The Significance of the Late Fall in Myocardial PCO₂ and Its Relationship to Myocardial pH after Regional Coronary Occlusion in the Dog


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SUMMARY. After acute regional coronary occlusion, myocardial tissue PCO₂, as measured by mass spectrometry, rises, reaches a peak, and then gradually falls. This late fall in myocardial tissue PCO₂ could be due to (1) a gradual increase in tissue blood flow (and hence improved carbon dioxide washout), (2) a gradual consumption of tissue bicarbonate, (3) a gradual reduction in the production of carbon dioxide due to progressive cellular damage, or (4) an artifact caused by the continued presence of the mass spectrometer probe in the ischemic tissue. To determine which of these four mechanisms is responsible for the late fall in myocardial tissue PCO₂, we subjected 27 anesthetized open-chest dogs to 3-hour occlusion of the left anterior descending coronary artery. Both myocardial tissue PCO₂ and intramyocardial hydrogen ion concentration were measured in the myocardial segment supplied by the left anterior descending coronary artery. Ten dogs (group 1) were killed after the occlusion (occlusion I), and 11 dogs (group 2) underwent reocclusion (occlusion II) at the same site after a 45-minute period of reflow. Regional myocardial blood flow was measured periodically by the intramural injection of 127Xe. Changes in myocardial tissue PCO₂ and hydrogen ion concentration were related to ultrastructural changes in the tissues adjacent to the myocardial tissue PCO₂ probe. Regional myocardial blood flow remained unchanged throughout the 3-hour occlusion, ruling out increased carbon dioxide washout as a cause for its late fall. Tissue hydrogen ion concentration, as measured by a new lead glass electrode, correlated well with myocardial tissue PCO₂, with the reduction in regional myocardial blood flow, and with ischemic damage assessed histologically. Myocardial hydrogen ion concentration also exhibited a late fall after the occlusion, from a peak of 199.8 ± 27.8 nmol/liter to 91.9 ± 12.1 nmol/liter (mean ± SEM). This ruled out consumption of tissue bicarbonate as the cause for the late fall in myocardial tissue PCO₂. Peak rise in myocardial tissue PCO₂ after occlusion II (71.2 ± 7.9 mm Hg) was significantly lower than peak myocardial tissue PCO₂ after occlusion I (116.7 ± 13.9 mm Hg, P < 0.001). The difference between these latter two values, as well as the magnitude of fall in myocardial tissue PCO₂ during occlusion I, related directly to the degree of histological damage observed. In six additional experiments (group 3), peak myocardial tissue PCO₂ in myocardial segments supplied by normal (unoccluded) coronary arteries remained unchanged over 3 hours, and hydrogen ion concentration in segments supplied by an occluded left anterior descending coronary artery exhibited a late fall, even in the absence of a mass spectrometer probe. We conclude that, following regional coronary occlusion, there is a late fall in both myocardial tissue PCO₂ and hydrogen ion concentration; this late fall probably is a reflection of progressive cellular damage which does not reverse after a 45-minute period of reflow. Myocardial tissue PCO₂ and hydrogen ion concentration curves following coronary occlusion reflect the metabolic viability of the ischemic myocardium. (Circ Res 56: 537–547, 1985)

ACCUMULATION of hydrogen ions, lactate, and carbon dioxide, are important features of regional myocardial ischemia (Opie, 1976). Until recently, there have been no reliable techniques for the quantification of interstitial accumulation of carbon dioxide in the ischemic myocardium. In 1972, Brantigan, Gott, and Martz described the mass spectrometer technique for measurement of myocardial oxygen (PmO₂) and carbon dioxide (PmCO₂) tension. Utilizing this technique, we have demonstrated that the magnitude of rise in PmCO₂ following regional coronary artery occlusion correlated well with the degree of underlying ischemic damage assessed by intramural ST segment changes (Khuri et al., 1975), and by changes in regional blood flow and tissue histology (Khuri et al., 1979a).

Our studies with the mass spectrometer have shown that after regional coronary occlusion, PmCO₂ rises, reaches a peak in 20–30 minutes and then falls gradually. This late fall in PmCO₂ has not been observed during global myocardial ischemia (McGregor et al., 1974; Lange et al., 1983). Neither did Case et al. (1979b), who utilized a different PmCO₂ measuring system, observe such a fall during re-
regional ischemia. Since we had observed this late fall, invariably, in all the regional occlusion experiments we had performed, we elected to examine this phenomenon further, aided by a new tissue pH electrode that we had evaluated in our laboratory (Khuri et al., 1979b).

Thus, the purpose of this study is to describe the changes in PmCO₂ during 3 hours of regional myocardial ischemia, to relate these changes to changes in hydrogen ion concentration, and to postulate a reason for the late fall in PmCO₂ following regional coronary occlusion.

