Cardiac output during exercise by the open circuit acetylene washin method: comparison with direct Fick

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Cardiac output during exercise by the open circuit acetylene washin method: comparison with direct Fick. J Appl Physiol 88: 1650–1658, 2000.—An open-circuit (OpCirc) acetylene uptake cardiac output (Q̇T) method was modified for use during exercise. Two computational techniques were used. OpCirc1 was based on the integrated uptake vs. end-tidal change in acetylene, and OpCirc2 was based on an iterative finite difference modeling method. Six subjects [28–44 yr, peak oxygen consumption (VO₂) = 120% predicted] performed cycle ergometry exercise to compare Q̇T using OpCirc and direct Fick methods. An incremental protocol was repeated twice, separated by a 10-min rest, and subsequently subjects exercised at 85–90% of their peak work rate. Coefficient of variation of the OpCirc methods and Fick were highest at rest (OpCirc1, 7%, OpCirc2, 12%, Fick, 10%) but were lower at moderate to high exercise intensities (OpCirc1, 3%, OpCirc2, 3%, Fick, 5%). OpCirc1 and OpCirc2 Q̇T correlated highly with Fick Q̇T (R² = 0.90 and 0.89, respectively). There were minimal differences between OpCirc1 and OpCirc2 compared with Fick: up to moderate-intensity exercise (<70% peak VO₂); however, both techniques tended to underestimate Fick at >70% peak VO₂. These differences became significant for OpCirc1 only. Part of the differences between Fick and OpCirc methods at the higher exercise intensities are likely related to inhomogeneities in ventilation and perfusion matching (R² = 0.36 for Fick – OpCirc1 vs. alveolar-to-arterial oxygen tension difference). In conclusion, both OpCirc methods provided reproducible, reliable measurements of Q̇T during mild to moderate exercise. However, only OpCirc2 appeared to approximate Fick Q̇T at the higher work intensities.

pulmonary blood flow; solubility; inhomogeneity; dead space

MUL TIPLE NONINVASIVE TECHNIQUES have been developed to assess cardiac output (Q̇T) at rest and during exercise. The most common include acetylene rebreathing, carbon dioxide rebreathing, Doppler, and electrical impedance cardiography (3, 6, 10, 13, 18, 25). Among these techniques, the acetylene rebreathing technique has been validated against invasive techniques and has gained wide acceptance (11, 26). A drawback to the method, however, is the build-up of carbon dioxide as a result of rebreathing and the resultant dyspnea. This is a particular problem at higher intensities of exercise or with the longer rebreathing times that may be necessary when equilibration of gases is prolonged, such as in patients with ventilation inhomogeneity (i.e., aging or obstructive airway disease) (12). Another drawback to the acetylene rebreathing technique is the potential change in the lung-rebreath bag volume due to a changing respiratory quotient and potential errors that can occur when trying to fill the rebreath bag with a precise volume (12).

Previous work by Stout et al. (24), Gan et al. (7), and Nielsen et al. (16) has suggested the use of an open-circuit washin method (OpCirc) to assess Q̇T at rest and during exercise. The technique is very similar to rebreathe, requiring two inert gases, one soluble and the other essentially insoluble, to be able to compute pulmonary blood flow and to correct for changes in lung volume and alveolar dead space, respectively. Because the method only requires a washin of 6–10 breaths of the two inert gases, there is no rebreathing, and rebreathing remains spontaneous (i.e., without change in breathing pattern or, in many cases, cognition on the part of the test subject). To date, the OpCirc method for assessing Q̇T has been compared with thermodilution in anesthetized, ventilated dogs and compared with the rebreath technique in humans (7, 16, 24). The present investigation compares methods described by Stout et al. (24) and Gan et al. (7) to direct Fick measurements of Q̇T at rest and during mild through heavy exercise in healthy humans. We propose that the OpCirc method compares closely with invasive measurements of Q̇T and is highly reproducible in mild through heavy exercise.

METHODS

Subjects. All aspects of the study were approved by the Mayo Clinic Institutional Review Board. Subjects consisted of staff clinicians or residents in the Department of Anesthesiology who reviewed and gave written, informed consent before participation. Usual activity levels varied among the subjects; four subjects were former athletes and maintained a moderate level of training during the time of the study. Before testing, subjects were instructed to fast for at least 2 h. The majority of studies were performed in the early morning, thus resulting in a fasting period ≥8 h. Subjects were also instructed to avoid exercise the day of testing and to avoid heavy exercise training the day before testing.

