

Early Estimation of Myocardial Damage in Conscious Dogs and Patients with Evolving Acute Myocardial Infarction

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ABSTRACT To estimate the ultimate extent of myocardial damage during evolving myocardial infarction in conscious dogs and patients, we analyzed early serum creatine phosphokinase (CPK) changes with nonlinear curve-fitting techniques. In experiments with dogs, serial serum CPK changes were fit to a log-normal function by the least squares method; the extent of the completed infarct was calculated by analysis of observed serum CPK changes and verified by measurement of myocardial CPK depletion 24 h after coronary occlusion. Early prediction of myocardial damage was based on projected serum CPK values from best fit curves based on data obtained during the first 5 h after initial elevation of enzyme activity. The correlation between predicted and observed values was close ($r > 0.96$, $n = 11$). In 11 additional conscious animals subjected to coronary occlusion, isoproterenol was administered continuously as soon as damage had been estimated from projected serum CPK values. The extent of the completed infarct was assessed by analysis of all serial serum CPK values and verified by analysis of myocardial CPK depletion 24 h after coronary occlusion. In each experiment the calculated completed infarct size exceeded infarct size projected before administration of isoproterenol (average increase = 44 ± 10 [SE]%). When similar calculations were applied in experiments with eight dogs treated with propranolol, myocardial salvage was detected in 50% of the animals.

In 30 patients with uncomplicated acute myocardial infarction the extent of the completed infarct, measured by analysis of CPK activity in serum samples obtained every 2 h, was compared with damage estimated from CPK values projected by the best fit log-normal curve derived from data obtained during the

first 7 h after the initial serum CPK elevation. The estimate of damage based on early data correlated closely with the extent of infarction calculated from all available serial serum CPK values ($r = 0.93$, $n = 30$). Thus, the extent of the completed infarct could be estimated accurately during the early evolution of infarction. In patients with spontaneous extension of infarction manifested by chest pain and electrocardiographic changes, the calculated extent of the completed infarct exceeded that predicted. Conversely, salvage of myocardium, after reduction of myocardial oxygen requirements by administration of trimethaphan, was reflected by reduction of the extent of the calculated completed infarct with respect to that predicted from early serum CPK changes.

INTRODUCTION

Rapidly accumulating evidence indicates that infarct size is an important determinant of morbidity and mortality after acute myocardial infarction (1-4). Furthermore, it appears that the extent of ischemic injury detected by myocardial creatine phosphokinase (CPK)¹ depletion (5) and ST-segment changes in epicardial recordings (5-7) can be modified favorably in experimental animals and in patients with coronary artery disease (5-7). Therefore, prompt and accurate prediction of ultimate infarct size would provide an improved basis for proper selection of patients for potentially hazardous therapeutic interventions and would facilitate evaluation of their efficacy.

Myocardial necrosis resulting from ischemia is not homogenous. Hence, quantification of infarct size based on morphologic criteria is laborious and susceptible to inaccuracy. In addition, quantitative

Received for publication 8 February 1973 and in revised form 5 June 1973.

¹ *Abbreviations used in this paper:* CPK, creatine phosphokinase; ISp, predicted infarct size; ISs, observed infarct size.

analysis of myocardial damage based on morphology alone is not readily applicable to patients. To quantify the extent of ischemic injury in conscious animals and in patients, we have previously utilized mathematical analysis of serial serum CPK changes (8). However, since this approach detects damage only after the fact, it cannot be used to predict the ultimate extent of the completed infarct during its early evolution.

In the present study, we have applied curve-fitting techniques to analysis of serial serum CPK changes in conscious dogs with coronary occlusion and in patients with spontaneous myocardial infarction in order to predict the extent of infarction. Anticipated serum CPK values were projected on the basis of changes occurring soon after the onset of ischemia; infarct size² was predicted from the projected values; predicted "infarct size" was compared with observed "infarct size" and the extent of myocardial salvage after physiological and pharmacological interventions was quantified.

METHODS

Experimental studies

Coronary occlusion was produced in 37 conscious dogs by constriction of an externalized snare placed around a branch of the left anterior descending coronary artery 1 wk earlier as previously described (8). CPK activity was measured serially for 24 h in serum samples obtained through an inlying jugular venous catheter at 60–90-min intervals after coronary occlusion. "Infarct size" was calculated from serum CPK changes (ISs) and compared with "infarct size" predicted (ISp) from CPK curves projected on the basis of early data points (see analysis of data). The animals were then killed and the extent of ischemic injury was measured directly by determination of myocardial CPK depletion (8, 9).

In selected animals, the extent of infarction was modified during its evolution by either the administration of isoproterenol in peanut oil (0.15 mg/kg, s.c.) beginning 14 h after coronary occlusion and repeated 3 h later or by the administration of propranolol (2 mg/kg, i.v.) beginning 5 h after occlusion and continued at a dose sufficient to maintain heart rate at less than 85 beats/min compared with the average heart rate of 100 beats/min in animals with coronary occlusion alone.

Clinical studies

Serum CPK activity was determined in serial samples obtained every 1–2 h from all patients admitted to the University of California, San Diego Myocardial Infarction Research Unit. Each patient included in this study had acute myocardial infarction manifested by at least two of the following three criteria: characteristic history of prolonged chest pain (> 1 h); characteristic serial changes in conventionally measured serum enzymes (serum glutamic-oxaloacetic trans-

² Since infarct size assessed morphologically has been compared previously with the extent of infarction estimated from myocardial CPK depletion and serial serum CPK changes, "infarct size" is used in this manuscript to refer to the extent of ischemic injury reflected by CPK changes in serum.

aminase, lactate dehydrogenase, and CPK); and evolution of electrocardiographic changes including Q waves diagnostic of transmural myocardial infarction. In each case infarct size was estimated from serum CPK changes as previously described. In all patients with initially normal serum CPK values (≤ 40 mIU/ml), infarct size was predicted from changes occurring within 7 h after the initial serum CPK elevation (see analysis of data section).