Methods

Twenty-seven adult mongrel dogs of both sexes weighing 15–20 kg were anesthetized with pentobarbital (30 mg/kg), intubated, and placed on a Harvard respiratory breathing room air. Tidal volume and ventilatory rate were adjusted to yield normal arterial blood gases and pH measured with an IL model 713 pH/blood gas analyzer. Arterial pressure was monitored continuously through a catheter placed in the left femoral artery and attached to a Gould-Statham P23ID pressure transducer. The external jugular vein was cannulated and used for administration of fluids and drugs. A left thoracotomy through the 5th intercostal space was performed, the pericardium was split, and the heart was suspended in a pericardial cradle. The left anterior descending coronary artery (LAD) or one of its branches was dissected and prepared for occlusion at a later time. In the myocardial segment supplied by the LAD to be occluded (which was identified by epicardial cyanosis following a transient 5-second occlusion), measurements of regional myocardial oxygen and carbon dioxide (PmO₂ and PmCO₂), regional myocardial hydrogen ion concentration ([H⁺]), and regional myocardial blood flow (RMBF) were made. Arterial blood gases and pH were analyzed at 1-hour intervals throughout each experiment to ascertain their stability.

Measurements of Regional Myocardial Gas Tensions

The Medspect II mass spectrometer (Chemetron Inc.) was used to monitor PmO₂ and PmCO₂ throughout each experiment. The mass spectrometer probe, described previously (Brantigan et al., 1972), consisted of Teflon-coated 22-gauge stainless steel tubing with a 19-mm sensing tip. The probe was inserted in the left ventricular wall after making a small epicardial nick; it was advanced gently to the endocardial surfaces. The other end of the probe was attached to a cannula connecting it to the mass spectrometer. The vacuum exerted by the mass spectrometer withdrew gas molecules across the Teflon membrane at a rate of 5 x 10⁻⁶ ml/sec. A constant readout of the tissue gas tensions in mm Hg was displayed on the mass spectrometer.

The electrode was inserted into the myocardium through a minute epicardial incision and then advanced until the sensing surface was at a level within the myocardial wall closest to the level of the adjacent mass spectrometer probe. The electrode was then fixed to the myocardial wall with a fine epicardial suture. The vacuum exerted by the mass spectrometer withdrew gas molecules across the Teflon membrane at a rate of 5 x 10⁻⁶ ml/sec. A constant readout of the tissue gas tensions in mm Hg was displayed on the mass spectrometer. The electrode was inserted into the myocardium through a minute epicardial incision and then advanced until the sensing surface was at a level within the myocardial wall closest to the level of the adjacent mass spectrometer probe. The electrode was then fixed to the myocardial wall with a fine epicardial suture. The mass spectrometer probe was calibrated before and after each experiment in a water tonometer into which known gas mixtures of oxygen, nitrogen, and carbon dioxide were bubbled at 37°C. The initial and 95% in vitro response times of the probe were 0.5 and 5.5 minutes, respectively.

Measurement of Intramyocardial pH

A miniature steel-jacketed glass electrode developed in our laboratory in conjunction with Ingold Electrodes, Inc. was utilized for the continuous measurement of intramural pH (Khuri et al., 1979b). The details of the electrode are illustrated in Figure 1. The electrode is steel-jacketed for more rigidity; its sensing glass membrane is 4 mm long and 1 mm in outside diameter. It is conical in shape and rounded at the tip to ensure minimal tissue damage during insertion. Each electrode was tested in vitro for its Nernstian response in buffer of pH 4.00 and 7.00 at 25°C. The in vitro reproducibility of each electrode was determined as the difference in electromotive force (emf) potential voltage readings in pH 7.00 buffer at 1-minute intervals after an in-between reading in pH 4.00 buffer. The electrodes utilized had a reproducibility which was within 0.5 mV, or 0.008 pH unit. The 95% in vitro response time to a step change in pH was 30 seconds.

The electrode was inserted into the myocardium through a minute epicardial incision and then advanced until the sensing surface was at a level within the myocardial wall closest to the level of the adjacent mass spectrometer probe. The electrode was then fixed to the surface of the heart with a fine epicardial suture. The silver chloride reference electrode, 40 mm long and 2 mm in outside diameter, was placed in a subcutaneous pocket through the edge of the thoractomy incision, i.e., at a site remote from the ischemic myocardium. With this system, the placement of the reference electrode at a site remote from the ischemic myocardium does not affect the magnitude of fall in pH during regional ischemia (Khuri et al., 1984). Both the pH and the reference electrodes were connected to a Corning model 610A pH meter via shielded coaxial cables. The output from the pH meter was recorded on a multichannel recorder (model D7 Polygraph, Grass Instruments). Since all these experiments were performed at constant normothermic myocardial temperatures, hydrogen ion concentration, expressed as [H⁺] in nanomoles...
per liter (nmol/liter), was calculated from the pH in accordance with the formula \( pH = -\log [H^+] \) (Davenport, 1967).

