Nonlinear Relationship Between Creatine Kinase Estimates and Histologic Extent of Infarction in Conscious Dogs: Effects of Regional Myocardial Blood Flow

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SUMMARY We examined the relationships between creatine kinase (CK) enzyme estimates of infarction (EE), histologic extent of infarction (HI) and regional myocardial blood flow (RMBF) over a wide range of infarct sizes in chronically prepared awake dogs.

Twenty-three mongrel dogs were prepared with catheters in the aorta and left atrium and snares on the left anterior descending coronary artery. Seven days later, myocardial infarction was produced by complete coronary occlusion. Serial serum samples were obtained for CK analysis over 72 hours and EE was calculated using the Shell formula, which assumes constant serum CK appearance ratio (K_a) from infarcted myocardium. Regional myocardial blood flow was measured before occlusion and at 15 minutes, 2, 6 and 24 hours after occlusion using 7–10-μm microspheres. Six days after occlusion, HI and RMBF were determined from 1–2-g samples of the entire left ventricle.

The histologic extent of infarction ranged from 1–54 g. Linear regression analysis between EE and HI demonstrated an r value of 0.75 and SEE of ±34 g, indicating a poor linear relationship. The relationship between EE and HI was described best by the power function equation EE = 18.6 HI^0.5 (r = 0.91), indicating relatively less CK appeared in the blood as HI increased. When the analysis was limited to infarcts of less than 20 g, a good linear relationship was observed (r = 0.94). When K_a was calculated from HI and the Shell equation, the relationship between K_a and HI over the entire range of HI was described by the equation K_a = 5.53 HI^0.54 (r = 0.93), suggesting CK release from infarcted myocardium was not constant, but decreased as a power function as HI increased. In the total group of dogs, the best linear relationship between EE and HI resulted from modifying the HI by subtracting regions that remained severely ischemic, RMBF < 0.1 ml/min/g during the interval of CK release (r = 0.81, ±20 g). This procedure reduced the HI size in the larger infarcts preferentially.

This study indicates that over a large range of infarction, the relationship between EE and HI is best described by a power function rather than a linear function. This relationship may be caused by progressive reduction in CK release from regions of severely reduced blood flow. Estimation of infarct size from serum CK measurements using a constant K, introduced an error that increased as infarct size increased.

IN 1971 Shell, Kjekshus and Sobel developed a mathematical model for estimating the extent of acute myocardial infarction using serial measurement of creatine kinase (CK). They reported close correlation between CK estimates of infarction and myocardial CK depletion. Roe, Cobb and Starmer examined the relationship between serum CK estimate and histologic extent of infarction in conscious animals undergoing permanent circumflex coronary artery occlusions. They showed a poor correlation between enzymatic estimates and histologic extent of infarction over a wide range of infarct sizes. Enzymatic estimates markedly overestimated histologic infarction in each animal. By restricting the analysis to histologic infarcts of less than 13 g, the correlation improved. The poor correlation between histologic and enzymatic extent of infarction appeared to result from the appearance of relatively less CK in the blood in the larger infarcts.

In this study we examined the relationship between serum CK estimates and histologic extent of infarction after permanent occlusion of the left anterior descending coronary artery (LAD) in conscious dogs. Regional myocardial blood flow was measured at selected intervals after acute infarction to determine to what extent blood flow to the ischemic region may account for the disproportionately small release of CK in large infarcts.

Methods

Animal Preparations

Twenty-three adult mongrel dogs that weighed 16–34 kg were anesthetized with an i.v. bolus of sodium thiamylal (30–40 mg/kg) and underwent left thoracotomy. Polyvinyl chloride heparin-filled catheters, 3 mm in diameter, were introduced into the ascending aorta through the left internal mammary...
artery and into the left atrial cavity through the left atrial appendage. The catheters were tunneled to a subcutaneous pouch at the base of the neck. To produce a wide range of infarct size, an adjustable snare-type polyethylene occluder, constructed in this laboratory, was positioned around the LAD either proximal or just distal to the first large diagonal branch. The snare was tunneled to a subcutaneous pouch in the anterior chest caudal to the incision. The thoracotomy incision was closed and the dogs were allowed to recover.

