Effects of Lymphatic Transport of Enzyme on Plasma Creatine Kinase Time-Activity Curves after Myocardial Infarction in Dogs

Gail L. Clark, Alice K. Robison, Douglas R. Gnepp, Robert Roberts, and Burton E. Sobel

SUMMARY Because creatine kinase (CK) appears in cardiac lymph after myocardial infarction, this study was undertaken to determine whether lymph inactivates CK in vitro and whether interruption of cardiac lymph flow influences estimation of infarct size based on plasma CK changes in conscious dogs. After the effects of incubation of canine myocardial CK in native, deproteinized, or sulfhydryl- fortified lymph and dialysates had been characterized; effects of interruption of cardiac lymph flow on plasma CK time-activity curves after coronary occlusion were assessed in 13 conscious dogs, seven of which had exteriorized occlusive snares around cardiac lymphatics as well as the left anterior descending coronary artery. Native and deproteinized lymph as well as lymph dialysate markedly inactivated CK in vitro with associated nonenzymatically mediated proteolysis detectable on SDS gels. CK released into blood after coronary occlusion compared to myocardial CK depletion was 50% less in dogs with lymphatic occlusion (P < 0.01) although CK loss in the centers of infarcts (73% and 69%) and overall CK depletion (18% and 20%) were similar in the two groups. Based on comparison of observed to projected plasma CK values prior to lymphatic occlusion, significantly less CK appeared in blood when coronary occlusion was followed by lymphatic occlusion (P < 0.01), although the rate of disappearance of CK from the systemic circulation was not altered. Thus, lymph inactivates CK in vitro, and plasma CK time-activity curves after coronary occlusion are influenced considerably by interruption of cardiac lymph flow, a factor that should be incorporated to refine enzymatic estimates of infarct size.

SINCE the amount of creatine kinase (CK) (EC 2.7.3.2) depleted from the heart in experimental animals with infarction correlates with independent criteria of necrosis, a mathematical model has been developed to estimate CK depletion from analysis of plasma CK time-activity curves. Parameters in the model formulated initially include the proportion of CK depleted from the heart that appears in the circulation, CK distribution space, and the rate of CK disappearance from the circulation. Since CK, as well as other enzymes, appears in lymph after myocardial infarction, development of physiologically rather than empirically based models describing CK time-activity curves requires characterization of CK transport and potential inactivation in lymph. Cardiac lymph flow is low, approximately 1–3 ml/100 g per hr in anesthetized animals. Thus, CK released into lymph may be exposed to it for prolonged intervals. Accordingly, if lymph not only transports but also influences CK activity, plasma CK time-activity curves following coronary occlusion might be affected markedly.

The present study was undertaken to characterize effects of canine cardiac lymph on canine myocardial CK in vitro and the effects of marked perturbation of cardiac lymph flow in vivo on plasma CK time-activity curves after infarction.

Methods

Reagents

Bovine serum albumin, fraction V, N,N'-bis(2-hydroxyethyl) glycine (BICINE), N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 2-(N-morpholino)ethanesulfonic acid (MES), and Trasylol were obtained from Calbiochem; N,N'-methylenebisacrylamide from Eastman Kodak; indocyanine green dye from Hyson, Westcott and Dunning; ammonium persulfate, bromophenol blue, and sulfosalicylic acid from J.T. Baker Chemicals; ethylenediaminetetraacetic acid, disodium salt (EDTA) from Matheson, Coleman and Bell; Coomassie brilliant blue from Schwarz/Mann; 2-mercaptoethanol, 5,5'-dithiobis-(2-nitrobenzoic acid) (Ellman’s reagent), di-dithiobiterol (DTT), ethylene-glycol-bis-(08-aminoethyl ether) iN,N'-tetraacetic acid (EGTA), glycine, sodium dodecyl sulfate (SDS), and tris(hydroxymethyl) aminomethane (Tris) from Sigma.

