The Nature of Disappearance of Creatine Kinase from the Circulation and Its Influence on Enzymatic Estimation of Infarct Size

BURTON E. SOBEL, JOANNE MARKHAM, RONALD P. KARLSBERG, AND ROBERT ROBERTS

SUMMARY Continued progress in estimating myocardial ischemic injury from analysis of plasma enzyme time-activity curves requires improved characterization of processes affecting release from the heart, transport, and disappearance from the circulation. To determine whether the true disappearance rate (k₃) of creatine kinase (CK) is accurately reflected by the rate of elimination (k₄) from blood, we evaluated time-activity curves in 40 conscious dogs after induced myocardial infarction, bolus injection, or slow intravenous infusion of CK extracted from myocardium and CK harvested from plasma. The two CK preparations were compared by cellulose acetate electrophoresis, radioimmunooassay, gel chromatography, and stability in vitro. Plasma CK time-activity curves after intravenous injections of CK conformed more closely to double than to single-exponential curves (with average standard deviations only 42% as large), suggesting distribution in at least one extravascular compartment. Parameters of a two-compartment model obtained from the double-exponential curve provided estimates of k₄ markedly greater than k₃. Calculations based on observed plasma values and these estimates of k₄ accounted for 2-3 fold more CK released from the heart after infarction than that accounted for by calculations utilizing k₃. The decline of plasma CK after myocardial infarction was 60% slower than the decline after intravenous injections of enzyme. The relatively slow decline after myocardial infarction appears to be due both to differences between enzyme extracted from the heart and enzyme released endogenously into plasma and to continuing release of CK from the ischemic heart relatively late after coronary occlusion.

INFARCT SIZE has been estimated from plasma creatine kinase (CK) time-activity curves with a simple model attributing serial changes in plasma CK activity to release from the heart and concomitant disappearance from the circulation. However, several factors are not encompassed by this approach including: (1) potential contributions of isoenzymes of CK with different disappearance rates to plasma CK activity after myocardial infarction; (2) potential variations of the true disappearance rate of CK within the same experimental animal or patient during the interval of study; (3) the relatively poor conformity of CK time-activity curves after intravenous injection to single-exponential fits; and (4) the possibility that CK, like many other proteins, is distributed in at least one extravascular compartment.

With the development of quantitative assays for CK isoenzymes, the first pitfall could be avoided by analyzing MB ('myocardial') CK time-activity curves rather than total CK curves in patients. In dogs, since myocardium contains only a modest amount of MB (<2%) and since plasma CK activity after infarction is attributable primarily...
to MM CK, errors introduced in enzymatic estimation of infarct size due to variance between disappearance rates of isoenzymes of CK are likely to be modest.

Effects of hemodynamic alterations simulating those associated with complicated myocardial infarction, myocardi al infarction itself, and of selected pharmacological interventions on CK disappearance have been evaluated experimentally. Although CK disappearance may be affected by these and other physiological disturbances, changes observed have been relatively small.

In studies in conscious dogs, the disappearance rate ($k_d$) of CK from the circulation, a parameter needed for estimates of infarct size, has been calculated from single-exponential fits to plasma CK values after intravenous injection of canine myocardial CK. In clinical studies, $k_d$ has been estimated from analysis of the terminal portions of time-activity curves after myocardial infarction. Because frequent intravenous injection of purified CK in patients is not practical. However, neither estimate of $k_d$ may be ideal. The rate of disappearance of enzyme activity after myocardial infarction may differ substantially from disappearance of enzyme activity after intravenous injection of partially purified myocardial CK. Furthermore, the observed rate of decline of plasma CK after infarction may not necessarily be the same as the true disappearance rate of enzyme activity because of continued release of CK from the heart or resupply of the vascular compartment from an extravascular distribution space. Accordingly, the present study was undertaken to compare disappearance of enzyme from the circulation after intravenous injection and after myocardial infarction. Because results after intravenous injection differed substantially with CK extracted from myocardium compared to CK extracted from plasma, the two preparations were compared by cellulose acetate electrophoresis, radioimmunoassay, gel chromatography, and assay of stability during incubation in vitro. Plasma time-activity curves after bolus injection of both types of preparation conformed more closely to double- than to single-exponential curves, suggesting that CK may be distributed in at least one extravascular pool, in keeping with evidence that after infarction, enzyme is distributed in lymph as well as blood. Results obtained with a two-compartment model utilizing parameters estimated from double-exponential fits provide estimates of CK disappearance that would account for more CK released from the heart and appearing in the blood after infarction calculated from activity in plasma than that accounted for previously.

