Acute and Chronic Lack of Oxygen: Consequences for the Lung and Carotid Body; A Journey with *Bacillus investigationis*, the Curiosity Bug

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For there are indeed many who openly confess that the greatest part of those things which we do know is the least of the things which we know not.

*(William Harvey, 1628)*

A life-long infection with the curiosity bug has been a great asset to me. It has been the driving force for a research life, has overcome disappointments and frustrations, sustained interest, and also overwhelmed those destructive impulses—the pursuit of fortune and distinction.

I was lucky to have studied physiology in Ernest Starling’s laboratory. It was an exciting place. Starling had died early, but we learned his tradition of controlled artificial circuits for circulatory studies from Charles Lovatt Evans. A. V. Hill ran a biophysics laboratory that attracted brilliant people from all over the world. Wallace Fenn was one of them; Hill had won the Nobel Prize with Otto Meyerhof for studies on muscle heat production and metabolism (Fig. 1A). He taught us to do everything ourselves and make our own apparatus when we could. Bernard Katz, a future Nobel laureate, had just escaped from Nazi Germany; he worked late into the night looking at end-plate potentials with early cathode ray tubes. Tom Lewis of ECG fame taught simple bedside “clinical science.” Contact with these great men...
aroused our enthusiasm and set standards for a lifetime.

It is more than 360 years since Harvey demonstrated that the whole cardiac output flows through the lungs, though he knew nothing of their function and would never see a capillary. His deductions from observations were fantastic. In my student days (1935–42) there was still great ignorance about the pulmonary circulation, and after the Second World War scientists still argued as to whether a membrane separated the alveolar capillaries from air. In 1946 von Euler and Liljestrand cannulated the pulmonary artery directly in cats and showed that pressure rose during ventilation with low O\textsubscript{2} or high CO\textsubscript{2} mixtures. Their great contribution was to suggest that these gases caused vasoconstriction in the lung as opposed to causing a dilator effect, as they did in other organs. Von Euler and Liljestrand’s conclusion was speculation because they could not measure cardiac output; if cardiac output had risen with these stimuli, the result would have been the same. One must be right for the right reason and have supporting evidence, yet speculation is a great stimulus to research. It was soon shown that the Scandinavians had been correct; in controlled isolated perfused lung preparations hypoxia and hypercapnia both caused vasoconstriction. This phenomenon, not yet explained, has been studied from that day to this.

My husband was offered a university post while racing across Europe with the tanks in the final stages of World War II. Thus we arrived in postwar Oxford, and I soon got a job in the Nuffield Institute for Medical Research. This period in Britain has been called a “golden age.” There was no difficulty in funding research; science was valued. In the Nuffield Institute, Geoffrey Dawes and his team were studying the fetal circulation (Fig. 1B). Geoffrey had been named director of the institute before he turned thirty. Under him this laboratory became a centre to which physiologists, pediatricians, and obstetricians came from all over the world; clinical
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problems were considered side by side with basic physiology.

After the Second World War right heart catheterization made possible direct measurement of pulmonary arterial pressure in humans. Clinicians measured pulmonary vascular resistance as the ratio of pulmonary artery pressure to cardiac output or that of pulmonary artery-wedge pressure to cardiac output. However, the important work of Alan Burton taught us that this ratio is an unreliable guide to haemodynamic changes. One needs to measure the relation between pressure and flow over a wide range; if the line is curved or forms an intercept on the pressure axis, the ratio will vary passively due to simple geometry. If there is a true vaso-motor or mechanical change, then the whole line will shift.39

We began to study pulmonary haemodynamics in the anaesthetized open-chest cat.* We inserted a tall column into the left pulmonary artery (Burton’s method)39 and allowed it to fill with blood and then empty through the lung. From the pressure at the base of the column and the cross sectional area of the tube, we could calculate blood flow. Figure 2 shows some results; the line moved from A to B in a parallel fashion when we raised tracheal pressure.12 The young Dr. Grant Lee remarked perceptively that the shift “looked like an obstruction in the vascular bed.” In a nearby laboratory Banister and Torrance were perfusing isolated cat lungs. They showed that, when tracheal pressure was raised above left atrial pressure, flow was unaffected by left atrial pressure until it exceeded tracheal pressure. In this circumstance tracheal pressure became the downstream pressure for flow, which now depended on pulmonary artery pressure-tracheal or alveolar pressure and not pulmonary artery-left atrial pressure. They introduced the idea of sluice flow.2 Per-mutt and Riley in Baltimore were also showing that the driving pressure for blood flow through the lung was not always pulmonary artery-left atrial pressure. They showed that small collapsible vessels with muscle tone could act like the resistors used by Starling in his heart-lung preparation.40 Starling’s resistors consisted of a collapsible rubber tube inside a glass tube. The space between the tubes could be raised to any “surrounding pressure,” and this formed the outlet or downstream pressure; the inner tube fluttered open and shut with flow. The great contribution of the Baltimore group was to show that small muscular vessels could behave like Starling resistors and that tone in their walls could act as the surrounding pressure, if it was effectively greater than left atrial pressure. They called this “waterfall flow” and showed that it corresponded