Exercise protocol. Subjects reported to the General Clinical Research Center Exercise Core Laboratory on two occasions for exercise studies. During the first session, subjects were familiarized with the testing equipment and performed a maximal cycle ergometry test to volitional exhaustion. Dur-
During the second session, catheters were placed in the pulmonary and radial arteries and measurements of \( Q_t \) were assessed using the direct Fick and OpCirc methods. Pulmonary artery catheters (7 Fr, 65 cm, double-lumen pulmonary artery catheter; Arrow International, Reading, PA) were inserted via an 8-Fr introducer catheter (Arrow International) under local anesthesia through an antecubital vein during continuous electrocardiogram and pressure monitoring. The correct position of the pulmonary artery catheter was verified by the wedge position and pressure tracings. Flexible, 20-gauge, PVC radial artery catheters were inserted percutaneously under local anesthesia and taped in place to allow multiple arterial blood-gas samples to be taken. Both catheters were continuously flushed with heparinized saline (3 U/ml heparin; 3 ml/h) to maintain patency. Measurements were obtained at rest and during three submaximal steady-state work intensities (20, 40, and 60% of peak power). Each work intensity lasted 7–9 min, the time required to reach steady state as followed by two repeat sets of measurements. After 2–3 min at constant power to allow steady state in oxygen consumption (\( V_{O2} \)), arterial and mixed venous blood samples were drawn simultaneously, whereas oxygen consumption was measured over approximately a 30-s period. This was followed by an OpCirc measurement (8–12 breaths of acetylene-helium gas mixture). Allowing 1–2 min at constant power setting of the ergometer to wash out the inert gases from the subject’s lungs, we repeated the measurements of \( V_{O2} \) and OpCirc. On termination of the second OpCirc data collection, power output of the ergometer was increased to the next level and the procedures were repeated. After completion of the three submaximal work loads, a rest period of 5–10 min was taken, and the three work levels were subsequently repeated. When the second set of samples was completed, most subjects had an additional rest period, followed by a final work bout at –80–85% of the peak power achieved on their initial exercise test. Measurements of \( V_{O2} \), blood sampling, and OpCirc were taken again, similar to the submaximal work load measurements. One subject was unable to complete these last measurements.

OpCirc technique \( Q_t \) was measured noninvasively using the OpCirc inert gas washin method. The technique has been described previously (7, 16, 24). Figure 1 shows the valve setup for the washin of inert gases. A pneumotachograph (Hans Rudolph, Kansas City, MO, 381 series; transducer, Celecso, \( \pm 2 \) cmH\(_2\)O) was connected to a nonbreathing \( Y \) valve (Hans Rudolph, 273C), whose inspiratory port was connected to a pneumatic switching valve with low resistance and low dead space (Hans Rudolph, P0271, controller 4285A), allowing switching between room air and the \( Q_t \) gas mixture (0.7% \( \text{C}_2\text{H}_2 \), 21% \( \text{O}_2 \), 9% He, balance \( \text{N}_2 \)) on the inspired port with minimal dead space (<50 ml). Gases were sampled via a mass spectrometer (Perkin-Elmer, 1100), with a phase delay of 0.27 s. Throughout the resting and exercise breathing, the subjects were encouraged to breathe in a regular rhythm, avoiding coughs, swallows, and partial breaths, if possible. The subjects were allowed to breathe normally from the valve, and the operator switched the pneumatic valve to change the inspiratory air source to the \( Q_t \) mixture. The washin of \( \text{C}_2\text{H}_2 \) and helium was observed on the computer screen by the operator on a breath-by-breath basis, until eight breaths had been obtained, as shown in Fig. 2. Data analysis was performed immediately after each maneuver using the relatively rapid calculation method (OpCirc1) described by Gan et al. (7) and Stout et al. (24), as outlined in the APPENDIX. In addition, data were analyzed after the exercise session using a finite difference modeling method (OpCirc2), also outlined in the APPENDIX. For both techniques, we used all eight breaths. OpCirc1 calculated the uptake of \( \text{C}_2\text{H}_2 \), taking breath pairs, with 28 possible pairs. As a part of the data-smoothing process, outlier solutions of the breath pairs (>98th percentile) were removed and the \( Q_t \) was reaveraged. OpCirc2 examined all breaths and included a minimization technique to minimize differences between modeled and actual end-tidal gas concentrations (see APPENDIX).
Early in the development of the washin technique, we found it important to minimize valve and breathing apparatus dead space so that full-scale change in inspired acetylene was observed on the first breath of the washin. In addition, it was critical to eliminate small leaks in the tubing, gas reservoir, and nose clip, around the mouthpiece, and in old valves. We redsigned the pneumatic valve to minimize airway resistance during exercise while keeping the valve dead space low. We checked the mass spectrometer response time to assure that no significant changes occurred over the course of the study and that the response time was adequate to assess acetylene gas concentrations associated with high breathing frequencies.

