diffusion coefficient a priori is regarded as constant, but there was no discussion of the mechanism of specific activity that Bohr considered, particularly with regard to possible alternatives to secretion.

In 1909, Christian Bohr published the magnum opus that would become his scientific testament. In this work, he once again stated that the lungs play an active role in oxygen uptake (8):

Since 1888, results reported from this laboratory have established that the lungs play an active role in gas absorption. This fact is obviously of far-reaching importance for the understanding of respiratory processes in general, and especially for the understanding of the means employed by the organism for its regulation.

This is a very peculiar statement under the circumstances, as the work was evidently influenced by the Kroghs’ experimental results to the contrary, although these results had not yet published. Therefore, the main purpose of the work may have been for Bohr to argue that the oxygen diffusion coefficient is too low to drive a passive oxygen transport in conditions where oxygen consumption is increased, for example, by muscular activity (8). Of course, after the Kroghs’ measurements there could no longer be any doubt that the oxygen tension in alveolar air is higher than in the pulmonary venous blood of the lungs. Bohr therefore raised the more difficult question of the adequacy of the passive diffusion of oxygen, but without mentioning the Kroghs’ results:

The magnitude of the actual gas diffusion through the alveolar wall remains uncertain, despite several investigations directed towards this issue. Yet, only with this information is it possible to clarify the quantitative extent of the specific activity of lung tissue during the changing situations of the organism.

The essence of Bohr’s review from 1909 is the famous mathematical derivation, which led to the familiar formula for concentration and tension profiles in capillaries in terms of measurable physiological quantities. The treatment is characteristic of this era of great equations. It is based on the fact that oxygen tension gradually increases in the blood during its passage through the pulmonary capillaries, as shown in Fig. 6. The magnitude of this increase, and therefore also the kinetics underlying the expression for the average tension, are both determined by the pressure-dependent diffusion in each capillary element, which is a simple passive diffusion of oxygen from a higher to a lower tension. It applies both to each diminutive capillary element and to the capillary bed as a whole:

\[ \dot{V}_O = DlO_2(\text{P}_{O_2} - \text{P}_{CO_2}) \]  

where \( \dot{V}_O \) is the volume of oxygen transported per unit time; \( DlO_2 \) is the “hydraulic” conductivity of the diffusion barrier, commonly known as the pulmonary diffusion capacity in North America but in Europe called the “transfer factor” [which is written as \( TlO_2 \) (\( TlCO \) for carbon monoxide)]; and \( (\text{P}_{O_2} - \text{P}_{CO_2}) \) is the average pressure gradient, or tension difference, between alveoli and capillaries. The present text adopts the term “diffusion coefficient,” which is the convention used by both Bohr and the Kroghs, although it is now obsolete.

To assess the adequacy of passive diffusion, it is necessary to know both the diffusion coefficient and pressure gradient. Because the actual oxygen transport determines, as well as depends on, the concentration difference between pulmonary venous blood and pulmonary arterial blood, the apparent diffusion coefficient depends on the oxygen consumption and can therefore be calculated by equating the two expressions for the net transport of oxygen:

\[ DlO_2 = \frac{F}{\alpha} \left( \frac{C_v - C_a}{\text{P}_{O_2} - \text{P}_{CO_2}} \right) \]  

where F is the blood flow rate and \( C_v \) and \( C_a \) are the pulmonary venous and arterial concentrations of oxygen, respectively. As the equation applies to every diminutive capillary element, it can be transformed into an integral equation, which summarizes the overall impact of transport during oxygen’s passage through the capillary bed:

\[ \frac{DlO_2}{F} = \frac{C_v}{\alpha} \left[ \frac{dC}{\text{P}_{\alpha} - \text{P}(C)} \right] \]  

where \( P_\alpha \) is the alveolar tension and \( P(C) \) is the oxygen pressure in a capillary segment when the oxygen concentration is C. This equation has the famous solution known as Bohr’s Equation:

\[ \frac{DlO_2}{F} = -\alpha \ln \left( \frac{\text{P}_{\alpha} - \text{P}_V}{\text{P}_V - \text{P}_a} \right) \]  

