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Research Article

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Quantitative Assessment of the Extent of Myocardial Infarction in the Conscious Dog by Means of Analysis of Serial Changes in Serum Creatine Phosphokinase Activity

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ABSTRACT This study was designed to develop a method for quantitative assessment of infarct size in the conscious animal based on serial changes of serum creatine phosphokinase (CPK) activity. From 11 experiments in which myocardial CPK was injected intravenously in conscious dogs, the average CPK distribution space and average CPK fractional disappearance rate from serum were found to be 11.4% of body weight and 0.48% min respectively. In other experiments, myocardial infarction was produced in 22 conscious dogs by constriction of a left coronary artery snare and serum CPK activity was determined at frequent intervals for 24 hr. Since myocardial CPK depletion reflects infarct size, infarct size was determined directly by analysis of myocardial CPK content in the same animals 24 hr after coronary artery occlusion. CPK released from the infarct was determined from observed changes in serum CPK activity analyzed according to a model taking into account the fraction of CPK released from an infarct and the rates of appearance and disappearance of CPK activity from serum. Infarct size was calculated on the basis of observed changes in serum CPK and compared to infarct size determined directly by analysis of myocardial CPK depletion. Agreement was close and results from all experiments fit the equation: [infarct size (g) determined from serum CPK] = 1.13 × [infarct size (g) determined from myocardial CPK] − 1.3, $r = 0.96$, $n = 22$. The method described is useful for accurate assessment of infarct size in the conscious animal and for detection of modification of infarct size produced by pharmacologic interventions.

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INTRODUCTION

Quantification of the extent of myocardial infarction in the conscious experimental animal and in man remains an important unsolved problem (1). The incidence of myocardial infarction with shock, a major cause of death in patients with acute myocardial infarction, is related to the extent of myocardial necrosis (2, 3), and it appears that infarct size can be modified with pharmacologic and physiologic interventions in the acute experimental preparation (4). Accurate means for determining infarct size would provide information necessary for correlating changes in ventricular performance and hemodynamics with the extent of myocardial damage. Such information would be useful for grouping patients with acute myocardial infarction according to infarct size thus allowing more meaningful assessment of the value of potentially therapeutic interventions.

Myocardial injury has been assessed by analysis of tissue from animals previously subjected to coronary artery occlusion with histologic, histochemical, electron microscopic, and biochemical techniques (1, 5–12). We have demonstrated that myocardial creatine phosphokinase (CPK)[1] depletion provides an accurate index of infarct size in the rabbit (13) and in the dog (4). However, all of these methods require sacrificing the experimental animal and analyzing myocardium at necropsy.

[1] *Abbreviation used in this paper:* CPK, creatine phosphokinase.

Results of several investigations suggest that there is a relationship between the magnitude of changes in serum enzymes and the extent of infarct size after coronary artery occlusion (14–19), but the lack of close correlation has been disappointing. There are several reasons for this: (*a*) enzyme activity appearing in serum after myocardial infarction may reflect release not only from myocardial cells but also from other components in the heart participating in the inflammatory response, skeletal muscle, liver, and other parenchymal tissues (20, 21); (*b*) the magnitude of peak serum enzyme elevation depends not only on infarct size but also on the rate of release of enzyme into the circulation, the enzyme distribution space, and the rate of enzyme disappearance from the circulation (20, 22); and (*c*) current morphologic techniques for measuring infarct size unavoidably lack precision (18, 23, 24) limiting the validity of conclusions dependent upon anatomic criteria.

Since myocardial CPK depletion has been shown to be proportional to infarct size (13), and since the fraction of CPK released from the center of an infarct is relatively constant (13, 4), the present study was designed to develop a quantitative index of infarct size in the conscious dog based on changes in serum CPK activity. Accordingly, the kinetics of serum CPK disappearance after injection of purified enzyme were studied; a model comprising terms for CPK release into and disappearance from the circulation was developed; constants for the model were obtained from independent studies of the disappearance rate of injected CPK; and infarct size was estimated by means of serial changes in serum CPK after coronary artery occlusion in the conscious animal. Infarct size estimated by this method was compared with infarct size determined directly by measurement of total myocardial CPK depletion 24 hr after coronary artery occlusion in the same animal. In order to determine whether this method detects modifications of infarct size experiments were performed in which isoproterenol was administered in doses previously shown to augment infarct size in the open-chest dog (4). Infarct size after isoproterenol administration to conscious animals with coronary artery occlusion was estimated by analysis of serum CPK and compared to infarct size measured directly in the same animal.

METHODS

Animal preparations

Coronary artery occlusion was produced in 22 conscious dogs by constriction of a left anterior descending coronary artery snare placed around the vessel during operation 1 wk earlier. At the time of operation, animals were anesthetized with sodium thiamylal, 10 mg/kg, and anesthesia was maintained with 0.5% fluothane. A left thoracotomy was performed, the pericardium opened, and a 1-silk suture placed loosely around a branch of the left anterior descending coronary artery and exteriorized through a polyethylene tube (PE 240) secured to the epicardium, subcutaneous tissue, and skin. Jugular venous catheters were implanted for obtaining blood samples later and a left atrial catheter was inserted and secured for subsequent determination of left atrial pressure.

3–5 days after the operation, coronary artery occlusion was produced. Base line blood samples were drawn, animals were premedicated with lidocaine 1 mg/kg intravenously, and the coronary artery snare was occluded by traction from the external surface and secured to the skin. Blood samples were obtained at 60- to 120-min intervals for serial CPK determinations. 24 hr after coronary artery occlusion, each animal was anesthetized with injection of sodium pentobarbital, 30 mg/kg, and the heart excised. Myocardial CPK was measured in biopsies from normal posterior myocardium and grossly ischemic regions on the anterior surface, as well as in homogenate from the entire left ventricle.

In experiments in which the effects of isoproterenol were studied, the drug was suspended in peanut oil and administered subcutaneously, 0.015 mg/kg, beginning 14–17 hr after coronary artery occlusion.