Biochemical procedures

CPK activity was determined in dog serum, dog myocardial homogenates, and human serum by a spectrophotometric kinetic method as previously described (8, 9). Protein was measured by the biuret procedure (10).

Analysis of data

A. *Determination of infarct size by analysis of myocardial CPK content.* Myocardial CPK depletion was determined by analysis of CPK concentration in normal myocardium and total left ventricular CPK content in each animal as well as measurement of left ventricular weight. We have previously demonstrated that the extent of myocardial CPK depletion after sustained or interrupted coronary occlusion correlates with infarct size assessed morphologically (9, 11, 12). Accordingly, in the present study, infarct size was determined from myocardial CPK depletion as previously described (8).

B. *Determination of infarct size by analysis of serial serum CPK changes.* After myocardial infarction the rate of change of enzyme activity in serum (dE/dt) depends on at least two competing phenomena: release of enzyme from the heart ($f(t)$) and clearance of enzyme from the blood ($k_d E$) (8). Thus, $dE/dt = f(t) + k_d E$. We have shown previously that the cumulated amount of CPK released from the heart (CPK_r) appearing in 1 ml of serum can be calculated from

$\int_0^T f(t) dt$ (IU/ml). The value of this expression is proportional to the amount of CPK released from the heart. Its magnitude reaches the same ultimate value whether the rate of release of CPK from the heart is slow or rapid as long as the total amount of CPK released is the same. Since the fractional disappearance rate of serum CPK activity can be approximated by a constant, k_d , which has been measured experimentally (8) and since dE/dt and E can be measured directly, $\int_0^T f(t) dt$

can be obtained readily in each study. $\int_0^T f(t) dt$ was found

by use of the expression $\sum_0^T f(t) dt = \sum_0^T (\Delta E/\Delta t + k_d \bar{E}) \Delta t$ in

which E is observed enzyme concentration at time t and \bar{E} is the mean E between t_2 and t_1 . As $t \rightarrow \infty$ and enzyme release

ceases, $\sum_0^T f(t) dt$ approaches a constant. In the animal experi-

ments the dogs were killed 24 h after coronary occlusion since depletion of myocardial CPK is virtually complete at this time

(8). In patients $\sum_0^T f(t) dt$ was evaluated until it had remained

constant for several hours usually for 48–96 h after initial serum CPK elevation.

The proportion of CPK lost from myocardium that appears in serum, the volume into which CPK is distributed after release from myocardium, and CPK depletion per gram in the center of infarcts have been shown to be relatively constant

(8, 13). Thus, infarct size

$$= K \times \int_0^T f(t) dt. [K = \frac{(BW) \times (DV)}{(D) \times (CPK_N - CPK_I)}];$$

BW = body weight in kilograms; DV = CPK distribution volume; D = the proportion of depleted myocardial CPK activity appearing in serum; CPK_N = CPK activity in normal myocardium; and CPK_I = CPK activity in the center of infarcts at a specified time after coronary occlusion]. K and k_d for dogs have been found to be (6.1 × 10⁻¹) × (BW) and 0.0045 ± 0.001 (mean ± SD) min⁻¹. Corresponding values in man are (4.5 × 10⁻¹) × (BW) and 0.0010 ± 0.0005 min⁻¹ (8, 1). Despite marked hemodynamic perturbations in the conscious experimental animal, including acceleration of heart rate, elevation of central venous pressure, diminution of cardiac output, and reduction of renal blood flow, we have observed that k_d remains within the mean ± 1 SD (8, 14). In the dog, the proportion of CPK depleted from myocardium appearing in the serum (D) is virtually constant (8, 12-15). Since this value cannot be measured directly in man we have assumed it to be constant by analogy (8, 1).

Prediction of infarct size from projected serum CPK curves. Predicted infarct size (ISp) was calculated from CPK values projected from the best fit curves based on early serum CPK changes. Nonlinear Gauss-Newton stepwise iterations were employed in the curve fitting procedure (16). On the basis of preliminary analysis, serial serum CPK changes after uncomplicated myocardial infarction were assumed to fit a log-normal distribution:

$$E = \frac{b}{t} e^{-\left(\frac{\ln t - c}{d^2}\right)^2} / 2. \quad (1)$$

(E = serum CPK concentration at time t; b, c, d = nonlinear constants; e = the base of the natural logarithm.)

A nonlinear least squares fit was performed on the parameters b, c, and d to obtain an equation for E in each case. The partial differentials of the parameters used in the normal equations were:

$$\frac{\partial E}{\partial b} = \frac{1}{t} e^{-\left(\frac{\ln t - c}{d^2}\right)^2} / 2 \quad (2)$$

$$\frac{\partial E}{\partial c} = \frac{b}{d^2 t} \left(\frac{\ln t - c}{d^2}\right) e^{-\left(\frac{\ln t - c}{d^2}\right)^2} / 2 \quad (3)$$

$$\frac{\partial E}{\partial d} = \frac{2b}{td} \left(\frac{\ln t - c}{d^2}\right)^2 e^{-\left(\frac{\ln t - c}{d^2}\right)^2} / 2. \quad (4)$$

With the use of the log-normal function and these partial differentials, the function E was evaluated with a BMDX85 computer program (17) for nonlinear least squares fit. The best fit log-normal function and its 95% confidence limits and overall standard error can be directly solved by the computer with the use of the residual mean square difference. Predicted infarct size was calculated from the expression: Infarct size (IS) = K ∫₀^T f(t) dt = K ∫₀^T (dE/dt + k_dE) dt. Therefore, IS = K [E(T) + k_d ∫₀^T E dt] in which E(t) is the enzyme concentration as a function of time obtained from the best fit log-normal function, k_d is an approximation of the mono-exponential decay constant and K is the constant defined in section B. ∫₀^T E dt is found from the following relationships

(17, 18):

$$\int_0^T E dt = \sqrt{2\pi} b d^2 P(x)$$

in which x = (ln t - c)/d²

$$P(x) = \int_{-\infty}^x Z(x) dx$$

and Z(x) = exp (-x²/2)

Then, using a series approximation:

$$P(x) = \sqrt{2\pi} - Z(x)(g_1 w + g_2 w^2 + g_3 w^3) + \epsilon(x)$$

in which w = 1/(1 + hx), and g₁ = 0.4361836; g₂ = -0.1201676; g₃ = 0.9372980; h = 0.33267; |ε(x)| < 10⁻⁵.

