Measurement of regional myocardial perfusion and mass by subselective hydrogen infusion and washout techniques: a validation study

CINDY L. GRINES, M.D., G. B. JOHN MANCINI, M.D., MARK J. MCGILLEM, B.S., KIM P. GALLAGHER, PH.D., AND ROBERT A. VOGEL, M.D.

ABSTRACT  A technique was developed for measuring regional myocardial perfusion by intracoronary infusions of hydrogen (H2)-saturated saline. H2 concentration was detected during washout in the pulmonary artery by means of the voltage response of a platinum-tipped electrode. Regional myocardial perfusion was calculated from the H2 exponential desaturation curve according to the Kety-Schmidt principle. In 16 anesthetized open-chest dogs, validation of this technique was performed at baseline, reduced (stenosis), and hyperemic (dipyridamole) flow states by means of the radionuclide-labeled microsphere reference withdrawal technique. There was an excellent quadratic correlation between microsphere and H2 washout techniques (range 0.11 to 3.15 ml/min/g) (n = 33, r = .92, y = -0.12x² + 0.89x + 0.20, SEE = 0.20 ml/min/g; p < .0001). Hyperemic regional myocardial perfusion was underestimated by H2 washout and severely ischemic regional myocardial perfusion was overestimated. When regional myocardial perfusion values greater than twice normal were excluded, a strong linear correlation was present between H2 and microsphere measurements (n = 27, r = .89, y = 0.76x + 0.22, SEE = 0.18 ml/min/g; p < .0001). The H2 washout method was further tested in 18 additional open chest dogs for calculations of the mass of an arterial perfusion bed according to the principle that the mass of the bed (g) equals coronary blood flow (ml/min) divided by regional myocardial perfusion (ml/min/g). With electromagnetic flow probes to measure regional coronary blood flow, the estimate of perfusion bed mass in vivo (range 5 to 65 g) showed a strong linear correlation with the postmortem amount of myocardium stained by subselective intracoronary infusion of dye (n = 25, r = .95, y = 0.90x + 3.27, SEE = 4.93 g; p < .0001). Thus clinical estimates of regional myocardial perfusion may be feasible with the inexpensive, inert tracer H2 and subselective coronary catheterization. Measurement of coronary blood flow simultaneous with that of regional myocardial perfusion may be used for the determination in vivo of the mass of a coronary perfusion bed.


SELECTIVE CORONARY ANGIOGRAPHY has been performed clinically for more than 25 years and is regarded as the "gold standard" for the diagnosis of ischemic heart disease. However, the severity of coronary stenoses as estimated by angiography is plagued by marked interobserver and intraobserver variability.1-3 Incorrect angiographic determinations of the significance of a coronary stenosis have been documented by postmortem studies4, 5 as well as by physiologic testing.6, 7 Ischemic heart disease may be more accurately defined by direct measurements of adequate blood flow and myocardial perfusion. In addition, knowledge of the amount of myocardium perfused by a coronary artery is of clinical importance in determining the physiologic impact of stenoses and the need for revascularization procedures. Currently, there are no good techniques applicable to humans that can provide absolute measures of coronary blood flow, regional myocardial perfusion, or perfusion bed mass.

The use of inert gases for measuring blood flow was first introduced by Kety and Schmidt8 in 1945 and has since found wide applications to various organ systems. The technique is based on the Fick principle: the total uptake or release of any substance by an organ is the product of blood flow to the organ and the arteriovenous concentration difference of the substance.
Hydrogen (H₂) is metabolically inert and is present in minimal concentrations in body tissues. Because of its low water:gas partition coefficient of 0.018, the substance is rapidly removed from blood by the lungs, thus preventing recirculation. H₂ has the additional advantage of being detected by a platinum electrode as well as by gas chromatography. Therefore H₂ fulfills major criteria for tracer clearance studies of blood flow.

Washout of H₂-saturated tissue by local blood flow was found by Aukland et al. to have an exponential desaturation curve. When plotted semilogarithmically against time, the desaturation curves were linear and the slopes were inversely proportional to absolute perfusion. Tissue perfusion (F, ml/min/g) could be calculated from the half-time (t½) of the monoexponential desaturation curve with the formula F = ktp, where k is the clearance constant 0.693 divided by t½ and p is the tissue-blood partition coefficient for H₂, 1.0 ml/g.

Several studies have documented that mean global myocardial perfusion may be measured accurately by H₂ desaturation of coronary venous blood after inhalation of H₂. However, measurement of regional rather than global perfusion is necessary in the evaluation of individual coronary arteries. In addition, cannulating the coronary sinus is sometimes technically difficult and does not allow for evaluation of all of the myocardium supplied by the right coronary artery.

