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References

Myocardial Tissue Recruitment in the Dog as Determined by Double Tracer Dilution Method

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SUMMARY The amount of tissue perfused, as determined from the difference in volume of distribution between a diffusible indicator (14C-antipyrine) and an intravascular indicator (151-I-albumin) was measured at different values of coronary flow, perfusion pressure, and vasomotor tone in the working left ventricle of an open-chest dog. Coronary pressure and flow were regulated independently from the systemic circulation and coronary vasomotor tone was reduced by dipyridamole. At each flow vasomotor tone was assessed by using as a reference the maximal vasodilatation induced by arrest of flow. Measured tissue space was considered to be related to the capillary surface area available for tracer diffusion and therefore to the number of perfused capillaries per volume of muscle. A relationship between coronary blood flow and tissue volume was observed. It was found to be independent of vasomotor tone. Vasodilatation was found to increase available exchanging capillary surface at a constant perfusion pressure.

THE PRESSURE-VOLUME characteristics of the left coronary vascular system have been measured, but it has not been determined whether the observed increase in vascular volume that follows an increase of coronary flow and perfusion pressure is the consequence of vascular distention, recruitment of closed vessels, or a combination of both mechanisms. We reasoned that if distention were the primary mechanism, and we measured tissue volume, it should remain constant as coronary flow increased. If recruitment of closed vessels occurred, tissue volume should increase and then reach a plateau when all vessels were perfused. By use of the double indicator technique described by Chinard et al., we measured the extravascular tracer distribution space of the canine left coronary system through a range of pressures and flows with autoregulation both intact and absent. Our data suggest that recruitment does occur throughout the physiological range of pressure and flow, and that autoregulation is an important factor in the control of the number of tissue units perfused.

Methods

Seven mongrel dogs were anesthetized with sodium pentobarbital (30 mg/kg, iv) and ventilated with a Harvard respirator. A double-lumen steel cannula provided with two crossing wire loops projecting 3 mm from the tip was passed via the carotid artery into the left main coronary artery. The purpose of the wire loops was to prevent the cannula from occluding either the circumflex or anterior descending branches. A bypass circuit took blood from the aorta into a thermostated reservoir (37°C) from which it was pumped by a calibrated roller pump (Sarns model 3500) into one lumen...
of the cannula. The other lumen was used to measure coronary pressure. Flow and pressure oscillations due to the pump were removed by the use of a windkessel between the pump and the cannula. Through a small thoracotomy, a special clamp was placed around the left coronary mainstem containing the cannula, so that the left coronary circulation was sealed from the systemic circulation. By use of a flowmeter the flow pattern in the coronary cannula was found to be similar to that observed in an intact coronary artery. Care was taken to ensure even perfusion of all of the branches of the left coronary artery by repeated injections of Evans blue dye. The perfusion of the septal artery was verified by visual inspection of the septum after a final dye injection at the end of the study. A second double-lumen, nonocclusive, steel cannula was passed via the right jugular vein into the midportion of the coronary sinus. At the tip of this cannula a 1.5-cm wire loop ensured a fixed position of the sampling site from 0.5 to 1.0 cm from the ostium of the coronary sinus. Flow through the cannula was shunted to the left jugular vein, and during each measurement period the pressure in the coronary sinus was ascertained to be the same as right atrial pressure. The volume of the cannula system from the input to the sampling site was 2.5 ml. The input of indicators was through a stopcock on the coronary artery cannula. A second blood reservoir was incorporated into the bypass circuit to permit perfusion of the coronary cannula with indicator-free blood while measurements of transit time were made; i.e., recirculation was excluded. Pressure was measured with Statham Db transducers and recorded on an Electronics for Medicine DR 8 recorder.