In experiments in which the effects of reduction in cardiac output and renal blood flow on disappearance of injected CPK from serum were studied, cardiac output was reduced by constriction of a snare placed around the inferior vena cava and renal perfusion was reduced by constriction of a snare placed around the left renal artery 1 wk earlier. Continuous measurements of cardiac output were performed in animals with caval constriction with the use of an electomagnetic flow meter around the aorta as previously described (25, 26).

Reagents

Chemicals were of highest commercially available grade and were formulated in doubly glass-distilled water. CPK standard was obtained from Worthington Biochemical Corp., Freehold, N. J. Cysteine-hydrochloride, phosphoenol pyruvate, creatine, reduced nicotive adenine dinucleotide, and bovine serum albumin were obtained from Sigma Chemical Co. Creatine phosphate, glucose-6-phosphate dehydrogenase, reduced glutathione, nicotine adenine dinucleotide phosphate, sodium adenosine diphosphate, sodium adenosine triphosphate, sodium adenosine monophosphate, pyruvate kinase, lactic dehydrogenase, and hexokinase were obtained from Calbiochem. Isoproterenol hydrochloride was obtained from Winthrop and suspended in peanut oil (USP).

Preparation of myocardial homogenates

24 hr after coronary artery occlusion, each animal was sacrificed, and the heart rapidly excised and weighed. Full wall thickness biopsies (approximately 1 g) were obtained from four grossly normal areas for CPK analysis. The observed activity of CPK in these biopsies was used as the value for normal left ventricular CPK in that specific animal. Whole left ventricular homogenates were prepared by combining the homogenates from the normal biopsies with homogenate prepared from the remainder of the left ventricle. Ventricular myocardium was minced with scissors, homogenized in a Waring Blendor (half speed, four 15-sec bursts) in 25 ml/g of homogenizing medium consisting of 0.25 M sucrose, 0.001 M neutralized sodium ethylenediaminetetraacetic acid, and 1×10^{-3} M mercaptoethanol. The homogenate was then centrifuged at 16,000 *g* for 10

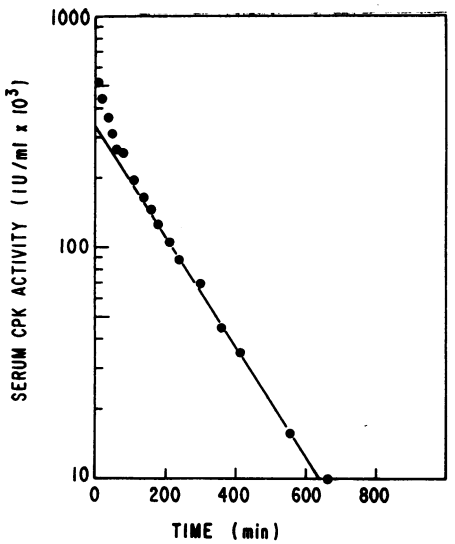

FIGURE 1 Changes in serum CPK activity after intravenous injection of partially purified CPK in the conscious dog. Serial serum CPK activity determinations were performed after injection of purified enzyme intravenously. The disappearance conformed to a biphasic curve as described in the text. The fractional disappearance rate (k_d) was calculated from the slope of the monoexponential portion of the curve.

min at 0°C and the supernatant fraction removed and centrifuged at the same speed for 10 min. Ventricular biopsies were processed similarly except that homogenization was performed in a Virtis 45 homogenizer, speed setting 5, in four 15-sec bursts.

Biochemical procedures

Protein contents of homogenates and of tissue fractions were determined by the biuret procedure (27). CPK in serum and tissue fractions was assayed spectrophotometrically as described by Rosalki (28). Results were expressed as International units (IU)/ml, IU/mg protein, or IU/g myocardium. CPK activity was heat and acid labile, entirely dependent on substrate, and not influenced by inhibitors in the tissue as shown by mixing experiments using standard purified enzyme. Recovery of myocardial CPK activity in the supernatant fraction varied between 82 and 86%.

CPK activity in serum was assayed in 50-μl aliquots in a final volume of 1.0 ml. When serum CPK activity was very high, appropriate dilutions were made in 0.01 M trihydroxymethylaminomethane (Tris), pH 7.4, containing bovine serum albumin, 0.2%. In all experiments, the amount of CPK assayed in any given sample was chosen such that activity was within the range in which the assay was linear.

In experiments designed to assess the kinetics of disappearance of injected CPK from serum of conscious dogs, myocardial CPK was obtained from freshly excised dog hearts and purified according to Noda, Kuby, and Lardy (29). In this procedure, tissue is extracted with 0.9% sodium chloride, and CPK is concentrated with ammonium chloride and ethanol. In our hands, enzyme purification led to a 100-fold increase in CPK specific activity, and a yield of 6–10% in the final lyophilized product. This concentrated CPK fraction was resuspended in 0.9% NaCl before intravenous injection into the animals. The isoenzyme profile of this fraction was determined with the use of cellulose-acetate electrophoresis and fluorescence scanning and was found to be virtually identical with that of CPK in whole homogenates of the dog heart. The dominant isoenzyme was the MM band in both. In addition, both whole homogenate and the enzyme fraction contained approximately 20% of a more rapidly migrating isoenzyme, MB. In all experiments concerning CPK kinetics, whether after injected CPK or after coronary artery occlusion, studies were not performed until base line serum CPK activity was stable and less than 0.1 IU/ml.

Analysis of data

Direct determination of infarct size. As demonstrated previously (13) the extent of myocardial CPK depletion in the whole left ventricle 24 hr after coronary artery occlusion is related to infarct size. CPK depletion within an infarct is heterogeneous (4) as is the distribution of tissue necrosis according to other criteria (10–12, 30, 31). Thus, total left ventricular CPK depletion is the sum of partial depletion in some areas of ischemic injury and maximal depletion in other areas. In the present study, CPK depletion in the center of large infarcts 24 hr after coronary artery occlusion was consistently reduced to 25% of the concentration in normal myocardium. In each experiment, total left ventricular CPK depletion 24 hr after coronary artery occlusion was determined. Infarct size (in grams) was calculated as total CPK depletion (IU) divided by 75% of CPK concentration (IU/g) in normal myocardium as shown in section A of the Appendix.

