Myocardial Infarction Size from Serial CPK: Variability of CPK Serum Entry Ratio with Size and Model of Infarction

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SUMMARY To study the variability of the fraction of CPK released from the infarcted heart which enters the serum (serum entry ratio, or SER) with size and model of infarction, nine dogs underwent homogeneous infarctions (LAD ligation) of varying sizes, and 10 dogs underwent scattered infarctions (left coronary embolization). In homogeneous infarcts there was an inverse linear relationship of SER to infarct size (IS) (SER = \(-0.8514\%\) LV + 0.345, \(r = 0.98\)). Such a relationship was found for scattered infarcts. CPK Kd (exponential disappearance constant for CPK) was not significantly different in homogeneous (\(-0.00178\) min\(^{-1}\)) vs scattered infarcts (\(-0.00195\) min\(^{-1}\)). Although similar IS was produced in each (homogeneous 19.9\% LV, scattered 18.4\% LV) cumulative CPK serum entry (CPK\(_{\text{SER}}\)) was much lower in homogeneous (4175 mIU/ml) vs scattered infarcts (7,296 mIU/ml). SER was also much lower in homogeneous (17.7\%) vs scattered infarcts (29\%) (\(P < 0.025\)). Cumulative CPK plateau occurred significantly later in homogeneous (15.8 hours) vs scattered infarcts (11.7 hours) (\(P < 0.01\)).

Further corrections to the serial CPK equations for IS determination are indicated. The method may not be applicable in some infarct situations, e.g., scattered infarction.

THE SERIAL CPK TECHNIQUE was developed by Sobel and Shell for the noninvasive estimation of myocardial infarction size.\(^1,2\) The disappearance of CPK from the serum is monoexponential,\(^1,3\) and it is therefore possible to determine total serum entry of CPK from serial serum samples and from knowledge of the distribution volume. Actual infarct size in grams of myocardium is then determined from knowledge of the normal myocardial CPK content, the fractional depletion of CPK from homogeneously infarcted myocardium, and the fraction of CPK depleted from the heart which actually enters the serum (serum entry ratio, or SER). The work of Sobel and Shell in dogs revealed an SER of 15\%, a figure which has been widely used in dog studies and which has been extrapolated for use in humans.\(^2\) We reasoned that the small proportion of CPK depleted from the heart which eventually enters the serum might be a source of large errors in calculation of infarct size — a 3\% change in local destruction of CPK would produce a 20\% change in serum entry of CPK. Roe and coworkers have previously noted relatively less total enzyme release in larger as compared to smaller infarcts.\(^4\) They speculated that small regions of infarction might release CPK in amounts equivalent to large zones of infarction because of better collateral perfusion in the smaller zones. They noted the wide variability of SER from one dog to another in the original work of Shell.\(^5\)

The escape of CPK from a zone of infarction depends in part on the access of the CPK to perfused myocardium. We postulated that the larger a zone of infarction, the slower would be the escape of CPK from its center, allowing more time for local destruction. We further postulated that the model of infarction might influence the SER. The CPK from multiple scattered small infarcts should have greater access to the circulation than that from zones of homogeneously infarcted myocardium. We studied the relationship of SER to the size of homogeneous infarcts, and the variability of SER in homogeneous vs scattered infarcts.

Methods

Ligation Dogs (Homogeneous Infarct Model)

Mongrel dogs weighing 22–27 kg (mean 24 kg) were anesthetized with sodium pentobarbital 30 mg/kg I.V. intubated, and anesthesia was maintained with a mixture of N\(_2\)O, O\(_2\) and 0.5\% fluothane, using a modified Boyle K anesthetic machine. The heart was exposed through a left thoracotomy and a silk snare was placed about the left anterior descending coronary artery (LAD) at some point between the first and fourth diagonal branches, depending on the size of infarct desired. The chest was closed and the snare enclosed in a subcutaneous pouch. Preoperative penicillin and dihydrostreptomycin, and postoperative (four days) ampicillin were administered I.V. A heparin-filled external jugular vein catheter was placed and the dog was allowed to recover. Serum CPK samples were obtained every 4–6 hours for 48 hours, and then twice daily for five to seven days. At this point, the dog was again anesthetized with pentobarbital 30 mg/kg, the snare was pulled tightly and...
secured, and the dog was allowed to regain consciousness. Three-milliliter blood samples were obtained from the jugular catheter q1hr × 36 hr, and q4hr for 12 more hours. Plasma volume was maintained with equal aliquots of physiological saline. Serum was removed and frozen at 20°C. At 48 hours post-ligation, the dog was sacrificed (pentobarbital plus 20% KCl I.V.) and the heart was quickly removed.

