Rate of Rise of Myocardial PCO₂ during Early Myocardial Ischemia in the Dog

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SUMMARY We have investigated the rate of rise of myocardial PCO₂ (PmCO₂) after coronary artery occlusion using a new method for this measurement. Previous studies of PmCO₂ have been limited by the slow response of the only available method, and no increase in PmCO₂ prior to 3 minutes after occlusion has been found. We have implanted a miniature PCO₂ electrode, with a 63% response time of 14 seconds, into the left ventricle of 14 open-chest dogs. After abrupt coronary occlusion, PmCO₂ began to rise in 13.6 ± 1.1 seconds in heparinized dogs and in 7.5 ± 0.7 seconds in unheparinized dogs. The subsequent magnitude of the increase in PmCO₂ was 24, 88, 171, and 222 mm Hg at 2, 5, 10, and 15 minutes after occlusion. The rate of rise of PmCO₂ was essentially linear from 1 minute to 10 minutes at a rate of 18.3 mm Hg/min. The rate of rise was slower during the first 30 seconds after occlusion (6.1 mm Hg/min) and also from 30 seconds to 1 minute (9.7 mm Hg/min). This rate of rise is much greater than that previously observed and reflects the severe myocardial acidosis developing during ischemia. A rise in PmCO₂ is one of the earliest metabolic changes that has been observed during myocardial ischemia.

MYOCARDIAL PCO₂ (PmCO₂) was measured directly for the first time 6 years ago (Brantigan et al., 1972a), with the remarkable finding that PmCO₂ could reach values in excess of 300 mm Hg during prolonged ischemia (Brantigan et al., 1972a) or anoxia (Brantigan et al., 1972b). Such an extreme value indicates the presence of a severe degree of underlying acidosis at this time. Since acidosis has a major depressant effect on myocardial contractility (Steenbergen, et al., 1977) and since myocardial contractility rapidly falls during ischemia (Pirzada et al., 1975), we have attempted to obtain a more exact estimate of the rate of rise of PmCO₂ after coronary artery occlusion. The only method available for measurement of PmCO₂ has been the mass spectrometer technique (Brantigan et al., 1972a), which has the disadvantage of having a very slow response time. The earliest rise in PmCO₂ reported after coronary occlusion, using this method, began at 3 minutes (Brantigan et al., 1972a). The question arises as to whether this relatively late rise in PmCO₂ is the result of the slowly responding method or whether an increase in PmCO₂ is, in fact, a late phenomenon. To examine this problem, we have used a miniature PCO₂ electrode, which responds to a change in PCO₂ almost immediately and reaches 63% of full response in 14 seconds. We have previously reported the details related to insertion of this electrode into the myocardium (Case et al., 1979) for the purpose of measuring PmCO₂.

Methods

Description of Electrode*

The design of the miniature PCO₂ electrode used in these studies has been reported previously (Neumark et al., 1975). The electrode was conceived for continuous measurement of intravascular PCO₂, which reflects accurately (Coon et al., 1976). We have adapted its use to the myocardium and have previously (Case et al., 1979) described details of its calibration, response time, insertion into the myocardium, and the relationship of the measured PmCO₂ to coronary venous PCO₂. The electrode is basically a miniature Sevringhaus electrode and consists of a pH sensing electrode encased in a synthetic membrane. A small quantity of aqueous bicarbonate medium is held between the membrane and the pH electrode. CO₂ can diffuse readily across the membrane, but the membrane is impermeable to the electrolyte. A change in PCO₂ outside of the membrane thus is reflected through a change in the pH of the contained bicarbonate medium. However, external changes in pH alone are not reflected by the electrode. An Ag/AgCl reference electrode is in contact with the bicarbonate solution. The active portion of the electrode is 10 mm long (as a result of the length of the diffusing membrane) and is situated at the end of a 10-cm shaft, which terminates in a shielded cable, containing a single conductor. The shaft of the electrode is 1 mm in diameter, approximately that of a no. 20F needle, and the active portion is slightly less. The signal was amplified through an Instrumentation Laboratories.
millivolt meter with the output recorded on a Beckman oscillograph.

We have described elsewhere (Case et al., 1979) the characteristics of this electrode and its response to changes in PCO₂. It is linear on a semilogarithmic plot over a PCO₂ range of 35–720 mm Hg. The onset of response to a sudden change in PCO₂ requires less than 0.3 seconds; 63% of maximal response occurs in 14 seconds and 95% of full response occurs in 45 seconds. Analysis of stability and sensitivity yielded the following data: (1) electrode sensitivity—mean maximal change in slope over a period of several hours, involving repeated insertions into the myocardium 1.8 ± 0.7%; (2) electrode drift—this was always unidirectional; mean rate of change 10.7 ± 2.2%/hour. The electrode was calibrated using a tonometer at 37°C and the electrode response measured against several equilibrated PCO₂ solutions, containing from 5 to 100% CO₂.