*All experiments on living animals were conducted under full anesthesia.
with Burton's "critical closing pressure."15,35

Waterfall or sluice flow is a useful explanation but not necessarily the sole explanation for events in the pulmonary circulation. In an artificial circuit, the pressure-flow line can be shown to change its slope or resistance if the tubing is narrowed with a screw clip but to shift in a parallel manner, as in Figure 2, if the surrounding pressure in a Starling resistor is raised.

Changes in Pulmonary Vascular Resistance in Collapsed Lung

Geoffrey Dawes's team was showing that blood flow through the fetal lung varied tremendously when the ewe was ventilated with high CO₂ or low O₂ mixtures. He suggested that it would be interesting to compare the adult collapsed lung with the fetal unexpanded lung. We needed a reliable instrument to measure blood flow, and our physicist, Derek Wyatt, designed an electromagnetic flow meter that has played a crucial role in our research and has never been equaled or surpassed by commercial instruments.52 We inserted this flow meter into a loop in the left pulmonary artery of cats. The two lungs were separately ventilated, the left with O₂. When the left tube was occluded, the gases were rapidly absorbed and the lung became solid like liver in about one minute. The rapidity of collapse dismayed visiting pediatricians, for they were still using high O₂ mixtures for babies. Figure 3 shows that the blood flow to the collapsing lung fell rapidly when ventilation ceased.4 Pressure-flow lines were obtained by progressively occluding first the right and then the left pulmonary artery; they moved to and fro with collapse and reexpansion (Fig. 3, right). We tried to estab-

Figure 3. Reduction in blood flow in a collapsed lung and changes in pressure/flow lines. Left: Blood flow in the left pulmonary artery of an anaesthetized cat, measured with an electromagnetic flow meter, falls rapidly when ventilation to the left lung ceases. Right: Pressure/flow line measured by progressive occlusion of first the left and then right pulmonary artery shifts when the lung is collapsed, returns on reexpansion, and shifts again on recollapse. (Compiled from reference 4).
lish the cause of flow reduction. Was it due to gas tensions in the unchanged venous blood flowing through the lung, to mechanical changes, or to release of vasoactive substances?

**Hypoxia and Lung Vessels**

There was intense interest at the time in hypoxic pulmonary vasoconstriction. It had been demonstrated to be a local mechanism in isolated perfused cat lungs under controlled conditions. We began to study the phenomenon in anaesthetized cats in vivo. The left lung was supplied with blood by a connection to the left carotid artery, in which flow and pressure were measured. An adjustable clip reduced the pressure to pulmonary arterial levels and enabled us to keep blood flow constant. Thus, if left atrial pressure remained constant (and it did), rises in pressure in the loop meant that vasoconstriction was taking place in the lung. Ventilation of the cat with 10% O₂ caused sustained and reproducible increases in pulmonary arterial pressure, which still took place after removal of the adrenal glands (Fig. 4, top). Vasoconstriction caused by hypoxia is surprising, because lack of O₂ is usually associated with negative effects. We set out to see whether vasoconstriction was caused by release of a transmitter, which in turn would cause vascular smooth muscle to contract. We found that several "α catecholamine in-

![Graph showing hypoxic vasoconstriction effects](image)

**Figure 4:** Hypoxic Vasoconstriction: Effect of α Catecholamine "Blockade" Anaesthetized cats. Left pulmonary artery supplied from left carotid artery; pressure reduced to pulmonary levels, and blood flow, measured with an electromagnetic flow meter, kept constant. Above: On ventilating cat with 10% O₂ between arrows, pulmonary arterial pressure rose, also after adrenalectomy. Below: Pressure rises during hypoxia, during bars, but this effect is slowly lost after 10 mg/kg phenoxybenzamine. (Compiled from reference 4.)
hibitors” (the terminology used at that time) abolished hypoxic vasoconstriction (phenoxybenzamine in Fig. 3, bottom). Also, α inhibitors decreased and β inhibitors increased pulmonary vascular resistance; we were excited and thought we had found the transmitter. Yet we had trouble with the paper on this work, which we had submitted to an eminent journal. It was returned with rude remarks. We were rescued, however, by a timely visit from Dr. A. P. Fishman, whose fine literary talent helped make it acceptable.

