Inert Gas Measurements of Myocardial Perfusion in the Presence of Heterogeneous Flow Documented by Microspheres

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SUMMARY Inert gas measurements of flow per unit weight (F/W) incorporating even saturation of heterogeneously perfused areas and two-decade resolution of coronary venous desaturation curves have been compared with radioactive microsphere measurements of F/W in closed-chest dogs with modest heterogeneity of F/W. Coefficients of variation for microsphere measurements in 96 left ventricular segments revealed global heterogeneity of F/W, of similar degree, in dogs with and without an abdominal aortocava l fistula (0.18 ± 0.07 vs 0.15 ± 0.04; p > 0.3). Endocardial-epicardial flow ratios were lower in the fistula dogs (0.77 ± 0.11 vs 1.05 ± 0.08; p < 0.01), reflecting transmural as well as nontransmural heterogeneity of F/W. Inert gas measurements of average F/W, derived from the Kety-Schmidt equation using dissolved hydrogen (H₂) as tracer, agreed within ± 20% of average microsphere F/W in 18 of 20 comparisons in fistula and nonfistula dogs. Semilogarithmically plotted H₂ desaturation data were curvilinear in both settings, but arbitrarily derived "slow-compartment" H₂/F/W agreed with average microsphere endocardial F/W only in the fistula dogs. We conclude that 1) methodologically adequate inert gas measurements give accurate values for average F/W in the presence of moderate heterogeneity of perfusion; and 2) although the presence of heterogeneous perfusion can be appreciated from the shape of inert gas desaturation curves, compartmental analyses of curves cannot ordinarily be interpreted in a specific transmural or other spatial sense.

QUANTITATIVE STUDIES of myocardial blood flow in man frequently involve inert gas techniques, usually with the aim of obtaining an average value for flow per unit weight (F/W) for the entire left ventricle or some segment thereof. In some situations, attempts are made to derive an estimate of flow distribution within the area under study, e.g., by multieponential compartmental analysis. Various potential theoretical and methodologic problems are well recognized when flow is distributed heterogeneously in any spatial sense.¹ Studies validating inert gas measurements in the presence of abnormal heterogeneity of flow are limited, and usually involve preparations with wide extremes of regional perfusion, e.g., after total occlusion of a major coronary arterial branch.² In addition, almost all validation studies in either normal or abnormal states have attempted the availability of the radioactive microsphere technique,³ which has become the experimental standard for defining flow heterogeneity and provides an independent basis for evaluating inert gas estimates of flow distribution. The present studies were therefore undertaken to evaluate the degree of agreement between simultaneous inert gas and microsphere measurements of average F/W in the presence of modest transmural and nontransmural heterogeneity of flow, and to test the degree to which this heterogeneity, as defined by the microsphere technique, can be appreciated in the inert gas data.

Methods

Thirteen closed-chest dogs weighing 20–35 kg were anesthetized with sodium pentobarbital, ventilated through a cuffed endotracheal tube with a Harvard respirator and prepared for simultaneous measurement of myocardial blood flow by inert gas and microsphere techniques. The closed-chest preparation was chosen to avoid surface gas transfer, which can seriously complicate inert gas measurements in open-chest preparations.⁴ Under fluoroscopic control, a #8F catheter was advanced from a carotid artery retrogradely into the left atrium for microsphere injection. A #4F catheter was inserted through the other carotid artery and positioned in the proximal portion of the left anterior descending coronary artery (LAD) for infusion of dissolved inert gas. A #7F Shirey catheter was inserted into the coronary sinus from an external jugular vein and advanced into the great cardiac vein for sampling of coronary venous inert gas concentration. A thoracic aortic catheter was used to monitor pressure and to sample systemic arterial blood during inert gas infusion. Two additional aortic catheters were used for microsphere reference arterial samples, as recommended by Heymann.⁵

Six dogs were studied without additional instrumentation. On the basis of previous studies,⁶ moderate spatial heterogeneity of left ventricular F/W was expected, i.e., values of F/W in 0.5–1.0-g segments were expected to vary appreciably in a random fashion throughout all ventricular regions and layers. In the remaining seven dogs, the abdomen was opened and a fistula created between the abdominal aorta and inferior vena cava below the renal arteries, using a #32 Bardex tube fitted with an adjustable external screw.

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clamp. Transmural as well as nontransmural heterogeneity of F/W, with a reduced endocardial-epicardial flow ratio, was expected in this setting.\textsuperscript{7} All dogs were given heparin systemically to facilitate processing of blood samples.