The integral is evaluated to a point in time (T) chosen to coincide with the time at which enzyme release is zero assuming a monoexponential decay (k_d). The log-normal equation approaches zero as t approaches infinity but not monoexponentially. However, over the interval of experimental and clinical interest, the rate of decay described by the log-normal function [(d² - c + ln t)/d²t] closely approximates k_d. After the point in time when the decay described by the log-normal function deviates from k_d, the contribution of k_dE to infarct size is too small to change the calculated value for infarct size appreciably because E is approaching zero.

The curve-fitting technique described permits prediction of infarct size on the basis of early observed serum CPK changes. Naturally, the correlation between infarct size predicted by application of this method and observed infarct size can be ascertained only by actual experimental observations.

RESULTS

The correlation between infarct size estimated from best fit serum CPK curves and infarct size calculated from observed serum CPK changes in the conscious dog. Serial serum CPK changes after myocardial infarction in a representative conscious dog are illustrated in Fig. 1A. Fig. 1B depicts a representative example of a CPK curve obtained by curve fitting all actual serum CPK data in the same animal. As can be seen, the curve depicting observed values and the best fit curve derived from observed values are almost identical. Infarct size calculated from the observed data was 25.6 CPK-g-eq³ compared with 25.7 estimated from the best fit curve. Thus, in this example the actual CPK curve is closely approximated by the best fit curve conforming to a log-normal distribution.

When successively fewer real data points are used as the basis for obtaining the best fit curve, predicted infarct size can be estimated from the CPK values

³ 1 CPK-gram-equivalent (CPK-g-eq) is that quantity of myocardium undergoing necrosis exhibiting total CPK depletion equivalent to depletion in 1 g of tissue undergoing homogeneous necrosis in the center of an infarct.

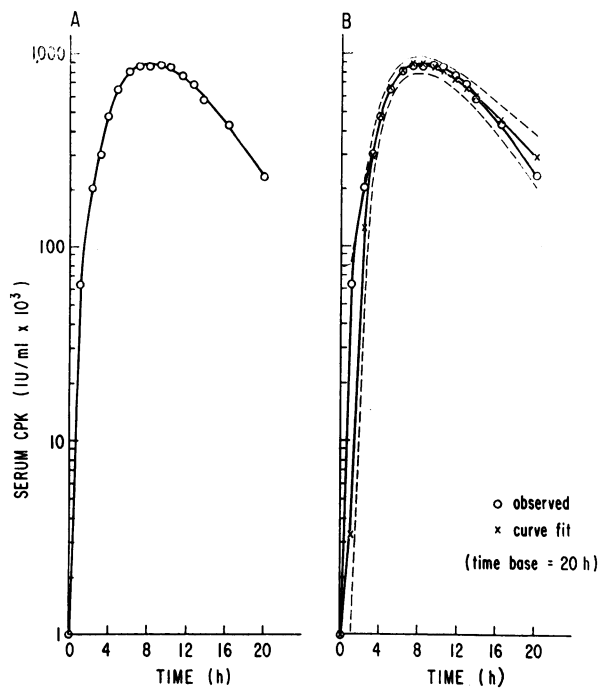


FIGURE 1 (A) Changes in serum CPK activity after myocardial infarction produced by coronary artery occlusion in the conscious dog. Results illustrate the characteristic changes in serum CPK activity as a function of time. Base-line concentrations have been subtracted from all values in this and subsequent figures. (B) Serum CPK activity values conforming to the best fit log-normal function obtained by the least squares method compared with observed values. The best fit curve was obtained using the observed data from the entire duration of the experiment. In this and subsequent illustrations O = observed CPK activity; X = CPK activity values conforming to the best fit log-normal function; dashed lines = 95% confidence limits; and time base refers to the initial interval during which data used for the curve fit were obtained. Results illustrate the close correspondence between observed and curve fit data. The best fit curve falls within the 95% confidence limits for the projection.

projected. The relationship between calculated infarct size and infarct size predicted from CPK curves based on progressively fewer initial observed serum CPK values is illustrated in Table I which summarizes results obtained in one study in a conscious dog. 15 real data points were obtained in this experiment representing CPK changes observed during 1,210 min after coronary artery occlusion in a conscious dog. Infarct size predicted from all 15 points was 26.0 CPK-g-eq; that predicted from only the initial 10 points was 25.6, and that predicted from only the CPK changes occurring during the first 5 h (300 min) was 24.2. The correspondence between predicted infarct size and actual infarct size was close when data for at least the first 300 min were utilized as the basis for CPK curve fits. In addition, as can be seen in Table I,

the overall standard error remained small in curves fit from initial data as long as data from at least 300 min after coronary occlusion were used as the basis for curve fitting.

To examine the relationship between infarct size predicted from early serum CPK changes and infarct size calculated from observed serum CPK changes during the first 24 h predictions were made in 11 conscious dogs 300 min after coronary occlusion (IS_{300}) and compared with infarct size calculated from: (a) 24 h serial CPK analysis (IS_s) and (b) direct measurement of myocardial CPK depletion. Results are shown in Table II. As can be seen IS_{300} corresponded closely to IS_s and to infarct size measured directly. Close correlations were seen over a wide range of infarct size. The mean difference between infarct size measured directly and IS_{300} was $1.8 \text{ CPK-g-eq} \pm 0.4$ and the mean difference between IS_s and IS_{300} was 4.5 ± 1.5 . These represent mean percent differences of 6 ± 2 and $14 \pm 3\%$, respectively. We have previously shown that IS_s correlates closely with infarct size measured directly (8). As shown in Fig. 2A, the correlation between infarct size calculated from the best fit curve derived from 24 h serum CPK changes and infarct size measured directly was also close ($r = 0.98$, $n = 11$). Furthermore, when infarct size was estimated from CPK curves fit from enzyme changes during the first