To correct for the difference in response time between the mass spectrometer probe and the pH electrode, we developed an in vitro response curve for each, by placing them first in a solution of known pH and Pco2 and then moving into another solution with a different pH and Pco2. The difference in the 95% response time between these curves and used as the basis for the correction.

Measurement of Regional Myocardial Flow (RMBF)

RMBF in the area adjacent to the mass spectrometer probe was measured using the washout of the radioactivity of Xenon-127 injected into the myocardium, as described previously (Khuri et al., 1980). Briefly, a 23-gauge right-angle needle attached to 50 P.E. tubing was inserted intramurally and fixed to the epicardium with a fine suture. A scintillation single-probe detector with a 2-inch in diameter sodium iodide crystal and a straight-bore lead collimator was suspended over the site of insertion of the needle and connected to a rate-meter. For each RMBF determination, 0.1 ml of saline containing approximately 200 μCi of 127Xe was injected and flushed with 0.15 ml of saline. The 127Xe washout curve was recorded on a multichannel recorder (Grass Instruments) and later transcribed onto a semilog paper so that its half time could be determined. RMBF was then calculated according to the formula:

\[
\text{RMBF (ml/100 g per min)} = \frac{(0.693)(0.7)(100)}{T_m}
\]

where 0.693 is the natural logarithm of 2, \( T_m \) is the halftime expressed in minutes, and 0.7 is the partition coefficient of xenon gas between tissue and blood (Ingvar and Lassen, 1961). To ensure that RMBF is determined at the same level within the myocardial wall as that of the Pmco2 probe, the exact depth of the latter was determined after its insertion into the myocardium by echocardiography (Ekoline 20, Smith Kline Instrument Co.), and the xenon needle was bent so that the distance between the tip and right angle of the needle was equal to the depth of the probe. The needle then was inserted around 5 mm away from the probe.

Experimental Protocol

The 27 dogs studied were divided into three experimental groups. Group 1 experiments were performed first. In this group of 10 dogs, the LAD was occluded for 3 hours; the animal then was killed, and histological damage was assessed. The same continuous hemodynamic and metabolic measurements were made in group 2 as in group 1. During occlusion I, regional myocardial blood flow was measured at 10, 30, 90, and 165 minutes into the occlusion; during occlusion II, it was measured when the Pmco2 peaked before the animal was killed.

Group 3 experiments were performed in six dogs to investigate whether the changes in Pmco2 and \([H^+]\) observed in groups 1 and 2 were caused by the continued presence of the mass spectrometer probe per se in the ischemic tissue. In this group, no Pmco2 probes were placed in segments supplied by the LAD. Instead, one Pmco2 probe was placed in a remote segment supplied by the circumflex coronary artery, and a single pH electrode was placed in a segment supplied by the LAD. All six dogs underwent a 3-hour occlusion of the LAD. After the peak \([H^+]\) was reached in three dogs (nearly 40 minutes into the LAD occlusion), a second pH electrode was placed in the ischemic area supplied by the occluded LAD, and was kept until the end of the experiment. RMBF was not determined in this group, and no histological studies were performed. Blood pressure and heart rate were recorded continuously.

Assessment of Histological Damage

At the end of each experiment, three tissue samples, each 2 x 1 x 1 mm, were obtained from the myocardium 1-2 mm away from the distal end, the middle, and the proximal end of the endocardial surface of the mass spectrometer probe. Three other similar tissue samples were obtained from the myocardium 1-2 mm away from the sensing tip of the pH electrode. All tissue samples were immersed in 3% glutaraldehyde for 3 hours, after which they were washed in cacodylate buffer and postfixed with cold 1% osmic acid for 1 hour. Then, the tissue cubes were dehydrated in graded alcohol. Subsequently, over a period of 24 hours, they were placed successively in propylene oxide and 1:1 mixture of propylene oxide and Epon 812. At the end of the 24-hour period, they were embedded in Epon 812. Sections (1 μm thick) were cut and stained with toluidine-blue. The severity of ischemic injury was graded according to the following criteria: 0, normal myocardial cell; 1+, clumping of nuclear chromatin alone, or with occasional vacuoles plus myocardial relaxation as manifested by wide I-bands; 2+, further clumping of nuclear chromatin plus intramyofibrillar edema and more vacuoles; 3+, marked intracellular edema, numerous vacuoles, and the sarcolemmal membrane lifted off the myofilaments by the intracellular edema; and 4+, severe swelling and architectural disruption. The histological sections were examined and graded by one observer (RAK) who had no previous knowledge of the associated changes in myocardial gas tensions. For each mass spectrometer probe, the average value of the three histological sections were obtained and expressed as mean ischemic score (MIS). The methodology utilized in the assessment of histological damage is similar to that which we reported previously (Kloner et al., 1974, 1977a, 1977b; Khuri et al., 1979a).