where \( \alpha \) is the ratio of \( C/P \) at steady state, \( P_\alpha \) is the oxygen tension in pulmonary arteries, and \( P_V \) is the oxygen tension in pulmonary veins. Bohr immediately realized that the equation is not a reflection of the real diffusion coefficient because it is derived from the actually measured transport values, regardless of the transport mechanism. In this sense, the diffusion coefficient of this equation is simply a descriptive term for the apparent barrier permeability. However, with a minor modifi-
cation, the equation can be used to calculate the pressure gradient (8):

$$\Delta P = \frac{C_v - C_v}{P_a - P_v} \alpha \ln \left( \frac{P_a}{P_v} \right)$$

in which the accuracy is highly dependent on the difference between the oxygen tensions in alveoli and pulmonary veins, which, in turn, depends on the degree of equilibration, as shown in Fig. 6. In practice, therefore, the equation tends to overestimate the average pressure difference, as shown in Fig. 7.

The pressure difference is only one half of the argument. As for the second half, the real diffusion coefficient of oxygen, Bohr reasoned that it can be determined with carbon monoxide, which binds to hemoglobin to such an extent that the tension in the plasma can be assumed always to be vanishingly low (8):

$$... \text{the tiny amounts of carbon monoxide absorbed into the}$$
$$\text{blood exercise no measurable tension:...This means that the}$$
$$\text{pressure driving the diffusion, i.e., the mean pressure differ-}$$
$$\text{ence, in that case equals the tension of the carbon monoxide}$$
$$\text{in the alveolar air.}$$

The expression of oxygen’s diffusion coefficient can then be rewritten for use with carbon monoxide by ignoring the carbon monoxide tension in the plasma and the loss of carbon monoxide from hemoglobin:

$$D_{I,CO} = \left[ \frac{C_v(T)}{P_a} \right] \frac{V_b}{T}$$

where $D_{I,CO}$ is the diffusion coefficient for carbon monoxide and $V_b/T$ is the blood flow calculated as the volume of a blood sample ($V_b$) collected in the time ($T$) during which the accumulation [$C_v(T)$] has taken place.

The diffusion coefficient of oxygen can then be calculated when the ratio between the diffusion coefficients is constant, as previously believed to be the case. Bohr calculated the ratio to be 1.23, but the ratio, in fact, is not constant but depends on oxygen’s binding to hemoglobin, and thus on oxygen’s tension or concentration, and, in reality, is much higher [a ratio of 1.71 (16)] under conditions of normal oxygen tension. Bohr’s original version of the carbon monoxide method comes closest to what is today called “the steady-state method.” In the calculation, Bohr used measurements of carbon monoxide accumulation reported by Haldane and Smith (14), and the average value of four measurements of carbon monoxide was $14.1 \pm 0.9$ ml-min$^{-1}$-mmHg$^{-1}$ (mean $\pm$ SE) (not 13.1, as cited by August Krogh), or approximately half of the current value.

It was now possible for Bohr to attempt to calculate the total oxygen transport as the product of two numbers, the (constant) pulmonary diffusion coefficient and the average pressure difference, both of oxygen, for different degrees of muscular work performed by humans. The values of muscular work were those reported by Zuntz (34), who also published the values of pressures that formed the basis of the calculation of pressure differences. In contrast, as mentioned above, the diffusion coefficient was calculated from values published by Haldane and Smith (14). The result is shown in Fig. 8. The comparison assured Bohr that the oxygen consumption increases with the intensity of work but that the oxygen transport calculated from a constant diffusion coefficient not only remained relatively unaffected by the exertion but also proved to be less than the actual oxygen consumption when the rates of consumption are high (8):

$$...\text{Assuming a diffusion of the magnitude established here, it is}$$
$$\text{evident that the uptake of oxygen has happened with the}$$
$$\text{collaboration of the pulmonary cells, and it raises the question}$$
$$\text{that also concerns the secretion of carbon dioxide, of whether}$$
$$\text{the specific cellular activity implies an adjustment of the}$$
$$\text{oxygen tension to a value that enables the diffusion that}$$
$$\text{actually takes place, or whether an actual conveyance of oxy-}$$
$$\text{gen through the alveolar wall is the consequence.}$$

