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Larsen, Erik Hviid; Hoffmann, Else; Hedrick, Michael S.; Wang, Tobias

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August Krogh’s contribution to the rise of physiology during the first half of the 20th century

Erik Hviid Larsen, Else Hoffmann, Michael S. Hedrick, Tobias Wang

Abstract

August Krogh (1874–1949) was amongst the most influential physiologists in the first part of the 20th century. This was an era when physiology emerged as a quantitative research field and when many of the current physiological disciplines were defined; Krogh can rightfully be viewed as having introduced comparative physiology, epithelial transport and – together with Johannes Lindhard – exercise physiology as independent disciplines. With a unique ability to design and construct equipment, Krogh could address novel questions in both human and animal physiology with unprecedented precision. Krogh would characteristically focus on a given physiological problem over a couple of years, delineate the focal mechanisms, provide a solution to the major problems, and then move onto new academic ground. For each of his major research areas (respiratory gas exchange, capillary function, osmoregulation), he wrote comprehensive books or monographs that remain important resources for scholars today, and he engaged in the writing of physiology textbooks for the Danish high school. Krogh’s research appears to have been driven by curiosity to understand how animals (including humans) work, but he did not hesitate to apply his insight to societal and clinical problems throughout his long academic career.

1. Introduction

August Krogh was born in 1874 in Grenå, a small provincial town in Jutland with around 2000 inhabitants, and quickly gained a genuine fascination with natural history that was stimulated by William Sørensen (1848–1916), an influential zoologist, maverick, and friend of the family. Krogh was also very interested in technology and read books on mechanical and technical inventions that he replicated by building various equipment. He finished high school in Aarhus in 1893 and moved to Copenhagen for the medical preparatory exam, but decided to pursue his passion in zoology by enrolling for a magisterkonferens, the equivalent of a MSc, in 1895. On Sørensen’s advice, Krogh started to attend lectures in physiology by the eminent Christian Bohr (1855–1911) in the fall of 1895, and the first lecture concerned blood gas transport, including the mechanisms and quantitative methods. His choice of respiratory processes as his primary field of research owes to the precision with which O\textsubscript{2} and CO\textsubscript{2} could be investigated and analysed quantitatively (Bohr, 1891\textsuperscript{a}, 1891\textsuperscript{b}). Thus, when Krogh in 1897 entered Bohr’s physiological laboratory, he wrote his first paper on the influence of salicylic acid on gastric digestion of proteins, and completed an MD degree in 1890 on the lipid content in milk. He became renowned for his quantitative approach to physiological problems involving physical chemistry, and had a profound influence on the early development of physiology in Denmark. He was a genuine experimental biologist with attention to clear concepts and quantitative methods. His choice of respiratory processes as his primary field of research owes to the precision with which O\textsubscript{2} and CO\textsubscript{2} could be investigated and analysed quantitatively (Bohr, 1891\textsuperscript{a}, 1892). It was during his stay with Carl Ludwig (1816–1895) in Leipzig that Bohr switched focus to study respiratory properties of blood with quantitative investigations of haemoglobin’s O\textsubscript{2} binding capacity. His studies indicated lower binding capacity of venous blood as compared to that of arterial blood (Bohr, 1891\textsuperscript{b}) for which he introduced the concept of different types of haemoglobin. He also developed a keen interest in pulmonary gas exchange and adopted Ludwig’s view that both O\textsubscript{2} and CO\textsubscript{2} were secreted by ‘vital’ processes across the pulmonary epithelium (Bohr, 1891\textsuperscript{a}, 1891\textsuperscript{b}). Thus, when Krogh in 1897 entered Bohr’s

2. Training in Christian Bohr’s physiological laboratory

Christian Bohr had been appointed Professor in Physiology in 1890. He started his career under supervision of Peter Panum (1820–1885) at Copenhagen University, wrote his first paper on the influence of salicylic acid on gastric digestion of proteins, and completed an MD degree in 1880 on the lipid content in milk. He became renowned for his quantitative approach to physiological problems involving physical chemistry, and had a profound influence on the early development of physiology in Denmark. He was a genuine experimental biologist with attention to clear concepts and quantitative methods. His choice of respiratory processes as his primary field of research owes to the precision with which O\textsubscript{2} and CO\textsubscript{2} could be investigated and analysed quantitatively (Bohr, 1891\textsuperscript{a}, 1892). It was during his stay with Carl Ludwig (1816–1895) in Leipzig that Bohr switched focus to study respiratory properties of blood with quantitative investigations of haemoglobin’s O\textsubscript{2} binding capacity. His studies indicated lower binding capacity of venous blood as compared to that of arterial blood (Bohr, 1891\textsuperscript{b}) for which he introduced the concept of different types of haemoglobin. He also developed a keen interest in pulmonary gas exchange and adopted Ludwig’s view that both O\textsubscript{2} and CO\textsubscript{2} were secreted by ‘vital’ processes across the pulmonary epithelium (Bohr, 1891\textsuperscript{a}, 1891\textsuperscript{b}). Thus, when Krogh in 1897 entered Bohr’s
Physiological Laboratory in Bredgade in the center of Copenhagen, he became immersed in a laboratory milieu with numerous other younger researchers studying different aspects respiratory physiology on various animals, including fish, amphibians, birds, reptiles and mammals. Krogh’s time in Bohr’s laboratory lasted until 1907 when the two scientists stopped communicating because of severe disagreements on the mechanism of respiratory gas exchange between blood and alveoli of the lung (Schmidt-Nielsen, 1995).

