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

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EJM Campbell. Multum in parvo: Explorations with a small bag of carbon dioxide. Can Respir J 2001;8(4): 271-278.

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 (PCO_2) 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 PCO_2 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 PCO_2 climbed. Rebreathing also allowed increases in voluntary ventilation to be achieved without the development of alkalosis, leading to studies of maximal voluntary ventilation and respiratory muscle fatigue. Equilibration of PCO_2 during rebreathing was used to measure mixed venous PCO_2 during exercise and develop an integrated approach to the physiology of exercise in health and disease; alveolar-arterial disequilibrium in PCO_2 during exercise was uncovered. Equilibration of PCO_2, as well as PO_2, during rebreathing of carbon dioxide and nitrogen gas mixtures showed different time courses of venous gases at the onset of exercise. Starting with the rebreathing of carbon dioxide in oxygen mixtures in a small rubber bag, an astonishing range of topics in respiratory physiology was explored, with observations that remain valid, but in some respects unresolved, to the present day.

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

Condensé : Expériences menées avec un ballonnet de CO_2

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 CO_2 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 PCO_2 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 CO_2 (PCO_2) durant la réinhalation a conduit à une analyse de la capacité de storage du CO_2 par l'organisme. Ces connaissances sur le storage du CO_2 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 PCO_2 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 PCO_2. 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 PCO_2 durant la réinhalation a servi à mesurer la PCO_2 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 PCO_2 durant l'exercice a ainsi été découvert. L'ajustement de la PCO_2 et de la PO_2 durant la réinhalation d'un mélange gazeux de CO_2 et d'azote a donné lieu à des temps d'absorption

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différents des gaz veineux en début d'exercice. Débutant par la réinhalation de CO_2 intégré à des mélanges d'oxygène contenus dans des ballonnets de caoutchouc, l'expérience a permis l'exploration d'un étonnant éventail des différents aspects de la physiologie respiratoire; certaines de ces observations demeurent valides aujourd'hui, alors que d'autres nous échappent encore.

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 (PCO_2) (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 PCO_2 method, and the several lines of research that spun 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 story. 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 Rahn'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 PCO_2

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 PCO_2. Then, in 1956, Collier (3) described his rebreathing method for measuring mixed venous PCO_2. In this method, the patient rebreathes from a small bag primed with a mixture of carbon dioxide in oxygen, having a PCO_2 that is felt to be above the patient's mixed venous PCO_2. For example, in a healthy patient, an 8% carbon dioxide mixture might be used (PCO_2 of about 56 mmHg). The PCO_2 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 PCO_2 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 PCO_2 (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 PCO_2 (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 PCO_2, 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 PCO_2. 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 PCO_2 was relatively normal, but not in those with a high PCO_2. 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 equilibrium 'plateau' was obtained in the carbon dioxide analyzer tracing. Thus, the need for special carbon dioxide cylinders was obviated.

Second, we found that the PCO_2 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 technique in the laboratory at McMaster University for assessing patients with severe airflow limitation and sleep disordered 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 ^{3}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 blood lactate concentration is poor. It seemed clear to us that this was due in large part to the fact that sev-

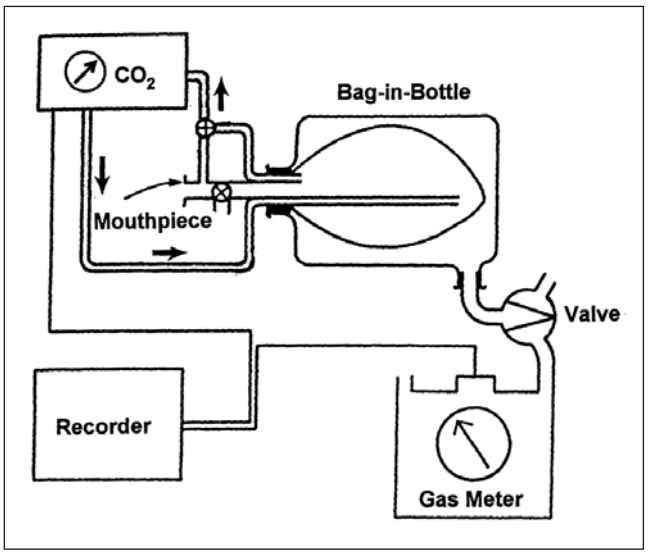

Figure 1) *Diagram of equipment used to measure the ventilatory response to carbon dioxide (CO_2)*

