Kinetics of Myoglobin Release and Prediction of Myocardial Myoglobin Depletion After Coronary Artery Reperfusion

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To better define the usefulness of blood myoglobin measurements in evaluating the effectiveness of attempted thrombolysis, we studied the kinetics of myoglobin entry into and removal from the circulation after coronary artery reperfusion and the relation between directly measured depletion of myocardial myoglobin after coronary occlusion and reperfusion and the amount of depletion predicted from plasma myoglobin concentration-time curves. Initially, canine myoglobin was administered to 11 dogs by both bolus injection and 40-minute infusion, and the subsequent disappearance patterns of myoglobin from plasma monitored by radioimmunoassay. A monoexponential regression line (corresponding to a one-compartment model) and a biexponential regression line (corresponding to a two-compartment model) were determined for each set of washout data, the kinetic parameters were calculated, and the goodness of fit of each model was assessed. Results were similar after both methods of myoglobin administration. In five of 11 animals, the one-compartment model described the myoglobin kinetics better; in the other six animals, the one-compartment model was statistically superior, but values for the volume of distribution and elimination rate constant differed by only 10% from the one-compartment estimates. After bolus administration of myoglobin and with a one-compartment model, the volume of distribution of myoglobin was determined to be 1,601±77 (SEM) ml, representing 6.8±0.2% of total body weight; the elimination rate constant averaged 0.132±0.006/min and corresponded to a mean half-time of disappearance of 5.5±0.2 minutes. In 16 additional animals undergoing a 2-hour occlusion and 4-hour reperfusion period of the mid left anterior descending coronary artery, myoglobin was rapidly released from the injured myocardium, with peak rate of myoglobin release occurring within 50 minutes of onset of reperfusion. The pattern of myoglobin release into the circulation was discontinuous ("staccato phenomenon") and persisted throughout the entire reperfusion period with 16% of total myoglobin release occurring after 2 hours. In 12 animals, the amount of myoglobin predicted by the one-compartment model to have entered the circulation was compared with directly measured myocardial myoglobin depletion that varied from 5 to 117 mg. The correlation coefficient for the relation (y=1.2 x –14.5) was 0.97 (p<0.01). (Circulation 1989;80:676–683)

The intracardiac protein myoglobin is rapidly released into blood after reperfusion of injured myocardium and appears useful for the noninvasive detection of reperfusion.1 Although blood myoglobin concentration peaks soon after coronary reperfusion, the kinetics of myoglobin entry into and clearance from blood are not well defined. In addition, myoglobin release from injured myocardium appears to be an ongoing process that may persist for several hours after the onset of reperfusion.1

The present study was undertaken to determine the kinetics of myoglobin appearance in and disappearance from blood and to evaluate the possibility that the amount of tissue myoglobin depletion resulting from myocardial injury can be estimated from the plasma myoglobin concentration-time curve. In an initial series of experiments, myoglobin kinetics were defined after systemic administration of myoglobin into anesthetized dogs. Analyses of plasma disappearance patterns were performed with one- and two-compartment models. Subsequently, patterns of
myoglobin entry into the circulation and quantification of myoglobin release after coronary artery occlusion and reperfusion were determined in other animals by plasma myoglobin concentration-time curves and appropriately determined kinetic parameters.

Methods

General Preparation

Mongrel dogs weighing 15–26 kg were anesthetized with sodium pentobarbital and ventilated with a Harvard positive pressure respirator (South Natick, Massachusetts). Under sterile conditions, a left thoracotomy was performed, and one or two pneumatic occluders were placed on the left anterior descending coronary artery (LAD) just distal to the first or second diagonal branch. Electrodes were sewn onto the epicardium in the distributions of the LAD and left circumflex coronary artery (LCx). A catheter was placed in the left atrium for myoglobin and drug administration; a second catheter was placed in the descending aorta for measurement of arterial pressure and collection of blood myoglobin samples. All animals were allowed to recover for at least 7 days so that elevated myoglobin levels related to skeletal muscle damage during surgery could return to baseline.

Characterization of Myoglobin Kinetics With One- and Two-Compartment Models

On the day of the acute study, each animal was anesthetized with thiamylal sodium and halothane. Aortic pressure, lead II of the standard electrocardiogram, and the LAD and LCX surface electrograms were measured continuously. After administration of 10,000 units i.v. heparin, 4.0 mg dog skeletal muscle myoglobin (Sigma Chemical, St. Louis, Missouri) dissolved in 5 ml normal saline was injected into the left atrium in 11 animals (bolus myoglobin injection), and arterial samples were drawn every 2 minutes for a 30-minute washout sampling period.