3. Measurements on Greenland revealed the increase in atmospheric CO$_2$ by fossil fuels

In 1902, Krogh was provided the opportunity to visit his friend Morten Porsild (1872–1956), a botanist and founder of the Arctic Station on Disko in West-Greenland, who would spend most of his career in Greenland. In preparing an expedition to South Western Greenland, Krogh designed equipment to measure O$_2$ and CO$_2$ in water and air samples to study respiratory gas exchange in polar organisms. However, he became more interested in global CO$_2$ balance. This issue had recently been studied by the Swedish chemist Svante Arrhenius (1859–1927), who suggested increased CO$_2$ in the atmosphere as an explanation for global warming (Arrhenius, 1896). During the summer of 1902, Krogh performed detailed CO$_2$ measurements in water and air, and reached the conclusion that although the oceans constitute an important buffer of CO$_2$, industrial combustion of coal would lead to significant elevations of atmospheric CO$_2$. In one of the two monographs that resulted from this expedition, Krogh warned almost prophetically: “The combustion of coal by man is an ever increasing factor that has in recent years reached very considerable magnitude — The world’s production of coal amounted in 1902 to 700 million tons giving by combustion 2.6 × 10$^{6}$ ton of carbonic acid or rather more than 1/1000 of the quantity present in the atmosphere. In the geological insignificant period of 1000 years the percentage of carbonic acid could therefore be doubled by this course alone, if other factors remained unchanged” (Krogh, 1904a). Needless to say, factors did not remain the same; in 2020, the CO$_2$ emission was on the order of 37 × 10$^{9}$ tons with an atmospheric CO$_2$ content above 0.04% as compared to 0.03% in 1902.

4. Doctoral thesis on pulmonary and cutaneous gas exchange in frogs

Krogh began his research career by addressing pulmonary and cutaneous gas exchange in frogs, and his many detailed measurements on the partitioning of CO$_2$ excretion and O$_2$ uptake would form the basis of his PhD in 1903. But Krogh also devoted considerable attention to other matters. With Vilhelm Maar (1871–1940), another of Bohr’s protégés, he translated the work of Niels Steensen (Nicolaus Stenonius, 1638–1686) from Latin to Danish (Krogh and Maar, 1902) and he embarked on a series of studies on the control of buoyancy in insect larvae where he perfected the ability to perform precise measurements of CO$_2$ and O$_2$ concentrations on very small gas samples. He used the same techniques with frogs, and Krogh subsequently defended what is equivalent to a PhD on midday Saturday October 10th, 1903, with the title ‘On the cutaneous and pulmonary respiration of the frog’. A paper with the same title was published in Skandinavisches Archiv für Physiologie shortly thereafter (Krogh, 1904b). True to form, Krogh published an accompanying comparison of cutaneous gas exchange in eel, frog, tortoise, and pigeon in the same issue, showing that the rate of cutaneous exchange, when expressed as quantity per time per surface area is highest in the species with the thinnest skin and the highest capillary density (Krogh, 1904c).