Unfortunately, soon after the paper was published, our hypothesis was shattered by two papers from Anton Hauge in Norway. They seemed to show conclusively that histamine was the transmitter in the rat. However, testing his hypothesis we found that the antihistamine (H1) inhibitor he had used in rats did not abolish hypoxic vasoconstriction in either cats or dogs. Indeed, given to rats during hypoxia, histamine caused dilatation. We then found that the α catecholamine inhibitors, so promising in cats, were ineffective in dogs and rats. So we had both been wrong. We became friends and learned the limitations of pharmacological “blockade.” Differences can be instructive! Another lesson was that species differ profoundly. Over the 45 years since this phenomenon was discovered many transmitters have been proposed, but all so far have been discarded. Yet the pharmacological inhibitors used have been relatively specific in that they abolished hypoxic but not other forms of pulmonary vasoconstriction. There is surely some clue here.

Hypoxia and the Lung in Health, Disease, and at High Altitude

In the 1960s I continued work on hypoxia in the Department of Medicine in Sheffield. Charles Stuart-Harris, head of the department, was studying chronic bronchitis and emphysema in that formerly very polluted city (Fig. 5). I still thought of this disease as smoker’s cough, but Stuart-Harris was showing the lethal nature of this terrible smokers’-pollution disease. It was valuable to compare physiological mechanisms in the lung with disordered function in disease. I owed my opportunity to Stuart-Harris’s vision that one should study patients and fundamental mechanisms side by side. Many of his patients were extremely hypoxic, so much so that one marveled at their survival; one patient walked into the laboratory, and his arterial PO₂ was measured as 28 mmHg, close to estimated value on the summit of Everest. A further widening of my horizons came when a mountaineering colleague, John Clegg, arrived with a hypobaric chamber in which we started to keep rats and mice. We began to consider hypoxia of the lung in many contexts, its role in the healthy fetus and in the adult and its effects on mountaineers and high-altitude dwellers and in our laboratory animals. We looked for similarities and differences. Why, for example, do the pa-
tients with hypoxic lung disease, aptly named “blue bloaters,” die, whereas dwellers at very high altitudes are capable of sustaining heavy exercise? What is the cause of the mountain sickness so common among climbers? Our laboratory animals were subjected to conditions that mimicked these states.

**Role of Blood-Gas Tensions in Unventilated Lung and Response Curves of Lung Vessels to Hypoxia and Hypercapnia**

We tried to find out whether hypoxia caused blood flow changes in collapsed lungs. In cats we measured blood flow in a loop in the left pulmonary artery, which had a connection to the left carotid artery. On occlusion of the left bronchus during normal perfusion with venous blood, the flow to this lung fell rapidly as before. When we switched to arterial blood at the same pressure, the control flow rate was almost restored, and it fell again on returning to the venous supply. Pressure-flow lines shifted during collapse, as in Figure 3, but returned nearly or wholly to the control position during continued collapse with arterial blood perfusion (Fig. 6, bottom). Thus some property of venous blood rather than a mechanical effect caused flow to fall; the low $P_{O_2}$ in venous blood was a likely candidate.

We set out to determine the quantitative relationship between blood flow and blood-gas tensions. We devised circuits in which the left lower lobe of lung was autoperfused under controlled conditions and separately ventilated with different gas mixtures. In dogs and cats we put a loop into the vein, draining this lobe in which we could measure flow and pressure and sample blood for gas tensions. When we ventilated this test lobe with decreasing $O_2$ concentrations or increasing $CO_2$ concentrations, each step led to a fall in blood flow (Fig. 7), but there was little or no change in pulmonary arterial pressure or left atrial pressure. From measurements of blood-gas tensions in the effluent blood we were able to plot the relationship between blood flow % control and both $PO_2$ and $PCO_2$. Hypoxia led to an S-shaped curve. There was a slow fall in flow above normal oxygen tensions followed by a steeper fall within the physiological range. The arteriovenous difference in $PO_2$ caused a mean 47% fall in flow in cats, rather less in dogs; this reduction would be expected in unventilated areas of lung. There was a curvilinear relationship between flow and $PCO_2$, but the arteriovenous difference was associated with only a 6% fall in flow. Thus one would expect hypoxia to be the most important factor in ventilation-perfusion matching.