Because of the possible existence of a second compartment with relatively long kinetic rate constants (which might be overlooked after bolus myoglobin injection), dog skeletal muscle myoglobin was also infused for 40 minutes (myoglobin infusion) at a constant rate (26.3 mg myoglobin in 50.0 ml normal saline at 0.687 ml/min) into the left atrium of the same 11 animals. Arterial samples for myoglobin determination were collected every 2 minutes during the final 10 minutes of the infusion and for 30 minutes after termination of the infusion.

Standard radioimmunoassay methods2–5 were used to determine plasma myoglobin concentration in each sample, and the washout curves were plotted with a Quasi-Newton optimization routine in the SYSTAT analytical package (SYSTAT, Evanston, Illinois). Both one- and two-compartment models were used (Figure 1), and the kinetic parameters were determined. An F test at the 0.05 significance level was performed comparing the two curves to determine whether or not the two-compartment model (i.e., biexponential least squares fit) described the washout points in a statistically superior manner.6

Myoglobin Kinetics After Coronary Reperfusion

In 16 other animals, myoglobin kinetics were studied after coronary reperfusion. After similar measurements of plasma myoglobin washout after bolus myoglobin injection, intravenous lidocaine (30 mg) was given to each animal, and the LAD occluder(s) was inflated for a 2-hour period. Successful occlusion was verified in each animal by a shift in the ST segment, loss of R wave voltage, or widening of the QRS complex on the LAD surface electrogram; within 15 minutes after reperfusion, the LAD surface electrogram reverted to baseline. In four animals, a pulsed Doppler flow probe (Triton Technology, San Diego, California) was placed on the LAD just distal to the occluders to verify the absence of flow during the occlusion period and the return of unrestricted flow after occlusion release. Baseline arterial samples for determination of plasma myoglobin concentration were obtained immediately before occlusion onset and just before occlusion release. During the 4-hour reperfusion period after occluder release, arterial myoglobin samples were drawn at 4-minute intervals for 60 minutes, at 10-minute intervals for 40 minutes, and at 20-minute intervals until completion of the study.

Myoglobin concentration-time curves for each animal were analyzed with one-compartment values for the volume of distribution (Vp) and elimination rate constant (k0) calculated for each animal from the preocclusion myoglobin washout study. Quantitative myoglobin entry into the circulation, Mb0(t), was assessed numerically by calculating a myoglobin release function (Equation 1, Appendix).
In 12 of the 16 animals, the heart was excised immediately after completion of the study. The right and left atria and right ventricle were discarded. A core area from the normal LCx area was removed and saved as was the entire injured LAD area (with wide margins) so that total myoglobin depletion could be determined. The cardiac tissue was weighed and then frozen in liquid nitrogen until it was analyzed for myoglobin content.

Plasma samples from each animal were analyzed as discussed above. The excised cardiac tissue was thawed and added to phosphate-buffered saline (pH 7.0), 1.0 ml/0.1 g tissue, and homogenized for 30 seconds in a Waring blender. The resulting suspension was centrifuged at 17,000 rpm for 30 minutes, and the clear supernatant was transferred and analyzed for myoglobin content with a standard radioimmunoassay technique.2-5

Comparison of Cardiac and Skeletal Muscle Myoglobin Kinetics

Because of the possibility of differing kinetics for cardiac and skeletal muscle myoglobin, both were injected into five other animals, and the respective kinetic parameters were determined with a one-compartment model. In three animals, 4 mg cardiac myoglobin was injected initially followed by a 30-minute washout sampling period. After an interval of 30 minutes, 4 mg skeletal muscle myoglobin was injected, and the washout was repeated. In the other two animals, the order of myoglobin injections was reversed. The volumes of distribution, VD, for cardiac and skeletal muscle myoglobin were the same (1,329±79 [SEM] vs. 1,325±71 ml, respectively; p>0.50, paired t test) as were the half-times of elimination (6.0±0.6 vs. 6.1±0.6 minutes, respectively; p>0.50, paired t test).

