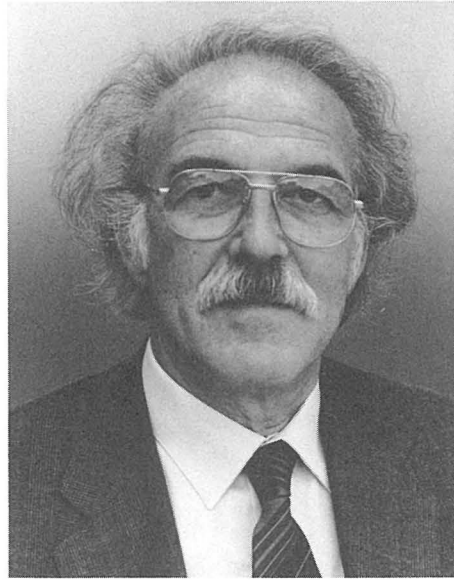


Exploring the Structural Basis for Pulmonary Gas Exchange

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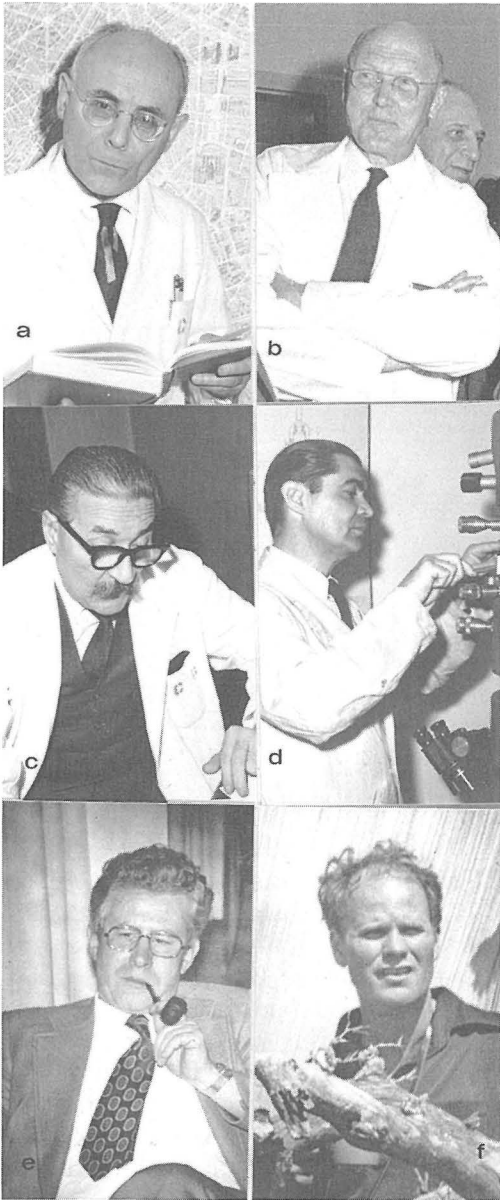
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My story begins in the summer of 1959 when I joined, as a research fellow, the group of André F. Cournand and Dickinson W. Richards in the famous “C 6” Cardiopulmonary Laboratory of the Columbia University Division at Bellevue Hospital in New York City. This job had been offered to me in February of the same year following a seminar on the anatomy of collateral circulation to the lung that I gave at Bellevue on invitation by André Cournand. Relations between myself and Cournand had been strained, because a year before I had irritated Cournand—who had been awarded the Nobel prize in 1956 and clearly knew his worth—by taking a fellowship with Averill A. Liebow at Yale University instead of with him. I had written to Cournand from Switzerland in 1957

but never got an answer because, apparently, the U.S. Postal Service could not find this famous man in New York to deliver my letter. By the time I got a positive reply I had already committed myself to spending two years at Yale conducting experiments on the development of collateral circulation to the lung, a project in line with my previous research at the anatomy department in Zürich under the direction of Gian Töndury.

André Cournand was not a man to give up. Following my seminar he called me into the office of Dickinson W. Richards, Director of the First Medical Division, and bluntly said that I should leave Yale prematurely and come to work at Bellevue. Surprisingly, they offered me a substantial supplement to my modest fellowship,



(a) André F. Cournand (1895–1989); (b) Dickinson W. Richards (1895–1973); (c) Domingo M. Gomez (1901–1978); (d) George E. Palade (1912); (e) Hans Bachofen (1938); (f) C. Richard Taylor (1939).

which was highly welcome because at Yale my wife and I lived in a shabby apartment without a bathroom and ate junk food like Chef Boyardee ravioli to make ends meet. But what really pushed me to take the

job—and, in turn, irritate Averill Liebow—was not the money but the task, for when I asked Cournand what he expected me to do, the reply was, “Do anything on the structure of the lung that is of interest for physiology.” My mind was immediately made up because that was such a tremendous challenge. Back at Yale I worked frantically to complete my experiments and write a manuscript,⁴⁷ and, in preparation for my work in New York, developed a method for lung fixation by formalin steam that would be “more physiological” than conventional fixation.⁶⁴ Then I bundled my things and traveled with my wife through the United States on a camping trip in our 1951 Nash (purchased for \$200). I took up my job at Bellevue after Labor Day 1959, ripped of my last penny.

Meanwhile, Domingo Gomez had arrived at the C 6 Laboratory. A Cuban refugee who had barely escaped with his life from Fidel Castro’s revolution and reign of terror, he found refuge with his old friend Cournand. They had been acquainted in their young years in France and then again when Gomez had worked with Homer Smith at New York University. Afterwards Gomez returned to Cuba on invitation by the then-dictator Fulgencio Batista to create a Cuban Heart Institute, a project that never materialized. Gomez would prove to be the genius behind the work in which I was about to engage. A son of Cuban peasants, he had been sent by Batista to medical school in Paris, where he became a cardiologist and developed his extraordinary skills in mathematical reasoning. This training made him a biophysicist in the true sense of the word, one who would seek a rational explanation for life processes such as the circulation of blood,²¹ the binding of O₂ to hemoglobin, and gas exchange in the lung—and later even for social problems in public health.

When I took on my new job I set up two laboratories, a small one in the pathology department to process specimens and serve as a link to morphology and one in

the C 6 Laboratory to serve as a link to physiology. My equipment was modest but adequate, and it was all brand new because the laboratory had not done any morphology. I had a microscope, a microtome, some glassware, and later on a calculator. I participated in some of the ongoing experiments and listened and talked to the many senior and junior physiologists bustling about the place. This gave me new perspectives but did not help me find a worthwhile morphological project “of interest for physiology.” Cournand himself would be in and out of the place. It was his time of glory, when he was in great demand as a lecturer all over the world. And he certainly enjoyed it, as he later confessed in his autobiography.¹⁰ He would come to the lab, stir up the crew, discuss all the projects, collect information and slides, and take off for the next lectures.

The first question that sent me on my track was put to me by Domingo Gomez who wanted to know how many alveoli there were in the human lung. The question was serious and pressing, for he needed the information to make calculations and predictions on gas exchange between air and blood. It also suggested the path for me to take: to provide sound quantitative information on the lung's structure because the customary meticulous anatomical descriptions would not be of sufficient interest for physiologists. But what we later came to call “morphometry” was not yet invented, so information was rare. When I searched the literature I found estimates of alveolar number to range from 66 to 725 million, not to mention even higher values. It was evident that I had to obtain my own estimate, but no method was available for counting such microscopic entities on histological sections. I found out only much later that such a method had indeed been worked out in 1925 by the Swedish mathematician Wicksell, but his paper⁷² was too theoretical to be known among anatomists at that time.

So, as a first project, Domingo Gomez and I set out to develop a method for counting alveoli on sections. We analyzed theoretically the sectioning process, which presents, on sections, profiles of alveoli whose diameter would generally be smaller than that of the alveolus. The number of alveolar profiles counted on the unit area of microscopic sections, N_A in contemporary symbolism, therefore depended not only on the number of alveoli in the unit volume, N_V , but also on their size. We devised a formula that would allow N_V to be calculated from a count of N_A and the estimation of the relative lung volume occupied by alveoli, V_V :

$$N_V = N_A^{3/2} / \beta \cdot V_V^{1/2} \quad (1)$$

The method required an estimation of the shape factor β , which related alveolar volume and mean cross sectional area. We derived a “reasonable” set of values for β for different geometric shapes and used linear integration to estimate relative alveolar volume. But we then wanted to verify the method on some model specimen of known composition. For that purpose we embedded a known number of peas and short segments of string beans into gelatin, cut the blocks with a sharp knife, and did the required measurements on the cut surfaces (Fig. 1): the method gave a correct result!

While we were doing this kitchen work in the laboratory next to the room where patients were being studied by cardiac catheterization, Cournand returned from one of his trips and stepped into the lab. He expressed utter consternation at our experimental object: “I did not hire you to study vegetable aspic,” he told us, and walked out. We were evidently in trouble, but when we eventually submitted our method and the first estimates of alveolar number for the human lung obtained by a sound morphometric method to the *Journal of Applied Physiology*,⁵⁹ we included a photograph of our “vegetable

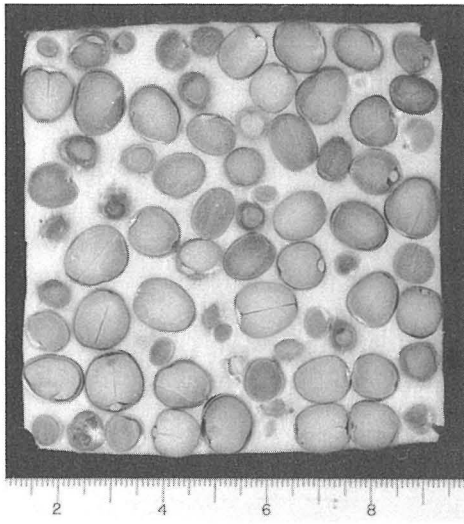


Figure 1. Cut surface of gelatine block containing peas used as models for spherical particles in testing particle counting method. (Reproduced with permission from reference 59.)

aspic” to support the method and got it published (Fig. 1). The number of alveoli we determined, about 300 million in an adult human lung, is still an accepted figure. Today we realize that our method is only approximate and not unbiased; better methods are now available,^{11,41} but these improved methods have still not been used to estimate the number of alveoli in the human lung.

