A Sensitivity Analysis of Enzymatic Estimation of Infarct Size

SHELL AND COWORKERS present a compartmental mathematical model representing a biological system (acute myocardial infarction) in which CPK appears in the blood and is eventually degraded. From this model they developed a method for estimation of the size of a volume of infarcted myocardium. This method would seem ideally suited for estimating the acute effect of an intervention on infarct size, and several investigators have proposed using it. Up to this time, the literature on enzymatic estimation of infarct size has emphasized the situations in which it should be used. However, there is little discussion of the potential limitation of this method. Therefore, we thought it would be useful to review the original work of Shell and to determine if any limitations existed, with particular emphasis on the sensitivity of infarct size estimation to the observed variations in model parameters.

Mathematical Model

A mathematical model relating serum creatine phosphokinase (CPK) to infarct size has been proposed by Shell and coworkers. The model depicts serum enzyme activity as a function of myocardial release and first order decay. In addition, Shell and coworkers assumed that the releasing function was an unknown function of time. They considered as parameters the decay rate, the fraction of CPK released, the fraction of the body weight equivalent to the enzyme distribution space, and the amount of CPK depleted per gram of myocardium. They then applied this model to laboratory and clinical evaluation by studying dogs subjected to sustained occlusion of the left anterior descending (LAD) coronary artery and patients with uncomplicated acute myocardial infarction.

The following is a brief review of the model as proposed and currently in use in several laboratories. Let \( f(t) \) be an unknown function of time, \( t \), representing myocardial CPK appearance in the distribution space, and let \( E(t) \) be the serum CPK value at time \( T \). In addition, let \( k_d \) be the first order decay parameter (which by convention is negative) which represents CPK disappearance. Then, the rate of change of serum CPK activity \( (dE/dt) \) at any time, \( t \), is equal to the difference between the amount appearing peripherally \( (f(t) - f(t)) \) and the amount eliminated from the distribution space \( (kdE) \):

\[
\frac{dE}{dt} = f(t) + k_d E
\]

The estimation of CPK released from the myocardium between time 0 and time \( T \) is then obtained by integrating \( f(t) \) from \( t = 0 \) to \( t = T \):

\[
\int_0^T f(t)dt = E(T) - k_d \int_0^T E(t)dt
\]

The enzyme appearance function, \( \int_0^T f(t)dt \), can be used to estimate grams of infarcted myocardium when knowledge of the distribution space, the fraction of cellular CPK which appears in blood, and the total amount of CPK contained in a gram of tissue is available. Shell indicated that the distribution space is proportional to the body weight, \( W \), where \( k_w \) is the proportionality parameter. The fraction of releasable
CPK is $k_\text{R}$ and the total CPK per gram of tissue is $CPK_D$. The estimated infarct size in grams is then:

$$\text{Size} = W \int_{0}^{T} f(t)dt \over k_\text{R} \cdot CPK_D$$

$$= W \int_{0}^{T} \left[ E(T) - k_\text{R} \int_{0}^{T} E(t)dt \right].$$

This equation contains four parameters $k_\text{R}$, $k_\text{D}$, and $CPK_D$, the values of which have been determined experimentally in dogs, and have been shown to display variation from subject. These considerations prompted an evaluation of the sensitivity of the calculated infarct size to changes in mean ± one standard deviation for each individual parameter. A one standard deviation change was chosen because it occurs frequently, about 68% of the time, if the parameter variation is gaussian. The effect of this variation is important when planning infarct size intervention studies.

**Application of the Model to Enzyme Data**

The calculations presented in this paper were based on data published in tables I — III of reference 2. For serial enzyme values obtained from table I, reference 2, the initial enzyme value of zero was assumed to occur at 60 minutes and the first nonzero value to occur at 100 minutes. Use of this model implies that when the integral is calculated over a time interval, it theoretically reaches a point at which no additional enzyme is being released into peripheral blood $(f(t) = 0)$. The accumulated enzyme activity then reaches a constant level and represents the total amount of enzyme released. $\int_{0}^{T} f(t)dt\dagger$ in published data does not uniformly become constant. Instead, it sometimes is still either increasing $(f(t) > 0)$ or decreasing $(f(t) < 0)$ when data collection is terminated. This is a reflection of the lack of fit between the theoretical model and the biological system. Since reference 2 did not specify excluding data associated with negative $f(t)$, all data were included in obtaining our integrated values of $f(t)$. Elimination of those intervals where $f(t) < 0$ did not alter discrepancies between reported infarct size estimation based on integration values from table II, reference 2, and those obtained from recalculation.