Results

Characterization of Myoglobin Kinetics with One- and Two-Compartment Models

For each of the 11 animals receiving bolus myoglobin injection and infusion, individual data points were fitted using one- and two-compartment models. The left panels of Figures 2 and 3 illustrate

![Figure 2](image-url)  
**Figure 2.** Plots of myoglobin washout kinetics for dog 3 after bolus myoglobin administration (left panel) and Mb infusion (right panel). In this particular animal, the elimination kinetics are better described by a two-compartment model (solid line).

![Figure 3](image-url)  
**Figure 3.** Plots of myoglobin washout kinetics for dog 9 after bolus myoglobin administration (left panel) and Mb infusion (right panel). In this particular animal, the elimination kinetics are described by a one-compartment model.
results from two different animals (dogs 3 and 9, respectively) after bolus administration of myoglobin. Least squares lines were fitted with a monoexponential function (corresponding to a one-compartment model) and a biexponential function (corresponding to a two-compartment model). For dog 3 in Figure 2, the biexponential model is a statistically better fit, whereas the one-compartment model better describes the data for dog 9 in Figure 3. Kinetic parameters after bolus myoglobin injection for all 11 animals are listed in Table 1. In six of 11 animals, the biexponential model is statistically better, whereas in the five other animals, there is no statistical difference between the one- and two-compartment models. In examining the six animals for which a biexponential fit is superior, the volume of distribution estimated from the one-compartment model (V_D) underestimates the total volume of the two-compartment model (V_1 + V_2) by 10±2.7% (SEM); similarly, the elimination rate constant for the one-compartment model (k_d) underestimates the elimination rate constant for the two-compartment model (k_1→2) by 9±2.4%.

The right panels of Figures 2 and 3 illustrate washout curves after a 40-minute myoglobin infusion in the same two animals. Again, least squares

### Table 1. Kinetic Parameters for One- and Two-Compartment Models After Bolus Myoglobin Injection

<table>
<thead>
<tr>
<th>Dog</th>
<th>V_D (ml)</th>
<th>k_d (min⁻¹)</th>
<th>V_1 (ml)</th>
<th>V_2 (ml)</th>
<th>k_1→2 (min⁻¹)</th>
<th>k_2→1 (min⁻¹)</th>
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<td>1,420</td>
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<td>1,125</td>
<td>382</td>
<td>0.184</td>
<td>0.093</td>
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<td></td>
<td></td>
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<tr>
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<td>5</td>
<td>1,325</td>
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<td>6</td>
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<td>1,579</td>
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<td>1,192</td>
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<td>1,296</td>
<td>393</td>
<td>0.192</td>
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<td>9</td>
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<tr>
<td>10</td>
<td>1,928</td>
<td>0.141</td>
<td>1,589</td>
<td>695</td>
<td>0.153</td>
<td>0.070</td>
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<td>11</td>
<td>2,148</td>
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</table>

Mean 1,601 0.132 1,344 459 0.152
SEM 77 0.006 80 68 0.013

V_D, volume of distribution; k_d, elimination rate constant; V_1 and V_2, volumes of distribution of compartments 1 and 2; k_1→2, k_2→1, kinetic rate constants.

*No statistical difference between one- and two-compartment fits.

### Table 2. Kinetic Parameters for One- and Two-Compartment Models After a 40-Minute Myoglobin Infusion

<table>
<thead>
<tr>
<th>Dog</th>
<th>V_D (ml)</th>
<th>k_d (min⁻¹)</th>
<th>V_1 (ml)</th>
<th>V_2 (ml)</th>
<th>k_1→2 (min⁻¹)</th>
<th>k_2→1 (min⁻¹)</th>
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<tr>
<td>1</td>
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<td>*One-compartment</td>
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<tr>
<td>2</td>
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<td>1,049</td>
<td>280</td>
<td>0.120</td>
<td>0.078</td>
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<tr>
<td>3</td>
<td>1,410</td>
<td>0.095</td>
<td>1,304</td>
<td>331</td>
<td>0.096</td>
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<td>4</td>
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<td>1,510</td>
<td>693</td>
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<td>0.043</td>
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<td>5</td>
<td>987</td>
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<td>934</td>
<td>445</td>
<td>0.153</td>
<td>0.165</td>
</tr>
<tr>
<td>6</td>
<td>2,389</td>
<td>0.082</td>
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<td>*One-compartment</td>
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<tr>
<td>7</td>
<td>1,007</td>
<td>0.109</td>
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<tr>
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<td>1,534</td>
<td>0.108</td>
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<tr>
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<td>1,722</td>
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<td>*One-compartment</td>
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<tr>
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<td>2,126</td>
<td>0.107</td>
<td></td>
<td></td>
<td></td>
<td>*One-compartment</td>
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<tr>
<td>11</td>
<td>1,178</td>
<td>0.115</td>
<td>1,054</td>
<td>364</td>
<td>0.117</td>
<td>0.108</td>
</tr>
</tbody>
</table>

