Multum in parvo: Explorations with a small bag of carbon dioxide

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A collection of 12 papers published between 1957 and 1972 are revisited. The papers had a common theme of the use of rebreathing carbon dioxide and explored a variety of topics in respiratory physiology. The first study established a method for the noninvasive and indirect estimation of arterial carbon dioxide pressure that was suitable for the routine clinical monitoring of respiratory failure and whose clinical utility remains to this day, but which also provided observations that were the stimulus for the studies that followed. The rate of rise in the partial pressure of carbon dioxide (PCO₂) during rebreathing led to an analysis of body carbon dioxide storage capacity. Knowledge of carbon dioxide storage led to a method for quantifying lactate production in exercise without the need for blood sampling. The changes in ventilation that accompanied the increase in PCO₂ provided the basis for a rapid method for measuring aspects of breathing control (Read’s method), which was later modified to measure the ventilatory response to hypoxia. The physiology of breath-holding was explored through observations of the fall in breath-holding time as PCO₂ climbed. Rebreathing also allowed in -

Key Words: Carbon dioxide storage; Cardiac output; Exercise; Lactate; Rebreathing; Respiratory control

Condensé : Expériences menées avec un ballon-net de CO₂

RÉSUMÉ : Une série de 12 articles publiés entre 1957 et 1972 sont ici passés en revue. Les articles avaient pour thème commun l’utilisation de la réinhalation du CO₂ et portaient sur divers sujets se rattachant à la physiologie respiratoire. La première étude présentait une méthode d’estimation non invasive et indirecte de la PCO₂ qui convenait à la surveillance clinique de routine de l’insuffisance respiratoire et qui est toujours utilisée en clinique de nos jours. On y fournissait par contre aussi certaines observations qui ont été le point de départ des études subséquentes. Le taux d’élévation de la pression partielle du CO₂ (PCO₂) durant la réinhalation a conduit à une analyse de la capacité de stockage du CO₂ par l’organisme. Ces connaissances sur le stockage du CO₂ ont donné naissance à une méthode de calcul de la production du lactate durant l’exercice, sans prélèvements sanguins. Les changements de ventilation associés à l’augmentation de la PCO₂ ont formé la base d’une méthode rapide de mesure des différents aspects de la régulation de la respiration (méthode de Read), par la suite modifiée pour permettre une mesure de la réponse ventilatoire à l’hypoxie. La physiologie de l’apnée a été étudiée par l’observation du déclin de la durée de l’apnée de façon inversement proportionnelle à la PCO₂. La réinhalation a aussi donné lieu à des augmentations de la ventilation volontaire, sans induction d’alcalose, à l’origine d’études sur la ventilation volontaire maximum et sur la fatigue des muscles respiratoires. La régulation de la PCO₂ durant la réinhalation a servi à mesurer la PCO₂ veineuse mixte durant l’exercice et à mettre au point une approche intégrée de la physiologie de l’exercice dans la santé et dans la maladie. Un déséquilibre alvéolo-artériel de la PCO₂ durant l’exercice a ainsi été découvert. L’ajustement de la PCO₂ et de la PO₂ durant la réinhalation d’un mélangage gazeux de CO₂ et d’azote a donné lieu à des temps d’absorption

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When I was approached to contribute to the series “Classics Revisited”, I was tempted to choose the paper that I wrote with Jack Howell that described a simple rebreathing method for the estimation of arterial partial pressure of carbon dioxide (PCO₂) (see below). However, I soon realized that I would not be able to do justice to the sequelae and full ramifications of that paper in the compass of a short essay. Still, the idea of telling the story of the PCO₂ method, and the several lines of research that spurn off from it, had taken hold and have led to the present paper.