5. S-shaped blood O$_2$ equilibrium curve and the Bohr effect

While studying gas exchange in frogs, Krogh also engaged himself in a characterisation of blood respiratory properties where, seemingly on request of Bohr, he took full advantage of his superb technical skills. As with every new step of Krogh’s scientific accomplishment, he designed novel experimental approaches and instruments. For answering the question concerning O$_2$ in blood, Krogh constructed an apparatus for mixing CO$_2$ and O$_2$ at desired partial pressures performing precise measurements of the partial pressures of both gases (Krogh, 1904d). It is likely that Bohr’s earlier studies on blood CO$_2$ and O$_2$ binding (Bohr, 1891b) directed the planning of the new experiments, which resulted in two papers. First, the single-authored technical paper based on horse blood, where Krogh, for the first time, shows that the blood-O$_2$ equilibrium curve (OEC) is S-shaped. In the second paper, Bohr, Karl Albert Hasselbalch (1874–1962; made immortal by using a logarithmic transformation in what is now known as the Henderson-Hasselbalch equation) and Krogh provided a detailed description of O$_2$ equilibrium curves of dog blood. The most important result of this early study was the disclosure that blood O$_2$ affinity is reduced upon addition of CO$_2$ (lowering pH), since then known as the “Bohr-effect” (Bohr et al., 1904), where the obvious link to facilitating unloading of O$_2$ in the tissues is now textbook wisdom. The cooperativity of blood O$_2$ binding, indicated by the S-shaped OEC, provided the concept of allosteric control of enzymatic processes and allosteric regulation of haemoglobin function, has been studied extensively in different species (e.g. Weber and Campbell, 2011). Recent studies emphasize that the Bohr effect enhances the sigmoidal shape of the O$_2$ equilibrium curve, and that the magnitude of the Bohr factor is directly related to O$_2$ affinity (Malte and Lykkeboe, 2018; Malte et al., 2021).

6. Pulmonary gas exchange by diffusion and diffusion alone

In the final paragraph of his thesis on gas exchange in frogs, Krogh concluded “that the cutaneous respiration is withdrawn from the direct influence of the nervous system and may be supposed to be effected solely by physical powers (diffusion), and that the pulmonary respiration, predominantly at least, is effected by secretory processes in the epithelium and is regulated through the nervous system.” This view obviously sided with Ludwig and Bohr arguing that O$_2$ (and CO$_2$) were secreted across the lung epithelium like a glandular process depending on ‘vital’ forces. However, Krogh would change his mind soon thereafter, and spark an unfortunate and irreconcilable conflict with his mentor, Christian Bohr, who introduced quantitative physiology in Denmark and, amongst other lasting accomplishments, derived the equation for dead space calculations, developed the Bohr integration, and the ‘Bohr effect’ (e.g. West, 2019).

The mechanisms for pulmonary gas exchange had been debated since the latter half of 19th century, with Ludwig, Bohr and John Scott Hal-dane (1860–1936) being the most influential proponents of involvement of ‘vital forces’. This view should not be confused with vitalism, and Bohr had actually made careful (but now known to be erroneous) measurements of the partial pressure of O$_2$ exceeding that of the lungs (Bohr, 1909), and he calculated that the pulmonary diffusive capacity for O$_2$ measured at rest, was much too low to explain pulmonary gas exchange in exercise. We now know that capillary recruitment explains the rise in pulmonary diffusion capacity as pulmonary blood flow increases during exercise (Savoy et al., 1980). Bohr’s views were also supported by numerous studies on frogs, tortoises and mammals. Seemingly showing that pulmonary gas exchange was under parasympathetic regulation (see Wang, 2011). Finally, Bohr found clear evidence for O$_2$ secretion in the fish swim bladder, now known to be caused by acidification of the blood in the gas gland rather than by metabolically dependent membrane transport.

Under the impetus of Bohr, who must have been very impressed by a talented student with extraordinary technical skills, Krogh was encouraged to study the mechanism of pulmonary gas exchange around 1906. First, Krogh realised that the method hitherto applied to measure pO$_2$ and pCO$_2$ of arterial blood provided erroneous results. The method used by Bohr and others was based upon the addition to the blood of an
air sample of known composition enclosed in a bubble of precisely known diameter, waiting for equilibration between the air sample and blood plasma, and then measuring the partial pressures and absolute volumes of the gas samples. To meet the low sensitivity of the gas analysis apparatus, Krogh calculated that the air sample had to be so large that equilibrium could not be obtained within the time frame of a normal laboratory experiment. This challenged him to develop a method with strongly enhanced sensitivity, now known as Krogh’s microtonometer, and a method by which he could operate with small gas samples enclosed in an air bubble of known micrometer-dimensions (Krogh, 1908a, 1908b). In letters to his parents, Krogh describes that the first experiments were proceeding well, and that professor Bohr had accepted to join him in further experiments. But, as described in Bodil Schmidt-Nielsen’s outstanding biography of her parents (Schmidt-Nielsen, 1995), very little is known about what happened next, and it seems that Krogh and Bohr did not exchange scientific views in this period. In 1910 August and his wife, Marie, published seven experimental papers, which they referred endearingly to “the seven little devils”, providing conclusive evidence for pulmonary gas exchange to be governed by lossal contribution to Scandinavian physiology.

