Diffusional Shunting in the Canine Myocardium

ALLAN C. ROTH AND ERIC O. FEIGL

SUMMARY A test for the existence of a diffusional shunt in the myocardium was performed in closed-chest, chloralose-anesthetized dogs. Coronary blood flow was varied from 0.21 to 1.17 ml/min per g with a roller pump via a stainless steel coronary perfusion cannula. Following intracoronary artery bolus indicator injection, the coronary sinus venous appearance times of hydrogen, plasma protein labeled with indocyanine green dye, and red blood cells labeled with 99mTc were measured. Venous appearance time was calculated, either as the time from injection until the indicator reached 5% of its peak venous concentration or the time from injection until 0.1% of the injected material arrived at the coronary sinus. At low coronary flow rates (0.4 sec dye appearance time) hydrogen appearance preceded by about 1 second the appearance of the intravascular 99mTc-labeled red blood cells and the dyed plasma, indicating that hydrogen traversed an extravascular shunt path from small arteries to veins. As flow was increased, this hydrogen precession was reduced, and at high flows detectable levels of hydrogen arrived in the coronary sinus after the intravascular indicators. The difference in appearance time between hydrogen and the intravascular indicators was related linearly to the dye appearance time (proportional to 1/flow), as would be expected with diffusional shunting. These data indicate that there is arterial-venous shunting of hydrogen in the myocardium. Furthermore, diffusional shunting may be important in the delivery of oxygen to, or removal of carbon dioxide from, the heart.


ARTERIOVENOUS diffusional shunting of oxygen around capillaries has been suggested as an explanation for the observation that myocardial tissue oxygen tension is lower than coronary venous oxygen tension (Whalen, 1971; Lössé et al., 1975; Grunewald and Sowa, 1978). Bassingthwaighte and co-workers observed differences in the indicator dilution curves of labeled water, antipyrine, and xenon through the coronary circulation and proposed that this could be due to a countercurrent diffusional shunt in the myocardium (Bassingthwaighte et al., 1970; Bassingthwaighte and Yipintsoi, 1970; Yipintsoi and Bassingthwaighte, 1970). However, Rose et al. (1977) argue that the above differences between antipyrine and water are due to capillary and sarcolemmal barriers in the heart, which also could explain the tissue-venous oxygen gradient.

There are anatomical descriptions of arterioles and venules in close proximity within the myocardium, an arrangement which might permit arteriovenous (or venular-arterial) diffusional shunting (Hellberg et al., 1972; Bassingthwaighte et al., 1974; Grayson et al., 1974). The possibility that arteriovenous diffusional shunting exists is supported by the demonstration of oxygen and carbon dioxide diffusion through the walls of small arteries and arterioles (Duling and Berne, 1970; Pittman and Duling, 1977). Diffusional shunting in the coronary circulation may be important in the delivery of oxygen to, or the removal of carbon dioxide from, the myocardium, and thus in the local metabolic control of coronary blood flow.

The objective of the present study was to test the hypothesis that diffusional shunting occurs in the myocardium by examining the coronary venous appearance times of simultaneously injected diffusible and intravascular indicators (Levy and Saceda, 1959; Kampp et al., 1968; Sejrsen and Tønnesen, 1972; Brodersen et al., 1973). Hydrogen gas was chosen as the diffusible indicator because it is inert and is neither produced nor consumed in the myocardium, and indocyanine green bound to plasma albumin and 99mTc-labeled red blood cells were used as the intravascular indicators. Two methods, 5% of peak and 0.1% of total area, were used to determine appearance times. Hydrogen appearance preceded intravascular indicator appearance in the coronary sinus at low coronary blood flows and followed the intravascular indicators at high flow rates, indicating a diffusional shunt.

Methods

General Preparation

Eleven dogs of either sex, weighing 26–33 kg, were studied with a closed-chest preparation. Each dog was sedated with morphine sulfate (2.5 mg/kg, sc) approximately 1 hour before an initial injection of α-chloralose (100 mg/kg, iv). Anesthesia was maintained by a supplemental α-chloralose infusion of 25 mg/kg per hour, iv. The dogs were maintained...
on positive pressure ventilation (Harvard 601) with a 5-cm H2O end-expiratory pressure, and tidal volume and rate were adjusted to keep end-expiratory CO2 levels between 4.5% and 5%, as measured by an infrared absorption meter (Beckman LB-2). Sodium heparin (750 U/kg plus 250 U/kg per hour, iv) was administered to prevent coagulation, and 150 mM sodium bicarbonate was infused (5 ml/kg per hour, iv) to prevent anesthesia-induced acidosis. Rectal temperature was held at 37°C with a thermostatically controlled heating pad (Yellow Springs 73A). Coronary flow was controlled by an external perfusion circuit, and myocardial venous effluent was sampled by a coronary sinus catheter without opening the chest, as shown in Figure 1.