**Influence of Variation in Parameters on Infarct Size Estimation**

The potential variation of infarct size estimates based on a formula containing four parameters with known standard errors, and therefore, known standard deviations (SD), should be considered critically before interpreting the results of such calculations in either the clinical or the experimental setting. The fractional disappearance rate of CPK ($k_\text{D}$) is a parameter which can impart considerable error into the estimate of the infarct size. For the canine model $k_\text{D}$ has been reported to be $-0.0048 ± 0.0003$ min$^{-1}$ (mean ± se, $N = 11$),\textsuperscript{2} although a value of $-0.0045$ min$^{-1}$ has been recommended as well.\textsuperscript{3} For the human model, $k_\text{D} = -0.0010 ± 0.0001$ min$^{-1}$ (mean ± se, $N = 24$) has been determined from the rate of disappearance of enzyme activity in patients with a single discrete curve of serum CPK activity.\textsuperscript{5} Table 1A depicts the effects of the published $k_\text{D}$ variations on a calculation based on the serial enzyme data of animal 7 (table I, reference 2). The reported values for integrals of CPK appearance and infarct size estimate for this animal disagree with the recalculated integral and infarct size based on the use of the recommended $k_\text{D} = -0.0045$ min$^{-1}$ and the mean $k_\text{D} = -0.0048$ min$^{-1}$.\textsuperscript{2} The range of $k_\text{D} ± 1 \text{sd}$ is $-0.0038$ min$^{-1}$ to $-0.0058$ min$^{-1}$, which results in a variation of the calculated infarct size of 46-66 gram-equivalents, or ±19% of the mean value. Using the reported mean $k_\text{D}$ value for the dog, the recalculated infarct size is 57 grams, not 27 grams, as reported. Similarly, $k_\text{D}$ determined in dogs with decreased cardiac output, isoproterenol (isuprel) administration, and pentobarbital anesthesia impart major alterations in infarct estimation when applied to the same animal's serial enzyme values (range 20-103 gram-equivalents). Using the same serial enzyme values in a 70 kg man and the reported human values of $k_\text{D} ± 1 \text{sd}$ results in a nine gram, or ±15% error from the mean value.

The value of $k_\text{D}$ is critical for an accurate assessment of damage. The determination of $k_\text{D}$ by injection experiments is extremely difficult due to elimination of enzyme material with short half-life during purification, but other factors contribute to this difference as well.\textsuperscript{4} In addition, when attempts are made to ascertain the value of $k_\text{D}$ from the enzyme curve of an individual patient or experimental animal, it must be restricted to those time intervals where $f(t)$ is 0, which corresponds to those times when myocardial enzyme release is zero. If this limitation is not observed, the derived $k_\text{D}$ will be in error since it was obtained from data which included enzyme release. More importantly, the influence of pharmacologic interventions (isoproterenol and pentobarbital) and pathophysiologic phenomena (decreased cardiac output) contribute to variation of $k_\text{D}$, which produces significant discrepancy in calculated infarct size. Knowledge of variations of the $k_\text{D}$ value as it is affected by pharmacologic agents and manipulations is, therefore, one of the prerequisites to the accurate assessment of infarct size.
The calculated infarct size is also sensitive to variation in the myocardial CPK depletion parameter (CPKD) (table 1B). That value in the human is 540 IU ± 38 IU (mean ± se, N = 5). This has a range of ±1 se of 455-625 I.U./g (mean ± 15.7%) with the corresponding span of infarct sizes of 52-71 gram-equivalents. This potential deviation of 15.7% from the mean applied to the mean value of 800 I.U./gm in an animal with all other parameters as mean values results in an infarct size range of 46-63 gram-equivalents. Variation in CPKD is due to two primary factors, namely, individual variation from one heart to another and technical variation related to homogenization and assay.

Calculation of the distribution space involves a parameter (kw) of 11.4% ± 0.1% (mean ± se, N = 11), which has a range for 1 se of 11.1% — 11.7%. This percent of body weight, in grams, represents the volume in which CPK is distributed. The range of infarct size based on enzyme values from animal 7 (table I, reference 2) is quite small, 52-55 gram-equivalents. A similar range is described when the factor is applied to human data.