We measured volume as the product of mean transit time and flow. The input function used to measure mean transit time was the ramp, as suggested by Zierler. The output of this function (Fig. 1) is a ramp whose linear extrapolation to the x axis is the mean transit time (t) of the indicator. The slope of the ramp is a measure of flow. Intravascular T was measured with 131I-labeled albumin, and intravascular plus tissue T with 125I-antipyrine (in one experiment tritiated water was used). Tissue T was calculated by subtracting T of 125I-albumin from T of 131I-antipyrine. The ramp was delivered by a servo-controlled Harvard pump, model 600-910/920-S, controlled by an operational amplifier. The duration of the ramp was at least 4 times T for the tissue indicator. At 30-second intervals, 5-ml samples were taken by syringe from the coronary sinus cannula. The sampled blood (2 ml) was pipetted into counting vials and the isotopes were differentially counted with a Beckman Biogamma counting system. Sufficient counts were collected to obtain a standard deviation of less than 1%. Values were graphed and mean transit time was measured (Fig. 1). Blood flow was corrected by dividing total pump flow by the mass of tissue perfused; this, in turn, was determined by injecting Evans blue dye in agar into the cannula at the end of the study and weighing the dyed myocardium. This was done to account for the differences in weight of the left coronary system of each dog. The stained myocardium included the entire left ventricle and left atrium and 10-20% of the right ventricle. Mean transit times were not corrected for delay in the sampling system. Tissue volume was measured as the difference between T of 125I-antipyrine and T of 131I-albumin times blood flow. Thus tissue volume excludes vascular volume. At the end of each ramp, flow was arrested for 30 seconds to test the degree of vasomotor tone. The absence of vasomotor tone (maximal vasodilation) was indicated by a constant coronary perfusion pressure before and after arrest of the flow.

![Figure 1](http://circres.ahajournals.org/DownloadedFrom/myocardial_tissue_recruitment_zerler277.png)

**Figure 1** Illustration of typical ramps used to calculate mean transit time (t) from a vascular 131I indicator (x) and extravascular 131I indicator (●). Time in minutes is on the x axis and output indicator concentration, expressed as percent of injectate, is on the y axis. Mean transit time is the intercept of the extrapolated outflow curve with the abscissa. Tissue transit time is t - t.
Up to six measurements of transit time were made at different coronary flows. Intentionally the sequence of flow changes was varied. In addition to "physiological" flows (corresponding to a coronary perfusion pressure equal or lower than the aortic pressure) "unphysiological" flows (perfusion pressure higher than the aortic pressure), also were used with the intention of reaching the point of maximal capillary surface area. Statistical regression analyses were performed with a stepwise multiple regression computer program.*

Results
All of the data are tabulated in Table 1. Measurements in each dog are arranged in order of decreasing coronary blood flow. Control measurements were first obtained before vasodilation was induced by dipyridamole (dogs c, d, and g). The coronary diastolic pressure following a 30-second arrest of flow is indicated for each flow as recorded during the ramp. The difference between coronary diastolic pressure and this pressure following a flow arrest is proportional to the degree of vasomotor tone. Complete or almost complete abolition of vasomotor tone occurred in many instances at the lowest flow and after dipyridamole (except dog g, sequence 4).

EFFECT OF CORONARY FLOW ON TISSUE MEAN TRANSIT TIME
The relationship between tissue mean transit time and flow is shown in Figure 2A. Tissue mean transit time (T), coronary flow (x) regression is statistically significant (r = 0.90 - 0.0027x; r = -0.85; P < 0.01). For the tissue compartment the linear term accounts for most of the relationship, and the addition of a quadratic term, the simplest choice to explore the presence of curvature,