In contrast to previous applications of the Kety-Schmidt principle to whole organ perfusion, we proposed to measure regional myocardial perfusion by subselective intracoronary H₂ infusion while monitoring the H₂ washout curve in the pulmonary artery. This approach allows for regional assessment and obviates the problem of coronary sinus cannulation. The purpose of this study was to validate the accuracy of this method during baseline, reduced, and hyperemic flow states and to determine whether measurement of coronary blood flow simultaneously with that of regional myocardial perfusion would enable determination of the mass of regional perfusion beds in vivo.

**Methods**

Validation of regional myocardial perfusion measurements. Sixteen mongrel dogs were anesthetized with sodium pentobarbital (35 mg/kg), intubated, and ventilated with a Harvard respirator through a cuffed endotracheal tube. A left thoracotomy was performed and the heart suspended in a pericardial cradle. The left anterior descending and circumflex coronary arteries were dissected free and encircled by appropriately sized and calibrated electromagnetic flow probes (Carolina Medical Electronics, Inc.), a soft rubber tie, and a screw occluder. Small side branches were ligated if necessary. A No. 5F high-fidelity micromanometer (Millar Instruments) was inserted through a stab wound in the ventricular apex.

All measurements were recorded on a Gould 2800S recorder. Continuous measurements of heart rate, systolic blood pressure, left ventricular end-diastolic pressure, dP/dt, and coronary blood flow were made. Flow probes were frequently rezeroed, and phasic arterial tracings, as well as reactive hyperemia after a 20 sec coronary occlusion, were recorded to ensure accurate measurements and probe fit. A No. 5F platinum-tipped, Teflon-coated standard bipolar pacing electrode (USCI) was inserted through a stab wound in the right ventricle and advanced into the main pulmonary artery. A subcutaneous steel electrode was used as the reference pole. The terminals of the platinum and reference electrodes were connected by cables to the Gould recorder (100 megaohm circuit). A standard external calibration with a pulse of 50 mV was routinely performed and recorded.

A 26-gauge infusion cannula was placed into the coronary artery just distal to the flow probe. H₂-saturated saline was produced by agitating H₂ gas with saline for 60 sec before each injection. This solution was infused at 15 ml/min for 30 sec into the coronary lumen. During reduced flow states, this infusion rate was decreased to 6 to 8 ml/min over 30 sec. H₂ concentration during saturation and desaturation phases was detected by voltage differences generated by the pulmonary arterial platinum electrode and the tissue ground. Three desaturation curves were obtained for each artery during each flow state. Curves with baseline drift greater than 10% of the total amplitude were discarded. Results from remaining curves were averaged. The declining or washout portion of the curves was plotted semilogarithmically. Half-time was determined from the slope of the monoeponential clearance and used to calculate regional myocardial perfusion by the Kety-Schmidt formula. If washout curves appeared to be biexponential, the logarithm of the second decade of desaturation was used to calculate regional myocardial perfusion. The value of regional myocardial perfusion for this "slow compartment" was derived from the usual equation for monoeponential desaturation, F = 0.693/t½, as previously described. Perfusion measurements were obtained at baseline, reduced (graded coronary occlusion to reduce coronary blood flow to 20% to 70% of baseline), and hyperemic (dipyridamole 0.1 mg/kg iv) flow states.

Commercially available microspheres, 15 μm in diameter, labeled with one of several isotopes (113Sn, 109Ru, 141Ce, 46Sc) were used to validate the method. Approximately 3 × 10⁶ microspheres (ultrasonicated and vortex agitated) were injected into the left atrium during each of the steady-state flow conditions. Reference withdrawal blood samples were obtained from both carotid and femoral arteries by Harvard withdrawal pumps. Hydrogen desaturation curves were obtained simultaneously with each microsphere injection.

