Classics is an occasional column, featuring historic publications from the literature. These articles, written by modern experts in the field, discuss each classic paper's impact on the field of biology and their own work.

Classics

THE STRUCTURAL CONDITIONS FOR OXYGEN SUPPLY TO MUSCLE CELLS: THE KROGH CYLINDER MODEL

Ewald R. Weibel discusses August Krogh's classic paper, 'The number and distribution of capillaries in muscles with calculations of the oxygen pressure head necessary for supplying the tissue', published in *The Journal of Physiology* in 1919.

The paper we are here discussing, published by August Krogh in 1919, deals with the architectural relationship between blood capillaries and muscle cells and the conditions that allow oxygen supply from the blood to all parts of the muscle cells. It is one of the most influential papers in terms of the concept and modelling of oxygen supply on the basis of structural considerations. Indeed, in the last 5 years this paper has drawn about 25 citations per year, three times as many as the average rate at which it was cited since its publication nearly a century ago. The year following this publication, 1920, August Krogh was awarded the Nobel Prize in Physiology and Medicine for his 'discoveries on capillary regulation', the topic not of this article but of the third of the three papers published in the same issue of *The Journal of Physiology* (Krogh, 1919a; Krogh, 1919b; Krogh, 1919c). In retrospect, one may wonder whether the third paper really was Krogh's most influential contribution.

Indeed, a decade earlier, August Krogh, in part with his wife Marie, had published a series of seven papers – called the 'seven little devils' – in which he unambiguously established that oxygen uptake in the lungs was adequately accomplished by passive diffusion (Krogh, 1910). He thus clearly rejected the notion that oxygen uptake involved active transport processes or 'secretion', a notion upheld mainly by Krogh's laboratory head Christian Bohr (Bohr, 1909) but also supported by other respiratory physiology luminaries of the time, notably J. B. S. Haldane. This rigorous demonstration was, indeed, a major breakthrough, but the Nobel Prize committee thought this was inferior to Krogh's more recent propositions about the regulation of capillary blood supply to the tissues: 'It is not this work, despite its great merit, which the Nobel Prize is intended to reward. Settling a question of dispute … should hardly be considered a discovery', said the presenter of the Nobel Prize, whereas 'the work by Krogh (to be considered) forms some sort of introduction to other investigations having as their aim the determination of the process by which the oxygen required of the tissue is satisfied': that is the regulation of capillary blood flow (Johansen, 1920).

After having settled the process of pulmonary respiration, Krogh turned his attention to internal respiration, the delivery of oxygen to the cells, of which capillary blood flow is an important aspect (Krogh, 1920). In 1915 he was engaged in a broad review of the literature for his book *The Respiratory Exchange of Animals and Man* (Krogh, 1916). As described by his daughter Bodil Schmidt-Nielsen in her biography of August and Marie Krogh (Schmidt-Nielsen, 1995), August Krogh was struck by the observations that oxygen tension in the tissues, particularly in muscles, should be very low at rest. In 1913 he had shown that oxygen consumption increased by 10-fold in muscle work and that this was accompanied by a large increase in blood flow (Krogh and Lindhard, 1913). What would be the role of capillaries in this process? How would they adjust to the different demands in oxygen supply and blood flow between rest and exercise? After discussions with Marie in December 1915 he seems to have immediately embarked on a systematic series of experimental studies on capillaries for which 'he needed many frogs, which his friend Barcroft was able to ship from England in spite of the war' (Schmidt-Nielsen, 1995). August Krogh approached the complex problem very systematically along three lines: (1) the physical problem of the rate at which oxygen diffuses into and through the tissues; (2) the anatomical problem of the number and distribution of capillaries with respect to the cells; and (3) the physiological problem of regulating the supply of blood and by that the availability of oxygen under the conditions of rest and in exercise. In January 1919, just 3 years after beginning his experiments, Krogh submitted three papers to the Editor of *The Journal of Physiology*, J. N. Langley, sending copies to Joseph Barcroft commenting: 'The two first are rather dull, though I hope useful, but I venture to believe that you will find the third one interesting.' Well, Krogh was right: it was the third of these papers – the 'interesting' one – that won him the Nobel Prize the following year. But in hindsight, the 'dull' papers were indeed 'useful', their effect sustained: the first one defined what we still call the 'Krogh diffusion coefficient', and the second one established the 'Krogh cylinder model', which is discussed here as a 'classic'. The three papers are tightly linked, the result of Krogh's clear conception of the various factors that affect oxygen supply to the cells, which may have evolved in the course of the 3 years of experimental work on this topic.

In the second paper, entitled 'The number and distribution of capillaries in muscle with calculations of the oxygen pressure head necessary for supplying the tissue', Krogh starts by saying: 'In a preceding paper I have recorded measurements of the diffusion constants for certain gases and especially oxygen in animal tissues.' That first paper (Krogh, 1919a) was indeed a most impressive work, in which Krogh had to build a new type of apparatus with which to obtain these measurements with accuracy. He then remarked that these data are useful only if one knows the rate of oxygen consumption and 'the average distance which an oxygen molecule has to travel from a capillary into the tissue before entering into chemical combination.' In order to study the relationship between cells and the capillaries that supply them, Krogh made 'the muscles the basis of my study', because, in contrast to most tissues, 'in the muscles we have a fairly regular distribution of capillary vessels, which are in the main parallel to the muscle fibres.' He describes in detail how the capillaries extend from the arterioles to the venules, coursing along the muscle fibres with few anastomoses. 'Thin transverse sections show the capillaries as small dots which are distributed with conspicuous regularity among the muscle fibres, and it becomes at once apparent that no serious error can be committed by supposing each capillary to supply oxygen independently of all the others to a cylinder of tissue surrounding it.' The cross-sectional area of such a cylinder is easily obtained by dividing the total area of the section by the number of capillaries counted in this area.

