The Exotic Gases, CO, O₂, and CO₂

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This chapter is a personalized report of one path of my research on respiratory gas exchange with all the warts. It is part biographical, part review, part current, and part prognosticatory. In other words something old, something new, something borrowed, and something blue. I, by accepted standards, am old, as is my research. Some of it is new, I proclaim, but almost everything that is new is borrowed one way or another from my collaborators and students. As for my prognostications, they are blue sky.

After World War II the leaders of respiratory physiology were not only extremely able but also gentlemanly (as were the ladies) to each other and their juniors, which gave the whole field a feeling of community. I have been blessed with colleagues who have taught and stimulated me. I have mentioned only a few in this chapter, those whose collaboration was pertinent to the thread of rapid reaction kinetics.

This research in respiratory physiology has primarily involved exchanges and reaction/diffusion kinetics of CO, O₂ and CO₂ among blood, gas, and tissue. These familiar gases may perform in extraordinary fashions and have exotic aspects. I will say only a modest amount about older work on CO and very little about O₂, about which Professor Piiper says more elsewhere in this volume, but most about CO₂ kinetics, my current major interest.

This chapter affords me the opportunity to expound those profound principles of the conduct of physiological research,
at least in the United States, which I have accumulated over a lifetime. I was reluctant to proffer these principles, as it seemed pompous and impertinent, but I have escaped this stigma because in the end I found that others had observed these principles before. The first principle is

Serendipity is the Dominant Strategy.

This is a humbling and apparently unacceptable idea to administrators who try to plan research, committees of learned societies, principle investigators of NIH program projects, and would-be captains of the biomedical industry. If one knows enough about a problem to budget large expenditures of resources and time economically, both of course someone else's, the effort becomes one of development rather than research. The inability of our brightest and best to predict new research revelations has been frequently documented. A fine historical account of the converse, that discoveries of critical importance to biomedical science have not been foreseen nor have they resulted from administrative planning, is given in the articles by Julius Comroe and Robert Dripps in *Science*. However, such is the seduction of large-scale research planning, that we repeat the errors of scientific history, although warned by Santayana that, “Those who cannot remember the past are condemned to fulfill it.” This is not to rule out the possibility of a stunning new and unexpected scientific finding uncovered by a committee; serendipity might even work through MBAs.

Principle I has a corollary in the dictum of Louis Pasteur, “Dans les champs de l'observation le hasard ne favorise que les esprits préparés.” Therefore, learning from the Boy Scouts and Sir Robert Baden-Powell, to do original science we should “Be prepared.”

As a medical student I did some pedestrian research on the treatment of hypertension with SCN (thiocyanate), which had a efficacy approximating that of garlic, simply because a faculty member gave me a lab and encouragement. It did result in my first publication. As a house officer at the Peter Bent Brigham Hospital in Boston, I applied an ear oximeter, borrowed from Glenn Millikan, who had taught me in pharmacology, to cardiac patients with Samuel A. Levine, a superb clinical cardiologist. I measured, probably for the first time, human pulmonary arterial temperature during a catheterization with Lewis Dexter. All of these forays into research occurred because of individuals and serendipitous opportunities.

Through the efforts of George Thorn, I was assigned to the Quartermaster Corps Climatic Research Laboratory in Lawrence, Massachusetts, during World War II, which became the alma mater of other respiratory physiologists of more note. Our mission was to protect soldiers under a wide range of climatic conditions; one aspect of this mission was the physiology of temperature regulation. Under the guidance of Cuthbert Bazett and Richard L. Day, the New York pediatrician, my research resulted in my second publication, the first paper in the first issue of the *Journal of Applied Physiology*, which may contain the first experimental evidence for countercurrent exchange, the cooling of arterial blood in the hand by returning venous blood. I was the last author. I continued in this field at Harvard with Eugene Landis, but when I was offered a faculty position at the Graduate School of Medicine at the University of Pennsylvania by Robert Dripps and Julius Comroe, I accepted without a qualm because Julius had taught and inspired me at Penn and because the school offered more money (a figure that Landis thought was indecently high). I was fascinated by the physiology of temperature regulation and might happily have spent my life in it but changed my field to respiratory physiology because that was the focus of Julius Comroe’s program (Fig. 1).
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Figure 1. Julius Comroe, Jr., about 1956 in Philadelphia.