7. Exercise physiology and establishment of Krogh’s own laboratory for zoophysiology

Krogh had been appointed lecturer in zoophysiology at Copenhagen University on the early spring of 1907, but had not been given his own laboratory. Thus, Marie Krogh had been at work behind the scenes, by making direct contact to the Ministry of Church and Education, and secured financial support for Krogh to establish his own Laboratory of Zoophysiology in Ny Vesteregade in the center of Copenhagen in 1910. Because the discussion on secretion versus passive diffusion in the lungs bore direct relevance to the issue of adequate diffusion capacity in exercise, it seemed logical that August Krogh would take interest in how the cardiorespiratory system provides for the increased “call for O2 in the tissues” during physical exertion. At the same time, Johannes Lindhard (1870–1947) was appointed professor in Theory of Gymnastics at The University of Copenhagen, but lacked laboratory space, so Krogh invited him to join the newly-established facilities. Krogh had met Lindhard some years earlier when Lindhard prepared to participate as a physician in the infamous 1906–08 Denmark Expedition to Greenland headed by Ludvig Mylius-Erichsen (1872–1907). The expedition aimed at exploring the largely unknown, hostile area of North East Greenland. Three members of the expedition including Mylius-Erichsen died of hunger and exhaustion at the end of 1907. Lindhard’s own sad experience during this expedition may explain his primary interest in studies of the physiology of the human body during strenuous physical demands, which was faithfully shared by Krogh. In Ny Vesteregade, Krogh and Lindhard embarked on a series of studies where they characterized the responses of metabolism, pulmonary ventilation and the cardiovascular system to physical exercise (Hellsten and Saltin, 2010). Krogh designed the famous exercise bike with exquisite control of workload and precise measurements of change in body mass (Krogh, 1913). Their experiments were carried out on themselves, as well as on Marie and the young boy, that delivered groceries to the apartment on the top floor, above the lab, where Marie and August lived with the children that were born in this period; Erik (1908), Ellen (1913), Agnes (1917) and Bodil (1918). Krogh and Lindhard established the concept of feed-forward regulation, i.e. that both heart rate and pulmonary ventilation increases prior to afferent feed-back from chemoreceptors (Krogh and Lindhard, 1913).

From precise measurements of the respiratory quotient and the body mass change they established the concept of ‘oxygen debt’, and that humans start exercise by burning carbohydrate followed by lipid oxidation. Krogh also developed the understanding of how the rate of venous return is of paramount importance for the rise in cardiac output during exercise (Joyce and Wang, 2021).

8. Capillary physiology: novel experimental approaches and mathematical modelling

Krogh’s studies of pulmonary physiology and whole body energy metabolism during exercise logically led to the investigation of the mechanism of O2 supply to working muscles. It was generally held that an increased oxygen demand during work is met simply by increased capillary perfusion rate. As always when starting on a new project, Krogh initiated his studies by applying his astute sense for classical physics to formulate an explicit working hypothesis. He reasoned that the increased capillary perfusion rate had to be wrong. Increasing the perfusion rate would be counter-balanced by the shorter residence time for the red cells in the working muscle. As a testable working hypothesis, Krogh proposed that the increased call for O2 by the working muscle would be met by a shorter diffusion distance between capillaries and muscle cells, which would mean that the total amount of blood in the muscle would increase in exercise. This mechanism would work if merely a small fraction of capillaries are open at rest, while a large number of capillaries open when the demand for O2 increases. This hypothesis raised the questions of the rate of O2 diffusion in the muscle, and the density and distribution of open capillaries in the oxygen consuming muscle. To answer the first question, Krogh designed a setup of two half chambers separated by a piece of muscle tissue exposed to solutions of different oxygen tensions (pressures). To simplify calculations, oxygen tension in the chamber prepared to be oxygen free was clamped to zero by adding red cells to the solution (Krogh, 1919b). By this approach, the diffusion constant for O2 was obtained from the rate of O2 diffusion driven by the known tension gradient, corrected for the tissue’s own O2 consumption during the measurements. Next, the quantitative relationship between O2 tension and O2 consumption in situ was studied by mathematical modelling. In collaboration with the mathematician Agner K. Erlang (1878–1929), a mathematical equation was derived, which met the requirement of bringing together the quantities measured by Krogh and the unknown tension of O2 of the muscle (see Fig. 1; Krogh, 1919a). Next, Krogh developed and tested new methods for direct observation of capillaries in vivo by microscopic examinations as well as stained capillary preparations in vitro (Krogh, 1919c). Krogh observed that the individual capillary opens and closes spontaneously in the resting muscle, and that the density of open capillaries increases with increasing work load. For example, in the diaphragm of guinea pig the density increased from 31 to 3000 mm−2 as result of a twenty-fold rise in O2 consumption corresponding to a capillary exchange area of 750 cm2/cm2. His calculations showed that this mechanism of regulating the capillary blood flow secured an O2 tension of the muscle at the level of the venous blood even during maximum work load. The studies also resulted in the novel observation of contractile Rouget cells envelop the individual capillaries supposedly to constitute the ‘capillarioromotor mechanism’ (Krogh, 1920). In the same year Krogh was awarded the ‘Nobel Prize in Physiology or Medicine for his discovery of the capillary motor regulating mechanism’. These early publications on capillaries spurred intensive research on mechanisms and regulation of solute and water exchange between capillary blood and tissue. Fifty years after Krogh’s initial work, at the Alfred Benzon Symposium held in Copenhagen in 1969 six modified versions of the Krogh-Erlang capillary-cylinder model were presented (Schmidt-Nielsen, 1995). For the most recent discussion of Krogh’s capillary recruitment theory, see the paper by Poole et al. (2021), which is included in this special issue of Comparative Biochemistry and Physiology, Part A.
showed that cell water volume is controlled by a dynamic volume
and Simonsen, 1989). They introduced the concepts of regulatory
different ion composition of cell water and extracellular fluid
cellular fluid of freshwater animals and the environment, as well as the
-9. Energy requirement of body fluid homeostasis