**Methods**

**BIOCHEMICAL PROCEDURES**

CK activity was assayed in tissue extracts and plasma samples spectrophotometrically. Canine myocardial CK was extracted and partially purified (at least 150-fold) by ethanol fractionation and batch adsorption with DEAE-Sephadex A-50 under conditions in which only MM CK is isolated, judging from electrophoresis of the final product on cellulose acetate. The exclusive extraction of MM is not surprising in view of the small proportion (approximately 2%) of canine myocardial CK contributed by isoenzymes other than MM. For experiments with CK obtained from plasma, enzyme was obtained from conscious donor dogs to avoid possible effects of anesthetic agents on circulating CK. Plasma CK was elevated by at least 10-fold in donor dogs as follows: After blood was anticoagulated with 0.005 M neutralized ethylene glycol bis (β-aminooethyl ether)N,N,N′,N′-tetraacetic acid(EGTA), 95% ethanol was added dropwise to plasma at 0–4°C (maintained for all preparative steps) to a final concentration of 50% and the mixture stirred slowly for 30 minutes and centrifuged at 2000 g for 15 minutes. Ethanol was added to a final concentration of 70% in the supernatant fraction, and this mixture was stirred for 30 minutes and centrifuged. The pellet, containing CK, was resuspended in 0.05 M Tris-HCl, pH 7.4, 0.001 M mercaptoethanol in a volume equal to the initial of plasma and centrifuged again. NaCl was added to the supernatant fraction to yield a concentration of 0.05 M prior to adsorption of the mixture for 40 minutes with DEAE-Sephadex A-50 (66 ml of gel-100 ml of initial plasma volume), previously swollen in 0.05 M Tris-HCl, pH 7.4, 0.05 M NaCl, and 1 mM mercaptoethanol. After filtration through Whatman no.1 paper, the filtrate was dialyzed for 48 hours against 0.01 M glycine-NaOH, pH 9.0. Precipitate formed during the dialysis was removed by centrifugation at 12,000 g and the supernatant fraction which contained CK was freeze-dried. The final specific activity of the preparation exceeded 100 IU/mg protein.

For use in other experiments, myocardial CK was obtained from crude extracts of normal myocardium and from zones of infarction identified by gross inspection 10 hours after coronary occlusion. Myocardium was excised within 10 minutes after the dog was killed with an overdose of intravenous sodium pentobarbital, and the tissue was squeezed through a muscle press. This crude homogenate was diluted in normal saline prior to intravenous administration to recipient dogs. This type of extract was used to avoid possible effects of purification on kinetics of myocardial CK injected into the circulation. Potential contribution of myokinase to apparent CK activity in plasma were excluded by evaluating plasma samples both with and without creatine phosphate as substrate.

For comparison in vitro, purified myocardial CK and CK extracted from plasma were analyzed by cellulose acetate electrophoresis. Sephadex gel chromatography (to obtain approximate values for molecular weight), radioimmunoassay, and stability at 37°C, pH 7.4, in serum that had previously been heat-inactivated at 55°C for 30 minutes.

**ANIMAL PREPARATIONS**

All studies of time-activity curves were performed in conscious rather than anesthetized dogs because of the marked inhibitory effect of anesthesia on CK disappearance in vivo. For studies of plasma CK time-activity curves after intravenous injection of myocardial CK or CK extracted from plasma of donor dogs, enzyme was injected through a jugular venous catheter. Blood samples were obtained every 2 minutes for 10 minutes, every 5
minutes for 20 minutes, every 20 minutes for 2 hours, and then every 30 minutes for 6-17 hours. Blood samples from the recipient dog were collected in 0.005 M neutralized EGTA and centrifuged at 2000 g for 10 minutes prior to addition of mercaptoethanol to the supernatant fraction in a final concentration of 0.005 M, stored at 0-4°C, and assayed within 24 hours.

For studies of plasma CK time-activity curves after myocardial infarction, an exteriorized snare was placed around the left anterior descending coronary artery 1 week before each study in dogs anesthetized with sodium thiopental, 10 mg/kg, and 0.05% fluothane. After plasma CK had returned to normal after surgery, coronary occlusion was produced in each conscious dog and plasma samples were obtained serially via an indwelling jugular venous cannula.