Left Coronary Artery Perfusion

A modified Smith stainless steel cannula (Smith et al., 1974; Mohrman and Feigl, 1978) was advanced through the right carotid artery into the ascending aorta and the balloon tip inflated. The cannula tip then was inserted into the left main coronary ostium with the balloon sealing the edges of the ostium. Coronary cannula pressure was kept below aortic pressure for all but four injections to avoid leakage from the cannula. The seal at the coronary ostium was checked periodically by stopping the inflow and measuring the distal pressure. The seal was considered satisfactory if the coronary pressure fell to 20 mm Hg. The cannula seal was tested further at the end of the experiment by injecting crystal violet dye into the perfusion line with the pump running and the heart beating. Leaks were demonstrated readily by dye stains in the aorta. Data from experiments with evidence of leaks were discarded. The crystal violet dye injection was used also to determine the weight of perfused left ventricle postmortem.

Blood from a femoral artery was supplied to the cannula via a roller pump (Sarnes 3500) at constant flow. Coronary flow was measured by an extracorporeal electromagnetic flow transducer (Zepeda SWF-3). Zero flow records were obtained repeatedly during the experiment, and the flowmeter was calibrated at the end of the experiment. Coronary flow was experimentally varied with the pump between 0.21 and 1.17 ml/min per g (Table 1).

Indicators and Bolus Injection

Two-Indicator Experiments

For seven dogs, indocyanine green (Hynson, Westcott & Dunning) was used as the single intravascular indicator and was equilibrated with 50 ml of blood to bind the dye to plasma albumin. The dye first was put into solution with the supplied aqueous solvent, and this solution was mixed with blood, giving a dye concentration of approximately 0.3 mg/ml. Hydrogen was bubbled through this blood at 37°C for at least 15 minutes.

Three-Indicator Experiments

Since it has been shown that red blood cells have a shorter mean transit time than plasma (Thomas, 1965; Groom, 1968; Goresky et al., 1969), it was necessary to demonstrate that early hydrogen appearance times were not due to preferential partitioning of hydrogen in red cells. Therefore, in four dogs the injected bolus contained three indicators; red cells labeled with 99mTechnetium pertechnetate, indocyanine-green-labeled plasma, and hydrogen. Two ml of red cells were labeled with 6 mCi 99mTc, using a Brookhaven kit containing freeze-dried stannous citrate and heparin, following the method of Smith and Richards (1975). These labeled red cells were mixed into 30 ml of indocyanine-dyed whole blood saturated with hydrogen. Prolonged bubbling of hydrogen caused some hemolysis; thus, at regular intervals, samples of the injectate were centrifuged, and the plasma and packed cells were counted with a NaI crystal scintillation counter. Data were acceptable only if the plasma activity was less than 7% of the red cell activity, and it was

![Figure 1 Schematic diagram of the closed-chest experimental preparation. The roller pump controlled the left coronary flow through a stainless steel cannula. The inset illustrates the balloon seal at the coronary sinus ostium, and the injection tube opening at the cannula tip. Coronary sinus blood was continuously sampled at 7.2 ml/min. Coronary pressure was measured and the bolus of labeled blood was injected at the tip of the perfusion cannula, as shown in the inset.](http://circres.ahajournals.org/)

Downloaded from http://circres.ahajournals.org/ by guest on April 4, 2017
below 3% in most cases. Also, the labeled red cells were examined under a microscope and appeared to be of normal shape.

The bolus of labeled blood was injected into the coronary perfusion cannula by a power injector (OMP thermodilution injector 3700) set at 50 psi injection pressure. The full 2-ml bolus was injected in less than 1 second by way of a separate injection tube with its port at the tip of the perfusion cannula at the ostium of the left coronary artery (Fig. 1).

**Indocyanine Green Measurement**

Coronary sinus blood was withdrawn via a Sones catheter (USCI no. 007538) which was inserted via the right jugular vein with fluoroscopy. The tip position, measured postmortem, ranged from 35 to 55 mm into the coronary sinus. In all experiments, blood was withdrawn at 7.2 ml/min with a syringe pump (Harvard model 968) (Fig. 1). The slow withdrawal rate and the tip placement avoided contamination with atrial blood (Koberstein et al., 1969). The dye dilution curves were measured by a densitometer (Waters XP302). The sensitivity of the detector to blood oxygenation was tested by comparing coronary injections of venous and arterial blood and was found to be negligible. The densitometer had a response time constant (1/e = 0.63) of 0.07 second but the output was filtered during data analysis to a time constant of 0.32 second.