**Inert Gas Measurements**

Dissolved hydrogen (H\textsubscript{2}) was chosen as the inert gas tracer because of its high degree of intrapulmonary elimination and its suitability for quantitation in coronary venous blood by gas chromatography. After drawing coronary venous H\textsubscript{2} "blank" samples, isotonic saline saturated with dissolved H\textsubscript{2} was infused into the LAD at a rate of 6.0 ml/min for 15–20 minutes, using a gear-driven infusion pump. Aortic blood was sampled near completion of each infusion, and recirculating H\textsubscript{2} could not be detected. At the termination of each infusion, a stopcock in the infusion line was closed abruptly, so that the LAD catheter was isolated from the infusate and arterial H\textsubscript{2} inflow ceased immediately. Fifteen to 20 2.0-ml coronary venous blood samples were collected in glass syringes during the last 2 minutes of each infusion and for 12–15 minutes thereafter; the frequency of sampling was more rapid during the initial phase of desaturation, and blood cleared from the catheter before sampling was continuously returned to the dog. All samples were analyzed for H\textsubscript{2} concentration by gas chromatography, using vacuum extraction of dissolved gases and a specially designed thermal conductivity unit, as described previously.\textsuperscript{9} H\textsubscript{2} concentration was quantitated from the height of the chromatographic H\textsubscript{2} peak and expressed as arbitrary units per 2.0-ml blood sample. Average F/W was calculated using the usual Kety-Schmidt equation

\[
F/W = 100 \lambda \cdot \frac{\Delta C_v}{\delta_{H_2} C_v dt} \tag{1}
\]

where \(\Delta C_v\) = the difference in coronary venous H\textsubscript{2} concentration between the onset and termination of desaturation, \(\delta_{H_2}\) = the area under the linearly plotted venous desaturation curve, and \(\lambda\) = the tissue-blood partition coefficient, here taken as 1.0 ml/g. Because arterial H\textsubscript{2} concentration is negligible throughout desaturation, it does not appear in the denominator, which represents the mean venous-to-arterial H\textsubscript{2} difference during desaturation. Coronary venous H\textsubscript{2} concentration-time curves were also plotted semilogarithmically over a 2-decade range. A weighted least-squares regression line\textsuperscript{9} was fitted to the logarithm of H\textsubscript{2} concentration vs time during the second decade of desaturation. A value of F/W for this "slow compartment" was derived from the usual equation for monoexponential calculation of F/W

\[
F/W = 100 \lambda \cdot \frac{0.693}{t_{0.5}} \tag{2}
\]

where \(t_{0.5}\) is the time required for venous H\textsubscript{2} concentration to be halved. Arterial H\textsubscript{2} concentration is again considered negligible from the onset of desaturation; unlike equation 1, tissue-blood diffusion equilibrium is assumed.

**Radioactive Microsphere Measurements**

Two minutes before the completion of each H\textsubscript{2} infusion, radiolabeled 9-\textmu m microspheres (3M Company, Minneapolis, Minnesota) suspended in 10% dextran with a few drops of Tween-80 were injected into the left atrium over a 20-second period, and the atrial catheter was flushed with 10 ml of saline over an additional 10-15 seconds. Approximately 3 \times 10\textsuperscript{7} spheres were present in each injection; tracers varied among experiments but included \textsuperscript{125}I, \textsuperscript{14}Ce, \textsuperscript{51}Cr, \textsuperscript{88}Sr, \textsuperscript{89}Nb and \textsuperscript{46}Sc. The injections were not accompanied by detectable changes in aortic pressure or heart rate. Collection of three 30-second samples from each of the two reference sample catheters was begun a few seconds before each injection of spheres; flow through each catheter was adjusted to approximately 12 ml/min and blood allowed to drip into weighed collection vials containing 1 ml of heparin. Volumes of the three successive samples varied by less than 15%.

The dog was sacrificed at the completion of the experiment and the heart removed and placed in 10% formalin for 4–6 days. After removing both atria and the free wall of the right ventricle, the left ventricle was cut into five sections parallel to the mitral valve ring. The apical section was discarded and the four remaining sections cut into eight wedges each. Each wedge was further subdivided into three transmural layers, giving a total of 96 segments for the entire left ventricle. Each tissue sample was weighed and placed in vials for gamma counting along with the arterial reference samples, using a 512-channel, multiple-region-of-interest system (Model 25601, Nuclear Chicago, DesPlaines, Illinois) and standard region-of-interest analysis.\textsuperscript{3} F/W for each of the 96 tissue samples was calculated from the usual formula

\[
F/W = 100 C_m \cdot \frac{RBF}{C_r} \tag{3}
\]

where \(C_m\) = counts per minute per gram of myocardium, \(C_r\) = counts per minute in the total reference sample, and RBF = reference sample flow rate (ml/min). Count rates per unit flow for the paired reference samples always agreed within 12% and were averaged for the final determination of F/W.