This narrative has as its central character a rubber bag that contains 4 to 5 L of gas when full. Its theme is the inventiveness and industry of a number of fellows and colleagues who worked with me at The Middlesex Hospital and Hammersmith Hospital in London, United Kingdom during the 1960s, and later at McMaster University in Hamilton, Ontario, in using the bag to study several different topics in physiology. I have chosen the key paper that first explored each topic, making a dozen papers in all, and have given less space to the papers that, in some cases, followed.

To clarify the storylines and to avoid prolixity, I have bent the rules of scientific writing (with the connivance of the editor-in-chief) in regard to the details of instrumentation and experimental design, and in terminology. Also, I have restricted the list of references to those that may be regarded as key to the storyline. Anybody wishing for an amplification of the accounts herein will have no difficulty in tracing their origins.

THE THREE-BREATH METHOD FOR MEASURING LUNG VOLUME

In 1955, as a fellow at Johns Hopkins, I collaborated with Dick Shepard and others (1) in a study of the effect of lung volume on anatomical dead space. Herman Rahm’s three-breath method was used to measure lung volume (2): a rebreathing bag is filled with a known volume of oxygen and then, starting from residual volume or functional residual capacity, the patient takes three deep breaths in and out of the bag, and the oxygen (or nitrogen) is measured. A simple dilution equation yields the lung volume.

It seems to me that in these days of rapid gas analysis, the method may still be competitive with those in current use. But that study was done in 1955; three years later I began what was to become a long-standing affair with the method of rebreathing from a small bag.

THE TWO-STAGE METHOD FOR MEASURING MIXED VENOUS PCO₂

In the 1950s, in an attempt to promote controlled oxygen therapy for patients with chronic airflow limitation who were in respiratory failure, I was stymied by the absence of an easy and practical method for measuring PCO₂. Then, in 1956, Collier (3) described his rebreathing method for measuring mixed venous PCO₂. In this method, the patient rebreathes from a small bag primed with a mixture of carbon dioxide in oxygen, having a PCO₂ that is felt to be above the patient’s mixed venous PCO₂. For example, in a healthy patient, an 8% carbon dioxide mixture might be used (PCO₂ of about 56 mmHg). The PCO₂ passing between the patient and the bag is recorded with a rapid analyzer, and within a few breaths, equilibration of carbon dioxide occurs, identified as a ‘plateau’ in the carbon dioxide record. This occurs before recirculation (ie, within 20 s) and, thus, signifies equilibrium of PCO₂ between the bag, the alveolar gas and, most importantly, the mixed venous blood entering the pulmonary capillaries. Subsequently, Collier’s group showed that the arbitrary subtraction of a venous-arterial difference of 6 mmHg yielded an acceptable estimate of arterial PCO₂ (4).

Jack Howell and I immediately appreciated the merits of Collier’s method. It required much less skill than the Riley bubble method that I had been using for the direct measurement of blood PCO₂ (this being some 10 years before the introduction of blood gas electrodes). Also, it obviated the need for arterial puncture, which, at that time, was frowned upon by senior medical staff. Howell and I borrowed an infrared carbon dioxide analyzer and a recorder with the intention of trying Collier’s method in patients with acute-on-chronic respiratory failure. In such patients, the high PCO₂, shallow breathing and poor intrapulmonary gas mixing might be expected to defeat a method that depends on an equilibration of carbon dioxide between the alveolar gas and the rebreathing bag. In a number of studies, often done at night with Howell ‘doing the rebreathe’ and me ‘doing the bubble’, we found a satisfactory agreement between the indirect rebreathing estimate and the directly measured arterial PCO₂. But, of much more importance, we also explored the method.

The Collier method had two disadvantages: it needed a rapid carbon dioxide analyzer, and it needed cylinders spanning a range of known, high carbon dioxide concentrations, and neither of them was readily available in the United Kingdom at that time. Almost accidentally, we surmounted these snags – in the reverse order.