Mean 1,456 0.111 1,111 388 0.113
SEM 137 0.007 100 69 0.071

V_D, volume of distribution; k_d, elimination rate constant; V_1 and V_2, volumes of distribution of compartments 1 and 2; k_1→2, k_2→1, kinetic rate constants.

*No statistical difference between one- and two-compartment fits.
lines were fitted with both monoexponential and biexponential functions. As summarized in Table 2, the two-compartment model provides a statistically better fit in five of 11 animals studied.

Myoglobin Kinetics After Coronary Reperfusion

The upper panel of Figure 4 illustrates a representative myoglobin concentration-time curve after coronary reperfusion of a 2-hour mid-LAD occlusion. As shown in this figure and summarized in Table 3, the time for myoglobin concentration to fall from its peak value to one-half peak value after reperfusion is longer than the disappearance halftime after myoglobin bolus injection (37.0±4.0 vs. 5.4±0.2 minutes; *p*<0.01), suggesting ongoing myoglobin entry into the circulation.

Also illustrated in Figure 4 (lower panel) is the calculated myoglobin release function determined with a one-compartment model in the same animal. Although it is apparent that myoglobin is rapidly released into the circulation after coronary reperfusion, a significant fraction of total myoglobin release occurs late in the reperfusion period. Figure 5 summarizes cumulative myoglobin release for all 16 animals. Peak rates of release occurred at 31±3.4 minutes (range, 10–50 minutes), but there was ongoing release throughout the 4-hour reperfusion period.

In the 12 dogs in which the left ventricle was excised and analyzed for myoglobin content, total myoglobin lost from the injured area varied from 5 to 117 mg (Figure 6). Myoglobin loss estimated from the myoglobin concentration-time curve with a one-compartment model was linearly related to myoglobin depletion directly measured from the excised heart (*y*=1.2 *x*−14.5; *r*=0.97; *p*<0.01).

**Discussion**

This study shows three potential advantages of myoglobin concentration-time curves in assessing coronary reperfusion: 1) myoglobin kinetics can be usefully described with a one-compartment model, 2) myoglobin is rapidly released from injured myocardium after coronary reperfusion, with peak release rate occurring within 50 minutes after vessel

![Figure 4](http://circ.ahajournals.org/)

**Figure 4.** Myoglobin concentration-time curves after coronary reperfusion of dog 6 (upper panel). In this particular animal, peak [Mb] occurred 32 minutes after occlusion release. The myoglobin release function (lower panel) shows that the rate of myoglobin entry is high during the 1st hour of the reperfusion period. However, a "staccato phenomenon" is present, and measurable myoglobin entry into the circulation continues throughout the 4-hour reperfusion period.

![Figure 5](http://circ.ahajournals.org/)

**Figure 5.** Plot of cumulative myoglobin release throughout the reperfusion period. Although peak myoglobin release occurs early in the reperfusion period, 16% of total release occurs after 2 hours.

**Table 3.** Comparison Between Half-time of Elimination After Bolus Myoglobin Administration and After Coronary Artery Reperfusion

<table>
<thead>
<tr>
<th>Dog</th>
<th>t₁/₂ after bolus myoglobin injection (min)</th>
<th>t₁/₂ after coronary reperfusion (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>6.2</td>
<td>48.9</td>
</tr>
<tr>
<td>13</td>
<td>3.7</td>
<td>21.3</td>
</tr>
<tr>
<td>14</td>
<td>5.0</td>
<td>21.0</td>
</tr>
<tr>
<td>15</td>
<td>5.0</td>
<td>34.0</td>
</tr>
<tr>
<td>16</td>
<td>6.5</td>
<td>21.0</td>
</tr>
<tr>
<td>17</td>
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<td>5.9</td>
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<td>21</td>
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<tr>
<td>22</td>
<td>6.0</td>
<td>18.4</td>
</tr>
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<td>23</td>
<td>4.4</td>
<td>46.7</td>
</tr>
<tr>
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<td>25</td>
<td>6.0</td>
<td>26.0</td>
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<tr>
<td>26</td>
<td>6.0</td>
<td>37.9</td>
</tr>
<tr>
<td>27</td>
<td>5.8</td>
<td>34.3</td>
</tr>
<tr>
<td>Mean ± SEM</td>
<td>5.4±0.2</td>
<td>37.0±4.0</td>
</tr>
</tbody>
</table>