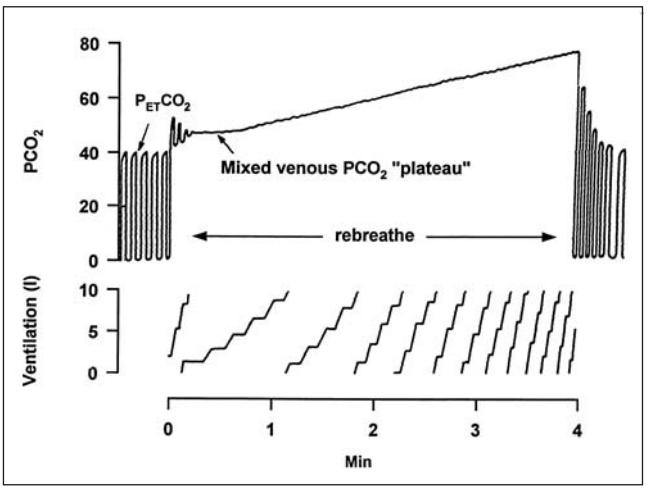

Figure 2) *Records obtained during rebreathing, showing the initial equilibrium plateau (mixed venous partial pressure of carbon dioxide [PCO_2]), followed by a linear increase in PCO_2 (at a rate of about 6 mmHg) and increasing ventilatory response. $P_{ET}CO_2$ End-tidal PCO_2*

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 PCO_2 was measured by rebreathing to reflect the change in 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 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 [VO_2]), carbon dioxide from the reaction between lactic acid (HLa) and bicarbonate ($HLa + Na^+HCO_3^- = NaLa + CO_2 + H_2O$), and changes in body stores (calculated from changes in mixed venous PCO_2). That is:

$$\text{Total } VCO_2 = \text{Aerobic } VCO_2 + CO_2 \text{ from HLa} \pm \text{Stored } CO_2$$

Because three of these terms are readily and noninvasively measurable (VCO_2, VO_2 and mixed venous 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 PCO_2 constant, but so was the rate of increase in ventilation (Figure 2). Hence, the ventilation to 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 (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 hypoventilation syndromes (18).

The ventilatory response to hypoxia: The success of Read's method was due to the fact that when an individual

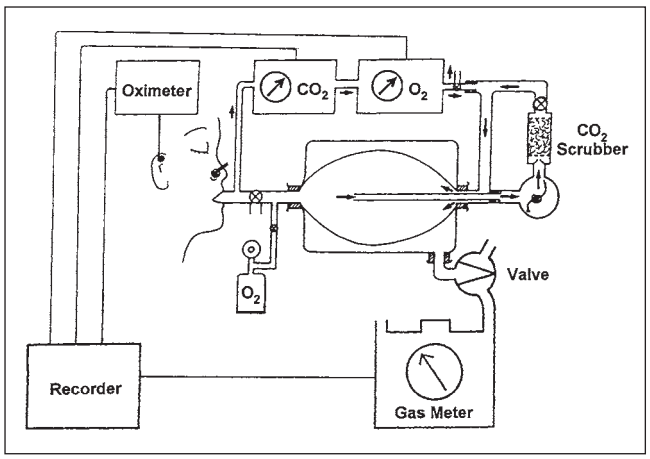

Figure 3) *Experimental set-up used to measure hypoxic responsiveness and maximal voluntary ventilation. CO_2 Carbon dioxide; O_2 Oxygen*

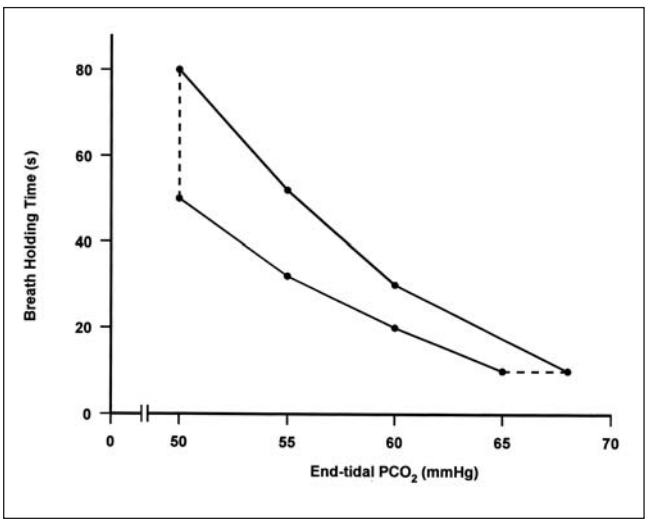

Figure 4) *The fall in breath-holding time with increasing partial pressure of carbon dioxide (PCO_2) observed by Godfrey and Campbell (19)*