The acetylene channel of some mass spectrometers may also have faster kinetics when primed with test gas immediately before a measurement is made. We tested this using our system and did not find prior priming of the channel to appreciably alter the acetylene kinetics. Since the technique required accurate assessment of breath-by-breath tidal volumes, we found it critical to develop careful calibration curves for the pneumotachograph and to eliminate or minimize any drift due to temperature, humidity, and electronics. The pneumotachograph was linearized using the technique of Yeh et al. (27) and calibrated using test gas before each study.

Blood gases. Mixed venous blood samples were drawn from the pulmonary artery catheter at the same time that arterial blood was drawn from the radial artery catheter, and measurements of \( V_2 \) were taken. The blood samples were analyzed for oxygen content using an IL 1306 co-oximeter and blood gases (\( P_{O2}, \ P_{H}, \text{ and } P_{CO2} \)) were analyzed by using an IL 482 blood-gas analyzer. \( Q_T \) was then determined using Fick (\( V_2 = \frac{V}{V_2} \times \text{arteriovenous oxygen difference} \)). All blood samples were collected anaerobically, agitated, and immediately chilled in crushed ice before analysis (usually within 5–10 min of collection). The co-oximeter and blood-gas analyzer were calibrated with standards over the broad range of oxygen and carbon dioxide values expected during the study. An in-dwelling, fast-response thermocouple at the end of the pulmonary artery catheter was used to assess temperature changes during exercise for correcting the blood-gas values before calculation of \( Q_T \).

Data analysis. \( Q_T \) at each work intensity was calculated using OpCirc1 and OpCirc2 methods for each subject. Each subject had four data points per work level, except at the highest work intensities, where only two data points were obtained per subject. This included repeat measurements of \( V_2 \) and \( Q_T \) during each of two exercise sessions at the lower work intensities and repeat measurements during one exercise session for the highest work intensity. For each subject, all data points were submitted to least squares regression using the equation \( Q_T = K_0 + V_2 \times K_1 + \frac{V_2}{2} \times K_2 \), where \( K_0, K_1, \text{ and } K_2 \) are the intercept, linear term, and squared term coefficients, respectively. From the regression data on each subject, \( Q_T \) was estimated at fixed levels of \( V_2 \), and the interpolated data were averaged for all subjects. The fit was very good (\( R^2 > 0.97 \) for OpCirc, \( R^2 > 0.93 \) for Fick). Paired \( t \)-tests were applied to the interpolated data to test for differences in techniques (\( P < 0.05 \)).

RESULTS

Subject characteristics. Five male subjects and one female subject were tested. Four of the subjects were moderately active, typically running or cycling several times per week. The peak \( V_2 \) obtained from the initial cycle ergometry test averaged 120% of age predicted. Peak heart rate averaged 102% of age predicted.

<table>
<thead>
<tr>
<th>Table 1. Subject characteristics</th>
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<tr>
<td><strong>Age, yr</strong></td>
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<tr>
<td><strong>Height, cm</strong></td>
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<tr>
<td><strong>Weight, kg</strong></td>
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<tr>
<td><strong>Peak ( V_2 ), ml·kg(^{-1})·min(^{-1})</strong></td>
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<td><strong>Peak power, W</strong></td>
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<td><strong>Peak HR, beats/min</strong></td>
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Subjects included 5 men and 1 woman. \( V_2 \), oxygen consumption; HR, heart rate.

Other characteristics of the group are also shown in Table 1.

OpCirc vs. Fick measurements of \( Q_T \). Figure 3 shows identity plots of OpCirc1 (A) and OpCirc2 (B) vs. Fick for all values obtained on all subjects. Each subject typically had four data points per work intensity, except at rest and at the highest work intensity, for which only two data points were obtained per subject. \( R^2 \) values for OpCirc1 and OpCirc2 vs. Fick for the entire data set were 0.90 and 0.89, respectively. The mean differences (±SD) between techniques were \(-0.9 ± 2.0 \) and \(-0.9 ± 1.9 \) l/min for OpCirc1 vs. Fick and OpCirc2 vs. Fick, respectively.

Table 2 compares mean values for OpCirc1 and OpCirc2 with mean Fick measurements at rest and over the four work levels. Other pertinent exercise and gas exchange data are also shown in Table 2. OpCirc1 and OpCirc2 did not differ from Fick at rest or over the
first two work intensities (37 and 54% of peak \( \dot{V}O_2 \)). However, OpCirc1 (but not OpCirc2) was less than Fick (\( P < 0.05 \)) over the highest two work intensities (71 and 91% of peak \( \dot{V}O_2 \)). Figure 4 shows the OpCirc1 and OpCirc2 data relative to direct Fick measurements at interpolated \( \dot{V}O_2 \) values. OpCirc1 was less than Fick between \( \dot{V}O_2 \) of 3.5 and 4.5 l/min (\( P < 0.05 \)), but no significant differences were noted otherwise. Therefore, the OpCirc2 calculation method more closely tracked Fick \( \dot{Q}T \) measurements with heavy exercise.