This brings me to the second principle:

Inertia is a Powerful Force in Scientists.

An investigator starts where he was taught and pushes on in small steps. He rarely makes a quantum jump into a new field but, stimulated by his observations, extends his horizon and may find in front of him, to his surprise, a new vista of discovery. If he does change fields, he is careful to do so under the umbrella of preceptors in his new area.

I was armed with experience in Experiment 26 of the medical school laboratory course in physiology at Harvard, long since gone by the board, as have so many. Run by John Pappenheimer and Baird Hastings, it taught students more about the alveolar air equation than they wanted to know and more than most chest physicians know today. Figure 2 is a charcoal drawing by John's son, Will, and suggests CO₂ and O₂ exchange among red blood cells, plasma, and alveolar gas, which became my forte.

It was humbling to my intellectualization of scientific research to realize in preparing this chapter that my work in respiratory physiology has been so dependent on the development of new apparatus for the measurement of rapid chemical reactions rather than as logical steps in the development of a master strategy to discover new physiology. The instruments were developed through small incremental innovations, the chronology of which is given in Table 1, borrowing from many and starting from what F. J. W. Roughton showed me in the early fifties.

CO Research

I found on arriving back at Penn a small laboratory in the bowels of the Old Medical School Building, now called the John Morgan Building after an original prominence, in which was a breath analysis mass spectrometer and an infrared CO analyzer, which Julius Comroe and Seymour Kety had ordered before I came. With the help of Ward Fowler, David Bates, and Bernard van Lingen we followed the disappearance of CO from alveolar gas at different PO₂ (Fig. 3) using the new mass spectrometer to measure helium and O₂ and the infrared meter to measure CO. This was certainly an early, maybe even the first, use of these instruments in physiology. Use of a mass spectrometer to measure respiratory gases had been reported in an abstract by Fred Hitchcock.

The raw data in Figure 3 shows that increasing alveolar PO₂ decreases the rate of CO disappearance in seconds. Only a drop in the rate of the chemical reaction of CO with intracellular hemoglobin, resulting from the competitive reaction of O₂, can occur this rapidly. Therefore this also means that the velocity of gas uptake by red cells in the alveolar capillaries can be rate limiting, a new concept at the time. To interpret the results quantitatively, we needed measurements of the rate of CO
uptake by human red cells at 37°C, so I wrote F. J. W. Roughton at Cambridge to see if he had such data. He did not but came to Philadelphia to obtain the measurements (Fig. 4), bringing with him a continuous-flow gas pressure driven rapid-mixing apparatus, having managed to avoid U.S. Customs with the help of the Air Transport Command of the U.S. Air Force. The mixing apparatus is shown in Figure 5. Its principle was developed by Hartridge and Roughton and is in its simplest form a tee-tube in which a solution of CO and a suspension of red cells at a given P O₂ are driven in each side arm and the turbulent reacting mixture flows out the central shaft at a rate about 0.2 cm/ms, so that by observing the HbCO/ HbO₂ at progressive distances down the tube, the course of the reaction can be followed. The beauty of this apparatus is that the analytical apparatus, or observer, can have as slow a response time as convenient, limited only by the volume of reactant available.

Roughton brought with him a reversion-spectroscope, an ingenious optical instrument built by Hamilton Hartridge (who had learned fluid mixing from work on airplane carburetors in World War I) that reverses and superimposes the two absorption bands of HbCO or HbO₂ in the green (540 to 568 µm). As [HbCO] increases, the peak of HbO₂ at 576 µm moved toward that of HbCO at 578 µm and this movement can be quantitated by superimposing the two bands. It was a subjective instrument requiring experience and rapport to use, so that only Roughton operated it, turning reactant flows on and off with hemostats while peering into the spectroscope. On one historic occasion he...
Figure 2. Objective Participants. Gouache, pastel, and charcoal on paper by Will Pappenheimer, 1989. Dimensions 52 in by 41 in. Reproduced by permission of the artist.

