2929

From: ADVANCES IN PHYSIOLOGICAL RESEARCH
Edited by H. McLennan, J.R. Ledsome,
C.H.S. McIntosh and D.R. Jones
(Plenum Publishing Corporation, 1987)

THE AUGUST KROGH LECTURE: THE WORLD AS A LABORATORY

Physiological Insights from Nature's Experiments

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August Krogh, the Nobel laureate from 1920 to whom this lecture is dedicated, epitomized the very essence of comparative physiology in his famous statement (Krogh, 1929): "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".

I profess as many before me that the animals' environment and the constraints it offers should also be a paramount consideration in organismic physiology.

In this context I cannot help recalling a discussion I overheard years ago in the Arctic between Larry Irving and some fellow physiologists. The discussion got heated when Larry Irving refused to recognize the white laboratory rat as an animal. He argued that the white rat with food and water ad libitum and a thermostatted cage placed in a regulated light-dark cycle for literally thousands of generations could and should not qualify as an animal. I think Irving won the discussion.

On this basis and these premises I will in the following give a few examples of my research in comparative environmental physiology.

Regulation of skin blood flow

What probably has struck me the most as a biologist in the Arctic and Antarctic has been that the homeotherms there, the birds and mammals, are so strikingly well insulated. This perhaps should not surprise anyone, but in reality this fact shifts the main physiological problem for the polar homeotherm from an easily recognized one of conserving heat in perhaps -40°C to one of getting rid of heat when the polar animal during physical activity increases heat production by a factor of 10 or more. It is easy to reason that polar animals must depend on using selected areas of the skin as dissipating surfaces for heat during increased heat production. Regulation of skin blood flow hence becomes a primary target for study. These surfaces will have to be less insulated by fur, blubber or feathers than the rest of the animal, a fact which makes them vulnerable for costly heat loss at rest or for tissue injuries from freezing when ambient cold becomes severe. Circumvention of these threats must also rest with effective regulation of blood flow. Skin as an organ is not readily isolated from other tissues for direct blood flow measurement without grossly interfering with the integrity of the preparation, and indirect methods for flow measurement will always give uncertainties in interpretation.

For me the ideal preparation for studying skin blood flow became the extremities including the tail of mammals and the feet of birds.

Measuring blood flow to the tail of muskrats at rest and provoked to exercise on a small treadmill in Alaska, using a plethysmographic technique, revealed that tail blood flow could increase 350 times when a heat pad was placed on the trunk or during exercise.

Nerve block of muskrat tails prevented the colossal increase in tail blood flow and it was proposed that skin is endowed with a neurogenic vasodilator mechanism, much as skeletal muscle was known to have (Johansen, 1962).

Next the target animals for studies of skin blood flow and its regulation became Antarctic birds, primarily the giant petrel, Macronectes giganteus, and some species of penguins.

The body trunks of these animals have a formidably high insulation but their large naked feet consist mainly if not only of skin. The Macronectes foot with its large swimming web gives easy access to placement of small thermistors inside arteries and veins as well as intracutaneously. Web arterial and venous pressures can be monitored as can blood flow to the leg directly using an electromagnetic flow probe placed on the metatarsal artery.

If these feet were immersed in ice water, an experiment Nature does whenever the bird lands on water, we see a prompt onset of vasodilatation causing a several fold increase in foot blood flow and a temporary rise in arterial blood temperature. No primary vasoconstriction as in the typical homeotherm occurs.

This work (Johansen and Millard, 1973) disclosed that the neurogenic component of the vascular control involved both a tonic constrictor component most likely regulated by an α-adrenergic mechanism and a vasodilator component demonstrated both by nerve section and nerve stimulation. At the time we identified the latter as a cholinergic vasodilator mechanism. Later workers (Murrish and Guard, 1977) concluded that the vasodilatation was dependent on β-adrenergic nerves. Over the years many workers have taken an interest in the cutaneous vasodilatatory mechanism. McGregor (1979) among others has refuted the early suggestion that the neurogenic vasodilatation is cholinergically based, also he has eliminated histamine as a potential neurotransmitter; this amine has been implicated in several attempts to explain cutaneous vasodilatation in mammals. Purinergic as well as peptidergic nervous control of skin vasodilatation also receives scant support from the literature.

The nature of skin neurogenic vasodilatation, which seems to be present in all homeotherms to a different degree, must thus still await its final explanation.