rebreathes from a small bag, the response does not affect the stimulus; so-called 'open loop' conditions obtain. Tony Rebuck applied the same principle to the measurement of the ventilatory response to hypoxia. This required several modifications to the equipment and procedure (Figure 3). First, an adjustable carbon dioxide 'scrubber' circuit containing soda-lime had to be added to maintain isocapnia. Second, a method of measuring arterial oxygenation that was both accurate and prompt was needed to avoid risky degrees of hypoxia (an ear oximeter was found to fit the bill). Third, to accelerate equilibration, the bag was initially filled with 7% carbon dioxide and 24% oxygen, and rebreathed until arterial saturation fell to about 50%.

Rebuck and Campbell (16) used this system in normal patients and observed the well known hyperbolic relationship between increases in ventilation and falls in alveolar PO_2 that had been subjected previously to mathematical treatments of varying complexity. However, he also observed that the increase in ventilation was *linearly* related to the fall in *oxygen saturation*. Accepting that the ultimate chemoreceptor stimulus is PO_2, it is not unreasonable to suggest that in a highly metabolic tissue such as the carotid body, the PO_2 is governed by the amount, rather than the pressure, of oxygen in its blood supply.

Despite the qualms of some purists, Rebuck's method has taken its place as the most widely used method for evaluating hypoxic responsiveness (14). As well as finding a relationship between responsiveness to carbon dioxide and hypoxia, Rebuck also found a very wide range in the normal hypoxic responsiveness that was in keeping with Read's results, a finding that was intriguing, physiologically sobering and clinically disappointing.

The physiology of breath-holding: During studies of rebreathing, it became well known that people can rebreathe for far longer than they are able to hold their breath, implying that there is some sort of interaction between chemical and mechanical stimuli during breath-holding. Simon Godfrey 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) PCO_2 and the length of time for which patients could voluntarily hold their breath was linear: the higher the PCO_2, the shorter the time, with a decline in breath-holding time to zero at a PCO_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 PCO_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_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_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_2. They include the need for high initial PCO_2 in the rebreathing bag, an appropriate bag volume to obtain rapid mixing without too much shrinkage, the recognition of a 'plateau' in PCO_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 PCO_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 might occur very rapidly during heavy exercise and, thus, before an equilibrium of PCO_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:

$$(\text{Venous} - \text{expired } PCO_2) = (\text{venous} - \text{arterial } PCO_2) + (\text{arterial} - \text{expired } PCO_2)$$

Now, (venous – arterial PCO_2) is the denominator in the Fick equation:

$$\text{Cardiac output} = (CO_2 \text{ output}) \times (\text{venous} - \text{arterial } CO_2 \text{ content})$$

And (arterial – expired PCO_2) is the numerator in the Bohr equation for the dead space/tidal volume ratio (V_D/V_T):

$$V_D/V_T = (\text{arterial} - \text{expired } PCO_2) \times (\text{arterial } PCO_2)$$

Thus, arterial PCO_2 is common to both equations, and given mixed venous and expired PCO_2 knowledge of arterial PCO_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_2 could only imply that both of these components were normal. This is because for a normal PCO_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_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:

$$\text{Expired } PCO_2 = VCO_2 \times VE$$

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

$$\text{Arteriovenous } CO_2 \text{ content difference} = VCO_2 \times \text{cardiac output}$$

The upper left quadrant was used to relate expired PCO_2 to arterial PCO_2 through a modification of the Bohr equation:

$$\text{Arterial } PCO_2 = \text{expired } PCO_2 \, (1 - V_D/V_T)$$

So far, so good. A final graphical relationship between the venous-arterial PCO_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_2 values, originating at points where mixed venous PCO_2 equalled arterial PCO_2 and, obviously, the difference in the carbon dioxide content was zero. For any given mixed venous PCO_2, an intersection of the curve by a vertical line for the equivalent arterial PCO_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 alinear (26).