**Embolic Dogs (Scattered Infarct Model)**

Mongrel dogs weighing 21–29 kg (mean 24.8 kg) were anesthetized with pentobarbital 30 mg/kg and intubated. A heparin-filled external jugular vein catheter was placed in each dog. A small incision was made over the right femoral artery and a pre-shaped 7-F polyurethane catheter was introduced over a guidewire into the femoral artery. The catheter was advanced to the left coronary artery and a small hand injection of Renografin 76 (meglumine diatrizoate) (2–3 ml) was used to demonstrate selective catheterization of the left coronary artery with visualization of the LAD and circumflex vessels. An injection of 300 μ carbonized plastic microspheres (1–2 mg/kg body weight) suspended in 15–20% dextran (0.5 ml) was then made and flushed in with physiological saline (1 ml, four or five times). A repeat injection of Renografin 76 (2–3 ml) was made to ensure that selective catheterization of the left coronary artery without significant washout had been maintained. Control experiments using peripheral injection of microspheres ensured that release of CPK from noncardiac tissues as a result of escape of microspheres would not contribute significantly to total CPK entry to serum. The catheter was removed, the femoral artery was repaired, and the dog was allowed to recover. Serial blood samples were obtained as above to 48 hours, when the dog was sacrificed and the heart removed.

**Tissue**

The heart was immediately placed on aluminum foil in an ice-filled container. The ventricles were separated from the atria and opened. The visual distribution and size of infarction was noted. All possible fat and connective tissue was removed, and 300–500 mg biopsies were cut from areas of infarcted left ventricle and right ventricle. Thirty to 60 mg biopsies were taken from the central zones of densely infarcted myocardium. Atrial biopsies were also taken. The left ventricle and right ventricle were then separately cut into several large pieces of 20–40 g and weighed. All tissue was frozen at −20°C. On a subsequent day, the muscle was thawed, and each piece was minced with scissors and placed in 4 volumes of ice-cold KCl 10 mM/1 with 2 mercaptoethanol 1 mM/1. The tissue was homogenized using two 30-second bursts at maximum speed setting on a VirTis 23 homogenizer. Optimal dilutions, time, and speed settings were determined from multiple preliminary investigations. The homogenate was centrifuged at 2,000 g for 20 minutes and the CPK content of the supernate was determined using the SKI bulk reagent modification of the Rosalki method at 37°C. It was thus possible to calculate for each dog preinfarction CPK content of the left ventricle and right ventricle using the uninfarcted myocardial biopsies and the ventricular weights. Total residual CPK content of the left ventricle and right ventricle was determined from the homogenization of the entire ventricles, and total myocardial CPK depletion was calculated from the difference. A mean value for residual CPK content at the center of homogeneously infarcted myocardium was obtained in each of the nine ligation dogs. This value was related to the total CPK depletion in each dog to permit expression of total infarct size quantitatively in grams of homogeneously infarcted myocardium. In embolization dogs the total CPK depletion was determined as above, but CPK depletion from the centers of infarcted zones was assumed equal to the mean obtained from ligation dogs, to circumvent the impossibility of obtaining a biopsy of completely infarcted tissue in the setting of scattered small infarcts.

**Serum Samples**

Serum samples were kept frozen at −20°C until the completion of sampling when they were thawed and CPK concentrations were determined in duplicate as a batch for each dog. Control CPK concentration was subtracted from each value, which was then plotted on a log y-axis against time on the x-axis. The curve of CPK values was visually inspected and once the steep and linear downslope began, all subsequent points were used to determine Kd and r² (where Kd = exponential disappearance constant for CPK and r² = correlation coefficient) from the least squares approximation technique. A computer program was used to determine points on the curve of cumulative serum entry of CPK. Cumulative entry of CPK per milliliter of serum was determined from the plateau of the cumulative CPK curve, and an assumed plasma volume (PV) of 0.05 × body weight was used to determine total serum entry of CPK.