Figure 3. The effect of altering the O\textsubscript{2} and CO\textsubscript{2} tensions in inspired gas on the rate of disappearance of CO from the alveolar gas. FA\textsubscript{CO} is alveolar CO concentration in \% atmosphere. (Subject: Ward S. Fowler).

Figure 4. R. E. Forster II (left) and F. J. W. Roughton (right).
groped for a hemostat but instead seized a pair of scissors, severed the rubber tubing, and rapidly disposed of two bottles of reactants that had taken half a day to prepare. At this point, Bill Briscoe and I decided an automatically recording photoelectric instrument was needed and developed the split-beam two-color analyzer shown in Figure 5 using a prism purloined from Britton Chance. By measuring the changes in the difference of absorption by the cell suspension at two colors, most of the artifacts produced by light scattering were eliminated. Using this apparatus Roughton, Briscoe, Ferdinand Kreuzer, and I measured the rate of uptake of CO by human red blood cells (θ) at 37°C with increasing \( \text{PO}_2 \) and, with Leon Cander, the diffusing capacity \( (D_L) \) at different alveolar \( \text{PO}_2 \). Summing the resistances to gas CO transport in the alveolar capillary, which are the reciprocals of the conductances or diffusing capacity, we obtained the convenient relationship:

\[
\frac{1}{D_L} = \frac{1}{D_M} + \frac{1}{\theta V_C},
\]

where \( D_L \) is the diffusing capacity of the whole lung and \( D_M \) is the diffusing capacity of the alveolar membrane alone, both in \( \text{ml} \ \text{CO/min/mmHg} \); \( \theta \) is in \( \text{ml} \ \text{CO/min/mmHg/ml normal blood} \); and \( V_C \) is the volume of the capillaries in ml.

These measurements of \( D_L \) and \( \theta \) as a function of \( \text{PO}_2 \) plus the theoretical interpretation permitting calculation of \( D_M \) and \( V_C \) were contained in four papers in the Journal of Applied Physiology.
1957 by Roughton and myself; the senior authorship was divided equally but arbitrarily. This demonstrates a minor principle concerning the order of authorship designated the “Gilbert and Sullivan rule.” Things are never what they seem; skim milk masquerades as cream. The papers in which I was senior author were primarily the work of Roughton, and vice versa. I believe this is still the only method to measure pulmonary capillary blood volume.

The next improvement was the introduction of a fine platinum teflon-covered PO$_2$ electrode into the reacting stream to measure the disappearance of [O$_2$] with time when a suspension of deoxygenated red cells was mixed with an O$_2$ solution. This was the first use of an electrode in a rapid reaction apparatus and had the potential theoretical artifact that the inevitable stagnant layer on the teflon surface would be delayed in comparison to the average in the stream cross section so that the measured PO$_2$ would be correspondingly later in time. Fortunately this did not turn out to be an important error, so we were encouraged to use other electrodes later, such as pH and PCO$_2$. The response time of the electrodes was measured in seconds to tens of seconds, so large volumes of reactants were consumed to obtain a measurement, and the development of more rapidly responding electrodes became a high priority. In this PO$_2$ electrode reaction apparatus we also used for the first time in our experiments motor-driven pistons to deliver the reactants (Glenn Millikan had done the same at Cambridge in the 1940s).

The reason for using a PO$_2$ electrode rather than continuing to use photospectroscopy was heightened sensitivity, since a mmHg PO$_2$, which is easily measured, represents a very small Δ[HbO$_2$]. With this apparatus, we (Bishop, Staub and I) measured the rate of O$_2$ uptake by red cells starting at a high HbO$_2$ saturation. The apparatus also provided an entree into red cell CO$_2$ kinetics because we could measure the displacement of O$_2$ by CO$_2$ in the Bohr shift.