ANALYSIS OF RESULTS WITH A TWO-COMPARTMENT MODEL

Despite the convenience of evaluating plasma CK time-activity curves with a one-compartment model, the behavior of several proteins, including CK, may be better described with multiple compartmental models, since the proteins may be distributed in extravascular as well as vascular pools. In the present study, we compared fits with single and double exponentials. The double-exponential function was used to obtain parameters for a two-compartment model (Fig. 1) assuming distribution of CK in an extravascular compartment with first order transfer rates between blood and extravascular compartments and a first order elimination rate from blood.

With this model, the amount of CK present in the vascular and extravascular compartments as a function of time can be described by the following system of differential equations in which g(t) is any input function:

\[
\frac{dE_v(t)}{dt} = \lambda_{ve}E_v(t) - (\lambda_{ev} + \lambda_v)E_v(t) + g(t)
\]

\[
\frac{dE_e(t)}{dt} = \lambda_{ev}E_v(t) - \lambda_vE_e(t).
\]

The solution to this system for the vascular pool under conditions in which CK is administered by bolus intravenous injection and g(t) is the function accounting for maintenance of normal plasma CK activity is of the form:

\[
E_v(t) = A e^{-\alpha t} + B e^{-\beta t} + C
\]

where C is the normal plasma CK level and the variables A, B, \alpha, and \beta are functions of the rate constants and the initial conditions, i.e., the amount of CK present in the compartments at time zero. The four parameters of the solution can be obtained experimentally from double-exponential fits to the plasma CK time-activity curves following bolus injections. From these fits, the rate constants can then be calculated from the following formulas:

\[
\lambda_\alpha = \frac{\alpha \beta (A + B)}{A\beta + B\alpha}
\]

\[
\lambda_{ve} = \frac{A\beta + B\alpha}{A + B}
\]

\[
\lambda_v = \frac{A\alpha + B\beta}{A + B} - \lambda_v.
\]

To compare behavior of CK extracted from heart muscle and CK extracted from plasma of dogs with experimentally induced myocardial infarction, parameters in this model can be obtained from best-fit double-exponential curves after intravenous injection of each preparation. The two-compartment model provides an estimate of the disappearance rate of CK from the circulation that is substantially greater than the observed rate of decline of activity in serial samples. This is because the model takes into account continuing supply of the vascular pool with enzyme from the extravascular compartment. Since enzymatic estimates of infarct size depend on the true disappearance rate as one parameter, the two-compartment model would account for more CK released from the heart based on calculations with a given plasma curve than the one-compartment model.

Results

CHARACTERIZATION OF CK DISAPPEARANCE WITH A TWO-COMPARTMENT MODEL

In our initial approach to enzymatic estimation of infarct size in experimental dogs, we estimated \( k_v \) from single-exponential fits to plasma CK values obtained after intravenous injection of partially purified canine myocardial CK. However, CK values during the first hour after infarction conformed poorly to a single exponential, and
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As can be seen in Figure 2, the plasma CK time-activity curve after intravenous injection of purified myocardial CK conforms much more closely to a double-exponential than to a single-exponential fit. Results from six experiments are summarized in Table 1. As can be seen, the overall standard deviation of the fit curves from the data are only 42% as large on the average with double-exponential compared to single-exponential fits.

As shown in Table 2, values for the parameter \( \lambda_v \), the true disappearance rate of CK, differ substantially from the values of the slope of the observed plasma CK time-activity curve because resupply of the vascular pool from an extravascular compartment is taken into account by the model.

**PLASMA CK TIME-ACTIVITY CURVES AFTER MYOCARDIAL INFARCTION**

Construction of the left anterior descending coronary artery in a conscious dog results in a plasma CK time-activity curve that peaks typically within 7-14 hours and declines slowly generally reaching a value of 10% of peak or less within 30 hours. Results in Table 3 were obtained by analyzing the declining portion of such curves from 18 conscious dogs subjected to coronary occlusion that survived for at least 24 hours. The estimate of the rate of disappearance was obtained from the slope of the best-fit straight line (least squares method) from a plot of ln CK vs. time. The correlation coefficient of the fit in each case exceeded 0.91. In the fitting procedure, the initial value selected was 80% of peak CK and the last value used was

**TABLE 1** Overall Standard Deviation between Data and Fit Curves*

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>Single-exponential fit (%)</th>
<th>Double-exponential fit (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20.8</td>
<td>3.6</td>
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<tr>
<td>2</td>
<td>10.4</td>
<td>6.7</td>
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<tr>
<td>3</td>
<td>15.9</td>
<td>5.6</td>
</tr>
<tr>
<td>4</td>
<td>18.2</td>
<td>7.3</td>
</tr>
<tr>
<td>5</td>
<td>15.4</td>
<td>10.0</td>
</tr>
<tr>
<td>6</td>
<td>10.3</td>
<td>5.2</td>
</tr>
<tr>
<td>Mean</td>
<td>15.2</td>
<td>6.4</td>
</tr>
</tbody>
</table>

* Expressed as the percentage of the data point values in each case.