On our medical ward, there was always a cylinder of 7% carbon dioxide in oxygen for use in the emergency treatment of patients with coal gas poisoning. This served well as the bag mixture for patients whose PCO₂ was relatively normal, but not in those with a high PCO₂. However, we found that if the bag of 7% carbon dioxide was rebreathed for 1 to 1.5 min, the carbon dioxide concentration climbed sufficiently high that, when rebreathed again after a few minutes of rest, a satisfactory equilibration ‘plateau’ was obtained in the carbon dioxide analyzer tracing. Thus, the need for special carbon dioxide cylinders was obviated.

Second, we found that the PCO₂ of the gas remaining in the bag after 20 to 30 s of the second rebreathe was always
within 1 mmHg of the plateau value. Thus, a simple chemical carbon dioxide analyzer could be used to measure the bag \( PCO_2 \) instead of the rapid infrared machine. For this, I simplified the otherwise daunting Haldane analyzer (used as ‘gold standard’ analysis of oxygen and carbon dioxide) to measure carbon dioxide to within 0.2% (1 to 2 mmHg).

Subtracting 6 mmHg from the mixed venous value to estimate the arterial \( PCO_2 \) had always irritated me because the carbon dioxide dissociation curve is not linear, implying that such a correction should increase with increasing \( PCO_2 \). Much later, McEvoy et al (5) formally studied the effect of changes in \( PCO_2 \) on the difference and showed that multiplying the mixed venous \( PCO_2 \) by 0.8 led to a more precise estimate of arterial \( PCO_2 \) (which was logically more pleasing, as well).

In 1962, the two-stage method for measuring mixed venous and arterial \( PCO_2 \) was born (6). For a few years, it was very popular in the United Kingdom, until the introduction of the Severinghaus-Bradley carbon dioxide electrode. Nearly 20 years later, Powles and Campbell (7) elegantly argued that the combination of mixed venous \( PCO_2 \) and oxymetry could be used to assess both alveolar ventilation and systemic oxygen delivery, without recourse to all the plumbing that is commonplace nowadays in critical care facilities. The method still has a place in the rapid, noninvasive assessment of alveolar ventilation, particularly in outpatients; it remains a routine ordered breathing.

Our initial experiences with rebreathing led to a number of projects that preoccupied many of my colleagues and myself during the 1960s and 1970s that fit into the broad categories of body carbon dioxide stores, ventilatory control, breathing capacity and exercise.

**Body carbon dioxide storage capacity:** While developing the two-stage method, Howell and I had noticed that when one continued to rebreathe after the equilibrium plateau had been reached, the \( PCO_2 \) rose linearly at a rate of about 6 mmHg/min; incidentally, we had not yet realized how long one can rebreathe carbon dioxide from a small bag as long as it contains enough oxygen! A rate of 6 mmHg/min is much greater than is predicted from the buffering capacity of body fluids, suggesting that the carbon dioxide storage capacity is much less than was thought at the time. An obvious explanation was that it took time for carbon dioxide to be distributed between those organs with a high metabolic rate (the ‘fast carbon dioxide space’) and those with a lower metabolic rate (the ‘slow space’). However, such a situation should be reflected in a slackening of the rate of rise at a high \( PCO_2 \), when the difference in \( PCO_2 \) between the two spaces increases, whereas the rate appeared linear.

A good candidate for the slow space in a resting subject was muscle, which, at rest, comprises a large volume of low metabolic rate that is perfused with little blood flow. When they modelled body carbon dioxide stores, Fahri and Rahn (8) had found it necessary to exclude resting muscle in their predictions of the effects of rebreathing, and, even then, the fit between their model and the results of rebreathing experiments was not good, as shown later by Fowle and Campbell (9).

Fowle and Campbell (9) confirmed that the rate of rise in \( PCO_2 \) during rebreathing at rest was 6 mmHg/min and was constant over 4 to 6 min. Carbon dioxide production was measured to calculate the ‘immediate’ carbon dioxide storage capacity, and an average value of 0.5 mL/mmHg/L was obtained. Using the known value for the slope of the blood carbon dioxide dissociation curve gave a volume of 10 L, half of which was presumably blood. Fowle also studied rebreathing during exercise, which was associated with an increase in the rate of rise in \( PCO_2 \) to above 40 mmHg/min but a smaller increase than expected in the apparent immediate storage capacity.