The fourth parameter, the proportion of enzyme from myocardium (kn) which appears in the distribution space has been determined (0.30 ± 0.02, mean ± se, N = 22). The range for 1 se of this mean is 0.21-0.39. Applying these values to the serial enzymes of animal 7 (table I, reference 2), with all other parameters assigned mean values, produces an infarct estimate ranging from 41-76 grams. The same analysis in the patient weighing 70 kg is an infarct measuring from 46-86 grams. Table 2 illustrates the variation in fractional release as recalculated from the data in reference 2. This proportion (CPKR/CPKD = kR) can be determined from the published serial enzyme values, integrals, body weights, grams infarct (based on myocardial depletion), and the recommended mean parameters.

From these data, estimation of infarct sizes and peripheral recovery of enzyme (CPKR/CPKD) can be determined. The proportion of enzyme which appears peripherally, as recalculated from published data, is 32.2% ± 7.6% (mean ± 1 se), with a range of absolute recoveries of 20%-46%. However, when ∫₀⁰ tf(t)dt is calculated from the available data, a significant discrepancy can be noted between the published and the recalculated integral values for dogs 4-8. The correct values in these animals are associated with the most significant differences in the calculation of the CPKR/CPKD. The mean ± 1 se is equal to 45.7% ± 17.3%, with a range of values extending from 23%-76% recovery in the distribution space.

The recalculated CPKR/CPKD was determined without utilizing the percent recovery parameter, and the computation of ∫₀⁰ tf(t)dt involved only the reported serial enzyme values and the recommended kn of -0.0045 min⁻¹. Therefore, to achieve the lower integrated values from the published serial enzyme values, it would be necessary to use a kn less than the reported mean minus 1 se. The possibility of a wide variation in CPKkn/CPKD suggests that disproportionate amounts of CPK may be released compared to the actual amount of damage sustained by the myocardium. In figure 1, both the published and the recalculated infarct size estimates are compared with the extent of necrosis determined by myocardial CPK depletion. The coefficient of correlation is 0.95 for

Table 1

| Table 1 |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| **Effects of Reported Variation in Constants on Infarct Size Quantitation** |
| **Canine** | **Human** | **Mean - 1 se** | **Mean + 1 se** | **20-25% C.O.** | **Isuprel** |
| **Reported** | **Recalc.** | **Stated mean** | **Mean - 1 se** | **Mean + 1 se** | **Isuprel** |
| kₙ | kₙ | kₙ | kₙ | kₙ | kₙ |
| Inf. size (g) | Inf. size (g) | Inf. size (g) | Inf. size (g) | Inf. size (g) | Inf. size (g) |
| CPKₕ | CPKₕ | CPKₕ | CPKₕ | CPKₕ | CPKₕ |
| Mean | Mean | Mean | Mean | Mean | Mean |
| 800 | 540 | 540 | 540 | 540 | 540 |
| 674 | 455 | 455 | 455 | 455 | 455 |
| 926 | 625 | 625 | 625 | 625 | 625 |
| 53 | 51 | 51 | 51 | 51 | 51 |
| 63 | 69 | 69 | 69 | 69 | 69 |
| 46 | 52 | 52 | 52 | 52 | 52 |

*Serial enzyme data and all constants utilized in these calculations are those of animal 7 (table I, reference 2). †C.O. = cardiac output decreased. ‡kₙ values, CPKₙ/g = 540, 11.4% × body weight, and 30% appearance ratio obtained from reference 3. §sd of CPKₙ/g infarct = 800 derived from 15.7% variation from mean for human CPKₙ/g = ± sd.
Table 2

<table>
<thead>
<tr>
<th>Animal</th>
<th>C-1</th>
<th>C-2</th>
<th>C-3</th>
<th>C-4</th>
<th>C-5</th>
<th>C-6</th>
<th>C-7</th>
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<th>C-10</th>
<th>C-11</th>
</tr>
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Calculations from Published Data

\[ \int_{0}^{\infty} f(t) \, dt \]

Inf. size (g) | 16 | 29 | 32 | 5 | 8 | 7 | 27 | 23 | 55 | 44 | 14 |
CPK<sub>R</sub>/CPK<sub>DT</sub> (% recovery) | 34 | 26 | 46 | 20 | 39 | 27 | 31 | 33 | 36 | 39 | 23 |
Mean ± 1 sd (32.2% ± 7.6%) Range of values 20-46%

Recalculations from Published Data

\[ \int_{0}^{\infty} f(t) \, dt \]

Inf. size (g) | 16 | 31 | 63 | 8 | 15 | 15 | 53 | 44 | 56 | 40 | 14 |
CPK<sub>R</sub>/CPK<sub>DT</sub> (% recovery) | 34 | 27 | 55 | 33 | 76 | 56 | 62 | 63 | 37 | 35 | 23 |
Mean ± 1 sd (45.7% ± 17.3%) Range of values 23-76%

*Values from table II in Reference 2.
†CPK<sub>R</sub>/CPK<sub>DT</sub> is calculated from: CPK<sub>R</sub> = \[ \int_{0}^{\infty} f(t) \, dt \times [11.4\% \text{ Body Weight}] \]; CPK<sub>DT</sub> = 800 IU × gm

Infarct Size (depletion estimate from table III, Ref. 2). Quotient = % of CPK<sub>DT</sub> released into the distribution space.

