Depressed Myocardial Creatine Phosphokinase Activity Following Experimental Myocardial Infarction in Rabbit

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ABSTRACT

Since creatine phosphokinase (CPK) is found predominantly in myocardial and skeletal muscle cells, in contrast to cells participating in the inflammatory response, it was considered likely that measurement of activity of this enzyme in the heart would provide a sensitive and relatively specific index of the extent of ischemic injury following acute coronary artery occlusion. Accordingly, CPK activity was measured serially following coronary artery occlusion in extracts from rabbit myocardium with gross infarction and from normal rabbit left ventricle. In addition, myocardial CPK activity was assayed in extracts from various portions of dog hearts 24 hours after ligation of the coronary artery. CPK activity of rabbit myocardium with infarction was uniformly depressed within 6 hours following coronary occlusion. After 24 hours, activity declined from 15.5 ± 0.9 (mean ± SE) to 3.4 ± 0.3. CPK activity in whole left ventricular extracts was depressed and in general, the extent of depression was proportional to the size of the gross infarct. CPK depression in various regions of the dog heart 24 hours after coronary occlusion correlated with the extent of reduction of blood flow determined with the use of radioactively labeled microspheres. Results suggest that depression of myocardial CPK activity may be useful in estimating the extent of tissue damage following experimental coronary artery occlusion and the effect of prophylactic and therapeutic measures on the survival of myocardium in this setting.

ADDITIONAL KEY WORDS

myocardial enzymes myocardial ischemia infarct size coronary artery occlusion myocardial necrosis

The accurate assessment of early cell damage following myocardial infarction has been elusive. Although histochemical and biochemical indices reflecting changes in metabolism have been employed in identification of myocardial ischemia (1-8), there is as yet no convenient quantitative means for determination of the amount of tissue with infarction relatively soon after coronary occlusion. The activity of many enzymes in the heart is rapidly affected by ischemia culminating in cell death. However, most of these enzymes are not confined to myocardial cells and changes in activity are influenced by the appearance of other cells participating in the inflammatory reaction following acute myocardial infarction (9-13). Although creatine phosphokinase (CPK) activity has been found in several tissues, the enzyme is found predominantly in skeletal muscle and myocardial cells (14) in which it plays a role in the metabolism of high energy phosphate compounds intimately related to the process of muscle contraction. Accordingly, it was considered likely that myocardial CPK activity would be a sensitive and relatively specific indicator of the extent of ischemic injury following acute coronary occlusion. The experiments described in this communication
were undertaken to assess the value of CPK activity as an index of cell damage following acute myocardial infarction induced by coronary occlusion in rabbits.

**Methods**

**PROCEDURES IN ANIMALS**

**Production of Myocardial Infarction.**—Male rabbits weighing 2.5 to 3.0 kg were anesthetized with sodium pentobarbital, 30 mg/kg, intravenously. Positive pressure respiration was maintained with a Harvard ventilator connected to an endotracheal tube. A left thoracotomy was performed. Myocardial infarction was produced by ligation of the left circumflex artery with a silk 5.0 suture 11 to 14 mm from the aortic root. The site of ligation was verified post mortem. Care was taken to avoid ligation of the adjacent coronary vein. Ischemia in myocardium supplied by the ligated vessel was manifested by a blue color appearing within minutes following ligation. In all experiments control tissue was obtained from sham-operated animals including pericardiotomy and isolation of the corresponding coronary artery but no ligation.

Additional experiments were performed using myocardium obtained from dogs anesthetized with 30 mg/kg sodium pentobarbital and subjected to ligation of the left anterior descending coronary artery. Tissue extracts were prepared from biopsies obtained 1 hour after occlusion of the vessel.

In experiments designed to examine the effect of increased afterload on myocardial CPK activity, supravalvular aortic constriction was produced in guinea pigs weighing 250 to 400 g as previously described (15) with the use of a Ted lion clip (i.d. 2.0 mm) placed just distal to the aortic valve. CPK activity was measured in hearts from normal controls, from sham-operated animals and those with aortic constriction 24 hours after operation.

In experiments designed to examine the relationship between myocardial blood flow and myocardial CPK activity, studies were performed using dogs whose left anterior descending coronary artery was ligated 24 hours before sacrifice. Myocardial blood flow was measured 24 hours later with the use of radioactively labeled microspheres (16). Carbonized plastic microspheres (25 ± 5μ) labeled with Ytterbium-169 (149Yb), 16 μCi, in 10% dextran were injected through an indwelling catheter into the left atrium. Relative blood flow was measured by counting the radioactivity in microspheres contained in full-wall biopsies (0.5 g) of various portions of the left ventricle. CPK activity was measured in homogenates of the same biopsies.