Estimation of infarct size based on serial changes in serum CPK activity after coronary artery occlusion. The major goal of this study was to determine whether myocardial CPK depletion after coronary artery occlusion could be determined accurately by analysis of serial changes in CPK activity in serum. Accordingly, the following mathematical model was employed. We assumed that the instantaneous change in serum CPK activity was due to an appearance function, i.e., release of CPK from the heart into the circulation, and a disappearance function, i.e., removal of CPK from the circulation by a variety of mechanisms. As shown in section B of the Appendix, based on these assumptions, one can derive a value for the absolute amount of CPK released from the heart into the circulation based only on observed serial changes in serum CPK activity. To use this model operationally one needs to know: (*a*) the effective distribution space of CPK (i.e., the actual volume in which CPK is distributed after injection into the circulation); (*b*) the fractional disappearance rate (k_d) of CPK from the circulation; and (*c*) the observed values of serum CPK activity after coronary artery occlusion. In the present study, CPK distribution space and k_d were determined in separate experiments in which partially purified myocardial CPK was injected into conscious dogs. In other experiments, serial changes in serum CPK activity were determined in conscious animals subjected to coronary artery occlusion. The data were analyzed according to the equations in section B of the Appendix. Thus, a value for total CPK released into the circulation was obtained in each experiment in which an animal was subjected to coronary artery occlusion.

CPK depletion in myocardium undergoing necrosis is due to at least two phenomena: release of enzyme into the circulation and denaturation of enzyme locally (13). In order

to calculate infarct size from myocardial CPK released into the circulation, one needs to know what fraction of myocardial CPK depleted in an infarct is released. That fraction of depleted myocardial CPK which was released and thus appeared in the circulation was similar in all experiments (see Results). Accordingly, infarct size was calculated from serial changes in serum CPK activity as shown in section C of the Appendix.

RESULTS

Disappearance of injected CPK from serum. In 11 experiments partially purified myocardial CPK was injected intravenously into conscious dogs. Changes in serum CPK activity after injection showed a characteristic biphasic pattern (Fig. 1): an initial decay representing dilution into the effective distribution space, followed by a sustained monoexponential decay with a fractional disappearance rate (k_d) of 0.0048 min^{-1} ±0.0003 (mean ±SE, n = 11). This pattern is similar to that seen in studies of other enzymes in the circulation (20, 22).

Effective CPK distribution space was calculated by extrapolating the monoexponential portion of the curve to zero time, and dividing the value for CPK concentration at this time into the known amount of CPK injected. The effective distribution space in 11 experiments was 11.4 ±0.1% of body weight (mean ±SE, n = 11). Thus, effective distribution space was found to be almost 3 times plasma volume (32).

In experiments designed to calculate infarct size by means of analysis of changes in serum CPK, results would be altered only if k_d varied markedly, for example, by more than 2-fold. Factors potentially capable of influencing k_d in animals with myocardial infarction include several, among which are decreased cardiac output and altered renal function. In two experiments cardiac output was reduced by 20 and 25% in conscious dogs by partial occlusion of the inferior vena cava for 6 hr. In two additional experiments renal blood flow was decreased by complete occlusion of the left renal artery with a snare. These interventions did not change k_d markedly when injected CPK was administered to the animals (average k_d = 0.007 min^{-1} in animals with diminished cardiac output 0.006 min^{-1} in animals with decreased renal perfusion). However, it should be noted that in all of five experiments in which anesthesia was maintained with sodium pentobarbital for 16 hr k_d decreased by approximately 80% in the presence of adequate ventilation.

Decrease of myocardial CPK activity after coronary artery occlusion. In 11 dogs myocardial CPK activity in the center of an infarct was determined after coronary artery occlusion. In these experiments, the coronary artery snare was occluded at the time of operation while the animals were anesthetized. The center of the infarct was determined with the use of epicardial electrocardiographic maps of ST segment elevation as previously described (4). Since we have shown that the mapping procedure predicts the area in which CPK depression will be maximal 24 hr later, the map was used in the present study to locate this region. Serial biopsies (approximately 200 mg) were obtained from this region after coronary artery occlusion, and CPK activity and protein determined in homogenates of each biopsy. CPK activity in serial biopsies from nonischemic regions from the same dog heart did not vary appreciably in any of six experiments. The time course of CPK depletion in the center of infarcts is shown in Fig. 2. During the first 5–6 hr CPK activity remained relatively constant. Between 6 and 14 hr after coronary artery occlusion myocardial CPK activity decreased markedly. 24 hr after coronary artery occlusion approximately 70% of initial CPK activity was lost. This pattern of depletion of enzyme activity in ischemic tissue is similar to that reported in studies of other enzymes (5) and in studies of CPK depletion in the rabbit (13). In nonischemic areas, myocardial CPK content was consistently within 5% of the average left ventricular CPK content in normal dogs (n = 32).

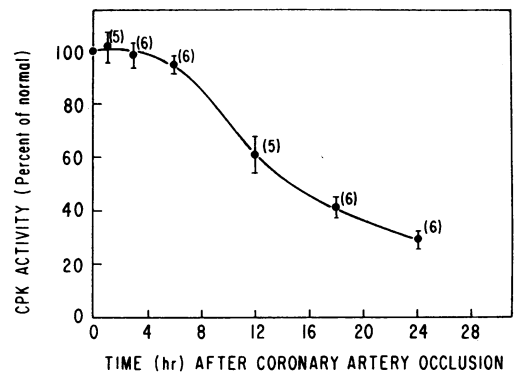

FIGURE 2 Loss of myocardial CPK activity in the center of infarcts produced by coronary artery occlusion. Determinations of myocardial CPK activity were performed as described in the text. Results expressed are means ±SE with the number of dogs studied at each time interval indicated in parentheses.

Changes in serum CPK activity after acute coronary artery occlusion in the conscious dog. Results of serial studies of serum CPK activity after coronary artery occlusion in 11 conscious dogs are shown in Table I. An example of the data from one animal is illustrated in Fig. 3. In general, serum CPK activity did not begin to increase for several hours after coronary artery occlusion. Subsequently, it increased rapidly to a peak. The delay in enzyme elevation corresponded with the delay in myocardial CPK depletion and the period of rapid rise of serum CPK activity correlated with the period of rapid depletion of myocardial CPK activity.