Analysis of Data

We calculated a rate-pressure product, using the mean blood pressure and heart rate. All summary data were expressed as mean ± SEM. Linear regression was used to establish the relationships between parameters which exhibited a biological continuum. Data representing nonbiological continuum were analyzed by multivariate analysis. Where applicable, Student's t-test for paired and unpaired data was used to evaluate the significance of changes observed.
TABLE 1
Summary of Data Obtained in Group 2

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>At Pmco2max (occlusion I)</th>
<th>3 hr post occlusion I</th>
<th>45 min post reflow</th>
<th>At Pmco2max (occlusion II)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HR (beats/min)</td>
<td>115 ± 6</td>
<td>130 ± 5</td>
<td>121 ± 7</td>
<td>109 ± 8</td>
<td>97 ± 8*</td>
</tr>
<tr>
<td>Mean BP (mm Hg)</td>
<td>136 ± 7</td>
<td>130 ± 4</td>
<td>131 ± 7</td>
<td>121 ± 8</td>
<td>121 ± 6</td>
</tr>
<tr>
<td>Rate pressure product</td>
<td>19885 ± 1477</td>
<td>17390 ± 1089</td>
<td>16149 ± 1516</td>
<td>13385 ± 1891</td>
<td>10851 ± 1164*</td>
</tr>
<tr>
<td>Pmco2 (mm Hg)</td>
<td>23 ± 4.3</td>
<td>2.6 ± 1.2</td>
<td>3.8 ± 1.9</td>
<td>49.7 ± 11.3</td>
<td>4.5 ± 2.1</td>
</tr>
<tr>
<td>[H+] (nmol/liter)</td>
<td>38.2 ± 1.8</td>
<td>116.7 ± 13.9</td>
<td>63.7 ± 5.7</td>
<td>33 ± 2.7</td>
<td>71.2 ± 7.9*</td>
</tr>
<tr>
<td>RMBF (ml/100 g per min)</td>
<td>46.9 ± 4.5</td>
<td>184.6 ± 34.9</td>
<td>89.6 ± 14</td>
<td>44.3 ± 2.5</td>
<td>95.8 ± 13.6*</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM. Pmco2max = peak value during occlusion.
*When compared to occlusion I, differences are significant (P = or <0.001).

To determine whether the Pmco2 values obtained postocclusion reflected a level of CO2 accumulation over and above that which would be expected on the basis of decreased washout only, we utilized a mathematical model which predicted the postocclusion Pmco2 assuming no change in the rate of CO2 production between the pre- and the postocclusion states. Hence, under the assumption of a constant preocclusion rate of CO2 production (Co) following a step change in coronary flow from Q1 ml/min to a reduced but steady flow of Q ml/min, the concentration of CO2 at time T [C(T)] was predicted by

\[ C(T) = \left[ \frac{C_0 - \frac{M + QC_1}{Q}}{1 + \frac{M + QC_1}{Qv}} \right] e^{-\frac{Q}{V}T} + \frac{M + QC_1}{Q} \]

where C0 = concentration of CO2 in the coronary arterial blood,

\[ M = C_0Q_1 - C_1Q_1 = Q_1(C_0 - C_1), \]

and V is the CO2 space in the myocardium modeled as a well-mixed compartment of 75 cm³.

Results

In all three groups, heart rate and mean blood pressure did not change significantly throughout the initial 3-hour coronary occlusion. Data obtained in groups 1 and 3 were similar to those obtained in group 2, and are shown in Table 1. In group 2, heart rate (but not mean BP) during occlusion II was significantly lower than during the initial 3-hour occlusion. Consequently, there was a significant difference in rate-pressure product between the two occlusions (Table 1).

Myocardial Gas Tension Changes

During the initial 3-hour LAD occlusion in groups 1 and 2, Pmco2 rose to peak and then invariably fell, in all the animals studied. Pmc2 fell to a nadir and remained unchanged. A typical curve obtained is shown in the upper panel of Figure 2, and data are summarized in Tables 1 and 2. In group 2, following the release of occlusion I, Pmco2 rose continuously throughout the whole period of reflow, reaching a peak level which was significantly higher than preocclusion value (P = 0.005). Following occlusion II, Pmco2 fell again and was not significantly different from the nadir value during occlusion I (Fig. 2, lower panel; Table 1). Following occlusion II, Pmco2max was significantly lower than Pmco2max during occlusion I, but was not significantly different from the Pmco2 value recorded 3 hours after occlu-

![Figure 2](http://circres.ahajournals.org/)