Evidently the transport of oxygen matches the demand for oxygen. One of the explanations considered by Bohr was an activity of cells that maintains oxygen tensions at levels con-

Fig. 7. Average pressure differences between alveoli and pulmonary capillaries calculated by Bohr (8) for different degrees of oxygenation of pulmonary arterial blood (called venous blood by Bohr) from measurements by Zuntz (34) at different degrees of walking exercise. Lower oxygen concentrations in pulmonary arterial blood are associated with greater pressure differences, but the pressure differences vary somewhat less than the oxygen content of arterial blood. The average pressure differences can be read for the different combinations of oxygen concentration in pulmonary arterial and venous blood. At greater oxygen consumptions, the oxygen contents decline in blood returning to the lungs, and the tension differences between alveoli and capillaries rise. Note that Bohr accidentally reversed the normal use of the terms for pulmonary arteries and veins, according to which pulmonary arteries carry deoxygenated blood from the heart and pulmonary veins carry oxygenated blood to the heart (8).
sistent with the required oxygen transport. Expansion of the diffusion area is one such activity, although it was not so considered by Bohr. Whether this mechanism is called a specific cell activity or an increase in the apparent diffusion coefficient depends on the researcher’s philosophical bend. Real diffusion coefficients are not affected by much more than the temperature, and Bohr can be said to have been entitled to think of the transport facilitation as a kind of work. The difference between the calculated passive transport and the measured oxygen consumption is shown in Fig. 9.

Bohr realized that a particular or specific activity that goes beyond passive diffusion modifies the diffusion capacity when oxygen demand increases (8):

Both the elimination of carbon dioxide and the uptake of oxygen must therefore be regarded as secretory processes regulated by the activity of the cells of the lungs and not determined entirely by the composition of the alveolar air or the composition of the blood flowing to the lungs.

The theory of the specific cell activity now rested on the details of quantitative physiological reasoning. August Krogh, however, did not accept the underlying numerical values or the assumptions on which they rested. Krogh had probably long known of Bohr’s calculations before he met them in the printed version, but he did not find the intermixture of values from different authors under different experimental conditions compelling.

On experimental grounds, the arguments presented in the seven Little Devil papers disputed Bohr’s quantitative reasoning. The effect was striking, and the major review of 1909 did, in fact, become Bohr’s scientific testament: on the morning of Friday, February 3, 1911, less than 2 mo after the issue of the last Little Devil paper, Christian Bohr was found dead at his desk. The day before, he had worked until 10 PM. That day, Bohr’s son, Niels, who received the Noble Prize in Physics in 1922, had announced that he had completed the thesis that his father had greatly worried about. The procrastination had been a source of concern in the family, so the father had sent Niels to a cottage in North Zealand with the order to not to come home without a completed thesis. Niels returned home with the finished thesis the day before his father’s death, on February 2, 1911.

Fourth Act (1910–1920)

In which Marie Krogh uses the carbon monoxide method of measuring the pulmonary oxygen diffusion capacity and August Krogh is awarded the Nobel Prize for having revealed that the oxygen supply to muscles is regulated by a specific cellular activity known as capillary recruitment.

In the first five Little Devil papers, Marie and August Krogh summarized the results of experiments conducted before the publication of Bohr’s major review of 1909. In the last two Little Devil papers, the authors distanced themselves more argumentatively from the results of Bohr’s quantitative reasoning, but the chronology is somewhat uncertain because of the speedy publication practice of physiological periodicals in those days: Bohr’s review was submitted in April 1909, and already in November and December 1909, Marie and August Krogh handed over their seven submissions to the same journal.

In the sixth Little Devil paper, the authors now claimed that Bohr’s calculation of a diffusion coefficient with the use of Haldane and Smith’s values of carbon monoxide uptake (14) was based on arbitrary assumptions (19):

Bohr himself has determined in a very elegant manner the tension-differences which will govern the absorption of oxygen by diffusion, but which we could not accept as convincing diffusion-constants, based as they then were…exclusively during muscular rest and upon assumptions regarding some of the necessary constants…which appeared arbitrary…

With new measurements from the carbon monoxide method applied to humans (Fig. 10), the Kroghs showed that the diffusion coefficient actually increases with muscular work. For these measurements, the Kroghs used a new version of the carbon monoxide method, now known as the “single-breath method” (19):