TABLE I

Serum CPK Activity after Coronary Artery Occlusion in the Conscious Dog

Experiment	Time after coronary artery occlusion (min)															
	60–100	100–200	200–300	300–400	400–500	500–600	600–700	700–800	800–900	900–1000	1000–1100	1100–1200	1200–1300	1300–1400	1400–1500	1500–1600
1	57	221	358	450	467	410	377	269	230	127	103	72	54	—	—	
2	62	247	384	473	589	748	795	748	411	400	347	319	271	260	—	
3	40	179	550	900	1276	1338	2452	2500	2418	1405	1249	1270	—	676	—	
4	20	65	160	248	299	288	218	180	161	—	—	—	46	27	—	
5	91	204	280	296	361	332	307	276	249	185	148	121	101	93	75	
6	20	150	218	389	410	423	345	340	281	215	123	99	72	—	—	
7	63	200	470	804	858	872	848	737	579	426	—	—	232	—	—	
8	8	31	157	693	937	1070	1103	1090	962	793	620	—	—	442	359	
9	0	24	216	386	571	1270	1624	1876	1300	795	570	550	—	—	200	
10	100	772	1285	1380	2230	2354	1312	907	508	303	—	—	—	—	—	
11	8	32	95	148	260	353	437	306	252	153	—	—	—	84	—	

The apparent decay of serum CPK activity after myocardial infarction is not the same as the decay of serum CPK activity after injection of partially purified enzyme. The latter is monoexponential as has been found in studies of other serum enzymes (20, 22). However, CPK activity disappears from serum at a different rate after an infarct because apparent decay is probably affected by continued release of enzyme into the circulation during a substantial portion of the first 24 hr after coronary artery occlusion. Such an interpretation is supported by analysis of the integrated value for CPK released into the circulation as a function of time after coronary artery occlusion (Fig. 4). As can be seen, total CPK released rises relatively rapidly initially, and then continues to increase slowly at a progressively diminishing rate. The characteristics of this curve are consistent with continued slow release of myocardial CPK after a relatively discrete release of enzyme approximately 3–12 hr after coronary artery occlusion.

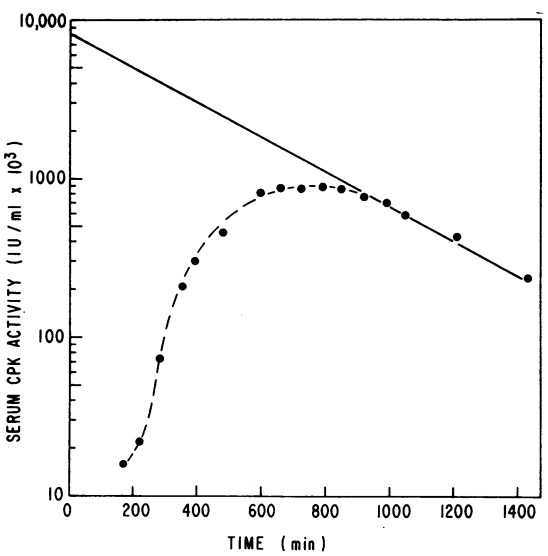

FIGURE 3 Changes in serum CPK activity after myocardial infarction produced by coronary artery occlusion in the conscious dog. Coronary artery occlusion was produced by constriction of a previously placed snare as described in the text. Results plotted demonstrate the characteristic changes in serum CPK activity observed as a function of time after coronary artery occlusion. The terminal portion of the curve has a slope differing from the slope in Fig. 1 because decay of enzyme activity in serum after infarction is influenced by the continued appearance of CPK released from the heart. Thus, the observed serum CPK disappearance curve reflects simultaneous decay of activity of enzyme in serum and release of additional activity from myocardium.

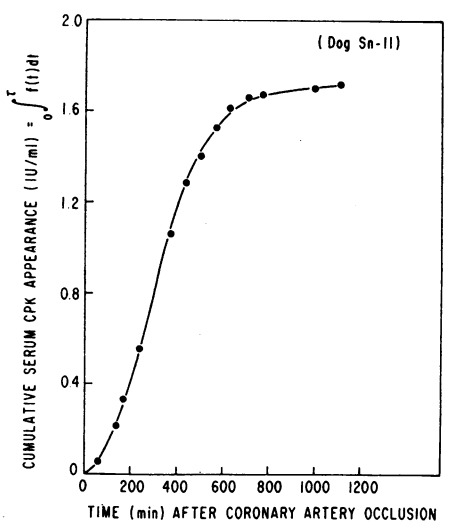

FIGURE 4 Integrated CPK appearance function [$\int_0^t f(t)dt$]. Serial serum CPK determinations were performed in the conscious dog after coronary artery occlusion and $\int_0^t f(t)dt$ calculated as described in the text and section B of the Appendix. As can be seen, this index approaches a maximum which represents the total CPK released from ischemic myocardium which would have appeared in 1 ml of serum if no simultaneous loss of CPK activity from serum were occurring.

TABLE II

Infarct Size Based on Serum CPK Changes

Experiment	Body weight	Integrated appearance function	CPK released	Total myocardial CPK depleted	Infarct size
	g	*IU/ml**	*IU‡*	*IU§*	*g‖*
C-1	23,000	1.465	3,841	12,803	16
C-2	22,000	2.779	6,969	23,230	29
C-3	16,000	6.841	12,477	41,590	52
C-4	17,500	.564	1,128	3,760	5
C-5	22,000	.744	1,860	6,200	8
C-6	22,000	.692	1,730	5,767	7
C-7	35,000	1.616	6,448	21,493	27
C-8	21,000	2.314	5,540	18,467	23
C-9	25,000	4.605	13,124	43,747	55
C-10	16,000	5.756	10,499	34,997	44
C-11	25,000	1.185	3,392	11,307	14
I-1	24,000	0.197	539	1,797	2
I-2	22,000	4.505	11,299	37,633	47
I-3	20,000	4.096	9,339	3,113	39
I-4	24,000	0.111	302	1,007	1
I-5	24,000	0.854	2,337	7,790	10
I-6	23,000	0.279	732	2,440	3
I-7	19,000	0.166	360	1,200	2
I-8	19,000	0.074	160	533	1
I-9	12,000	8.495	11,854	39,511	49
I-10	29,000	1.015	3,356	11,187	14
I-11	20,000	2.681	6,113	20,377	26

* $\int_0^t f(t)dt$.