Studies were performed 7–14 days after the initial surgery, when the dogs were active, fully recovered from surgery, and free of anemia and fever. On the morning of the study each dog received 10 mg of morphine sulfate intravenously in 1-mg boluses over 20 minutes to produce mild sedation. The subcutaneous pouches were infiltrated with 2% lidocaine hydrochloride, and the catheters and snare were brought to the exterior through two small incisions. The aortic and left atrial pressure catheters were connected to Statham P23Db transducers, which were referenced to the mid-chest level. The subcutaneous area over the left external jugular vein was infiltrated with 2% lidocaine hydrochloride, and a 3-mm polyvinyl chloride catheter was passed through the vein into the superior vena cava to facilitate frequent venous sampling.

Study Protocol

The laboratory was dimly illuminated and kept free of noise and extraneous activity. After the dogs had become accustomed to the laboratory conditions, control hemodynamic and electrocardiographic measurements were recorded. Phasic and mean left atrial and aortic pressures and the ECG were recorded on a direct-writing oscillograph (Hewlett-Packard model 8800) and an eight-channel magnetic tape recorder (Hewlett-Packard model 3917-A).

Regional myocardial blood flow was determined by injecting 7–10-μ carbonized microspheres labeled with the gamma-emitting radionuclides 141Ce, 85Cr, 89Sr, 48Sc, and 125I into the left atrium. Microspheres were obtained as 1.0 mCi of each nuclide in 10 ml of 10% dextran. This stock solution was diluted with 10% dextran so that 1.0 ml contained 3 million microspheres. Before injection, the microspheres were mixed by alternate agitation for at least 15 minutes in an ultrasonic bath and a Vortex agitator (Scientific Industries, Inc.). Dispersion of microspheres was verified by examining a drop of microsphere suspension with a light microscope. In each dog, blood flow measurements were made with 1.0 ml of suspension injected over 5 seconds into the left atrial catheter, and the catheter flushed with 5 ml of normal saline. A reference sample of arterial blood was collected from the aortic catheter at a constant rate of approximately 17 ml/min using a withdrawal pump, beginning with the microsphere injection and continuing for 90 seconds.

After the dog had become adjusted to the labora-

tory conditions a control measurement of regional blood flow was made. Lidocaine hydrochloride, 2 mg/kg, and morphine sulfate, 2–4 mg, were then injected intravenously, and the snare occluder was tightened gradually over a 5–10-minute period to produce total occlusion. Dogs with normal baseline ECGs and ST-segment elevation, an increase in heart rate, and/or an increase in left atrial pressure in response to complete occlusion were included in the study. Morphine sulfate was administered intravenously in 2–3-mg dosages during the first 30 minutes after occlusion to minimize discomfort resulting from the occlusion. The dose of i.v. morphine sulfate used in each dog was 20 mg (10 mg were given at the onset of the study or approximately 60 minutes before occlusion). Intravenous lidocaine hydrochloride, 2 mg/kg, was administered as bolus injections at 15-minute intervals during the first hour. Additional regional blood flow measurements were made 15 minutes and 2, 6 and 24 hours after occlusion. Analgesics and antiarrhythmic drugs were not administered after the initial 1 hour of occlusion. Venous blood samples were obtained at hourly intervals during the first 12 hours at 2–3-hour intervals for the next 24 hours, 6–13-hour intervals for an additional 48 hours, and daily thereafter. The dogs were sacrificed 5–6 days after occlusion to allow time for the infarcted and noninfarcted myocardium to become clearly delineated by routine histologic technique.

Measurements of Regional Myocardial Blood Flow

At postmortem examination the heart was removed; the snare was checked to ascertain that complete occlusion had occurred and that the sutures stabilizing the base of the snare were intact. The left ventricle was sectioned into four transverse rings from base to apex as previously described.4,9 Rings 1 and 4 were divided into four circumferential regions and rings 2 and 3 into six circumferential regions. The six regions of each circumferential ring of the left ventricle consisted of anterior free wall, anterior papillary muscle, septum, posterior free wall, posterior papillary muscle and lateral free wall. Each transmural region was further divided into four layers from the epicardium to the endocardium; sample size was 1–2 g. The samples were weighed and placed into individual plastic vials filled with buffered formalin to preserve the tissue for later histologic section.