**Experimental Procedure**

A left thoracotomy was performed on dogs anesthetized with pentobarbital (30 mg/kg, iv) while on positive pressure ventilation. The pericardium was opened widely and supported; the left anterior descending and/or circumflex coronary arteries were dissected free, approximately 1–2 cm from their origin; and a ligature was placed under each artery. The electrode was inserted into the myocardium, as we have described previously (Case et al., 1979), as follows. A tract was prepared for the electrode by insertion of a solid no. 20F needle into the myocardium at a site free of obvious vessels and thereafter passed parallel to the surface of the heart at an estimated mid-myocardial level to a distance of 25 mm. The electrode then was inserted to a distance of 20–25 mm, and its shaft supported. The final position of the electrode was parallel and near to a major branch of the artery to be occluded, either the left anterior descending or circumflex coronary artery, with the electrode tip near the main vessel. It was also well within the cavitary area produced by a brief occlusion of the coronary artery. We previously have noted (Case et al., 1979) that, after electrode insertion, the Pmco₂ rises rapidly to 100–150 mm Hg and then slowly subsides within 10–15 minutes to reach a stable level very close to that of coronary venous PCO₂. After this equilibrium was reached, and all values were steady, the effect of occlusion was observed. If there was any active bleeding from the insertion site after the initial puncture, the site was abandoned. The electrode was removed frequently for recalibration and then reinserted into the same site or a new one. Two to four sites were used in each left ventricle. Temperature, as measured by a thermometer in the chest cavity, was kept at 37.0–37.5°C. Measurements were made also of arterial pressure. Following insertion of the electrode, as described above, and a stable Pmco₂ recording, the coronary artery was occluded abruptly with a snare, for periods of 30 seconds to 2 minutes, while recording Pmco₂ at a chart speed of 100 mm/min. To maintain a sensitivity to small PCO₂ changes and yet record large changes, Pmco₂ was recorded at three different sensitivities by placing the output from the electrode-millivolt meter into a parallel circuit entering into three different channels of the oscillograph, with resultant Pmco₂ ranges of 55–80, 35–200, and 35–720 mm Hg.

The occlusion was repeated with a 15- to 30-minute recovery period between occlusions, and the electrode was withdrawn for recalibration and inserted thereafter into the same or a new site. Finally, the artery was ligated and observations were made for a 17-minute period. The first six dogs were heparinized (5000 U/hour) to maintain catheter patency, and the subsequent nine were not heparinized so as to remove a factor that might affect the rate of rise of Pmco₂.

The effect of coronary artery occlusion on temperature of the myocardium was examined in two dogs by insertion of a thermocouple with a diameter of 0.25 mm into the myocardium near the PCO₂ electrode. Continuous recordings were made of Pmco₂ and myocardial temperature during coronary occlusion, and the Pmco₂ was corrected for this temperature change, using an experimentally determined relationship between temperature of an equilibrated fluid and electrode response.

**Mathematical Correction of PCO₂ Electrode Response**

Since the apparent rise of the Pmco₂ after occlusion was diminished effectively by the time constant of the electrode, a calculated correction was applied to the recorded Pmco₂ values in data from one experiment in order to estimate the actual PCO₂ value at the surface of the electrode tip. This estimate was made as follows:

The electrode transfer function was determined by applying a step change in PCO₂, measuring the resultant electrode response, and then dividing the Laplace transform of that response by the Laplace transform of the step input (Brown and Nilsson, 1962), according to the relationship \( G(s) = C(s)/R(s) \). Here, \( G(s) \) is the electrode transfer function in the Laplace plane, and \( C(s) \) and \( R(s) \), respectively, are the Laplace transforms of the electrode output and input. It was found that the electrode response could be represented accurately by the equation \( PCO₂ = 56.6 - 21e^{-23t} \), where \( t \) is the time following the abrupt PCO₂ change.

A typical myocardial electrode response curve resulting from coronary occlusion then was fitted to a third-degree polynomial, and the Laplace transform of that polynomial was divided by the electrode transfer function, \( G(s) \), to determine the Laplace transform of the actual electrode input. The inverse transform was taken to determine the cor-
The early effect of coronary artery occlusion on myocardial $P_mCO_2$ which begins to increase 4 seconds after occlusion. Note the steady base line value of $P_mCO_2$ in this high sensitivity recording.