THE AVIAN BROOD PATCH

A remarkable skin vascular organ

Using the world as a laboratory must not be taken to suggest that we never work at home. In the line of skin blood flow and its control recent experiments have been done (Midtgård, Sejrsen and Johansen, 1985) in

Copenhagen on skin circulation in the brood patch which most birds develop during their breeding season. An area on the breast and abdominal skin surface defeathers and develops a profuse vascularization serving to heat the incubated eggs. This vascularization develops with the hormonal changes in the breeding season. Evidence is suggestive that the brood patch occurs as a "seasonal organ". If the vascularization indeed should prove to develop de novo and regress when the breeding season terminates, we have a truly disposable organ such as the placenta, the antlers of reindeer and other ungulates, marvellous model organs for the study of angiogenesis: the formation of blood vessels.

Blood flow through the brood patch is an important factor in the control of egg temperature during incubation. Brood patch blood flow was measured by wash out of ^{133}Xe labelled into the brood patch skin. The egg temperatures could be changed by using artificial metal eggs which could be perfused selectively or collectively with water from a thermostatted system.

When the brood patch was cooled, skin blood flow promptly increased. Importantly, only the area directly cooled showed this cold vasodilatation. Cooling of skin adjacent to the brood patch caused a decreased blood flow and reduced skin temperature. It is certain that the response is strictly local, which will heat individual eggs in a clutch in relation to their respective temperatures and in this way diminish temperature differences between the eggs and have important survival value in synchronizing hatching and successful development of the clutch.

BODY TEMPERATURE REGULATION IN THE TROPICS

Life in a thermostat

We shall now discuss some aspects of body temperature regulation in the tropics. The setting will be in equatorial East Africa, specifically a subterranean habitat about 3 feet underground. There lives a most unusual mammal, the rodent Heterocephalus glaber, the naked mole rat, completely hairless and nearly blind. It lives in an intricately arranged system of burrows, feeding on roots and other plant material.

Unique physiological features of Heterocephalus include its low core temperature of about 30°C, a temperature corresponding very closely to the

air temperature in its dark humid habitat where diurnal and seasonal temperature variations are 1 to 2 degrees different from that of the mole rat. This mammal must be one of very few which literally lives in a thermostat set to its own core temperature. Expectedly Heterocephalus has a very low heat production and a high thermal conductivity. The mole rat thus effectively has no resistance to heat flow (Johansen, Maloiy and Kornerup, 1986).

What is it about this animal's ability to thermoregulate? Does it have the sensors and effectors we know as integral parts of homeotherm thermoregulation? If it does, maybe Nature in the naked mole rat has afforded us an animal capable of showing how accurate body thermoregulation may develop when resistance to heat flow develops and becomes dependent on ambient temperature (T_A).

Deep body temperature (T_B) varies rapidly with time when Heterocephalus is taken out of the "thermostat" and reaches near ambient temperature within 2 hours. This results in a nearly linear relation between T_A and T_B when steady state has been reached, usually within 1-2 hours after a change in T_A.

When the mole rat is removed from its "thermostat" and exposed to lower ambient temperatures interesting responses occur. Its strong social behaviour becomes intensified and huddling becomes conspicuous. If T_A is lowered to 15-20°C, shivering particularly of the forepart of the animal is very striking.

Consistently during cold exposure the head skin around the brain case, particularly in the occipital region, was clearly the warmest part of the body, even warmer than the rectal temperature. This higher head temperature correlated with large deposits of brown fat particularly accumulated around the posterior portion of the skull.

It is felt that the experiment Nature has done by placing a mammal in a thermostat set at its core temperature may give us a potential model animal for studies about how regulatory mechanisms may develop. We were strengthened in this view when a colony of mole rats brought from Kenya to Denmark and kept at stable air temperatures of about 24°C rather than their Kenyan habitat temperature of about 30°C, showed a trend for T_B to be kept higher than T_A for longer than observed in the Kenyan animals.