Later, Fowle collaborated with Christine Matthews, a mathematician working in the Medical Research Council’s Cyclotron Unit at the Hammersmith Hospital, to measure arterial and expired gas concentrations of labelled \( ^{11} \) carbon dioxide and \( ^8 \) water after central venous administration (10). The results suggested that both carbon dioxide and water reached equilibrium very rapidly with extracellular fluid and more slowly with intracellular fluid. They concluded that during rebreathing at rest, carbon dioxide leaving tissues with a high rate of carbon dioxide production is rapidly distributed throughout the extracellular fluid but accumulates only slowly with the intracellular fluid of the tissues with a low carbon dioxide production; Fowle (9) had previously suggested that a lack of carbonic anhydrase could be responsible. Therefore, the rapid rate of rise in \( PCO_2 \) during rebreathing is due to the limited buffering of the extracellular fluid (especially interstitial fluid with its absence of buffers). Subsequently, using rebreathing with labelled carbon dioxide and analog computer modelling, Matthews et al (11) found that the distribution of carbon dioxide was incompatible with the hypothesis that the only factor limiting carbon dioxide distribution in the body is blood flow. The suggestion was made that a lack of carbonic anhydrase-like activity in intracellular fluid might explain the slow equilibration with intracellular fluid.

I am still unsure whether the rise in \( PCO_2 \) during rebreathing is a trivial matter capable of a simple explanation or an important matter whose understanding may be of fundamental importance and perhaps utility. We have learned a lot in the past decade about the determinants of carbon dioxide concentrations in various body compartments by considering Stewart’s physicochemical approach (12), which shows that interstitial fluid has no capacity to store carbon dioxide, and the low bicarbonate concentration in muscle also limits storage there.

**BODY CARBON DIOXIDE STORES, THE CARBON DIOXIDE BALANCE DURING EXERCISE AND ESTIMATION OF LACTATE ACCUMULATION**

Since the 1930s, an increase in the respiratory exchange ratio (RER) during exercise has been taken as an indication of lactic acid accumulation. However, the relationship between RER and lactate concentration is poor. It seemed clear to us that this was due in large part to the fact that sev-
eral factors influence RER during exercise in addition to lactate accumulation, and that changes in carbon dioxide ‘stores’ in the body could contribute in a major way, either by lowering RER if carbon dioxide stores increased or by increasing RER should carbon dioxide be ‘washed out’. To quantify these effects, it was necessary to understand the body’s capacity to store carbon dioxide in exercise, and to find an index of change in stores.

Clode and Campbell (13) studied normal patients during exercise in a steady state, and had them increase their ventilation voluntarily to wash out carbon dioxide, which was measured in Douglas bags, or rebreathe to retain carbon dioxide. Mixed venous $\text{PCO}_2$ was measured by rebreathing to reflect the change in $\text{PCO}_2$ of the stores, and it was found that the washout or retention of 1 mL carbon dioxide/kg body weight was accompanied by a 1 mmHg decrease or increase, respectively, in mixed venous $\text{PCO}_2$. That is, the carbon dioxide storage capacity was 1 mL/mm/kg, a value slightly less than twice the resting value found by Fowle and Campbell (9).