Bohr (1909)

Fig. 9. Bohr’s calculation of the necessary increase of diffusion capacities for carbon monoxide and oxygen during work (8).
The influential American circulation physiologist Seymour S. Kety praised Marie Krogh’s use of the carbon monoxide method, but he did not mention Bohr’s essential contribution. Particularly, Kety did not believe that Bohr had shown sufficient insight into the equilibration of oxygen between alveoli and capillaries (17):

What Bohr did not realize, however, and what even Murray and Morgan failed to anticipate (since they stopped their graphs short of the most critical region) is that normally pulmonary blood comes very close to absolute equilibrium with alveolar air before it leaves the lung.

In contrast, on the subject of Marie Krogh’s insight, Kety stated that (17):

Marie Krogh (2) brilliantly side-stepped the difficulties inherent in the Bohr equation and measured the diffusion coefficient of the lungs by means of CO. She reasoned that the capacity of blood for this gas was so great that small amounts of it dissolved in blood would exert a negligible tension…

Yet, it appears that Kety misunderstood Bohr’s calculations: Bohr used his equation to calculate the pressure gradient and his carbon monoxide method to calculate the diffusion coefficient. The two calculations were quite independent.

Kety’s influential publication drew the world’s attention to August and Marie Krogh’s single-breath version of the carbon monoxide method. The point of departure of Marie Krogh’s Doctoral Thesis from 1915 was August Krogh’s detection of a tension difference between alveolar air and pulmonary venous blood. Marie Krogh reopened the issue of the adequacy of oxygen’s diffusion coefficient, which had been the cornerstone of Bohr’s review in 1909 (26):

...If on the other hand the tension of oxygen in the blood is always lower than in the air, as found by A. Krogh and the writer (1), and also by Hartridge (2), the second question arises: Is diffusion quantitatively sufficient to explain the intakes of oxygen measured under the most adverse conditions and especially during muscular work at low oxygen pressures?

And she summarized the principles underlying the use of the carbon monoxide method of determination of the pulmonary diffusion coefficient:

The principle, according to which the diffusion can be estimated (5) and has been estimated by A. Krogh and the writer (1), takes as its starting point the assumption that an essentially indifferent gas, like carbon monoxide, must pass through the alveolar epithelium by diffusion alone—an assumption which has never been questioned by anybody…

Here, Ref. 5 refers to findings by Bohr. Using the single-breath version of Bohr’s method, Marie Krogh reported the pulmonary diffusion coefficient of different groups of subjects. As shown in Fig. 11, it was only somewhat higher than values previously published by Christian Bohr and August Krogh. Marie Krogh reaffirmed the position that oxygen’s diffusion coefficient rises during exercise, as shown in Fig. 12.

In the end, Marie Krogh asked whether the diffusion coefficient may be related to changes in blood flow that affect the time available for air exchange (26):

In many cases, namely during rest and with a constant oxygen consumption in the tissues, the $O_2$ absorption taking place in the lungs during the diffusion experiment would be an index of the changes in blood flow as pointed out by A. Krogh and Lindhard...
This effect is not passive, as the heart does the work required to reduce the average oxygen tension in pulmonary capillaries, and it does not explain the increase of the diffusion coefficient when it is measured with the steady-state version of the carbon monoxide method.

The explanation had to wait several more years. Five years later, August Krogh was awarded the Nobel Prize. Before 1920, Krogh’s work was not well known, even among physiologists, but he won the Nobel Prize after as few as three nominations. The first nomination arrived in 1919, and the Chairman of the Nobel Committee for Physiology or Medicine, Prof. J. E. Johansson, wrote a cautious but positive report on Krogh’s work. After a visit to Krogh, he wrote a new and totally supportive report, and he nominated Krogh for the prize the following year. The award was given for Krogh’s detection of the mechanism of regulation of oxygen supply to muscles at rest and during exercise, which can only be explained by capillary recruitment (23):

Through comparing various researches, some by Verzár, some by Lindhard and myself, into the utilization of oxygen by muscles and its dependence, partly on the oxygen pressure in the blood, partly on muscular activity, I came, in 1915, to the conclusion that, either there must be very serious errors in the researches concerned, or the circulation in the capillaries must otherwise occur in an entirely different way from that which one normally visualized.