The search for oxygen

We shall now divert your interest to adaptive properties of blood in respiratory gas exchange. In Goethe's famous play Faust, Mefistopheles says to Faust: "Blut ist ein ganz besonderer Saft". (Blood is a remarkable juice). Nothing could be more true. Adaptive relationships between blood O_2 affinity and environmental factors are common and reflect long term as well as acute changes in environmental factors. August Krogh was a pioneer also in this field. Today we know that such adaptive changes in blood respiratory properties can be traced to molecular properties in the haemoglobins. Also modulations of blood O_2 affinity are typically due to ligand or cofactor interaction with the O_2-Hb binding. Short term alterations in O_2 affinity may become manifest within minutes after the environmental or behavioural changes set in, probably reflecting hormonal control of cofactor-Hb interaction. Recent work has also disclosed that the classical Hill plot expressed by the n-value or cooperativity coefficient is not the straight line it used to be but depends on the O_2 saturation level, a phenomenon likely traceable to the state of aggregation of the haemoglobin molecules (Lykkeboe and Johansen, 1978). An increase in n-value with increasing saturation has now been demonstrated in species from all vertebrate classes including a mammalian species (R. Holland, personal communication). The physiological significance of this n-value increase can be most important particularly for low affinity bloods.

We shall first stop in the huge fresh water ocean known as the Amazon. Among the 1500 known species of fish living in the vast region, we shall look at three. Depending on season and location large areas of the Amazon confront the aquatic life with conditions of hypoxia which would require mountains twice as high as Mount Everest to match.

Two of the species I will discuss belong to the relict family Osteoglossidae, which includes only 5 living species of very limited phyletic diversity. These "living fossils" can be found in the same habitats of the Amazon. One, Osteoglossum bicchirosum, (growing to about 30-50 cm) is an exclusive waterbreather. The other, Arapaima gigas, the world's largest fresh water fish, coexists with Osteoglossum, but is an obligatory airbreather and will drown unless it has access to atmospheric air to inhale into its specialized swimbladder lung.

If we compare the O_2-Hb dissociation curves for Osteoglossum and Arapaima at similar temperature and pH we find a much higher affinity in the waterbreathing Osteoglossum expressed by a P_{50} of 6.1 mm Hg compared to 21.0 mm Hg in Arapaima (Johansen, Mangum and Weber, 1978b). When this information is placed in the context of the water P_{O_2} at the site in the Amazon where these studies were done ranging from 15 mm Hg at night to 30 mm Hg during daylight, it becomes clear that the obligatory dependence on airbreathing in Arapaima is an absolute requirement for survival. If the fish had to depend on waterbreathing with gills, its arterial O_2 saturation could not exceed 10-15%. Osteoglossum, however, can rely on gill breathing for saturation of its blood. Interestingly, the O_2 uptake of Arapaima was more than twice that of Osteoglossum in similar sized specimens. The much lower affinity of Arapaima blood is consistent with the much higher O_2 availability in air than water. The low affinity blood will also allow for more efficient O_2 unloading and in this way support a higher level of O_2 uptake.

Notably, Arapaima had a very low gas exchange ratio of its swimbladder lung (about 0.15) suggesting that CO_2 excretion predominantly takes place to the water in the gills or skin as is typical of bimodally breathing fishes. How the fish avoids losing O_2 from blood to water during passage through the gills must depend on a physiological shunt mechanism in the gill and the different diffusion rates and solubility for O_2 and CO_2 in water.

A third species from the Amazon I will discuss is the teleost fish, Synbranchus marmoratus. Synbranchus is a facultative airbreather implying that the entire O_2 requirement can be supported by either water or airbreathing. Synbranchus also voluntarily makes long excursions on land moving in a snake-like fashion. The species can also estivate for long periods in moist soil. Blood respiratory properties in Synbranchus, when in well aerated water and acutely air exposed, show a marked reduction in O_2 affinity with air exposure. This is caused both by reduced pH from retention of CO_2 and from an increase in the red cell concentration of the trinucleoside phosphates ATP and GTP. These data show that blood respiratory properties adapt to the principal mode of gas exchange in a bimodal breather. In distinction to the osteoglossids, the adaptive response of Synbranchus depends on quick onset and reversible ligand changes (H^+ ion and trinucleoside phosphates) and not on intrinsic molecular properties as in Arapaima and Osteoglossum (Johansen, Mangum and Lykkeboe, 1978a).

We may conclude that the time course of adaptation may be influenced by the selection of a particular mechanism to modulate O_2-Hb binding properties. Short-term and often transient changes in environmental oxygen levels appear to induce rapid and reversible changes in oxygen affinity of the blood by alterations in the concentrations of metabolically labile cofactors within the red cell. On the evolutionary time scale, the adoption of airbreathing is accompanied by more profound and genetically fixed changes in oxygen binding, effected by less easily reversible changes in the structure of the haemoglobin molecule.