Reproducibility of \( \dot{Q}T \) measurements. Within-session (a given work intensity) and between-session (repeat work intensity after a period of rest) coefficients of variation (CV) of OpCirc1, OpCirc2, Fick, and \( \dot{V}O_2 \) are shown in Table 3. The greatest variability for all measurements was observed at rest. The within-session and between-session CV for OpCirc1 and OpCirc2 averaged <4% for all work intensities and were similar to or less than the mean CV observed for Fick and \( \dot{V}O_2 \) at each work intensity (Table 3). The reproducibility of OpCirc1 and OpCirc2 was comparable in both within- and between-session measurements at all work intensities. Thus it did not appear that one calculation method was superior in regards to reproducibility.

Among individual subjects, the within-session CV ranged from 1 to 5% for light to moderate exercise and from 1 to 6% for heavy exercise but was generally uniform, with no subjects having uniquely variable data. Between-session CV was \( \leq 1-4\% \) at the lower work intensities and between 1 and 6% with moderate exercise. Repeat measurements with different sessions were not available for the heaviest work intensities.

**DISCUSSION**

The focus of the present investigation was to compare the OpCirc washin technique using acetylene to the direct Fick method for determination of \( \dot{Q}T \) at rest and during exercise. We used two computational techniques, OpCirc1 and OpCirc2, to quantify the uptake of acetylene and to account for changes in lung volume, dead space ventilation, and breath-by-breath variability. We found that both methods compared favorably with direct Fick measurements of \( \dot{Q}T \), particularly at light to moderate exercise intensities. There was a tendency for both OpCirc1 and OpCirc2 to underestimate Fick at the higher work intensities; however, this
only reached significance using the OpCirc1 method. Both OpCirc1 and OpCirc2 were found to be highly reproducible, particularly during exercise.

Computational techniques and assumptions. Both computational methods are based on a simplified conceptual model of the lung that includes a single dead space compartment and a single well-mixed gas-exchanging compartment, the "alveolus." Modeling studies and studies in challenged or diseased lungs of animals and humans have shown that both ventilation inhomogeneity and ventilation-to-perfusion ratio (V/A/Q) mismatching will cause QT and lung volumes to be underestimated using rebreathing (4, 14, 19) and OpCirc techniques (16). Acetylene uptake is dependent on lung perfusion, which essentially carries the acetylene away from the lungs; however, acetylene can only be carried away as delivered via ventilation. Thus the technique is clearly dependent on the ventilation-to-capillary perfusion ratio (V/A/Qc). Any gas uptake measure will be affected by VA/Qc inhomogeneity, leading to underestimation of blood flow. If the test gas is delivered from the ventilation side, the technique will give more information about high VA/Qc regions and could miss information about low VA/Qc regions, whereas techniques that deliver the gas by blood (e.g., multiple inert-gas elimination technique) will obtain good information about low VA/Qc regions. Further study is needed to settle this issue. In other words, the OpCirc technique will, effectively, see low VA/Qc regions as shunt and may underestimate QT.

It is known that VA/Q inhomogeneity increases with exercise (8), although the cause of this increase is not fully known. In our studies, most subjects showed an increase in alveolar-to-arterial oxygen tension difference (A-aDO2), consistent with an increase in VA/Q inhomogeneity (Table 2), although diffusion limitation and shunt cannot be ruled out. There was a significant correlation between A-aDO2 and the Fick-OpCirc difference for OpCirc1, but not for OpCirc2 as shown in Fig. 5. However, a correlation with OpCirc2 may be evident with more subjects, as there were three outliers that reduced the correlation in the present study. We conclude from these considerations that it is likely that the OpCirc method may underestimate QT in the presence of VA/Q inhomogeneities, possibly reducing its usefulness in presence of pulmonary disease (14). Further work is necessary to explore this in patients with obstructive airway changes and known VA/Q abnormalities.

Anatomic shunts (right to left) can also contribute to the differences observed between the OpCirc methods and the Fick technique. Although not fully known, it is estimated that 1–2% of the QT may be shunted away from the gas exchange units of the lung (through the thebesian and bronchial circulations), and it has been proposed that this may contribute to a substantial portion of the widened A-aDO2 during exercise. The influence of the shunt becomes particularly significant at the reduced mixed venous PO2 observed during heavy exercise (8). Right-to-left shunts will typically be measured by the Fick method but not by measurements of pulmonary blood flow with gas uptake techniques such as OpCirc with acetylene. Regions of the lung that have perfusion but no or little ventilation (low or zero VA/Qc), would also give an effective right-to-left shunt. Left-to-right shunts are less common but could occur with a patent foramen ovale depending on the cardiac pressure gradients. However, the left-to-right shunts would be measured by both the Fick and acetylene methods for assessing QT.