Biochemical Procedures

Canine myocardial isoenzyme of MM creatine kinase was isolated and enriched by ethanol fractionation to a specific activity of approximately 400 IU/mg protein. Creatine kinase activity was assayed spectrophotometrically as previously described. Polyacrylamide disc gel
Electrophoresis with and without SDS was performed in the usual way.12,13

Assay of Creatine Kinase Activity in Lymph in Vitro

To evaluate effects of canine lymph, plasma, and whole blood on canine MM CK activity in vitro, 1 IU of CK was added to 2 ml of each, and enzyme activity was assayed serially during incubation at 37°C for 1-5 hours. Since a substantially greater loss of activity was observed in lymph than in whole blood, lymph fortified with exogenous CK was modified in several ways to simulate plasma or blood prior to incubation. In some experiments, albumin, known to protect enzymes incubated in vitro, was added so that the concentration of protein in native lymph or a lymph supernatant fraction was equivalent to that in canine plasma (5.6 ± 0.07 (SE) g/dl).7 In other experiments, the buffering capacity of canine lymph was controlled to approximate that of plasma or whole blood by addition of 0.05 M HEPES (pH 7.4). In other experiments, BICINE (pH 9.05), or MES (pH 6.1) was employed, or hydrogen ion concentration was modified by equilibrating canine lymph with CO2 at selected partial pressures. The effect of washed erythrocytes and erythrocyte ghosts on CK activity was assessed by adding each preparation to canine lymph.

As discussed in the Results section, lymph inactivated CK in vitro. To determine whether the inactivation was accompanied by proteolysis of CK, incubations were performed in lymph deproteinized by heating at 100°C for 10 minutes, as well as in native lymph, each fortified with canine CK with and without Trasylol. In addition, samples of the incubation medium were analyzed serially by SDS polyacrylamide gel electrophoresis to determine whether alterations in physical properties of CK occurred during incubation.

Since some loss of CK activity incubated in lymph persisted despite fortification of lymph with albumin and buffer, and since enzymatically mediated proteolysis did not appear to be responsible, factors such as thiol protection, known to be important in maintaining CK enzymic activity, were examined. In preliminary experiments, the sulfhydryl content of canine lymph was 75% less than that of plasma.14 Accordingly, incubations were performed with canine lymph fortified with 2-mercaptoethanol with and without HEPES buffer, 0.05 M, pH 7.4. Since exogenous thiol detectable by titration14 declined during incubation, perhaps because of interactions with trace metals, some incubations were performed with 10 mM EDTA.

Since EDTA did not prevent loss of titratable sulfhydryl groups in lymph fortified with 2-mercaptoethanol, equilibrium dialysis of canine lymph was performed to determine whether a low molecular weight substance other than a heavy metal bound by EDTA may have been involved in thiol oxidation or inactivation in lymph. Lymph was first dialyzed against distilled water for 16 hours at 25°C in a 1-ml equilibrium dialysis cell. The contents of both chambers were saved. Canine CK then was incubated in native lymph, distilled water, the contents of the lymph side of the dialysis cell, or the contents of the water side of the cell with and without DTT.

Effects of Occlusion of Cardiac Lymphatics on Plasma CK Time-Activity Curves in Vivo after Myocardial Infarction

As previously reported, plasma CK time-activity curves reflect the amount of CK depleted from the heart after myocardial infarction produced by ligation of the left anterior descending coronary artery in conscious dogs.3 Under controlled conditions, observed plasma CK values can be projected from serial data during the first 5-hour interval after the initial CK elevation.15 Since canine lymph inactivated CK in vitro and since CK in cardiac lymph is markedly elevated in excess of plasma CK after myocardial infarction,5,6 we used the conscious dog to determine whether interruption of cardiac lymph flow at a selected interval after coronary occlusion leads to deviation of observed plasma CK values from those projected prior to occlusion of the lymphatics.

Canine cardiac lymph traverses three interconnectedplexuses:16-22 subendocardial, myocardial, and subepicardial. Ultimately, all three channels drain via the subepicardial route in trunks accompanying the coronary arteries to form two lymphatic vessels in the right and left atrioventricular grooves, most commonly uniting at the base of the pulmonary artery to form a single trunk entering the cardiac node, which generally lies between the superior vena cava and the innominate artery. Often the trunk divides, with branches reaching the cardiac node via the pretracheal node.