At the end of each experiment, the dog was killed and the heart was excised. The coronary artery was cannulated with polyethylene tubing at the site of previous H₂ infusion and ligated just proximal to the site. Subselective intracoronary infusion with triphenyltetrazolium chloride (TTCA), phosphate buffered at 38°C, was performed simultaneously with aortic root infusion of Evans blue dye at 100 mm Hg pressure. The heart was sliced into transverse rings of 1 cm thickness. The myocardium within the perfusion bed of interest was sliced into several pieces of approximately equal thickness and divided into endocardial, midmyocardial, and epicardial segments. Tissue samples were also obtained from the right and left kidneys, right ventricular inflow and outflow tracts, and left ventricular free wall opposite to the H₂ perfusion bed to confirm adequate dispersal of microspheres (< 15% variability within each perfusion bed). Each block of tissue was blotted to absorb excess fluid and then weighed on a Mettler analytic balance.
Reference blood samples were hemolyzed with KOH and desiccated at 70°C for 3 to 5 days. Radioactive counts were determined with a Tracor (Model 1185) gamma scintillation counter and myocardial blood flow was calculated as $Q_m = (C_m \times Q_r)/C_r$, where $Q_m = \text{myocardial flow (ml/min)}$, $C_m = \text{counts in the tissue sample (counts/min)}$, $Q_r = \text{withdrawal rate of the reference arterial sample}$, and $C_r = \text{counts in the reference arterial sample}$. Flow per gram of tissue was calculated by dividing the blood flow by the weight of the sample.  

Validation of estimate of perfusion bed mass. A separate set of 18 dogs were instrumented as above but microsphere determinations were not performed. Coronary arteries and branches of varying sizes were selected to obtain different sized myocardial perfusion beds. Flow probes were accurately sized and calibrated and coronary blood flow was measured simultaneously with H$_2$ infusion and washout. In very small arterial branches, the infusion rate of H$_2$ saturated saline was reduced to 6 to 8 ml/min over 30 sec to reduce its effect on baseline coronary blood flow. Under baseline flow conditions, mass (g) of the myocardial perfusion bed was estimated as CBF (ml/min)/RMP (ml/min/g), where CBF is coronary blood flow and RMP is regional myocardial perfusion. Two to four measurements were obtained per artery, and when more than one curve was stable, results were averaged. At the completion of the experiment, the heart was excised and subselective coronary staining was performed as above. In nine dogs it was possible to obtain perfusion and mass measurements in two different coronary arterial beds. In these dogs, subselective intracoronary staining was performed with TTC in the first artery, Evans blue in the second artery, and saline perfusion in the aortic root. This allowed good definition of the different perfusion beds. The heart was sliced and perfusion in interest were excised and weighed.

Perfusion measurements obtained during unstable flow conditions were excluded, as were all desaturation curves with significant baseline drift (> 10% of total amplitude). In a total of 93 hydrogen desaturation curves obtained in 17 dogs, 23 were excluded because of drift (29%). Repositioning the ground electrode eliminated the drift in many cases. Two additional animals were excluded because of difficulty in staining techniques, one with coronary air emboli and one because of loss of reference withdrawal bloods.

Results from the two protocols were analyzed by linear and polynomial regression analyses. Reproducibility was assessed by determination of the intraclass correlation coefficient.  

Results were considered significant at $p < .05$.

**Results**

Subselective infusion of H$_2$-saturated saline was associated with slight coronary hyperemia as measured by electromagnetic flow probe (5 ml/min) in most arteries under baseline and dipyridamole-induced hyperemic flow states. However, regional myocardial perfusion did not appear to be altered by infusion of H$_2$-saturated saline. At baseline flow, microsphere determination of regional myocardial perfusion revealed no differences in the bed perfused by H$_2$ compared with the opposing perfusion bed (1.08 ± 0.24 vs 1.12 ± 0.30 ml/min/g, mean absolute difference = 0.09 ± 0.08; $p = .54$). As expected, hyperemia was not observed in the presence of a flow-limiting stenosis. No changes in systolic blood pressure, left ventricular end-diastolic pressure, or dP/dt were observed during intracoronary H$_2$ infusion.

Hydrogen saturation and desaturation curves were found to have amplitudes of approximately 50 mV (figure 1). Despite prolonged use of the platinum detector, a reduction in sensitivity was not observed. The declining portion of the curve, representing myocardial desaturation, produced a straight line when plotted on semilogarithmic paper (figure 2) and was therefore treated as a monoexponential function during baseline and hyperemic flow states.

Endocardial:epicardial flow ratio measured 1.06 ± 0.2 under baseline conditions, 1.05 ± 0.35 during hyperemia, and 0.34 ± 0.20 during reduced flow states by the microsphere technique. Although changes in regional myocardial perfusion were expected to generally parallel any changes in regional epicardial coronary blood flow, a greater than 50% reduction in epicardial coronary blood flow was required to produce a measurable reduction in regional myocardial perfu-
sion in most arteries. In six arteries, induction of a coronary stenosis resulted in marked diminution of coronary blood flow (50% to 84% reduction from baseline) but caused moderate to no change in regional myocardial perfusion by either H₂ washout (0 to 37% reduction) or microsphere (0 to 50%) methods. Despite a flow-limiting stenosis, the fact that myocardial perfusion remained normal in some cases implied the presence of collateral flow, detectable by H₂ washout.