**Calculations**

The approaches of Shell et al. and Roberts et al. with appropriate modifications were used:

1. \[ CPK_t = E_t + Kd \int_0^t \frac{E_{t-x} + E_t}{2} \cdot dt \]

where \( CPK_t \) = cumulative CPK serum entry/ml, \( E_t \) = serum CPK at time t, \( Kd \) = exponential disappearance rate determined for each dog (see also Discussion section),

\[ \frac{E_{t-x} + E_t}{2} = \text{average CPK value during preceding time interval } x \text{ (generally 60 min), and } \]

\( dt = \text{exact time interval in min (generally 60 min)} \)
2. \( CPK_R = CPK_x \times DV \)
where \( CPK_R \) = total CPK serum entry, \( DV \) = distribution volume, \( PV = 0.05 \) BW (ref. 2, 3, 8)
where \( PV \) = plasma volume and \( BW \) = body weight.
3. \( CPK_{D(LV)} = ([CPK_{N(LV)}] - [CPK_{M(LV)}]) \) LV weight.
4. \( CPK_{D(RV)} = ([CPK_{N(RV)}] - (CPK_{M(RV)})) \) RV weight
where \( CPK_{D(LV)} \) and \( CPK_{D(RV)} \) = CPK depleted from LV or RV, \( [CPK_{N(LV)}] \) and \( [CPK_{N(RV)}] \) = CPK concentration in normal LV and RV (from biopsies), \( [CPK_{M(LV)}] \) and \( [CPK_{M(RV)}] \) = mean residual CPK concentration in LV and RV (from homogenization of entire ventricle).
5. \( CPK_{R(LV)} = CPK_R \times \frac{CPK_{D(LV)}}{CPK_{D(LV)} + CPK_{D(RV)}} \)
where \( CPK_{R(LV)} \) = total CPK serum entry attributable to LV infarction.
6. \( SER_{LV} \times \% = \frac{CPK_{R(LV)}}{CPK_{D(LV)}} \times 100 \)
where \( SER_{LV} \times \% \) = SER calculated from LV infarction.
7. \( IS_{LV} = \frac{CPK_{D(LV)}}{([CPK_{N(LV)}] - [CPK_{M(LV)}])} \)
where \( IS_{LV} \) = infarct size in grams of LV, \( [CPK_{N(LV)}] \) = mean CPK concentration in the centers of homogeneously infarcted areas of LV, ligation dogs.
\( IS_{LV} \times \% = \frac{IS_{LV}}{LV_{wt}} \times 100 \)
where \( IS_{LV} \times \% \) = infarct size as \% of LV.

Example Calculation (dog 1 from table 1A):
1. \( CPK_r = 1332 \) mIU/ml
2. \( CPK_R = 1332 \times 0.05 \times 22.7 = 1512 \) IU
3. \( CPK_{D(LV)} = (1835 - 150148) 84.4 = 4726 IU \)
4. \( CPK_{D(RV)} = (1614 - 50205) 31.1 = 0 \)
5. \( CPK_{R(LV)} = 1512 \times \frac{4726}{4726 + 0} = 1512 \) IU
6. \( SER_{LV} \times \% = \frac{1512}{4726} \times 100 = 32\% \)
7. \( IS_{LV} = \frac{4726}{1835 - 0.34(1835)} = 3.90 \) g
\( IS_{LV} \times \% = \frac{3.90}{84.4} = 4.62\% \)

### Results

The homogeneous infarction study included nine dogs who underwent ligation of the LAD. The scattered infarction study included 10 dogs who had embolization of the left coronary artery. Myocardial necrosis was well circumscribed in the area perfused by the occluded artery in the ligation dogs and generally was transmural at the apex, with subendocardial extension proximally (fig. 1A). In the embolization dogs necrosis was randomly scattered throughout the left ventricle (fig. 1B) and could be present in the myocardium at any level from endocardium to epicardium. Necrosis was confined to the left ventricle in ligation dogs, but there was significant right ventricular infarction present in the embolization dogs. Comparisons were made in terms of the degree of left ventricular infarction found, and in embolization dogs plateau CPK values were corrected to determine what serum CPK could be attributed to left ventricular necrosis.