To evaluate the efficacy of lymphatic occlusion, 1.25 mg of indocyanine green dye were injected with a 23-gauge needle into the left ventricular apical myocardium in two dogs subjected to thoracotomy. Dye appeared in the paracoronary lymph channels within seconds and at the cardiac node within minutes. Egress of dye from the injection site was complete within 30 minutes. Subsequently, both the cardiac and pretracheal nodes were occluded distally with mass ligatures and the thoracotomies were closed. Forty-eight hours later the thoracotomies were reopened and injection of dye repeated. Dye was still visible at the injection site 2 hours later (Fig. 1), indicating that effective lymphatic obstruction persisting for at least 48 hours had been accomplished. Since indocyanine green interferes with spectrophotometric assay of CK activity, as shown in preliminary experiments, in experiments concerned with assay of myocardial CK, lymphatic occlusion was verified by gross inspection rather than by injection of dye when the dogs were killed to confirm that the lymphatic snare was tight distal to the cardiac and pretracheal nodes and that the nodes were swollen.

Conscious Animal Preparations

Thirteen male mongrel dogs (20-25 kg) were subjected to left lateral thoracotomy through the 4th intercostal space under sodium thiopental (10 mg/kg, i.v.) and 0.05% halothane anesthesia.

To produce coronary and phased lymphatic occlusion,
externalized silk snares were placed around the left anterior descending coronary artery below the first diagonal branch and around the soft tissue distal to both the cardiac and pretracheal lymph nodes in seven of the dogs (Fig. 1). In six control dogs, only coronary snares were used, but the lymph channels were dissected in a sham procedure. Location of the lymph nodes was determined by injection of indocyanine green dye at the apex of the left ventricle in all dogs prior to closure of the thoracotomy.

When the dogs had recovered fully and plasma CK was $<90$ IU/liter, coronary occlusion was produced. Conscious dogs were used to avoid the effects of anesthesia which decreases CK disappearance rates from blood by as much as 80%, and to avoid potential effects of anesthesia in decreasing the rate of lymph flow substantially. In addition, acute preparations would not have been suitable because thoracotomy itself causes release of extramyocardial CK. CK activity was determined in four samples from normal, grossly nonischemic myocardium, five samples from the center of the apparent zone of infarction, and a sample of the whole homogenate of the left ventricle.

![Figure 1](http://circres.ahajournals.org/figlink/l12e7)

**Figure 1** The diagram on the left (modified from Miller) illustrates the paracoronary location of cardiac lymphatic vessels, the route of lymphatic efflux from the heart, and the location of occlusive snares used in the present study around the left anterior descending coronary artery and distal to the cardiac and pretracheal nodes identified by intramyocardial injection of indocyanine green dye at the apex. The photograph on the right illustrates canine preparation 48 hours after lymphatic and coronary occlusion and shows retention of indocyanine dye at the apex 2 hours after injection, confirming lymphatic occlusion.

Results

**Effects of Lymph on Creatine Kinase Activity in Vitro**

When canine myocardial CK was incubated in canine blood, plasma, or lymph, activity declined. An average of 78%, 22%, and 4% remained after 5 hours of incubation (Fig. 2). When lymph was modified to simulate conditions in blood more closely, the decline of activity was diminished but not precluded. Deproteinated and native lymph, both fortified with albumin, retained 85% of exogenous CK activity after 2 hours of incubation in contrast to an average of 13% (range 3–38%) in lymph without albumin. Thus, fortification of lymph with albumin in concentrations comparable to those in plasma afford substantial but incomplete protection of CK activity.

Unbuffered stored canine lymph had a pH of approximately 9.0. When the buffering capacity was increased, retention of CK activity during incubation occurred but only when pH was maintained near the physiological
range. After 1 hour of incubation, only 9% of CK activity persisted with BICINE buffer at pH 9.0, but 85% persisted with HEPES at pH 7.4. Since the moiety responsible for thiol inactivation might be less soluble or complexed with OH\(^{-}\) groups at alkaline pH, as is the case with many heavy metals, and since the equilibrium between S-S groups in CK and SH groups shifts toward S-S at alkaline pH, potentially decreasing the likelihood of thiol inactivation, these results are compatible with the possibility that inactivation of CK in lymph is due primarily to interactions involving thiol groups. In control experiments, 100% activity of the canine myocardial CK preparation was maintained during incubation at 37\(^\circ\)C in H\(_2\)O or HEPES buffer (pH 7.0, 7.4, and 8.0) for 6 hours. Eighty-five percent and 65% of activity were maintained after 3.5 hours of incubation at 37\(^\circ\)C in BICINE buffer (pH 8.9 and 9.2).