**TABLE 2** Parameters Estimated from One- and Two-Compartment Models

<table>
<thead>
<tr>
<th>Dog no.</th>
<th>( \lambda_v )</th>
<th>( \lambda_s )</th>
<th>( \lambda_x )</th>
<th>Extravascular space( * ) (%)</th>
<th>( k )*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.023</td>
<td>0.018</td>
<td>0.012</td>
<td>79</td>
<td>0.005</td>
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<tr>
<td>2</td>
<td>0.021</td>
<td>0.008</td>
<td>0.009</td>
<td>37</td>
<td>0.006</td>
</tr>
<tr>
<td>3</td>
<td>0.005</td>
<td>0.003</td>
<td>0.006</td>
<td>46</td>
<td>0.005</td>
</tr>
<tr>
<td>4</td>
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<td>0.021</td>
<td>0.011</td>
<td>56</td>
<td>0.005</td>
</tr>
<tr>
<td>5</td>
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<td>0.006</td>
<td>0.009</td>
<td>38</td>
<td>0.007</td>
</tr>
<tr>
<td>6</td>
<td>0.020</td>
<td>0.007</td>
<td>0.006</td>
<td>35</td>
<td>0.005</td>
</tr>
<tr>
<td>Mean</td>
<td>0.020</td>
<td>0.010</td>
<td>0.009</td>
<td>48</td>
<td>0.005</td>
</tr>
</tbody>
</table>

* \( \lambda_v \) in this model estimates the true disappearance rate (k); \( \lambda_s \) and \( \lambda_x \) are exchange rates from vascular to extravascular and from extravascular to vascular pools.

**TABLE 3** The Decline of Plasma Creatine Kinase (CK) Activity after Myocardial Infarction

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Plasma CK* at 80% of peak (IU/liter)</th>
<th>Slope† (min⁻¹)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>1403</td>
<td>0.00258</td>
</tr>
<tr>
<td>2</td>
<td>1040</td>
<td>0.00163</td>
</tr>
<tr>
<td>3</td>
<td>1544</td>
<td>0.00188</td>
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<tr>
<td>4</td>
<td>1347</td>
<td>0.00127</td>
</tr>
<tr>
<td>5</td>
<td>1132</td>
<td>0.00113</td>
</tr>
<tr>
<td>6</td>
<td>1089</td>
<td>0.00155</td>
</tr>
<tr>
<td>7</td>
<td>2565</td>
<td>0.00111</td>
</tr>
<tr>
<td>8</td>
<td>636</td>
<td>0.00125</td>
</tr>
<tr>
<td>9</td>
<td>2128</td>
<td>0.00240</td>
</tr>
<tr>
<td>10</td>
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</tr>
<tr>
<td>11</td>
<td>288</td>
<td>0.00188</td>
</tr>
<tr>
<td>12</td>
<td>890</td>
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</tr>
<tr>
<td>13</td>
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<td>0.00168</td>
</tr>
<tr>
<td>14</td>
<td>259</td>
<td>0.00120</td>
</tr>
<tr>
<td>15</td>
<td>479</td>
<td>0.00312</td>
</tr>
<tr>
<td>16</td>
<td>1729</td>
<td>0.00167</td>
</tr>
<tr>
<td>17</td>
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</tr>
<tr>
<td>18</td>
<td>338</td>
<td>0.00365</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>0.0018</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td>0.0007</td>
</tr>
</tbody>
</table>

* These values are included to indicate the wide range of apparent infarct size.
† Obtained from the plot of ln CK vs. time by least squares approximation.
the last value obtained or the first value with activity 
<100 mIU/ml. As can be seen in Table 3, the average 
rate of decline of CK activity after myocardial infarction 
is substantially less than 0.005 min⁻¹, the average rate of 
decline, calculated in the same fashion, after bolus injec-
tion of partially purified myocardial CK (Table 2). As 
can be seen in Figure 3, in experiments in the same dog, 
the disparity between the slope of the curve after myocar-
dial infarction and the rate of decline of plasma CK 
activity after bolus injection is striking.