**Materials.**—All chemicals were of the highest grade commercially available and were formulated in glass distilled water. CPK standard was obtained from Worthington Biochemical Corporation. Cysteine-hydrochloride, phospho-creatine, creatine, reduced nicotinamide-adenine dinucleotide (NADH), and bovine serum albumin were obtained from Sigma Chemical Company. Creatine phosphate (CP), glucose-6-phosphate dehydrogenase, reduced glutathione (GSH), nicotinamide-adenine dinucleotide phosphate (NADP), sodium adenosine diphosphate (ADP), sodium adenosine triphosphate (ATP), sodium adenosine monophosphate (AMP), pyruvate kinase, lactate dehydrogenase, and hexokinase were obtained from Calbiochem. Microspheres labeled with 149Yb were obtained from Minnesota Mining and Manufacturing Company.

**Determination of Myocardial CPK Activity.**—Rabbits were killed by stunning 6 to 40 hours after coronary ligation or at corresponding times following sham operation. All preparatory procedures were performed at 0 to 4°C. The heart was rapidly excised, and the entire left ventricle including the intraventricular septum was isolated as a unit. In some studies, the gross area of myocardial infarction identified by the hemorrhagic border on the endocardial surface was excised and the ischemic and nonischemic residual tissue were processed separately. In other experiments, serial slices through the myocardium were utilized to better define the border of the infarct at several depths within the wall. The areas with infarction in each slice were excised separately and recombined for preparation of tissue extracts.

Ventricular myocardium was minced with a scissors and homogenized in 25 vol/g of 0.25M sucrose, 0.001M neutralized ethylenediaminetetra-acetic acid (EDTA) and 0.1 M mercaptoethanol in a VirTis "45" homogenizer with two 15-second bursts at a speed setting of 5. The homogenate was then centrifuged at 10,000 X g for 10 minutes and the supernatant fraction removed and centrifuged at the same speed for 10 minutes. CPK activity in the supernatant fraction was assayed by several methods after appropriate dilutions of sample were made in a buffer containing 0.2M bovine serum albumin (BSA) and tris(hydroxymethyl)aminomethane (Tris) 0.01M, pH 7.4. Protein was determined by the biuret method (17).

The CPK assay method generally used was the spectrophotometric, kinetic procedure described by Rosalki (18). Samples were assayed at 30°C in a reaction mixture containing Tris, 50 mM; ADP, 1 mM; creatine phosphate, 10 mM; glucose, 10 mM; MgCl2, 30 mM; NADP, 0.8 mM; AMP, 10 mM; cystein-HCl, 5 mM; hexokinase, 0.8 IU; and 1.0 mg homogenate. CPK activity was measured in homogenates of the same biopsies.
glucose-6-phosphate dehydrogenase, 0.3 IU; and supernatant fraction protein, 0.3 to 0.5 μg in a final volume of 1.25 ml. Results were expressed as international units (IU) (micromoles of substrate converted per minute) per milligram of supernatant fraction protein. Enzyme reaction rates were linear for at least 15 minutes after an equilibration period of 5 minutes, were proportional to the quantity of supernatant protein added, and were reproducible within a range of ±3. The kinetics of the reaction are characteristically not first order during the equilibration period (18), probably because of the influence of build-up of concentration of glucose-6-phosphate in the early stages of the reaction. There was no apparent enzyme activity after heating the sample at 55°C for 5 minutes, or treating it with 1.2N perchloric acid, indicating that reaction rates were not influenced by tissue components such as adenosine triphosphate. The reaction was entirely dependent on substrate. Mixing experiments using standard enzyme and extracts from normal rabbit myocardium and that with infarction demonstrated the absence of an inhibitor in either tissue. The supernatant fraction protein per gram of tissue was similar in homogenates from nonischemic and ischemic rabbit heart muscle. In addition, the percent recovery of CPK activity in the supernatant fraction varied only modestly and inconsistently. Thus, results expressed as enzyme activity/milligram supernatant fraction protein can be considered to be independent of variation in myocardial protein content or extraction. This assay procedure measures the so-called "back" reaction, i.e., catalysis by CPK of CP + ADP \(\rightarrow\) ATP + creatine, and results obtained with it are referred to accordingly in the text and tables.