Native lymph exposed to room air had a PCO\(_2\) of approximately 5.0 mm Hg. In lymph equilibrated to increase PCO\(_2\) to 33 mm Hg, resulting in a pH of 7.5, 68% of CK activity was retained after 1 hour of incubation. Addition to lymph of washed erythrocytes or hemoglobin in concentrations analogous to those in whole blood conferred protection on CK so that more than 90% of activity persisted after 2 hours. In contrast, only 9% of activity persisted in lymph fortified with red cell ghosts. Thus, the protective effects of red cells seem to be due to the improved buffering capacity provided.

Inactivation of CK incubated in lymph did not appear to be due to enzymatically mediated proteolysis. Thus, CK activity declined even more rapidly in deproteinized lymph, to 1% of initial activity after 90 minutes than in native lymph. Trasylol (10,000 U/ml) a potent inhibitor of enzymatic proteolysis, also failed to protect CK incubated in lymph. However, proteolysis with nonenzymatically mediated alteration in the physical properties of CK incubated in lymph accompanied loss of enzyme activity. As shown in Figure 3, material migrating more

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**Figure 2** Creatine kinase activity in whole blood, plasma, and lymph. Results expressed are mean percentages of initial creatine kinase activity present during incubation at 37\(^\circ\)C in whole blood (n = 4), plasma (n = 4), and lymph (n = 21) for 1–5 hours.

**Figure 3** Sodium dodecyl sulfate (SDS) gel electrophoresis of incubation medium containing creatine kinase (CK). Creatine kinase was added to the water side of a lymph dialysis cell after equilibrium had been reached and was incubated for up to 22 hours at 37\(^\circ\)C. The CK band (black arrow) on SDS gels decreased in intensity in samples obtained progressively later during the incubation period in contrast to the lower molecular weight bands which increased in intensity. The Dalton scale of molecular weights to the right of the SDS gels was derived using the following standard marker proteins on identical gels according to the method of Weber and Osborn: bovine serum albumin (68,000 daltons); catalase (58,000); aldolase (40,000); chymotrypsinogen (25,700); myoglobin (17,250); and lysozyme (14,300). These findings suggest that the physical state of the CK molecule became altered during incubation.
rapidly on SDS gels appeared and the CK protein migrating slowly decreased progressively during incubation of CK in lymph for 22 hours. Assay of CK activity run in parallel to the SDS gel electrophoresis experiments demonstrated a decline of CK activity coincident with the diminution of the CK band.

Protective Effects of Thiol Groups

Incubation of CK in the contents of both the water side and the lymph side of a dialysis cell after equilibrium had been reached showed <3% of initial CK activity remaining in either medium after 6 hours (Fig. 4). CK added to native lymph in a parallel experiment was even more markedly inactivated. DTT added to the contents of the water side of the dialysis cell along with CK did confer some protection of CK activity (43%), although in other experiments loss of titrable thiol content in lymph was marked, with more than 80% lost within 2 hours. Therefore, a low molecular weight, freely dialyzable material appeared to be associated with both loss of CK enzymatic activity and loss of titrable thiol in lymph during incubation. This suggests that CK inactivation in lymph may be due to inactivation of thiol groups. The presence of additional protein, such as albumin, may minimize this effect by virtue of binding the moiety responsible for inactivation.

Since some protection of CK in lymph in vivo is likely to occur because of the proximity of lymph to blood with its high buffering capacity, it is difficult to extrapolate from the results in vitro. Accordingly, we performed additional experiments in which purified CK was injected into an isolated segment of a peripheral lymphatic vessel and serial 10-μl samples were obtained for analyses of CK activity. Although this set of conditions deviates from conditions in which lymph flow is not interrupted, the conditions do permit continued juxtaposition of lymph to the vascular space in situ. The decline of CK activity in the isolated segment of the lymphatic circulation averaged 55% in 95 minutes (n = two experiments), generally paralleling the decline observed in experiments in vitro but with some apparent protection of activity in situ.