**PLASMA CK TIME-ACTIVITY CURVES AFTER 
INTRAVENOUS INJECTION OF ENZYME IN CRUDE 
EXTRACTS OF MYOCARDIUM OR CK HARVESTED 
FROM DONOR DOG PLASMA**

Because the disparity between decline of CK after 
infarction and decline after injection of purified myocar-
dial CK could have been due to alterations in CK associ-
ated with purification, additional experiments were per-
formed with CK in crude extracts prepared from normal 
myocardium and zones of infarction. As can be seen in 
Figure 4, the rate of decline of plasma CK activity after 
bolus injection of a crude extract of normal myocardium 
was similar to that after bolus injection of purified my-
ocardial CK in the same dog studied 1 week later and 
substantially more rapid than the average rate of decline 
calculated from descending limbs of plasma CK time-
activity curves after myocardial infarction in 18 other 
dogs (Table 3). Similar results with an average \( k_d \) after 

![Figure 3](image-url)  
**Figure 3** Disappearance of plasma creatine kinase (CK) activity after myocardial infarction (MI) and after bolus injection of purified myocardial CK 1 week previously in the same conscious dog. The disappearance curve after infarction plotted is the termi-
 nal portion of the plasma CK time-activity curve obtained follow-
 ing coronary occlusion beginning with the first CK data value 
which was less than 80% of the peak CK value. Similar results 
were obtained in each of three other experiments, regardless of 
whether the bolus injection preceded or succeeded myocardial 
infarction.

Injection of 0.0082 were obtained in five analogous exper-
iments with injection and infarction in the same dog. In 
two of these experiments, extracts were prepared from 
zones of infarction rather than from normal myocardium. 
These results suggest that purification of myocardial CK 
does not account for the difference in disappearance after 
intravenous injection compared to the rate seen after 
infarction.

On the other hand, release of CK into the plasma from 
ischemic myocardium in vivo may alter the molecule such 
that slower disappearance is observed. To evaluate this 
possibility, we harvested CK in plasma from donor dogs 
subjected to myocardial infarction and injected the plasma 
into recipients. To obviate possible effects of anesthesia 
in the donor plasma, infarction was produced in conscious 
dogs that then were bled when plasma CK activity was 
markedly elevated. Five hundred to 1000 IU of CK 
concentrated from plasma in a volume of 10 ml (\( n = 4 \)) 
or in larger volumes were administered to recipient dogs, 
after which plasma CK time-activity curves were analyzed. 
Ten-milliliter volumes were utilized to avoid effects of 
marked expansion of vascular volume on results. In an-
other experiment, an injectate of larger volume (114 ml 
containing 1000 IU of CK) was used to avoid possible 
effects of concentration of CK in the injectate on the 
kinetiles of CK in the circulation of the recipient. Prepara-
tions of CK extract were used within 12 hours after 
storage at 0-4°C. The average rate of decline of plasma 
CK activity in all four experiments with 10-ml injectates, 
calculated from the slope of the best-fit line of the plot of 
\( \ln \) CK vs. time, was 0.004 ± 0.002 (SD) min⁻¹, less then 
50% the rate of decline of activity after bolus injections 
of purified myocardial CK in the same dog in each case. 
Slow disappearance (<50% of that seen after injections 
of myocardial CK) was seen also in the experiment with 
the 114-ml injectate. These results suggest that native CK 
released into the circulation and harvested from plasma 
behaves differently from CK prepared from myocardium.
THE SHAPE OF DISAPPEARANCE CURVES OF CK HARVESTED FROM PLASMA

As can be seen in Figure 5, disappearance curves of CK extracted from plasma and CK extracted from myocardium injected intravenously in the same recipient dog 1 week later exhibit similar shapes, although the rate of disappearance is substantially less with CK extracted from plasma. Data after both injections are fit better by a two-exponential, compared to a single-exponential curve. In each of two other dogs, similar results were obtained but the injection of myocardial CK was performed first. Estimates of the true disappearance rate ($\lambda_c$) of CK harvested from plasma and injected intravenously obtained with the two-compartment model were 44% less than the value for the same parameter after intravenous injection of purified myocardial CK in these three dogs.