### Myocardial CPK Content

CPK content of normal left ventricular myocardium (ligation dogs) was 1865 IU/g ± 148 (mean ± SD) (table 1A). This value did not differ significantly from that for normal right ventricular myocardium, which was 1748 IU/g ± 214. The CPK content of atrial myocardium (990 IU/g ± 358) was markedly less than that of left ventricular myocardium (\( P < 0.001 \)).

CPK depletion at the center of homogeneously infarcted myocardium was 81.5% ± 11.8 (mean ± sd) for ligation dogs (table 1A). The identification of a homogeneously infarcted zone large enough to biopsy in embolization dogs was difficult, and the CPK depletions observed (66.7% ± 13.7) (table 1B) probably represented assay of a mixture of infarcted and normal tissue.

### Serum Entry Ratio

An eight-fold range of infarct size expressed as percent of left ventricle infarcted was produced in the ligation dogs (4.62 - 34.9% of left ventricle) (table 1A). This range was even greater in terms of absolute weight of left ventricle infarcted (3.9 - 66.5 g). SER varied inversely as infarct size (fig. 2) (SER = -0.08514 (%LV) + 0.345, \( r = 0.98 \)).

A similar range of infarct size was produced in embolization dogs (3.4 - 42.2% of left ventricle, or 3.98 - 62.5 g) (table 1B). There was no significant relationship of serum entry ratio to infarct size in the embolization dogs (fig. 2).

The mean infarct size was not significantly different in ligation dogs (19.9% left ventricle ± 10.3, mean ± sd) vs embolization dogs (18.4% left ventricle ± 13.1) (table 1A and 1B). The Kd for CPK did not differ significantly between ligation dogs (−0.00178 min⁻¹ ± 0.00049, mean ± sd) and embolization dogs (−0.00195 min⁻¹ ± 0.00053) (tables 1A and 1B). Mean \( CPK_{R(LV)} \) (cumulative CPK serum entry/ml at
Table 1A. Ligation Dogs

<table>
<thead>
<tr>
<th>Dog</th>
<th>Kd min⁻¹</th>
<th>r²</th>
<th>CPKₘₑₘ (mIU/ml)</th>
<th>BW (kg)</th>
<th>CPKₘₑₘ IU</th>
<th>[CPKₘₑₘ (LV)] IU/g</th>
<th>LV wt IU/g</th>
<th>[CPKₘₑₘ (LV)] IU/g</th>
<th>CPKₘₑₘ (LV) IU</th>
<th>[CPKₘₑₘ (LV)] IU/g</th>
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=±0.0049 =±0.0040 =±1770 =±1.9 =±148 =±32.4 =±214

**FIGURE 1.** Gross appearance of ligation and embolization ventricles at 48 hours postinfarction.  A) Ligation infarction. The left ventricle has been opened, with the septum at left. Necrosis is confined to the apex. The endocardial surface in this area is hemorrhagic (black) in contrast to the pale normal endocardium. On cut surface of myocardium, necrotic muscle is pale and transmural and confluent.  B) Embolization infarction. Necrosis is scattered throughout the ventricle. Various black hemorrhagic foci are noted within the pale background of the endocardial surface. On the cut surface of myocardium, necrotic muscle is pale, and is patchy and randomly distributed from endocardium to epicardium. Normal muscle is dark on cut surface.

tributable to the left ventricle), however, was much lower in ligation dogs (4175 mIU/ml ± 1767, mean ± sd) vs the embolization dogs (7,296 mIU/ml ± 5400), although because of considerable range of embolization infarct size, the difference did not quite reach statistical significance at the 0.05 level (0.06 > P > 0.05) (tables 1A and 1B). The mean SER was 17.7% ± 9 for ligation dogs vs 29.0% ± 12.7 for embolization dogs (tables 1A and 1B and fig. 2), a sharp difference indicating a much lower fractional entry of CPK to the serum in ligation vs embolization infarction (P < 0.025).