I soon found out that Domingo Gomez was not interested in the number of alveoli but merely wanted this number to calculate the lung’s inner gas exchange surface. The question was whether this parameter could be measured directly. I then discovered that metallurgists were using a simple method for estimating interface area between grains in alloys, a method developed by the Russian metallurgist Saltikov in 1945.³⁶ It consists of drawing test lines on the section and counting the number of intersections they form with the surface trace. The intersection density per unit test line length, I_L , is directly proportional to the surface density in the unit tissue volume: $S_V = 2 \cdot I_L$. The application of this

method gave almost the same result as that obtained from the number of alveoli, but it was much easier to use and was free of assumptions about shape.

We then also needed to estimate the amount of capillary blood on the alveolar surface, which was difficult to do because the light microscope did not offer adequate resolution. I developed a rather cumbersome method based on a model of the capillary network and painstaking measurements of capillary segments obtained on en face images of alveolar septa. The calculations could be made only with a powerful digital computer, a huge machine that occupied a whole building but was less powerful than today’s personal computer.

Two social events then occurred that had some significance for the future. The first helped give what we were doing a name. Domingo Gomez and I had usually described our enterprise as the study of the “architecture” of the human lung,⁶⁰ but that term was not satisfactory because it did not reflect the investigative process. One Sunday morning I woke up from a dream and the term “morphometry” surfaced. That afternoon I was invited to a barbecue at Dickinson Richards’ home and immediately broke the news to him; he did not like it but later was the first to use the term in a lecture! The second event occurred a few weeks later, when Hans Elias, the anatomist from Chicago, visited me on his way home from Europe. I told him about “morphometry,” and he informed me that he had himself just invented the term “stereology” to describe the methods of measuring three-dimensional structures on two-dimensional sections—precisely what we were doing—and had already founded the International Society for Stereology. This was a significant development because it would, over the next years, bring together morphologists from various disciplines to share methods of study that applied equally well to metals, rocks, lungs, or cells. We collaborated intensively over the years.⁵⁶

After two years at the Cardiopulmonary Laboratory I had obtained as much information as I could with the methods and specimens I had available; the basic methods of morphometry were worked out, and a first data set “useful for physiology” was available. I collected and fixed five normal human lungs from victims of accidents or violence—of which there were plenty in New York at the time. I then studied the factors by which lung tissue shrinks on preparation for histology and estimated that, in the lung *in vivo*, the alveolar and capillary surface areas should be on the order of 75 m², and the capillary blood volume about 180 ml. Thus the most important parameters for estimating the effect of structure on pulmonary gas exchange were available (Table 1). When I presented these data to groups of established respiratory physiologists, my estimate of capillary volume particularly met with disbelief because the Roughton-Forster method gave a much lower value. But that is what I had found. I then soon came to realize that the estimates of gas exchange surface were probably too low because the light microscope did not allow the surface to be adequately resolved.⁵⁰

I could of course have proceeded to collect more human lungs than the five I had fixed and could even have extended the studies into pathology. But this was

made very difficult—to say it very politely—by some of my colleagues in the pathology department who did not appreciate my approach and its apparent success. Thus I found that the cadaver lungs that had been assigned to my study would almost invariably be damaged, “inadvertently” of course, by smaller or larger cuts, which then precluded their adequate fixation by the steam technique. So I decided to expand the approach in two directions: first, to study the morphometry of the airway tree and, second, to study the fine structure of the alveolar septum, its capillaries and the tissue barrier, by using the electron microscope to overcome the limits set by the resolving power of the light microscope.

Studying the Human Airway Tree

The study of the airway tree was motivated by Domingo Gomez’ interest in calculating the change of flow velocity of air as a breath of fresh air was drawn deep into the lung. He had predicted that there might be a critical point along the airways, owing to design, where flow velocity dropped so low that O₂ diffusion in the gas phase became the dominant means for ventilating alveoli, and he suspected that distortion of these critical airways was the

Table 1
Morphometric Data Describing the Human Pulmonary Gas Exchanger, as Originally Estimated by Light Microscopy in Weibel⁴⁸ and Subsequently by Improved Electron Microscopic Techniques by Gehr et al.¹⁶

	1963	1978/87	
Age	34–74	19–40	years
Body mass	—	74 ± 4	kg
Alveolar surface area	75	130 ± 12	m ²
Capillary surface area	70	115 ± 12	m ²
Capillary blood volume	180	194 ± 30	ml
Tissue barrier thickness*	(0.46) [#]	0.62 ± 0.04	µm
Plasma barrier thickness*	—	0.15 ± 0.01	µm

*Harmonic mean barrier thickness.

[#]estimated on rat lung

major disturbance in centrilobular emphysema.²² Gomez and I began by setting up a model of the airway tree that we separated into conducting, transitory, and respiratory zones (Fig. 2). We assumed that dichotomous branching prevailed, which established a hierarchical order of the airways from the trachea to the terminal branches. This model also determined that the number of branches would double with each generation so that in generation z it was $N(z) = 2^z$. The first task, then, was to estimate over how many generations the human airway tree branched to reach the terminal airways, the alveolar sacs. I

used our new counting method to obtain the number of alveolar ducts and sacs in my five human lungs and found that it ranged from 12 to $16 \cdot 10^6$, from which we calculated that there must be, on average, about 23 generations of airway branching.⁶⁰

The next task was to estimate the dimensions of airways, the diameter and length of the segments in relation to their position in the airway tree. For that purpose I borrowed one of the excellent plastic casts of the bronchial tree that Averill Liebow had produced while I was working with him at Yale. By painstaking measurement using a fine needle caliper, my devoted assistant Barbara Frank and I mapped the dimensions of well over 1,000 airways on a pedigree chart and then calculated the diameter and length ratios of paired daughter branches, the length-to-diameter ratio, and, finally, the distribution of lengths and diameters for each generation. We also compared the data obtained on the cast with in vivo bronchograms to check on whether our preparation faithfully represented the airway tree. Then, Domingo Gomez came into play when we went about to derive models of the human airway tree from all this information. We realized that the dichotomous airway branching was irregular but that it also revealed a basic progression in the airway dimensions from the trachea out to the peripheral airways. So we synthesized our data into two models: model "A," which reported the dimensions in relation to "regular dichotomy," where all branches in one generation had the same characteristic dimension, and model "B," which expanded the model to consider the irregularities of branching. Subsequently it turned out that model "A" was useful for many applications and is still widely used, whereas the more realistic model "B" was largely overlooked.

The main result of this analysis was that with each generation the average diameter of the airways decreased according

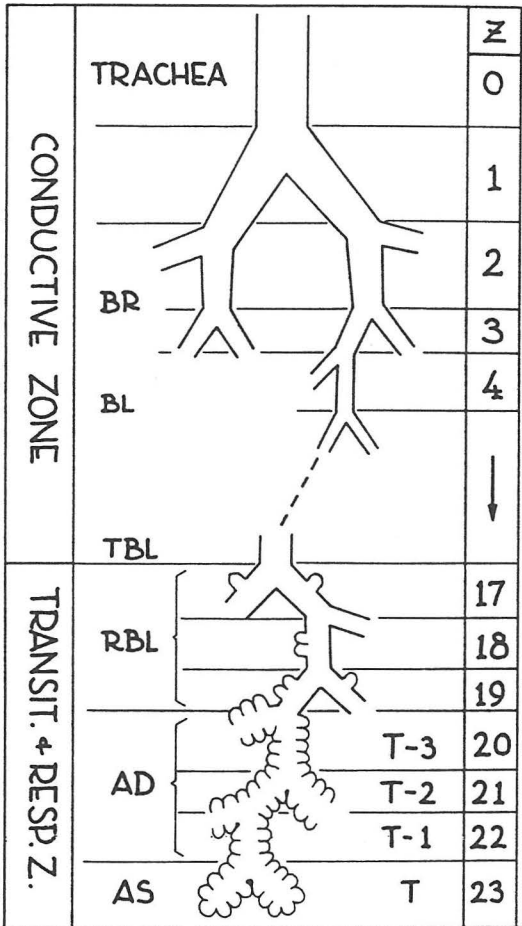


Figure 2. Model of airway tree described by generation z of dichotomous branching. (Reproduced with permission from reference 48.)

to an interesting law (Fig. 3). We found that conducting airways reduced their diameter by a factor of cube root of $1/2$, which led to the conclusion that the loss of energy due to frictional resistance in mass air flow was minimized in the conducting tree; in contrast, the peripheral transitory and respiratory airways retained a larger diameter, which we interpreted as favorable for diffusion of O_2 in the gas phase, as it must prevail at this level.⁶⁰ As a consequence, the total airway cross section increases dramatically along the tree and reaches about 1 m^2 in the most peripheral alveolar ducts. And, because the distance from one branch point to the next decreases from a few centimeters to less than 1 mm, the airways resemble a trumpet with a wide bell.