| TABLE 1 Hemodynamic and Volume Results for Seven Dogs |
|-------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Dog | Seq | Heart rate | Aortic systolic pressure | Aortic diastolic pressure | Coronary systolic pressure | Coronary diastolic pressure after flow arrest | Left atrial mean pressure | Coronary blood flow | Tissue T | Tissue volume |
| a  | 1   | 170        | 132               | 100              | 140             | 88              | 46               | 10             | 155         | 0.47        | 73           |
|    | 2   | 170        | 104               | 76               | 134             | 86              | 38               | 7              | 105         | 0.57        | 60           |
|    | 3   | 160        | 96                | 72               | 116             | 76              | 32               | 7              | 72          | 0.64        | 46           |
|    | 4   | 155        | 75                | 50               | 57              | 25              | 23               | 9              | 36          | 0.86        | 31           |
| b  | 5   | 158        | 96                | 82               | 178             | 144             | 70               | 6              | 157         | 0.40        | 63           |
|    | 1   | 180        | 110               | 90               | 164             | 126             | 60               | 7              | 128         | 0.50        | 64           |
|    | 4   | 168        | 102               | 80               | 152             | 118             | 50               | 6              | 111         | 0.60        | 67           |
|    | 2   | 170        | 106               | 88               | 136             | 98              | 40               | 6              | 89          | 0.66        | 59           |
|    | 3   | 165        | 85                | 55               | 58              | 30              | 30               | 5              | 44          | 0.73        | 33           |
| c  | 1   | 164        | 110               | 70               | 160             | 124             | 48               | 6.5            | 200         | 0.40        | 80           |
|    | 2   | 155        | 100               | 60               | 154             | 116             | 42               | 6.5            | 150         | 0.53        | 78           |
|    | 3   | 156        | 104               | 62               | 110             | 80              | 30               | 6.5            | 100         | 0.73        | 73           |
|    | 5*  | 130        | 90                | 60               | 28              | 25              | 6                | 6              | 80          | 0.68        | 54           |
|    | 4   | 140        | 95                | 62               | 58              | 28              | 22               | 6              | 50          | 0.80        | 40           |
| d  | 1   | 173        | 116               | 82               | 175             | 132             | 35               | 7.5            | 158         | 0.46        | 73           |
|    | 2   | 160        | 106               | 70               | 135             | 100             | 33               | 7              | 102         | 0.58        | 60           |
|    | 3   | 175        | 125               | 94               | 66              | 32              | 25               | 10             | 62          | 0.74        | 45           |
|    | 5*  | 160        | 118               | 90               | 100             | 50              | 40               | 9.5            | 177         | 0.42        | 74           |
|    | 6*  | 152        | 82                | 50               | 60              | 33              | 33               | 9              | 102         | 0.54        | 55           |
|    | 4   | 142        | 72                | 42               | 47              | 27              | 25               | 8.5            | 62          | 0.73        | 45           |
| e  | 1   | 145        | 110               | 84               | 208             | 170             | 58               | 5              | 181         | 0.44        | 79           |
|    | 2   | 160        | 130               | 100              | 144             | 102             | 52               | 5              | 116         | 0.60        | 68           |
|    | 3   | 150        | 128               | 104              | 82              | 53              | 46               | 14             | 81          | 0.62        | 50           |
| f  | 2   | 132        | 112               | 68               | 180             | 135             | 76               | 5              | 133         | 0.47        | 57           |
|    | 1   | 155        | 108               | 70               | 144             | 106             | 44               | 10             | 71          | 0.47        | 34           |
|    | 3   | 126        | 94                | 52               | 84              | 56              | 36               | 5              | 42          | 0.78        | 33           |
| g  | 3   | 90         | 102               | —                 | 198             | 177             | 56               | 5              | 114         | 0.58        | 66           |
|    | 1   | 98         | 98                | 60               | 170             | 150             | 36               | 6              | 80          | 0.80        | 64           |
|    | 2   | 90         | 104               | 54               | 72              | 44              | 30               | 10             | 52          | 1.04        | 54           |
|    | 4*  | 90         | 106               | 73               | 190             | 138             | 74               | 6              | 247         | 0.36        | 89           |
|    | 5*  | 90         | 106               | 73               | 190             | 138             | 74               | 6              | 195         | 0.36        | 70           |
|    | 6*  | 120        | 88                | 66               | 62              | 34              | 34               | 8              | 80          | 0.83        | 66           |

Indicators used were 111-albumin, 111-antipyrine, and tritiated water (dog d). Seq = the sequence in which volumes were measured. Flow is in ml/min per 100 g of perfused myocardium; mean transit time (T) is in minutes; volume is in ml/100 g of perfused myocardium.

* Animals received 0.1 mg dipyridamole and are maximally vasodilated.
improves the fit only slightly. The decrease in variance of the tissue mean transit time accounted for by the independent variable (x) is about 3%, passing from the linear to the quadratic equation (y = 1.03 - 0.0051x + 0.00001x²).

**EFFECT OF CORONARY FLOW ON TISSUE VOLUME**

Figure 2B depicts the relationship between tissue volume and coronary flow. As would be expected from the fact that volume = flow times T, a statistically significant linear regression with a high correlation coefficient applies (y = 0.25x + 31.9; r = 0.87; P < 0.01). The addition of a quadratic term decreases the variance of the dependent variable accounted for by the independent variable by 4.5% (y = 0.51x + 18.1 - 0.001x²). The regression line was carried out to include all values because the dipyridamole-induced vasodilation (circle with dot) did not seem to alter the relationship. Despite the use of flows corresponding to a perfusion pressure higher than the aortic pressure, a plateau (maximal organ capillary surface) is not obvious. This finding will be discussed later.