Alveolar-arterial PCO_2 differences and the 'downstream experiment': When it was thought that the rebreathing method for measuring mixed venous PCO_2 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 PCO_2 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 PCO_2 between alveolar gas and capillary blood, and the venous carbon dioxide content was unchangeable, it appeared that the PCO_2 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 PCO_2 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 PCO_2, 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. Diffidently, 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 PCO_2 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

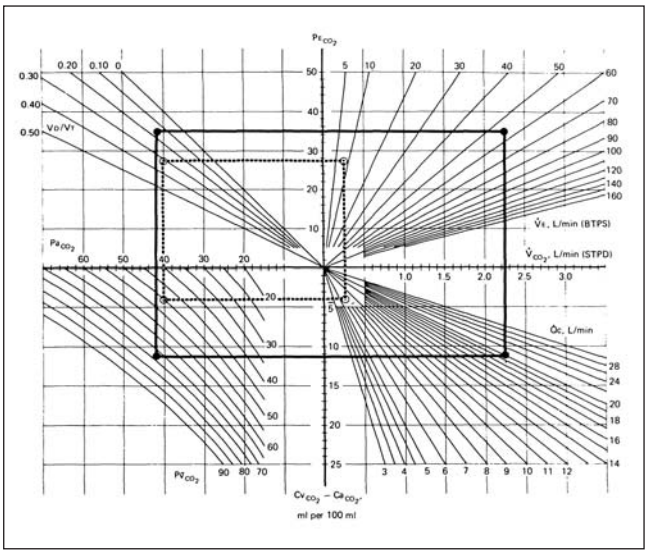

Figure 5) *McHardy's quadrantic diagram, showing values obtained at rest and during exercise in a normal subject. Note that the responses to the approximately 10-fold increase in carbon dioxide production with exercise include a three-and-a-half-fold increase in cardiac output (lower right quadrant), threefold widening of the veno-arterial carbon dioxide difference in content and pressure (lower left quadrant), no change in arterial PCO_2, 50% fall in V_D/V_T ratio (upper left quadrant), 30% increase in the PCO_2 of mixed expired gas (vertical axis) and sevenfold increase in ventilation (upper right quadrant). The diagram allows changes in responses associated with cardiorespiratory disorders to be explored quantitatively (26)*

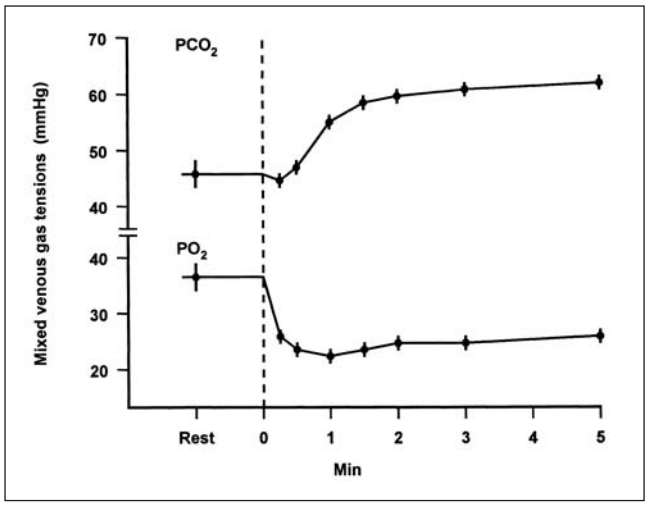

Figure 6) *Changes in mixed venous partial pressure of carbon dioxide (PCO_2) and partial pressure of oxygen (PO_2) at the onset of exercise. The main results of the study by Edwards et al (32), showing the differences in rates of change in PCO_2 and PO_2 in mixed venous blood, measured indirectly by rebreathing*

still present for carbon dioxide (but not for oxygen) (31). A by-product of this study was that it became possible, with sufficient attention to technique, to obtain mixed venous plateaus for both carbon dioxide and oxygen.

Mixed venous blood gases at the start of exercise: Most studies of exercise have concentrated on the 'steady state', and little attention had been paid to the transient changes at the start of exercise. Furthermore, the short duration of most daily exercise means that a steady state is seldom attained. The rates at which most mechanisms change at the onset of exercise will have a major effect on the mixed venous oxygen and carbon dioxide. For these reasons, Edwards and his colleagues (32) undertook a series of studies of mixed venous PCO_2 and PO_2 using rebreathing of carbon dioxide in nitrogen. They found that there was a delay of 30 s after the onset of exercise before PCO_2 began to rise and the steady-state value was not achieved until 5 min (Figure 6). On the other hand, the PO_2 started to fall in 15 s and reached a minimum in 1 min. They attributed the changes in PCO_2 to hyperventilation and the changes in PO_2 to a lag in the response of the tissue circulation to tissue oxygen consumption.

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.

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