*tl/₂, half-time.*
reopening, and 3) with a one-compartment model, plasma myocardium levels reflect actual cardiac tissue depletion of myoglobin.

Our myoglobin kinetics data can be compared with values obtained in human subjects into whom 125I-labeled myoglobin was injected.7 In healthy individuals and in patients with acute myocardial infarction, 125I-radioactivity was measured for 48 hours after bolus injection, and a washout curve was constructed. In all cases, the semilogarithmic washout curves were clearly biexponential with elimination rate constants as long as 0.67±0.22/hr in healthy patients and 0.80±0.37/hr in patients with acute myocardial infarction. Because myoglobin concentration was determined by measuring 125I radioactivity rather than immunoreactive myoglobin levels, the curvilinearity of these particular semilogarithmic washout curves most likely reflects recirculating radioactive fragments of the parent 125I-labeled myoglobin molecule (caused by normal renal catabolism of the myoglobin molecule), rather than true two-compartment kinetics of the parent molecule (see Figures 1 and 3 in reference 5).

In evaluating the possible existence of a second compartment with relatively long rate constants, the infusion rate (26.3 mg in 50 ml infused at a rate of 0.687 ml/min) was chosen so that steady-state plasma myoglobin levels would be in the same approximate range as peak values after bolus myoglobin injection. As summarized in Table 2, the kinetics after myoglobin infusion are adequately described by a one-compartment model in five of 11 animals. If, in the six animals in which the two-compartment model is statistically superior, the one-compartment values for \( V_D \) and \( k_d \) are used instead of the corresponding two-compartment parameters, the error introduced in calculating the myoglobin release function varies between 4.2% and 11.8%. Thus, a one-compartment model provides a good estimate of true myoglobin kinetics and was used in our calculations in the subsequent studies of reperfusion.

To understand where myoglobin is likely to be located once it leaves the injured myocardial cells, the magnitude of the volume of distribution of myoglobin obtained in the present study (6.8±0.19% of total body weight) needs to be compared with actual body fluid compartments. Although we did not measure plasma volumes, estimates of plasma volume in dogs have ranged from 4.3% to 5.8% of total body weight (Table 1-1 in reference 8), with simultaneous estimates of intravascular volume varying from 7.8% to 10.3% of total body weight. Because myoglobin does not enter red blood cells, myoglobin appears to have an effective volume of distribution that exceeds plasma volume by approximately 25%. At the present time, we cannot determine whether or not this effective volume of distribution of myoglobin includes some transfer of the protein into the interstitial space of some or all organs.

Our myoglobin findings contrast with canine studies of MM creatine kinase kinetics, in which disappearance curves after bolus creatine kinase injection conform better to a two- than a one-compartment model.9 In these animals, the MM creatine kinase disappearance rate constant for a one-compartment model consistently underestimated the true rate constant by 44%, contrasting with the present 9% difference between one- and two-compartment estimates for myoglobin. A second important difference between MM creatine kinase and myoglobin kinetics is the rate of protein clearance from the circulation. For MM creatine kinase, disappearance rate constants averaged 0.009/min, corresponding to a half-time of disappearance of 77 minutes; this is much longer than the mean disappearance half-time of 5.4 minutes for myoglobin. Thus, myoglobin is cleared approximately 15 times faster from the circulation than MM creatine kinase, suggesting that plasma myoglobin concentration-time curves reflect patterns of protein entry into the circulation more directly than does MM creatine kinase.