ESTIVATION AND TORPOR

The lungfish and the hummingbird

Among those of Nature's experiments I have found most rewarding to study are prolonged estivation and daily torpor. These adaptations are indispensable for many animals during drought and starvation. A most striking example is the estivating lungfish. Nocturnal torpor I would like to discuss briefly in the context of the hummingbird. Hummingbirds have such a phenomenally high metabolic rate when active during the day that night-time torpidity for many species when access to food is curtailed becomes obligatory.

The African lungfish, Protopterus, Kamongo in Swahili, is a fish so well known from the fascinating papers and other writings of Homer Smith (Smith, 1930). Since that time many scientists have been interested in the estivating lungfish. The works of A.P. Fishman and his group in Philadelphia among others have been highlighting the subject in recent years (Fishman, Delaney and Laurent, 1985).

It has been my privilege to work on Kamongo with Geoffrey Maloiy in Kenya and with my colleague Lomholt in Denmark on fishes brought there from Kenya.

We were fortunate to find access to an area near Malindi on the Kenya coast where a number of estivating lungfish could be removed intact in the soil in which they had been embedded by Nature about one year earlier. One specimen in particular became unforgettable. A chunk of mud equipped with

a Fleisch head connected to a pneumotachograph was sitting on my desk for more than 7 years after removal from the site at Malindi. This was truly a revelation in suspended animation for me, regularly watching the periodic excursion on a recorder reflecting Kamongo's unbelievably small tidal volumes and low breathing effort. One day a foul smell told us that Kamongo had given up waiting for the rains.

Only a few points from our work on estivating lungfish will be brought up here.

With estivation there occurs a most dramatic increase in blood O_2 affinity correlated with a conspicuous reduction in the ratio of red cell organic phosphates to haemoglobin concentration (Johansen, Lykkeboe, Weber and Maloiy, 1976).

We originally reasoned that this dramatic increase in blood O_2 affinity was an adaptive response to an internal hypoxic state such as earlier demonstrated for a number of fishes. When later a needle was placed into the lung space of estivating lungfish, mass spectrometer readings revealed that lung O_2 tension was not at all low as we had surmised it to be. In the meantime, Delaney, Lahiri, Hamilton and Fishman (1974) had also demonstrated that arterial P_{O_2} in Protopterus induced into artificial estivation was high.

Estivation in lungfish is attended by a very large reduction in aerobic metabolism, which can be as low as 5% of the awake non-starved value. Starvation of lungfish in the awake state also reduces O_2 uptake markedly, as it does in ectotherms in general.

In the context of starvation we must of course ask what it is that the tissues have in short supply. Substrate is obviously one commodity although as long as you can fuel metabolism from your own tissues this may not be limiting. Could O_2 be another? If arterial O_2 tension is not reduced in starvation what could be limiting for the tissues in terms of their O_2 supply? Obviously blood flow, which we know will reduce tissue O_2 uptake, for example in diving animals. Also we could speculate that the capillary-to-tissue P_{O_2} gradient could be a limiting factor in tissue O_2 delivery. This gradient will be very much reduced if O_2 is unloaded from

Hb at very low O_2 tensions such as those resulting if blood has a very high O_2 affinity.

We may then perhaps turn around our original and incorrect interpretation of the high O_2 affinity in estivation. We turn cause and effect in reverse, and speculate that some specific factors influencing red cell phosphate metabolism directly may bring about the very high blood O_2 affinity, which in turn will affect the capillary-to-tissue P_{O_2} gradient and cause reduced tissue O_2 uptake.

BEIJA FLOR

The one who kisses the flowers

Hummingbirds, Beija flor in Brazilian, are unique metabolic machines. Their metabolic rate (O_2 uptake) can vary by a factor of more than 300 between torpidity and active flight, a transition which may occur in less than 30 minutes. Nocturnal torpor is a necessity for most hummingbirds, especially species experiencing cold nights, because the high active metabolic rates cannot be sustained when nectar feeding is interrupted and ambient temperature has fallen.

Let us first examine how the respiratory properties of blood may support such high and rapidly changing metabolic rates. In general the O_2 affinity of hummingbird blood is low and the O_2 capacity is high. Three species studied in detail had P_{50} values at pH 7.40 and temperature 39°C between 41.0 and 44.0 mm Hg. The average Bohr factor was -0.39. The thermal sensitivity of the blood O_2 binding was rather low expressed by a ΔH value at P_{50} of -7.65 Kcal.mole^{-1} compared to more than twice that value for the purified haemolysate. A non-linearity of the Hill transformation expressed by increasing n-values at higher O_2 saturations demonstrated that n-values could exceed 7.0 for O_2 saturation 80% or higher (Johansen et al., 1986).