We had problems with this paper as well. A referee for one eminent journal asked, “Why did you try two species?” Such miseries have to be borne. Another important journal took the paper at once. Figure 8 shows similar curves measured during hypoxia in ferret lungs in vivo. On the left are flow-$PO_2$ lines, and on the right are lines relating pulmonary arterial pressure to $PO_2$ in a second preparation; the lobe was perfused with blood drawn from the right atrium at a constant flow rate. These curves show the expected changes in pressure if the whole lung becomes hypoxic, as in an hypoxic environment at high altitude or when insufficient oxygen enters the body because of lung disease. The curves on the left, like those in Figure 7 for the cat, show how local hypoxia may divert blood away from hy-
Figure 6. Effect of collapse of a lung perfused with arterial or venous blood: Above: Anaesthetized cat. Measurements of blood flow and pulmonary arterial pressure in a loop in the left pulmonary artery. A connection to a carotid artery permits perfusion with arterial blood. Blood flow falls on bronchial occlusion, while the lobe receives its normal pulmonary arterial supply (venous blood). A switch to carotid arterial blood at the same pressure restores flow, while a return to venous blood again reduces flow. Below: Anaesthetized dog. Pressure/flow lines measured during ventilation or collapse during perfusion with arterial or venous blood as in cat, above. Ventilated, arterial (○), venous (●), Collapsed, venous (■), arterial (●), venous (□). Reexpanded, venous (■).
Figure 7. Relation between blood–gas tensions and local blood flow. Anaesthetized cats. Blood flow and gas tensions were measured in a loop in the left lower lobe pulmonary vein: pulmonary arterial pressure was measured in the main pulmonary artery. Above: A, test lobe ventilated with increasingly hypoxic gas mixtures (at arrows); flow fell at each change. B. PO$_2$ in effluent blood, measured at 1, 2, 3, etc., is plotted against blood flow, % control. Below: A, test lobe ventilated with progressively higher concentrations of CO$_2$ in air (at arrows); B, PCO$_2$ is plotted against flow, % control. (Reproduced with permission from reference 11.)
Figure 8. Local hypoxia causes flow diversion; general hypoxia raises pulmonary artery pressure: ferrets. A: 6 ferrets. Blood flow % control related to alveolar PO₂ (measured by mass spectrometry), circuit as in Figure 6; flow is diverted to better ventilated lung in local hypoxia. B: 4 ferrets. Venous blood from right atrium is pumped at constant flow to left lower lobe pulmonary artery. Lobe ventilated with decreasing O₂ concentrations. Pressure rises with each change in PO₂ as in generalized hypoxia in the lung. (Reproduced with permission from Barer et al. J. Physiol 281: 40–41P, 1978.)

We compared the stimulus-response curves to hypoxia and hypercapnia with the changes seen in collapsed lungs (Fig. 9). If lungs were ventilated with oxygen briefly before collapse, we noticed in both cats and dogs that the reduction in flow after bronchial occlusion took place in two phases. In phase 1, PO₂ remained high as there was a store of oxygen, whereas PCO₂ rose and pH fell; phase 2 coincided with a fall in O₂ tension. We measured gas tensions in the two phases and compared that reduction in flow at these times with the reductions caused by hypoxia or hypercapnia of similar degree in the same lobe. The lower part of Figure 7 shows that the flow changes in phase 1 of collapse fitted well with changes caused by hypercapnia, whereas on the right a good correlation is shown between the reductions in flow in phase 2 of collapse and those due to hypoxia. It is probable, therefore, that the effect of collapse under these experimental conditions is due to the combined effects of hypoxia and hypercapnia.

Is this mechanism important in adult humans? At first thought, the very low PaO₂ seen in patients with hypoxic lung disease suggests that it is not. However, the damage to their lungs is so widespread that a shutdown of all affected areas would not be possible; the whole cardiac output must pass through the lung. Marshall and Marshall were later to show in dogs that hypoxic diversion of flow becomes less effective the larger the area that is made hypoxic; it is replaced by a rise in pressure, as we saw in Figure 8, right. Also, in these patients bronchoconstrictor drugs that are also vasodilators (aminophylline, isoprenaline) sometimes reduce arterial PO₂; this could be due to an increase in blood flow through poorly ventilated areas of lung. Yet the view is held by some that hypoxic vasocostriction in adults is only a vestige of an important fetal mechanism; in the fetus it diverts blood flow away from the non-
ventilated lung through the ductus arteriosus and towards the umbilical artery. Yet diversion of blood flow from hypoxic lung regions has been clearly shown in humans, and in certain postures changes in ventilation-perfusion measurements during hyperoxia suggest redistribution of flow. In humans generalized hypoxia certainly leads to a rise in pulmonary arterial pressure. In hypoxic sleep apnoea, pressure also rises. In our experiments the changes in blood flow with gas tensions were of the same order as those found in fetal lambs. So there is probably some redistribution of flow in favour of better ventilated regions, although calculations have shown that the gain of the mechanism is not great. Without evidence I am predisposed to think that when we doze in lectures, some lung units may close and their blood flow diminish, to open again when we yawn.