To achieve the maximum fidelity in counting the samples, separate vials with approximately 10,000 counts/min of a single radionuclide-labeled microsphere were placed in the counting chamber of a Packard Model A5912 gamma spectrophotometer. The energy counting windows were set for each isotope using a video display to optimize the number of counts while minimizing the spill-over contaminant activity from the other isotopes. A matrix was constructed using the counts recorded in all windows from each isotope. This matrix considers the fraction of contaminant counts contributed to each window by the other isotopes. Raw counts of the individual
isotopes were obtained in each of the myocardial and reference blood samples. A system of simultaneous linear equations was solved with an IBM model 1130 digital computer to correct the raw counts for contaminant activity from the other isotopes. Flow to each area of the myocardium was calculated using the formula

\[ Q_m = Q_r \cdot C_m/C_r \]  

(1)

where \( Q_m \) = myocardial flow (ml/min), \( Q_r \) = reference blood flow (ml/min), \( C_m \) = count activity in the tissue sample, and \( C_r \) = count activity in reference blood flow samples. Myocardial tissue sample blood flow (ml/min) was divided by the appropriate sample weight and expressed as ml/min/g.

**Correction of Ischemic Blood Flow Measurements for Microsphere Loss**

The interpretation that infarcted myocardium effects microsphere loss is based on the observation that in the absence of myocardial infarction, regional myocardial blood flow is relatively homogeneous in the left ventricle, i.e., the ratio of blood flow in the circumflex to anterior descending coronary artery equals one, but after infarction the ratio of preocclusion regional blood flow in ischemic/nonischemic regions decreases. The reduction in the ratio may represent a combination of microsphere loss and/or tissue swelling. The infarcting process should affect the microspheres present in a myocardial sample in a similar fashion; the relative loss of microspheres injected before and after occlusion but before microsphere loss begins should be comparable. Percent apparent microsphere loss for each sample in the ischemic zone was calculated using the equation

\[ \% \text{ apparent microsphere loss} = \frac{\text{MCF} - \text{CFI}}{\text{MCF}} \times 100 \]  

(2)

where CFI = preocclusion blood flow in an individual sample of the region subjected to ischemia and MCF = mean flow in nonischemic samples from the same layer. If CFI is greater or less than MCF in equation 2, the percent of apparent loss appears as a negative or positive value, respectively. A positive value indicates a loss and a negative value an apparent gain. The following equation was used to correct the ischemic blood flow measurements in each sample:

\[ \text{MBF}_c = \text{MBF}_u \cdot \frac{100}{100 - \% \text{ microsphere loss}} \]  

(3)

where \( \text{MBF}_c \) = corrected blood flow value, \( \text{MBF}_u \) = uncorrected blood flow value, and \% microsphere loss = the percent loss from equation 2.

**Quantitation of Infarction**

After analysis for radioactivity, each sample was sectioned for histologic examination. A minimum of four step-sections were taken through each small tissue block and stained with hematoxylin-eosin. The extent of infarction in each section was traced on paper using a projection microscope. Infarcted myocardium at this point was sharply delineated from intact myocardium and was characterized by complete or partial cellular dissolution, inflammatory cell infiltrates, and loss of normal cellular architecture. The percentage of infarction in each section was calculated using a sonic digitizer interfaced to an IBM 1130 computer. The total weight of the infarcted tissue was calculated from the sum of the weight of infarcted tissue in individual samples.

**Enzyme Analysis and Quantitation**

CK enzyme determinations were performed at 37°C by the modified Rosalki method using Spin-Chem reagent (Smith Kline Instruments) on the Centrifichem programmable analyzer (Union Carbide Corp.), which has a tested kinetic precision within 3 milliopitical density units. Enzyme estimates of the extent of infarction (EE) were determined using the basic formula described by Shell:

\[ \text{EE (gEq)} = \frac{T}{\text{CKD} \cdot K_r} \]

where \( T \) represents the enzyme appearance function which is the amount of CK in 1 ml of the distribution volume at time t. The function defined as \( f(t) = \text{de} / \text{dt} + K_a F \), where \( \text{de} / \text{dt} \) is the derivative of the enzyme value at time t and \( K_a (-0.0045 \text{ min}^{-1}) \) is the proportional disappearance rate constant which, by convention, is negative and expresses the percent per minute decay rate of serum enzyme levels. \( K_a (0.114) \) represents the proportion of body weight (BW) in which released enzyme is distributed. \( \text{CKD} (1366 \text{ IU/g}) \) represents the estimated amount of enzyme depleted per gram of infarcted myocardium, and \( K_r (0.30) \) is the ratio of total enzyme appearance in the blood to total CK disappearance from infarcted myocardium. The value used for CK background activity was 68 IU/l. The values for CKD of background activity were modified from activity at 30°C to that at 37°C using the thermal conversion factor 1.708.