POTENTIAL CONTRIBUTORS TO THE OBSERVED DIFFERENCES IN DISAPPEARANCE RATE

Biochemical Differences

Disappearance rates of several purified proteins injected intravenously differ markedly in comparison to the physiological turnover rates of the corresponding proteins. In general, these differences cannot be attributed to changes in primary structure, but appear to reflect subtle alterations in conformation of prosthetic groups. In the present study, results of several experiments in vitro indicate that CK extracted from plasma remained quite similar to CK extracted from myocardium. Addition of EGTA (added to plasma) to myocardial CK preparations did not change results. The ratio of enzyme activity to displacement binding of plasma myocardial CK in a recently developed radioimmunoassay system was virtually identical with an average of 57% and 53% with 0.16 IU/liter; and 83% and 82% with 1.6 IU/liter with purified compared to native enzyme ($n = 4$ experiments with each). Electrophoretic mobility of both preparations was the same on cellulose acetate at pH 8.8. The elution profile of CK enzyme activity on G-150 Sephadex columns was the same when preparations extracted from plasma were compared to those extracted from myocardium (Fig. 6), with approximate molecular weight of CK in the two preparations of 85,000 daltons.

We previously have shown that CK activity declines more slowly when enzyme is incubated in plasma or serum in vitro compared to the rate of decline in vivo after bolus injection intravenously or after experimentally induced myocardial infarction. When CK extracted from plasma was incubated in heat-inactivated serum in vitro, the rate of decline of activity (10% in 2 hours) was comparable to that seen with CK extracted from myocardium incubated under the same conditions.

Thus, the striking disparity between rates of disappearance in vivo of CK extracted from plasma compared to CK extracted from myocardium is not accompanied by gross changes in binding to antibody, charge and migration in an electrophoretic field, molecular weight, or lability in vitro.

Slow Filling of an Extravascular Compartment

CK disappearance after myocardial infarction might be slowed by resupply of the vascular pool form an extravascular compartment that fills slowly. To simulate conditions that might be required for filling of such a compartment, CK was injected repeatedly intravenously in the same normal conscious dog prior to evaluation of the plasma CK time-activity curve. Purified myocardial CK (>110 IU/mg) was injected in doses of 120 IU/kg body weight every hour for 10 hours with persistent and marked elevations of plasma CK resulting throughout the interval. CK disappearance was evaluated beginning at the time when plasma CK activity was maximum after the last injection (1433 mIU/ml) and continuing for 7 hours and until CK activity had returned to <150 mIU/ml. The rate of disappearance observed under these conditions was 0.006 min$^{-1}$ (based on a single-exponential fit), substan-

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**Figure 5** A = The disappearance of purified, myocardial CK injected in a conscious dog; B = the disappearance of CK extracted from plasma injected intravenously in the same recipient dog.

**Figure 6** The mobility of CK harvested from plasma and CK purified from myocardium on Sephadex G-150 in relation to markers of known molecular weight. As can be seen, apparent molecular weight, estimated from mobilities of the preparations in 0.05 M Tris, pH 7.5, and 0.1 M HCl at a flow rate of 12 ml/hr was identical for CK in the two preparations. (BD, LDH, and CYT C = blue dextran, lactic dehydrogenase, and cytochrome C).
Potentially, contributions of CK released late after myocardial infarction to plasma CK time-activity curves

One possible reason for the slower rate of decline of plasma CK activity after myocardial infarction compared to that seen after intravenous injection of enzyme is continued release of CK from ischemic myocardium relatively late during the evolution of infarction. Since canine myocardium contains CK activity of approximately 2000 IU/g,25 and since peak plasma CK activity in a 20-kg dog with myocardial infarction is of the order of 1200 IU/ml, continued release from even a small region of myocardium would distort the descending limb of the curve substantially. For example, continuing release after occurrence of peak plasma CK from only 3 g of myocardium would reduce the slope of the descending limb of the plasma CK time-activity curve by approximately 50% if the true disappearance rate were 0.005 min⁻¹.

Administration of zymosan intravenously in conscious dogs reduces the decline in plasma CK activity after intravenous injection of partially purified myocardial CK.10 In the present study, to detect continuing release when plasma CK activity was declining after myocardial infarction, we injected zymosan intravenously (10 mg/kg) at selected intervals after the occurrence of peak plasma CK activity. As can be seen in Figure 7, administration of zymosan more than 8 hours after peak CK activity had occurred slowed disappearance. When zymosan was administered earlier after peak plasma CK, not only was disappearance slowed, but in addition, plasma CK increased. Similar results were obtained in four other experiments, two each with zymosan given within 4 hours of peak or more than 8 hours after peak CK (data not shown). This appears likely to have been due to continuing release from the heart for a considerable interval after the occurrence of peak CK, with the rate of release exceeding the rate of disappearance persisting in the face of zymosan.