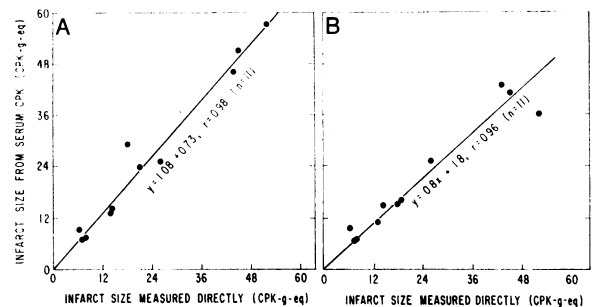


FIGURE 2 (A) The relation between infarct size calculated from observed serum CPK changes and infarct size estimated by direct measurement of myocardial CPK depletion. Infarct size is expressed as CPK-g-eq (see text). Infarct size calculated from observed serum CPK activity (ordinate) correlates closely with infarct size calculated from myocardial CPK analysis (abscissa). (B) The relation between infarct size predicted from the best fit curve derived from data obtained during the first 5 h after initial serum CPK elevation and infarct size calculated from direct measurement of myocardial CPK depletion 24 h after coronary artery occlusion. Predicted infarct size, represented on the ordinate, was calculated as described in the text and compared with infarct size calculated from measurement of myocardial CPK content (abscissa). Infarct size predicted on the basis of data obtained during the first 5 h after coronary occlusion correlates closely with infarct size calculated from direct analysis of myocardium 24 h after occlusion.

TABLE I
The Relationship between Calculated Infarct Size and Infarct Size Predicted from CPK Curves in One Study of a Conscious Dog*

Number of hourly samples	Time before prediction	IS (CPK-g-eq)	Overall standard error (SE)	Fit parameters		
				<i>b</i> ($\times 10^{-3}$)	<i>c</i>	<i>d</i> ($\times 10$)
	<i>min</i>					
15	1,210	25.98	45.92	5.074	6.5	5.94
14	990	26.01	45.92	5.077	6.5	5.94
13	830	25.98	45.92	5.070	6.5	5.94
12	770	25.96	45.93	5.063	6.5	5.95
11	700	25.79	46.45	5.013	6.5	5.98
10	630	25.58	48.19	5.949	6.5	6.01
9	570	25.19	53.9	5.829	6.5	6.09
8	500	25.71	63.7	4.694	6.5	6.17
7	440	24.48	69.1	4.630	6.5	6.20
6	360	24.91	82.5	4.487	6.5	6.27
5	300	24.20	92.5	4.398	6.5	6.35
4	240	17.80	247.9	3.070	6.5	7.06

* The CPK curves are based on progressively fewer initial observed serum CPK values.

300 min and compared with infarct size measured directly, a linear relationship was evident: $(IS_{300} = 0.8 [\text{infarct size measured directly}] + 1.8, r = 0.96, n = 11)$ (Fig. 2B). These data indicate that in the conscious dog infarct size calculated from myocardial CPK depletion, 24 h after coronary occlusion, can be predicted accurately as early as 5 h after occlusion

from log-normal CPK curves fit from initial serum CPK changes.

C. *Modification of the extent of infarction.* Isoproterenol augments infarct size in anesthetized dogs subjected to coronary occlusion who develop myocardial infarction without congestive heart failure (5). To determine whether augmentation of infarct size can be recognized

TABLE II
*Examination of the Relationship between Infarct Size Predicted from Early Serum CPK Changes and Infarct Size Calculated from Observed Serum CPK Changes in 11 Conscious Dogs**

Dog number	IS measured directly (CPK-g-eq)	IS calculated from all serum CPK values (CPK-g-eq)	ISp from CPK changes during the first 300 min (CPK-g-eq)	The difference between ISp and ISs measured directly (CPK-g-eq)	The difference between ISp and ISs from serum CPK changes (CPK-g-eq)
1	14.2	14.4	14.6	0.4	0.2
2	18.0	29.0	15.2	-2.8	-13.8
3	52.0	57.0	46.0	-6.0	-11
4	7.2	7.3	7.6	0.4	0.3
5	6.4	9.0	9.3	2.9	0.3
6	7.9	7.2	7.4	-0.5	0.2
7	25.6	26.9	24.5	-1.1	-2.4
8	21.0	24.4	15.6	-5.4	-8.8
9	45.0	51.0	41.0	-4.0	-10.0
10	44.0	46.0	42.3	-1.7	-3.7
11	14.1	13.0	12.1	-2.0	-0.9
Mean	23.2	25.9	21.4	-1.8	-4.5
SE	5.0	5.5	4.5	0.8	1.6

* This was observed 300 min after coronary occlusion.

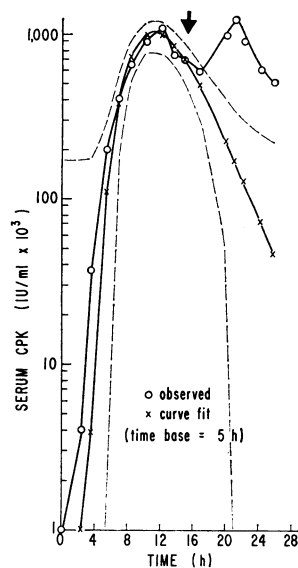


FIGURE 3 Changes in observed serum CPK activity after isoproterenol administration compared with values for serum CPK activity conforming to the best fit curve obtained from data before the intervention. The best fit curve was obtained from data during the first 5 h after coronary artery occlusion. Administration of isoproterenol was initiated 17 h after occlusion (↓). The observed and fit values for CPK activity agreed quite closely before drug administration. However, within 4 h after isoproterenol administration was initiated, observed CPK values exceeded the 95% confidence limits of the best fit curve based on early changes, and calculations of observed and predicted infarct size indicated extension of infarction.

by the CPK curve-fitting technique, we utilized isoproterenol in the present study. Isoproterenol did not elevate serum CPK in normal conscious dogs ($n = 6$) nor did it alter the disappearance rate of intravenously injected, partially purified myocardial CPK ($n = 3$) (8, 14). However, when isoproterenol was administered to conscious dogs beginning 14 h after coronary occlusion, subsequent observed serum CPK values exceeded the 95% confidence limits of CPK curves fit from changes occurring before administration of isoproterenol during the initial 5 h. Results of a typical experiment are shown in Fig. 3. Similar results were seen with each of 11 dogs. The augmentation of infarct size ($ISs/IS_{300} \times 100$) was $144 \pm 10\%$, and ISs was significantly greater than ISp ($P < 0.001$). Since ISs was virtually identical to infarct size measured directly in each of these experiments, spurious results due to variation in CPK clearance from the circulation can be excluded. These results are presented not primarily to confirm the deleterious effect of isoproterenol on infarct size but rather to demonstrate that augmentation of infarct size can be quantified in the individual conscious dog by comparison of observed to predicted infarct size.