Average F/W for the entire ventricle and for each of the three transmural layers was also calculated. To characterize the distribution of F/W in venous outflow, which is weighted toward higher values of F/W,\textsuperscript{1} the fraction of total outflow originating from each segment was calculated by expressing absolute outflow for the segment (F/W \times W) as a fraction of summated absolute outflow for all segments.

**Results**

Figure 1 illustrates linear and semilogarithmic plots of coronary venous H\textsubscript{2} desaturation curves from a representative dog with an aortocaval fistula. Figure 2
Coronary venous desaturation in a dog with an aortocaval fistula. Average flow per unit weight (F/W) is calculated from the linearly plotted \( H_2 \) desaturation curve, shown on the left, using the Kety-Schmidt equation. The same data are plotted semilogarithmically on the right. A weighted least-square fit has been applied to the logarithms of data points in the second decade of desaturation to obtain an arbitrary "slow-compartment" \( F/W \), using a monoexponential function.

- **Figure 1.**

- **Figure 2.** Comparison of average flows per unit weight \( (F/W)'s \) obtained with \( H_2 \) and microspheres.

- **Figure 3.** Semilogarithmically plotted \( H_2 \) desaturation curves for the first 6 minutes of desaturation in dogs with and without aortocaval fistulas.

- **Figure 4.** Distribution of flow per unit weight \( (F/W) \) from microsphere measurements in 96 left ventricular segments in a fistula dog. For simplification, \( F/W's \) have been grouped arbitrarily in increments of 10 ml/min/100 g. The ordinate shows the percentage of total left ventricular outflow originating from segments having the \( F/W's \) shown on the abscissa. Note the preponderance of low \( F/W's \) in the subendocardium. **EPI** = epicardial; **MID** = midmyocardial; **ENDO** = endocardial.
in the nonfistula dog (fig. 5), F/W’s are similar in all three transmural layers. This pattern was consistent in all dogs studied.

Figure 6 compares arbitrary “slow-compartment” H$_2$ F/W’s derived from the second decade of H$_2$ desaturation with corresponding microsphere values of average F/W for the inner third of the ventricle. Nine of the 11 measurements in fistula dogs agree within ± 15%, while the same agreement occurs in only one of nine measurements in nonfistula dogs.

Figure 7 illustrates similar curvilinearity of two H$_2$ desaturation curves with theoretical venous outflow curves generated from the 96 microsphere values of F/W. A monoexponential desaturation was calculated for each of the 96 segments, and a composite 96-compartment curve was generated by weighting the individual segments for their contribution to venous outflow and summing them. Curves similarly generated from microsphere data in other experiments were also curvilinear when plotted semilogarithmically.

Discussion

These studies indicate that values for average F/W derived from H$_2$ and microsphere measurements agree closely in the presence of heterogeneous perfusion,
and that heterogeneous perfusion is reflected in the shape of inert gas venous desaturation curves. In considering the findings, several features of the experimental preparation must be considered.

The choice of H₂ as inert gas tracer was intended to ensure negligible systemic arterial tracer concentrations during desaturation. H₂ leaving the myocardium in coronary venous blood was diluted, presumably by more than 90%, as it mixed with systemic venous return in the right heart. In addition, because of the low solubility of H₂ in blood (0.015 ml/ml/760 mm Hg), the H₂ concentration in mixed venous blood should have been reduced by an additional 95–98% as the blood was exposed to alveolar air. The absence of H₂ in systemic arterial blood sampled during H₂ infusion verified that arterial H₂ concentration was indeed negligible throughout desaturation. The 15–20-minute duration of the H₂ infusion was intended to achieve even saturation of areas with widely different perfusion rates. The gas chromatographic analysis of H₂ concentration allowed venous H₂ levels to be resolved to within 1–2% of those present at the onset of desaturation. As discussed previously, both these precautions are intended to guarantee that areas of low F/W are fully represented in the measurement of average F/W, and that the latter is therefore not underestimated.

In applying the radioactive microsphere technique, the precautions recently summarized by Heymann et al. were followed. The agreement of count rates per unit flow from the two arterial reference samples indicated that appropriate mixing of spheres and blood was achieved in the preparation used. More than 1000 spheres reached each of the 96 left ventricular segments in 19 of the 20 measurements performed. The decision to use all 96 segments in the calculation of average F/W was made after we found no systematic difference between F/W calculated for all 96 segments and F/W calculated only for segments in the anterolateral ventricular wall (p > 0.3, paired t test). This agreement also supported the use of H₂ desaturation derived from LAD infusion as a reflection of F/W in the entire ventricle. The selective LAD infusion minimized the volume of fluid that had to be administered during the relatively long saturation period. In three cases, microsphere measurements of average F/W were repeated at the termination of H₂ desaturation; each agreed within 10% of the value obtained immediately before desaturation.