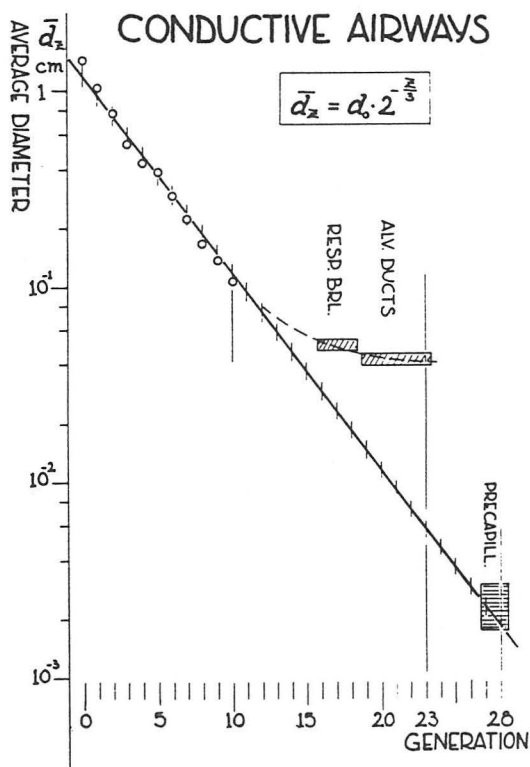


Figure 3. Average diameter of conductive airways, respiratory bronchioles, alveolar ducts, and precapillaries plotted against generations of dichotomous branching. (Reproduced with permission from reference 60.)

In recent years, I have reexamined the dimensional analysis of the peripheral airways using newer techniques, but this reexamination has largely confirmed the old data, except for a few details, which were refined.²³ Yet our model analysis has been challenged: first, by using an alternative way of ordering airways by “orders up” starting at the periphery²⁶ then by considering the airways as a fractal tree.⁷¹ It still appears, however, that the simple model “A” is useful for many applications.

Moving to a New Dimension

During my second year at Bellevue Hospital I realized that the light microscope did not allow the precise study of the pulmonary gas exchanger because the dimensions of the gas exchange barrier were at the limit of its resolving power. When discussing the progress of my work with Cournand and Richards, I said that I needed to learn electron microscopy to study the gas exchange barrier with adequate resolution. They asked me where I would like to do that, and I replied, without hesitation, that I would like to have George E. Palade at Rockefeller Institute as my mentor. Palade was one of the stars in the development of this then new technique, and I knew that he was interested in capillary endothelium. But he was also not easily accessible. That, however, was no problem for my famous mentors, and they quickly arranged for me to meet George Palade over lunch at Rockefeller, and he agreed to take me on. This move was facilitated by my appointment the year before as career investigator of the Health Research Council of the City of New York. This position allowed me to work anywhere in New York City, but it also created a problem: my salary was too high for Rockefeller, so I had to accept a pay cut to be admitted to Palade’s lab. I also had to learn electron microscopy by

working with one of his associates on a project on the spleen. This was more difficult to swallow because I found the project uninteresting and because I could not forget the lung. Although I had no problems abandoning collateral circulation when I moved to Bellevue, I was now completely hooked on morphometry of the lung. So I would sneakily also fix lung specimens along with the spleens of our experimental animals. Finally George Palade generously let me do what I wanted, although it was not in his line of interest.

At Rockefeller I had the unusual opportunity of joining a group that was at the forefront of the newly emerging field of cell biology. I was also completely ignorant of the new developments in molecular biology, but the environment at the institute was so stimulating that I made a special effort to get into the picture. This experience would have a great influence on the further development of my research interests, which henceforth always included some aspects of cell biology. Here, together with Domingo Gomez and Bruce W. Knight, a young mathematician at Rockefeller, I worked out methods for electron microscopic morphometry that allowed a precise analysis of the alveolar septum. I learned, for example, that the estimates of alveolar surface area I had obtained by light microscopy (Table 1) were probably underestimates.⁵⁰ We also developed methods for estimating the thickness of the air-blood barrier and realized that the conductance of the pulmonary gas exchanger was dependent on the harmonic mean of the tissue barrier rather than on its arithmetic mean, which estimates tissue mass⁶¹. The harmonic mean thickness is one third of the arithmetic mean, which is the result of alternating thin and thick parts of the barrier. The thick parts contain cells and fibers and hence serve a support function, whereas the thin parts act as the main gas exchanger between air and blood (Fig. 4).

At the end of this period I could write

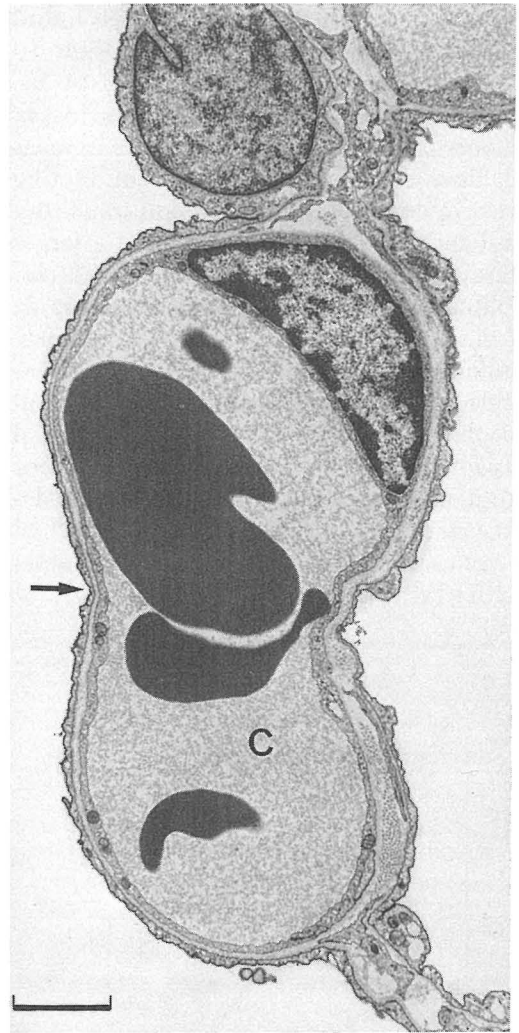


Figure 4. Electron micrograph of alveolar capillary (C). The alveolar epithelial surface (arrow) is exposed to the alveolar space. Scale marker: 2 μm .

my monograph *Morphometry of the Human Lung*,⁴⁸ which summarized the methods and results of nearly four years of research in two centers of excellence of biomedical research. I wrote this book because, on returning to Switzerland in 1963, I had to submit a thesis in view of my pending academic appointment. I do not think I would have written this book without this external pressure, but I certainly do not regret it. It concluded a fasci-

nating period of apprenticeship with great masters, a period of freedom and stimulation that I would never experience again. This was made possible thanks to postdoctoral research fellowships, first from the Swiss Academy of Medical Sciences and then through an exchange arrangement between the U.S. National Institutes of Health and the Swiss National Science Foundation. When, in subsequent years, I served on the Swiss National Research Council as chairman of the Swiss Medical Research Council, I did all I could to promote the fellowship system because I believe it is important to allow promising young scientists first to be exposed to leading masters and then to give them freedom to pursue and develop their own ideas. This is how an investigator is made.

Developing a Structural Model for Pulmonary Gas Exchange

One of the questions that had remained unsolved was how the morphometric information obtained could be used to establish a link with pulmonary physiology—in other words, how this information could be shown to be of interest to physiology. Maintaining contact with Domingo Gomez, I eventually worked out a model that allowed pulmonary diffusing capacity DL_{O_2} to be calculated from morphometric data.⁵¹ This was based on the classical model of Roughton and Forster³⁵ that broke DL_{O_2} into two sequential components, the membrane-diffusing capacity DM_{O_2} and the blood where the capillary volume V_C and a chemical reaction velocity θ_{O_2} determined O_2 binding capacity:

$$1/DL_{O_2} = 1/DM_{O_2} + 1/\theta_{O_2} \cdot V_C \quad (2)$$

It is evident that DM_{O_2} depends on the design of the barrier, on the area over which O_2 diffuses from the air into the

blood, i.e., on the alveolar and capillary surface, and on the thickness of the tissue and plasma barriers. The model we had originally developed for the gas exchange barrier⁶¹ suggested that the overall barrier thickness is to be estimated as the mean of reciprocal local thicknesses, i.e., as its harmonic mean. The morphometric model therefore comprised three serial conductances for tissue (Dt_{O_2}), plasma (Dp_{O_2}), and blood whose reciprocals had to be added:

$$1/DL_{O_2} = 1/Dt_{O_2} + 1/Dp_{O_2} + 1/\theta_{O_2} \cdot V_C \quad (3)$$

Dt_{O_2} and Dp_{O_2} were defined in terms of the corresponding surfaces and thicknesses.⁵¹

This method was first applied mostly to animal lungs because the measurements had to be obtained by electron microscopy. We had to await the availability of well-fixed human lungs before we could estimate the diffusing capacity of the human lung.¹⁶ The current estimate based on the best available data (Table 1) suggests that the diffusing capacity of the human lung is about $200 \text{ mlO}_2 \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$, a value that is about 10 times higher than the conventional physiological estimates obtained at rest. Is this a meaningful result? I believe so, for several reasons. First, if physiological DL_{O_2} is estimated in the exercising individual, values up to $100 \text{ mlO}_2 \cdot \text{min}^{-1} \cdot \text{mmHg}^{-1}$ are reached; this indicates that the “capacity” of a functional system, i.e., its upper limit of performance, can be larger than what we measure physiologically. Second, it has now been shown repeatedly in animal experiments that morphometric DL_{O_2} is a realistic estimate of functional capacity, which is, however, normally not completely utilized but includes a reserve that is exploited only in cases of very large O_2 demand, such as in top athletes, or in hypoxia.

By virtue of its structural design the lung is a very good gas exchanger, and the factors responsible for this are that (1) it maintains a very large surface, which is

ventilated through the airways; (2) it keeps this surface loaded and perfused with a reasonable amount of blood, just one red cell layer thick; and (3) it maintains a very thin barrier of tissue as support for the capillaries. The problem is how this is achieved. Supporting a surface nearly the size of a tennis court by means of a tissue sheet 50 times thinner than a sheet of air mail stationery is no trivial matter. Mechanical problems must be addressed, and the question is to what extent they find their solution in properties of structural design.