The computational methods of both Stout et al. (24) and Gan et al. (7) make a number of additional simplifying assumptions, most importantly, an estimate of soluble gas uptake at the capillary during the breath cycle that involves two such assumptions. The first is that alveolar gas concentration during the inspiratory phase of the breath cycle is obtained by linear interpolation of end-expiratory points from the previous and current breaths; the second is that driving pressure for diffusion is constant during inspiratory and expiratory phases of the breath. Because of the increasing rate of absorption of soluble gas as VO2 and QT increase, this approximation could lead to errors in QT estimation at high levels of exercise. Our more complete computational method (OpCirc2) used all data points acquired at 8-ms intervals and thus did not include this approximation. The results from this method were closer to Fick QT measurements at the high intensities of exercise, and, in fact, the statistics were not significantly different from the Fick method. The disadvantage of this technique is its computational complexity, requiring several minutes of computer time for each analysis. However, it appears to be more accurate at higher intensities of exercise.

The gas solubility used in calculations is a critical assumption. We used the values found by Cander (5) for blood and tissue [0.74 and 0.76 ml C2H2 (STPD)·ml blood−1·atm−1, respectively]. Because solubility is a function of lipid and cell content of the blood, it would ideally be determined at the time of the study on each
subject, potentially improving the accuracy of the method (5, 9).

Because the OpCirc method critically depends on the measurement of gas uptakes per breath, the time delay between gas concentration signals and flow measurements must be accurately determined so that the two signals may be properly aligned in time (21). By adjusting this parameter in the analysis program, we found that Q̇T would be underestimated if the time delay were underestimated. The time delay can be obtained in individual subjects by having them perform a rapid inspiratory maneuver after a prolonged expiration. At onset of inspiration, gas concentration should remain at the end-expiratory value until the dead space of the breathing valve has been cleared by the inspired gas, after which gas concentrations should abruptly increase to the inspired value. The time delay we used, 0.27 s, was obtained by averaging time delays from a number of laboratory personnel who performed the rapid inspiratory maneuver during preliminary experiments and was confirmed by spot checking during the course of the study.

Another important determinant of the calculation of Q̇T using the OpCirc technique was the time response characteristics of the acetylene channel of the mass spectrometer. Clearly, a slow response time will result in an underestimation of Q̇T, especially at the higher frequencies of breathing. The 10–90% rise time of our transient was ~0.06 s. We included a correction for the slow rise time of our mass spectrometer that was described by Gan et al. (7). This potential error was tested in preliminary studies by having subjects breathe at two frequencies (30 and 60 breaths/min) at a given work intensity. In a small number of subjects, Q̇T did not fall with the higher breathing frequency and was, in fact, slightly augmented, consistent with increased work of breathing (1).

Advantage of the OpCirc technique relative to rebreathe, breath hold, and vital capacity maneuvers. Previous studies have found a good correlation between the rebreathe method for the determination of Q̇T relative to invasive measurements in animals and humans at rest and during exercise (11, 15, 17). Similarly, in a limited study performed at rest, Q̇T assessed by a single vital capacity breath of acetylene followed by a slow complete exhalation compared favorably to thermodilution measurements (23). However, the washin technique offers several advantages over these methods. Significant problems during rebreathing are the buildup of carbon dioxide, especially during heavy exercise, and the difficulty of matching the rebreathe tidal volume to the patient tidal volume without inhibiting breathing. In addition, subjects are often asked to transiently alter breathing patterns during rebreathing (augment breathing, breathe deeper or faster), which likely alters Q̇T, especially in some patient populations. There are also potential errors during rebreathing because of a change in bag volume over time due to a changing relationship between carbon dioxide production and V̇O₂ (VCO₂/V̇O₂), unless two tracer gases are simultaneously evaluated (12). Similarly, the vital capacity maneuver or a breath hold followed by a slow expiration may introduce errors due to alterations in intrathoracic pressure and is difficult to perform during exercise. In contrast, using the OpCirc method, subjects are switched into a large reservoir containing the gas mixture without altering gas exchange or breathing pattern. In most of our studies, subjects were switched into the Qt gas mixture with little or no awareness, even near maximal exercise.

A theoretical advantage to the rebreathe method has been the assumption that rebreathing to equilibration of an insoluble gas, such as helium, helps eliminate potential problems with ventilation distribution. However, both rebreathe and OpCirc should be affected similarly by ventilation and V̇A/Q̇ inhomogeneity. In both methods, a well-mixed, constantly inspired concentration of gas is inhaled with each breath. Inspired gas is delivered to well-ventilated regions, and the exhaled concentration is a weighted average of concentrations from all lung units contributing to ventilation.