The supply of oxygen is maintained when a specific cellular activity increases the diffusion capacity in parallel with the increased oxygen demand, as Krogh concluded in his Nobel Lecture in 1920:

The conclusion to which I was forced, in order to reconcile the results of earlier researches, was this, that in a resting muscle the blood could not flow through all of the capillaries, but only through a small percentage of them, which, further, must be more or less regularly distributed throughout the muscle, as is shown in Fig. 2, which is a wholly schematic representation of capillaries in a cross-section of muscle.

Figure 2 mentioned in the quote above is the schematic representation of muscle capillaries, shown here in Fig. 13. Capillaries take turns to open, depending on the need for oxygen. The greater the need, the more capillaries remain open at the same time, as shown in Fig. 14 (23).

And so the argument came full circle. Diffusion is passive, but additional factors help regulate the diffusion capacity under conditions of varying oxygen demand. In practice this means...
that the pressure difference between capillaries and tissues is kept fairly constant. Regrettably, the contestants reached no agreement while Bohr was alive, but August Krogh sent his mentor a grateful thought when he spoke at the banquet in Stockholm and declared: “I know that a substantial part of the honor bestowed on me at this time belongs to my outstanding teacher Christian Bohr.”

Epilogue

Is capillary recruitment an “active” and “specific” property in the sense that Bohr intended? In 1984, Burggren and Moalli (9) reported evidence of what they called “‘active’ regulation of cutaneous gas exchange by capillary recruitment in amphibians,” thus hinting at the conclusion that capillary recruitment represents an active form of diffusion facilitation. They contrasted this evidence to the then prevailing view that cutaneous respiration in amphibians is “poorly regulated” and “passive.” Since the system would then be diffusion limited, and since the partial pressure of the respiratory medium would be comparatively constant over short periods of time, they argued that cutaneous gas exchange could be increased only by adjustments in the partial pressure of blood in the capillaries. This debate, of course, is reminiscent of the disagreement that divided Bohr and Krogh 75 yr earlier.

Now, 100 yr later, the facts are that the lung-diffusing capacities for carbon monoxide, nitric oxide, and oxygen, the membrane-diffusing capacity for carbon monoxide, and the capillary volume all increase linearly with cardiac output from rest to peak exercise, without ever reaching a maximum. The number of perfused alveolar capillaries increases with the perfusion pressure, and the recruitment of diffusing capacities is due to several mechanisms, including unfolding and distension of alveolar septa, opening and distension of capillaries, increased capillary hematocrit, and more homogeneous distribution of erythrocytes in capillaries (15).

The works of Bohr are still cited, as listed in current citation indexes. Thus, Bohr (6) has been cited more than 173 times since 1945. References to works from the end of the 19th century and the beginning of the 20th century can be difficult to ascertain, and it is not unheard of that authors borrow from other writers or guess at the pagination, but the trail can be followed in citation indexes. About 20% of the references are wrong, mostly those that refer to the name of the journal, where citations vary from “Skandinavisches Arch,” “Arch Physiol,” and “Skand Arch J Phy” to “Lungenathmung Skand.” Other errors include the volume number (“62”), page numbers (p. 248, 263, 68, or 2236), and year (1890 or 1981?!).

The Bohr effect (7) has been cited more than 355 times since 1945, but most of the references are incorrect. Thus, 298 citations refer to the journal as “Scand Arch Physiol” rather than the correct “Skand Arch Physiol,” which perhaps is a reflection of the number of authors who actually read the work they cite. Others refer to the wrong volume (vol. 6, 15, 17, or 18), wrong pages (p. 41, 104, 401, or 409), or wrong year (1892, 1905, or 1907).

Bohr’s scientific testament (8) has been cited more than 189 times since 1945. Most of the references are correct, which perhaps shows that it is of genuine interest to the many authors who refer to this particular work. Again, the journal name causes trouble, and “Scand Arch Physiol,” “Arch F Physiol,” and “Scand Arch Phys” occur interchangeably. Nobody has mistaken the volume number, but the page numbers (p. 211, 240, 251, or 261) and year (1900) are often wrong.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

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