Time to the plateau of the cumulative CPK curve was significantly longer in the ligation dogs (17.02 hours ± 3.93, mean ± sd) vs the embolization dogs (11.7 hours ± 2.17) (P < 0.005) (table 2).
TABLE 1A. (Continued)

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<th>RV wt</th>
<th>[CPK_{RV}] IU/g</th>
<th>CPKD(RV) IU</th>
<th>CPK(LV) milU/ml</th>
<th>SER(LV) %</th>
<th>CPK(LV) %</th>
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Relationship of Infarct Size to Cumulative Serum CPK

8. Infarct size = \( \frac{\text{CPK}_r \times \text{DV}}{P_{\text{CPK}} ([\text{CPK}_n] - [\text{CPK}_i])} \) (ref 2)

where \( \text{CPK}_r \) = cumulative serum CPK/ml, \( \text{DV} \) = distribution volume, \( = 0.05 \text{ BW} \) (ref. 2, 3, 8), \( P_{\text{CPK}} \) = proportion of CPK depleted from heart which enters serum (SER), \( [\text{CPK}_n] \) = concentration of CPK in normal myocardium, \( [\text{CPK}_i] \) = concentration of CPK in center of homogeneously infarcted myocardium, \( [\text{CPK}_n] - [\text{CPK}_i] = [\text{CPK}_n] \times 0.815, = 1865 \text{ IU/gm} \times 0.815 \) (from table 1A): Infarct size in grams of left ventricle may be expressed as % LV infarction \( \times \text{LV wt.} \)

The inverse relation of SER to % LV infarction (fig. 2) permits expression of SER as a function of %LV infarction:

9. SER = \(-0.8514 \cdot \text{(%LV)} + 0.345\)

![Figure 2. Relationship between serum entry ratio (SER) and infarct size. Left: ligation dogs. SER in each is plotted against infarct size expressed as %LV (% left ventricle infarcted) depletion. SER = \(-0.8514 \cdot \text{(%LV)} + 0.345\); \( r = 0.98 \). Right: Embolization dogs. SER in each is plotted against infarct size expressed as %LV depletion. There is no significant linear correlation. The regression line for ligation dogs is represented by the dashed line. The SER/%LV ratio exceeds values on this line in all but two dogs.](http://circ.ahajournals.org/Download)
TABLE 1B. Embolization Dogs

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<th>Dog</th>
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<th>r²</th>
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<th>BW (Kg)</th>
<th>CPKr (IU)</th>
<th>[CPKRES(LV)]</th>
<th>LV wt (g)</th>
<th>[CPKRES(LV)]</th>
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<td>1617</td>
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<td>7.9482</td>
<td>24.8</td>
<td>17.86</td>
<td>1786</td>
<td>123.1</td>
<td>0.0053</td>
<td>+203</td>
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</tr>
</tbody>
</table>

Mean ± SD: 0.0053 = ±0.0245; 0.5787 = ±2.6; 0.181 = ±8.3; 0.283 = ±180

Difference from ligation dogs: NS

Abbreviations: Kd = exponential disappearance constant for CPK; r² = correlation coefficient; CPKm = CPK entry/ml serum; BW = body weight; CPKr = total CPK entry to serum; [CPKm(LV)] = normal CPK concentration in LV; CPKRES(LV) = total CPK depleted from LV; [CPKm(RV)] = normal CPK concentration in RV; CPKRES(LV) = total CPK concentration in RV; CPKRES(LV) = mean residual CPK content of LV; [CPKm(SER)] = mean CPK content in center of infarcted zone of LV as % of normal; [CPKm(SER)] = CPK concentration in center of infarcted zone (mean CPKm(LV) of 18.5% in ligation dogs used in embolization dogs); IS(LV) = left ventricle infarct size.

Therefore,

10. CPKr = \( \frac{\text{IS} \times 1685 \times 0.815 \times \text{SER}}{\text{BW} \times 0.05} \)

\( = \frac{\%\text{LV} \times \text{LV} \times 1685 \times 0.815}{\text{BW} \times 0.05} \)

\( \times (-0.8514 \%\text{LV} + 0.345) \)

(from 8 and 9 above)

For a dog with LV/BW ratio of 4.99 g/kg (mean from ligation dogs — table 1A):

11. CPKr = \( \%\text{LV} \times 152 \times 10^9 \)

\( (-0.8514 \%\text{LV} + 0.345) \)

Therefore, for every infarct size, anticipated CPKr may be calculated and plotted as a function of %LV infarction (fig. 3).