Most authors commenting on such n-value increases, particularly beyond values of 4.0, which raise conflict with conventional views on cooperativity in tetramer vertebrate haemoglobins, do not discuss the physiological implications of this change in O_2-Hb equilibrium. If arterial O_2 tension in hummingbirds ranges between 80.0 and 90.0 mm Hg,

values reported typical of other species of resting birds (Kawashiro and Scheid, 1975), a value of 80 mm Hg would give an arterial O_2 saturation of 97.5% in the hummingbird Melanotrochilus. By comparison a linear Hill transformation from the Hill coefficient at P_{50}, would give a saturation of only about 82.0%. Conversely, if the n-value prevailing at P_{50} was applied to the entire equilibrium curve, an arterial P_{O_2} exceeding 140.0 mm Hg would be needed to arterialize the blood to 97.5% at pH 7.40.

Hummingbirds spend nearly all of their awake time in flight, which calls for near maximal efficiency in gas exchange and blood gas transport for extended periods. The saturation dependent increase in n-value may help offset a possible diffusion limitation in the parabronchi during exercise and thus prevent arterial desaturation (Scheid, 1978). Submaximal O_2-Hb loading from reduced residence time of blood in the parabronchial blood capillaries during exercise would also be offset by a steeply rising affinity at the higher saturations.

Summarily, when the Hill plot curves upward in a low affinity blood such as for hummingbird blood at higher levels of saturation, arterial O_2 saturation will be safeguarded while the inherently low affinity of the blood will allow most of the possible O_2 unloading to take place at P_{O_2} values conserving an effective P_{O_2} gradient from capillary blood to cellular sites. It can be calculated that a 40% unloading of O_2 from arterial blood saturated to 90% with O_2 can occur with a P_{O_2} gradient between arterial and venous blood of less than 22.0 mm Hg; an unloading efficiency likely to exceed the maximum possible for any mammalian blood.

The nocturnal torpidity of most hummingbirds may cause deep body temperature to fluctuate by 20 to 25°C within a very short time during entry and arousal from torpor. Such rapidly fluctuating body temperatures will influence pulmonary gas exchange and internal blood gas transport in important ways, including shifting the O_2-Hb and O_2-Mb equilibrium curves. Since arterial P_{O_2} during torpidity is likely to drop due to a marked decrease in ventilatory effort, arterial desaturation can only be prevented if a left shift in O_2-Hb equilibrium attends the reduced body temperature. A ΔH value of about -8.0 Kcal.mole $^{-1}$, such as we measured, may be an ideal compromise between safeguarding arterial saturation and maintaining high venous O_2 tensions at reduced body temperatures. Again the change in n-value at higher saturation will be of major importance.

During regulated torpor at for instance a body temperature (T_B) of 15°C, the O_2 uptake of a hummingbird may be as high as that of the same bird in a homeotherm resting state with a T_B of 40°C. This implies that respiratory gas exchange and blood gas transport must operate with similar efficiency over a body temperature difference of at least 25°C, a situation not matched in any other homeotherm.

In regard to the potential energy saving by torpor in hummingbirds, calculations for one species, Melanotrochilus fuscus, show that 15% of the homeotherm energy expenditure suffices to sustain the energy needs at ambient temperatures between 15 and 20°C (Berger and Johansen, 1986).

Before leaving the Beija flor let us estimate the potential performance of their heart and blood circulation during the maximal energy expenditure of flight. We start with a small species weighing 2.5 grams, have an O_2 uptake rate in flight of 40 ml $O_2 \cdot g \cdot hr^{-1}$. Blood O_2 capacity is set to 22 vol% and blood volume to 8% of body weight. If now the utilization of O_2 from the circulating blood is 60% and we use the Fick principle to calculate cardiac output, we arrive at the figure of 12.6 $ml \cdot min^{-1}$. This is a staggering 5 times the body weight of the bird per minute at a heart rate of up to 1400 beats per minute. Blood volume for the bird calculated as 0.2 ml, will give an average circulation time of about 1 second for the blood volume. This figure raises the obvious question if the residence time of circulating blood in the lung and tissue capillaries is long enough for the blood to serve its functions. The answer is, of course, yes; the small hummingbirds have long demonstrated that.