Before proceeding to an experimental estimation of the number of capillaries, Krogh describes his model by means of a formula worked out for him by the mathematician Mr K. Erlang, which describes the oxygen tension at any distance x in the tissue surrounding the capillary as a function of the oxygen tension (P_{O_2}) in the capillary, the diffusion constant for oxygen in muscle tissue (Krogh, 1919a), the rate of oxygen consumption, and the radii of the cylinder (R) and of the capillary (r). Considering the case x=R, this formula 'gives us the maximum tension difference necessary to supply the muscle with oxygen' at any point along the capillary. 'If this difference is found to be larger than the O_2 tension of venous blood it follows that some portions at least of the muscle (near the venous end of capillaries) must suffer from want of oxygen, while if it is smaller the oxygen tension must be positive everywhere within the muscle.' This may occur because capillary P_{O_2} falls from arterial to venous P_{O_2} as blood flows along the capillary while oxygen is extracted.

With this model, Krogh defined the O_2 diffusing capacity of muscle capillaries, and thus set the basis for the quantitative study of the effect of structure on the limitation of O_2 supply to the muscle cells. By doing so, Krogh raised the model of internal respiration to the level of pulmonary gas exchange where, in 1909, Christian Bohr had defined the pulmonary diffusing capacity in terms of the diffusion constant and the structural parameters barrier thickness and surface; together with the P_{O_2} difference between alveolar air and capillary blood, this determined oxygen uptake by diffusion (Bohr, 1909). Bohr then obtained physiological measurements of O_2 partial pressures that surprisingly showed P_{O_2} to be larger in arterial blood than in alveolar air, which led him to conclude that O_2 uptake by diffusion does not suffice, but active transport must be involved. However, in his 'seven little devils' of 1910, Krogh obtained better estimates of the O_2 partial pressures and thus showed Bohr's conclusion to be wrong. Bohr's model of diffusing capacity, however, stood the test of time, so that his 1909 paper is also a classic that is still frequently cited – and his erroneous conclusions are simply overlooked.

It is worth reading Krogh's second paper in the original for its beautiful narrative style when he describes the problems he encountered obtaining specimens where all of the capillaries were injected with India ink so that he could obtain an accurate estimate of the cylinder radius in different specimens. It is also a pleasure to read his approach to testing his model in a comparative study of muscles of different animals (cod, frog, horse, dog and guinea pig), relating his – limited – measurements to the animal's metabolism, concluding: 'The number of capillaries per square mm. of the transverse section of striated muscle appears to be a function of the intensity of the metabolism, being higher in small mammals than in larger forms.'

In 1922 August Krogh was invited by Yale University to deliver the Silliman Lectures, an assignment that brought the commitment to write the lectures up in the form of a monograph to be published by Yale University Press (Krogh, 1922). In 11 lectures, Krogh spoke about his broad studies of the 'anatomy and physiology of capillaries'. The first lecture, which concerned 'the distribution and number of capillaries in selected organs', now looked beyond muscles. After several lectures on the regulation of capillary function, he discussed in Chapters IX and X 'the exchange of substances through the capillary wall'. Here he noted the limitation of the structural information that was available to him, so he ended Chapter I with 'a plea for the study of quantitative anatomy'. The anatomists did not listen. About 40 years elapsed before stereology provided anatomists with the tools necessary to obtain accurate quantitative information on structure that could interest physiologists (Weibel & Elias, 1967). These tools, combined with electron microscopy, yielded significant advances in the study of the structural basis of energy metabolism and capillary oxygen supply of muscles that would undoubtedly have pleased August Krogh (Hoppeler et al., 1973; Hoppeler et al., 1981a; Hoppeler et al., 1981b). They also provided the insight that the muscle microvasculature uses structural adaptations to avoid the regions with 'want of oxygen' near the venous end of capillaries, as described above, by gradually increasing the capillary density between the arterial and the venous end (Wolff et al., 1975), so that, in muscle, the Krogh cylinder looks more like a cone (Weibel et al., 1981).

I should not close this review without briefly discussing the 'interesting third paper' of this series (Krogh, 1919c), where Krogh shows that under normal conditions, i.e. when oxygen demand is not at its maximum, many capillaries are not perfused. The Krogh cylinder characterizes the limit of oxygen supply: at lower oxygen demand, fewer capillaries suffice to supply a larger cylinder, and the remaining capillaries are shut. In this paper, Krogh describes his studies on the regulation of capillary perfusion that is an intrinsic property of the capillary wall, studies he performed to a large extent on the tongue of the frog, which he observed under his microscope while stimulating with fine needles. Again, it is worth reading the original article to capture the struggle of the keen experimenter to understand the object of his study while progressing through various experiments. The paper also reflects a culture of scientific writing that is personal and emotional, and honestly reports failures and successes – very different from our contemporary style. We come to experience the important finding that capillaries are not passive tubes, but are capable of restricting their perfusion by actions of the vessel wall. Indeed, this paper is full of challenges and so the reasoning of the Nobel committee – that this work 'forms some sort of introduction to other investigations' – becomes convincing, although the same could also have been said of the 'classic' Krogh cylinder paper!

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