Previous studies using the OpCirc method. Becklake et al. (2) examined the use of steady-state N₂O uptake after washin was complete and compared this to a dye dilution estimate of Q̇T during light and moderate steady-state exercise. By using this technique, a repeat value generally ran within 20% of the initial measurement and compared favorably to the dye dilution estimates of Q̇T (~20%) difference. The present OpCirc method offers several advantages over the methods described by Becklake et al. (2). It does not require steady-state conditions, no assumptions are made about the magnitude of the lung volumes, and blood flow may be determined in the presence of breath-by-breath changes in functional residual capacity (FRC). In addition, the method variance is likely reduced using C₂H₂ instead of N₂O, as the soluble gas, because the smaller solubility coefficient of N₂O decreases the slope of the disappearance curve, making it slightly more susceptible to experimental noise.

A technique similar to that described by Stout et al. (24) and, subsequently, by Gan et al. (7) was used in the present study, with some modifications, for the OpCirc1 method. Stout et al. (24) developed a model that regards the total pulmonary inert gas uptake as the sum of dead space, alveolar, lung tissue, and pulmonary blood flow uptakes. Analysis of any two breaths during breathing of the inert gas mixture yields two simultaneous equations with two unknowns, pulmonary blood flow and tissue volume. Various summation techniques were evaluated for averaging the breaths and reducing the breath-by-breath variation in measurements. This model was compared with computer simulation of respiratory gas exchange for N₂O and N₂, and comparisons were made with direct Fick in five anesthetized dogs. Agreement was observed within ±20%. Limitations to the study, in regards to practical use, were the use of anesthetized, paralyzed, and ventilated animals. Under these controlled, steady-state conditions with low breathing frequencies, reproducible values would be expected.
Gan et al. (7) used computational methods that were similar to those used by Stout et al. (24) for the calculation of OpCirc1 $\dot{Q}T$ but refined them by adding several smoothing techniques. The two smoothing methods described by Gan et al. (7), which we used for OpCirc1, included smoothing the end-tidal gas concentrations during washin by fitting to a polynomial and a method for culling out outliers obtained by solving all pairs of breaths for $\dot{Q}T$ and tissue volume. After the outlier points were excluded, average $\dot{Q}T$ from all possible remaining pairs of solutions were reported. An additional smoothing technique described by Gan et al. (7), but not used in the present study, effectively reduces noise in gas uptake measurements caused by breath-by-breath variations in inspiratory and expiratory volumes. Instead, we included the measured differences between inspiratory and expiratory volumes in our calculations. It is not clear if the differences in calculation methods of Gan et al. (7) and our OpCirc1 technique would have any impact on accuracy or reproducibility of the technique.

Unlike the OpCirc1 method, our OpCirc2 has not been previously described. For the OpCirc2 method, each data point acquired every 8 ms was used to calculate gas uptake at the alveolar level in a homogenous lung model that included measured dead space (see APPENDIX). We developed this model to determine if the simplifying assumptions behind the OpCirc1 method had an impact on calculated $\dot{Q}T$, particularly at high exercise intensities, for which gas uptakes are larger. We found that OpCirc2 was not significantly different from Fick $\dot{Q}T$, even at high exercise intensities, whereas OpCirc1 results were lower. This suggests that nonlinearities in the OpCirc1 solution become important as gas fluxes increase with exercise. However, further work would be needed to prove this point. It must be stressed that even the OpCirc2 method assumes homogenous gas distribution; therefore, calculated $\dot{Q}T$ may be affected by $V\dot{A}/Q\dot{c}$ mismatch in subjects with lung disease, similar to what has been shown for $\dot{Q}T$ determined by the rebreathe technique (14, 19).

More recently, Nielsen et al. (16) compared the washin technique with rebreathing in 10 healthy subjects. They found reproducibility of the OpCirc method to be less than the rebreathe method. Although we did not directly compare OpCirc to rebreathe, the CV in OpCirc reported here is less than one-half of that reported by Nielsen et al. (16) and is comparable to or slightly less than what we have reported for rebreathe in other studies (22). We do not have an explanation for the difference in reproducibility between our data and that of Nielsen et al. (16), although with our experiences in both rebreathe and OpCirc, we feel that OpCirc is equivalent to or better than rebreathe for assessment of $\dot{Q}T$ during moderate to heavy exercise.

**APPENDIX**

Definitions of symbols and terms in the appendix are found in the glossary.