Clode and Campbell (13) used this value for storage capacity to construct a classical metabolic balance for carbon dioxide that incorporated total carbon dioxide output, carbon dioxide produced from aerobic metabolism (estimated from the volume of oxygen consumption per unit of time [VO2]), carbon dioxide from the reaction between lactic acid (HLA) and bicarbonate ($\text{HLA} + \text{Na}^+\text{HCO}_3^- = \text{NaLa} + \text{CO}_2 + \text{H}_2\text{O}$), and changes in body stores (calculated from changes in mixed venous $\text{PCO}_2$). That is:

$$\text{Total VCO}_2 = \text{Aerobic VCO}_2 + \text{CO}_2 \text{ from HLa + Stored CO}_2$$

Because three of these terms are readily and noninvasively measurable ($\text{VCO}_2$, VO2 and mixed venous $\text{PCO}_2$), the fourth (lactate accumulation in the blood) could be derived. The ‘fit’ between estimated and directly measured lactate concentration during exercise was good, indicating that the balance approach improved the noninvasive estimate of lactate. Although this quantitative approach was far more precise than any of the methods that followed (‘anaerobic threshold’), all of which remained qualitative in terms of the extent of lactate increase, the technique appears not to have been adopted by other groups.

The ventilatory response to carbon dioxide: David Read came to work at the Hammersmith Hospital and Postgraduate Medical School of London from Sydney. He realized early on that the increase in ventilation during rebreathing might be used to measure the ventilatory response to carbon dioxide. He placed the rebreathing bag securely in one opening of a bell-jar and, through the other opening, connected the bell-jar to a spirometer, which recorded the excursions of the bag (Figure 1); the so-called ‘bag-in-bottle’ arrangement became one of the most widely used simple methods in respiratory physiology of the 1960s and 1970s, and remains so to the present time (14).

When Read had normal patients rebreathe 7% carbon dioxide in oxygen, not only was the rate of rise of $\text{PCO}_2$ constant, but so was the rate of increase in ventilation (Figure 2). Hence, the ventilation to $\text{PCO}_2$ ratio was also a constant and, thus, Read’s rebreathing method was born (15). It soon replaced other methods, chiefly because it fulfilled ‘open loop’ conditions in which the ventilatory response did not affect the stimulus ($\text{PCO}_2$). Other methods using fixed concentrations of carbon dioxide breathed in a steady state did not meet this important control engineering feature and were time consuming as well. Read’s study, and many that followed, showed the ventilatory response to carbon dioxide to have a very wide (eightfold) range in normal patients, a physiologically sobering and clinically disappointing fact. Nevertheless, carbon dioxide responsiveness was shown to be related to the responses to hypoxia (16) and exercise (17), and it remains a useful tool in the investigation of hypventilation syndromes (18).
realized that Read’s rebreathing method, with its ‘open loop’ property, offered a means of elucidating this interaction. First, he found that the relationship between alveolar (and arterial) \( P_{CO_2} \) and the length of time for which patients could voluntarily hold their breath was linear: the higher the \( P_{CO_2} \), the shorter the time, with a decline in breath-holding time to zero at a \( P_{CO_2} \) of 70 to 80 mmHg (19) (Figure 4). Second, at the end of an initial breath-hold, even a single breath from the bag permitted the breath-hold to be resumed. The implication is that a nonchemical motor and/or sensory stimulus builds up during breath-holding, which, although powerful, may be cancelled by a single act of breathing, despite the fact that this act serves no ventilatory purpose.

**The sustained maximum breathing capacity:** For decades, the maximum breathing capacity (MBC) was conventionally measured with a large spirometer and by having patients ventilate maximally for 15 s. In terms of a respiratory exercise, it was a ‘sprint’, and the relevance of the results to the maximum ventilation that could be sustained over several minutes, such as required during exercise, was uncertain, rather like using the time for a 100 m dash to predict performance in a mile race. The short length of time used to measure MBC was constrained by the effects of hyperventilation with prolonged breath-holding; of course, carbon dioxide could have been added to the inspirate, but that would have been very clumsy.

Freedman devised an ingenious circuit in which the subject partly rebreathed from a small bag and partly breathed the surrounding air; by adjusting the proportion of each, it was possible to maintain end-tidal \( P_{CO_2} \) constant. The total ventilation was recorded by a gas meter, and patients could target their breathing to any desired level by superimposing a motor driven pointer, allowing the researcher to measure how long patients could maintain a given ventilation.