The close agreement between H₂ and microsphere values for average F/W (fig. 2) further validates the use of the inert gas approach in the presence of spatial heterogeneity of flow. Although both techniques have been compared with direct measurements of coronary venous outflow in right-heart bypass preparations, the previous inert gas validation during "abnormal" states involved an extreme degree of heterogeneous perfusion, produced by coronary arterial occlusion. The present studies with microspheres allow evaluation of inert gas measurements against an appropriately validated standard in the closed-chest setting, where potentially serious difficulties related to surface gas exchange are avoided. The two points in figure 2 located outside the ± 20% agreement lines were obtained in the same dog; no specific reason for the lack of agreement was identified.

Microsphere and inert gas studies using prolonged periods of saturation and desaturation have indicated that myocardial perfusion is heterogeneous in normal as well as abnormal states. The current studies provide additional verification of this observation. The variability of microsphere F/W values in our dogs is similar to that reported by Falsettii and Bassingthwaighte. The first two of these studies suggest that a substantial portion of variability in local F/W is related to moment-to-moment changes at the microcirculatory level, without any change in total arterial inflow. Bassingthwaighte concluded that the distribution of F/W, as measured by microspheres in 250 segments of conscious baboon ventricles, is similar under various physiologic conditions. In the Falsettii studies, and the present studies in nonfistula animals (fig. 5), the variability of F/W in endocardial, midmyocardial and epicardial layers is similar to that for the entire ventricle, i.e., the spatial heterogeneity of F/W is primarily nontransmural. The present studies in fistula dogs (fig. 4) verify that heterogeneity of F/W within individual layers persists even when average F/W for the layer is altered in relation to average F/W for the entire ventricle.

Although it is still uncertain how precisely microsphere measurements reflect flow variation in small segments of tissue, the curvilinearity of semilogarithmically plotted desaturation curves generated from microsphere data (fig. 7) confirms that nonlinearity of semilogarithmic H₂ curves does, at least in part, reflect heterogeneous perfusion. Additional factors that can also potentially affect the shape of desaturation curves obtained with diffusible tracers have been discussed elsewhere. The arbitrarily calculated "slow-compartment" measurements in figure 6 show that compartmental analysis of diffusible tracer desaturation curves involves major simplification of in vivo perfusion patterns. In the fistula dogs, values of F/W calculated for the monoeponential slow compartment agree reasonably well with average microsphere F/W for the 32 segments in each endocardial layer. At the same time, the heterogeneity of endocardial F/W revealed by the sphere data cannot be appreciated in the H₂ measurements. Without independent knowledge that the lower values of F/W originate from the endocardium, it would be premature to suggest that the H₂ data reflect "average" F/W in the endocardium, i.e., epicardial F/W is frequently lower than endocardial F/W in conscious dogs with slow heart rates. The lack of agreement of H₂ slow compartment F/W's and subendocardial microsphere F/W's in nonfistula dogs additionally illustrates that spatial heterogeneity of F/W in all myocardial layers cannot be distinguished from transmural heterogeneity on the basis of an inert gas curve. Thus, attempts to estimate flow in a presumably hypoperfused endocardial layer from the
“slow compartment” of a venous (or precordial) inert gas desaturation curve are not ordinarily warranted.

Although a variety of more complex compartmental and noncompartmental approaches for deriving flow distributions from desaturation data have been suggested, they seem unlikely to circumvent the difficulties just outlined. When we subjected our data to the analysis suggested by Van Liew, standard deviations of F/W’s derived from semilogarithmically plotted H₂ curves agreed poorly with those available from microsphere measurements. We have also expressed desaturation data as Laplace transforms of flow distributions and attempted to use numerical procedures to invert the transforms and reconstruct the distributions. As was the case with attempts to recover ventilation-perfusion ratio distributions from measurements of inert gas elimination, this approach is ill-conditioned mathematically. Qualitative changes in the shape of a distribution often produce minor changes in the desaturation curve, and qualitatively incorrect distributions are recovered when these changes lie within the bounds of experimental error. Thus, while curvilinear inert gas desaturation curves can reasonably be interpreted as indicating overall heterogeneity of perfusion, specific interpretations about the pattern of heterogeneity are not possible with presently available techniques.

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