The expression for the electrode output after occlusion was $P_{CO_2} = 60.8 + 10.54t^2 - 2.49t^3$, and the derived expression for the electrode input for this output response was $P_{CO_2} = 60.8 + 6.567t + 8.22t^2 - 2.49t^3$, ($R^2 = 0.995$).

All summary data in this paper are expressed as the mean value ± SEM.

Results

Early Changes in $P_mCO_2$

It was found possible to record $P_mCO_2$ at a high sensitivity and with a stable value, so that very early changes in $P_mCO_2$ could be detected (Fig. 1). Myocardial $P_{CO_2}$ rose promptly in all instances following an abrupt coronary artery occlusion. In 53 occlusions in 14 dogs, $P_mCO_2$ began to increase at 9.6 ± 0.7 seconds after the occlusion. The mean delay before $P_mCO_2$ began to rise following 18 coronary occlusions in the six heparinized dogs was 13.6 ± 1.1 seconds. A more rapid rise was observed in the unheparinized dogs; in 35 occlusions in these eight dogs, the mean onset of $P_mCO_2$ rise was 7.5 ± 0.7 seconds. The difference between these two groups was significant ($P < 0.001$). Variability existed from dog to dog and site to site in the time of rise, with the range of 0.6–24 seconds (Fig. 2), which suggested that the variation in delay was related to conditions pertinent to the myocardial-electrode interface at any particular site. In general, however, there was a consistent response in the rate of rise and magnitude of response with repeated occlusions at the same site or at different sites. Since a delay of less than 4 seconds was found on several occlusions in different hearts, it is evident that a rise in $P_mCO_2$ can begin this early, and suggests that longer delays are related to variability in CO2 transmission to the electrode.

A transient dip in $P_mCO_2$, lasting 6–38 seconds (mean = 16.7 sec), occurred after occlusions in one-third of the dogs but always in association with an arrhythmia at the time of occlusion. These points were not used in the analysis of the time and rate of $P_mCO_2$ rise. The magnitude of this $P_mCO_2$ dip was 0.4–2.2 mm Hg (mean = 1 mm Hg), and its precise cause was unknown, with the exception that it was found when multiple premature beats occurred at the time of occlusion. All the records from which the $P_mCO_2$ data for this paper were taken did not contain this dip and were similar to that observed in Figure 1. Once $P_mCO_2$ began to rise, it increased steadily thereafter, as long as the occlusion was maintained. After releasing a brief occlusion, $P_mCO_2$ continued to rise in most instances, with an overshoot varying from 0 to 14 mm Hg, an average of 3.6 mm Hg. The rate of fall of $P_mCO_2$ was somewhat slower than the rise; 2.6 minutes were necessary to return to control value after a 2-minute occlusion. Thereafter, $P_mCO_2$ fell below con-

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

**Figure 2** Distribution of time of rise of $P_mCO_2$ for all coronary occlusions. The time of rise in unheparinized dogs (stippled bars) is less than that in the heparinized dogs. The time of rise was less than 2 seconds in several instances.
Effect of Prolonged Occlusion on PmCO₂

When observed over a more prolonged period of occlusion, PmCO₂ was found to increase steadily and to reach extreme values, as in Figure 3, where PmCO₂ is seen to reach 300 mm Hg at 10 minutes and 400 mm Hg after 17 minutes of occlusion. It also was of interest that the appearance of ventricular fibrillation had little effect on the rate of PmCO₂ rise. In the experiment in Figure 4, ventricular fibrillation developed 3.5 minutes after coronary occlusion, by which time PmCO₂ had already more than doubled. Without subsequent intervention, PmCO₂ continued to rise at the same rate, and perhaps even faster, exceeding 400 mm Hg after 15 minutes of occlusion.

Magnitude of PmCO₂ Rise after Occlusion

When the mean PmCO₂ increase after occlusion was averaged at specific time intervals for all occlusions in unheparinized dogs (Table 1), it is seen that

| Table 1 Mean Increase in PmCO₂ after Coronary Artery Occlusion |
|------------------|-----------------|----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Time (min) after occlusion | 0.5 | 1.0 | 2.0 | 5.0 | 10.0 | 15 |
| No. of occlusions | 37 | 37 | 7 | 6 | 5 | 5 |
| No. of dogs | 9 | 9 | 6 | 6 | 5 | 5 |
| Mean PmCO₂ rise (mm Hg) | 2.28 | 7.15 | 24.13 | 88.3 | 171.6 | 222.2 |
| SEM | ±0.18 | ±0.57 | ±5.17 | ±16.5 | 36.8 | ±47.7 |
there was initially a small rise (2.3 mm Hg) at 0.5 minutes, which reached 24 mm Hg at 2 minutes. The rate of rise seen in this table is similar to that observed in Figure 1 for a single occlusion. At 15 minutes, there was a mean increase of 222 mm Hg.