**Chronic Effects of Hypoxia on the Pulmonary Circulation**

In the 1970s we began to study the effects of prolonged exposure to hypoxia in rats and mice. We hoped to throw light on changes in patients and highlanders and perhaps to find means of preventing or reversing them. We used either a hypobaric chamber at half an atmosphere or a normobaric chamber at 10% or 12% O₂; CO₂ levels were kept normal. We detected no difference between results with the two...
chambers. We looked first to see whether the arteries had become more muscular. There seemed to be a change in wall thickness in very small vessels, but we could not pinpoint it in routine sections. After more than a year of effort, a pathology technician suggested an elastic stain might be helpful. We used one and immediately saw that the small pulmonary arterioles, which are normally thin-walled with little or no muscle and only one elastic lamina, had developed a second internal elastic lamina bounding a new muscular coat. Later measurements with the lung perfused with contrast medium at known pressures showed that the lumen was narrowed 10–14%, a change with profound haemodynamic consequences. We sent a slide to Professor Donald Heath, who replied that the changes closely resembled those he had found in his bronchitic patients. This was the first of many fruitful exchanges. The crucial observation was that there was peripheral extension of new muscle to tiny vessels beside alveolar ducts and among alveoli. The proportion of these “thick-walled peripheral vessels” increased several fold in chronically hypoxic rats and mice. Also the right ventricle became hypertrophied, the packed cell volume increased, and pulmonary arterial pressure rose fully developed after 10 days hypoxia but was still raised after 6 weeks in air; after 20 weeks recovery we could still detect some residual abnormalities in these vessels. So there was rapid remodeling but slow resolution, which has implications for disease states and recovery. Yet the right ventricular hypertrophy and polycythaemia resolved more quickly. It seems that different growth factors and stimuli cause different changes. This is an area of great current interest and importance. During the events and illnesses of life our bodies are subject to changes of which we are quite unaware.

Some diverse observations on these rats may bear fruit in the future. Growth changes observed during hypoxic exposure might be relevant to hypoxic episodes during growth in humans. The lung and chest grew abnormally fast relative to body size, for skeletal growth and weight increase were retarded. In young animals, lung size did not return to “normal” after recovery in air. Then, with possible relevance to clotting problems at high altitude, platelet volume was increased, a change associated in other circumstances with increased platelet activity. Also the megakaryocytes in bone marrow from which platelets are derived showed an increase in polyploidy. We also found that the endocrine cells in the conducting airway and alveolar walls contained more calcitonin-gene-related peptide than normal. We have no idea what this could mean; indeed we have no notion of the function of these endocrine cells.

Haemodynamic consequences of hypoxic exposure were demonstrated by measurement of pressure-flow relations in isolated blood-perfused rat lungs; left atrial pressure was zero. Figure 9 shows mean lines for groups of rats measured during air ventilation (look only at lines CS and CHS; the other lines are described below). Note that by this time we plotted the lines correctly, with flow as the independent variable on the abscissa!
Compared with control rats, line CS, the line for chronically hypoxic rats, CHS, is steeper and has a larger projected intercept on the pressure axis. We attribute the intercept to activity of the small, newly muscularized vessels in a state of tone, which now forms the downstream pressure for flow. The increased slope, which is a measure of resistance in larger vessels as opposed to resistor properties of small collapsible vessels, could have several causes. We found arterial compliance reduced; the vessels were stiffer, which could be due to deposition of new connective tissue in the walls of larger vessels. Alternatively the volume of the vascular bed could be smaller. Pressure in the rat and human pulmonary circulations is similar, although the flow in the latter is at least 100 times greater. However, vascular volume measured with $^{125}$I-labeled serum albumin was similar to normal rats; this is an expression of the relatively large lung size in these rats. In these tests, both groups of lungs were perfused with normal blood of normal hematocrit; in chronic hypoxia there was polycythemia, which made a substantial contribution to vascular resistance, as we found when we varied the hematocrit artificially.\(^8\)

Like others, we tried to find drugs that would prevent or reverse the high pressure and structural changes of chronic hypoxia. We had some success with $\alpha$-methyl dopa, which, given daily during exposure, attenuated pulmonary hypertension, right ventricular hypertrophy, and muscularization of arterioles.\(^28\) The basis of this effect is obscure. More exciting was a drug brought to us by Professor Yingnian Cai from Beijing. It was ligustrazine, synthesized principle of an ancient Chinese herbal remedy grown in peasants' gardens and used for more than 2,000 years for "heart disease." It proved to be a potent pulmonary vasodilator. When given daily during hypoxic exposure, it attenuated the right ventricular hypertrophy, pulmonary hypertension, and vascular changes.\(^16\) Figure 10 shows the results. There were four groups of rats: controls treated with saline (CS), controls treated with ligustrazine (CL), chronically hypoxic rats treated with saline (CHS), and chronically hypoxic rats treated with ligustrazine (CHL). The saline-treated