In figure 3 the actual values of CPKr, measured in ligation dogs (normalized for LV/BW = 4.99) are plotted against the %LV infarction (determined from myocardial homogenization) and may be compared to
TABLE 1B. (Continued)

<table>
<thead>
<tr>
<th>CPKRES(RV)</th>
<th>RV wt IU/g</th>
<th>[CPKM(RV)] IU</th>
<th>CPK(LV) IU</th>
<th>[CPKI(LV)] IU</th>
<th>SER(LV)</th>
<th>CPK(LV) IU</th>
<th>[CPKI(LV)] IU</th>
<th>IS(LV) g</th>
<th>IS(LV) %</th>
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<td>35.4 g</td>
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<td>33.3</td>
<td>24.3</td>
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</tr>
</tbody>
</table>

± 8.2       ± 5400         ± 12.7      ± 13.7      ± 20.2      ± 13.1

NS         .06 > P > .05   P < 0.025   P < 0.025   NS         NS

FIGURE 3. Cumulative serum CPK (CPKr) calculated (from SER = -0.8514 %LV + 0.345) for various theoretical and experimental infarct sizes. The dashed line represents values of CPKr calculated from the equation CPKr = %LV × 152 × 10^6 (-0.8514 %LV + 0.345), for various theoretical infarct sizes in dogs with LVW/BW = 4.99 g/kg (mean of ligation dogs), and plotted against the corresponding theoretical infarct size. The values of CPKr rise to a maximum at infarct size of 20.3 %LV and then begin to fall. Each dot is a CPK value calculated from serial CPK measurements in an actual ligation dog experiment, normalized for LVW/BW = 4.99 (CPKr × LVW/BW), and then plotted against the corresponding measured infarct size. All values except that for dog six agree closely with the theoretical relationship. Abbreviations: %LV = % left ventricle infarcted; LVW = left ventricle weight; BW = body weight.

The theoretical curve. Agreement is generally close, except for dog 6.

If SER were a constant whatever infarct size, then CPKr would vary linearly with infarct size — an assumption which has been made previously (fig. 4A). However, if there were an inverse linear relationship of SER to infarct size, then a given increment of infarct size would produce a lesser increment of CPKr (fig. 4B). If the inverse relationship were sufficiently steep, as in the present study, the expected curve would be that of figure 3 (duplicated fig. 4C). For each CPKr, there are two infarct sizes lying within the range.
of infarct sizes at which survival is possible. In fact, in the situation illustrated in figure 4B, there would also be two infarct sizes calculated for each CPKr, but the larger value would be an infarct size at which survival is impossible and therefore only one real solution would emerge.

In a noninvasive study to determine infarct size as %LV from CPKr, knowledge of LV weight and BW would permit calculation of infarct size by solution of a quadratic equation of the form:

12. \(a(\%LV)^2 + b(\%LV) + CPKr = 0\) (derived from 10 and 11 above)

Figure 3 is the plot of this equation when LV/BW = 4.99 g/kg. Thus, when infarct size is calculated from CPKr for each ligation dog using this formula, two values are derived (table 3). The value agreeing most closely with that measured from myocardial CPK assays, and most consistent with the clinical course was plotted against the measured value (ISc = 0.931 ISa - 0.772, \(r = 0.93\)) (fig. 5).

**Discussion**

It has been noted since the initial work of Shell et al. that during the course of acute myocardial infarction in dogs, the ratio of the amount of CPK entering the serum compared to the amount of CPK depleted from the myocardium (SER) was rather low. The value was initially reported as 0.3 ± 0.02.1 This figure was subsequently modified to 0.15.2 It has been shown that cardiac lymphatics are the major escape route of myocardial enzymes to the distribution space.6,10 It has been further shown that Kd of CPK in lymphatic fluid is very high,11 and that occluding cardiac lymphatic drainage results in lower fractional escape (SER) of CPK to the plasma.12 It would be reasonable to postulate that prolonged contact of CPK with cardiac lymph during slow transport from the center of a large zone of homogeneous infarction might lead to extensive local destruction. SER would then be low. Conversely, more rapid lymphatic transit over shorter distances from the center of a small infarct would result in relatively less local destruction and therefore a higher SER. Such mechanisms may account for the inverse ratio of SER to infarct size observed in the ligation dogs in the present study.