The absence of an evident effect of vasomotor tone on the flow-tissue volume relationship implies that for a constant flow vasodilation is able to maintain the same volume of tissue perfused despite the associated decrease of perfusion pressure, and, on the other hand, that at the same perfusion pressure vasodilation and the consequent increase of flow are associated with an increase of tissue volume. This is more obvious if one looks at the relationship between tissue volume and coronary perfusion pressure.

**EFFECT OF CORONARY DIASTOLIC PRESSURE ON TISSUE VOLUME**

Because coronary blood flow is, in the main, diastolic flow, we examined the relationship between coronary diastolic pressure (x) and tissue volume (y). Data are plotted in Figure 2C. The linear regression for all the values is statistically significant (y = 0.19x + 42.8; r = 0.59; P < 0.01). If values for dipyridamole-induced vasodilation (except dog g, sequence 4) are excluded, the linear regression equation becomes y = 0.29x + 27.8 (r = 0.87; P < 0.01) and the vasodilated group remain above the regression line. An increase in perfusion pressure appears to be followed by an increase in tissue volume; vasodilation appears to influence this relationship. At low values of perfusion pressure, once maximum vasodilation has been reached, small changes in pressure result in large changes in tissue perfused.

**Critique of the Method**

To measure indicator mean transit time we selected the ramp as the input function, following Zierler’s suggestion because of its theoretical greater accuracy. Once recirculation is removed, the linearity of the output function represents the major advantage over other input functions. Also, by considering only the tissue transit time (the difference between the two measured transit times) we eliminated errors due to timing and catheter distortion. In this study there usually was agreement between blood flow as calculated from the slope of the output reference indicator curve and the known pump flow. This, in our opinion, was a good check of the propriety of the slope chosen and the insignificance of coronary sinus contamination with blood from the right coronary artery or right atrium. The agreement between known and calculated flow is also equivalent to observing complete tracer recovery. However, on several occasions blood flow as calculated from the diffusible tracer was higher than the known pump flow, that is, recovery of indicator was not complete and its output slope was divergent from the reference slope (Fig. 1). This was observed in spite of the fact that the output ramp showed a
linear rise for periods as long as 5 minutes, even at high flow, which according to Zierler's theory should indicate a complete equilibration of the indicator.

For a ramp input, "equilibration" means that the rate of rise of concentration of a diffusible indicator is uniform throughout the perfused volume. It is important to note, however, that any systematic proportional error (such as measurement of the concentration of injectate) in the ramp data will affect only the slope and not the time intercept which is \( t \). If a measurement error or a selective loss of diffusable tracer in the heart cavities is excluded, a possible explanation for the difference in slope could be the presence of long diffusion distances, in the perfused bed. It is obvious that when a large portion of capillaries are closed, the remaining ones must serve a much larger perfusion volume, with longer diffusion distances and therefore a greater equilibration time. An extended input such as the ramp would provoke a continuing flux of dye into the less accessible areas, and this loss of indicator could be interpreted as an increased flow. Although the same problem is implicit in the use of a bolus input function, it will be less apparent, even in absence of recirculation, because of the difficulty of determining the entire tail of the curve. Zeiger and Goresky* used a bolus as the input function and delayed recirculation in a similar preparation and showed changes in the shape of the downslope of curves for diffusible indicators that were related to the rate of perfusion and observed incomplete recovery especially at low flows.

In this study the tissue mean transit time always was computed as the distance between the extrapolation of the two output functions to the abscissa. In case of divergent slopes the transit time computed at the completion of the ramp indicated in many instances a calculated volume much larger than the physical volume of the heart. For this reason, the divergence was interpreted as an artifact, an error affecting only the slope. Thus the intercept, rather than some average horizontal distance between the nonparallel ramps, was considered to be the best measure of \( t \).

Also, we tested the adequacy of the coronary sinus sampling site by injecting indicators into the right coronary artery and right atrium and determining that they were not recovered from the coronary sinus cannula during measurement periods.