The next advance in kinetic apparatus was the application of the stopped-flow rapid mixing apparatus developed by Quentin Gibson, to the exchanges of red cells (an example is given in Figure 6). In this instrument the two reactants are propelled by pistons into a mixing chamber; the mixture flows into a third syringe whose plunger hits a stop, causing all flow to cease in a millisecond. In 1954 Roughton and I talked with Gibson in his home in Sheffield about the possibility of using the apparatus for cells but worried about stagnant layers of fluid developing around the cells immediately after the flow was stopped, slowing the reaction measurements. Sirs and Roughton at Colloid Science in Cambridge built a stopped-flow spectrophotometric apparatus, a copy of which Lawson, Holland, and I used to measure the rate of egress of O$_2$ from red blood cells by mixing an oxygenated cell suspension with a solution containing dithionite, which reduces the O$_2$. Because the dithionite will penetrate to the surface of the cells, we hypothesized that any stagnant layer effect would be minimal. Holland and I measured the rate of O$_2$ uptake by human red cells and concluded the values we obtained were not significantly different from those obtained with a continuous-flow instrument. With this agreement we were confident that the stopped-flow apparatus could be used on cell suspensions. There were reports that the stagnant layer around the cells did retard the reactions but the conditions were extreme and we still thought the artifact was negligible. More recently, however, we (Krawiec, Gottliebsen, Fish and Lin, unpublished) compared stopped-flow and continuous-flow photocolorimetric measurements of ΘO$_2$ and found the stopped-flow values were about half those from continuous-flow measurements. Not all investigators agree with us, however.
Figure 6. Diagram of a stop-flow rapid-mixing apparatus set up to measure the rate of deoxygenation of human red cells. The two lower syringes are pushed upward, and reacting mixture flows until the third, or upper, syringe hits the stop, halting flow in less than a millisecond. The white light beam passes through the reacting mixture, is split, and each half passes through a 559 mµ or 573 mµ interference filter and onto two separate photomultiplier tubes, the difference of whose output is amplified and fed into a cathode ray tube. By taking the difference of the two transmitted colors, most of the changes in output resulting from light scattering are eliminated. The output is essentially linear with [HbO₂].

Masaji Mochizuki from Sapporo came to Philadelphia to help me in the early 1960s, and we measured the facilitated flux of CO through a thin layer of hemoglobin solution absorbed on to a millipore membrane, stimulated by analogous studies of Pete Scholander. In this form of transport, CO reacts to form HbCO on one surface, and this liganded form diffuses to the other surface where the CO dissociates, contributing to the total diffusion of CO. We demonstrated that the facilitated flux rate was limited by the velocity of the dissociation of HbCO so that this flux could be used to measure the reaction velocities of CO and Hb. Ian Longmuir, Woo, and I applied this to the facilitated diffusion of CO₂. Unfortunately, because [H+] as well as [CO₂] and [HCO₃⁻] vary, it is impossible to obtain an analytic solution, so the method was of limited usefulness until Donaldson and Quinn modified the experiment to measure the exchange of isotopically labeled CO₂ at chemical equilibrium in which [H+] is constant, thereby permitting a useful analytic solution.