**Figure 2.** Myocardial gas tension (Pmco2 and Pmco2) curves recorded in the course of a typical experiment in group 1 (upper panel) and group 2 (lower panel). In group 1, the animal was killed at the end of a 3-hour occlusion of the LAD. In group 2, the 3-hour occlusion was released, 45 minutes of reflow were allowed, and the occlusion was re instituted; the animal was killed after Pmco2 reached its peak during occlusion II.
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TABLE 2
Summary of [H⁺], Pmco₂ and Pmco₂ Data Obtained in Group 1

<table>
<thead>
<tr>
<th></th>
<th>Pmco₂</th>
<th>Pmco₂</th>
<th>[H⁺]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stabilization time after probe or electrode insertion</td>
<td>43.2 ± 2.9 min</td>
<td>43.2 ± 2.9 min</td>
<td>12 ± 3.4 min</td>
</tr>
<tr>
<td>Baseline value</td>
<td>16.2 ± 1.3 mmHg</td>
<td>52.8 ± 3.3 mmHg</td>
<td>45.6 ± 2.0 nmol/liter</td>
</tr>
<tr>
<td>Time of initial change after CO*</td>
<td>1.0 ± 0.1 min</td>
<td>2.7 ± 0.3 min</td>
<td>14 ± 0.8 sec</td>
</tr>
<tr>
<td>Peak or nadir value reached after CO</td>
<td>0.2 ± 2.0 mmHg</td>
<td>190.8 ± 22.0 mmHg†</td>
<td>199.8 ± 27.8 nmol/liter</td>
</tr>
<tr>
<td>Time at which peak or nadir value was reached</td>
<td>9.4 ± 2.5 min</td>
<td>28.1 ± 4.4 min</td>
<td>37.7 ± 5.2 min</td>
</tr>
<tr>
<td>Value at 3 hr into the CO</td>
<td>0.2 ± 3.0 mmHg</td>
<td>84.4 ± 7.1 mmHg‡</td>
<td>91.9 ± 12.1 nmol/liter</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM.
* CO = coronary occlusion.
† Pmco₂max.
‡ Pmco₂3h.

sion I (Table 1). For each of the two occlusions in group 2, the time of initial rise of Pmco₂ and its maximal rate of rise (ΔPmco₂/Δtmax), as well as Pmco₂max and the time it took to reach Pmco₂max are all shown in Figure 3 and summarized in Table 3. Note that the differences between both occlusions in all four parameters were highly significant. To determine whether, in group 2, the fall in Pmco₂max during occlusion II was causally related to the fall in rate-pressure product (Table 1), the difference in Pmco₂max between occlusions I and II was correlated to the difference in rate-pressure product between the two occlusions. A poor linear correlation between both parameters was observed (r = 0.027), suggesting that the fall in Pmco₂max during occlusion II was not primarily due to the reduction in rate-pressure product.

In group 3, Pmco₂ recorded from six probes placed in segments supplied by a normal circumflex coronary artery remained unchanged throughout the 3-hour LAD occlusion (Table 4).

Changes in Myocardial [H⁺] and Their Relationship to Pmco₂, RMBF, and Tissue Histology

Changes in [H⁺] throughout the 3-hour occlusion were similar to the changes in Pmco₂ in that both parameters rose after the occlusion, reached a peak, and then fell gradually throughout the rest of the occlusion (Tables 1 and 2). The rise and subsequent fall in [H⁺] was highly significant (P = 0.0005). In group 2, mean peak [H⁺] during occlusion II was also significantly lower than during occlusion I (P = 0.005), but not significantly different from the mean [H⁺] recorded 3 hours after occlusion I (Table 1). In group 3, myocardial [H⁺] was determined in a segment supplied by an occluded LAD in the absence of a mass spectrometer probe. Data from these experiments, shown in Table 4, were no different from [H⁺] data obtained in groups 1 and 2. In the three animals in this group in which a second pH electrode was placed in the ischemic segment nearly 40 minutes after occlusion, only a fall in [H⁺] was observed (Table 4).

Because of the difference in response time between the mass spectrometer probe and the pH electrode, the initial rise in [H⁺] following coronary occlusion was observed significantly earlier (P < 0.001) than the initial rise in Pmco₂ (Table 2). Correcting for the difference in response times between the pH electrode and the Pmco₂ probe, cumulative data were calculated from the [H⁺] and the Pmco₂ curves just before the occlusion, and at 5-minute intervals throughout the occlusion. These data are
### TABLE 3
Differences in the Characteristics of the Pmco2 Curves between the Two Coronary Occlusions in Group 2

<table>
<thead>
<tr>
<th></th>
<th>Occlusion I</th>
<th>Occlusion II</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time of initial Pmco2 rise following CO* (min)</td>
<td>2.4 ± 0.13</td>
<td>4.55 ± 0.51</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>ΔPmco2/Δtmax (mm Hg/min)</td>
<td>10.4 ± 2.2</td>
<td>2.7 ± 0.4</td>
<td>&lt;0.005</td>
</tr>
<tr>
<td>Minutes after CO at which point c was reached</td>
<td>8.2 ± 0.6</td>
<td>11.1 ± 0.7</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Minutes after CO at which Pmco2max was reached</td>
<td>18.3 ± 24.4</td>
<td>41.4 ± 8.4</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Pmco2max (mm Hg)</td>
<td>116.7 ± 13.9</td>
<td>63.7 ± 5.7</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SEM.