The Hotelling t² test was used to compare hemodynamic measurements after coronary artery occlusion to control values.

**Hemodynamic Measurements**

Hemodynamic data from the 23 dogs are presented in table 1. Mean heart rate was 74 ± 3 beats/min (mean ± SEM) before occlusion. Heart rate increased to 108 ± 7 beats/min after occlusion. Heart rhythm immediately after occlusion and 2 and 6 hours later was sinus tachycardia with occasional premature ventricular complexes. The predominant rhythm was ventricular tachycardia 24 hours after occlusion. Dogs that developed ventricular fibrillation were excluded from the study. Aortic systolic pressure increased from 119 ± 3 mm Hg to 132 ± 4 mm Hg after occlu-
Enzyme Estimate vs Histologic Extent of Infarction

The relationship between enzyme estimate (gEq) and histologic extent of infarction is shown in figure 1 and table 2. The histologic extent of infarction varied from 0.7-54.4 g, or from 0.7-44% of left ventricular weight. In each dog the enzyme estimate was larger than the histologic extent of infarction, with enzyme estimates varying from 14.1-169.4 gEq. Considering the entire range of infarction, linear regression analysis between CK estimates and histologic extent of infarction demonstrated a correlation coefficient of 0.75 and see of ± 23 g (p < 0.01), indicating a very poor linear relationship between the two variables. Relatively less CK appeared in the serum as the histlogic extent of infarction (HI) increased (fig. 1). The equation that best described the data was a power function:

\[ EE = 18.6 \times HI^{0.45} \; (r = 0.91). \]

Although the correlation coefficient of the above power function equation was good, the variability between CK estimates and histologic extent of infarction was considerably greater in dogs with more than 20 g of infarction. Figure 1 and table 2 illustrate that for similar CK estimates the extent of histologic infarction varied widely (dogs 10, 16, 19 — EE 76.9-79.3 gEq, HI 18.2-38.8 g; dogs 20, 21 — EE 81.4 and 79.8 gEq, HI 24.7 and 42.6 g). Conversely, for similar size histologic infarcts, the enzyme estimates varied widely (dogs 7, 17, 20 — HI 23.9-25.8 g, EE 57.8-104.3 gEq; dogs 15, 18 — HI 51.2 and 54.4 g, EE 169.4 and 67.6 gEq). The result of limiting the analysis of enzyme estimate vs histlogic extent of infarction to the dogs with histlogic size less than 20 g is shown in figure 2. If the upper range of infarction in the present study had not exceeded 20 g, an excellent linear relationship between enzyme estimate and histlogic infarction would have been observed (r = 0.94).

Variability of Calculated \( K_r \)

\( K_r \), the ratio of CK entering the serum to that released from the infarcted tissue, can be derived using the Shell equation, the histlogic extent of infarction (HI), and the serum CK appearance function:

\[ K_r = \frac{\int_0^T f(t)dt \cdot KW \cdot BW}{CK_s \cdot HI} \]

The calculated \( K_r \) for each dog is shown in table 2, and the relationship between calculated \( K_r \) and histlogic extent of infarction is shown in figure 3. The equation that best described the relationship between \( K_r \) and histlogic infarction was \( K_r = 5.53 HI^{-0.44}, r = -0.93 \). As histlogic size increased, calculated \( K_r \) decreased as a power function. This relationship demonstrated a relative decrease in appearance of enzyme activity in the serum as infarct size increased. The values of \( K_r \) greater than 1 resulted from the marked overestimation of infarct size by the enzyme estimate method.
Regional Myocardial Blood Flow