**CO₂ Research**

Roughton and colleagues had tried to estimate the physiological rate of the carbonic anhydrase catalyzed hydration of CO₂ in red cells by measuring the rate at increasing concentrations of hemolysate and extrapolating to the normal intracellular concentrations. They could not measure the reaction at carbonic anhydrase concentrations greater than one-third of the normal intracellular value; the reaction was too fast for the mixing time of their apparatus. We saw the possibility of using a PCO₂ electrode in a continuous-flow instrument to measure CO₂ uptake by human red cells. While the concentrations of carbonic anhydrase and hemoglobin were physiological in each red cell, the suspension was dilute (1/20), so the rate of change of PCO₂ in the reacting mixture was easily measured. Typical results are shown in Figure 7. The initial rate of CO₂ uptake, calculated from the initial slope of the PCO₂ curve, was, however, only 60% of the value predicted by extrapolation from in vitro measurements on hemolysate. We concluded that the reaction was most likely impeded by the accumulation of end products, particularly H⁺. This finding demonstrates that one cannot measure correctly the rate of CO₂/HCO₃⁻ reactions inside an intact lipid membrane, which is impermeable to H⁺ by any method involving a net formation or consumption of CO₂.

Figure 7 also shows the effect of a sulfonamide carbonic anhydrase inhib-
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**Figure 7.** Decrease in \( \text{PCO}_2 \) at 37°C with time after mixing a 1:10 suspension of normal human blood in physiological buffer at \( \text{PCO}_2 \) of 2 Torr, \( [\text{HCO}_3^-] \) of 25.8 mM, and pH 8.66 with a physiological saline solution containing a \( \text{PCO}_2 \) of 71 Torr, \( [\text{HCO}_3^-] \) of 29.4 mM, and pH 7.25 (upper curve). In the lower curve, the cell suspension contained in addition 350 \( \mu \text{M} \) acetazolamide, 20,000 times \( \text{Ki} \). The dashed line represents the extrapolated uncatalyzed reaction (modified from 18).

...itor, sufficient in concentration to fully eliminate the enzyme activity. The remaining uncatalyzed rate should be linear on the graph; the initial upward convexity is produced by the formation of hemoglobin carbamate. This stimulated us to go on later and measure the kinetics of the \( \text{CO}_2 \) reaction with hemoglobin,\(^\text{18}\) and this in turn brought Research Principle III to my attention. About 1979 a distinguished American scientist complimented me on this paper saying it provided the first experimental results that convinced him that hemoglobin carbamate really existed. This was in spite of the fact that Meldrum and Roughton had published data on this topic in 1933 and the results had been in physiological and some biochemical textbooks for decades. I assume from this conversation that many scientists had not accepted their conclusions but did not make the public aware of their skepticism.

The third research principle is

*Don’t Forget the Silent Majority*

The egalitarian and permissive mores of the American scientific community dictate that one does not disagree in public with another scientist’s conclusions with the same strength as one’s private convictions. However, these convictions will still surface in Study Sections and at Site Visits.

The next modification of the stopped-flow apparatus was to introduce an electrode, a glass pH electrode, into the reacting mixture to follow rapid changes in \( [\text{H}^+] \). We were not optimistic that the instrument would work because in the first place pH electrodes are notoriously slow in their response.\(^\text{40}\) Part of this lag we thought resulted from their high electrical resistance, which we hoped could be overcome by modern high impedance DC amplifiers. Second, we were concerned that the inevitable stagnant layer on the electrode surface would increase on cessation of flow and magnify the artifact. We were pleased to find that, in the stop-flow apparatus with the reactant mixture directed as a jet against the glass tip, the response time was as little as 0.005 seconds. We concluded that the slow response of the ordinary laboratory glass pH electrode was in...
large part due to a failure to change the fluid on the glass surface fast enough with any test solution. The stopped-flow apparatus does this more successfully. We—Chow, Lin, and myself (unpublished)—found that the response time decreased with increasing buffer concentration in the test solution. We surmised that the buffer facilitates the transport of H⁺ to the active surface.