* Coronary occlusion.

shown in Figure 4. [H+] and Pmco2 at these points in time were compared within each experiment by linear regression analysis. The correlation coefficients ranged from 0.77 to 0.99 and averaged 0.89. P was <0.001 for all correlations.

There was a direct linear relationship in group 1 between the peak rise in [H+] following the occlusion and the mean ischemic score assessed histologically (Fig. 5A). A linear relationship was also observed between peak [H+] and the fall in regional myocardial blood flow (Fig. 5B).

### Regional Myocardial Blood Flow

RMBF data in group 1 are shown in Figure 4. Before occlusion, RMBF was 61.9 ± 10.6 ml/100 g per min. At 10, 30, 90, and 165 minutes into the occlusion, it was 11.6 ± 5.3, 10.6 ± 3.1, 11.1 ± 3.6, and 11.6 ± 4.3 ml/100 g per min, respectively. The fall in RMBF following the occlusion was highly significant (P < 0.001), but there were no significant differences between the RMBF values obtained throughout the occlusion. In group 2, there was no significant difference between RMBF during occlusion II and mean RMBF during occlusion I (Table 1).

### Relationship of Pmco2 and Pmoo2 Changes to the Degree of Histological Damage

Since the mean ischemic score (MIS) was not a biological continuum, this relationship was elucidated by grouping Pmco2 and Pmoo2 values over three ranges of MIS. The difference between the maximal Pmco2 value reached during the occlusion, and the value reached at the end of a 3-hour occlusion (Pmco2max — Pmco2(3hr)) represented the magnitude of late fall in Pmco2. During the first occlusion, there was a direct relationship between the magnitude of rise and the magnitude of late fall in Pmco2 and the degree of histological damage observed (Fig. 6). In group 2, two additional direct relationships were noted (Fig. 7). The magnitude of rise in Pmoo2 after the release of occlusion I (probably a manifestation of a reactive hyperemic response) related directly to the degree of underlying ischemia assessed both by Pmco2max during occlusion I (r =

### TABLE 4
[H+] and Myocardial Gas Tensions Data in Group 3

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>[H+] in LAD (ischemic area)</th>
<th>Pmoo2 in CCX (normal area)</th>
<th>Pmco2 in CCX (normal area)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before CO</td>
<td>40 min post-CO</td>
<td>180 min post-CO</td>
</tr>
<tr>
<td>1</td>
<td>78</td>
<td>759</td>
<td>204</td>
</tr>
<tr>
<td>2</td>
<td>117</td>
<td>1288</td>
<td>1096</td>
</tr>
<tr>
<td>3‡</td>
<td>68</td>
<td>126</td>
<td>89</td>
</tr>
<tr>
<td>4‡</td>
<td>55</td>
<td>295</td>
<td>145</td>
</tr>
<tr>
<td>5</td>
<td>115</td>
<td>389</td>
<td>126</td>
</tr>
<tr>
<td>6‡</td>
<td>56</td>
<td>245</td>
<td>162</td>
</tr>
<tr>
<td>Mean</td>
<td>81.5</td>
<td>492.6</td>
<td>275.0</td>
</tr>
</tbody>
</table>

LAD = left anterior descending coronary artery. CCX = circumflex coronary artery. CO = coronary occlusion.

* nmol/liter.

† mm Hg.

‡ In experiments 3, 4, and 6, a second pH electrode was inserted in the ischemic area after a peak in [H+], recorded by first pH electrode, was reached.
Observed vs. Predicted Postocclusion PmCO₂

Figure 8 compares the observed postocclusion PmCO₂ values (at the points in time during which RMBF measurements were made) to those predicted assuming a constant rate of CO₂ production equal to the preocclusion rate. The highly significant differences between both curves indicated that CO₂ production had increased following the occlusion. Note that, in the predicted curve, no significant difference in PmCO₂ was observed between 30 and 165 minutes postocclusion. In contrast, a highly significant fall in the observed PmCO₂ was noted during this period, confirming a decrease in PmCO₂ production which occurred beyond 30 minutes of occlusion.