![Figure 10. Altered pressure/flow lines in chronically hypoxic rat lungs: the effect of ligustrazine. Mean pressure/flow lines from four groups of rats measured in isolated blood-perfused lungs. (1) CS 5 control rat lungs, treatment with 0.9% saline (placebo); (2) CL 5 control rats treated with ligustrazine; (3) CHS 11 rats exposed to 10% O$_2$ for 2 weeks treated with saline; (4) CHL 11 rats similarly exposed treated with ligustrazine. Note steeper lines and higher intercept on pressure axis in CHS compared with CS rats. Ligustrazine has no effect in control rats, but in chronically hypoxic rats the intercept is normal, the slope unaltered. (Reproduced with permission from reference 16.)](image-url)
lines have already been described; they are similar to those always found in normal and hypoxia-exposed rats. The controls treated with the drug had an unchanged line, but the chronically hypoxic treated rats had a line whose slope was unchanged but whose intercept had returned to control values. Thus only one feature of the remodeled vascular bed was altered, and we expected that this would prove to be the muscularization of arterioles. Indeed our count of thick-walled peripheral vessels in the ligustrazine-treated chronically hypoxic rats was significantly less than that in the saline-treated group. This very interesting finding requires further confirmation. The unchanged slope suggests that changes in larger vessels, perhaps stiffening due to connective tissue proliferation, was undiminished.

**Reactivity of the Pulmonary Circulation in Chronically Hypoxic Rats**

We looked to see whether the chronically hypoxic rat pulmonary circulation had abnormal responses to natural stimuli and drugs. In the perfused lung of normal rats, almost no tone can be demonstrated, as in the normal human lung—that is, vasodilator drugs have little or no effect unless there is preconstriction. In the chronically hypoxic rat, as in human hypertensive pulmonary disease, there is resting “tone,” and dilatation is easily shown. We perfused the lungs with blood and defined reactivity as the rise or fall in pulmonary arterial pressure at constant flow. This is a superficial definition, but it describes the change the right heart experiences during systole. Responses to vasoconstrictor substances (angiotensin I and II, PGF2α), dilator substances (isoprenaline, adenosine) and substances with mixed effects (bradykinin, ATP, histamine, arachidonic acid, platelet-activating factor) were enhanced. Increased muscularity, increased tone, and narrowed lumen could account for most of these changes. However, there were peculiar changes in hypoxic vasoconstriction. We found in young rats of our strain an increased response to hypoxia over a wide range of oxygen tensions, as in Figure 11. However, the group in Denver and Dr. Kentera in Belgrade found exactly the opposite. Responses to hypoxia were attenuated, despite more muscular vessels. Once again differences stimulated further research, and it became clear that enhancement or attenuation depended on the age and strain of rat and on the length of hypoxic exposure. An exaggerated response was always found after a few days’ recovery and a diminished response after only 36–48 hours of hypoxic exposure. It seems that there is a balance between the effects of increased muscle and metabolic changes. Metabolic changes could be due to changed activity of the endothelium adjacent to the new muscle, to special properties of the new muscle, or to altered numbers or expression of receptors. Reactivity changes could have important consequences during development of pulmonary vascular disease.

**Oedema of Hypoxic Exposure**

We tried hard to reproduce the forms of oedema associated with hypoxia. Mountaineers and high-altitude residents are sometimes afflicted by pulmonary oedema that can be fatal. Patients with hypoxic lung disease frequently develop systemic oedema. Our rats exposed to 10 or even 8% O2 did not develop oedema, nor, when we measured fluid and sodium balance, could we detect retention of either. However, when we tried to mimic the circumstances in which mountaineers and highlanders get pulmonary oedema, we had partial success. Highlanders who spend some time at sea level and then
Figure 11. Changed reactivity to hypoxia in chronically hypoxic rats. Relation between effluent blood $P_{O_2}$ and pulmonary arterial pressure in control (open symbols, solid line) and chronically hypoxic rats (closed symbols dashed line) in isolated blood-perfused lungs during ventilation with increasing hypoxia; four $O_2$ mixtures, represented by different symbols, were used. Points represent mean pulmonary arterial pressure and mean $P_{O_2} \pm$ SEM for each level of hypoxia. (Reproduced with permission from reference 20.)

return home are prone to pulmonary oedema. We kept rats for several weeks in hypoxia, allowed them to recover in air for a few days, and then returned them to the hypoxic environment. They developed oedema of the alveolar wall but not florid oedema in alveolar spaces. Mountaineers are susceptible to oedema when they first ascend to high altitude. So we placed unadapted rats in severe hypoxic surroundings for a few hours and found that the endothelium of alveolar capillaries became detached and formed bullae that obstructed the capillaries. Professor Heath’s group had also made this observation.