In normal subjects, Freedman (20) found that maximum voluntary ventilation fell off during the first 4 min, but could then be sustained at a level of about 70% of the 15 s ‘sprint’ or MBC value but with a wide variation from 40% to 90%.
The fall-off was attributed to respiratory muscle fatigue. In view of the prevailing definition of muscle fatigue (failure to maintain the required or expected force), that attribution is rather tautological. Subsequently, a study by Clark et al (21) found no difference between the 15 s MBC and the 4 min maximum voluntary ventilation in patients with severe airflow obstruction. With hindsight, one might suggest that in normal patients, the mechanical properties of the lungs and thorax permit a ventilation that can exceed the aerobic metabolic capacity of the respiratory muscles, but this is not true of patients with airflow obstruction in whom the mechanical limitations restrict the permissible work. In other words, the normal but not the ill patients could generate forces that were accompanied by 'high frequency' fatigue of the respiratory muscles.

**Mixed venous PCO\textsubscript{2} in exercise:** When I went to the (now Royal) Postgraduate Medical School at the Hammersmith Hospital in 1961, I attempted to develop a comprehensive method of analyzing the cardiorespiratory responses to exercise in health and disease that was based on the transport line for carbon dioxide rather than oxygen. There were theoretical and technical reasons for this, as will emerge below, but central to both was the measurement of mixed venous PCO\textsubscript{2}. A parallel objective was the development of a method to explore and present findings.

Exercise poses a number of problems in the use of rebreathing to estimate mixed venous PCO\textsubscript{2}: They include the need for high initial PCO\textsubscript{2} in the rebreathing bag, an appropriate bag volume to obtain rapid mixing without too much shrinkage, the recognition of a 'plateau' in PCO\textsubscript{2} indicating that the bag carbon dioxide breathed in is expired unchanged and the shortened circulation time during exercise. Jones and his colleagues met these problems, in order, by developing a recipe for the initial bag carbon dioxide breathed in is expired unchanged and the shortened circulation time during exercise. Jones and his colleagues met these problems, in order, by developing a recipe for the initial bag PCO\textsubscript{2} that depended on the workload, finding the required volume to be 1.5 times the patient's tidal volume, developing criteria for a 'true' plateau and by a combination of techniques showing that significant recirculation did not occur before 15 s of rebreathing (22). The last finding was fortunate because it had been a concern that recirculation did not occur before 15 s of rebreathing (22). The last finding was fortunate because it had been a concern that recirculation might occur very rapidly during heavy exercise and, thus, before an equilibrium of PCO\textsubscript{2} could be obtained.

**The four-quadrant diagram – A display of integration between physiological mechanisms:** Central to the thinking about carbon dioxide transport and excretion was an appreciation of the following relationship:

\[
\text{Venous – expired PCO}_2 = (\text{venous – arterial PCO}_2) + (\text{arterial – expired PCO}_2)
\]

Now, (venous – arterial PCO\textsubscript{2}) is the denominator in the Fick equation:

\[
\text{Cardiac output} = \frac{(\text{CO}_2 \text{ output}) \times (\text{venous – arterial CO}_2 \text{ content})}{\text{arterial – expired PCO}_2}
\]

And (arterial – expired PCO\textsubscript{2}) is the numerator in the Bohr equation for the dead space/tidal volume ratio (\(V_D/V_T\)):

\[
V_D/V_T = (\text{arterial – expired PCO}_2) \times (\text{arterial PCO}_2)
\]

Thus, arterial PCO\textsubscript{2} is common to both equations, and given mixed venous and expired PCO\textsubscript{2} knowledge of arterial PCO\textsubscript{2} would allow solution of both equations. But, it was argued further: given the usual changes in cardiac output and \(V_D/V_T\) with exercise, a normal difference between venous and expired PCO\textsubscript{2} could only imply that both of these components were normal. This is because for a normal PCO\textsubscript{2} difference, a low cardiac output would imply a vanishingly small dead space, and a high \(V_D/V_T\) would imply an impossibly high cardiac output. This logic suggested that arterial PCO\textsubscript{2} could be estimated, and most of the classical physiological variables calculated, without recourse to catheters and blood samples. Later studies, in brief, showed that this was right.