Fig. 1. Krogh’s cylinder model of the diffusion of O₂ to the muscle. (A). The
graph of a capillary surrounded by O₂ consuming muscle tissue, where the
O₂ diffusion constant, p is the same everywhere governed by the O₂ con-
sumption, p likewise independent of x. R is half the distance between neigh-
bouring capillaries each of radius, r and O₂ tension of T₀. All of these four
variables were experimentally obtained by Krogh. (B) The Krogh-Erlang
equation assumes that an individual capillary by diffusion supplies O₂ to the
‘Krogh cylinder’. The O₂ tension at distance x from the capillary, Tₓ could
not be obtained by experiment but was computed by the Krogh-Erlang equation
shown here.

9. Energy requirement of body fluid homeostasis

In the 1930s Krogh returned to the question of driving forces for mass
transport across biological membranes, which was presented to him by
Christian Bohr at the beginning of the century. He chose the frog as
experimental animal and developed methods for studying ion uptake
from diluted solutions of NaCl with ‘salt depleted frogs’ (Krogh, 1937a).
The new experiments were designed in such a way that demonstration of
salt uptake would prove that the ions moved in the direction opposite to
that governed by external physico-chemical forces (diffusion). This
novel type of mass transport was termed ‘active’ as opposed ‘passive’
transport. In his Croonian Lecture elaborating on this finding in October
1946, Krogh concluded that the different ion composition of the extra-
cellular fluid of freshwater animals and the environment, as well as the
different ion composition of cell water and extracellular fluid “has not to
do with a true equilibrium, but with a steady state maintained against a
passive diffusion and requiring the expenditure of energy” (Krogh, 1946). As
discussed by Kay and Blaustein (2019), this fundamentally novel insight
became embedded in the pump-leak theory of stationary- and time
independent states of volume and cation concentrations of red cells
(Tosteson and Hoffman, 1960). The Tosteson-Hoffman theory was
generalized by Else Hoffmann and co-workers for cells in which chloride
is above thermodynamic equilibrium, with the first studies carried out
on non-polarized Ehrlich ascites tumor cells (Hoffmann, 1978; Hoff-
mann and Simonsen, 1989). They introduced the concepts of regulatory
volume increase (RVI) and regulatory volume decrease (RVD), and
showed that cell water volume is controlled by a dynamic volume
regulated anion channel in parallel with a Na⁺ gradient-driven co-
transporter energized by the sodium-potassium pump. The key player in
RVD, the ‘Volume-Regulated Anion Channel’ (VRAC) is expressed in all
vertebrate cells investigated (Hoffmann et al., 2009; Pedersen et al.,
2015).