In eight other dogs, propranolol was administered beginning 5 h after coronary occlusion when infarct size had already been predicted. The animals were killed 24 h after coronary occlusion and infarct size was verified by determination of myocardial CPK content. As shown in the representative experiment illustrated in Fig. 4, serum CPK values fell below the confidence limits of the projected CPK curve within 4 h after administration of propranolol commenced. In this example predicted infarct size was 47 CPK-g-eq, ISs was 32 CPK-g-eq, and infarct size measured from myocardial CPK analysis was 35 CPK-g-eq. Thus, propranolol resulted in salvage of 32% of the anticipated infarct. The ratio between CPK activity lost from myocardium and that accounted for in serum remained constant (0.3 ± 0.02) in all eight experiments performed with propranolol, indicating that the intervention was not merely altering clearance of CPK from the circulation. In four dogs, propranolol did not modify infarct size favorably as indicated by failure of predicted infarct size to exceed ISs.

Clinical applications: prediction of infarct size in patients with myocardial infarction. Typical changes

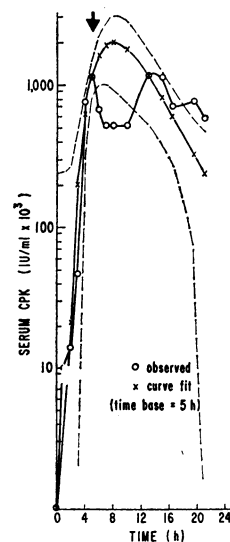


FIGURE 4 Changes in observed serum CPK activity after propranolol administration compared with projected serum CPK activity obtained by curve fitting. The best fit curve was derived from CPK changes during the first 5 h after coronary artery occlusion. Propranolol was administered beginning 5 h after occlusion, and subsequently the observed CPK values deviated from those projected. This was followed consistently, as shown in this example, by apparent late extension of infarction and additional CPK release despite continued administration of propranolol even though left atrial and systemic blood pressure remained constant. In this example predicted infarct size was 47 CPK-g-eq and infarct size calculated from all observed serum CPK values was 32 CPK-g-eq reflecting apparent salvage of myocardium equivalent to 32% of predicted infarct size during the 24 h observation period.

in serum CPK activity in a patient with acute myocardial infarction are illustrated in Fig. 5A. We have previously shown that infarct size calculated from analysis of such changes correlates with prognosis (1). Preliminary analysis indicated that serum CPK curves exhibit the same general shape in most patients with uncomplicated myocardial infarction and that best fit log-normal CPK curves approximate observed curves closely. Fig. 5B illustrates the best fit curve corresponding to the observed curve in Fig. 5A.

Best fit curves derived from only early data approximated observed curves in patients with uncomplicated myocardial infarction just as they did in conscious dogs subjected to coronary occlusion. Data shown in Table III were obtained from one patient. They illustrate the relationship between best fit curves projected from early data and observed curves when progressively fewer serum CPK values from the same patient were used as the basis for curve fitting. Infarct size calculated from all observed serum values was found to be 14 CPK-g-eq. When the best fit log-normal curve (obtained from CPK changes during a 56 h interval) was used to calculate infarct size, the value obtained was 13.7 CPK-g-eq. Infarct size predicted from the best fit curve based on data from the first 46 h was identical. Infarct size calculated from the best fit curve based on progressively fewer points remained quite similar as long as data from at least 7 h (420 min) were used for

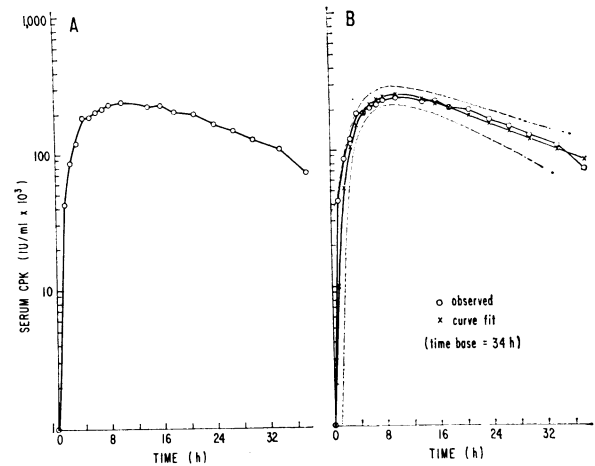


FIGURE 5 (A) Changes in serum CPK activity associated with acute myocardial infarction in man. Results illustrate characteristic serial serum CPK changes as a function of time. Base-line concentrations have been subtracted from all values in this and subsequent examples. Zero time is taken as the time when serum CPK activity was last within the normal range. In the patient selected for this example chest pain began 4 h before zero time. It occurred from 2 to 14 h before zero time in other patients included in this investigation. (B) Changes in values for serum CPK activity conforming to the best fit log-normal function derived from all observed serum CPK values by the least squares method compared with observed changes in serum CPK activity. Results illustrate the close correspondence between observed and curve fit data.