When the data from Table 1 are plotted, it may be seen that the rate of rise of Pmco2 is essentially linear from 1 minute to 10 minutes after coronary occlusion, and that the calculated rate of rise over this period of time is 18.3 mm Hg/min. During the 1st minute, the rate of rise is slower, particularly during the first 30 seconds. As shown in Table 1, the rate of rise from 0.5 to 1 minute is only 9.7 mm Hg/min; from 7.5 to 30 seconds (assuming that Pmco2 rise begins at an average of 7.5 seconds), the rate of rise is only 6.1 mm Hg/min.

**Correction of Pmco2 for Temperature Change**

The effect of coronary occlusion on myocardial temperature change was observed in two dogs. The decrease in temperature began at the time of occlusion, reaching a fall of approximately 1.5°C at 5-10 minutes (Fig. 5), but thereafter there was little change. When the simultaneously measured Pmco2 reading was corrected for this temperature fall, using a previously determined temperature-Pmco2 relation for this electrode, it was apparent that the change in myocardial temperature had no important effect on the Pco2 as recorded by the microelectrode (Fig. 5).

**Correction of Pco2 Reading for The Time Constant of Electrode**

To obtain a better estimate of Pco2 at the tissue-electrode interface, the Pmco2 recording for one occlusion was corrected through a calculated estimate of the actual Pco2 input necessary to achieve the given Pmco2 reading (see Methods). Data for the actual Pmco2 observed following the coronary occlusion and the calculated correction are shown in Figure 6. It may be seen that the estimated Pco2 at the electrode interface is approximately 5-10% higher than that actually recorded. It is apparent that the rate of rise in Pmco2 after coronary occlusion is slow enough so that the applied correction does not make an important adjustment and that, in this setting, the use of the electrode alone will give a reasonably correct estimate of the actual Pmco2 at the surface of the membrane on the electrode.

**Discussion**

Direct measurement of Pco2 in the myocardium has added a new dimension to the understanding of the sequelae of myocardial ischemia. The progressive increase in Pmco2 after coronary occlusion, reaching extreme values, provides continuous evidence of the severe degree of myocardial acidosis which is developing at this time. Since the original description of the direct measurement of Pmco2 (Brantigan et al., 1972a), subsequent studies have confirmed the increase of Pmco2 during myocardial ischemia or anoxia (Brantigan et al., 1972b; Furuse et al., 1973; Khuri et al., 1975a, 1975b; MacGregor et al., 1974; O'Riordan et al., 1977a, 1977b). Even with lesser degrees of ischemia, it was observed (O'Riordan et al., 1977b) that Pmco2 provides a much more sensitive index of ischemia than myocardial PO2 measurements, since Pmco2 changes more extensively; the magnitude of Pmco2 rise also...
was related to the degree of S-T segment change (O’Riordan et al., 1977a, Khuri et al., 1975c).

All of the studies of Pmco2 that have been published to date (with the exception of our single miniature electrode study) have been performed with the mass spectrometer method. With the mass spectrometer technique, a Teflon-covered cannula is inserted into the myocardium, and by means of a vacuum, CO2, continuously but slowly, is withdrawn from the myocardial tissue and passed through the mass spectrometer where the concentration of CO2 molecules is detected. A major problem with this technique is its inordinately slow rate of response to tissue Pco2 change. After an abrupt change in Pco2, there is a 1-minute delay before any change can be sensed; thereafter, a 63% response occurs in 3 minutes, and a full response requires 10 minutes (Brantigan et al., 1972a). The earliest information we have available regarding the earliest time reported is after 5 minutes of occlusion. Since we have found that Pmco2 actually begins to rise at 7 seconds after occlusion, it is clear that the delayed rise found in previous studies was a result of the slowly responding methodology. It might be expected that the difference in results with these two techniques would become less apparent with longer periods of occlusion, but this was not the case over a 15-minute period of observation. Khuri et al. (1975c) found a mean increase in Pmco2 at 5 and 15 minutes of 28 and 90 mm Hg, whereas our data for these periods shown are 88 and 171 mm Hg increase. They also observed a peak increase in Pmco2 at 30 minutes after occlusion, with a progressive decline thereafter. Our data do not extend to this period of time, but we have never observed a fall in Pmco2 with a continued occlusion. It is possible that their decrease may be a result of the continuous sampling of CO2 from that point in the myocardium.