We used the stopped-flow pH apparatus to measure the effective permeability of the red cell membrane to OH⁻ and to HCO₃⁻/Cl⁻ exchange.¹ In considering the classic diagram of red cell gas and ion exchanges in the capillary beds of Roughton,³³ we suddenly realized that the necessary cyclic changes of [H⁺] in the plasma could not be produced by the movements of HCO₃⁻ and Cl⁻ across the cell walls but were produced by the hydration of CO₂ and dehydration of HCO₃⁻ in the plasma. Some years later I found that Roughton had pointed this out in his 1935 review.³³ Since there was no carbonic anhydrase in the plasma, these reactions were necessarily slow, could not be completed in the capillary transit time, and might never be complete as the blood cells cycled through lung and peripheral capillary beds. There the matter should have rested if at least three groups of investigators⁸,¹₄,²⁴ had not looked for the predicted disequilibrium between plasma [CO₂] and [H⁺][HCO₃⁻]. They could only find this in the presence of carbonic anhydrase inhibitors, demonstrating the presence of carbonic anhydrase available to the capillary plasma. There is a large amount of carbonic anhydrase in the lung, among which are several different isozymes (Fig. 8), including a carbonic anhydrase IV isozyme type, which is generally found in membranes and could be on the capillary endothelium.

Clearly we needed a method of measuring carbonic anhydrase activity or, rather, the velocity of CO₂/HCO₃⁻ reactions uninhibited by end products. In 1977 Nobutomo Itada and I applied the ¹⁸O exchange method of Mills and Urey²²,²⁸ to this purpose. The principle is illustrated in Figure 9. When ¹⁸O labeled HCO₃⁻ is added to water, labeled CO₂ is produced and reaches almost complete isotopic equilibrium with H¹⁸O¹⁶O₂⁻. The ¹⁸O exchanges with ¹⁶O in water more slowly and is enormously diluted so that

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**Figure 8.** Cartoon of lung showing location of possible functions of its carbonic anhydrase isozymes. The leftmost broad arrow represents the major flux of CO₂ transported by blood. The dashed arrow indicates the flux of CO₂ arising from uncatalyzed dehydration of HCO₃⁻ in plasma. The third set of arrows indicate the flux of CO₂ that may be facilitated by carbonic anhydrase in the endothelium and in the epithelium. The last arrows on the right indicate the possible production of CO₂ from HCO₃⁻ exchanged with plasma.
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Figure 9. Cartoon of the exchange of ¹⁸O, solid O's, in CO₂ and HCO₃⁻ with those of H₂O. The oxygens in HCO₃⁻ are symmetrical, so when the molecule is dehydrated, the probability of an ¹⁸O forming labeled H₂O is 1/3, while the probability of its forming labeled CO₂ is 2/3.

C¹⁸O¹⁶O essentially disappears, and at an exponential rate that can be measured conveniently in a mass spectrometer. From this rate, the unidirectional velocity constants can be calculated. Experimental results on red cells and hemolysate are shown in Figure 10. Hemolysate accelerates the disappearance, and the effect of carbonic anhydrase can be quantified. The results with an intact red cell suspension showed a “step,” or first a rapid and then a second slower exponential disappearance, a double exponential process. This occurs because CO₂ can diffuse easily into the cell where the ¹⁸O exchange with water occurs 17,000 times faster than outside, and [C¹⁸O¹⁶O] drops because the extracellular labeled [HCO₃⁻] carbonic anhydrase which is 21 times the [CO₂] and thus 95% of the ¹⁸O in the solution in the absence of carbonic anhydrase, cannot dehydrate to form C¹⁸O¹⁶O fast enough. This also means that the intracellular labeled HCO₃⁻, which is in nearly complete isotopic equilibrium with C¹⁸O¹⁶O, is much less than extracellular HC¹⁸O¹⁶O₃⁻. This transmembrane gradient causes the flux of labeled HC¹⁸O¹⁶O₃⁻ into the cell providing a measure of membrane permeability to HCO₃⁻ as well as the activity of carbonic anhydrase in the cell. Silverman and colleagues independently developed a similar method, and, on the basis of their finding that carbonic anhydrase was not inhibited in intact cells to the same extent as in solution, concluded that red cell membrane resistance to CO₂ diffusion was rate limiting. 38,39 We believe our disagreement can be explained by the relative impermeability of the red cell membrane to the sulfonamides used.