If, however, we seek information from the literature about the kinetics of the reactions in red cells between haemoglobin and its ligands and consider the diffusion rates and boundary conditions of red cell gas exchanges, we will find that the smallest hummingbirds are at a size approaching limitation by cardiovascular performance. The rate of blood flow should thus be added to other well known factors which set the lower limit for the size of homeotherms (Schmidt-Nielsen, 1984).

ON BEING TALL

Cardiovascular function in the giraffe

The physiology of scaling has rightfully enjoyed widespread interest among physiologists, certainly among comparative physiologists. The hummingbirds being so small have understandably defended an important role in scaling discussions. Scaling refers to correlations with weight, volume and linear dimensions. One dimension which to my mind has been neglected in this interesting discussion is that of length. Both horizontal and vertical lengths of animals are of great interest in physiology, particularly in cardiovascular physiology. Length in the vertical direction is height. If this dimension is large the animal is tall. I want to allude briefly to the physiology of being tall.

In a museum in Berlin, there is a skeleton of a Brachiosaurus, an extinct sauropod that lived 65 million years ago. The skeleton reaches nearly 12 metres high and gives any cardiovascular physiologist awesome thoughts about the hydraulic engineering of such a creature.

Today we can watch living giraffes reaching nearly 6 metre heights as survivors of Nature's experiments on being tall.

Alan Burton's wonderful reading on the Physiology and Biophysics of the Circulation (Burton, 1965), chapter 9, starts with the quotation (Lyly, 1580): "The truth, the whole truth and nothing but the truth". Burton goes on and now I quote him: "It is no harder, in the circulation, for blood to flow uphill than downhill." If this is nothing but the truth, it is also the truth that a full-grown giraffe, 5-6 metres tall, has a systemic blood pressure at heart level about double that of normal man and most mammals. Since the days of Poiseuille and perhaps before, our understanding of haemodynamics has been conceived by clear minds working with rigid glass tubes and coloured water. Much later a pump in lieu of the heart was placed in the glass models, but this pump was typically not pulsatile and if it was, flow in the large major tubes leaving the pump did not come to a halt or reverse between beats such as in the living organism (Spencer and Greiss, 1962).

With collapsible tubes and passive valves between the intermittently beating pump and the tube system, and flow records at the base of the aorta showing reversed and later zero flow in diastole (which does not imply that flow in the lesser arteries and capillaries is halted), "the whole truth and nothing but the truth" must be that when systole starts, the heart must overcome the pressure keeping the valves shut, which must include the height of the fluid column resting on them, a possible back pressure from a more peripheral windkessel function, the resistance of the microcirculation and possibly collapsed veins, before blood returns to the heart by a waterfall effect.

If we next turn to the other end of the giraffe, to the legs where arterial, venous and probably microvascular pressures must far exceed those at heart level and use this to reason about tissue fluid balance, we find that August Krogh did it before us.

What Krogh asked the giraffe is an example of his so-called "visual thinking". Krogh wrote: "It would be extremely interesting to know just how the giraffe avoids filtration oedema in its feet". Krogh tried to find means to study the subject, but only came as far as watching giraffes in the Copenhagen Zoo and verified that they did not have oedema in their feet, except in one instance when he recalled that a giraffe after standing still for a considerable length of time did show some swelling of its feet.

Fifty years later our group asked the same question (Hargens, Millard, Johansen, Gershuni, Pettersson, Burroughs, Meltzer and Van Hoven, 1986). We were able in 1985 to work with the giraffe in Nature's laboratory.

First let us briefly comment on morphological specializations of interest to the question of fluid balance. Peripheral arteries in the extremities have a phenomenally high muscular component giving the arteries a pinhole lumen and a very thick muscular wall. This picture extends down to the microvasculature.

Williamson, Vogler and Kilo (1971) demonstrated that the capillary membrane thickness in giraffe legs is more than twice that typical of mammals. Importantly, this may reduce capillary permeability when going from the neck to the leg. These morphological features may be important in reducing capillary pressures, reducing the effective surface area, the hydraulic conductivity and increase the reflection coefficient for proteins, thus impeding excessive filtration.

In addition, there is a conspicuous subcutaneous connective tissue fascia along the extremities and neck, presumably having an important G-suit effect. The G-suit was thus not at all invented by aviation medicine but by the giraffe. The connective tissue G-suit may also serve to reduce filtration by increasing interstitial tissue pressure and reduce venous capacitance. A well developed lymphatic system will also serve to prevent oedema formation.