Discussion

Source and Measurement of Tissue Carbon Dioxide in Regional Myocardial Ischemia

In regional ischemia there is a reduction in both substrate (oxygen) and blood flow to certain segments of the myocardium, resulting in altered metabolic pathways (Scheuer and Brachfeld, 1966; Opie, 1976). Experimental studies on the beating heart in situ have shown that, even with total occlusion of a coronary artery, tissue flow in the center of the ischemic zone is never totally interrupted, and oxidative metabolism continues, albeit at a very slow rate (Opie, 1976). Anaerobic metabolism predominates and results in accumulation of H⁺, lactate, and CO₂ among other metabolic products (Opie, 1976). The CO₂ measured in an ischemic segment is thus the result of production of CO₂ and its decreased washout. Two processes are thought to lead to CO₂ production in regional ischemia: (1) retention of respiratory CO₂ (since, in contrast to anoxic states, there is a continued but reduced provision of oxygen) (Opie, 1976; Gevers, 1977) and (2) buffering of H⁺ with bicarbonate (Khuri et al., 1975; Gevers, 1977). Although the relative contribution of these
two processes to the overall CO₂ production has not been investigated, it is accepted that the source of CO₂ is closely linked to the source of [H⁺] in the ischemic process. Gevers (1977) has postulated a number of sources for the H⁺ in the ischemic process, the most important of which is the breakdown of ATP. Other sources that he has postulated include continued mitochondrial respiration with CO₂ production and subsequent cytoplasmic acidification, hydrolysis of triglycerides, and increased glycogen turnover. Chance and Mela (1966) have postulated that H⁺ can also be produced by the increased uptake of Ca²⁺ in the ischemic state.

The sources of H⁺ in regional ischemia are theoretically sources of CO₂, pending the availability of tissue bicarbonate. Since CO₂ is freely diffusible across cell membrane, measuring it in the extracellular tissue space should be fairly representative of the overall CO₂ pool in the ischemic myocardium. Brantigan et al. (1972) first described a Teflon probe-mass spectrometer system for the on-line measurement of tissue PCO₂. Utilizing this system, we have shown that during regional myocardial ischemia the magnitude of rise in PmCO₂ correlated with intramural S-T segment changes (Khuri et al., 1975), with reduction in regional myocardial blood flow, and with histological evidence of ischemia (Khuri et al., 1979a). The measurement of PmCO₂ with mass spectrometry has been useful not only in quantifying the degree of regional myocardial ischemia, but also in assessing the efficacy of pharmacological interventions in ameliorating ischemic damage (Hillis, 1979, 1981; Rude, 1980).

Our experiments with the mass spectrometer (Khuri et al., 1975, 1979a) have consistently shown what the present study demonstrates—namely, that, after acute coronary ligation in the canine heart in situ, PmCO₂ rises, reaches a peak, and then gradually declines. Case described a different method for the measurement of PmCO₂ which employed what was essentially a miniature Sevringhaus electrode (Coon, 1976; Case, 1979a). Utilizing this electrode, he observed a rise in PmCO₂ 9.6 ± 0.7 seconds (mean ± SEM) after regional coronary occlusion (Case, 1979b). He stated that he did not observe a subsequent fall in PmCO₂ with prolonged occlusions, although his observations in that study did not extend beyond 15 minutes postocclusion (Case, 1979b).

**Quantification of Regional Myocardial Ischemia by Measurement of Tissue pH**

Benzing, Gebert, and Strohm were the first to utilize glass microelectrodes to monitor intramural pH changes in the dog myocardium during ischemia (Benzing et al., 1971; Gebert et al., 1971). Similar observations were made by Hicks et al. in 1976. Cobb and Poole-Wilson (1980) have described a new membrane tissue electrode with which they assessed the severity of acidosis in myocardial ischemia. These groups demonstrated prompt and significant regional pH changes in response to coronary occlusion, but they did not attempt to compare the magnitude of these changes to other quantitative parameters of myocardial ischemia. Walters et al.
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Khuri et al. have described a miniature glass electrode housed in a 21-gauge needle for the measurement of intramyocardial pH. They did not relate their interstitial pH measurements to regional myocardial blood flow or to tissue histology, but they did demonstrate that these measurements reflected intracellular metabolism during elective arrest of the heart.

In the present study, we utilized a new glass electrode which we developed in our laboratory in conjunction with Ingold Electrodes, Inc. We assessed the ability of this electrode to quantify regional myocardial ischemia in a manner similar to our previous assessment of the Pmcoon probe (Khuri et al., 1979a). Changes in the extracellular [H⁺] in the ischemic myocardium related directly to the degree of histological damage incurred (Fig. 5A), to the magnitude of fall in regional myocardial blood flow (Fig. 5B), and to the magnitude of rise in Pmcoon (r = 0.89). These data prompt us to conclude that changes in intramyocardial pH can be as reliable as changes in Pmcoon in reflecting the degree of underlying ischemic damage.