‡ $\int_0^t f(t)dt$ [11.4% of body weight (i.e. CPK distribution space)] = CPK_r.

§ $CPK_r/0.3$ (i.e. fraction of myocardial CPK depleted that appears in serum) = CPK_d.

‖ CPK_d/800 IU per g.

C refers to animals with coronary artery occlusion alone; I refers to animals with coronary artery occlusion to whom isoproterenol was administered as described in the text.

Infarct size estimated from changes in serum CPK compared with infarct size determined directly by analysis of myocardial CPK depletion. Total CPK released from the heart was calculated as discussed earlier (section B of the Appendix) in 22 dogs after coronary artery occlusion. The correlation between estimated CPK released from the heart determined by serum CPK analysis and infarct size determined by myocardial CPK analysis is shown in Fig. 5. Results from all experiments fit the regression line: CPK released (IU) equal 272 × grams infarct − 318, $r = 0.96$, $n = 22$. The standard deviation of the slope of this line was 18. Values employed in calculations used to estimate infarct size from serial changes in serum CPK are shown in Table II.

It is likely that some of the diminution of myocardial CPK activity after coronary artery occlusion reflects local degradation and denaturation of enzyme activity without corresponding release of CPK into the circulation. In the present study, the fraction released was determined in animals with coronary artery occlusion alone and those treated with isoproterenol after coronary artery occlusion. This fraction was found to be relatively constant with a value of 0.30 ±0.02 (mean ±SE, $n = 22$). Thus, only approximately 30% of myocardial CPK depletion was reflected by CPK appearance. The result is in agreement with previous observations in the rabbit (13) comparing loss of CPK activity from incubated but not perfused myocardium with CPK depletion in the center of myocardial infarcts *in situ*. Table III shows the results of comparison of infarct size, expressed both in grams and as per cent of left ventricular weight calculated on the basis of myocardial CPK depletion and

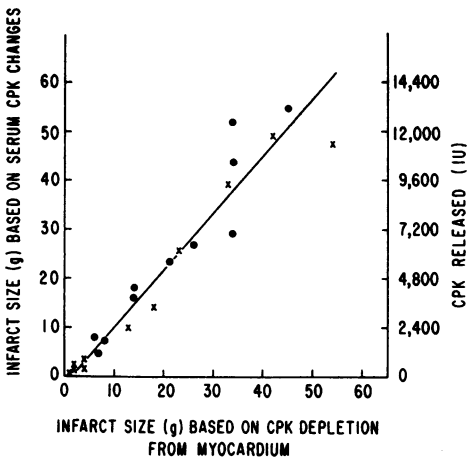

FIGURE 5 The relation between infarct size determined on the basis of changes in serum CPK activity and infarct size determined on the basis of depletion of myocardial CPK activity 24 hr after coronary artery occlusion. Results from experiments in which animals were subjected to coronary artery occlusion alone are indicated by (●), while those from experiments in which animals were given isoproterenol after coronary artery occlusion are indicated by (×). Infarct size (in grams), determined by analysis of myocardial CPK activity as described in the text, is represented on the abscissa while infarct size (in grams), calculated on the basis of serial changes in serum CPK activity, is represented on the left ordinate. Total CPK released (CPK_r) calculated as described in the Appendix, is represented on the right ordinate. Results from all experiments fit the following regression lines (least squares method):

1. [infarct size (grams) (determined from serum CPK change)] = 1.13 × [infarct size (grams) (from tissue CPK)] − 1.3, $r = 0.96$, n = 22, SD of slope = 0.07.
2. CPK_r (I.U.) = 22 × [infarct size) (grams) (from tissue)] − 318, $r = 0.96$, n = 22.

infarct size estimated from CPK released into the circulation and corrected for fractional myocardial CPK release (0.30).

As can be seen, there is good correspondence between per cent infarct calculated and estimated in each case over a range of infarct size from 300 mg to 54 g. Results of this correlation are depicted graphically in Fig. 5 and fit the regression line: infarct size (serum) (grams) = 1.13 × infarct size (tissue) − 1.3, $r = 0.96$, n = 22.

Other indices of infarct size based on changes in serum CPK activity after coronary artery occlusion in the conscious dog. The peak serum CPK value obtained in experiments in which coronary artery occlusion was produced in the conscious animal correlated roughly with the size of the myocardial infarct in the same animal measured directly by myocardial CPK depletion (Fig. 6). However, there was considerable scatter in these data and the correlation was not nearly as close as that between the integrated value for total CPK released and

TABLE III

Comparison of Infarct Size Based on Serum CPK Changes and Myocardial CPK Depletion

Experiment No. 1	Infarct size based on serum CPK changes		Infarct size based on myocardial CPK depletion	
	g	*% LV weight*	*g*	*% LV weight*
C-1	16	13	14	12
C-2	29	19	34	22
C-3	52	61	34	40
C-4	5	6	7	9
C-5	8	9	6	7
C-6	7	9	8	10
C-7	27	19	26	18
C-8	23	23	21	21
C-9	55	52	45	43
C-10	44	56	34	44
C-11	14	15	18	19
I-1	2	3	2	3
I-2	47	34	54	39
I-3	39	43	33	36
I-4	1	1	2	2
I-5	10	13	13	16
I-6	3	4	4	5
I-7	2	2	4	5
I-8	1	1	1	1
I-9	49	52	42	45
I-10	14	11	18	14
I-11	26	33	23	29

C refers to animals with coronary artery occlusion alone; I refers to animals with coronary artery occlusion to whom isoproterenol was administered as described in the text.