Regional myocardial blood flow was measured during control condition before occlusion and 15 minutes, 2, 6 and 24 hours after occlusion. Table 3 lists mean blood flow values ± SD before and after coronary occlusion in myocardial samples in which blood flow was 0.20 ml/min/g or less, 15 minutes after coronary artery occlusion. The blood flow values after occlusion are tabulated as uncorrected values and after correction for microsphere loss. The blood flow values before occlusion have been normalized to nonischemic region blood flow to illustrate the mean microsphere loss in these samples. The greatest microsphere loss occurred in dogs with the greatest histologic infarction. In two dogs with small infarcts, blood flow values before occlusion were higher in samples that later became ischemic than in nonischemic regions, resulting in a calculated apparent microsphere gain. The mean of the normalized flow measurements for the group was 0.80, indicating an average microsphere loss of 20% in these regions (range 27% gain to 63% loss). Correction for microsphere loss increased the ischemic blood flow values as a function of the degree of microsphere loss. Mean blood flow for the group was 0.09 ± 0.03 before and 0.11 ± 0.04 ml/min/g after correction. Correction increased ischemic flow greater than 0.04 ml/min/g in only two dogs. Thus,

**TABLE 2. Body Weight, Left Ventricular Weight, Histologic Extent of Infarction, Percent of Left Ventricle Infarcted, Modified Histologic Extent of Infarction Serum CK Measurement and Calculated K_r in 26 Dogs**

<table>
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<th>BW (kg)</th>
<th>LV (g)</th>
<th>HI (g)</th>
<th>% LV</th>
<th>Modified HI</th>
<th>EE (g-Eq)</th>
<th>K_r</th>
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Abbreviations: BW = body weight; LV = left ventricular weight; HI = histologic extent of infarction; %LV = percent of left ventricle infarcted; HI = histologic extent of infarction; EE = serum CK measurement of extent of infarction; K_r = ratio of total enzyme appearance in the blood to total CK disappearance from infarcted myocardium.

**FIGURE 2.** The relationship between serum CK enzyme estimate of infarction and histologic extent of infarction for dogs with histologic infarct size less than 20 g. The relationship was linear:

\[ EE = 3.87(HI) + 17.58 \] (r = 0.94).

**FIGURE 3.** The relationship between the calculated K_r and the histologic extent of infarction is a power function with

\[ K_r = 5.53 HI^{0.44}, r = -0.93. \]
although apparent microsphere loss did affect the ischemic blood flow measurements in these dogs, the magnitude of the effect was very small expressed as a change in absolute blood flow. In comparison, coronary artery occlusion resulted in markedly reduced blood flow in these regions.

The blood flow measurements during ischemia were analyzed to determine whether regional blood flow during the period of CK appearance accounted for the variable and disproportionately small appearance of CK. The larger infarcts were characterized by larger regions which remained severely ischemic during the period of greatest CK appearance in the blood. Most the CK activity from infarcted myocardium is degraded within the infarcted myocardium and does not appear in the blood, so we reasoned that areas that were severely ischemic during the interval of CK appearance may release a disproportionately small amount of CK. The ischemic zone was subdivided into regions of varying degrees of ischemia, i.e., 0–0.05, 0–0.10, 0–0.15, 0–0.20 ml/min/g, and the weight of tissue perfused by each range of blood flow was calculated at each interval after occlusion. The histologic infarct size was modified by subtracting the weight of infarcted tissue in a given blood flow range

from the total weight of infarcted tissue. Linear regression analyses between CK estimates and the modified histologic infarct sizes were carried out. The best linear correlation between CK estimates and modified histologic infarction resulted from subtraction of tissue in the 0–0.10 ml/min/g flow range at 24 hours ($r = 0.81 \pm 0.20$ SEE). The use of blood flow values corrected for microsphere loss did not improve the regression analyses. Table 2 illustrates the effect of modifying the infarct size by subtracting tissue perfused by 0.10 ml/min/g at 24 hours. The extent of the ischemic region in each dog that remained severely ischemic is the difference between the modified and nonmodified histologic infarction. None of the animals with less than 10 g of histologic infarction had tissue samples with flow less than 0.10 ml/min/g at 24 hours. Although there was significant variability between dogs as the infarct size increased, the regions of severe ischemia increased (table 2). Consequently the extent of modification of infarction was greater in the larger infarcts. Figure 4 is a plot of the CK estimate and modified histologic infarct size. Significant variability in the linear relationship remained as a result of the larger infarcts.