**Hydrogen Measurement**

A hydrogen electrode was fabricated from 1.5-cm sections of 0.4 mm diameter platinum wire. The electrode was cathodized and plated in a 5% solution of chloroplatinic acid (J.T. Baker Chemical Co.) with a current of approximately 60 µA for 10 minutes. This plating technique resulted in a grey shaded, sensitive electrode. The electrode was connected to a low impedance balancing circuit (Neely et al., 1965), and the circuit was completed with a reference calomel electrode connected to the withdrawal tube by a saline bridge. The electrode system was calibrated by passing samples of saline equilibrated with known hydrogen gas mixtures (0.5, 1.1, and 2% H₂ in N₂) through the detector system. The electrode was linear (±6% of full scale) in this range of hydrogen concentrations, and the range was larger than that observed during an experiment. The response time constant (1/e) for the electrode system, which was measured by balancing the circuit to zero current for 0% hydrogen and then quickly immersing the electrode in 2% hydrogen-saturated saline, was 0.41 second.

**Red Cell Measurement**

The ⁹⁹ᵐTc-labeled red cell dilution was measured by detecting the γ emission of the venous outflow with a flow-through NaI scintillation counter. The pulses from an Ortec pulse height analyzer were fed into Canberra scalers, and these pulses were counted for 0.1 second. At the end of each counting period, the digital data were transferred to magnetic tape, using a buffer-tape controller system described by Knopp et al. (1972). The magnetic tape was then analyzed using a CDC 6400 computer with the output containing the point-by-point accumulated time and associated counts describing the dilution curve. The pulse height analyzer was used in the discriminator mode with the window set to detect γ radiation with energy levels between approximately 70 and 380 keV. This window permitted detection of the peak ⁹⁹ᵐTc gamma emission at 140 keV but eliminated most of the thermal noise at the lower energy levels. The scintillation detector was calibrated with serial dilutions of the labeled red cells and was found to be linear (±4% of full scale) in the range of interest.

**Measurement of Catheter Transit Time and Instrument Lags**

The transit delay time for catheter transit to the densitometer was determined by presenting a step input of dye at the tip of the catheter and recording the densitometer output while withdrawing fluid at 7.2 ml/min. The transit time was measured from injection time to the point where the response reached 5% of the peak value, and was approximately 5 seconds in the various experiments. All dye appearance times reported have been corrected for catheter transit time.

Since there was a physical separation of the detectors in the withdrawal circuit, there was a built-in appearance time delay due to geometry (Δtₗ). In addition, differences in the instrumentation time delay of the densitometer electronics (Δ₉) and the platinum hydrogen electrode (Δ₉₂) also would cause appearance time differences (Δ₉ - Δ₉₂). A

**Table 1** Summary of Hemodynamic Data

<table>
<thead>
<tr>
<th>Quartile</th>
<th>Flow range (ml/min per g)</th>
<th>Average flow (ml/min per g)</th>
<th>Dye appearance (sec)</th>
<th>Mean aortic pressure (mm Hg)</th>
<th>Mean coronary pressure (mm Hg)</th>
<th>Heart rate (beats/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.21–0.37</td>
<td>0.30 ± 0.05</td>
<td>3.80 ± 0.80</td>
<td>93.3 ± 26.4</td>
<td>52.8 ± 18.5</td>
<td>122.7 ± 36.9</td>
</tr>
<tr>
<td>II</td>
<td>0.37–0.47</td>
<td>0.42 ± 0.03</td>
<td>3.25 ± 0.54</td>
<td>113.2 ± 20.2</td>
<td>67.4 ± 19.4</td>
<td>110.2 ± 26.2</td>
</tr>
<tr>
<td>III</td>
<td>0.47–0.65</td>
<td>0.55 ± 0.06</td>
<td>2.53 ± 0.57</td>
<td>112.5 ± 19.5</td>
<td>86.1 ± 23.3</td>
<td>131.8 ± 35.3</td>
</tr>
<tr>
<td>IV</td>
<td>0.65–1.2</td>
<td>0.82 ± 0.14</td>
<td>2.33 ± 0.56</td>
<td>119.7 ± 14.0</td>
<td>96.5 ± 16.8</td>
<td>138.1 ± 26.0</td>
</tr>
</tbody>
</table>