To evaluate the disparity between some of the findings in the present study and those reported in one earlier investigation (10), CPK activity was determined by two additional methods. Both of these were used to assay the forward reaction (creatinine + ATP \(\rightarrow\) CP + ADP). One procedure used was the Büchler coupled enzyme method, performed under conditions exactly as described by Braasch et al. (10). This assay will be referred to in the text and the tables as the forward reaction. The second procedure used was that described by Noda et al. (19). In the latter method, CPK activity is determined by measurement of inorganic phosphate released from CP formed during the reaction. This method is entirely independent of coupling enzymes and hence also independent of the effect of tissue constituents which might influence them, contrary to the Büchler method used to measure the forward reaction employed by Braasch and coworkers. In some studies in which the forward reaction was assayed by the coupled enzyme method, tissues were homogenized in the same medium and by the same procedure as that described by Braasch et al. (10).

**Results**

Myocardial CPK Activity Following Coronary Artery Occlusion.—The activity of CPK in extracts from tissue in the grossly identifiable center of the ischemic area following coronary artery occlusion is illustrated in Figure 1. Six hours after coronary ligation the activity was significantly depressed to 9.3 ± 1.0 (mean ± SE, n = 4) IU/mg protein compared to the control value of 15.5 ± 0.9 (n = 9). After 24 hours, activity was further reduced to 3.4 ± 0.2 (n = 8). By 42 hours following coronary artery occlusion, there was only a minimal additional reduction in CPK activity in extracts from the center of the tissue with infarction.

The decline of myocardial CPK activity was measured in nonperfused hearts incubated in closed containers at 37°C as shown in Figure 1. The decrease in activity was striking. However, after 24 hours, reduction in CPK activity in homogenate from the entire incubated but nonperfused left ventricle was only 34% of that in the center of an infarct. Since increased enzyme activity characteristically appears in the systemic circulation following myocardial infarction, it is likely that some enzyme released from necrotic cells is liberated into the circulation. This would account for the greater diminution in enzyme activity remaining in the heart in situ compared with that in the nonperfused, incubated heart.

The time course of depression of CPK activity in homogenate derived from the whole left ventricle following coronary occlusion is shown in Figure 2. There was a modest change after 6 hours, while 24 hours after coronary occlusion whole left ventricular homogenate CPK activity was markedly diminished compared to that in sham-operated controls. Again, the subsequent change from 24 to 42 hours following coronary occlusion was much less marked.

Since myocardium surrounding an area of
infarction may be subjected to an increased work load, CPK activity was measured in nonischemic tissue peripheral to the infarction in rabbits and in myocardium from sham-operated guinea pigs and pigs subjected to a degree of aortic constriction previously shown to produce marked ventricular hypertrophy (15). In presumably nonischemic tissue peripheral to areas of infarction in the rabbit, CPK activity did not differ from that in sham-operated animals (15.2 ± 0.5, n = 7; and 15.5 ± 0.9, n = 9, respectively). Severe aortic constriction, previously shown to produce cardiac hypertrophy and failure (15), did not alter myocardial CPK activity in guinea pig whole left ventricular homogenates within 24 hours. Values obtained following aortic constriction were 11.7 ± 0.5 (n = 6) IU/mg protein compared to 12.2 ± 0.6 (n = 4) IU/mg protein in sham-operated controls.

The Relationship Between Myocardial CPK Depression and Infarct Size.—The relationship between the size of a myocardial infarction and the reduction of CPK activity in whole left ventricular homogenate is illustrated in Figure 3. The tissue with infarction was identified grossly 24 hours after coronary occlusion, excised along the outer margins of the hemorrhagic zone on the endocardial surface, and weighed. In some experiments, infarcts were excised on the basis of the gross border in several sections through the left ventricular wall, rather than simply the hemorrhagic border on the endocardial surface. The percent of tissue with infarction was calculated from the weight of the infarct/left ventricular weight. Following occlusion of the left circumflex artery, a range of infarct weight from 10 to 55% of left ventricular weight was obtained. Attempts to produce larger infarcts failed because of mortality associated with acute left ventricular dilatation. Standardization of the distance from the aortic root to the site of ligation produced infarcts averaging 34% of left ventricular weight. CPK activity was determined in whole homogenates prepared from both the tissue with infarction and the left ventricle without infarction. As can be seen, there is a direct relationship between the extent of CPK depression and the percent of tissue with infarction. In some hearts, CPK activity was slightly depressed in a border zone adjacent to the infarct in extracts from apparently grossly normal tissue. This suggests

![Image](http://circres.ahajournals.org/)

**Figure 1** Myocardial CPK depression following coronary artery occlusion. CPK activity (mean ± SE) in ischemic tissue (o) obtained 6, 24 and 42 hours after ligation of the left circumflex coronary artery and in hearts incubated in closed containers at 37°C (x). n is indicated in parentheses.