Changes in blood flow to the total ischemic region and to subzones of the ischemic region between each interval, i.e., 15 minutes to 24 hours, 2–24 hours, and 6–24 hours after occlusion, were analyzed. Blood flow changes in subgroups of dogs with similar CK estimates and dissimilar histologic infarct sizes, and similar histologic infarct sizes and dissimilar CK estimates were analyzed. There was no consistent relationship between changes in flow to the total or subzones of the ischemic regions and the variability between CK estimates and histologic extent of infarction.

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

**Figure 4.** The relationship between serum CK estimate of infarction and the histologic infarct size modified by subtraction of tissue perfused by flow less than 0.10 ml/min/g at 24 hours:

$$EE = 2.7 \ (\text{mod. } HI) + 27.8 \ (r = 0.81)$$
Discussion

In 1971 Shell et al. proposed a mathematical model for the estimation of infarct size using serial serum CK measurements. Infarction was produced by permanent occlusion of the LAD in conscious dogs, and the extent of infarction was determined by myocardial CK depletion at 24 hours. They reported a linear relationship between enzyme estimate and extent of infarction determined by cardiac CK depletion. Roe et al. produced permanent occlusions of the circumflex coronary artery and sampled serum CK at frequent intervals. Infarct size was determined by histologic examination 6–7 days after occlusion. They found that CK enzyme estimate markedly overestimated histologic infarct size in all dogs. Histologic extent of infarction ranged from 1–27 g, and there was very poor correlation between CK estimates and histologic infarction in the total group of animals. The best correlation was obtained with the Shell formulation, when the analysis was limited to infarcts with a histologic size of less than 13 g, \( r = 0.78 \). Vatner et al. examined the relationship between histologic extent of infarction and enzyme estimate by the Shell formulation. In dogs with permanent occlusion of either the circumflex or LAD, the relationship between enzyme estimate and histologic extent of infarction was linear \( (r = 0.94) \). Vatner et al. also compared serum CK appearance in dogs undergoing reperfusion after 1 or 4 hours of total coronary occlusion. Reperfusion compared with permanent occlusion did not alter the linear relationship \( (r = 0.90) \), but did result in relatively more CK release into the blood, suggesting that regional myocardial blood flow plays an important role in the amount of CK release from infarcted myocardium.

There are several differences in methods between the study of Vatner et al. and the present study. In the study of Vatner et al., certain animals with infarcts less than 10 g were excluded, they were sacrificed 24 hours after occlusion, and infarction was estimated by gross examination and by myocardial CK analysis. In the present study all dogs with hemodynamic and electrocardiographic response to occlusion were included in the analysis, dogs were sacrificed at 5–6 days to allow sharp delineation between infarcted and noninfarcted myocardium, and infarction was quantitated from multiple histologic sections of the entire ischemic region.

The basic formulation of Shell has been modified by Norris et al. and Roberts et al. Norris et al. recommended that an individual \( K_a \) derived from the downslope of the CK curve be used, rather than the constant \( K_a \) of the Shell formulation. Roberts and associates further modified the enzyme estimate equation by assigning new values to the constants \( K_r \) and \( K_w \) and adopting the individualized \( K_a \). Roe et al. compared the values for infarct size obtained by the Shell formulations with those calculated using the modifications of Norris and Roberts and showed no improvement in the correlations of enzyme estimate with histologic extent of infarction. Modification of a given measurement resulted in additive or multiplicative effects on the final estimate of infarct size, but did not change the basic relationship between enzyme estimates and infarct size. Thus, the relationship between CK estimate of infarction and the extent of myocardial necrosis and the variables that may influence the relationship is still controversial.