Values shown are average ± 1 SD.
method of short path calibrations was used to determine the magnitude of the corrections required. Hydrogen-saturated dyed blood was injected into the functioning withdrawal circuit 4 cm from the detectors (Fig. 1). The difference in appearance times with forward flow through the detectors (ΔtF) of dye and hydrogen was measured by subtracting the time of 5% peak hydrogen response (tH2) from the time to 5% of the dye response (td). The forward time difference (ΔtF) was broken into its component parts (Fig. 2a): ΔtF = td — tH2 = t1 + Δtg — δd — (t1 + δd + δH2) = Δtg + (δd — δH2), where t1 is the transit time to the hydrogen electrode. When the short segment of the withdrawal circuit containing the densitometer and the hydrogen electrode was rotated 180° so that the order of the detection path was reversed (Fig. 2b), then the appearance time difference for reverse flow through the detectors (ΔtR), measured as above was: ΔtR = t2 — tH2 = δg — (t2 + Δtg — δH2) = [δg — δH2] — Δtg, where t2 is the transit time to the densitometer. Then if ΔtF and ΔtR are added and subtracted, an independent calculation of delays will be obtained: ΔtF — ΔtR = Δtg + (δd — δH2) — [(δd — δH2) — Δtg] = 2(δd — δH2). Therefore, the overall correction for the measured appearance time difference can be calculated as: ΔtF — ΔtR = Δtg — δH2, which should be the same as ΔtF. These values were determined for each two-indicator experimental setup. The average value for Δtg was 0.24 second, and the average value for (δd — δH2) was 0.27 second. For the three-indicator experiments, the instrument lags were determined as above, since the reverse flow calibration was not feasible with the gamma counter.

Experimental Protocol

A series of 2-ml bolus injections of the labeled blood at different pumped coronary flow rates were recorded on an oscillograph and magnetic tape recorder. Adenosine was infused in one experiment to achieve a high flow rate. Prior to each injection, the coronary flow was set at the desired level with the infusion pump, and the detectors were zeroed. The time of injection was recorded as a spike on the coronary pressure trace and on an event marker channel. At the conclusion of each trial at one flow rate the injection tube was flushed with saline and the process was repeated at a different flow rate.

Data Analysis

The recordings of the hydrogen electrode and densitometer outputs were digitized at 10 samples a second for analysis on a PDP-8 computer. Two algorithms for determining the appearance time were used. The first technique defined the time (with linear interpolation) at which the indicator curve had reached 5% of its peak value, a method similar to that of Perl and Chinard (1968). Appearance times for the two-indicator experiments were also calculated by computing the time required for 0.001 of the total indicator to pass the detector; that is, a percentage of the cumulative efflux of indicator from the heart. H(t) (Zierler, 1962). The fractional cumulative efflux is: H(t) = \int_0^t h(t')dt', where h(t) (the frequency function) is the fraction of indicator leaving the system per unit time at time t, defined as:

\[ h(t) = \frac{c(t)}{\int_0^\infty c(t)dt} \]

Therefore, h(t) is the indicator curve normalized to a total area of unity, and its integral H(t) is a time-dependent function that increases to a maximum value of unity. H(t) was determined by integrating the entire indicator curve by trapezoidal rule technique and then finding the time it took for the accumulated area to reach 0.001 of the total.

For the 99mTc-labeled red cells, the 10-Hz digital output from the scintillation counter was smoothed by calculating a three-point moving average, and appearance times were calculated as 5% of peak value with a linear interpolation between points. The signal-to-noise level of the γ-ray detection system was not adequate to permit analysis by the 0.001 of area method.

Statistical Analysis

The statistical analysis of the data was carried out using the Statistical Package for the Social Sciences (Nie et al., 1970). Dye appearance time was used as the independent variable in all analyses because it is a direct measure of transit time, and tests of a linear nature could be performed. Had coronary flow been used instead of appearance time, the curves would have been hyperbolic and, there-
fore, very difficult to test. If there were no dependence of the differences in appearance times (intravascular indicator minus hydrogen) on coronary flow rate, a plot of the difference vs. dye appearance time would have a slope of zero. Therefore, the slope of the regression (y on x) of the appearance times for each dog was tested against a zero slope using a t-test for significance of slope (Snedecor and Cochran, 1967). In addition, the regression of the data for all dogs was tested with the same t statistic against a slope of zero. To account for dog-to-dog variability, the mean of the individual slopes for each dog also was tested against a slope of zero.

A statistical test of the possibility of hydrogen preferentially partitioning into red blood cells was to test the slope of hydrogen appearance times versus red blood cell appearance time against a slope of unity. If hydrogen was carried in red blood cells, then the appearance times of hydrogen and red cells would be expected to be nearly equal and thus plot as a line with a slope equal to one.