Effects of Reduction of Lymph Flow in Vivo on Plasma CK Time-Activity Curves after Myocardial Infarction

Since lymph is capable of inactivating CK in vitro, and since some CK released from ischemic myocardium appears in cardiac lymph in vivo, it appeared likely to us that marked reduction of lymph flow from the heart might influence plasma CK time-activity curves. For example, one might anticipate that decreased exposure of CK to lymph during lymphatic occlusion would increase the overall amount of CK appearing in blood compared to that lost from the heart. On the other hand, if transport in lymph is an obligatory pathway for egress of some CK from the heart into the circulation, one might anticipate that occlusion of lymph flow would decrease the amount of enzyme ultimately appearing in blood. Stasis of CK in lymph might facilitate its inactivation and again diminish the amount appearing in blood.

To examine these and other possibilities, we performed studies in conscious dogs. In dogs with coronary occlusion without lymphatic occlusion, the mean ratio of CK released into the circulation (calculated from plasma CK time-activity curves) to CK depleted from the heart (measured directly) (Table 1) was 0.19 ± 0.02 (se). In contrast, in dogs with lymphatic occlusion 5 hours after the initial CK elevation following coronary occlusion, this ratio was markedly decreased to 0.09 ± 0.02, P < 0.01. The difference could have been due to less CK reaching the circulation in dogs with lymphatic occlusion or to more CK depletion from the heart.

The percentage of CK depleted from the center of a zone of infarction, determined from values in samples from the hearts of dogs with coronary occlusion alone, was virtually identical to that in dogs with lymphatic occlusion (73% and 69%, Table 2). Likewise, total myocardial CK depletion was comparable in the two groups (18% and 20%). Thus, lymphatic occlusion after coronary occlusion did not alter the amount of CK lost from the heart.

To determine whether less CK reached the circulation, it first was necessary to determine whether the elimination rate of CK from blood was altered in dogs with lymphatic occlusion. The elimination rate was calculated from the slope (least squares approximation) of the declining portion of the plot of log plasma CK activity vs. time during the 7-hour interval after peak CK activity occurred. Results among the seven dogs with both coronary and lymphatic occlusion [0.0016 ± 0.0002 (se) min⁻¹]
TABLE 1  Total CK Released into Blood (CKr) Compared to Total CK Depleted from the Heart (CKj) after Coronary Occlusion

<table>
<thead>
<tr>
<th>CK,</th>
<th>9,883</th>
<th>2,523</th>
<th>9,778</th>
<th>7,360</th>
<th>7,780</th>
</tr>
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<tbody>
<tr>
<td>Mean</td>
<td>6711</td>
<td>0.19 ± 0.02 (SE)</td>
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* IU of CK.

and the six dogs with coronary occlusion alone (0.0015 ± 0.0002) were not significantly different and corresponded to elimination rates calculated in a similar fashion and reported previously.27 It should be noted that elimination rates calculated in this fashion differ from disappearance rates calculated from serial samples after intravenous injections of purified enzyme.28

Since there was no significant difference in the apparent elimination rate of CK that had reached the circulation, and since CK depletion from the infarct was not altered, it appeared likely that lymphatic occlusion was preventing some CK from reaching the blood, thereby reducing the ratio of CK appearing in blood to that lost from the heart.

Figure 5 illustrates representative plasma CK time-activity curves. One is from a dog with coronary occlusion alone, and the other from a dog with coronary occlusion and subsequent lymphatic occlusion. In both cases, projected CK values, based on curves fit from data during the first 5 hours, are compared to observed values. As can be seen with coronary occlusion alone, the observed values of CK in plasma slightly exceeded those projected. However, when coronary occlusion was followed by lymphatic occlusion, the observed plasma CK values deviated well below those projected prior to lymphatic occlusion. Although the peak CK activities evident on the two curves selected for illustrative purposes appear similar, the mean peak activity of plasma CK in dogs with coronary occlusion alone (1497 IU/liter) differed significantly from dogs with coronary occlusion followed by lymphatic occlusion (836 IU/liter) (P < 0.01). This difference appears to reflect the comparable elimination rates but disparate rates of delivery of enzyme from the heart to the blood in the two groups. As shown in Table 3, which summarizes results of several experiments, CK appearing in blood estimated from the observed plasma CK time-activity curve exceeded CK anticipated from the projected curves by an average of 17 ± 10% (SE) in dogs without lymphatic occlusion. In contrast, observed accumulation of CK in blood was 30 ± 12% (SE) less than that anticipated based on projected values in dogs with lymphatic occlusion. This directionally opposite difference was significant at the 0.01 level, indicating that a smaller proportion of CK lost from the myocardium reached the blood when cardiac lymph flow was occluded 5 hours after coronary occlusion.