The advantages of the method are:
1. It is carried out at chemical equilibrium so [H⁺] is constant.
2. It can measure carbonic anhydrase activity inside a membrane, which other methods cannot.

Figure 10. In the left panel semilogarithmic graphs of the abundance of ¹⁸O in CO₂ any time $t$, $\alpha_1$, minus its final equilibrium value, $\alpha_e$ (solid lines), or the abundance of ¹⁸O in HCO₃⁻ at any time $t$, $\gamma_1$, minus its final equilibrium value, $\gamma_e$ (dashed lines). In the left panel human hemolysate was added at the arrow. In the right panel, a suspension of human red cells was added at the arrow. The lines before the additions represent the uncatalyzed exchange of ¹⁸O with oxygen in H₂O.
3. It can measure \( \text{HCO}_3^- \) self-exchange across the cell membrane.

4. It can be modified to give the most sensitive method for carbonic anhydrase activity.

At the same time the method has disadvantages; it requires expensive equipment and is slower than some other techniques.

Using the \( ^{18}\text{O} \) exchange method we found the same carbonic anhydrase activity, 17,000 times the uncatalyzed rate at 37°C in intact and lysed human red cells. This answers the original question of Roughton as to whether the enzyme activity of carbonic anhydrase in the highly concentrated milieu of red blood cells is the same as in a dilute solution. The answer is yes; the intracellular activity is proportional to the concentration of enzyme and can be extrapolated from values in dilute solutions.

\( ^{18}\text{O} \) exchange between \( \text{CO}_2 \) and water can also be used to measure carbonic anhydrase activity in a test tissue exposed to labeled \( \text{CO}_2 \) in a gas phase. This modification was used by Shoko Nioka to determine the average carbonic anhydrase activity in an isolated perfused guinea pig lung rebreathing a physiological \( \text{CO}_2/\text{O}_2 \) mixture containing \( ^{18}\text{O} \) labeled \( \text{CO}_2 \). The \( \text{CO}_2 \) equilibrates with the \( \text{HCO}_3^- \) buffer system in the pulmonary parenchymal tissue in several seconds. The rate of \( ^{18}\text{O} \) loss from the labeled \( \text{CO}_2 \) in the alveolus gas to the pool of unlabeled oxygen in the tissue water is slower but can be used to measure carbonic anhydrase activity in the parenchymal fluid. By adding sulfonamide inhibitors of different membrane permeability to the lung perfusate, she was able to show that almost all of the carbonic anhydrase was present within the cells of the lung and not on the endothelium of the capillaries. This agreed with the results of Henry et al., who homogenized rat lung and measured the carbonic anhydrase activity in the different cellular and subcellular fractions.

One other technique that can measure the velocity of the reversible reactions of \( \text{CO}_2 \) and \( \text{HCO}_3^- \) is nuclear magnetic resonance (NMR) with \( ^{13}\text{C} \) labeled \( \text{CO}_2 \). The broadening of the nuclear magnetic resonance peak indicates quantitatively the speed of interchange of the labeled atoms between \( \text{CO}_2 \) and \( \text{HCO}_3^- \). However this measurement must be done deep inside a strong magnet and is impractical for many purposes.

In 1980 we embarked in a new and exciting direction, the result again of serendipity in the laboratory. Leena Mela was investigating the effects of hypoxia on mitochondrial function. The mass spectrometer and reaction chamber/inlet system was available, so Susanna Dodgson and Bayard Storey measured the carbonic anhydrase activity of these subcellular particles, finding there was considerable activity in liver mitochondria (Fig. 9). The “step” in the record of the intact mitochondria shows that there is carbonic anhydrase activity inside the inner membrane and that \( \text{HCO}_3^- \) is less permeable than \( \text{CO}_2 \). The mitochondrial carbonic anhydrase turned out to be a new isozyme, now designated carbonic anhydrase \( V \).