**Results**

**Two-Indicator Appearance Times by 5% of Peak Criterion**

Simultaneous indicator curves for diffusible hydrogen and intravascular indocyanine green for one dog are shown in Figure 3. At a low flow, the foot of the hydrogen indicator curve appeared before the indocyanine green curve, consistent with diffusional shunting of hydrogen. At a higher flow, the hydrogen appeared later. The hydrogen indicator curve had a much longer tail than the indocyanine green curve, reflecting the large volume of distribution for hydrogen in the myocardium.

As determined by the 5%-of-peak method, the difference in appearance times between hydrogen and dyed plasma proteins vs. indocyanine green dye appearance time for the seven two-indicator experiments is plotted in Figure 4. The indocyanine green appearance time is related reciprocally to coronary blood flow rate. These data indicate the strong dependence of the difference in appearance times for dye and hydrogen on coronary blood flow rate, with hydrogen preceding indocyanine green dye by over 1 second at low flows but with dye precession at high flow rates. Hydrogen appearance after dye at high flow rates indicates that there was little intravascular hydrogen throughput. The dependence (slope) of the regression line of the difference in appearance times vs. indocyanine green dye appearance time was tested against the null hypothesis (zero slope) by the t statistic and was found to be significant ($P < 0.001$) for each dog except one, in which only a narrow range of flows was obtained. The slope for all seven animals (Fig. 4) was significantly different from zero ($P < 0.001$). In addition, the mean of slopes for the individual dogs was significantly different from zero ($P < 0.001$).

**Two-Indicator Appearance Times by 0.001 of Area Criterion**

The fractional cumulative efflux, $H(t)$, of hydrogen and indocyanine green dye from the myocardium is calculated as the area under each indicator curve relative to the peak value. The area for hydrogen was always greater than that for dye, with the difference in appearance times (H vs. D) plotted against the coronary flow rate. This difference was significant ($P < 0.001$) for each dog, indicating a strong dependence of the difference in appearance times on coronary blood flow rate.
Summary of the effects of changing coronary flow rate, expressed as dye appearance time, on the difference in appearance times of hydrogen and dye for seven dogs with 5% of peak as the criterion for appearance. Hydrogen appearance before dye is shown as a positive value on the ordinate. Note that, as dye appearance time was increased (coronary flow reduced), hydrogen appearance moved ahead of dye appearance. The regression line is a least squares fit $y$ on $x$. The null hypothesis that hydrogen precession was independent of coronary blood flow rate would be a horizontal line on this plot. The fractional cumulative efflux, $H(t)$, is a more conservative measurement of appearance time than the percentage of peak method and gives smaller values for appearance time differences. This is because a given value of $H(t)$ will occur much later on the dilution curve for hydrogen than for indocyanine green due to the large volume of distribution for hydrogen. For comparison, the 0.001 level of $H(t)$ point for indocyanine green averaged 3.0% of the peak value for this intravascular indicator, whereas the comparable average value for hydrogen was 12.9% of peak. That is, in the average curve, 12.9% of the peak value of the hydrogen curve had to be reached before 3.0% of the peak value of the indocyanine green curve for a positive appearance difference (hydrogen precession) to be recorded by the 0.001 $H(t)$ method. The important observation was the flow dependence of the difference in appearance times rather than the exact magnitude of that difference, which will be dependent on the criterion used, e.g., 10%, 5% of peak or 0.0005, 0.001 fractional cumulative efflux.
Summary of the effects of changing coronary flow rate (expressed as dye appearance time) on the differences in appearance times of hydrogen and dye for seven dogs with 0.001 of total area, H(t), as the criterion for appearance time. Hydrogen appearance before dye is shown as a positive value on the ordinate. As dye appearance time is increased (coronary flow decreased), hydrogen appearance moved from after to before dye appearance. The regression line is a least squares fit on x.

Three-Indicator Appearance Times by 5% of Peak Criterion

Examples of the simultaneous indicator curves of labeled red blood cells, indocyanine green, and hydrogen at two different coronary flow rates are given in Figure 7. At the low flow, the hydrogen appeared first, followed by red blood cells, then the labeled plasma proteins. When the coronary flow was raised, the order of appearance changed. At high flow the labeled red blood cells appeared first, followed by dye and hydrogen. A comparison of appearance times for several flows (=1/dye appearance time) for the same animal is shown in Figure 8. It can be seen that the difference in appearance times between hydrogen and dye, and hydrogen and red blood cells, was similar. The difference in appearance times between red blood cells and dye was nearly constant. A summary graph of the four three-indicator experiments is shown in Figure 9. The slopes of both the dye-hydrogen difference and red blood cell-hydrogen difference vs. dye appearance time (=1/flow) were positive and significantly different from zero (P < 0.001). The mean of the slopes for individual dogs was also significantly different from zero (P < 0.001). The slope of the dye-red blood cell difference curve was slightly positive and was significantly different from zero for the grouped data (P < 0.01), but the mean of the slopes for individual dogs was not significantly different from zero, indicating a large dog-to-dog variability.