These relationships were embodied in a rather formidable equation that provided rigour but little insight. Then the idea was had of 'doing for carbon dioxide' what Barcroft (23) had 'done for oxygen' in his marvelous 1934 book *Features in the Architecture of Physiological Function* to develop a graphical display in four quadrants, of the integration between processes and variables involved in gas transport between air and exercising muscle. In Barcroft's diagram (23), each of the quadrants depicted variables involved in each of the four major oxygen transport processes – diffusion, blood flow, blood oxygen capacity and hemoglobin flow. The remarkable feature of the diagram was that each quadrant shared an axis with the one next to it, enabling integration (or disintegration) to be graphically and quantitatively displayed. The major, or starting, independent axis was the metabolic oxygen consumption. In McHardy's diagram, the analogous axis was metabolic carbon dioxide production. This was plotted on the right-sided horizontal axis (Figure 5), with the upper quadrant expressing the responsive ventilation to carbon dioxide output:

\[
\text{Expired PCO}_2 = \text{VCO}_2 \times \text{VE}
\]

and the lower expressing the Fick equation applied to carbon dioxide as the following:

\[
\text{Arteriovenous CO}_2 \text{ content difference} = \text{VCO}_2 \times \text{cardiac output}
\]

The upper left quadrant was used to relate expired PCO\textsubscript{2} to arterial PCO\textsubscript{2} through a modification of the Bohr equation:

\[
\text{Arterial PCO}_2 = \text{expired PCO}_2 (1 – \frac{V_D}{V_T})
\]

So far, so good. A final graphical relationship between the venous-arterial PCO\textsubscript{2} difference and the venous-arterial difference in carbon dioxide content was needed to combine the equations of Bohr and Fick. The carbon dioxide 'dissociation' curve needed to be described, but how? McHardy eventually produced the solution, which was a remarkable breakthrough.

McHardy (24) constructed partial carbon dioxide dissociation curves for a range of mixed venous PCO\textsubscript{2} values, originating at points where mixed venous PCO\textsubscript{2} equalled arterial PCO\textsubscript{2} and, obviously, the difference in the carbon dioxide content was zero. For any given mixed venous PCO\textsubscript{2}, an intersection of the curve by a vertical line for the equivalent arterial PCO\textsubscript{2} yielded the veno-arterial content difference. We were very proud of this effort (25), and for many years our weekly physiological seminars were based on the four-quadrant diagram. In this age of computers, it has little
to offer as an aid to calculation, but it remains very revealing when used ‘à la Barcroft’ to explore and display integration, particularly when relationships become anilnear (26).