TABLE III
Calculated IS Based on Curves Projected from Early Serum
CPK Changes in One Study of a Patient

Number of hourly samples	Time before prediction	Calculated IS (CPK-g-eq)	Overall standard error (SE)	Fitted parameters		
				b ($\times 10^{-5}$)	c	d_1 ($\times 10$)
	<i>min</i>					
21	3,240	13.65	17.0	2.24	7.18	9
20	3,000	13.70	17.0	2.25	7.19	9
19	2,760	13.66	17.0	2.25	7.18	9
18	2,280	13.63	17.0	2.24	7.18	9
17	2,040	13.70	17.0	2.25	7.19	9
16	1,800	13.65	17.0	2.24	7.18	9
15	1,620	13.57	17.0	2.23	7.18	9
14	1,441	13.42	17.2	2.20	7.17	9
13	1,260	13.24	17.5	2.17	7.16	9
12	1,080	12.80	19.1	2.08	7.13	9
11	960	12.48	20.7	2.03	7.11	9
10	840	12.16	22.5	1.97	7.10	9
9	600	12.04	23.2	1.95	7.10	9
8	420	12.04	23.2	1.95	7.10	9
7	360	12.23	22.1	1.98	7.10	9
6	300	12.86	20.1	2.08	7.10	9
5	240	13.88	23.6	2.25	7.10	9

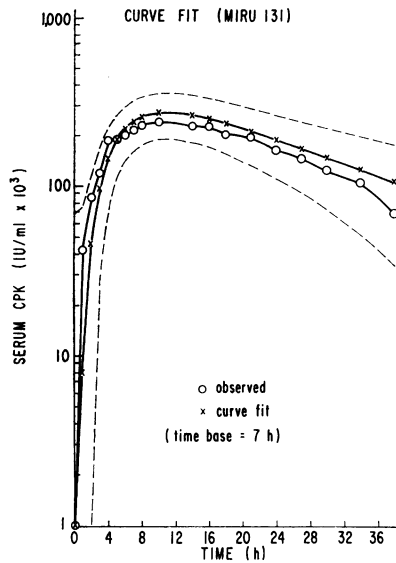


FIGURE 6 Changes in values for serum CPK activity conforming to the best fit log-normal function based on data from only the first 7 h after initial CPK elevation compared with observed serum CPK values in a patient with acute myocardial infarction. The correspondence between observed and projected CPK values is quite close.

the fit. In this particular case, correspondence was obtained even when a shorter interval was used for the prediction. The values of the fit parameters (b, c, d) remained quite similar as progressively fewer data points were used for curve fitting indicating that the computer program converged to reproducible minima.

The CPK curve projected from serum CPK changes during only the first 7 h in this patient is illustrated in Fig. 6. As can be seen, the actual values were approximated closely by points on the best fit-projected curve and fell within the 95% confidence limits of the fit curve. Thus, in this example, infarct size could be predicted accurately within 7 h after serum CPK values initially became elevated. The overall standard error of the projection was small and the confidence limits for the best fit curve were narrow.

The relationship between infarct size calculated from observed serial serum CPK changes and infarct size predicted 7 h after the initial serum elevation in 30 patients with uncomplicated myocardial infarction is shown in Table IV. In these 30 patients, the mean difference between calculated infarct size and predicted infarct size was $2 \text{ CPK-g-eq} \pm 2$ ($n = 30$), and the average percent difference over a wide range of infarct size (1–101 CPK-g-eq) was only 5%. In one patient, (patient 9), observed values were substantially greater than those on the projected curve derived from data during the first 7 h after the initial serum enzyme elevation for reasons not entirely clear. This patient

represents an example of either a failure of the method to predict infarct size accurately or an occult extension of infarction.

Best fit CPK curves based on all available serum CPK values closely approximated actual curves. Thus, as shown in Fig. 7A, infarct size calculated from best fit curves = $1.01 \times \text{ISs} + 8.99$ ($r = 0.99$, $n = 30$). In addition, projected curves based on data from serum CPK changes (during the first 7 h) were similar to actual curves and as shown in Fig. 7B, infarct size calculated from curves based on early data (ISp) = 0.99

TABLE IV
The Relationship between Infarct Size Calculated from Observed Serial Serum CPK Changes and Infarct Size Predicted 7 h after the Initial Serum CPK Elevation in 30 Patients with Uncomplicated Myocardial Infarction

Patient number	IS calculated from all observed serum CPK values (CPK-g-eq)	ISP from serum CPK changes during the first 420 min (CPK-g-eq)	The difference between ISp and ISs (CPK-g-eq)
1	12	16	4
2	48	61	13
3	9	17	8
4	50	57	7
5	23	20	-3
6	24	32	8
7	19	10	-9
8	84	81	-3
9	44	87	43
10	101	97	-4
11	3	3	0
12	1	1	0
13	106	106	0
14	11	8	-3
15	3	3	0
16	27	36	9
17	59	62	3
18	21	31	10
19	51	30	-21
20	37	66	29
21	3	3	0
22	39	21	-18
23	29	27	-2
24	14	14	0
25	10	8	-2
26	5	6	1
27	17	14	-3
28	40	32	-8
29	7	10	3
30	92	92	0
Mean	33	35	2
SE	5	6	2

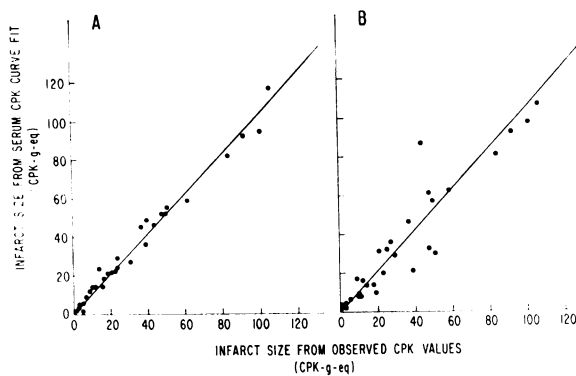


FIGURE 7 (A) The relation between infarct size calculated from the best fit log-normal function and infarct size calculated from observed serial serum CPK changes. The best fit log-normal function was obtained by a Gauss-Newton least squares fit (see text) using all available observed data for the fit (ordinate). Infarct size calculated from analysis of observed serial serum CPK changes is plotted on the abscissa. There is a close correlation and a linear relationship between infarct size calculated from best fit curves and infarct size calculated from observed serial serum CPK changes ($IS_{curve\ fit} = 1.01 \times IS_{obs} + 8.99$, $r = 0.99$, $n = 30$). (B) The relation between predicted infarct size and completed infarct size calculated from observed serial serum CPK changes. Each best fit function was based on data obtained only during the first 7 h after initial CPK elevations. Predicted infarct size was calculated from CPK values conforming to the best fit curve (ordinate) and correlated closely with completed infarct size calculated from all observed CPK values (abscissa). ($IS_p = 0.99 \times IS_s + 2.44$, $r = 0.93$, $n = 30$).