### Glossary

- $V_{ti}$: Tissue volume; static volume that acetylene dissolves in
- $\dot{Q}c$: Pulmonary capillary blood flow
- $V_{Acet}^n, V_{He}^n$: Uptake volumes of acetylene and helium (ml STPD), respectively, for the nth breath of the washin maneuver. These are obtained by integrating the gas concentration $\times$ flow $\times$ time product for each 8-ms sample over each breath
- $RV_{Acet}^n, RV_{He}^n$: Residual volumes of acetylene and helium, respectively, left in the lungs due to difference between inspired and expired volume of the nth breath
- $F^n_{e,Acet}, F^n_{e,He}$: End-expiratory fractional concentrations of acetylene and helium respectively, for the nth breath
- $F^n_{e,Acet}, F^n_{a,He}$: Fractional concentrations of alveolar gas for acetylene and helium, respectively. In practice, alveolar gas concentrations were obtained from end-expiratory results
- $K_{EE}$: Barometric pressure (mmHg)
- $\alpha_{1,Acet}$, $\alpha_{2,Acet}$: Tissue and blood solubilities of acetylene, respectively. Although these two terms were carried through in the derivation, in practice, only one value, 0.74 ml ml tissue$^{-1}$ atm$^{-1}$, was used for both
- $P_b$: Inspiratory and expiratory times, respectively, of the nth breath
- $V_{SD} V_{D,vl}$: Volume of serial dead space and volume of the breathing valve (ml)
- $VA$: Alveolar volume

#### OpCirc1

Mass balance principles are applied to both acetylene and helium uptake. With each breath, the amount leaving the lungs on expiration subtracted from the amount that entered on inspiration must equal the increase in concentration in the gas and tissue spaces plus the amount taken up by the blood. This leads to a system of three equations with two unknown parameters representing $V_{ti}$ and $\dot{Q}c$ (7):  

$$z^n = V_{ti} \cdot u^n + \dot{Q}c \cdot v^n$$  

where  

$$z^n = V_{Acet}^n - K_{EE} \cdot (V_{He}^n - RV_{He}^n) - RV_{Acet}^n$$  

$$u^n = (F^n_{e,Acet} - F^{n-1}_{e,Acet}) \cdot (P_b - \alpha_{1,Acet})$$  

and  

$$v^n = \alpha_{1,Acet} \cdot [(\rho^n - F_{g_{Acet}})T^n_p + (F^n_{a,Acet} - F_{g_{Acet}}) \cdot T^n_p]$$

where $F_{g_{Acet}}$ is the mixed venous fraction concentration of acetylene, estimated from end-expiratory fractional concentration of the breath immediately preceding start of washin.

The quantity $K_{EE}$ is a ratio of end-tidal concentration differences of breaths $n$ and $n-1$  

$$K_{EE} = \frac{F^n_{e,Acet} - F^{n-1}_{e,Acet}}{F^n_{e,He} - F^{n-1}_{e,He}}$$

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The symbol $\rho^n$ is an approximation of the mean alveolar concentration of acetylene over the inspiratory portion of the breath

$$\rho^n = \frac{F_{\text{A,Acet}}^n \cdot V_{\text{SD}}}{V^n_i} \left(1 - \frac{F_{\text{A,Acet}}^n - F_{\text{A,Acet}}^{n-1}}{V^n_i}ight)$$

(VSD) is found from gas dilution of helium

$$V_{\text{SD}} = \frac{F_{\text{He}} - F_{\text{ex}}}{F_{i,\text{He}} - F_{\text{ex}}} \cdot V_E$$

where $V_E$ is end-expiratory volume.

These $V_{\text{SD}}$ values are only averaged for breaths with an end-expiratory value $<90\%$ of the inspiratory value to avoid an unstable solution due to small numbers in the denominator.

Two data-smoothing techniques outlined by Gan et al. (7) were used in the calculations. First, the end-expiratory gas concentrations for each breath were adjusted slightly by fitting end-expiratory concentrations vs. time to a third- or fourth-order polynomial equation, and then replacing each value with the value calculated from the equation. This process was justified by Gan et al. (7) by pointing out that the end-tidal concentrations vs. time should follow an exponential approach to an equilibrium value. From Taylor’s theorem, an exponential curve can be approximated by a polynomial series. Second, in calculating the uptake volumes of each gas by integrating flow and gas concentration over the breath, the response of the gas analyzer (mass spectrometer) was corrected using a first-order differential correction.

With values for $\rho^n$, $V^n_i$, and $V^n$ calculated for each breath, pairs of breaths are solved using Eq. 1 to find a solution for $V_{\text{SD}}$ and $Q_c$ for each pair, producing a list of $n \times (n-1)$ solutions, where $n$ = total number of breaths. The mean and standard deviation are then found for the set of solutions, outlying solutions (>98th percentile) are removed, and the average is retaken. This process of culling out outliers is only performed once.

**OpCirc2**

This technique is more involved computationally, but results in a more precise solution. The lungs are considered to be one well-mixed alveolar compartment separated from the inhaled gas bag by an anatomic dead space. Gas transport in each unit of time, $\Delta t$ (the 8-ms sampling period of the data), is governed by the following mass balance considerations at the alveolar level.