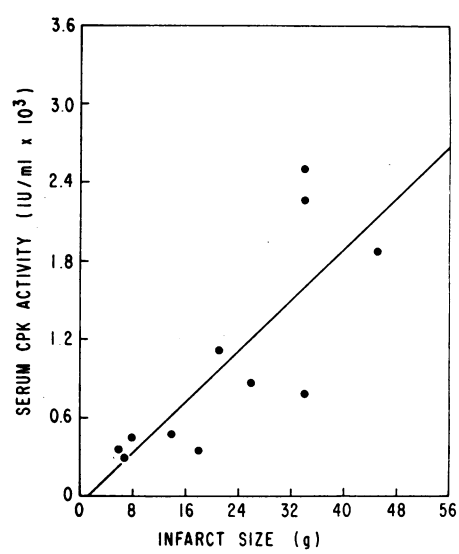

FIGURE 6 The relation between peak serum CPK activity and infarct size determined by analysis of myocardial CPK content 24 hr after coronary artery occlusion. Peak serum CPK is represented on the ordinate and infarct size (in grams), determined from myocardial CPK depletion, is represented on the abscissa. As can be seen, there is a general relation between peak serum CPK activity and infarct size, but the correlation is not close for reasons discussed in the text.

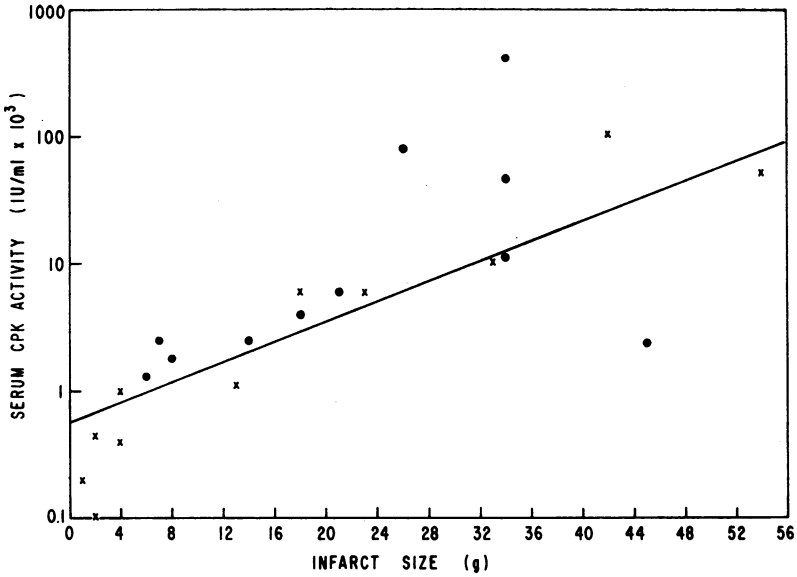

FIGURE 7 The relation between the extrapolated value for serum CPK activity (CPK_0) and infarct size determined by analysis of myocardial CPK depletion. Results from experiments in which animals were subjected to coronary artery occlusion alone are indicated by (●), while those from experiments in which animals were given isoproterenol after coronary artery occlusion are indicated by (×). In experiments in which more than one peak of serum CPK elevation occurred, the extrapolation to time zero was performed by using the descending portion of the last peak. Although there is a general relation between CPK_0 and infarct size, the correlation is not close for reasons discussed in the text.

infarct size. Results fit the regression equation: peak serum CPK (mIU/ml) = 50 × (infarct size [from tissue CPK] [grams]) − 88, r = 0.8, n = 11.

The time to peak serum CPK activity after coronary artery occlusion was 726 ±30 min (mean ±SE, n = 11). Since the slope of the terminal portion of the CPK serum decay curve after myocardial infarction was consistent, and since peak serum CPK activity occurred approximately 12 hr following coronary artery occlusion, it appeared likely that extrapolation of the terminal portion of the curve in any given experiment to the onset of rise time would provide an empiric index of infarct size. The value for CPK obtained by this extrapolation (CPK_0) would be expected to represent the hypothetical CPK concentration at zero time assuming that all the CPK released into the circulation was released as a bolus and diluted into the CPK distribution space. However, the correlation between CPK_0 with infarct size measured directly by myocardial CPK depletion (Fig. 7) was not nearly as close as that between integrated value of total CPK released and infarct size.

Alteration of infarct size by administration of isoproterenol after coronary artery occlusion in the conscious dog. In order to evaluate the validity of using changes in serum CPK activity to quantitatively assess modification of infarct size in the conscious animal, isoproterenol was administered to 11 animals with coronary artery occlusion. Administration of this agent increases infarct size and extent of myocardial CPK depletion in the open-chest, anesthetized animal (4). Results of an illustrative experiment in the present study demonstrating changes in serum CPK activity after isoproterenol administration to a conscious animal previously subjected to coronary artery occlusion are shown in Fig. 8. Administration of isoproterenol was followed by a substantial secondary rise in serum CPK activity suggesting that additional enzyme was released from the myocardium. This secondary rise in serum CPK activity after isoproterenol administration was seen in 10 of 11 experiments (Table IV). In the 11th, serum CPK activity did not increase after coronary artery occlusion alone, most likely because of the rich collateral network in this particular dog. However, after isoproterenol administration serum CPK became elevated suggesting that myocardial necrosis occurred after isoproterenol administration in the presence of a coronary artery occlusion by itself insufficient to produce an infarct. Based on the integrated value for total CPK released, and the proportion of total CPK released after isoproterenol administration compared to that released after coronary artery occlu-

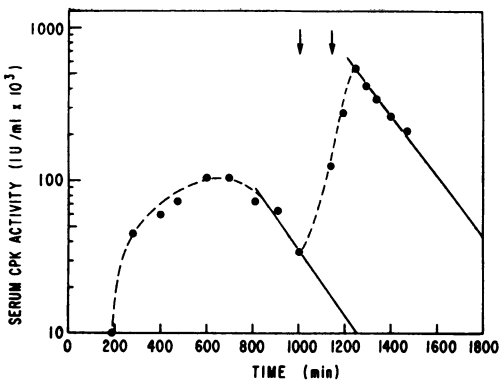

FIGURE 8 Changes in serum CPK activity after isoproterenol administration to a dog with coronary artery occlusion. Isoproterenol was administered at the times indicated by arrows. As can be seen, a substantial secondary peak of elevated serum CPK activity distorted the decay portion of the serum CPK curve after coronary artery occlusion alone.

sion alone, administration of isoproterenol augmented the extent of infarct size from 15 to 240%.