We have observed (Case et al., 1979) that Pco2 value in the myocardium and in coronary venous blood corresponds closely, and that this relationship is maintained in the normally oxygenated heart as these values are varied by respiratory means, thus showing that there is little gradient in Pco2 between extracellular and vascular compartment of the myocardium. There is an extensive body of literature dating back to Fenn (1928), as well as to Wallace and Hastings (1942), who suggested that it is very unlikely that any gradient in CO2 tension exists between serum and tissue due to the high rate of CO2 diffusion, but there has been no prior direct confirmation of this concept.

The implication of the rising Pmco2 seen during ischemia is that it reflects a progressive decrease in myocardial pH at this time. It has been postulated (Khuri et al., 1975c) that the rise in Pmco2 results from local accumulation of lactic acid with displacement of the reaction,

\[ \text{H}^+ + \text{HCO}_3^- \rightleftharpoons \text{H}_2\text{CO}_3 \rightleftharpoons \text{CO}_2 + \text{H}_2\text{O}, \]

to the right. Another important source of CO2 accumulation could be its continued generation in the absence of a means for its efflux. Opie (1976) has suggested five possible sources for an increase in tissue H+ during ischemia. It is evident, however, that an accumulation of CO2 within myocardial tissue, whether a primary or secondary event, represents a myocardial acidosis. It is known through a variety of techniques that a significant decrease occurs both in extracellular and intracellular pH during ischemia. Technical aspects of myocardial pH measurement are difficult, with data primarily being obtained indirectly through use of frozen tissues or homogenates or with biochemical methods, such as the dimethyl-2,4-oxizolidine dione distribution technique (Steinbergen et al., 1977). Results of these measurements of pH during ischemia have been summarized (Opie, 1976), and all show a progressive decrease during myocardial ischemia.

The physiological importance of a developing acidosis during ischemia lies in the fact that myocardial contractility can be severely impaired by acidosis alone (Steinbergen et al., 1977; Wildenthal et al., 1968; Lorkovic et al., 1966). Although initially this was thought to result from a reduction in extracellular pH, the greater importance of intracellular pH recently has been recognized (Steinbergen et al., 1977). Pressure development in the rat ventricle is reduced by 80% using a perfusion fluid with a pH reduced to 6.7 by the use of CO2 in the presence of adequate oxygenation (Steinbergen et al., 1977).

Myocardial contractility begins to decrease within 3 (Pirzada et al., 1975) to 10 (Theroux et al., 1974) seconds following a coronary artery occlusion. No satisfactory explanation for this sequence of events has been provided, since the decrease in contractility precedes any metabolic disturbance yet described (Pirzada et al., 1975). ATP does not begin to decrease until 20 seconds after a sudden anoxic intervention, and ATP stores remain at 80% of control value after 1 minute (Williamson, 1966). Whereas it has been stated (Opie, 1976) that a direct link between ATP levels and contractility need not be found because of the possible compartmentalization of ATP, it seems reasonable to examine other possibilities. It has been postulated (Katz and Hecht, 1969) that the cause of the early loss of myocardial contractility was the development of intracellular acidosis, which would act by displacement of calcium ions from contractile sites. Evidence for development of an early acidosis during ischemia is limited (Benzing et al., 1971/72; Lai and Scheuer, 1975). In the present study, we have observed a rise in Pmco2 rapid enough to explain...
this early loss in contractility on the basis of acidosis, but further information is needed to establish a causal relationship.

The rise in Pmco2 observed here represents one of the earliest metabolic disturbances so far reported during myocardial ischemia or anoxia. Following coronary artery occlusion, myocardial lactate becomes elevated within 15 seconds, and creatine phosphate is decreased by this time; ATP begins to fall within 30 seconds (Dunn and Griggs, 1975). A rise in extracellular potassium has been reported to occur within 30 seconds (Hill and Gettes, 1977), and the earliest pH decrease was found at 15 seconds (Benzing et al., 1971/72). Myocardial adenosine is elevated at 5 seconds (Olsson, 1970), and a rise in coronary venous lactate was found with occlusions longer than 6 seconds (Olsson and Gregg, 1965). Following abrupt anoxia, creatine phosphate and ATP begin to decrease at 20 seconds, and myocardial lactate begins to rise at the same time (Williamson, 1966). The rise in Pmco2 at 7 seconds (and earlier) well precedes most of these changes, although it would be expected that tissue lactate is also elevated at this time if the Pmco2 rise is to be attributed to this substance.

The data presented here also provide supporting information that points to the overwhelming myocardial acidosis that occurs with ischemia and indicate that this is progressive, even in the presence of ventricular fibrillation. This suggests that additional efforts to control or reverse this acidosis during ischemia would be valuable.

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