The total acetylene volume in the alveolar compartment is given by $V_{\text{Acet}} = F_{\text{A,Acet}} \times (VA + \alpha_{t,\text{Acet}} \times Vt_i)$. The change in the acetylene volume per unit time is equal to the rate of disappearance into the blood plus the amount entering the alveolar volume by inspiratory via the anatomic dead space

$$\frac{d[V_A + \alpha_{t,\text{Acet}} \cdot V(t) - F_{\text{A,Acet}}]}{dt}$$

where $F_{\text{A,Acet}}$ indicates the fractional concentration of acetylene at the alveolar end of the dead space. This equation can be solved for $dF_{\text{A,Acet}}/dt$

$$dF_{\text{A,Acet}}(t) = \frac{-\frac{d}{dt} \left[ F_{\text{A,Acet}}(t) - F_{\text{A,Acet}} \right]}{VA + \alpha_{t,\text{Acet}} \cdot Vt_i}$$

$$= \frac{\left[ F_{\text{Acet}}^n - F_{\text{A,Acet}}(t) \right] \cdot \frac{dV}{dt}}{0 (\text{Exp})}$$

where $\text{Insp}$ is inspiratory and Exp is expiratory.

This finite difference equation is used at each time-sampling increment to update the fractional concentration of acetylene. The helium concentration is treated similarly, except that $\alpha_{t,\text{He}} = 0$ (negligible tissue solubility for helium).

The process starts with a calculation of $V_{\text{SD}}$, as above, and $V_A$, as follows

$$V_A = \frac{\sum_{k=1}^{n} \left(V^n_i - V^n_k \cdot F_{\text{Acet}}^n - F_{\text{Acet}}^{n-1}\right)}{F_{\text{Acet}}^n - F_{\text{Acet}}^{n-1}}$$

This equation is applied to helium data for all breaths where the ratio of $F_{\text{E}}/F_{\text{I}}$ is $<95\%$ to avoid unstable solutions.

A computer algorithm then sets up a dead space volume that consists of an ordered list of 1-ml units, the total volume equaling $V_{\text{SD}}$. The $V_A$ and each $V_{\text{SD}}$ element is initially filled with gas concentration equal to expired concentration of the breath immediately before the start of the washin maneuver, simulating end expiration. The computer then samples the first inspiratory data point, obtaining volume change, $\Delta V$, and values for gas concentration at the mouth from the raw data stream. At the mouth end of the dead space, the first $m = \Delta V$ dead space elements are set to the measured gas concentration. At the alveolar end of the dead space, $m$ elements are each added to the alveolar space, using Eq. 9 to update the alveolar concentration and increasing alveolar gas volume by $\Delta V$. As the process continues during inspiration, a front of gas

![Fig. 6. Model demonstrating the OpCirc2 method for calculation of $Q_r$. Thin line shows raw input data for acetylene concentration at the mouth. Thick black and thick gray lines show model results for mouth and alveolar concentrations, respectively (see Appendix for description of the model). In this study, OpCirc2 modeled the entire alveolar gas concentration curves throughout inspiration and expiration. This is in contrast to the work of Stout et al. (24) and Gan et al. (7), who examined end-inspiratory and end-expiratory alveolar gas concentration and estimated the mean alveolar values.](image-url)
moves through the dead space elements until inspired gas appears at the alveolar end of the dead space. Further inspiration adds inspired gas to the alveolar compartment. During expiration, Eq. 9 is again applied and the dead space elements are filled from the alveolar end with the current value for alveolar gas concentration. This process continues until the entire data stream has been used, and end-tidal values for each of the breaths is obtained from the model. The sum of squared differences between measured and modeled end-tidal concentration is obtained for use in an iterative search procedure that finds the best $Q$ and $V_{ti}$.

Taylor minimization (20) was used to find the best combination of $V_{ti}$ and $Q$ that minimized the sum of squared errors between modeled and actual end-tidal gas concentrations. Imagine a three-dimensional surface shaped like a large bowl with the value for sum squared errors as the height above a plane defining the ranges of values for $V_{ti}$ and $Q$. The algorithm finds the low point of this surface (bottom of bowl) by finding its local slope and descending the steepest path down the slope to the minimum. This process generally took 50–100 steps. A typical solution is shown in Fig. 6. The solution for $V_{ti}$ was occasionally unphysiological, and we were unable to find methods resulting in consistently reasonable values for it. The solution for $Q$ usually appeared reasonable despite the occasional unstable values for $V_{ti}$. Thus $V_{ti}$ values were not reported in this study.

We thank Kathy O’Malley, Candy Swee, and Darrell Loeffler for technical help during the study; Drs. Bradley Narr and David Seamans for expertise in placing catheters and blood-gas sampling; and Audrey Schroeder for preparation of the manuscript. Support for the study included The Mayo Foundation, Human Health Services Grant M01-RR00085, General Clinical Research Centers, Division of Research Resources, and National Heart, Lung, and Blood Institute Grants HL-52230 and HL-46493.

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Received 22 August 1999; accepted in final form 5 January 2000.

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