The Structural Basis of Lung Mechanics

Around 1960 much attention was devoted to surfactant as important in stabilizing the alveolar surface against surface tension effects. With the discovery by John Clements in 1962 that surfactant was a phospholipid, we could expect to see this material in electron micrographs because the lamellar bodies of type 2 cells from which surfactant presumably originated appeared made of pitch-black lamellae. After all, the fact that cellular membranes were so prominently visible resulted from fixation with osmium tetroxide, which became reduced on their phospholipid bilayer. But none of the electron micrographs of well-fixed lung tissue showed any traces of a phospholipid lining on the alveolar side (Fig. 4): the alveolar surface of the air-blood barrier was constituted of a bare cell membrane whose outer surface must be made of glycoproteins. But then, in studying the damage occurring in rat lungs poisoned with pure O_2 , we noted that alveolar edema fluid contained a peculiar structure made of osmiophilic lamellae in a characteristic pattern that we called "tubular myelin."^{31,66} Because small amounts of tubular myelin could also be found in normal lungs, we conjectured that this substance could be related

to surfactant. If that were so, then the absence of such a layer on the barrier surface could mean that our method of preparation did not retain it. Indeed, if the surfactant lining is fluid and loosely attached to the cell surface, then the instillation of fixative into the airways, the conventional procedure designed to preserve the content of the alveolar septum, would probably wash this lining off. The only hope to keep it in place would be to apply the fixative "from behind," that is through the capillary.

In the summer of 1967 I therefore fixed, together with Joan Gil, some rat lungs by perfusing a glutaraldehyde solution from the inferior vena cava through the pulmonary vasculature; the chest remained closed so that the lung was naturally air filled. On August 11, 1967, I looked at the first sections of one of these specimens and recorded the electron micrograph shown in Figure 5: it revealed an extracellular lining layer made of two parts, an amorphous hypophase topped by a strongly osmiophilic layer made of parallel lamellae strongly suggestive of a phospholipid nature. We repeated the experiment and confirmed the finding. We were convinced that we had demonstrated the existence of a duplex alveolar lining layer that was related to surfactant. I frantically wrote a paper, and by the end of the month it was submitted and rapidly accepted,⁵⁷ so I could finally go off for some holidays, which I had put off out of my excitement. The lining was not as perfect as we had expected it to be. So we improved the methods of fixation and preparation until what we saw fitted our concept of a continuous surface-active lining¹⁹: the hypophase-filled depressions in the alveolar epithelium thus smoothed the free surface, and the surface film was mostly smooth and made of a fine osmiophilic monolayer (Fig. 6). It was also associated with tubular myelin figures here and there.

The next question, evidently, was

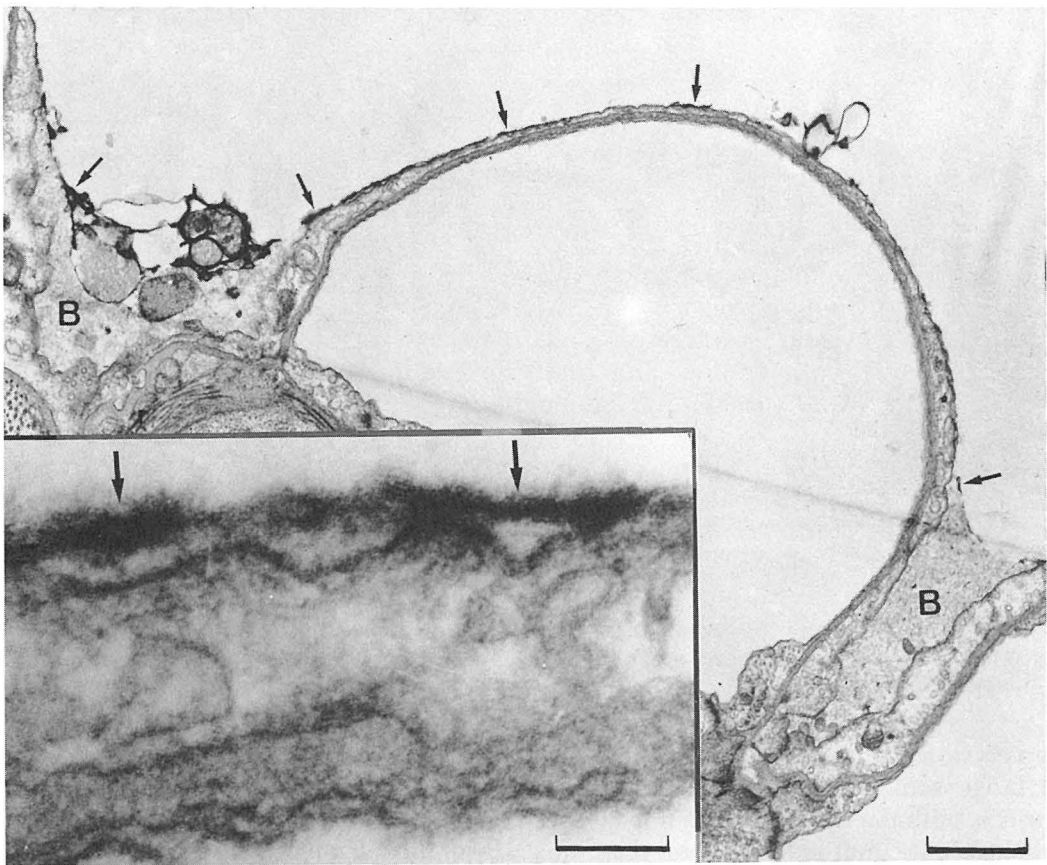


Figure 5. Electron micrographs of perfusion-fixed rat lung recorded on August 11, 1967, shows duplex lining layer with hypophase (B) and osmiophilic lamellae at surface (arrows), shown at higher magnification in inset. Scale markers: 1 μm , inset 0.1 μm . (Reproduced with permission from reference 57.)

whether this lining played a role in stabilizing the alveolar surface, i.e., whether we were really observing surfactant. We engaged in a series of experiments in which we attempted to fix lungs by vascular perfusion under various controlled inflation conditions, measuring the inflation pressure and then, on the micrographs, the mean surface curvature by a new stereological method developed by a metallurgist.¹² From this we described the structural changes associated with the pressure-volume hysteresis and even ventured to calculate the surface tension from the ratio of pressure to curvature: it appeared to fall, with deflation to values as low as

17 dyn/cm.²⁰ But these experiments were, from the point of view of lung mechanics, far from optimal. Real progress came when Hans Bachofen from the Pulmonary Division of our hospital, a disciple of Jack Hildebrandt, Leon Farhi, and Hermann Rahn, came to collaborate with us in a series of studies on structure-function relation of the lung's mechanical system.^{2,18,37}

With better physiological experimentation, the demonstration of the lining layer and its disposition on the alveolar surface improved (Fig. 6), and it appeared that in the inflation range of normal breathing the surface tension must indeed

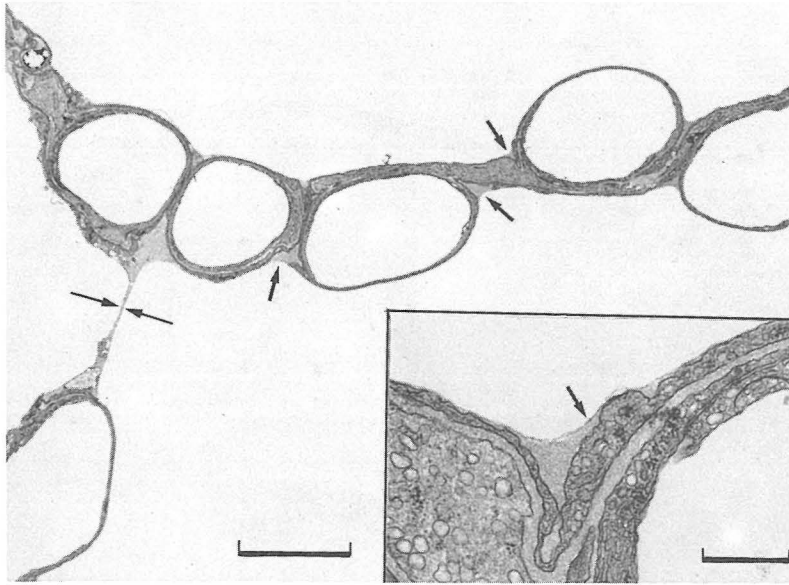


Figure 6. Demonstration of a smooth surface film by improved fixation method (arrows). The surface tension was very low as evidenced by bulging of capillaries and film of lining layer spanning interalveolar pore (paired arrows). Scale markers: 5 μm , inset 0.5 μm .

be very low. This allowed the capillaries to bulge somewhat toward the alveolar surface, with the result that the free alveolar surface was not much smaller than the tissue surface we had estimated by morphometry; accordingly, the existence of an extracellular lining layer reduced morphometric DL_{O_2} by no more than 25%.¹

Still the question remained of how important the surfactant lining is for stabilizing the gas exchange surface. Mead³⁴ had suggested that the tissue scaffold of lung parenchyma made adjacent alveoli "interdependent," which could prevent alveolar collapse. On the other hand, we had studied the connective tissue system of the lung and had come up with a model of a fiber continuum on which the capillary network was delicately supported within the alveolar septum, a continuum that extended, as axial fibers, from the hilum along the airway walls into the alveolar ducts and from there through the alveolar septa into the peripheral fibers that coursed through interlobular septa to the pleura.⁵⁸ Was this sufficient to support

the alveolar walls, or was surfactant needed? To answer this question Hans Bachofen compared normal lungs with air-inflated rabbit lungs depleted of surfactant by prior lavage with a detergent, fixing them by vascular perfusion at well-defined points of the pressure-volume curve.² Figure 7 compares specimens fixed at 60% of TLC on the deflation curve: in the air-filled normal lung the alveoli are patent pockets emanating from the alveolar duct, whereas in the surfactant-depleted lung the alveoli are collapsed. What is striking is that the free edges of the alveolar septa are stretched and pushed outward; the alveoli are therefore collapsed because the ring of strong connective tissue fibers that surrounds the alveolar mouths as the end part of the axial fiber tract cannot withstand the high surface tension generated at the margin of the alveolar septum if surfactant is missing. These studies brought enormous progress in our understanding of lung mechanics and its structural basis,^{3,37} which finally allowed us to design a new model