$\times IS_s + 2.44$, $r = 0.93$, $n = 30$. Thus, infarct size can be predicted accurately within 7 h after the serum CPK activity becomes elevated in patients with uncomplicated myocardial infarction.

Assessment of extensions of infarction and salvage of myocardium in patients. Extension of infarction and salvage of myocardium in patients with myocardial infarction was reflected by deviations of observed serum CPK values from those projected from the best fit log-normal curve derived from early serum CPK changes in a fashion similar to that utilized in studies in conscious dogs. An example of spontaneous extension of infarction manifested by recurrent chest pain and striking ST segment elevation is shown in Fig. 8A. Observed CPK values exceeded the confidence limits of the best fit-projected curve within 2 h after extension of infarction. In each of six patients with electrocardiographic and clinical evidence of extension, observed CPK values exceeded the 95% confidence limits of projected values within 4 h and observed infarct size (range 11–86 CPK-g-eq) exceeded predicted infarct size by $94 \pm 28\%$ (mean \pm SE).

Prediction of infarct size by curve-fitting techniques appears to be applicable to quantification of myocardial salvage after therapeutic interventions in

individual patients as well as to detection of extension of infarction. In the experimental animal, interventions which are thought to improve the ratio of myocardial oxygen supply to demand sufficiently early during evolution of ischemic injury appear to modify infarct size favorably (5). Accordingly, in the present investigation we attempted to modify infarct size favorably in hypertensive patients (blood pressure $> 150/90$) with myocardial infarction by reducing afterload to decrease ventricular wall tension and thereby reduce oxygen demand at a time when sufficient serum CPK data were available to predict infarct size by the curve-fitting technique. Afterload was decreased by intravenous administration of trimethaphan in doses sufficient to reduce systolic blood pressure by 25 mm Hg (5–10

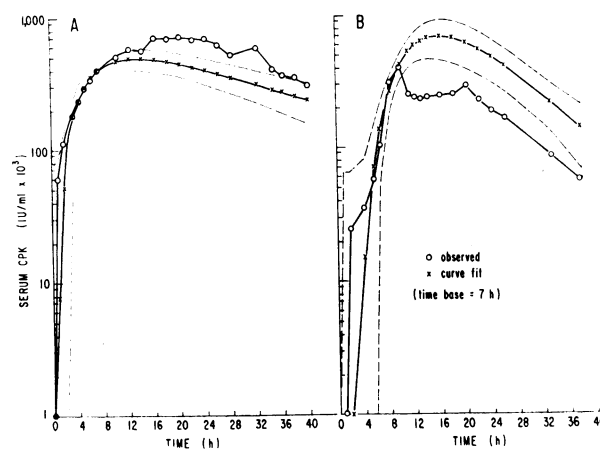


FIGURE 8 (A) Changes in observed serum CPK activity after spontaneous extension of infarction compared with values for serum CPK changes obtained from the best fit curve derived from early data. The initial bout of chest pain occurred 4 h before zero time, defined as the last time when serum CPK activity was within the normal range. Predicted infarct size was calculated from the best fit log-normal function based on serial serum CPK changes during the first 7 h after zero time. Recurrence of pain occurred 12 h after zero time and was associated with ST segment elevation on the electrocardiogram. Within 2 h observed serum CPK values deviated from those on the best fit curve derived from early data. (B) Changes in serum CPK activity after administration of trimethaphan compared with values for serum CPK activity conforming to the best fit log-normal function derived from early data. The initial episode of chest pain occurred 6 h before zero time. Predicted infarct size was calculated from the best fit curve derived from data obtained during the first 7 h after zero time. Administration of trimethaphan (500 mg/liter) was initiated to lower systolic blood pressure by 25 mm Hg. CPK activity fell below the 95% confidence limits of the best fit curve within a short time. During the latter portion of the observation period serum CPK values declined in parallel with the terminal portion of the best fit curve. Infarct size predicted from the log-normal function based on data before the intervention was 52.8 compared with the calculated value of 27.2 CPK-g-eq for the completed infarct based on analysis of all observed serum CPK changes.

$\mu\text{g}/\text{kg}/\text{min}$). As can be seen in the example depicted in Fig. 8B, observed serum CPK activity declined below the 95% confidence limits of projected values within 2 h after the onset of antihypertensive therapy. In this example, infarct size predicted on the basis of the CPK curve fit from data during the initial 7 h was 52.8 CPK-g-eq and infarct size calculated from observed serum CPK values through the time when antihypertensive therapy was discontinued was 27.2 CPK-g-eq. Thus, predicted infarct size was reduced by 49%. A similar result has been obtained in 9 of 11 patients evaluated and treated with this approach. Clearly, conclusions regarding the long-term benefit of such therapeutic interventions depend on analysis of many patients and on long-term follow-up. These results are presented only demonstrate that comparison of observed to predicted infarct size appears to be one useful means for evaluating potentially favorable therapeutic interventions in patients with acute myocardial infarction.

DISCUSSION

Results in this study demonstrate that infarct size can be predicted from serial serum CPK changes occurring during early evolution of infarction by means of nonlinear least squares curve-fitting techniques in the conscious dog subjected to coronary artery occlusion and in patients with spontaneous myocardial infarction. Since infarct size appears to be a major determinant of prognosis (1-4), accurate early prediction of infarct size in individual patients when favorable modifications may still be possible is of considerable potential value.