**Discussion**

Results obtained in this study indicate that canine myocardial CK is inactivated by lymph in vitro and that interruption of efflux of lymph from the heart in vivo reduces the proportion of CK lost from myocardium undergoing infarction that appears in blood. The potential importance of transport via lymph on kinetics of CK

**Table 2**  CK Activity in the Center of the Zone of Infarction

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<th>Group</th>
<th>Activity (IU/g)</th>
<th>Depletion (%)</th>
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</thead>
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<td>Coronary occlusion</td>
<td>612 ± 41</td>
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</tr>
<tr>
<td>Coronary plus lymphatic occlusion</td>
<td>704 ± 36</td>
<td>63 ± 3%</td>
</tr>
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</table>

Values expressed are means ± SE.

Values of CK activity in normal myocardium averaged 2228 IU/g.

**Table 3**  CK Activity in the Center of the Zone of Infarction

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Values of CK activity in normal myocardium averaged 2228 IU/g.
appearance in the circulation after myocardial infarction is underscored by several considerations. In general, proteins entering interstitial fluid are transported to the venous circulation via lymphatics, in part because they are too large to penetrate the basement membranes and tight endothelial junctions present in blood capillaries but absent from lymphatic vessels. It is true that some enzymes released from the heart enter the systemic venous circulation rapidly, apparently directly, as judged from negative arteriovenous differences. On the other hand, the role of their transport in lymph is emphasized by the findings that their activities in cardiac lymph are higher than in blood. After myocardial infarction, enzyme activity in cardiac lymph increases by several orders of magnitude, generally reaching peak elevations within 14 hours after the onset of ischemia. Based on quantitative considerations, it appears that between 30% and 50% of the elevation of transaminase activity in the canine systemic circulation after myocardial infarction can be attributed to enzyme reaching the circulation via transport in lymph.

Despite the apparent contribution of enzyme transported in lymph to plasma enzyme time-activity curves after myocardial infarction, little information is available regarding the fate of enzymes exposed to lymph in vivo. In the present study, inactivation of CK was readily demonstrable when enzyme was incubated in lymph in vitro. Although it is difficult to assess the extent of such inactivation occurring in vivo, in part because of its dependence on pH, it appears likely that some inactivation accounts for the relatively small proportion of CK appearing in blood compared to that lost from the heart itself. After myocardial infarction, pH in cardiac lymph changes, but appears to remain within the physiological range. In view of the fact that lymph flow remains low in absolute terms even though it may increase after myocardial infarction (to values in the range of 3 ml/100 g per hr), even a modest rate of inactivation could account for a substantial reduction of the overall amount of CK enzyme activity appearing in the systemic blood circulation.

Results of the present study suggest that transport of CK in lymph may be an important factor to take into account in the continuing effort to refine enzymatic estimation of infarct size based on analysis of plasma CK time-activity curves. It does not appear that the elimination rate of enzyme reaching the blood differs in animals with and without obstruction of the cardiac lymphatics. On the other hand, the proportion of CK depleted from the heart appearing in the systemic circulation is clearly reduced when cardiac lymphatic occlusion is imposed several hours after occlusion of the coronary artery. The extent to which this ratio varies from animal to animal or from patient to patient with myocardial infarction may be directly dependent on the variance of regional lymph flow as a function of time after the onset of infarction. Recent mathematical analyses of CK time-activity curves after myocardial infarction indicate that results conform more closely to biexponential functions fitting a two-compartment model. Distribution of enzyme in cardiac lymph may constitute the compartment represented mathematically in such a two-compartment model.