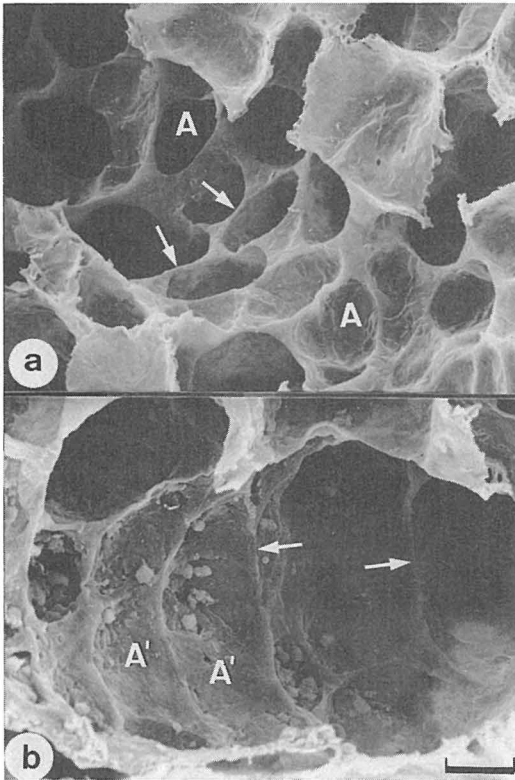


Figure 7. Scanning electron micrographs of normal air-filled (a) and surfactant-depleted (b) rabbit lungs fixed at 60% TLC on the deflation curve show alveoli to be open (A) in (a), collapsed (A') in (b). The alveolar duct is widened in the surfactant-depleted lung, resulting in a stretching of the fiber strands around the alveolar mouths (arrows). Scale markers: 50 μm . (Reproduced with permission from reference 73.)

of alveolar or, rather, acinar mechanics that involves both the fiber continuum and a surfactant lining, as shown in Figure 8.^{58,73}

This now establishes the link between lung mechanics and our attempts to characterize the pulmonary diffusing capacity from morphometric measurements. Mechanical factors are evidently important determinants of the configuration of the gas exchanger. The capillaries are supported on the fiber scaffold in such a way that one side of the barrier is very thin, made only of epithelial and endothelial

sheets (Fig. 4), whereas the other side contains a fiber tract. Capillary blood pressure tends to push the thin side out toward the alveolar space. This may be counteracted by surface tension, but surface tension appears low enough to allow some “crumpling” of the alveolar surface (Fig. 6), thus maintaining a large functional gas exchange surface. This may partially fail in high inflation, where surface tension is high, or under zone I or II conditions, where capillary pressure is low. In the range of physiological breathing it appears that at least 80% of morphometric DL_{O_2} is available for gas exchange.⁴

How Well Is the Lung Designed for Gas Exchange?

When, in 1970, I had set up a model for calculating DL_{O_2} from morphometric data, we had in hand the means for attempting some quantitative studies on structure-function correlation in the lung. The Bohr equation sets DL_{O_2} in relation to O_2 consumption:

$$\dot{V}_{O_2} = DL_{O_2} \cdot (PA_{O_2} - Pb_{O_2}) \quad (4)$$

The first hypothesis suggested by this relation is that DL_{O_2} should be matched to \dot{V}_{O_2} if the lung is well designed as a gas exchanger. And, evidently, variations in ambient P_{O_2} could also have an effect because they would affect the pressure head of the driving force, PA_{O_2} . So we engaged in three lines of research directed toward answering the question of whether DL_{O_2} was matched to conditions of O_2 supply and consumption. Peter Burri, who had studied lung growth,⁸ raised rats on the Jungfrauoch, our high alpine research station at 3,400 m altitude, and found them to develop a higher DL_{O_2} than control rats raised in Berne.⁷ We then discussed ways of modifying O_2 consumption. One day one of our medical students, Annemarie Geelhaar, returned from a shopping trip

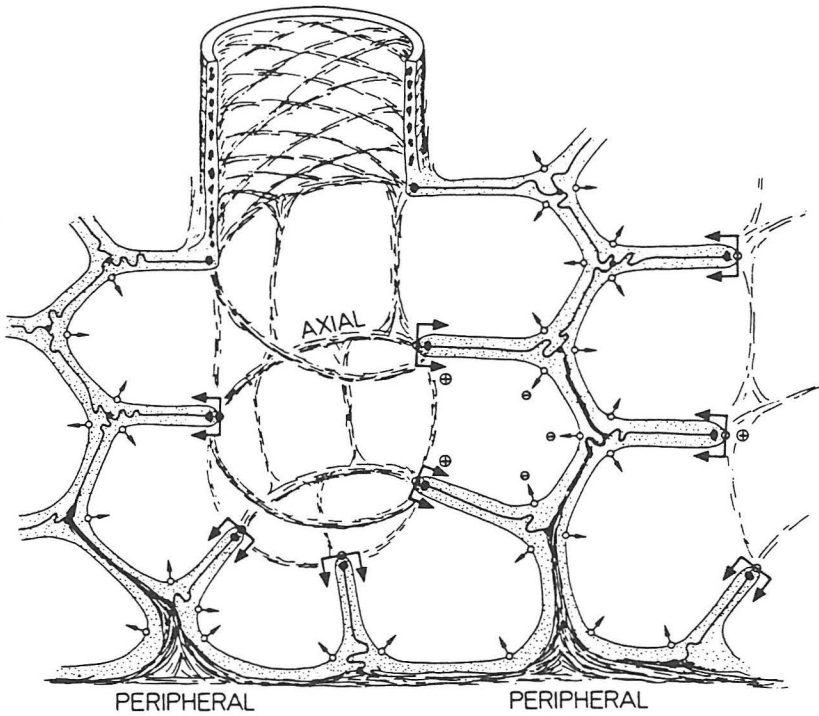


Figure 8. Model of the disposition of axial, septal, and peripheral fibers in an acinus showing the effect of surface force (arrows).

into town excited because she had discovered, in the window of a pet shop, the experimental animal we needed for that purpose: the Japanese waltzing mouse. We bought her some of these cute pets, which are continuously in motion, performing their waltz at a rate of nearly three turns per second. She measured their \dot{V}_{O_2} and found that it was considerably higher than \dot{V}_{O_2} in normal Swiss laboratory mice and that morphometric DL_{O_2} was proportionally higher.¹⁵ Of course, Japanese waltzing mice are genetically different from the Swiss mice, so this could not be interpreted as adaptation of the lung to increased O_2 demand by the body. But Peter Burri then succeeded in making artificial waltzing mice and found that they also had a larger diffusing capacity.²⁷ So it appeared that the size of the pulmonary gas exchanger was related to the O_2 needs of the body.

This suggested that we look for larger

variations in \dot{V}_{O_2} as they occur when body size varies. It is well known that \dot{V}_{O_2} increases with body mass to the power 0.75,³² which means that \dot{V}_{O_2} of a mouse should be three times higher than that of a dog. Does DL_{O_2} vary in parallel? Tenney and Remmers⁴⁵ had measured alveolar surface area in a large range of mammals, from shrew to whale, and found that it varied with O_2 consumption. Is this also true for DL_{O_2} ? We began to collect animals of different size, from the dog of 30 kg down to mice; we even got a colony of the smallest mammal, the Etruscan shrew, which weighs no more than 2.5 g. Because of its small size this fascinating animal pushes its metabolism to extremes: we recorded an electrocardiogram and found the resting heart rate to be over 1,000 min^{-1} and the respiration rate about 300 min^{-1} . Accordingly the metabolic rate per unit body mass was eight times greater in these shrews than in the rat.⁵⁵ The shrews

also have an enormous demand for food: they consume about six times their own body weight in insects every day, and, as we now know, when they are short of food they go into torpor; we may have inadvertently killed several of our Etruscan shrews because we found them “dead” when they presumably were in torpor. In all these different species we measured the morphometric lung parameters required to calculate DL_{O_2} and plotted these data against body mass and \dot{V}_{O_2} (Fig. 9): it turned out that DL_{O_2} was not proportional to \dot{V}_{O_2} but increased nearly linearly with body mass.⁵² On the other hand, free-living animals had a higher DL_{O_2} than captive animals of the same size, which still suggested that DL_{O_2} and O_2 needs could be related. I concluded that we were faced with an apparent paradox, with DL_{O_2} related to \dot{V}_{O_2} in one case, but not in the other.