Transcapillary fluid balance depends on the four "Starling pressures". Most importantly these are all dynamic and change with time, some more than others. We were able to measure the arterial, venous, and interstitial tissue pressures dynamically using telemetry techniques when giraffes were walking in their corral. Pressures on the neck were simultaneously recorded for comparison.

When these data are plugged into the Starling equation they reveal that in the neck region there should be no problem with oedema formation because a net reabsorption pressure prevails. In the legs, however, using data from a standing giraffe there is a gross imbalance of transcapillary pressures which would favour filtration. When oedema does not appear to happen it must relate to highly variable pressures with movement of the animal.

CEPHALOPODS

Very old members of the jet set

I surmise that more than 90% of all physiological research concerns mammals and more than 95% deals with the vertebrates. Perhaps more than 20% deals with the laboratory rat which Larry Irving refused to consider an animal. The vertebrates represent one animal phylum out of more than twenty. The Director of Research in Nature's laboratory must find this a highly biased choice of experimental subjects by us physiologists.

This striking imbalance must have its basis in the misconception that the mammal and man in particular has a special and important role to play as experimental subject in physiology. I take exception to this and again I would like to quote August Krogh from a lecture he gave at the International Physiology Congress in 1929. The title of the lecture was: "Progress in Physiology". Krogh said: "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 adaptations 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 modern investigations)". Another pertinent quotation goes as follows: "It is virtually a truism that the simplest organism that carries out the phenomenon in question will give the basic answer first".

I would like to deal briefly with some physiological aspects of the cephalopods - the most awesome and agile animals in the oceans. Some squids swim faster than most fishes and travel by jet. Cephalopods apparently represent the largest biomass in water. It is their blood that I want to discuss, blue blood it is, when its respiratory protein haemocyanin (Hcy) is oxygenated.

The O_2 affinity of cephalopod blood is like most bloods pH sensitive as expressed by the Bohr coefficient. No other group of animals, however, exhibits such a large variability in the Bohr factor between species. It may range from -1.80 as Redfield and Goodkind demonstrated 56 years ago for the squid Loligo, down to a modest -0.20 recently shown for Nautilus (Lykkeboe and Johansen, 1983).

The Bohr effect is generally accredited important physiological significance in blood gas transport in that metabolically produced CO_2 will promote unloading of bound O_2 in the capillaries. It is common to assume that this facilitation should be proportional to the size of the Bohr coefficient. However, closer scrutiny puts a large question mark by this generalization, because the Bohr effect is tantamount to an O_2-linked binding of protons (H^+ ions) to the respiratory pigment. This process is referred to as the Haldane effect. According to the linkage equation described by Wyman (1964), the Bohr coefficient and the Haldane coefficients are identical, i.e.

$$\frac{\Delta \log P_{50}}{\Delta pH} = \left\{ \frac{\Delta cRPH}{\Delta cRPO_2} \right\} pH$$

(RP = respiratory protein)

If now, as is the case in many cephalopods, the Bohr coefficient is numerically greater than -1.0 (i.e. the slope is steeper than -1.0), it

implies that more than 1 mmol of hydrogen ions will be bound to the pigment per mmol of O_2 unloaded. This is most important when our discussion is limited to hydrogen ions resulting from aerobic metabolism, since the CO_2 metabolically produced from aerobic metabolism can maximally yield 1 mmol of hydrogen ions per mmol of O_2 consumed, if the gas exchange ratio is unity. In fact if the Bohr coefficient (slope) is steeper than -1.0, perhaps -1.8 as in the case of Loligo, a pH increase rather than a decrease should accompany deoxygenation; i.e. venous blood should have a higher pH than arterial. This would align poorly with the usual reasoning that a large Bohr factor will aid O_2 unloading from the blood. A large Bohr factor may then be a detriment rather than an asset for O_2 unloading.

Or could it be that there exists another source of CO_2 or H^+ ions not anaerobically produced? If it is CO_2, it has to be O_2 linked, in other words be set free on deoxygenation, but independent of pH!!!

We have probed into this problem and can confirm that such a CO_2 binding exists (Lykkeboe, Brix and Johansen, 1980).

The question of whether pH can actually decrease during O_2 unloading even if a high ($\geq$-1.0) Bohr factor exists, may be answered affirmatively after our experiments. A prerequisite for this must, however, be the presence of an oxygen-linked CO_2 component large enough to generate a functional Haldane coefficient (mM CO_2/mM O_2 at constant pH) well below unity. This condition is likely to be species-dependent and relate to metabolic rate and metabolic scope. In Sepia latimanus, a moderately active cephalopod, the condition is marginally present, but in the case of Loligo pealei, a far more active species, it may be more developed.