Table 1 summarizes the microsphere and hydrogen determinations of regional myocardial perfusion in the basal (n = 10), hyperemic (n = 8), and stenotic states (n = 15). Although the mean values in the basal and stenotic conditions were not different, hyperemic flows were generally underestimated (p < .004) by the hydrogen method.

In six dogs, a marked reduction in coronary blood flow resulted in a biexponential washout curve with a "rapid and slow compartment." These were analyzed by monoexponential modeling of the "slow compartment" as previously described. Even with the slow compartment analysis, the regional myocardial perfusion by H₂ washout (0.45 ± 0.09 ml/min/g) overestimated mean perfusion as measured by microspheres.

**FIGURE 2.** H₂ desaturation curve plotted semilogarithmically. Half time of the monoexponential desaturation curve (t) is used to calculate regional myocardial perfusion (RMP) by the Kety-Schmidt principle.

**FIGURE 3.** Results of polynomial regression analysis comparing regional myocardial perfusion measurements by hydrogen washout with that of radiolabeled microspheres. Under hyperemic conditions, perfusion is underestimated by H₂ washout (0.23 ± 0.09 ml/min/g). In these cases, a severe stenosis resulted in virtually no subendocardial flow (0.06 to 0.12 ml/min/g). Therefore, H₂ washout represented midmyocardial and epicardial flows only, accounting for the overestimation (figures 3 and 4).

Microsphere validation of H₂ washout measurement of regional myocardial perfusion showed an excellent quadratic correlation (range 0.11 to 3.15 ml/min/g) (r

**FIGURE 4.** Excluding regional myocardial perfusion values greater than 2 ml/min/g, a strong linear correlation is present between the H₂ and microsphere methods of measuring perfusion.

**TABLE 1**

<table>
<thead>
<tr>
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<th>Observations</th>
<th>Hydrogen method</th>
<th>Microsphere method</th>
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<tbody>
<tr>
<td>Basal</td>
<td>10</td>
<td>1.09 ± 0.28</td>
<td>1.08 ± 0.24</td>
</tr>
<tr>
<td>Stenotic</td>
<td>15</td>
<td>0.59 ± 0.23</td>
<td>0.55 ± 0.38</td>
</tr>
<tr>
<td>Hyperemic</td>
<td>8</td>
<td>1.61 ± 0.34</td>
<td>2.40 ± 0.71^</td>
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^p < .004 vs hydrogen method.
Values greater than perfusion regional a strong ml/min/g; between of weight A = 
set flow states 

CDz was >- CD 0.76x 27) The estimated perfusion perfusion was simultaneously measured microsphere measurements (r = .89, y = 0.76x + 0.22, SEE = 0.18 ml/min/g; p < .0001, n = 27) (figure 4). Some overestimation in very low flow states is evident.

The estimated perfusion mass in vivo, calculated as simultaneously measured basal coronary blood flow/ regional myocardial perfusion from H2 washout in the second set of dogs, was found to range from 5 to 65 g. A strong linear correlation was found between the estimate of perfusion bed mass in vivo and the post-mortem weight of subselectively stained myocardium (r = .95, y = 0.90x + 3.27, SEE = 4.93 g; p < .0001, n = 25) (figure 5).

Repealed perfusion and mass estimations (54 observations in 21 perfusion beds) were highly reproducible. For mass calculations, the intraclass correlation coefficient was .92 and the root mean square error was 4.32 g. For perfusion measurements, the intraclass correlation coefficient was .79 and the root mean square error was 0.11 ml/min/g.

Discussion

Hydrogen is metabolically inert, present in body tissues in minimal amounts, quickly removed by the lungs and easily detected by a platinum electrode. The described technique is simple and inexpensive and may be easily applied to humans via a standard angioplasty catheter for subselective intracoronary infusion and a standard platinum-tipped pacing electrode in the pulmonary artery. Unlike other available methods, the hydrogen technique does not require expensive computers or radionuclides, does not significantly alter intrinsic flow, and does allow repeated determinations of regional myocardial perfusion. Combined with a means of measuring absolute coronary blood flow, the hydrogen washout method of measuring regional myocardial perfusion also allows determination of the mass of a coronary perfusion bed.