I did not know what to do with these intriguing results. So when on a sabbatical in 1974 I spent some time at Yale with George Palade working on the endothelial granules we had discovered when I worked with him as a fellow.⁶² I searched through the magnificent library at Yale Medical School for answers to my problem and fell upon a paper by one C. Richard Taylor, who had studied \dot{V}_{O_2} in animals of different body mass. Measuring both resting and maximal O_2 consumption elicited by exercise, he concluded that the ratio of maximal to resting \dot{V}_{O_2} was not constant but was highest in mid-size animals, such as dogs and lower in smaller and larger animals.⁴² I had measured resting or average \dot{V}_{O_2} . When I superimposed Taylor’s bell-shaped curve for $\dot{V}_{O_2} \text{ max}/\dot{V}_{O_2} \text{ rest}$ on my allometric plots of the relation between \dot{V}_{O_2} and DL_{O_2} , I immediately jumped to the conclusion that DL_{O_2} must be related to

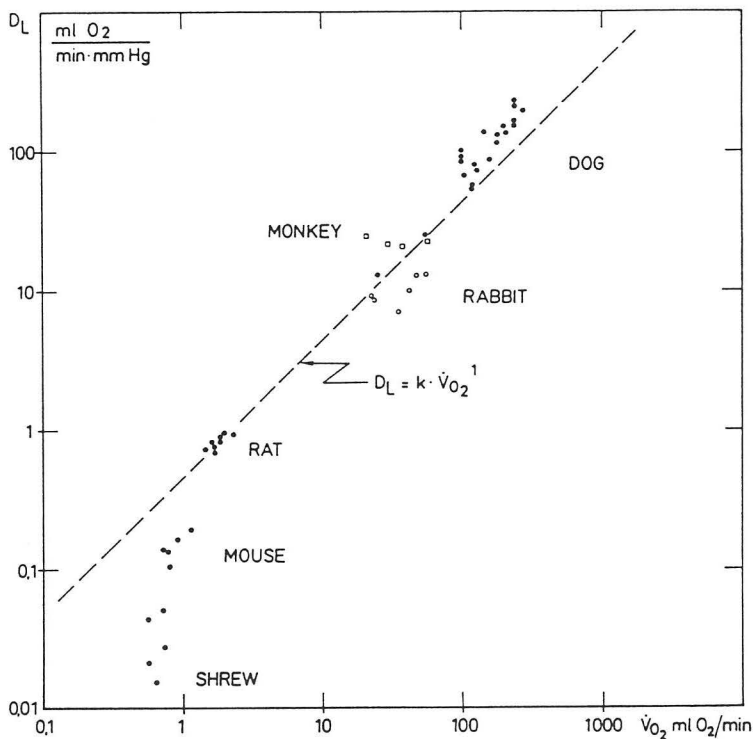


Figure 9. Morphometric pulmonary diffusing capacity DL_{O_2} plotted against (average) O_2 consumption. (Reproduced with permission from reference 52.)

\dot{V}_{O_2} max, which, of course, made a lot of sense anyway: if the lung is designed well its gas exchange capacity must be matched to aerobic capacity or \dot{V}_{O_2} max rather than to any arbitrary measure of metabolic rate.

I located C. Richard Taylor at Harvard and called him by phone, explaining my problem and its possible solution to him. A few days later I was in Boston, and Dick Taylor and I had lunch at the Harvard Faculty Club, together with Tom MacMahon. We had never met before, not even heard about each other, but halfway into lunch we had already decided that we would launch a joint expedition to Kenya to collect a set of free-living wild mammals, covering a large range in body mass, for which we would measure \dot{V}_{O_2} max and DL_{O_2} —hopefully to support the hypothesis that DL_{O_2} was related to \dot{V}_{O_2} max. This brief meeting triggered an extraordinary intense collaboration and close personal friendship and a refocusing of the research in both our labs on both sides of the Atlantic.

After our meeting, each of us wrote to his granting institution asking for a change in his research program and reallocation of funds, and we both got approval. Dick Taylor had worked in Kenya in 1969 and had good contacts, particularly with Geoffrey M. O. Maloiy, the professor of physiology and dean of the School of Veterinary Medicine at the University of Nairobi. He would offer us tremendous support, without which the study could not have been done. Dick had a treadmill built in Boston, large enough so that a small buffalo weighing half a ton could run on it. He shipped this heavy device to Kenya by sea, together with other equipment, and set it up at the Muguga field station outside Nairobi, where excellent facilities for housing animals existed as part of the East African Veterinary Research Organization. He took a sabbatical and in spring of 1977 went to Nairobi with a small group of young collaborators to work for 7 months.

For my part, I was unable to leave my job for a longer time, but I had a young zoologist, Peter Gehr, working with me who had done part of the morphometric studies on dog lungs as his thesis work. I dispatched him and my chief technician, Helgard Claassen, later also Odile Mathieu, to join Dick's group and eventually prepare the lung specimens for subsequent morphometric study in Berne. Our lab was set up in the Department of Veterinary Anatomy, then chaired by Wangari Muta Maathai, a beautiful and charming Kikuyu woman who helped us very generously in return for some lectures to her students. She would later get into political trouble but was just recently awarded the prestigious Africa Prize for her activities in the Green Belt Movement. She assigned some of her associates to work on our project, among them Deter Mwangi, who then spent a couple of years in Berne.

Using his many African contacts Dick had prepared our expedition well. But when he and the other members of the group arrived, an important political development occurred that nearly blocked our enterprise completely. The political powers had realized that tremendous damage was being done to African wildlife by ruthless commercial hunters, who nearly decimated the elephant population in amassing horrendous quantities of ivory. So the parliament of Kenya had passed a law that banned the capturing and hunting of wild animals, precisely what we had come for. A lot of diplomatic activity was necessary, particularly by Geoffrey Maloiy, until we could obtain permits and eventually get the animals we needed. But the whole enterprise retained a touch of illegality to the end, which, of course, only added to the excitement of this fascinating study. Indeed, one of the ministers told Dick that he could not protect him from being put into jail, but he promised to get him out fast! That was some consolation.

While this study on the relation of the

lung and maximal O_2 consumption was being set up in Nairobi, I acquired a new collaborator in Berne, Hans Hoppeler, who a few years before had done a remarkable thesis on the relation between muscle mitochondria and \dot{V}_{O_2} max in human athletes.²⁴ After completing medical school and a residency in surgery and obstetrics, he wanted to get back into research. When I went to Kenya to join the group for a few weeks I suggested that we expand the program to consider not only the lung as the supplier of O_2 to the body, but also the locomotor muscles as the chief O_2 consumers during maximal exercise. The study therefore became one of respiratory system physiology, more precisely on the design of the mammalian respiratory system in relation to function or its functional limits. The decision was rapidly made while we were all sitting under a tree behind the anatomy department on the Nairobi campus. We did not have much time for planning and thinking out detailed strategies because this was so new. What it required of my group was that, in addition to the lungs, samples from a variety of muscles had to be collected and fixed from all the animals, which would eventually be sacrificed after completion of the physiological studies. This was a tremendous additional load, and the working conditions were not optimal.

In Berne, I then reoriented my research program. Since my training period with George Palade at the Rockefeller Institute I had always maintained a research project in the field of cell biology in which we had explored the relation between cellular membranes and their enzymes in liver cells^{6,40,68}; one project also dealt with cell damage in the lung.⁵ In view of this new study I abandoned the liver cells and redirected the cell biology project to muscle and its mitochondria. The approach would be the same, namely, combing morphometric studies of cell composition with biochemical analyses in an integrated fashion, but the object was now the

membrane system of muscle mitochondria instead of the endoplasmic reticulum of liver cells.^{38,39} It also proved necessary to improve the stereological methods used to generate the extensive morphometric data that would now be needed. I had always maintained a personal interest in continuously developing this fascinating methodology with its beautiful mathematical appeal. This effort was greatly advanced by my friendship with Roger Miles, the eminent mathematician from Australia.^{49,53,56,65} When Luis Cruz-Orive joined our group in 1976, we acquired an “in-house theoretician” who would continuously contribute to improving and updating our methods of sampling and measurement in this rapidly advancing field.^{11,53}

The field study in Kenya lasted for 7 months, after which we had cratefuls of physiological data and tissue samples from lungs and 20 different muscles collected from over 20 different animals ranging in body size from 500 g to 250 kg. An important aspect of the study was that all the tissue samples had been obtained on the same animals that had been subjected to thorough physiological study. It took us over 3 years and involved some 20 investigators to analyze all this vast material. We finally produced nine papers, which we wanted to publish together in order to stress the systems physiology approach. We were fortunate to find in Pierre Dejours an editor of a renowned journal who was sympathetic to this idea; he eventually accepted the entire series for *Respiration Physiology*.⁶³ The papers had gone through an adequate peer review process, but Taylor got into some difficulties later with his granting agencies because some of them considered this “package of papers” as a non-peer-reviewed publication. The reason was that Pierre Dejours had generously named us “guest editors” for the issue of his journal in which our series was published, although he had personally handled the editorial process.

The Concept of Symmorphosis and the Lung

When all the data were assembled and first drafts of the manuscripts written, the whole group met in Berne for a week of intense discussions to develop a coherent picture of the study. At this time we realized that we had actually engaged in a study of the overall design of the respiratory system, of the “pathway for oxygen,”⁵⁴ and that this required some formalization. We first set up an analytical model of the respiratory system that would relate O₂ flow rate to a set of structural and functional parameters, and this from the lung to the mitochondrial O₂ sink (Fig. 10). As a second step, we formulated a hypothesis on structure-function correlation that we could subject to testing: the hypothesis of *symmorphosis*, which we defined as “a state of structural design commensurate to functional needs resulting from regulated morphogenesis, whereby the formation of structural elements is regulated to satisfy but not exceed the requirements of the functional

system.” We thought that symmorphosis—or economical design—should apply at all levels of the respiratory system so that it also meant that the lung’s structural parameters must be matched to those of the circulation of blood and of the muscle’s capillaries and mitochondria. The overall functional needs, in our case, should be the highest level of O₂ consumption that the organism can achieve, that is, \dot{V}_{O_2} max achieved by the muscle cells during running. We thus had collected the essential information for testing the hypothesis of symmorphosis.