The initial studies of Shell and Sobel demonstrated a linear correlation of peripheral CPK evolution and myocardial CPK depletion.1 Roe and Starmer have

---

**Table 3. Ligation Dogs**

<table>
<thead>
<tr>
<th>Dog</th>
<th>ISa (% LV)</th>
<th>Myocardial CPK assay</th>
<th>ISc (% LV)</th>
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<th>Solution B</th>
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<td></td>
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</table>

Abbreviations: ISa = actual infarct size; ISc = calculated infarct size; %LV = percent left ventricle infarcted; CPKr = cumulative CPK serum entry.
Deviation of the actual from the theoretical curve of CPKr vs infarct size may be explained by variation about the linear fit of SER vs percent left ventricular infarction \((r = 0.98)\), and by variations of normal myocardial CPK content, myocardial depletion ratio, and PV to body weight ratio. However, the pattern of rising CPK does not find a linear relationship of CPKr to histological infarct size, and concluded that determination of CPKr did not distinguish large from small infarcts. They speculated that a small region of infarction, well-perfused with collateral blood flow, might give rise to a CPK, equivalent to a larger infarct receiving less collateral flow. They pointed out several differences between their study design and that of Shell et al. Thus, in the Roe study, the circumflex artery was ligated rather than the LAD, infarct size estimate was based on myocardial histology rather than CPK depletion, the range of infarct size was 0.1–26.6 g rather than 6–45 CPK gram equivalents, and finally, the estimate was made 5–6 days postinfarct vs 24 hours. The present study, using LAD ligation, infarct size estimation from myocardial CPK depletion, infarct range from 3.9 to 66.5 CPK gram equivalents, and estimation at 48 hours postinfarct onset is analogous in design to the Shell study, yet the relationships of CPKr to infarct size are similar to those in the Roe study.

The embolization experiments resulted in widely scattered infarcts of varying size throughout the myocardium. A given infarct size in such an experiment is the sum of many smaller infarcts, and therefore one would anticipate more rapid transit of the CPK from the central zones of infarction than would be observed from a single homogeneous infarction of the same total size. The mean SER in embolized dogs was found to be significantly greater than in ligation dogs, supporting this reasoning. Further support is provided by the finding of more rapid completion of infarction as determined from the time to reach the plateau of this cumulative CPK curve for embolization dogs.

The SERs for embolization infarcts do not show the same inverse linear relationship to infarct size as in the ligation experiments. The distribution of microspheres is random, and they may clump and produce several large homogeneous areas of infarction in some dogs and more scattered, small infarcts in other dogs. The distribution pattern of infarcts varies for a given total infarct size from one dog to another, and therefore, one might not anticipate a close relationship of SER to infarct size.
The mean Kd for CPK was 0.00187 min\(^{-1}\) ± 0.00051 (mean ± sd) (ligation and embolization dogs were not significantly different). Shell and Sobel noted an in vivo Kd of 0.0045 min\(^{-1}\) ± 0.0010 for injected partially purified dog myocardial CPK.\(^{14}\) We found a closely similar value of 0.0047 min\(^{-1}\) ± 0.0009.\(^{3}\) Thus, the Kd of endogenous CPK is markedly lower than that of exogenous CPK. This phenomenon was first noted by Sobel and Shell\(^{1}\) and has been further commented upon by Roberts\(^{15}\) and by Roe.\(^{4}\) The use of the individual Kd values assumes that f(t) = 0, whereas one cannot be certain of this in the present study. However, the straight-line fit of the data throughout the CPK decay curve is so close, that if some slow ongoing release of CPK were occurring, it is likely to be very small in relation to total CPK.\(_r\). Additional explanations must be sought for the disparity between the Kds of endogenous and exogenous CPK. It may be that the injected CPK preparations are less stable than endogenous CPK.\(^{4}\) The 25% sd observed for injected CPK, and the marked difference from endogenous Kds, makes the use of a fixed mean Kd less desirable. We are using individually calculated Kds as advocated by Norris,\(^{16}\) but recognize the potential limitations.