![Image](http://circres.ahajournals.org/)

**Figure 2** CPK activity in whole left ventricular homogenate. Myocardial CPK activity (mean ± SE) in homogenates prepared from the whole left ventricles of sham-operated animals (o) and those with coronary occlusion (x) 6, 24 and 40 hours following operation. n is indicated in parentheses.
The correlation between myocardial CPK depression and infarct size. CPK activity was measured in whole left ventricular homogenates and infarct size was determined by weight as described in the text. Solid circles represent infarcts cut out on the basis of the endocardial hemorrhagic border. Open circles indicate those excised on the basis of multiple sections through the myocardium. The regression line (least squares method) is represented as a solid line; 95% confidence limits are shown. The means ± standard error of CPK activity in homogenates from normal tissue and from the center of infarcts are indicated by open circles and vertical lines. The broken line connecting these represents the hypothetical relationship between CPK activity and infarct size, assuming that this relationship is linear. As can be seen, the regression line derived from experimental data conforms quite closely to this hypothetical linear relationship.

Further evidence linking CPK depression with the extent of infarct size was obtained with the use of radioactively labeled microspheres. Infarcts were produced in two dogs by ligation of the left anterior descending coronary artery. Since marked ischemia of 24-hour duration can be assumed to produce tissue death, blood flow measurements obtained in 11 biopsies from each dog 24 hours after ligation of the left anterior descending coronary artery. Relative local blood flow (expressed as percent of normal based on nonischemic tissue from the same heart) was estimated by the distribution of radioactively labeled microspheres in the same biopsies. The regression line (least squares method) characterizing the relationship between depression of myocardial CPK activity and reduction of blood flow 24 hours following coronary artery occlusion is represented as a broken line.
TABLE 1
Comparison of Enzyme Coupled Forward and Back Reaction Assays of CPK Standard

<table>
<thead>
<tr>
<th>Dilution of standard</th>
<th>CPK activity (IU/ml sample)</th>
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<tbody>
<tr>
<td></td>
<td>Observed</td>
</tr>
<tr>
<td><strong>Forward reaction</strong></td>
<td></td>
</tr>
<tr>
<td>Undiluted</td>
<td>3.14</td>
</tr>
<tr>
<td>1:2</td>
<td>2.75</td>
</tr>
<tr>
<td>1:4</td>
<td>2.16</td>
</tr>
<tr>
<td>Without substrate</td>
<td>1.78</td>
</tr>
<tr>
<td><strong>Back reaction</strong></td>
<td></td>
</tr>
<tr>
<td>Undiluted</td>
<td>25.72</td>
</tr>
<tr>
<td>1:2</td>
<td>11.25</td>
</tr>
<tr>
<td>1:4</td>
<td>6.53</td>
</tr>
<tr>
<td>Without substrate</td>
<td>0</td>
</tr>
</tbody>
</table>

*Dilutions of purified dog heart CPK were made in 0.01M Tris, pH 7.4, containing 2 mg/ml of bovine serum albumin. In each experiment 50 ^l of sample were assayed at 30°C in a final volume of 1.25 ml in cuvettes with a 1-cm light path.

**t**Values were obtained by subtracting $\Delta AOD_{340}$ without substrate from total $\Delta AOD_{340}$ with substrate. Results are averages of duplicate determinations.

After 1 hour of ischemia. Since this finding differed from the observations of the present study, several control experiments were performed using the assay and homogenization procedures employed in the earlier work and using an assay system entirely independent of coupled enzymes. It should be noted that the reaction involving CPK measured in the forward reaction differs markedly from that measured in the back reaction in several important respects. These include pH optimum, reaction velocity, and Michaelis constants (20). In addition, the effect of inhibitors such as EDTA or thiol group activators varies depending on the reaction being used. Furthermore, tissue constituents may affect apparent activity of CPK measured with a coupled enzyme reaction by influencing the activity of the coupling enzymes, and yet not affect the CPK activity in the forward reaction measured by assay of CP formed or of inorganic phosphate released from it.