What did this study of African mammals tell us about the relation between O₂ needs and the lung? Did it prove our prediction that \dot{V}_{O_2} max should vary linearly with body mass? Did it thus resolve the apparent paradox that DL_{O₂} was matched to \dot{V}_{O_2} when animals of the same size were compared but not in an allometric comparison across the size range? It did neither. Dick Taylor and his collaborators found that \dot{V}_{O_2} max was about proportional to resting \dot{V}_{O_2} , perhaps with a slightly steeper allometric exponent, 0.81 instead of 0.75.⁴³

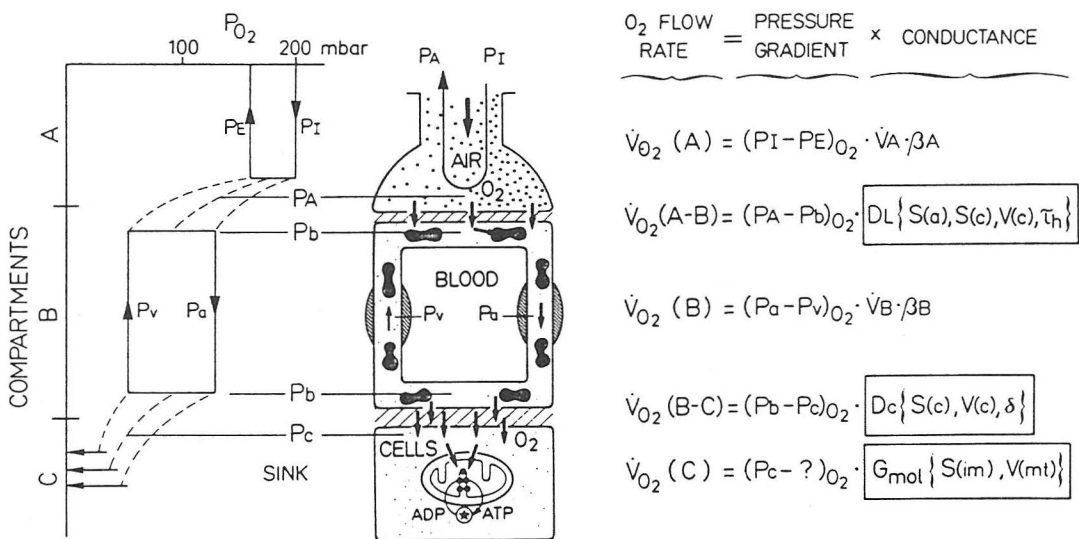


Figure 10. Model of respiratory system. (Reproduced with permission from reference 63.)

And our morphometric study of all these lungs revealed that morphometric DL_{O_2} was linearly proportional to body mass, thus confirming my original result.¹⁷ Thus \dot{V}_{O_2} max and DL_{O_2} have different allometric slopes when plotted against body mass (Fig. 11); as a consequence we had to conclude that the driving force for diffusive O_2 uptake, the alveolo-capillary P_{O_2} difference, must become smaller as animal size increases. We hypothesized that this could be the result of the larger size of acini in larger lungs, which could reduce alveolar P_{O_2} because of a longer diffusion path in the air phase or peripheral airways.⁷⁰ For comparison, it is worth noting that we did find a reasonable agreement between \dot{V}_{O_2} max and mitochondrial volume as well as for muscle capillaries.^{25,33} The hypothesis of symmorphosis was hence supported at these internal levels of the respiratory system, but apparently not in the lung.

Looking at Athletic Species

The variation of metabolic rate observed between species in relation to their body size is related to their basal or standard metabolism.³² Contrary to our expectations we found that animals of all sizes can apparently increase metabolic rate upon exercise by approximately the same relative amount, namely, by about tenfold, until they reach \dot{V}_{O_2} max. This so-called aerobic scope, however, can vary considerably according to the prowess of an animal or a species. Thus, athletic species such as the dog or the horse have an aerobic scope of 30–50 fold above standard metabolism, on average 2.5 times that of more sedentary species of the same body size such as goats or cows, respectively. Because we had seen that our “little athletes,” the Japanese waltzing mice, had a higher pulmonary diffusing capacity than their lazier counterparts, the Swiss

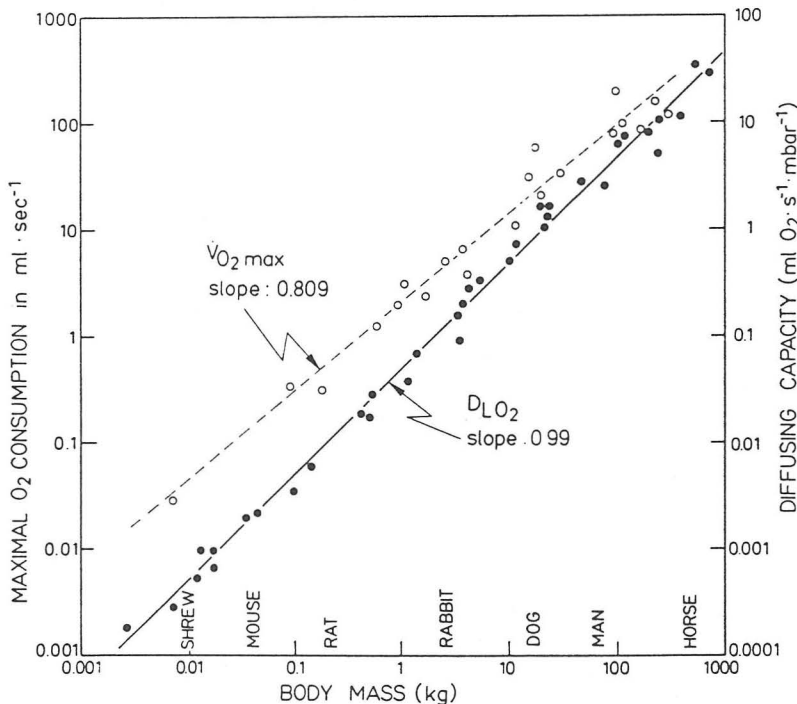


Figure 11. Allometric plots of pulmonary diffusing capacity DL_{O_2} and maximal O_2 consumption show different slopes. (Reproduced with permission from reference 54.)

mice, we wondered whether this adaptive variation in \dot{V}_{O_2} max observed in larger species was associated with corresponding variations in the design of the respiratory system.

Together with Dick Taylor and his group we undertook a second series of studies in which we wished to test the hypothesis of symmorphosis by comparing dogs with goats, and ponies with calves, studies that again occupied us for about 5 years and involved as many investigators as those on allometry. The resulting eight papers were also published as a coherent sequence in *Respiration Physiology*.⁴⁴ Because we now had a consistent model of the respiratory system, the study could be more complete; in particular we now included measurements of physiological parameters that characterize O_2 transport by the circulation of blood,³⁰ a part of the study brilliantly performed by a young Ph.D. student at Harvard, Richard H. Karas, as part of his thesis work. These studies were later complemented by the comparison of standardbred race horses with steers, both weighing about 500 kg, a study done at the Veterinary School in Uppsala, a collaboration between a Swedish, an American, and a Swiss group, each chipping away in its own area of expertise.^{9,28}

Table 2 provides a summary of the results of these studies; it compares the animals pairwise and calculates the ratio of parameters between the athletic and the sedentary species, e.g., dog/goat. We first observe that the ratio for body mass-specific \dot{V}_{O_2} max is 2.5 on average, as is the ratio for the total mitochondrial volume per unit body mass. From this we calculate that for \dot{V}_{O_2} max/ V (mi), the ratio of athletic to sedentary species is about 1, i.e., it is invariant with adaptive variation or with different levels of \dot{V}_{O_2} max. In other words, the total volume of mitochondria in skeletal muscle is higher in athletic species in strict proportion to \dot{V}_{O_2} max. This led to the conclusion that the unit volume

of mitochondria is capable of consuming the same amount of O_2 in athletic and sedentary species, which is to say that, in all species, muscle mitochondria are capable of the same maximal rate of oxidative phosphorylation, and this is also true in allometric variation. With respect to muscle capillaries, we must take into account that athletic species have a higher hematocrit (Table 2) and hence a higher O_2 capacity of the blood. The capillary volume is also higher, but it is the product of capillary volume and hematocrit, that is, the volume of capillary erythrocytes in the muscle that is proportional to the mitochondria of muscle cells and hence forms an invariant ratio to \dot{V}_{O_2} max. The differences in hematocrit also affect O_2 transport by the circulation, where we found the product of stroke volume (or ventricular volume) and hematocrit to be proportional to \dot{V}_{O_2} max, whereas maximal heart frequency is pairwise identical and depends only on body mass. But because maximal heart frequency varies with body mass it must be considered an additional functional factor.⁶⁹

Studying the lungs we found that the athletic species had generally larger morphometric parameters so that, in the end, DL_{O_2} was 1.7 times larger.⁶⁷ But this is clearly not proportional to the 2.5 times higher \dot{V}_{O_2} max (Table 2). In these studies we had obtained a reasonably complete set of physiological data so that we could have a closer look at what was happening in the lung during gas exchange (Fig. 12). By dividing alveolar capillary volume, obtained by morphometry, by cardiac output at \dot{V}_{O_2} max, obtained by the Fick principle, we could estimate the mean capillary transit time at \dot{V}_{O_2} max to be on the order of 0.3 s.²⁹ Alveolar P_{O_2} could be calculated by the alveolar gas equation; mixed venous and arterial P_{O_2} had been measured at \dot{V}_{O_2} max, and we had good estimates of the blood's O_2 reaction rate so that we could calculate the progression of capillary O_2 uptake by Bohr integration. This revealed, as shown

Table 2
 Adaptive Variation of the Pathway for Oxygen. Ratios of Morphometric and
 Physiologic Parameters of Muscle Mitochondria and Capillaries, and of Heart, Blood
 and Lung in Relation to $\dot{V}_{O_2 \max}$ in Three Species Pairs