Because of differences in methodology and response time between the mass spectrometer probe and the pH electrode, the exact temporal relationship between Pmcoon and [H⁺] changes following coronary occlusion cannot be elicited in this study. Furthermore, the temporal differences observed between the Pmcoon and the [H⁺] curves (Table 2) may not be due only to differences in the methods used to generate them. They may also reflect differences in solubility and diffusibility in the extracellular space between H⁺ and CO₂. For example, peak [H⁺] was reached well after peak Pmcoon, although the pH electrode had a faster response time. This, in itself, may reflect a more rapid washout of CO₂ due to its increased diffusibility in the extracellular space. It is clear, however, that elucidating the exact temporal and causal relationships between Pmcoon and myocardial [H⁺] during regional ischemia will have to await the development of a newer, more compatible measuring system.

Possible Causes for the Late Fall in Pmcoon Following Regional Coronary Occlusion

The late decline in Pmcoon observed during the occlusion can be theorized to be due to one or more of the following four mechanisms: (1) a gradual increase in tissue blood flow (and, hence, improved CO₂ washout) through the opening of new collateral channels; (2) a gradual consumption of tissue bicarbonate, since Pmcoon produced in ischemia is thought to be due, at least in part, to the buffering of hydrogen ions by bicarbonate; (3) an artifact caused by the continued presence of the mass spectrometer probe, as suggested by Case et al. Since the probe aspirates the gases from the surrounding tissues, albeit at a very slow rate, it may cause tissue depletion of CO₂ and, hence, a late fall in the Pmcoon curve; (4) a gradual reduction in the production of both H⁺ and CO₂ due to progressive cellular damage.

Evidence against the Blood Flow, Bicarbonate Consumption, and CO₂ Depletion Factors as Causes for the Late Fall in Pmcoon

To determine whether the late fall in Pmcoon following coronary occlusion related to a late improvement in the rate of washout, regional myocardial blood flow was measured from the washout curves of 127Xe injected intramurally at sites adjacent to the mass spectrometer probes. Regional myocardial blood flow at 10, 30, 90, and 165 minutes after coronary occlusion remained essentially unchanged, and no improvement in 127Xe washout was demonstrated (Fig. 4). Other investigators, utilizing the microsphere technique, also demonstrated no significant increase in collateral blood flow up to 6 hours after coronary occlusion in the dog (Opie, 1976).

In the present study, myocardial [H⁺] following
coronary occlusion exhibited a pattern similar to that of Pmco2; it rose soon after the onset of the 3-hour occlusion, reached a peak, and then gradually declined (Fig. 4). Within each study, the correlation coefficient between Pmco2 and [H+] averaged 0.89. During occlusion II in group 2, both Pmco2 and [H+] peaked at significantly lower levels than during occlusion I (Table 3). These observations rule out the possibility of the consumption of tissue bicarbonate as the cause for the late decline in Pmco2 during prolonged coronary occlusion. Had this been the case, the Pmco2 and [H+] curves would not have been similar, and a poor correlation between both parameters would have been observed.

The possibility of the late fall in Pmco2 being due to depletion of tissue CO2 as a result of the continuous sampling by the mass spectrometer was ruled out in group 3 experiments. Here, the continued presence of the probe in normal myocardial segments supplied by unoccluded coronary arteries, under stable conditions of blood pressure and heart rate, resulted in stable measurements over a period of at least 3 hours (Table 4). Furthermore, as demonstrated in Table 4, a late fall in [H+] in the ischemic segment was demonstrated, even in the absence of a mass spectrometer probe, and the addition of another pH electrode late after the occlusion demonstrated only a fall in [H+].

Evidence in Favor of Progressive Cellular Damage as the Cause for the Late Fall in Pmco2

By excluding the above three factors as causes for the late fall in Pmco2, the fourth possible cause remains: decreased production of CO2 secondary to progressive cellular damage. To postulate this, it is important to demonstrate an initial increase in the rate of postocclusion CO2 production as compared to the preocclusion rate, since one may argue that the rise in Pmco2 observed following coronary occlusion could be a manifestation of a steady state reduction in coronary flow without any net increase in the rate of CO2 production per se. To address this issue, we used a mathematical model which predicted for each experiment the postocclusion Pmco2 values had the preocclusion rate of Pmco2 production remained unchanged. Since RMBF was measured before and at 10, 30, 90, and 165 minutes after the coronary occlusion in group 1, predicted values could be calculated at four postocclusion points in time. As shown in Figure 8, the Pmco2 values observed at 10, 30, and 90 minutes following occlusion were significantly higher than those predicted on the basis of a steady state reduction in coronary flow alone. At 165 minutes, the observed Pmco2 was no different from the predicted value, indicating that between 30 and 165 minutes after the occlusion, a significant decrease in Pmco2 production had occurred.

Two additional observations made in this study suggested that the late fall in Pmco2 following coro-


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regional coronary occlusion allow the quantification of underlying ischemic damage and reflect the metabolic viability of the ischemic myocardium.

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