Results of calculation of infarct size when isoproterenol is administered to dogs with coronary artery occlusion would be altered if k_d varied markedly due to drug administration per se. However, in three experiments in which isoproterenol (0.015 mg/kg subcutaneously) was administered to conscious dogs without coronary artery occlusion and purified CPK was injected intravenously the average k_d was 0.009 min^{-1}. In addition calculated infarct size would be altered if isoproterenol administration per se led to an elevation of serum CPK activity. In four experiments in which isoproterenol (0.015 mg/kg subcutaneously) was administered twice at 3-hr intervals to conscious dogs without coronary artery occlusion there was no increase in serum CPK activity during a 24 hr observation period. Furthermore, if isoproterenol produced or exacerbated congestive heart failure in animals with myocardial infarction, k_d might be altered and/or CPK release from skeletal muscle might obscure changes in serum CPK due to release from myocardium. Accordingly, in six experiments mean left atrial pressure was determined immediately before, 15 min after, and 2 hr after isoproterenol administration in animals with coronary artery occlusion. In each instance, control values for mean left atrial pressure were less than 10 mm Hg. Administration of isoproterenol led to decreased mean left atrial pressure in all animals (4 ±0.5 mm Hg, mean decrease ±SE). This result is consistent with previous reports (33), and indicates that administration of isoproterenol in the dose used in the present study did not precipitate congestive heart failure and hence would not be expected to markedly influence serum CPK kinetics by changing hemodynamics.

DISCUSSION

A major cause of death in acute myocardial infarction in man is cardiogenic shock. Extensive necrosis may underlie the development of this syndrome (2, 3). Accordingly, the observation that infarct size can be favorably influenced by pharmacologic and physiologic interventions in the open-chest experimental animal after coronary artery occlusion is of particular interest (4), and the need for indices of infarct size in the intact experimental animal and in man is apparent.

This study was designed to develop an accurate, quantitative method for assessment of infarct size in the conscious experimental animal and for detection of modification of infarct size produced by pharmacologic and physiologic interventions. Techniques capable of accurately determining infarct size in the conscious animal would be useful in evaluating potentially therapeutic interventions. Application of such techniques to man would be useful in clarifying the relationship between infarct size and ultimate prognosis.

TABLE IV

Serum CPK Activity in the Conscious Dog after Coronary Artery Occlusion and Isoproterenol Administration

Experiment	Time after coronary artery occlusion (min)															
	60–100	100–200	200–300	300–400	400–500	500–600	600–700	700–800	800–900	900–1000	1000–1100	1100–1200	1200–1300	1300–1400	1400–1500	1500–1600
1	0	30	25	28	0	0	5	9	24	35	46	83	65	45	35	—
2	0	4	37	200	410	660	910	1108	756	600	620	1006	1260	920	620	520
3	0	60	100	260	615	840	860	780	660	520	340	665	795	520	255	246
4	22	25	26	30	32	13	7	7	0	7	23	15	0	—	—	—
5	0	0	0	0	0	0	0	0	50	166	510	382	237	211	140	109
6	6	7	71	75	80	95	52	7	0	10	24	9	0	—	—	—
7	23	34	17	12	0	0	0	0	13	36	47	42	36	28	—	—
8	0	38	40	28	22	22	0	0	26	20	0	—	—	—	—	—
9	0	200	870	1070	1320	1660	1535	1480	1235	540	590	670	670	770	600	560
10	2	9	15	—	73	103	103	85	72	63	33	123	520	335	235	—
11	10	56	147	366	500	640	800	500	394	394	360	370	360	326	170	149

Several studies have been concerned with utilizing changes in serum enzyme concentration to assess the extent of myocardial infarction in experimental animals (14–19). Lemley-Stone, Merrill, Grace, and Meneely (14), produced coronary artery occlusion in open-chest dogs and followed changes in serum transaminase for several days subsequently. There was a general relationship between per cent rise in transaminase and infarct size determined by gross anatomic changes at the time of postmortum examination. Similarly, Nydick, Wroblewski, and LaDue (15) produced coronary artery occlusion in closed-chest dogs and observed a general though inprecise relationship between infarct size determined by gross anatomic changes and peak serum transaminase. Nachlas, Friedman, and Cohen (18) used dehydrogenase staining techniques to measure infarct size after coronary artery occlusion in open-chest dogs and observed a gross relationship between peak serum lactate dehydrogenase activity and infarct size. Other investigators have confirmed the semiquantitative relationship between peak activity of several serum enzymes and infarct size (16, 17, 19). However, the correlation between infarct size and peak serum enzyme activity is not sufficiently close to permit accurate determination of infarct size in a given instance. This appears to be unavoidable, and reflects several factors among which are: difficulty in determining infarct size by gross and/or microscopic criteria, difficulty in excluding the contribution of enzyme activity in serum derived from skeletal muscle and other nonmyocardial components particularly in experiments in which animals are studied acutely, and variation in the rate of release of enzyme from myocardium undergoing necrosis in different animals.

The present study was designed to obviate several of these difficulties. Conscious animals were used in order to avoid changes in serum enzyme activity due to thoracotomy or other invasive procedures at the time of coronary artery occlusion. Serum CPK was chosen as the enzyme of interest since we have demonstrated that myocardial CPK depletion is a useful measure of the extent of myocardial necrosis (13) and relatively independent of contributions of nonmyocardial components such as cells participating in the inflammatory response. A model was developed to determine infarct size which took into account rate of release of myocardial CPK and simultaneous rate of disappearance of serum CPK. Infarct size calculated from changes in serum CPK activity according to this model was closely correlated with infarct size measured directly by analysis of myocardial CPK depletion. Substantial evidence indicates that myocardial enzyme depletion parallels irreversible ischemic cell damage (5–7, 13, 30). Although it is possible that some enzyme depletion could occur when cells are damaged but subsequently recover, infarct size, assessed by independent means, has been shown to correlate with myocardial CPK depletion in other experimental preparations (4, 13). Thus, it appears unlikely that irreversible cell damage estimated from serum CPK changes reflecting myocardial CPK depletion would differ appreciably from actual infarct size because of CPK release from ischemic cells that subsequently recover.