Roberts demonstrated a 50% reduction of Kd over the 3 hours after i.v. injection of pentobarbital 30 mg/kg.\(^{17}\) The half-life of pentobarbital in dogs is only about 3.5 hours,\(^{18}\) however, and reduction of Kd may therefore be of relatively short duration in relation to the total time of CPK release from the heart, which generally did not begin until 2.5 hours after ligation or embolization. However, the Kd calculated from the terminal portion of the exponential downslope of the serial CPK curve might be higher than the actual Kd at the time of pentobarbital action, and to the extent that this disparity exists, CPK entry into serum would be underestimated. Whatever the influence of the pentobarbital on Kd, it would be identical in the ligation and the embolization dogs, since each was anesthetized immediately before induction of myocardial infarction.

Roe and Starmer\(^{6}\) have emphasized the errors which may occur in the calculation of infarct size because of the variability of Kd, distribution volume (DV), CPK\(_D\) (CPK depletion from infarcted zones), and Ks (fractional entry of CPK to serum or SER or \(P_{CPK}\)). Variability of Kd in the present study emphasizes the necessity to determine this parameter in each dog.\(^{3, 17}\) DV has been shown to be equal to PV\(^3, 17\) which may be estimated from body weight.\(^{8}\) Consideration should be given to determination of PV during infarct size studies. Variability of CPK\(_D\) is a function of the variability of normal myocardial CPK content and of the CPK depletion in infarcted zones. The CPK\(_D\) cannot be estimated noninvasively. In dog ligation experiments, the SER varies inversely with infarct size. Therefore, the measured CPK, could be used to solve a quadratic equation yielding two values for infaract size (left ventricular weight would have to be estimated noninvasively). The clinical and hemodynamic course of the animal should generally permit selection of the appropriate infarct size. Such an approach gives rise to a relatively close relationship between infarct size determined from myocardial CPK depletion and infarct size calculated from CPK, \((r = 0.93)\) (fig. 4).

The cautions advised by Roe et al.\(^{4, 6}\) are warranted. However, their interpretation of the serial CPK data led them to conclude that the serial CPK technique did not distinguish large from small infarcts.\(^{4}\) The findings in the present study indicate that although large infarcts may give rise to cumulative serum CPK entry values no different from certain small infarcts, there is a predictable variation of CPK serum entry with infarct size, permitting calculation of correct infarct size from serial CPK data when the infarct is produced by coronary artery ligation.

The SER is unpredictable in the embolization experiments in the present study, and would also be unpredictable in reperfusion experiments because of washout of CPK.\(^{19, 20}\)

In humans, to the extent that spontaneous myocardial infarction may be considered analogous to ligation experiments, corrections might be made for variation of SER with infarct size. However, the correlation of rising CPK\(_r\) with worsening prognosis already reported in the literature\(^{10, 15}\) suggest that the phenomenon of decreasing values of CPK\(_r\) with larger infarction may not be observed in humans. It is possible that the peak of the CPK\(_r\) vs infarct size curve in humans occurs at infarct sizes at which survival is impossible, so that in patients who survive, infarct size has only a single value for each CPK\(_r\), (fig. 4B). On the other hand, human infarction may not be analogous to simple ligation. There is considerable data indicating that coronary artery thrombus may not underlie many infarctions,\(^{26}\) and therefore the kinetics of CPK release may be different from those in the dog ligation model.

A further problem in humans might arise because some myocardial infarctions, though small, may be diffuse and non-homogeneous,\(^{26}\) a situation analogous to the embolization experiments. Large SERs might occur and might account for the observation by us (unpublished data) and others\(^{25}\) in some patients of high CPK\(_r\) in the absence of power failure. Selection of an appropriate SER for infarct size calculation would not be possible in such a case. Aggregated platelets may be a factor in the causing of human infarction. There is experimental\(^{27}\) and human autopsy\(^{28}\) evidence that platelets may produce scattered myocardial infarction. Such a mechanism of infarction may be simulated by the microsphere embolization model.

Jarmakani et al.\(^{17}\) have considered the possible effects on CPK release of varying degrees of reperfusion in human infarction. Unpredictable changes in degree of coronary occlusion or establishment of collateral flow could produce variation of SER, distorting infarct size calculations.

The findings of our study have major implications for the calculation of infarct size in dogs and in man. We need to individualize, whenever possible, the various parameters of the infarct size equation. The great variation of SER with infarct size and model
necessitates more complex calculations for determination of infarct size and indicates that in some settings, meaningful quantitation may not be possible with this technique. The assumption that human infarction is analogous to coronary ligation infarction of the dog must be examined carefully.

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