These results suggest that continuing release of CK from the heart is one factor contributing to the relatively slow decline of plasma CK after infarction compared to CK disappearance after intravenous injection of myocardial CK or CK harvested from plasma of donor dogs.

Discussion

This study indicates the following: (1) plasma CK time-activity curves after injection of myocardial or plasma CK are fit by double-exponential better than by single-exponential curves; (2) estimates of the true CK disappearance rate based on a two-compartment model (with parameters obtained from double-exponential fits) are substantially higher than observed elimination rates; (3) disappearance of CK after infarction is substantially slower than disappearance after injection of either myocardial or plasma CK; (4) the relatively slow disappearance after infarction does not appear to reflect a different distribution volume compared to the volume after injection, judging from the rapid CK disappearance seen after repeated intravenous injections; and (5) both physical differences between extracted myocardial CK and continued release from the ischemic heart appear to contribute to the relatively slow disappearance seen after infarction.

Regardless of whether extracts from myocardium were prepared with ethanol fraction, ammonium sulfate precipitation, and batch adsorption to DEAE-Sephadex A-50, or whether they were prepared by simply squeezing intracellular fluid from broken cell preparations with a muscle press, disappearance rates of enzyme in the extracts were rapid after bolus intravenous injection. On the other hand, purification of CK in plasma with similar chemical manipulations did not lead to rapid disappearance after bolus injection. Thus, physical differences appear to be a contributing factor. Continued release appears to contribute to the relatively slow disappearance after infarction as well, judging from the experiments with zymosan.

Qualitatively, disappearance appears to be similar for myocardial or plasma CK with curves of both fit more closely by double-exponential than single-exponential curves. The good agreement between observed and fit values supports the two-compartment model described and suggests that the true CK disappearance rate is substantially higher than the observed elimination rate because of resupply of the vascular pool from the extravascular space.
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IMPLICATIONS REGARDING ENZYMATIC ESTIMATION OF INFARCT SIZE

With the one-compartment model utilized previously,1-5 calculations of infarct size depend on the value used for \( k_d \) in an interesting fashion. Thus,

\[
\text{infarct size (IS)} = (K) (BW) (CK_{Kn})
\]  

where BW is body weight, \( CK_{Kn} \) is cumulative CK released by the heart, and \( K \) is a proportionality constant. CK release from the heart is calculated from observed serial plasma CK values as

\[
CK_R = E(t) + k_d \int_0^t E(t) \, dt
\]  

where \( E(t) \) equals plasma CK activity at time \( t \), and \( CK_R \) is cumulative CK released by the heart up to time \( T \).6

The proportionality constant \( (K) \) relates \( CK_{Kn} \) to grams of myocardium undergoing infarction and can be expressed as

\[
K = \frac{DV}{P(CK_{N} - CK_{o})}
\]  

where \( DV \) is the distribution volume of CK per unit body weight, \( CK_{N} \) and \( CK_{o} \) are activity in homogeneous regions of normal myocardium and infarcts, and \( P \) is the proportion of CK released into blood compared to CK depleted from the heart assayed directly by measurements in myocardial extracts. This value is determined experimentally by assuming a value for \( k_d \) and measuring CK loss from the heart in animals with infarction.1 If the estimate of \( k_d \) used in such studies deviates from the true CK disappearance rate, the resulting estimate of \( P \) will deviate proportionally. Since \( k_d \) appears in the numerator and \( P \) appears in the denominator of Equation 3, errors in the estimate of \( k_d \) and \( P \) will be offsetting. These considerations apply as long as the same approach is used to estimate \( k_d \) in experiments in which infarct size is estimated by analysis of plasma CK time-activity curves and in experiments used to obtain estimates of \( P \).