10. Application of radioactive tracers for the study of epithelial
ion transport

Krogh’s identification in 1937 of active transport by frog skin coincided
with the appearance of the new revolutionary possibilities for
applying isotopes in the study of the dynamic state of biological systems,
-a brilliant idea conceived by the Hungarian physicist and Nobel Laureate
George de Hevesy (1885–1966) (Krogh, 1937b; Levi, 1985). In a
remarkable study, Hevesy, Hofer and Krogh studied the diffusion
permeability of frog skin to ‘heavy’ water (D₂O). They showed that the
D₂O permeability is independent of the direction of the flux of D₂O as
expected if the transport is due to simple diffusion. Surprisingly, how-
ever, in experiments studying the ‘permeability to ordinary water’
moving through the skin by osmosis, they found that the permeability of
ordinary water is five times that of labelled water. To the authors’
disappointment, they had to conclude that heavy water could not be
used as tracer for ordinary water, “which for the time being limits the
practical applicability of heavy water or any other indicator to comparative
experiments” (Hevesy et al., 1935). Almost 20 years later Hans H. Ussing
(1911–2000) repeated the experiment for measuring the ratio of uni-
directional water fluxes (Koefoed-Johnsen and Ussing, 1953). Under
the telling title “the contribution of diffusion and flow to the passage of D₂O
through living membranes”, the new results showed that water molecules
are not moving independently of one another, suggestive of bulk flow
through pores. This turned out to be the correct interpretation, solved by
Peter Agre (1949-) by his cloning of a ‘water channel protein’ denoted
aquaporin for which he received the Nobel Prize in Chemistry in 2003
(Agre et al., 1995).

It was Krogh’s intention that he himself should continue the 1937
study by using radioactive tracers to explore the membrane mechanism
of active ion transport. However, because of the German occupation of
Denmark he had to flee to Sweden in 1944. From his address in the
university town Lund in a letter dated 13th of March 1945, Krogh ur-
gently asked Ussing to take over the project on active ion transport by
frog skin. The technical details of the letter and focus of the study
explained in an enclosed Memorandum, did not leave any doubt about
his strong feelings for the project (see Larsen, 2009). Ussing agreed to do
so but was reluctant because his own investigations of body protein
turnover using deuterium-labelled amino acids were important to him at
the time. During the following decades Ussing’s ion and water transport
studies with isotope tracers resulted in generally accepted paradigms
that were applied not only to the skin but also to other transporting
epithelia (Larsen, 2002).

Despite the success of Ussing’s frog skin studies, comparative physi-
ologists have raised the concern that the outside of the skin of the
freshwater frog is bathed in Ringer’s solution when mounted in the
Ussing chamber. Larsen (2021) argues against this concern by pointing
out that the skin of amphibians on land is a respiratory epithelium, as
already disclosed by Krogh (1904). In agreement with this interpreta-
tion, the anuran skin has several structural, cellular and molecular
physiological properties in common with epithelia of conducting air-
ways of mammalian lung. The hypoosmotic cutaneous surface liquid
covering the amphibian like a coat and contained in the wet stratum
cornue corresponds with the airway surface liquid of upper airways.
This liquid layer is under continuous turnover due to the unavoidable
evaporation compensated for by the secretion from subepithelial mucous
glands and the reabsorption of the surplus of ions by the surface
epithelium. The ion mechanisms evolved to maintain the cutaneous
surface liquid (CSL) are energized by the sodium-potassium ATPase, for
ion secretion as well as for ion reabsorption. Calculation of the energy
expenditure in terms of the associated whole body O₂ uptake indicates
that the energy consumption due to the turnover of CSL is small
compared to the frog’s total energy metabolism (Larsen et al., 2014).

In a study of European anurans (R. esculenta, R. temporaria, Bom-
binator igneus) and urodèles (Tritonurus taeniatus, T. cristatus), leading to his
Thirty Nine Theses on the Water Economy of Amphibians and the Osmotic
Properties of the Amphibian Skin Ernest Overton (1865–1933) concluded
that water evaporates from the skin as from a free water surface
(Overton, 1904). The above interpretation of the skin function of am-
phibians on land agrees perfectly well with Overton’s classical study
published in the same year Krogh published his studies on the respira-
tory function of anuran skin.
11. Studies on exercising locust in Krogh’s private laboratory after retirement

Since the late 1930s, Krogh had wished to establish a small private laboratory for his retirement. However, this dream would not be realised until after the war when Krogh returned from Sweden where he had spent most of the final war-years in exile from the Nazi-occupation of Denmark. With support from the Scandinavian Insulin Foundation, Krogh equipped his basement in his house in Gentofte, a neighbourhood north of Copenhagen, to study insects. These experiments, which would be the last series of studies in his long career, were partially stimulated by a need to know more about the climatic events that determined locust migrations, but also bore direct relevance to Krogh’s early research on gas exchange and human exercise physiology. Torkel Weis-Fogh (1922–1975), who would later to lured to a full professorship at Cambridge University, played an important part in these studies, including how temperature, humidity and other climatic factors influenced migration in the locust. Krogh and Weis-Fogh constructed a round-about system of capillaries, through which the blood was flowing slowly when the insects were tethered by a string to their back, so they would fly in position for many hours while their velocity and changes in mass could be recorded (Krogh and Weis-Fogh, 1952). On single locusts, they recorded rates of gas exchange and, as with Lindhard many years earlier, used the respiratory gas exchange ratio to demonstrate that exercise was primarily fuelled by lipid-oxidation (Krogh and Weis-Fogh, 1951). They also followed up on Krogh and Zeuthen’s previous observations that a rise in body temperature occurred before the locust would initiate flying (Krogh and Zeuthen 1941), a discovery that still stands as one of the early demonstrations of endothermy in insects. Krogh’s final experiments, therefore embodied the merger of basic blue-sky research on the mechanisms that dictate how animals function with a desire to solve societal problems of locusts as a pest. Perhaps it was almost prophetical that Krogh had praised the value of such studies when he opened the world conference in physiology in 1929: “I want to say a word for the study of comparative physiology also for its own sake. You will find in the lower animals mechanisms and adaptions of exquisite beauty and the most surprising character, and I think nothing can be more fascinating than the senses and instincts of insects as revealed by the modern investigations” (Krogh, 1929).