In the present study, CPK standard obtained by purifying dog heart CPK 75-fold as described by Noda et al. (19) was assayed by several methods. As seen in Table 1, activity measured by the back reaction used in this study bore a close relation to anticipated activity over a fourfold enzyme concentration range and was entirely substrate dependent.

On the other hand, the apparent activity measured by the forward reaction, assayed under the same conditions as those employed by Braasch et al. (10), was not linear with respect to enzyme concentration and was not substrate dependent.

Results of experiments designed to examine recovery of activity of CPK standard added to the tissue extract before fractionation and dilution are shown in Table 2. In these experiments, rabbit muscle CPK was used as CPK standard. In studies of the forward reaction, the homogenization medium and homogenization procedure were the same as described by Braasch et al. (10); hence GSH was included in the homogenization medium. As shown in Table 2, recovery of standard was inconsistent and low when CPK activity was assayed by the forward reaction. On the other hand, when CPK activity was assayed by the back reaction, recovery of standard was close to that anticipated.

Results of experiments designed to examine recovery of activity of CPK standard added to the assay system are shown in Table 3. Again, recovery of standard was inconsistent and low when the forward reaction was used, while it was close to 100% when the back reaction was employed.

To compare the homogenization procedure...
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#### TABLE 2

<table>
<thead>
<tr>
<th>Recovery of CPK Standard Added to Tissue Homogenates</th>
<th>Normal myocardium</th>
<th>Nonischemic myocardium</th>
<th>Ischemic myocardium</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IU/mg</td>
<td>340/5 min</td>
<td>IU/mg</td>
</tr>
<tr>
<td>Forward reaction</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tissue alone</td>
<td>3.05</td>
<td>.072</td>
<td>3.19</td>
</tr>
<tr>
<td>Standard alone†</td>
<td>.033</td>
<td>.033</td>
<td>.033</td>
</tr>
<tr>
<td>Tissue and standard</td>
<td>.087</td>
<td>.077</td>
<td>.087</td>
</tr>
<tr>
<td>% recovery of standard</td>
<td>45%</td>
<td>21%</td>
<td>21%</td>
</tr>
<tr>
<td>Back reaction</td>
<td>18.5</td>
<td>17.66</td>
<td>17.66</td>
</tr>
<tr>
<td>Tissue alone</td>
<td></td>
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<td>.078</td>
</tr>
<tr>
<td>Tissue and standard</td>
<td>.391</td>
<td>.349</td>
<td>.349</td>
</tr>
<tr>
<td>% recovery of standard</td>
<td>93%</td>
<td>87%</td>
<td>87%</td>
</tr>
</tbody>
</table>

Tissue extracts were prepared from dog myocardium obtained from normal animals and 1 hour after operation from those subjected to coronary artery ligation. Nonischemic tissue extracts were prepared from biopsies in grossly normal areas surrounding the region supplied by the occluded vessel. Ischemic tissue was obtained from the grossly identified center of the region supplied.

*Values were obtained by subtracting AOD₃₄₀ without substrate from total AOD₃₄₀ with substrate. Results are averages of duplicate determinations.

† The AOD₃₄₀ given was measured by independently assaying the same amount of standard as that added in assays containing tissue and standard.

### TABLE 3

<table>
<thead>
<tr>
<th>Recovery of CPK Standard Added to Assay System</th>
<th>Normal myocardium</th>
<th>Nonischemic myocardium</th>
<th>Ischemic myocardium</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>IU/mg</td>
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</tr>
</tbody>
</table>

Tissue extracts were prepared as in experiments represented in Table 2.

*Values were obtained by subtracting AOD₃₄₀ without substrate from total AOD₃₄₀ with substrate. Results are averages of duplicate determinations.