When average values for \( k_d \) are used in enzymatic estimates of infarct size, the error in each individual case will depend on the variance of the true CK disappearance rate among individuals. In other words, the extent to which \( k_d \) in an individual differs from the average value of \( k_d \) used in obtaining an estimate of \( K \) will affect estimated infarct size since \( k_d \) influences the estimate of \( CK_{Kn} \) (Equation 2). With the data available (Table 2), only modest differences are evident in the variances between \( k_d \) calculated from a one-compartment model and the estimate of the true disappearance rate calculated from the two-compartment model. However, the two-compartment model accounts for recovery of substantially more CK release from the heart, as shown in Table 4. If estimates of the true CK disappearance rate were obtained under the same experimental conditions and the average value from the two models applied to estimates of infarct size, relative estimates of infarct size would not be affected substantially. However, the proportion of CK lost from the heart accounted for by analysis of plasma CK time-

| Table 4 Calculated Creatine Kinase (CK) Released (IU) |
|-----------------|-----------------|-----------------|
| Dog no. | Two-compartment model \( (\lambda) \) | One-compartment model \( (k_d) \) |
| 1     | 22,470 | 13,700 |
| 2     | 5,040  | 3,070  |
| 3     | 52,710 | 32,130 |

The \( \lambda \) and \( k_d \) values were obtained by analyzing the data obtained in six studies of plasma CK time-activity curves after intravenous injection of purified myocardial CK (Table 2). These parameters were used to calculate CK released from plasma CK time-activity curves in three conscious dogs subjected to coronary occlusion.

Unfortunately, plasma CK curves after myocardial infarction cannot be used directly to obtain parameters of the two-compartment model. This is because of probable contributions of continuing release of CK from the heart, and also because initial conditions (i.e., the amount of CK present in both compartments at the onset of the interval used for analysis) cannot be characterized directly. On the other hand, improved average estimates of \( k_d \), obtained from time-activity curves after intravenous injection of CK analyzed with a two-compartment model, can be utilized to obtain enzymatic estimates of infarct size that account for recovery of substantially more CK released from the heart than that accounted for in previous studies.

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References

Regional Variation of Lamb Blood Vessel Responsiveness to Vasoactive Agents during Fetal Development

CHE SU, JOHN A. BEVAN, NICHOLAS S. ASSALL, AND CHARLES R. BRINKMAN, III

SUMMARY Eight types of blood vessels were isolated from the fetal lamb and compared for their responsiveness to norepinephrine (NE), serotonin (5HT), and acetylcholine (ACh). Fetuses ranged from 53 days to term in gestational age, and from 0.05 to 4 kg in weight. The maximal contractile responses to the three agents were unequal among the vessels in the immature, premature, or mature periods of gestation. The vessels in the mature period were of three classes, increasing order of maximal response to NE per unit cross-sectional area of vascular wall: (1) thoracic aorta, ductus arteriosus, and pulmonary artery; (2) common carotid, ulnar, and mesenteric arteries; and (3) renal artery and saphenous vein. 5HT and ACh were less consistent than NE in the magnitude of their effect and often elicited dilation or failed to cause a response in the immature and premature vessels. The vessels also were categorized according to gestation-related changes in their maximal responses. The responses to NE and 5HT of all vessels, corrected for the cross-sectional area, either remained unchanged or increased at different rates. The ducal contractile response to ACh decreased toward term. The aorta, pulmonary artery, and ductus arteriosus were different from one another in their affinity to NE, although no change in the affinity occurred with progress in fetal maturation. It is concluded that quantitative and qualitative diversity in the response to NE, 5HT, and ACh exists among blood vessels from early prenatal life.

Effects of various autonomic drugs on the lamb fetal circulation have been extensively investigated. Among the fetal blood vessels, the ductus arteriosus is unique for being highly responsive to acetylcholine (ACh). However, a systematic investigation of the fetal vascular segments from different circulatory regions is yet to be undertaken.

The purpose of the present work was to determine whether fetal blood vessels are heterogeneous in their affinity for or maximal response to neurohumoral agents and, if so, whether such heterogeneity changes with gestational development.

Methods

Twenty-three ewes with dated pregnancies were used. Following spinal anesthesia with tetracaine, the fetus was delivered rapidly by cesarian section. The fetal head was covered immediately with a saline-filled rubber glove to prevent breathing. Under local anesthesia with lidocaine,

From the Departments of Pharmacology and Obstetrics and Gynecology, School of Medicine, Center for Health Sciences, University of California, Los Angeles, California. Supported by U.S. Public Health Service Grants HL 01755 and HL 08359.

Address for reprints: Dr. Che Su, Department of Pharmacology, UCLA School of Medicine, Los Angeles, California 90024

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