12. Visual thinking

In addition to being bestowed with a unique capacity to formulate critical questions, Krogh’s impressive contributions to physiology was in no small part due to his ability to develop new equipment that enabled measurements with unprecedented precision. In an autobiographical note, Krogh (1938b) ascribed this talent to what he called visual thinking and described, “I noticed as a small child that I could at will ‘see pictures on the ceiling’, pictures in which something happened and in which I might or might not take part myself. These pictures could be very clear and I could see any detail that I wanted”. Later in the same publication, Krogh continued, “A considerable part of my work was done in bed during the night when I would try to visualize the processes studied and the experiments to be carried out. I found I could visualize fairly complicated apparatus and all details of their working. The constructive ideas would come, apparently, out of nowhere, but the visionary examination of them was a conscious and rational affair. I never made, and even now never make, drawings, not even rough sketches, until the construction of an apparatus was complete, because I found that a drawing would hamper the free flow of ideas and bind me down to that particular solution of the problem”. In the same autobiographical note Krogh also mentions “cases of physiological processes that were visualized in essentially the same fashion. My work on the regulation of capillary circulation began when I was writing a monograph on Respiratory Exchange and had to consider the mechanism of internal respiration, especially in muscles where the demand for oxygen and material for combustion can be increased to more than 20 times the resting figure. The conception of a fixed system of capillaries, through which the blood was flowing slowly when the muscle was at rest, and at a tremendous rate when the muscle was working near the limit of its capacity, seemed very unsatisfactory from a teleological point of view and I came to visualize a functional arrangement in which the majority of capillaries would remain closed during rest while the open ones were equally spaced on the transverse section of the muscle. This again through teleological reasoning, led to the visualization of capillaries opening and closing in a sort of rotation proving a uniform supply of oxygen, when considered over a certain length of time, to all parts of the tissue. These general conceptions were substantially verified by observation, but in order to arrive at a real understanding elaborate measurements and determinations of the rate of diffusion of oxygen in animal tissues were necessary”. In the closing paragraph of the autobiographical note, Krogh concludes: “The problems which fascinate me in biological science are mainly those which give a free play to the faculties mentioned, but the attainment of a high degree of precision in measurements never fails to appeal to me and I have been a fairly skilled manipulative worker”.

13. Curiosity-driven research

Krogh’s research can be viewed as a progression through physiological problems. His initial interest in respiratory physiology was extended to cardiac function and then to capillary physiology and modelling of tissue gas exchange. With his emerging understanding of his understanding of capillary function, Krogh gained interest in fluid balance and turned his attention to osmoregulation. For each of his major areas of research Krogh wrote comprehensive books or monographs that remain important resources for scholars today (Krogh, 1916, 1922, 1939, 1941). These research transitions appear motivated by a genuine interest in understanding how living organisms work. They also bear witness of a talent for identifying critical problems worthy of investigation, and an equal ability to move on when having solved the problems at hand. Krogh would often cite the Danish polymath Piet Hein (1905–1996) who stated that “Problems worthy of attack, prove their worth by fighting back”, but also emphasised that a guiding principle for his research was to understand, “How do the organisms solve their problems? Or rather: How is the particular problem solved which (more or less accidentally) has caught my attention”.