There had been prior suggestions in the literature that liver mitochondria contained carbonic anhydrase, but there also were strong denials. We submitted the manuscript to an English biochemical journal, which rejected it rapidly because they said everyone already knew there was carbonic anhydrase in mitochondria so why publish old information. We then submitted it to an American biochemical journal, which criticized it because of published evidence that carbonic anhydrase was not present in liver mitochondria. This brings me to my fourth principle:

*Scientists Don’t Really Like New Ideas . . . Except Their Own.*

This sounds harsh, and I almost deleted it, but then found I am not the first
nor the most distinguished to point this out. "A new scientific truth does not triumph by convincing its opponents and making them see the light, but rather because its opponents eventually die and a new generation grows up that is familiar with it"—a statement of Max Planck.32

It is an accepted fact in the mitochondrial field that the inner mitochondrial membrane is impermeable to HCO$_3^-$.

While Figure 11 demonstrates that the liver mitochondrion is less permeable to HCO$_3^-$ self-exchange than CO$_2$, it is still permeable to the anion, albeit 1/10 as permeable as human red cell membrane.10 In spite of this low permeability, because a mitochondrion has about 1/200th the volume of a red cell, labeled HCO$_3^-$ should equilibrate between a mitochondrion and its ambient in a fraction of a second.

The most fascinating question concerning carbonic anhydrase V is its function. Our hypothesis was and continued to be that carbonic anhydrase V is present in liver mitochondria to produce HCO$_3^-$ from CO$_2$ fast enough to provide substrate for one or more of the syntheses that require this anion.12 In this context it is important to note that while the decarboxylation reactions of the mitochondrion produce CO$_2$, not HCO$_3^-$, it is the latter that is required to react with ATP and NH$_3$ to form carbamoyl phosphate in the urea cycle, to react with pyruvate to form oxaloacetate in gluconeogenesis, and to react with acetyl CoA to form malonyl CoA in fatty acid synthesis (Fig. 12).11,25

A major weakness in our hypothesis, at least for me, is that the uncatalyzed hydration of CO$_2$ should be rapid enough to provide the necessary HCO$_3^-$ from CO$_2$ without the acceleration of carbonic anhydrase and that, while carbonic anhydrase inhibitors reduce the rate of urea formation, they do not reduce it as much as expected.

Thus my interest in respiratory CO$_2$ exchanges has led me from the respiratory dead space to studying CO$_2$ handling by mitochondria, where we are now finding that either CO$_2$ is channeled among enzymes without being free to exchange with the ubiquitous CO$_2$/HCO$_3^-$ or there are gradients for CO$_2$/HCO$_3^-$ within a single mitochondrion.

**Acknowledgement:** I wish to recognize all my friends and colleagues who are not mentioned in this chapter. This chapter was not planned as a complete survey of my research and collaborators, but it is restricted to development of measurements of the rapid reactions of these exotic gases with tissue and red blood cells. I have resisted straying, which means

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**Figure 11.** Semilogarithmic graphs of mass 46 peak height-mass 46 peak height at final equilibrium against time. Dry NaHCO$_3$ 2% enriched with $^{18}$O was added to a solution of 300 mosM mannitol-sucrose at 25°C pH 7.4 to give a 25 mM solution. In upper panel, guinea pig liver mitochondria were added at the arrow to give the mgm protein/ml indicated on the curves. In lower panel freeze-thawed guinea pig mitochondria were introduced at the arrow to give the mgm protein/ml indicated on the curves. In curve 1, 10 µM acetazolamide was added to the initial solution before the freeze-thawed mitochondria were added in the same concentrations as in curves 2 and 3.
Figure 12. Pathways of synthesis of urea, glucose, and fatty acid that involve intrahepatocytic HCO₃⁻ (reference 11, reproduced with permission).

that other areas of my interests and many fellow workers and instructors may not be mentioned. Among the neglected research topics are temperature regulation; gas exchange in lungs, gills, and placentae; pulmonary function testing; pulmonary circulation; CO metabolism; and red cell permeability.

References


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