To test hydrogen partitioning in red cells, the slope of the hydrogen appearance times vs. dye appearance times was tested against a slope of unity. The composite slope and the mean-per-dog slope were significantly different from unity (P < 0.001), indicating that hydrogen did not travel preferentially with red cells.

Discussion

These data demonstrate an earlier venous appearance time for hydrogen than for two intravascular tracers, indocyanine green dyed albumin and 99m Tc-labeled red blood cells, after bolus intracoronary injection at low coronary blood flow rates. Further, the difference in appearance times between hydrogen and intravascular indicators was significantly greater at low coronary blood flow than at high flow rates. If other factors that might separate diffusible and intravascular indicator appearance times are ruled out, then this observation indicates that hydrogen travels by a path other than the intravascular route; that is, by a diffusional shunt.

Examples of the simultaneous indicator curves of labeled red blood cells, indocyanine green, and hydrogen at two different coronary flow rates are given in Figure 7. At the low flow, the hydrogen appeared first, followed by red blood cells, then the labeled plasma proteins. When the coronary flow was raised, the order of appearance changed. At high flow the labeled red blood cells appeared first, followed by dye and hydrogen. A comparison of appearance times for several flows (=1/dye appearance time) for the same animal is shown in Figure 8. It can be seen that the difference in appearance times between hydrogen and dye, and hydrogen and red blood cells, was similar. The difference in appearance times between red blood cells and dye was nearly constant. A summary graph of the four three-indicator experiments is shown in Figure 9. The slopes of both the dye-hydrogen difference and red blood cell-hydrogen difference vs. dye appearance time (=1/flow) were positive and significantly different from zero (P < 0.001). The mean of the slopes for individual dogs was also significantly different from zero (P < 0.001). The slope of the dye-red blood cell difference curve was slightly positive and was significantly different from zero for the grouped data (P < 0.01), but the mean of the slopes for individual dogs was not significantly different from zero, indicating a large dog-to-dog variability.

To test hydrogen partitioning in red cells, the slope of the hydrogen appearance times vs. dye appearance times was tested against a slope of unity. The composite slope and the mean-per-dog slope were significantly different from unity (P < 0.001), indicating that hydrogen did not travel preferentially with red cells.

Discussion

These data demonstrate an earlier venous appearance time for hydrogen than for two intravascular tracers, indocyanine green dyed albumin and 99m Tc-labeled red blood cells, after bolus intracoronary injection at low coronary blood flow rates. Further, the difference in appearance times between hydrogen and intravascular indicators was significantly greater at low coronary blood flow than at high flow rates. If other factors that might separate diffusible and intravascular indicator appearance times are ruled out, then this observation indicates that hydrogen travels by a path other than the intravascular route; that is, by a diffusional shunt.
Separation of hydrogen and dye by axial diffusion in the coronary vessels is certainly a possibility. The magnitude of this separation can be estimated by assuming an extended source and calculating the distance from the initial plane surface to 5% of peak concentration after a period of time. The calculated distance will be given by using a specific solution of the general diffusion equation, which yields the expression: \[ \frac{X}{(4Dt)^{1/2}} = 1.2 \]
where \(X\) is the diffusion distance in cm, \(t\) is time in seconds, and \(D\) is the diffusion coefficient in cm\(^2\)/sec (Crank, 1964). A worst-case calculation for the longest dye appearance time in this study, 6 seconds, using a hydrogen diffusion coefficient in water of \(5.5 \times 10^{-5}\) cm\(^2\)/sec (Ferrell and Himmelblau, 1967) and assuming a diffusion coefficient of zero for albumin or red cells gives a separation distance of: \(X = \frac{4 \times (5.5 \times 10^{-5}) \times 6}{1.2} = 0.044\) cm. Again, using a worst-case calculation with the lowest coronary blood flow rate used in this study of 50 ml/min, and assuming a coronary sinus radius of 0.2 cm, the calculated mean velocity at the sampling point in the coronary sinus is \((\text{flow/area}) \times 6.6\) cm/sec. Therefore, the maximum time separation due to axial diffusion can be calculated as: time separation = separation distance/sampling point velocity = 0.044/6.6 = 0.66 msec. This is a brief separation time compared to the separations of 1-2 seconds observed between hydrogen and albumin. Thus, intravascular axial diffusion of hydrogen ahead of albumin or red cells is unlikely to contribute significantly to the hydrogen precession found in this study.