After coronary reperfusion, myoglobin rapidly enters and is rapidly cleared from the circulation.1 Myoglobin entry is apparent within a few minutes of occlusion release, and in this study, peak concentrated myoglobin occurred at 34±3.8 minutes into the reperfusion period. With Equation 1 (Appendix) and a one-compartment model, a myoglobin release function was numerically calculated in which MB(t) is the rate of myoglobin entry over the period of time dt, \( k_d \) is the disappearance rate constant determined from bolus myoglobin injection with a one-compartment model, \( Q_{Mbol} \) is the quantity of myoglobin present in the compartment at time t (and is equal to the product of the volume of distribution and myoglobin concentration), and \( dQ_{Mbol}/dt \) is the incremental change in the quantity of myoglobin in the compartment over time period dt. For the animal shown in Figure 4, the peak rate of myoglobin release occurred approximately 30 minutes into

![Figure 6](https://example.com/figure6.png)

**Figure 6.** Regression plot of appearance of myoglobin in plasma as a function of cardiac depletion of myoglobin. See text for details.
the reperfusion period; for all 16 animals, peak myoglobin release rates occurred between 10 and 50 minutes.

A second feature of myoglobin release in the lower panel of Figure 4 relates to the so-called “staccato phenomenon.” During at least the first 60 minutes after coronary reperfusion, myoglobin appears to enter the circulation as several discrete bursts rather than in a continuous fashion. A similar pattern of myoglobin entry was noted in all 16 animals subjected to the occlusion-reperfusion protocol. Whether this pattern reflects varying flow after occluder deflation, reopening of different portions of the microcirculatory vasculature at different times after reperfusion, continuing episodes of cell injury and myoglobin release, or some other phenomenon is presently unclear.

A third feature of myoglobin kinetics after coronary reperfusion is ongoing release that persisted for the full 4 hours in these studies. Although the myoglobin release rate peaked early in the reperfusion period, myoglobin entry continued throughout the entire reperfusion period: 16±3.2% of total myoglobin release occurred after 2 hours and 7±1.7% after 3 hours. Groth et al computed a similar myoglobin entry function in 33 patients suffering from acute myocardial infarction in whom reperfusion was not attempted. Myoglobin was first detected in plasma 2.2 hours after the onset of symptoms; peak myoglobin release rate occurred at 4.6 hours, and myoglobin release continued for at least 36 hours. These observations further confirm that myoglobin release occurs substantially earlier when reperfusion is achieved than in acute infarction. However, with or without reperfusion, myoglobin appears to continue to enter the circulation for some time.

Ongoing myoglobin release is further corroborated by the data in Figure 4 and Table 3. The half-time of disappearance of myoglobin after the early reperfusion peak noticeably exceeds that after systemic myoglobin administration, further suggesting that myoglobin enters the circulation over a prolonged period of time rather than as a discrete bolus. In addition, prior work from our laboratory showed persistent coronary venous-arterial concentration gradients of myoglobin lasting throughout an 11-hour reperfusion period in animals subjected to a similar occlusion-reperfusion protocol.

The cause of ongoing myoglobin release is presently unclear. Although large vessel opening probably occurs within a short period of time, it is possible that reperfusion of the microcirculation is uneven and variable over time. This may especially be true if coronary flow to the reperfused segment is inadequate and diminishing with time (i.e., the “no-reflow” phenomenon). Because flow was not measured directly, we also cannot exclude the possibility that occluder deflation was less abrupt than presumed. Another possibility is that continuing myoglobin release is a consequence of ongoing cardiac cell membrane failure, as shown in studies with antimyosin antibodies to quantify cell membrane injury. Such ongoing myoglobin release may reflect so-called “reperfusion injury,” that is, irreversible damage to ischemic but viable tissue after restoration of blood flow after coronary occlusion.

In the 12 animals in which tissue levels of myoglobin were directly measured, there was a direct relation between the total amount of myoglobin appearing in plasma estimated from the myoglobin concentration-time curve and the amount of tissue myoglobin depletion (Figure 6). Our nearly entire recovery of myoglobin in plasma contrasts with creatine kinase measurements in which approximately 30% of the enzyme depleted from tissue was actually recovered in plasma. In estimating plasma myoglobin recovery, values for $k_d$ and $V_p$ were determined with a one-compartment model. Because hemodynamics were stable in these animals, there is little reason to suspect a change in elimination kinetics in the kidneys. This may not be true in animals or humans with large amounts of tissue injury and cardiac failure or in the presence of renal dysfunction where the elimination half-time of myoglobin would be prolonged.