**Discussion**

Many authors\(^2, 9, 10\) have postulated that there is recruitment of myocardial vascular units after an increase in coronary pressure and flow. Scharf and Bromberger-Barnea\(^11\) observed changes in myocardial weight, and thus in intravascular blood content of the isolated heart, with changes in coronary flow and pressure in both the coronary artery and coronary sinus, and these changes could be accounted for by recruitment or distention or by extravascular fluid, i.e., edema.

Martini and Honig* and Bing et al.\(^12\) provided direct evidence of recruitment by visualization of perfused vessels. Zeiger and Goresky* showed by multiple indicator-dilution technique an increase of the permeability surface product (that is proportional to the capillary surface area) as flow was increased. Duran et al.\(^13\) using the first circulation multiple tracer-dilution method, proposed recruitment mediated by vasodilation, on the basis of their observation of an increase in capillary surface area available for metabolic exchange. In our study the same assumption was made; the amount of tissue perfused, as measured by the extravascular diffusion space of a water indicator, is proportional to the capillary surface subserving exchange and therefore to the number of open capillaries.

To obtain the most accurate measurement of the extravascular transit time we used a known flow, the ramp, as the input function and we excluded tracer recirculation.

The problems encountered in the use of the ramp have been discussed in the preceding section. Our data can be summarized as follows: increasing coronary blood flow results in an increase in the quantity of tissue perfused. A plateau indicating complete recruitment was not obvious from our data, despite the fact that we used high perfusion pressures in an attempt to demonstrate it. The explanations for this may be multiple: the insufficient number of observations and the larger scattering of values that make difficult a curvilinear vs. a linear fit; the existence of a few vascular units with high opening pressures; the opening of collateral channels to the right coronary system of the heart; the development of myocardial edema at high flows. The relationship between flow and tissue volume was not significantly affected by vasomotor tone. Vasodilation, as proposed by Renkin\(^14\) for the skeletal muscle and by Duran et al.\(^15\) for the heart, appears to increase the capillary surface area, as shown by the larger tissue volume recorded after dipyridamole-induced vasodilation at a comparable perfusion pressure (Fig. 2C).

A wider range of pressures, with maximum vasodilation, was not explored because of the very large flows required and the corresponding shortening of mean transit times that result in relatively large errors in their determination. However, vasomotor tone appears to control, in the main, the number of tissue units perfused and to balance the effect of coronary pressure lowering, probably making accessible additional capillaries when those with a lower closing pressure are shut off.

There are several important implications in our data. It was observed by Gregg and Shipley\(^16\) and others\(^17, 18\) that increasing coronary pressure or flow increases myocardial oxygen consumption. Gregg,\(^16\) proposed several possible mechanisms which might explain this observation, including recruitment of tissue units. Scharf and Bromberger-Barnea\(^11\) observed that increasing coronary sinus pressure was as effective in increasing myocardial oxygen uptake as was increasing coronary artery pressure. This suggests that recruitment of closed units can occur from either end of the capillary net. Whalen et al.\(^19\) observed that in skeletal muscle decreasing blood flow decreased oxygen consumption without hypoxia becoming apparent. It is possible that a variable component of myocardial and skeletal muscle oxygen consumption is the number of perfused tissue units functioning, and this may be independent of external work.

Another important implication relates to the interpretation of isotope washout slopes from the myocardium using...
either single detectors or multiple detectors. As Zierler has indicated, such undimensioned slopes are rate-constant, i.e., the inverse of mean transit time, of flow per unit of volume, with the latter measurement receiving its dimension of flow (mL/100 g per min), from the equilibrium constant of the indicator. Because volume, the denominator, is dependent on flow, it is possible that observed changes in slope may reflect changes in perfusion volume rather than in flow, or changes in both. It is essential therefore that such washout curves be interpreted with caution, recognizing that they are not dimensioned and that both components, flow and tissue volume, are variables within the physiological range of pressure and flow.

The volume of perfused tissue, as computed in this study, obviously represents an average value. Both Sestier et al. and Falsetti et al. have described altered temporal and spatial heterogeneity of perfusion in the canine myocardium, and Klassen has observed altered spatial and temporal heterogeneity as a component of coronary artery disease. The size of these vascular tissue units, their regional distribution, the forces and chemical mediators involved in both closure and opening, and the periodicity of opening and closing remain to be investigated.

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