The coronary sinus withdrawal catheter was part of the venous detection system, and it is possible that the indicators separated while in the catheter. The maximum separation of indicators by axial diffusion in the Sones withdrawal catheter can be calculated in a manner similar to that used above. Given a 5-second catheter transit time, a tube diameter of 0.165 cm, and a withdrawal rate of 7.2 ml/min, a time separation of 7.7 msec is calculated. A parabolic velocity profile in the catheter is likely because of the low Reynolds number of approxi-
FIGURE 10 Schematic of two possible circulatory models with diffusional shunting which would be compatible with the results found in these experiments. Panel A shows a countercurrent circulatory loop with the intravascular path \( a-b-c-d-e \), and the path \( a-b-d-e \) shunting the capillary. The diffusible substance would exchange in a countercurrent fashion, shunting the capillary. Panel B is a two-component model with concurrent flow, where the shunt is from the arterial end of one capillary (k) to the venous end of an adjacent capillary (r). The intravascular path would be \( j-k-m-n \), while the diffusion shunt path would be \( j-k-r-q \). Approximately 35. Under these conditions, Taylor diffusion would be expected (Lassen and Crone, 1970). In Taylor diffusion, the more diffusible substance would diffuse from the higher velocity center stream to the slower-moving area close to the wall, and the effect would be to retard the appearance time of hydrogen with respect to dye or red blood cells. Therefore, Taylor diffusion would tend to diminish early hydrogen appearance caused by diffusional shunting. A test of hydrogen precession or retardation with respect to plasma protein in the coronary sinus withdrawal catheter was made by comparing hydrogen and dye appearance times with and without (short path—see Fig. 1) the catheter. In 17 trials, dye preceded hydrogen by an average of 0.04 second (Taylor effect), a difference that was not statistically significant.

Red cells have shorter vascular transit times than plasma because of axial migration of the red cells into the faster-moving center stream of the vasculature. Experimental evidence for red cells preceding plasma in the heart has been presented by Thomas (1965). Given the results of the two-indicator experiments, an argument could be made that hydrogen was partitioned preferentially in the red blood cells and appeared before the indocyanine green in the venous effluent without hydrogen diffusional shunting. However, the results of the three-indicator experiments with 99mTc-labeled red cells, shown in Figures 7-9, indicate that the appearance of hydrogen was independent of red blood cells, even though red cells did appear earlier than plasma. Hydrogen did not appear with red cells but was the first of the three indicators at low flows, and it appeared last at high flows.

Since the coronary pressure for each injection was different due to constant flow perfusion, the possibility for widely different vasodilation levels at each run exists. Therefore, perfusion heterogeneity probably exists over the range of coronary flow rates used in this study. If there were differences in blood velocity in neighboring capillaries, there could be a separation in tracer by diffusional shunting from a fast to a slow capillary. However, this shunting would cause the hydrogen to move into the lower velocity capillaries and therefore retard its appearance time.

Similar evidence for diffusional shunting has been observed in the kidney (Levy and Sauced, 1959), intestine (Kampp et al., 1968), skeletal muscle (Sejrsen and Tønnesen, 1972), and brain (Stosseck, 1970; Brodersen et al., 1973). The appearance time measurements in the present study provide evidence for diffusional shunting in the myocardium. However, such time measurements following bolus indicator injections provide no information on the magnitude of diffusional shunting during steady state conditions.

At least two simple circulatory models with diffusional shunting may be used to explain the results reported here. Panel A of Figure 10 shows a loop model with countercurrent flow similar to schemes suggested by Bassingthwaithe (1977) and Perl and Chinard (1968) and supported by anatomical evidence (Bassingthwaithe et al., 1974). After bolus injection of hydrogen and dye, the two tracers take separate paths: the dye path is \( a-b-c-d-e \), and the hydrogen path is \( a-b-d-e \), shunting an undefined vascular segment including the capillary. The appearance times can be described as the sums:

\[
\begin{align*}
  t_{\text{dye}} &= t_{\text{an}} + t_{\text{cap}} + t_{\text{vein}} \\
  t_{\text{H}_2} &= t_{\text{an}} + t_{\text{Diffusion}} + t_{\text{vein}}
\end{align*}
\]

where \( t \) represents the appearance time, and

\[
t_{\text{dye}} - t_{\text{H}_2} = t_{\text{cap}} - t_{\text{Diffusion}}
\]

Therefore, if we calculate the difference in appearance times:
and if $t_{cap}$ is assumed to be some fraction, $\alpha$, of $t_{dyne}$, then:

$$t_{dyne} - t_{H_2} = \alpha t_{dyne} - t_{Diffusion},$$

which is a linear equation of the form $y = ax + b$. The data from Figures 4 and 9 fit this linear model quite well, and even the $y$ intercept of approximately 2 seconds seems a reasonable estimate of the time required for hydrogen to diffuse the estimated 15- to 100-μm distance from arteriole to venule in the myocardium (Bassingthwaigte et al., 1974).