† The AOD₃₄₀ given was measured by independently assaying the same amount of standard as that added in assays containing tissue and standard.

used in the present study to that used in earlier work (10), rabbit myocardium was homogenized and fractionated as shown in Table 4. CPK activity was measured in several fractions. As can be seen, there were no striking differences with respect to recovery of activity in given fractions between the two homogenization procedures used. Since others have reported an increase in dog heart CPK 1 hour after ischemia (10)
when the enzyme was assayed by the coupled enzyme forward reaction, control experiments represented in Table 5 were performed. The forward reaction was assayed by a method independent of coupling enzymes. As shown in the table, dog myocardial CPK activity does not increase in nonischemic or ischemic tissue 1 hour after ligation of the coronary artery. This is the case whether the back reaction or the forward reaction assayed by phosphate release from CP produced is studied. Furthermore, results with the phosphate-generating reaction confirmed those obtained with the coupled enzyme back reaction in rabbit myocardium. CPK activity in extracts from hearts with infarction is markedly decreased 24 hours
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after ligation of the coronary artery according to results obtained with both assay procedures.

**Discussion**

The results of this study show that myocardial CPK activity is reduced in whole left ventricular homogenates within 24 hours after experimental coronary occlusion. Activity of other myocardial enzymes such as those involved in oxidative and glycolytic metabolism declines to 30 to 70% of control values in tissue with infarctions (9, 11). This variability reflects contributions to total activity from various cell types involved in the inflammatory process. Accordingly, it has been difficult to use enzymatic indices as a measure of the extent of myocardial necrosis. CPK is unusual in this regard. High levels of CPK have been detected primarily in skeletal and heart muscle cells (14, 21) in which it is closely associated with energy requirements related to the contractile process. As shown in the present investigation, the activity of CPK in heart muscle with infarction falls markedly to 22% of control values within 24 hours. The reduction in activity begins very shortly after coronary artery occlusion, before marked inflammatory changes are apparent (1). Thus, it appears that reduced CPK activity is an index of myocardial cell destruction relatively uninfluenced by other cellular components.

The data demonstrating the correlation between decreased myocardial blood flow and depressed CPK activity support this view. Since the index of reduced blood flow was obtained 24 hours after coronary artery occlusion, it can be assumed that persistent ischemia was present in those areas which were found to be underperfused. Accordingly, the generally linear relationship between depression of CPK activity and diminished perfusion suggests that a decline in myocardial CPK activity 24 hours after coronary artery occlusion reflects the extent of tissue necrosis. It is well known that the marginal zone is comprised of both normal and ischemic cell populations (22). The data shown in Figure 4 are compatible with the view that CPK activity in biopsy material from this region reflects the contributions of both populations.

Braasch et al. have reported that CPK activity increases in the center and periphery of tissue with infarction as well as in the normal, nonischemic surrounding myocardium 1 to 2 hours following coronary artery occlusion in the dog (10). The increases they observed were variable, and were relatively similar in tissue from the center of the infarct and the apparently nonischemic tissue surrounding it. This finding was not confirmed in the present study (Table 5). The disparity is difficult to reconcile but may reflect several factors. It is possible that variation between homogenization procedures used may have contributed to the differences in results. However, when the two homogenization procedures were compared, no appreciable difference was observed with respect to recovery of CPK activity in various fractions obtained by differential centrifugation (Table 4). It is well known that CPK activity is dependent on thiol group activation (23). Oxidation of thiol groups may have influenced the values of CPK activity in previous work (10). Although GSH was included in the homogenization medium used by Braasch and co-workers, this compound is easily oxidized, and depending on the final dilution of tissue extract used, the dilution of GSH in the final assay medium may have been insufficient to afford complete activation of the enzyme. Since the redox potential is lower in ischemic tissue, relative thiol group protection may be anticipated there and an apparent increase in CPK activity might result. The importance of this is emphasized by results of experiments performed in the present study using the forward reaction. CPK activity measured by this method was 91% inhibited by 10 mM GSH in the reaction vessel, while it was 57% activated by 0.1 mM GSH. Braasch et al. do not indicate the exact dilutions used in their experiments. However, final GSH concentrations might have varied appreciably depending upon the dilutions of sample made, the nature
of the diluent used, and the extent of oxidation of GSH.