The present study examined the relationship between serum CK estimates and histologic extent of infarction after permanent occlusion of the LAD in a large group of dogs. Considerable care was taken to ensure that all animals were treated identically. All dogs were healthy and free of infection and anemia. No antiarrhythmic agents or analgesics were given after the first hour of the occlusion. Occlusion of the LAD at various distances from the origin resulted in a wide range of histologic infarction, e.g., 0.7–54 g. The equation that best fit the relationship between CK estimate and histologic extent of infarction was a power function, \( r = 0.91 \) rather than a linear function, \( r = 0.75 \), \( r^2 = 0.56 \). As the extent of infarction increased, i.e., > 20 g, appearance of CK activity in the serum was variable and relatively less than in the smaller infarcts. An excellent linear relationship was observed by limiting the analysis to animals with less than 20 g of infarction \( (r = 0.94, r^2 = 0.88) \). Using either the Norris or Roberts modifications in the enzyme estimate equation did not improve the correlation between CK estimates and histologic extent of infarction in the total group or the group with less than 20 g infarcts. There are certain differences in the present and previous studies carried out in this laboratory. In the previous study, linear regression analyses between CK enzyme estimates and histologic extent of infarction demonstrated an \( r^2 \) value of 0.06, compared with 0.56 in the present study. Regression analyses in dogs with histologic infarction of less than 13 g demonstrated an \( r^2 \) of 0.42 in the previous study as compared to an \( r^2 \) of 0.88 in the dogs with less than 20 g of infarction in the present study. In both studies, CK enzyme estimates overestimated histologic infarction and there was relatively less appearance of CK activity in the blood in the larger infarcts. There are certain differences in the present and previous study that may have contributed to differences in the data. In the previous study the circumflex coronary artery was occluded and in the present study the LAD was occluded. We know of no previous data indicating that the relationship between myocardial infarction and CK appearance in the blood is different. A larger group and a wider range of infarct size was analyzed in the present study; 12 dogs in the present study and five in the previous study had histologic infarction greater than 13 g. The technique for serial blood sampling was improved in the present study. Blood samples were obtained from a catheter positioned in the superior vena cava in the present study and from repeated vein punctures in the previous study. In contrast to the previous study, intramuscular injections were not done and dogs that required cardioversion were excluded. The techniques for CK enzyme analysis and quantitation of histologic infarction were the same in both studies.
Despite the differences in methods, both studies support the conclusion that over a wide range of histologic infarction, it was not possible to adequately quantitate histologic infarction in an individual dog from serum CK estimates and that the major factor contributing to the poor linear correlation was inclusion of dogs with large myocardial infarcts.

The parameter $K_r$ in the enzyme estimate formula represents the ratio of the serum appearance of CK to the amount of CK released by infarcted myocardium. An assumption implicit in the formulation of Shell, Norris and Roberts is that $K_r$ is constant. This assumption can be tested by calculation of $K_r$ in individual animals. The basic formula can be rearranged to solve for $K_r$ if the serum appearance function of CK and the histologic infarct size are known. A calculated $K_r$ was obtained for each animal. This calculated $K_r$ was then compared with infarct size. The curve that best fit the relationship between $K_r$ and histologic infarct size was a power function with a correlation coefficient of $-0.93$, suggesting that the fraction of CK activity appearing in the serum from infarcted myocardium decreased as the infarct size increased.

Regional myocardial blood flow was measured before and after coronary artery occlusion using $9 \pm 1 \mu$ radioisotope-labeled microspheres. Recent studies have suggested that infarcted myocardium may effect microsphere loss, which alters interpretation of ischemic blood flow measurements.\(^5\)\(^-\)\(^7\) The interpretation of microsphere loss is based on the observation that in the absence of myocardial infarction, regional blood flow is relatively homogeneous in the left ventricle; the ratio of circumflex to LAD region blood flow equals 1,\(^6\) but after infarction of the ratio of preocclusion blood flow in infarct/noninfarct regions decreases.\(^6\)\(^-\)\(^7\) The reduction in the ratio indicates the apparent microsphere loss effected by the process of infarction. Capurro et al.,\(^6\) using $15 \pm 5\mu$ microspheres, observed losses of approximately 30% in the center of the ischemic region. The maximum loss occurred at 24 hours in the endocardial regions and at 48 hours in the epicardial regions. Jugdutt et al.\(^6\) used 7–10-$\mu$ microspheres and observed an average loss of 19% after left circumflex coronary artery occlusion; the apparent loss of microspheres in animals sacrificed at 12, 24, 48 and 72 hours was comparable. They observed no loss 6 hours after coronary artery occlusion. The microsphere loss was directly related to the extent of necrosis. In the present study the apparent microsphere loss averaged 20% in the ischemic regions in which regional blood flow was 0.20 ml/min/g or less 15 minutes after occlusion (table 3). The infarcted process should affect all microspheres present in a sample in similar fashion; the relative loss of microsphere injected before and after occlusion should be comparable. In recent studies\(^6\) we have used the microsphere loss determined from the preocclusion blood flow measurement to correct the ischemic blood flow measurements in each sample of the ischemic zone. It is appropriate to correct blood flow measurements obtained before the onset of microsphere loss, i.e., less than 6 hours after occlusion, but once loss begins, correction will overcorrect the ischemic blood flow values. Correction of blood flow values 24 hours after coronary occlusion would be excessive because events that would effect microsphere loss have already begun, or may have been completed in certain areas.\(^6\)\(^-\)\(^8\) In any event, the effects of microsphere loss on the ischemic blood flow measurements are so small in most instances that one may question whether the change in blood flow is biologically significant. In the present study mean blood flow to regions receiving 0.20 ml/min/g or less flow 15 minutes after occlusion was $0.09 \pm 0.03$ before and $0.11 \pm 0.04$ after correction for microsphere loss. Correction for microsphere loss resulted in greater than 0.04 ml/min/g increases in blood flow in only two dogs.