	Mitochondria			Blood	Capillaries		Heart		Lung		
	$\dot{V}_{O_2 \max}/M_b$	$V(mt)/M_b$	$[V(mt)]/\{\dot{V}_{O_2 \max}\}$	$V_v(ec)$	$V(c)/M_b$	$[V(c) \cdot V_v(ec)]/\{\dot{V}_{O_2 \max}\}$	f_H	Vs/M_b	$[Vs \cdot V_v(ec)]/\{\dot{V}_{O_2 \max}/f_H\}$	DL_{O_2}/M_b	$[DL_{O_2}]/\{\dot{V}_{O_2 \max}\}$
	$ml \cdot s^{-1} \cdot kg^{-1}$	$ml \cdot kg^{-1}$	$ml \cdot ml^{-1} \cdot s$		$ml \cdot kg^{-1}$	$ml \cdot ml^{-1} \cdot s$	min^{-1}	$ml \cdot kg^{-1}$	$ml \cdot ml^{-1}$	$ml \cdot s^{-1} \cdot mmHg^{-1} \cdot kg^{-1}$	$mmHg$
<i>25–30 kg</i>											
Dog	2.29	40.6	17.7	0.50	8.2	1.79	274	3.17	3.16	0.118	0.052
Goat	0.95	13.8	14.5	0.30	4.5	1.42	268	2.07	2.92	0.080	0.084
D/G	2.4*	2.9*	1.2	1.68*	1.8*	1.26	1.02	1.53*	1.08	1.48*	0.61*
<i>150 kg</i>											
Pony	1.48	19.5	13.2	0.42	5.1	1.45	215	2.50	2.54	0.079	0.053
Calf	0.61	9.2	15.1	0.31	3.2	1.63	213	1.78	3.21	0.050	0.082
P/C	2.4*	2.13*	0.9	1.35*	1.6*	0.89	1.02	1.40*	0.79	1.57*	0.65*
<i>450 kg</i>											
Horse	2.23	30.0	13.5	0.55	8.3	2.05	202	3.11	2.58	0.108	0.048
Steer	0.85	11.6	13.7	0.40	5.3	2.49	216	1.52	2.58	0.054	0.064
H/S	2.6*	2.6*	1.0	1.4*	1.6*	0.82	0.94	2.1*	1.00	2.0*	0.76*
Ath/Sed	2.5*	2.5*	1.03	1.5*	1.7*	0.99	1.0	1.7*	0.96	1.7*	0.67*

Note. Data from references 9,28,43. Last line presents overall ratios for athletic/sedentary species. Asterisk denotes ratios significantly different from 1.⁶⁹
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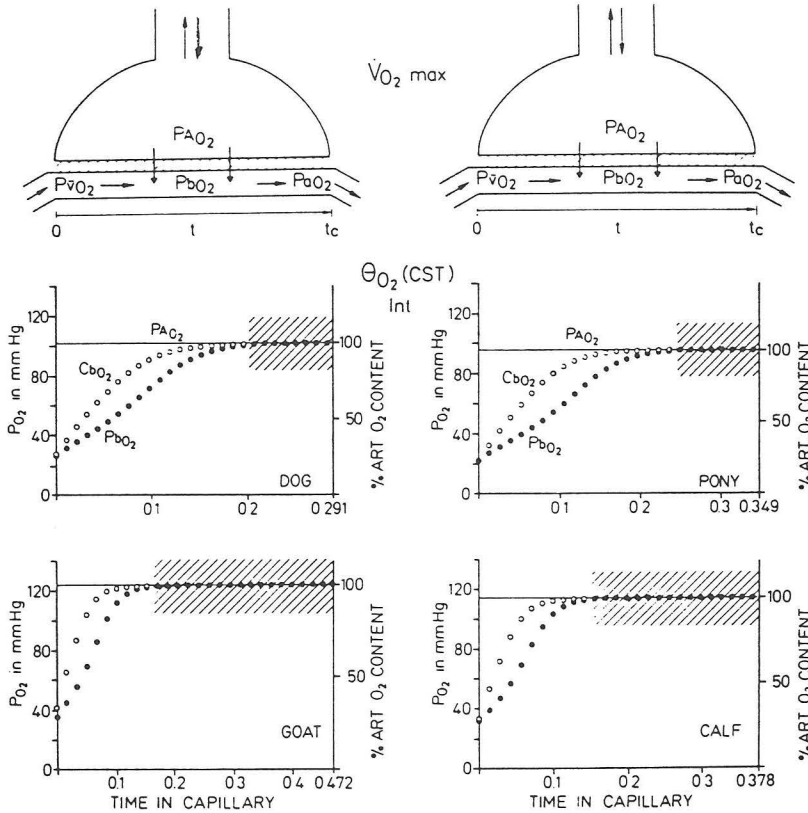


Figure 12. Increase in O_2 concentration, C_{O_2} and P_{O_2} of the blood during the time it transits the capillary bed. Athletic species (dogs and ponies) are compared with more sedentary goats and calves. The shaded areas indicate the “redundant” parts of the capillary path, which is shorter in athletic species. (Reproduced with permission from reference 29.)

in Figure 12, that equilibrium between capillary blood and alveolar air was completed, on average, before the blood left the capillary bed even at \dot{V}_{O_2} max. Thus we must conclude that the pulmonary gas exchanger maintains some redundancy or reserve diffusing capacity, but this redundancy appears to be smaller in the athletic than in the sedentary species.²⁹ This finding was confirmed when comparing horses and steers.⁹ To check whether this conclusion is justified we subjected goats to a hypoxia test on the treadmill and found that they can run and maintain their \dot{V}_{O_2} max down to inspired O_2 fractions of 15%.²⁹ In contrast, we never succeeded in having dogs run near \dot{V}_{O_2} max when in-

spired O_2 was only slightly reduced; their small redundancy in DL_{O_2} may not give them enough reserve capacity. With respect to the human lung, where we had found a redundancy of about 50% (Table 1), it has been shown that the human lung can support high levels of \dot{V}_{O_2} even at high altitude but that it can become the limiting factor for \dot{V}_{O_2} max in highly trained athletes who apparently also exploit some of this redundancy when they train for higher performance.^{14,46}

Thus it appears that the pulmonary diffusing capacity is crudely matched to maximal metabolic rate but that it may not need to follow the O_2 demands of the body because it maintains a certain degree of

redundancy, which can be a comfortable 50% in sedentary species, a value we had also found for the human lung (Table 1). Considering that the lung forms the interface to the environment where P_{O_2} can vary, a “safety factor” of 2 is, in fact, reasonable and could conform well to what an engineer would build into such a vital organ, particularly because it appears that the lung is incapable of easily repairing any damage to its gas exchanger without a residual loss in functional capacity. And our environment, indeed, offers many opportunities for ill effects on this delicate structure.

Conclusions

I have spent over three decades probing into the lung with all sorts of microscopes as well as with physiological and mathematical methods. I conclude that the beauty of this delicate and intricate structure is matched by the secrets it has not yet revealed. It still remains an exciting object of investigation. I have had the good fortune of a fantastic apprenticeship with great masters in the fields of morphology, respiration physiology, and cell biology, masters who have taught me to look beyond structure, as exciting as structure is for someone deeply committed to design and aesthetics. I have also had the opportunity of being put in charge of a sizable teaching and research department at the young age of 37 through which I could involve, in time, a large number of young and enthusiastic investigators. And, finally, I was more than lucky to find, at all times, partners for my research endeavors who brought in skills and expertise I did not have. Even the Atlantic Ocean and differences in the size and potential of the participating institutions and countries are no barrier to most fruitful collaboration—otherwise a partnership between the small University of Berne and the great Harvard University could not have

worked. All that counts is an open mind and a generous attitude toward one’s partner. If you and your partner do not become friends it does not work.

The greatest lesson I have learned is that exciting research needs partnership because it is, in the long run, not productive to remain enclosed in one’s narrow field. In my case, it would not have been productive to limit study to morphology or even morphometry; this tells only half the story, or less. Such studies must be closely combined with functional investigations true to the task I was given in 1959: “to do anything on the structure of the lung that is of interest for physiology.” But I would like to now widen this task and ask morphologists and physiologists together to do anything on the structure and function of living creatures that is of interest for the understanding of life.

Postscript

The preceding sounds like a happy success story, but for the sake of honesty and completeness I should perhaps mention that it was twice overshadowed by troubling experiences of scientific unfairness—to be more explicit: of persons who plagiarized but were never held responsible for their actions.

Before I moved from Columbia University to Rockefeller Institute in 1961 I introduced another fellow to the methods of lung morphometry that I had developed so that he could use them in the study of pathological specimens. I also gave him a copy of the draft of the methods section of “Morphometry of the Human Lung.” By coincidence I found out in 1962 that he had written, based on this privileged information, a paper on these methods, which he submitted for publication one year after he left the laboratory. Although I tried to delay the publication of this paper until my own was published, it appeared in 1962 whereas mine carries

the date of 1963. At the time I was shattered, for the date of publication sets the priority. I had been scooped.

A few years later I was working on O₂ toxicity in the lung, under contract with the U.S. Air Force Medical Research Division. Together with Peter Caldwell, then serving as captain in the air force, we exposed rats to pure O₂ breathing at Wright-Patterson Air Force Base. The analysis of tissues was conducted in my laboratory in Switzerland.³¹ I was required to submit detailed quarterly reports to my contracting agency, giving results of our study to an air force captain who would review them and would serve as “monitor” of my contract. One day I received a paper for review that had been submitted to a prestigious journal by this captain, and to my dismay I found that it reported not only the general findings but also the main morphometric results of our study. We later found out that this officer had ordered a sergeant-technician, whom we had trained, to repeat our experiment on a few rats; given a contract to someone else to produce a few electron micrographs that matched our data; and written the paper based on our original confidential report. When I informed the journal’s editor about the circumstances, the paper was rejected, but I was not allowed to inform air force authorities of this flagrant case of scientific fraud, because the paper

I had received for review was “privileged information.” The paper was then published, unaltered, in another journal. Some 15 years later, around 1980, this very person was involved in a major case of scientific fraud in the United States. The scenario closely resembled my own experience: a journal editor inadvertently referred the fraudulent manuscript for review to the original author of the abused data. In this instance, the story became public, and the brilliant if not soaring career of the man came to an abrupt end. What strikes me in this case is the pattern of repetition, and I wonder how much of this happens and remains unprosecuted, as it has in my own two cases. At the time of these incidents I was a young man with no established career, and these experiences hurt me deeply. In retrospect it appears, however, that persistence in honest work has greater rewards than fraudulent appropriation of another person’s ideas.

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