**Alveolar-arterial PCO₂ differences and the ‘downstream experiment’:** When it was thought that the rebreathing method for measuring mixed venous PCO₂ during exercise had been perfected, a study of the exercise responses in normal patients was undertaken. When the Fick equation was applied to carbon dioxide for the calculation of cardiac output, the suggested values were lower than published values. So we embarked on a difficult study to compare the cardiac output determined by the rebreathing method with results obtained using the gold standard ‘direct’ Fick oxygen method, which required arterial and central venous blood sampling. I say ‘difficult’ advisedly because at one point, Norman Jones had to telephone my wife to inform her that I would not be home that night, having developed transient atrial fibrillation while a catheter was being floated into my pulmonary artery. Again, the ‘indirect’ carbon dioxide cardiac outputs were low, by about 25% (22). The implication of the discrepancy was that the alveolar ‘plateau’ value of PCO₂ during rebreathing was not related to the central venous carbon dioxide content through the (static) blood in vitro dissociation curve; the derived content was too high and, thus, arteriovenous carbon dioxide content difference was wider than expected. Hence, because the plateau showed an equilibration of PCO₂ between alveolar gas and capillary blood, and the venous carbon dioxide content was unchangeable, it appeared that the PCO₂ during rebreathing decreased when leaving the lungs, en route not only to the arterial blood a few seconds later but also to the blood gas electrodes several minutes later. It seemed that this possibility might be tested by sampling arterial blood while rebreathing was going on, and so we fitted a series of three-way taps together and found that we were able to take up to six samples during the rebreathe, ‘downstream’ from the lungs. In eight normal patients, ourselves and colleagues, we found that the plateau PCO₂ was systematically higher than the corresponding arterial value, the difference increasing with increasing exercise and carbon dioxide output from less than 2 mmHg at rest to as much as 10 mmHg in heavy exercise (22). Later we found that the difference was closely related to the actual equilibrium PCO₂, explaining much of the interindividual variation (27).

Various explanations were offered for this phenomenon; some of our critics felt that it was due to technical error, such as not allowing for a higher temperature in the lungs than in the measuring electrodes. We satisfied ourselves that the measurements were as immaculate as was possible; measurement of esophageal temperature showed that temperature was not a factor. The discussion of possible mechanisms reached exotic levels, including an ionic effect at the capillary endothelium due to flowing blood – the Wein effect (28). There was a debate in the *Journal of Applied Physiology* between Gail Gurtner, who had found an alveolar arterial difference in an isolated dog lung preparation (29), and Robert Forster, the ultimate guru of pulmonary gas exchange and blood gas kinetics (30), but to this day, the controversy has not been resolved (nor have the experiments been repeated!).

My favorite explanation is an ‘upstream’ mechanism, in which blood carbon dioxide in all its forms may not attain equilibrium in the tissue (particularly the muscle) capillaries and is still changing by the time blood reaches the lungs. Differently, I put this suggestion to Robert Forster; he said that he had an open mind: while the primary exchanges and reactions affecting carbon dioxide are probably complete in the course of the tissue capillaries, many other substances are exchanged whose reactions are slower but may secondarily affect carbon dioxide. A candidate is chloride exchange through the red cell membrane, which is slow enough to affect bicarbonate equilibration between plasma (having no carbonic anhydrase) and erythrocytes. The mixed venous and possibly even arterial blood may not be as stable as is commonly assumed, particularly during heavy exercise.

One of the possible explanations for the high equilibrium PCO₂ was that it was due in some way to the oxygenation of hemoglobin in the pulmonary capillaries. To test this hypothesis, Norman Jones had patients rebreathe carbon dioxide in nitrogen to obtain equilibration of oxygen as well as carbon dioxide. Throughout this work, we were fortunate to have one of the first respiratory mass spectrometers and an exceptionally talented technician, Ted Davies, to keep its many vacuum pumps and electronics in tip-top shape so that tracings of the highest quality were obtained for carbon dioxide, oxygen and nitrogen. We showed that even in the absence of oxygenation, the alveolar-arterial difference was...
The main results of the study by Edwards et al (32), showing the differences in rates of change in $P_{CO_2}$ and $P_{O_2}$ in mixed venous blood, measured indirectly by rebreathing

**REFERENCES**


**FINAL WORDS**

Of course, there is a lot of unfinished business. The mechanisms and significance of many of the matters that I have summarized here are still unclear, including the kinetics and mechanism of carbon dioxide storage, the variability of the ventilatory response to hypercapnia and hypoxia, the discomfort of breath-holding and its relief by a single breath, the mechanism and importance of respiratory muscle fatigue, the downstream phenomenon and unsteady-state exercise.

Anyone wishing to venture into these fields would be well advised to include in their grant application a few dollars for a small rubber bag.