The present study suggests that regional blood flow to the ischemic region influenced the appearance of CK. Previous studies have demonstrated an inverse relationship between the extent of infarction and blood flow\(^4\) and that acute changes in blood flow may influence total CK appearance in the blood.\(^6\) Regional blood flow was measured serially after acute myocardial infarction and was analyzed to determine whether reduced flow, or a combination of reduced flow followed by increased flow to the ischemic region, could explain the disproportionately small release of CK. The large infarcts were characterized by large regions that remained severely ischemic during the interval of greatest CK release, i.e., 24 hours or less after acute coronary occlusion. A significant amount of the CK depleted from infarcted myocardium does not appear in the blood,\(^3\) so the CK released from areas of severe ischemia may undergo accelerated degradation, or a decreased release from the heart, and thus contribute less to the total appearance of CK in the blood. Also, once tissue is infarcted, greater increments in blood flow to the infarcted tissue may cause disproportionate CK appearance.\(^6\) The best linear correlation between CK estimate and histologic extent of infarction was obtained by subtraction of the weight of the tissue samples in which regional blood flow remained less than 0.10 ml/min/g at 24 hours from the total infarct weight ($r = 0.81 \pm 0.02$). Thus, although this procedure preferentially reduced infarct size in the larger histologic infarcts it did not entirely explain the disproportionate CK appearance in the larger infarcts. Other flow ranges and intervals were analyzed but did not improve the linear relationship. The greatest variability between CK estimate and histologic extent of infarction occurred in the larger infarcts. Analysis of the severity of ischemia or changes in flow to the ischemic region in dogs with similar histologic infarction but dissimilar CK estimates did not consistently explain the disproportionately small CK appearance.

Certain characteristics of the infarct may have contributed to disproportionate release of CK. The small infarcts were characterized by patchy areas of infarcted tissue, and the large infarcts by extensive areas of confluent infarction. The interface between noninfarcted and infarcted tissue would be relatively larger
in the smaller infarcts. The infarct pattern will determine the diffusion distance and thus may affect the time that CK is exposed to factors that effect degradation of enzyme activity. Finally, previous studies have reported that cardiac lymphatics transport a major portion of the released myocardial CK to the distribution space.\textsuperscript{18,19} The $K_a$ of CK in lymphatic fluid is much higher than the $K_a$ in serum. The time that CK remains in the lymph vessels may determine appearance of CK activity in the blood. Clark et al.\textsuperscript{14} reported that interruption of cardiac lymph flow by occlusive snares 5 hours after coronary occlusion reduced the total CK activity released into the blood. The effects of extent of infarction and/or degree of ischemia on cardiac lymph flow are not known, but variable and/or reduced lymph flow in the larger infarcts could contribute to the variable and disproportionate appearance of CK in the blood.

In the present study, linear regression analysis between CK estimates and histologic infarction resulted in a correlation coefficient of 0.75 and see of $\pm 23$ g (p < 0.01), indicating a poor linear relationship between the two variables. The equation that best described the relationship was a power function, indicating a disproportionately small increase in CK appearance as infarct size increased. The calculated ratio of CK appearance in the blood from infarcted myocardium, $K_r$ rather than being a constant measurement, decreased as a power function as histologic infarct size increased. The use of a mathematical formula using a constant $K_r$ for the serum CK estimate of infarct size introduced an error that increased as infarct size increased. The larger infarcts were characterized by central zones of severely ischemic myocardium during the interval of CK release which may have contributed to the disproportionate CK appearance.

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