We never succeeded in causing systemic oedema but made observations that might provide a clue for future research. The prevailing view has been that the systemic oedema of chronic hypoxic lung disease is due to right heart failure secondary to pulmonary hypertension. Yet the cardiac output remains normal until near death, and the hypertension is mild. The Sheffield group found in the 1950s that these patients had an unexplained reduction in renal plasma flow. An alternative hypothesis emerged that fluid and sodium retention could be due to disturbance of mechanisms that control water and sodium balance. Honig had shown in cats that hypoxia caused a diuresis and natriuresis, which was due to a carotid body reflex; the efferent limb of the reflex seemed to involve release of a hormone. He also deduced from the literature that in severe hypoxia there is, by contrast, water
and sodium retention. Karim et al. have shown this in dogs and think it also due to a carotid body reflex, which in this case causes a reduction in renal blood flow through sympathetic nervous activity. In the 1970s we had been introduced to the extraordinary drug almitrine, which has the unique property of mimicking the positive actions of hypoxia both at the carotid body, where it causes reflex stimulation of respiration, and in the pulmonary circulation, where it causes vasoconstriction (though this action is more complex). Honig had shown that almitrine also caused natriuresis in cats. In our group P. Bardsley extended these observations and showed that almitrine causes a reflex diuresis and natriuresis abolished by section of the carotid nerve in rats. Because the effect is seen in rats with only one transplanted and therefore denervated kidney, the effect must be attributed to a hormone. Could it be, in severe hypoxic disease, that the natriuretic reflex is overwhelmed by the one in which there is reflex neural reduction in renal blood flow?

The Carotid Body in Hypoxia: Structure and Function

The carotid body and the pulmonary vessels are the only sites known where hypoxia causes a positive change, an increase in ventilation and vasoconstriction. Both tissues are derived from branchial arches, and several functional links between them have been detected whose importance remains obscure.

In those humans with hypoxic lung disease or who live at high altitude, the carotid body is abnormally large. We found similar enlargement in rats exposed either to hypobaric or normobaric hypoxia. Initially we did this work with Donald Heath’s group. Figure 12 shows gross enlargement in a chronically hypoxic rat. The blood vessels are greatly dilated. Using morphometric techniques derived from the work of Ewald Weibel, we found other important changes. The number of Type I and endothelial cells and the amount of connective tissue increased; angiogenesis occurred. The harmonic mean distance between the capillaries and glomus tissue, a measure of diffusion distance, was reduced.

While I was reporting this work at a symposium on the carotid body, someone shouted out, “You are wrong. Type 1 cells are neurons; they can’t divide.” I am glad to say that we identified cell division in Type 1 cells after 1–4 days hypoxia by arresting mitosis in metaphase with vincristine, as shown in Figure 9. The cells are identified by their dense-cored vesicles, which contain dopamine. We do not know what these growth changes mean, but they suggest that the carotid body is a labile organ. Its size was reduced but not normal after 4 weeks in air.

A characteristic of long-term residents at high altitude is that they have a blunted ventilatory response to hypoxia. Figure 13 shows that our rats, immediately after removal from the hypoxic chamber, also had a response curve to hypoxia that was lower than that of the controls. Moreover, the relationship between almitrine dose and ventilation was also lower. We were excited about this, as it seemed to reproduce the high-altitude state. Disappointingly, the hypoxic response returned to normal after a few days in air, so we do not know if the reduced response is the beginning of “blunting.” We did, however, observe that dopamine, an inhibitor of carotid body stimulation by hypoxia, was present in increased quantities in the carotid bodies of these rats. When we gave normal and chronically hypoxic rats a dopamine 2 inhibitor, domperidone, ventilation increased in both groups. The increase was greater in the chronically hypoxic group. The difference between chronically hypoxic and control rats was wiped out, and response curves to
both hypoxia and almitrine were superimposed. Several explanations have been advanced for the blunted ventilatory response to hypoxia at high altitude; a local dopaminergic mechanism is yet another.

Site of Hypoxic and Almitrine Vasoconstriction in Normal and Chronically Hypoxic Rats

In the 1980s we examined the effect on hypoxic vasoconstriction of inflating the lung to high alveolar pressures. One must admit that the motive for conducting experiments is often evident in retrospect when, at the time, one was just following one's nose. Looking back I believe we thought that, when alveolar pressure exceeds left atrial pressure, any vasomotor effects detected would be occurring in upstream vessels. We measured pressure-flow lines in isolated perfused rat lungs during lung inflation to an alveolar pressure of 5 and then 15 mmHg while the left atrial pressure was ≤ 0.50 Figure 14 shows mean lines for groups of normal rats on the left and chronically hypoxic rats on the right. The solid lines, measured during normoxia, shift about 10 mmHg in a parallel fashion when the alveolar pressure is

Figure 12. The rat carotid body in chronic hypoxia. Left: A, light photomicrograph of normal rat carotid body. B, section of carotid body from rat exposed to 10% O₂ for 4 weeks (Note enlargement and dilatation of capillaries). Right: Electron micrograph of Type I cell from carotid body of rat exposed 2 days to 10% O₂, identified by dense-cored vesicles (small arrows). Chromosomes are lined up on spindle fibres (s). Mitosis arrested in metaphase by vincristine. (Compiled from references 13 and 17.)
Figure 13. Attenuated Ventilatory Responses to hypoxia and almitrine in chronically hypoxic rats. Ventilation measured under anesthesia immediately after removal of rats from hypoxic chamber (exposure 2–5 weeks at 10% O2) and in control rats of comparable age. Initial ventilation was similar in the two groups; % increase in $V_E$ with the stimulus is plotted. For each experiment the control rats showed greater increases in ventilation (upper lines). Above: relation between PO2 during progressive nonisocapnic hypoxia and increase in ventilation in 8 control and 10 chronically hypoxic rats. Below: relation between almitrine dose (cumulative doses during continuous infusion) and increase in ventilation in the same groups of rats, breathing air, CO2 not controlled. (Modified from reference 49.)