Inhibitors of the forward reaction such as ADP and inorganic phosphate are increased in ischemic tissue. These may contribute to variable results when the forward reaction measured by the coupled enzyme technique is used. Accordingly, in the present investigation, homogenates were prepared and CPK determinations were performed using the back reaction under conditions in which thiol activation was optimal. Thus, difference due to redox potential of the tissue, or oxidation of thiol groups during preparative procedures were minimized, and the influence of constituents expected to increase in ischemic tissue was minimized. The superiority of the back reaction in assays of CPK in the purified dog heart is demonstrated by results shown in Table 1. The back reaction shows greater dependence on substrate, and gives results which are virtually linear over a fourfold concentration range. On the other hand, the forward reaction shows poor dependence upon added substrate and a lack of linearity of observed activity as a function of enzyme concentration. A further comparison of the methods is provided by results shown in Tables 2 and 3. As can be seen, recovery of standard added to tissue extract or to the assay medium was inconsistent when the forward reaction was used. On the other hand, recovery of standard approximated anticipated values when the back reaction was employed. Perhaps most important assay of rabbit myocardial CPK by the phosphate-generating reaction, a measure of the forward reaction which is entirely independent of coupling enzyme, confirms the findings of depressed activity in extracts from hearts with infarction assayed by the back reaction (Table 5). Thus, results of these experiments support the view that myocardial CPK activity is characteristically depressed in ischemic tissue.

It might be argued that changes in CPK activity could result from altered work load imposed on the residual normal left ventricular tissue following myocardial infarction. Accordingly, CPK was measured in the myocardium of animals subjected to severe aortic constriction 24 hours following operation and found to be virtually identical to that observed in whole homogenates from the left ventricle of normal and of sham-operated animals.

The time course of changes in CPK activity in human serum following myocardial injury (24, 25) corresponds quite well with the time course of the depression of CPK activity in rabbit heart following coronary artery occlusion. This supports the prevalent view that myocardial injury leads to increased permeability (6) and subsequent release of CPK and other constituents into the blood. However, when one calculates anticipated peak serum values of CPK following myocardial infarction in man based on the assumption that all of the myocardial CPK in the tissue with infarction is released into the blood, the estimate is in excess of the observed peak serum values, even when one considers the role of renal excretion, distribution space, and clearance by the reticuloendothelial system of enzymes of similar molecular weight previously studied (26). Results in the present study may help to resolve this apparent paradox. In myocardium incubated in vitro for 24 hours, in which no perfusion occurs, CPK activity decreases by approximately 33% of that initially present (Fig. 1). On the other hand, in the grossly identifiable center of tissue with infarction remaining in situ for 24 hours following coronary artery occlusion, in which some perfusion persists as shown in Figure 4, myocardial CPK activity decreases by 78% of that initially present (Fig. 1). Accordingly, it is likely that a significant fraction of the reduction of myocardial CPK activity seen in tissue with infarction remaining in situ may be accounted for by denaturation and that not all of the reduction is due to washout from the myocardium into the systemic circulation.

The extent of depression of myocardial CPK activity is marked in the center of an area of infarction 24 hours following coronary artery occlusion (Fig. 1). Accordingly, it is possible to detect changes in activity of this enzyme in...
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homogenates from the whole left ventricle (Fig. 2). An estimate of the proportion of tissue with infarction can be made based on CPK depression in whole left ventricular homogenate. In general, although depression of CPK activity (Fig. 3) is linearly related to the proportion of tissue with infarction estimated by weighing the excised necrotic area, there is considerable scatter in the data demonstrating this relationship (Fig. 3). This may well reflect the difficulty of excising tissue with infarction in a precise manner based on gross morphologic changes. Furthermore, the extent of ischemic damage may be quite heterogeneous in part because of variations of blood flow within any given gross area of infarction. For these reasons, the difficulties in validating any proposed index of infarct size based on measurement of a chemical moiety in homogenates of whole left ventricle are formidable. Despite these inherent difficulties, the data obtained in this investigation suggest that myocardial CPK depression may be useful as an index of tissue damage following coronary artery occlusion. This view is supported by consideration of the regression line shown in Figure 3. The extrapolation of this line to a hypothetical 100% infarct (on the abscissa) provides a hypothetical CPK specific activity in whole left ventricular homogenate (on the ordinate) which corresponds quite closely to the CPK specific activity actually observed in homogenate from the center of tissue with infarction. As noted earlier, the difficulties entailed in validating estimates of infarct size based on chemical determinations make such estimates hazardous when applied to any given heart. However, since depression of CPK activity in groups of hearts subjected to coronary artery occlusion appears to correlate with myocardial damage, its measurement has potential value in assessing the effect of therapeutic and prophylactic measures on the extent of myocardial necrosis following experimental coronary artery occlusion.

References

KJEKSMUS, SOREL


Depressed Myocardial Creatine Phosphokinase Activity Following Experimental Myocardial Infarction in Rabbit
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