Several physiological factors may modify cardiac lymph flow in vivo including: age-related thickening of endocardial portions of the heart; variation in systemic venous pressure with elevations diminishing lymph flow in the thoracic duct and, by implication, in cardiac lymph channels draining in the superior mediastinum expansion of extracellular volume in association with congestive heart failure or from other etiologies; and lymphatic obstruction related to myocardial injury. As can be seen in Table 2, the marked reduction of lymph flow induced in the present study would lead to underestimation of the extent of infarction based on analysis of plasma CK time-activity curves by as much as 47%. The variance in lymph flow in vivo has not yet been characterized, nor are techniques currently available to assess it conveniently in intact experimental animals or patients. On the other hand, preliminary experience with externally detectable radioactively labeled tracers suggests that at least qualitative noninvasive assessments of overall cardiac lymph flow may be possible under selected conditions. These will permit characterization of the variance of cardiac lymph flow after infarction and possible incorporation of a parameter reflecting lymph flow in refined enzymatic estimates of infarct size.

One might argue that some of the results in the present

<table>
<thead>
<tr>
<th>Coronary occlusion alone</th>
<th>Coronary occlusion followed by lymphatic occlusion after</th>
<th>% difference</th>
<th>% difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CKr(O)*</td>
<td>CKr(P)*</td>
<td>% difference</td>
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</tr>
<tr>
<td>9,883</td>
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</tr>
<tr>
<td>2,523</td>
<td>1,787</td>
<td>+29%</td>
<td>-5%</td>
</tr>
<tr>
<td>2,944</td>
<td>3,259</td>
<td>-11%</td>
<td>-8%</td>
</tr>
<tr>
<td>9,778</td>
<td>4,416</td>
<td>+55%</td>
<td>-83%</td>
</tr>
<tr>
<td>7,360</td>
<td>6,624</td>
<td>+10%</td>
<td>-48%</td>
</tr>
<tr>
<td>7,780</td>
<td>6,380</td>
<td>+19%</td>
<td>-20%</td>
</tr>
</tbody>
</table>

Mean ± SE: +17% ± 10% -30% ± 12%

Results expressed are IU of CK released into blood calculated from observed plasma CK values (CKr(O)) or from predicted values (CKr(P)). The percent difference between these two estimates in each dog provides a measure of agreement between observed and predicted plasma CK time-activity curves.
study, particularly those concerned with plasma CK time-
activity curves in vivo and their response to occlusion of
cardiac lymph flow, may have been due to alterations in the
evolution of infarction induced by lymphatic occlusion
itself. However, this seems unlikely because the maximum depletion of CK observed in samples of the heart from within the central zone of infarction 48 hours after occlusion was comparable in dogs with and without occlusion of the cardiac lymphatics. In addition, the overall extent of myocardial CK depletion determined by analysis of whole homogenates of the left ventricle was comparable for the two groups. On the other hand, it is quite likely that marked reduction of cardiac lymph flow may influence the evolution of infarction later in its course. Thus, estimates of the extent and distribution of infarction based on morphological analysis were similar in dogs with and without lymphatic obstruction during the first 2 weeks after experimentally induced myocardial infarction, but morphologically larger infarcts were evident 6 weeks after coronary occlusion in dogs with occluded lymphatics. These late infarcts appeared to have enlarged with areas of recent necrosis surrounding older scars suggesting a “role of the lymphatics in restricting the extent of the necrotic process invoked by myocardial ischemia.”

Results of the present investigation indicate that a smaller proportion of CK lost from the myocardium reaches the blood when lymph flow from the heart is occluded 5 hours after coronary occlusion in intact, conscious dogs. Since the apparent rate of elimination of CK from blood is unchanged in dogs with lymphatic occlusion, and since the amount of CK depleted from the heart is comparable whether or not lymph flow from the heart is retarded, the decreased ratio of CK appearing in blood compared to that lost from the heart suggests that transport of CK in lymph contributes appreciably to the kinetics of appearance of the enzyme in the systemic blood circulation. Accordingly, it appears likely that characterization of the variance of lymph flow at selected intervals after coronary occlusion from animal to animal and from patient to patient and estimation of the extent of lymph flow in vivo would provide parameters useful in refining enzymatic estimates of infarct size.

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References

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