In the light of these ideas and results it is suggested that we re-evaluate the physiological consequences and significance of the Bohr and Haldane effects. We must expand the traditional and still prevalent viewpoint that metabolically produced CO_2 promotes dissociation of O_2 bound to the respiratory pigment and thus facilitates tissue O_2 transport in proportion to the magnitude of the Bohr coefficient. If large Bohr coefficients are present deoxygenations of the pigment will primarily promote the removal of CO_2 with a minimal change in hydrogen ion concentration (isohydric CO_2 transport). Thirdly, the magnitude of the Bohr effect will be decisive for the influence of a pH change on the O_2 affinity (Lykkeboe and Johansen, 1983).

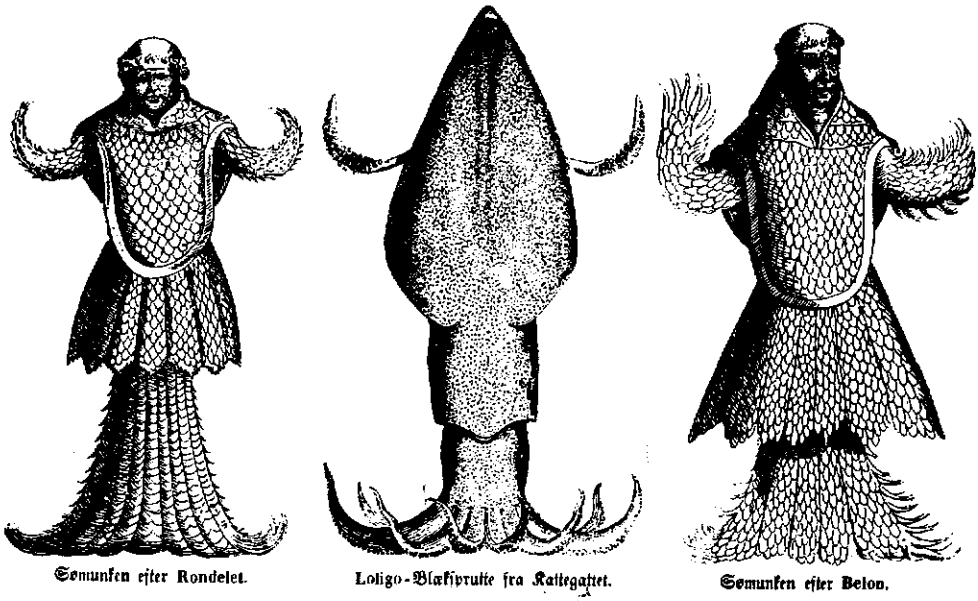

Fig. 1. After J. Steenstrup, 1855.

EPILOGUE

It is my impression that in our day and age the domain of politics dominates everything, also science unfortunately. It has not always been like this. Let me use my fascination with cephalopods to illustrate my point.

In the year 1550 a strange creature was caught in Øresund, the narrow strait separating present day Sweden and Denmark. The creature, nearly dead, was brought before the King (Fredrik II). He had a head like a man and a monk's crown, a bald head with a ring of hair. He also wore a coat like monks did. The King buried the creature with full royal honours. The famous author Holberg (born and raised in Norway, but claimed by Danes as theirs) wrote in his book: The History of Denmark, that the sea monks were the result of the Lutheran reformation when the monks became unwanted and had to take refuge in the oceans. The Danish King Fredrik II must have had similar thoughts because he had sent a drawing of a sea monk to the Spanish Emperor Karl and reassured him that the Catholic faith still blossomed in northern Europe in spite of Luther's reformation. Historians now tell us that because of this reassurance a treaty was signed between Emperor Karl and the Kings of Scotland and Denmark. The treaty is said to have given important stability and peace in Europe at the time.

In our time we as biologists have no influence on political decisions. Nature will go on making experiments, however, and as scientists in that laboratory we will always be behind and will never be out of a job. I wonder what a mermaid is?

ACKNOWLEDGEMENT

I would like to express my gratitude to the Nordic Insulin Laboratory in Copenhagen for their support and funding of the August Krogh Lecture. It is no happenstance that the Insulin Laboratory wished to make this dedication to August Krogh who played such an important role in promoting the understanding of insulin research and the production of insulin.

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