Several previous studies have shown the validity of determining global myocardial perfusion with H2 inhalation and H2 washout measurements from the coronary sinus. One concern with this experimental method is the flammability and explosiveness of pure H2 gas, especially in combination with small amounts of oxygen. With a water:gas partition coefficient of 0.018, very little H2 gas is used for saturation of 10 ml saline, and in its dissolved state, H2 is not flammable. Second, previous studies have required cannulation of the coronary sinus, which can be difficult and precludes measurement of right coronary artery perfusion. Third, H2 inhalation causes global myocardial saturation and desaturation; however, measurement of regional perfusion is desirable in the evaluation of coronary artery disease. This provided the motivation for the use of subselective intracoronary infusion, which eliminates H2 contamination of the systemic vasculature, obviates the need for coronary sinus cannulation, allows for relatively simple H2 detection in the pulmonary artery, and gives an estimate of regional perfusion.

Potential limitations. Baseline drift of the platinum electrode was a frequent problem, occurring in nearly one-third of all measurements. Repositioning the ground electrode was helpful in some cases. The use of a silver chloride electrode17, 18 may also help in overcoming the problem.

Nonselective sampling in the pulmonary artery assumes that myocardial venous effluent remains a constant fraction of the cardiac output during the sampling time; thus this technique requires steady-state coronary flow and cardiac output during the sampling interval.

Although prolonged (20 to 25 min) administration of H2 may better achieve homogeneous saturation,13, 14 the time requirement was thought to be unreasonable for potential clinical applications. To avoid prolonged subselective intubation of the coronary artery during angioplasty applications, a bolus technique of H2 infu-

FIGURE 5. Results of linear regression analysis comparing the estimate of perfusion bed mass in vivo (mass = coronary blood flow [CBF]/regional myocardial perfusion [RMP]) to the weight of the subselectively stained perfusion bed segment.
sion over 30 sec was chosen for investigation. Despite the known potential limitations of bolus infusions, reasonable desaturation curves were obtained.

Other investigators have reported that hydrogen washout deviated from a single exponential in the setting of heterogeneous perfusion. In this study, the clearance of H₂ was monoexponential except in the instance of virtually absent subendocardial flow and resultant marked transmural flow heterogeneity. Since the hydrogen saturation and desaturation was limited to a single perfusion bed rather than the entire heart, it is postulated that the degree of heterogeneity was reduced to the point that it was not apparent in most of the washout curves. However, electrical drift commonly seen with a platinum detector makes small changes in H₂ concentration in the tail of the washout curve difficult to detect. In addition, it is possible that the bolus technique of hydrogen administration did not achieve homogeneous saturation of myocardium, resulting in inadequate representation of low flow areas in the H₂ washout curve. Therefore it is possible that desaturation may have been multiexponential but not detected by this technique. However, our data as well as the results of others suggest that monoexponential analysis of such tracer curves is adequate.

As with other inert gases, H₂ washout detected from coronary venous outflow is unable to distinguish epicardial from endocardial blood flow. The measurements obtained represent a flow-weighted average of the blood flow across myocardium that is perfused by the H₂ solution. In the presence of very low subendocardial flow or occlusion of a distal branch, actual transmural myocardial perfusion may be overestimated by the H₂ desaturation technique.

As expected, myocardial perfusion greater than twice normal was underestimated by H₂ washout. This has consistently been reported with all inert gas washout techniques and may represent the time lag required for equilibration between myocardium and venous blood. Additional delay may be required for the oxidation of H₂ to 2H⁺ and its measurement by the platinum electrode. The inability to accurately measure regional myocardial perfusion greater than twice normal precludes the use of H₂ washout for precise determinations of reactive hyperemia.

Under baseline conditions, simultaneous measurement of regional myocardial perfusion and coronary blood flow allowed an accurate estimate of perfusion bed mass in vivo. Estimation of mass was validated only during baseline conditions; applications during abnormal flow states may not be as precise because of the limitations described above.

Despite the potential limitations common to all inert gas techniques, this subselective H₂ infusion and washout method is simple and relatively accurate in determining regional myocardial perfusion under 2 ml/min/g. The described technique is inexpensive, does not significantly alter coronary flow, and allows repeated determinations of regional myocardial perfusion. Further studies will be required to determine whether this technique can be applied easily to humans undergoing catheterization studies and subselective coronary cannulation. Because the proposed method allows examination of an individual coronary artery, it may be used to assess the effect of mechanical or pharmacologic interventions on regional myocardial perfusion. In addition, since H₂ washout assesses myocardial capillary blood flow, it may also be useful in examining ischemia in patients with normal epicardial coronary arteries or small-vessel disease and for the estimation of collateral flow. With a recently developed transarterial method of determining absolute coronary blood flow, the H₂ washout measure of regional myocardial perfusion may also allow accurate determination of the mass of the coronary perfusion bed in humans. This information may be helpful in determining the need for a revascularization procedure or for establishing the potential functional impact of a coronary stenosis on ventricular performance.

References

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