Raised from 5 to 10 mmHg, although the chronically hypoxic rats have steeper slopes and higher intercepts. Note that in the controls the intercepts are very close to alveolar pressure, which therefore forms the downstream pressure. In chronically hypoxic rats some muscular action must be causing the higher intercepts. During hypoxic vasoconstriction, dashed lines, the lines at the lower alveolar pressure, become steeper, with a higher intercept in both groups of rats attributable to muscle action. At the high inflation pressure of 15 mmHg, the two groups respond very differently. In controls the line shifts by much less than 10, whereas in chronically hypoxic rats it shifts by greater than 10 mmHg.

Virtually identical results were found during almitrine vasoconstriction. A possible explanation is illustrated in a diagram that we owe to a most helpful correspondence with Dr. Richard Riley. It shows that, in a normal rat, hypoxia or almitrine may cause constriction in small, collapsible vessels upstream from alveoli to form Starling resistors and become the downstream pressure. A rise in alveolar pressure is not transmitted to pulmonary arterial pressure until it is high enough to open up the Starling resistor and become once more the downstream pressure. Note that the intercept has come back to about 15 mmHg in the control group. However, in the chronically hypoxic rat, the new smooth muscle extends down to tiny pre-capillary vessels, which are surrounded by alveoli. During vasoconstriction these vessels form Starling resistors and create the downstream pressure. The high alveolar pressure sums with the muscular activity in these vessels and in some way enhances it; this pressure is fully transmitted to pulmonary arterial pressure. This explanation fits the facts and implies that in chronic hypoxia the main site of hypoxic and almitrine vasoconstriction has moved peripherally to the newly muscularized arterioles. However, some anomalies remained, which are evident in our paper.50 The implications of this change for pulmonary function cannot be imagined at present. In hypoxic lung disease and also in chronically hypoxic rats,9 the lung is held at a higher volume than normal so that
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Moncada and colleagues have shown that the endothelial-derived relaxant factor, nitric oxide, is continuously released and attenuates pressure. Is it responsible for the low vascular tone of the normal pulmonary circulation? Some years back it was suggested that this low tone might be actively maintained. Our results so far suggest that continuous release of nitric oxide cannot be the cause. Synthesis of nitric oxide from L-arginine in endothelial cells can be inhibited by L-arginine analogues. Two such analogues had trivial effects on pulmonary arterial pressure in normal rat lungs ventilated with air but raised pressure substantially in lungs of chronically hypoxic rats. Perhaps some change in the chronically hypoxic hypertensive rat lung stimulates release of nitric oxide, which then attenuates pressure through a negative feedback. If endothelium is damaged in pulmonary vascular disease, could the loss of this restraining effect of nitric oxide be responsible for the precipitate rises in pressure sometimes encountered? In severe pulmonary vascular disease, dilator therapy may be required, for example, in preparation for lung transplantation. We may need dilator drugs that are endothelial independent. Our Chinese herbal remedy proved to be endothelial independent!

Vale!

As the journey nears its end one should take stock. Luck, help, the patience of great teachers, and constant support of a life partner have made a research life possible. Above all it has been fun, but has anything substantial been achieved? Individually very little, but with the efforts of fine colleagues and good friends the world over, we have made progress. We have not solved hypoxic vasoconstriction in the past 45 years, but we no longer think that alveolar capillaries are in direct contact with air. Looking back, what was better
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and what was worse 40 years ago? Apparatus is now more complex and difficult to maintain and design oneself. Statistics, which then took weeks to calculate, now take minutes, but the maxim remains true that in biology if an effect is "genuine" statistics are unnecessary. Free exchange of ideas, so crucial to science and medicine, was once taken for granted. I believe it must now be defended. It retreats a little before severe competition for grants and jobs; equipment is much more expensive, and it is tempting to keep one's ideas secret to be "first." Then in Britain there is ALF. The Animal Liberation Front attracts criminals prepared to kill humans, steal animals, and "liberate" them in midwinter, only to be destroyed by predators. But it also attracts civilized people who teach us to consider animal suffering as we do our own. In every experiment human and animal rights must be balanced.

The curiosity bug will never be satisfied, and our ignorance remains very great.

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