Application of this method of assessment of infarct size depends on use of appropriate values in the model employed (sections B and C, Appendix) for k_d, CPK distribution space, and the fraction of CPK released from a myocardial infarct into the circulation. The fractional serum CPK disappearance rate, k_d, may be influenced by changes in circulatory dynamics affecting excretion of enzyme and/or inactivation of enzyme in vivo. BUN and serum creatinine did not change in animals included in the present study (data not shown) during the course of the experiments. However, in applying this method in other circumstances, such as in experimental preparations exposed to anesthesia during the course of study, caution must be exercised to determine that k_d remains at least relatively constant. CPK distribution space in the conscious dog is 11.4% of body weight, and the fraction of CPK released averages 0.30. These values may differ in other species, and if so, application of this method to them would require appropriate modification.

Despite these qualifications, consideration of the data obtained after isoproterenol administration to animals with coronary artery occlusion indicates that estimates of infarct size based on changes in serum CPK activity conformed quite closely to actual infarct size, measured directly with the use of myocardial CPK determinations in animals in whom the extent of infarction was apparently modified by a pharmacologic intervention. In each experiment, isoproterenol was administered after the integrated value for total CPK released had become virtually constant. In all experiments, a substantial secondary serum CPK peak occurred. Calculation of the final total CPK released indicated that additional CPK release after isoproterenol administration was 15–240% of the amount accounted for by coronary artery occlusion alone. This result is in keeping with a recent finding that isoproterenol administration augments infarct size in an open-chest preparation (4). In the present study, estimated infarct size based on serum CPK changes after coronary artery occlusion and isoproterenol administration including those changes contributing to secondary peaks, correlated quite closely with total infarct size determined 24 hr after coronary artery occlusion by measurement of myocardial CPK depletion (Table III). Although isoproterenol administration may increase the rate of myocardial CPK depletion, the total CPK released estimated with the mathematical model used is independent of rate and correlated well with

total CPK depleted measured directly. In previous studies we have demonstrated that even after isoproterenol administration, myocardial CPK depletion correlates with infarct size assessed by independent criteria in the dog (4) and in the rabbit (13, unpublished observations). Thus, estimates of infarct size based on serial changes in serum CPK are probably not spuriously influenced by isoproterenol administration. Inclusion of these data concerning isoproterenol is not meant to imply that administration of this agent would necessarily augment infarct size in all circumstances. In fact, it is likely that the effect of isoproterenol on infarct size depends on the prevailing hemodynamic state and extent of impairment of ventricular performance, as well as direct action of the drug on myocardial contractility (13). These data are included simply to illustrate that estimates of infarct size based on serum CPK determinations alone conform quite closely to the actual infarct size, measured directly by myocardial CPK depletion in animals subjected to a pharmacologic intervention soon after coronary artery occlusion.

The data presented in the present study demonstrate that assessment of serial changes in serum CPK activity provides an accurate index of the extent of myocardial infarction in the intact conscious experimental animal subjected to coronary artery occlusion. This method is suitable for assessment of infarct size over a wide range and for detection of modification of infarct size produced by pharmacologic and physiologic interventions.

APPENDIX

A. Calculation of infarct size based on direct measurement of myocardial CPK content. In equations in section A, unbracketed symbols refer to absolute amounts, and symbols in brackets refer to concentrations.

$[CPK_N]$ = myocardial CPK concentration (IU/g) in normal left ventricular muscle determined by biopsy of nonischemic tissue in each animal.

$[CPK_I]$ = myocardial CPK concentration (IU/g) in the center of infarcts 24 hr after coronary artery occlusion.

CPK_E = total left ventricular CPK (IU) expected in the homogenate from a normal ventricle of known weight.

CPK_M = total left ventricular CPK (IU) measured in the homogenate from a heart subjected to coronary artery occlusion 24 hr earlier.

CPK_D = total amount of CPK (IU) depleted from a given heart subjected to coronary artery occlusion.

$CPK_E = [CPK_N]$ [left ventricular weight (g)].

$CPK_D = CPK_E - CPK_M$.

$$\text{Infarct size (g)} = \frac{CPK_D}{[CPK_N] - [CPK_I]}.$$

The calculated value for infarct size represents the amount of myocardium undergoing complete infarction which would account for the CPK depletion observed.

B. Calculation of amount of CPK released from the heart after coronary artery occlusion based on serial determinations of serum CPK activity.

$CPK_{D.S.}$ = CPK distribution space (ml).

E = serum CPK activity (IU/ml) at any given instant.

t = time after coronary artery occlusion (min).

f(t) = CPK appearance function (rate of appearance of CPK activity in serum) (IU/ml/min).

k_d = fractional CPK disappearance rate from serum (min^{-1}).

CPK_r = total CPK released from the heart (IU).

$dE/dt = f(t) + k_dE$.

$f(t) = dE/dt - k_dE$.

$$\int_0^t f(t)dt = \int_0^t [dE/_{dt} - k_dE]dt.$$

This value of this integral $\left[\int_0^t f(t)dt\right]$ represents the total amount of CPK released from the heart appearing in 1 ml of serum and is referred to in the text and Table II as the integrated appearance function.

$$CPK_r = \int_0^t f(t)dt \times [CPK_{D.S.}].$$

C. Infarct size estimated on the basis of CPK released from the heart (CPK_r).

$[CPK_N]$, $[CPK_I]$, and $[CPK_D]$ are defined in section A.

CPK_r = CPK released from the heart after coronary artery occlusion (IU) (see section B of Appendix).

0.3 = that fraction of the total CPK activity lost from myocardium after coronary artery occlusion that appears in the CPK distribution space.

$CPK_d = CPK_r/0.3$ = total left ventricular CPK depleted (IU) estimated from changes in serum CPK.

$$\text{Infarct size (g)} = \frac{CPK_d}{[CPK_N] - [CPK_I]}.$$

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