The relationship between infarct size determined on the basis of changes in serum CPK activity (ordinate) and infarct size determined on the basis of depletion of myocardial CPK activity 24 hours after coronary artery occlusion (abscissa). Infarct sizes obtained by recalculation from serum enzyme data in reference 2 are indicated by (○). Infarct sizes from serum enzyme data as reported are indicated by (●). Infarct size based on CPK<sub>DT</sub> values are obtained from table III, reference 2. The theoretically perfect correlation is indicated by the solid line between the two axes. Results from published and recalculated infarct sizes fit the following regression lines (least squares method): Infarct Size estimate (serum CPK) = 1.29 × CPK<sub>i</sub> - 3.54, r = 0.95, N = 11 for published values and Infarct Size estimate (serum CPK) = 1.24 × CPK<sub>i</sub> + 4.43, r = 0.82, N = 11 for the recalculated values.

The release of disproportionate amounts of CPK compared to anatomically determined infarct size following acute coronary occlusion has been observed in both reperfused and permanently occluded animals. Reperfusion of an occluded coronary artery produces an immediate and rapid increase in serum enzymes characterized by myocardial isoenzyme patterns. Prediction of infarct size from the early portion of the curve would indicate an extremely large infarct. In fact, such a release profile is associated with a small infarct by anatomic estimation. Rapid release of enzymes following reperfusion of damaged myocardium has received little attention, and is generally considered an insignificant "wash-out" phenomenon with no clinical relevance. However, it is associated with relatively small volumes of infarction.
studies have suggested that a significant proportion of enzymes released from myocardium following coronary occlusion escapes to the vascular space via coronary lymphatic drainage. The mode of enzyme escape from myocardium and the factors which affect this release are important for an understanding of changes in serum levels. A recent study by Gervin et al. has clearly demonstrated that cardiac lymphatics are the major escape route of myocardial enzymes to the distribution space. In animals subjected to temporary occlusion of the LAD with collection of cardiac lymph during and following reperfusion, the enzyme and isoenzyme levels of CPK and LDH in samples obtained from peripheral vascular sites failed to reflect myocardial necrosis for up to three hours following reperfusion. Simultaneous analysis of cardiac lymph revealed marked elevations of enzyme levels and diagnostic isoenzyme patterns for myocardial necrosis after only 30 minutes of coronary occlusion, yet prior to reperfusion. Upon reperfusion, CPK in the diverted coronary lymph increased within two hours to peak levels as high as 100,000 I.U./L, while no significant change was noted in peripheral blood. The influence of coronary perfusion on interstitial fluid flow from the myocardium is then obvious. If lymph flow were not diverted from the circulation, the anticipated result would be a sudden and extreme increase in serum levels. This "wash-out" would then be associated with a limited anatomic infarct. Mathematical estimation or prediction of infarct size in this context overestimates, in a nonpredictable manner, the actual extent of necrosis.

It is, therefore, conceivable that a successful mechanical or pharmacologic intervention may be associated with deceptive elevations of serum enzyme levels. The proportion of CPK escaping from the ischemic tissue would then be significantly greater than 30% of that released into the myocardial interstitium, and would be associated with less anatomic damage. The sudden appearance of released enzymes in serum could signify more than diffusion from the tissue. It, logically, represents more adequate perfusion of the ischemic region rather than the accepted interpretation of extension of damage.

The model on which the formulation is based is, then, an oversimplification of a highly complex biologic system. Since the mathematical model is so sensitive to variation in the model parameters and fails to fit the biologic system, the calculation of infarct size should be applied with the understanding that, at best, only qualitative information will be produced. However, it must also be realized that therapeutic interventions themselves provide significant distortion that would produce erroneous clinical interpretation. More knowledge is necessary regarding perturbations of \( k_d, CPK_R/CBK_D \), and the other parameters to validate infarct size estimation before prediction of infarct size calculated from initial serial enzyme data becomes appropriate.

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C. Frank Starmer, Ph.D.

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