Panel B of Figure 10 shows an alternative concurrent flow model which could explain the observed results. This model, with offset capillary start and end points and a diffusional shunt from the arterial end of one capillary to the venous end of another, is similar to the proposed capillary models of Levitt (1971) and Grunewald and Sowa (1978) and is supported by some anatomical evidence (Hellberg et al., 1972). The dye path is now $j-k-m-n$, whereas the hydrogen path is $j-k-r-\overline{q}$, with the capillary unit on the left being shunted. The calculation for difference in appearance times of the tracers would be identical to those above, with the same linear equation as the result.

The use of diffusible tracers such as hydrogen or xenon in measuring coronary flow may be complicated by increased initial washout caused by diffusional shunting. If there is arterial-venous shunting of oxygen, the venous levels of oxygen would be higher than tissue levels, as has been measured (Whalen, 1971; Lössе et al., 1975; Schubert et al., 1978; Grunewald and Sowa, 1978). However, a diffusion barrier could lead to the same condition. Also, if coronary flow were reduced, there might be a disproportionate reduction in oxygen uptake due to a diffusional shunt. By the same reasoning, the levels of carbon dioxide in the myocardium would be higher than venous levels, and the washout of any excess carbon dioxide may be delayed by a shunt mechanism. Since either a lack of oxygen or a surplus of carbon dioxide has been shown to cause coronary vasodilation (Case et al., 1978), there is a possibility that a diffusional shunt may play a role in the control of coronary blood flow by influencing the distribution and washout of these vasoactive gases.

Extrapolation of the present results with hydrogen to the behavior of oxygen and carbon dioxide in the heart must be made with caution. However, a comparison of the physical characteristics of these molecules is instructive. Altman and Dittmer (1971) give the diffusion coefficients in water at 37°C of oxygen and carbon dioxide as $2.0 \times 10^{-5}$ and $1.5 \times 10^{-5}$ cm²/sec, respectively, which compares with hydrogen at $5.5 \times 10^{-5}$ cm²/sec. The fat solubility also is a useful index to assess the net flux of a substance through tissue. Altman and Dittmer (1971) give the olive oil solubility coefficient at 37°C of hydrogen, oxygen, and carbon dioxide as 0.055, 0.112, and 1.25 ml/ml, respectively. Thus the potential for diffusional shunting in the heart may be the greatest for carbon dioxide.

In summary, the present results indicate arteriovenous diffusional shunting of hydrogen in the myocardium. Diffusional shunting may be important in the delivery of oxygen to, or removal of carbon dioxide from, the heart.

Acknowledgments

We are indebted to Drs. James Bassingthwaigte, Carl Goresky, and David Mohrman for helpful discussions in designing these experiments. Dr. Wil Nelp and Peter McGrath generously assisted with the Technetium labeling. Richard Kern assisted with the radiation counting, using equipment generously loaned to us by Dr. Bassingthwaigte. Stephanie Lathrop and Feller Smith provided expert technical assistance.

References


Grunewald WA, Sowa W (1978) Distribution of the myocardial tissue PO₂ in the rat and the inhomogeneity of the coronary bed. Pfluegers Arch 374: 57-66


Downloaded from http://circres.ahajournals.org/ by guest on April 4, 2017
Losse B, Schuchhardt S, Niederle N (1975) The oxygen pressure histogram in the left ventricular myocardium of the dog. Pfluegers Arch 356: 121-132
Stosseck K (1970) Hydrogen exchange through the pial vessel wall and its meaning for the determination of the local cerebral blood flow. Pfluegers Arch 320: 111-119
Thomas HW (1965) On the difference between the clearance curves of labelled red cells and of labelled plasma from the circulatory bed of the heart and lung. Biorheology 3: 36-40
Diffusional shunting in the canine myocardium.
A C Roth and E O Feigl

doi: 10.1161/01.RES.48.4.470

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1981 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circres.ahajournals.org/content/48/4/470