Two points relating to study design should be noted. Coronary blood flow was measured with a Doppler flow probe in only four of 16 animals subjected to the occlusion-reperfusion protocol. Although myoglobin kinetics were the same in all animals studied, we cannot be totally confident that the occlusion was complete or reflow was unrestricted in the animals in which flow was not actually measured. Also, we cannot assume from the data in this study that plasma myoglobin levels reflect the amount of actual tissue necrosis occurring despite reperfusion because no independent measurement of infarct size (either by histologic sections or with triphenyltetrazolium chloride staining) was undertaken.

In summary, myoglobin kinetics can be described by a one-compartment model with a volume of distribution of approximately 6.8% of total body weight and an elimination half-time of 5.2 minutes. Coronary reperfusion is associated with an early, rapid release of myoglobin from injured myocardium. Although the peak rate of myoglobin entry into the circulation occurs within 10–50 minutes, myoglobin release appears to occur as repeated discrete bursts and to persist for at least 4 hours after the onset of reperfusion. Finally, analysis of myoglobin concentration-time curves with a one-compartment model allows estimation of cardiac depletion of myoglobin occurring after reperfusion of an occluded coronary artery.

**Appendix**

**Mathematical Analysis of One- and Two-Compartment Models**

Because protein kinetics (e.g., creatine kinase) may be better described with a multicompartment
model, mathematical analyses in this study were performed with both one- and two-compartment models (Figure 1).

For a one-compartment model, conservation of mass requires that the rate of myoglobin entry into the compartment, \( M_{b1}(t) \), be equal to the rate of elimination plus the rate of accumulation:

\[
M_{b1}(t) = k_d \cdot Q_{Mb(t)} + \frac{dQ_{Mb(t)}}{dt} \tag{1}
\]

where \( Q_{Mb(t)} \) is the quantity of myoglobin present in the compartment at time \( t \), and \( k_d \) is the elimination rate constant. When myoglobin is no longer entering the system (e.g., after bolus administration or after termination of the Mb infusion), \( M_{b1}(t) = 0 \), giving

\[
0 = k_d \cdot V_D \cdot [Mb(t)] + V_D \cdot \frac{d[Mb(t)]}{dt} \tag{2}
\]

where \( V_D[Mb(t)] \) has been substituted for \( Q_{Mb(t)} \) in Equation 1. Equation 2 describes the washout kinetics for a single compartment. The mathematical solution to this differential equation is of the form

\[
[Mb(t)] = [Mb(0)] \cdot \exp(-k_dt) \tag{3}
\]

where \([Mb(0)]\), the concentration of myoglobin present at time 0, and \( k_d \), the elimination rate constant, are determined by fitting a least squares monoexponential curve to the washout data points of \([Mb(t)]\) plotted as a function of \( t \).

For a two-compartment model (with first order kinetics describing the transfer of myoglobin), there are five parameters \((V_1, V_2, k_{1-1}, k_{1-2}, k_{2-1})\) to be determined (Figure 1, right panel). Conservation of mass in each compartment allows two differential equations to be written. For compartment 1

\[
M_{b1}(t) + k_{2-1} \cdot Q_{Mb(0)} = k_{1-2} \cdot Q_{Mb(0)} + \frac{dQ_{Mb(0)}}{dt} \tag{4}
\]

For compartment 2

\[
k_{1-2} \cdot Q_{Mb(0)} = k_{2-1} \cdot Q_{Mb(0)} + \frac{dQ_{Mb(0)}}{dt} \tag{5}
\]

The solution to these simultaneous differential equations when \( M_{b1}(t) = 0 \) (i.e., after a bolus injection or after termination of the myoglobin infusion) is of the form

\[
[Mb(t)] = Ae^{-\alpha t} + Be^{-\beta t} \tag{6}
\]

where \([Mb(t)]\) is the concentration of myoglobin in the central (i.e., blood) compartment at time \( t \), and \( A, \alpha, B, \) and \( \beta \) are parameters that are determined by fitting a biexponential curve to the actual washout data points \([Mb(t)] \) vs. \( t \). After determination of \( A, \alpha, B, \) and \( \beta \), the rate constants \((k_{1-1}, k_{1-2}, k_{2-1})\) and the volumes of distribution of each compartment \((V_1, V_2)\) can be computed with standard two-compartment analysis techniques.19

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References

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