14. The ‘Krogh Principle’ in comparative physiology

Krogh is often characterized as the ‘inventor’ of the Krogh Principle, that is “for a large number of problems there will be some animal of choice, or a few such animals, on which it can be most conveniently studied”. This famous, and equally beloved quote, stems from Krogh’s opening speech at the 13th International Physiological Congress on August 19th 1929 at the Harvard Medical School in Boston, published the same year in American Journal of Physiology under the title of ‘Progress in Physiology’ (Krogh, 1929). The comment on the right choice of animal model was made in direct connection with Krogh’s early studies on pulmonary gas exchange and Krogh explained, “Many years ago when my teacher, Christian Bohr, was interested in the respiratory mechanism of the lung and devised the method of studying the exchange through each lung separately, he found that a certain kind of tortoise possessed a trachea dividing into the main bronchi high up in the neck, and we used to say as a laboratory joke that this animal had been created especially for the purposes of respiration physiology”. Little did Krogh know that the German-born biochemist and Nobel Laureate Hans Krebs (1900–1981) would refer to proper choice of animal models as ‘The Krogh Principle’ in a review article (Krebs, 1975). Krebs himself had used the red breast muscle of pigeons as basis for his description of the citric-acid cycle. However, as pointed out by C. C. Barker Jørgensen (1915–2007), another of Krogh’s hand-picked young scientists, and later professor of Zoophysiology at University of Copenhagen, the discussion of the best animal model should in fact be ascribed to Claude Bernard. In his book from 1865, Bernard strongly advocated the not yet established ‘experimental medicine’ for raising medical laboratory practice from its purely descriptive level to an experimental
hypothesis-driven discipline on par with experimental physics and chemistry (Bernard, 1865). Also, rather than placing focus on the selection of a particular animal model, Krogh favored the view that “the route by which we can strive toward the ideal is by the study of the vital functions in all their aspects throughout the myriads of organisms” (Krogh, 1929). Krogh elaborated on the importance of comparing physiological mechanisms in a wide array of organisms, “we will find out before very long the essential mechanisms of mammalian kidney function, but the general problem of excretion can be solved only when excretory organs are studied wherever we find them and in all their essential modifications” (Krogh, 1929). Jorgensen (2001) therefore concludes that Krogh endorsed the comparative approach as a means to establish general insight to physiological problems.

15. Entrepreneur and contributor to solving societal problems

 Whilst Krogh’s research was curiosity-driven, he had a keen eye for the application of physiology. Already in 1903, he took initiative to investigate the death of two workers when one of them fell into a well. Krogh could show, contrary to the public opinion of poisonous gases, that the air in the bottom of the well lacked O₂ and that the workers had succumbed to asphyxiation. This analysis was written and published to alleviate similar problems in the future and Krogh would write many small publications, often in Danish, for local clinical journals on his physiological research. Krogh likely saved the life of Marie and their newborn son Erik by converting one of the heating cabinets from the laboratory to an incubator.

Krogh’s most significant direct societal impact was, however, in endocrinology, an area of physiology where he did no primary research himself. When visiting the USA to give the Seligman Lectures following his Nobel Prize, Krogh was informed of the discovery of insulin at The University of Toronto and took immediate interest, because Marie Krogh had been diagnosed with diabetes some years earlier. Before insulin treatment, diabetes had had a variable and usually dire outcome. Krogh negotiated the right to produce and distribute insulin in Scandinavia. He and the physician H. C. Hagedorn founded the Nordic Insulin Laboratory and the Scandinavian Insulin Foundation. In 1989 this company merged with Novo Therapeutic Laboratory to be come Novo Nordisk, the dominant biomedical company for production of insulin for treatment of both type 1 and type 2 diabetes. The Novo Nordic Foundation supports physiological research and is currently the largest research agency in Denmark. As the following quote from a Science article shows, already in 1939 Krogh had envisioned great future possibilities for cooperation between biological sciences and industrial entrepreneur- ship: “That industrial development is deeply influenced by scientific discoveries has long been recognized in the case of physics and chemistry, and I need only mention such names as Faraday, Oersted and Hertz and the electrical industry to make it clear to you how enormous the influence has been. The influence of biological sciences on what I would call biotechnical industries is a later development, but not less profound, and the relations between industry and biological science is, as all good and durable relations should be, by no means one-sided, but mutual, science deriving extremely valuable help and stimulus from the industries. I propose to discuss briefly such mutual relationships as illustrated by conditions in Europe and I shall go a little more into the relationships in Denmark to which I can claim some firsthand knowledge”. Toward the end of the article Krogh concludes, “Under the leadership of Dr. Hagedorn the Nordisk Insulin Laboratory has grown into a fairly large concern, maintaining its own hospital for the study of endocrine disorders and fulfilling, I believe, an important function in keeping insulin prices in Europe at a comparatively low level. The surplus is handed over to the Scandinavian Physiological Foundation composed of clinicians and physiologists from Denmark, Finland, Norway and Sweden, who distribute grants in aid of research. I think this a fair way of letting the exploitation of a scientific discovery help to further scientific research, but of course everything depends upon finding the right man to make such an undertaking a success” (Krogh, 1938a).

Declaration of Competing Interest

The authors claim no conflict of interest.

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