Results in the dog studies demonstrate that best fit serum CPK curves derived from data during a 24 h period correlate closely with actual CPK values ($r = 0.98$). Furthermore, infarct size determined from best fit-CPK curves projected from progressively fewer serum CPK values correlates closely with observed infarct size as long as data from at least the first 5 h are utilized (Table II).

A log-normal function was chosen empirically because of the similarity of observed plots of data to plots of best fit log-normal functions. It would be of considerable theoretical interest to derive an equation for E as a function of time on the basis of knowledge of the appearance constants ($f(1..n)$), disappearance constants ($k_d(1..n)$), distribution volumes, and rates of penetration of CPK into specific compartments or distribution volumes. However, information currently available is not sufficient to provide an adequate basis for such a derivation. The log-normal function was chosen empirically. Hence, its selection does not depend on any specific theoretical formulation.

The value of the log-normal function does not approach zero in parallel with a simple monoexponential

function, but rather at a rate given by the ratio, $\frac{d^4 - c + \ln t}{d^4 t}$. Over the clinically useful range (0-3,200

min for small infarcts and 0-9,000 min for large infarcts) k_d and the value of this ratio are virtually identical. Beyond this range, k_d deviates from the value of the ratio. However, the term kdE approaches zero since E is near zero. Thus, estimation of infarct size is not affected appreciably by the deviation of k_d from the ratio.

Observations in the clinical studies were analogous to those in the studies performed with conscious dogs. In 30 patients with uncomplicated acute myocardial infarction, best fit log-normal curves based on all available serum CPK values closely approximated observed curves. Thus, in patients with acute myocardial infarction, as in conscious dogs with coronary artery occlusion, serum CPK curves conform closely to best fit log-normal functions. In addition, in these 30 patients, infarct size predicted from best fit curves based on serum CPK data obtained only during the first 7 h correlated closely with infarct size calculated from all available serum values ($r = 0.93$). The mean difference between predicted and observed infarct size was only 4%. In the present study, predictions of infarct size were obtained only in patients with normal serum CPK values at the time of admission.

Application of nonlinear curve-fitting techniques to predict serum enzyme values and predictions of infarct size by this method require considerable caution. Nonlinear systems do not necessarily exhibit unique solutions. Moreover, the methods used to produce the least squares fit may result in convergence to biologically meaningless minima if initial estimates for parameters are inappropriate. The computer program used in these studies employed the Gauss-Newton approach to a least squares solution and applied standard linear techniques; an approach that assumes linearity of the normal equations over short ranges. It became clear early in these studies that the three (b, c, d) nonlinear parameters in the log-normal equation were related to aspects of the distribution of CPK activity as a function of time. Thus, b appeared to reflect both the time to peak CPK activity and the magnitude of peak activity; c to the natural logarithm of the time to peak activity, and d to the width (or standard deviation) of the distribution. Since the time to peak CPK activity was relatively constant in the dog, limits for c were established between 6.3 and 6.6. Since the time to peak activity in patients is a function of infarct size (1) (unpublished observations) limits for c were set between 7.1 and 7.6 in the clinical studies. Use of these limits facilitated convergence to a unique solution independent of specific initial estimates.

Several tests were utilized to assess the extent to which each fit curve conformed to a log-normal dis-

tribution. The overall standard error proved to be the most useful. It was employed to calculate confidence limits for the fit curve. In all control animal experiments and in all studies of patients with uncomplicated myocardial infarction, observed serum CPK values were within the 95% confidence limits of the best fit curve derived from data obtained during the initial 5 h (dog) and 7 h (man) period. Accordingly, deviation of actual serum CPK values from the confidence limits of the best fit curve suggests that extension of infarction or salvage of myocardium is responsible.

In this study k_d is assumed to be constant—an assumption requiring qualification. We have approximated the clearance of enzyme as a single exponential characterized by a constant fractional disappearance rate (k_d) since activity of partially purified dog heart CPK injected intravenously decays in a manner approximating that predicted by a monoexponential function (8). However, *a priori*, the actual disappearance of CPK released from the heart cannot be monoexponential since it is known that different isoenzymes of CPK disappear from serum at different rates and that myocardium contains more than one CPK isoenzyme. Thus k_d in the present formulation is an approximation. On the basis of experiments performed in conscious dogs, the average value of k_d was $0.0045 \pm 0.001 \text{ min}^{-1}$ (mean \pm SD, $n = 11$) (8). In patients, the value for k_d was approximated by measuring the decline of CPK activity during the terminal portion of CPK curves when release of enzyme from myocardium could be assumed to have ceased (k_d was found to average $0.001 \pm 0.0005 \text{ min}^{-1}$, $n = 24$) (1). In calculations of infarct size in patients, we elected to use this mean value for k_d although it entails a potential variation of infarct size from patient to patient of $\pm 30\%$ (with k_d varying by 1 SD). Evidence obtained in conscious dogs indicates that k_d remains essentially constant despite hemodynamic perturbations or impairments in hepatic or renal function (8, 14). The relative uniformity of k_d among patients requires verification. k_d could be measured by injection of CPK in patients and assay of disappearance of its activity or estimated by approximation from the best fit log-normal function from the term: $dE/dt_{\max} = e^{d^t - (1+c)/d^t}$. However, we elected to use a value for k_d based on the average observed disappearance after release had presumably ceased in our calculation of observed infarct size because it is applicable even when curve-fitting techniques are not utilized (1).

Use of the log-normal function offers a potential advantage for predicting infarct size or calculating it analytically, since the term $\int_0^T f(t)dt$ may be found directly. In general, we calculated observed infarct size by summing $\Delta E/\Delta t$ (see section B). When Δt is large,

potential inaccuracies are introduced. Predicted infarct size is found from the best fit log-normal function by a standard approximation to the integral (see section C) (17, 18).

Implications concerning extension of myocardial infarction or salvage of myocardium based on the relationships between predicted infarct size and observed infarct size might be erroneous if the amount of enzyme released into serum (CPK_r) represented a changing proportion of the amount of enzyme activity depleted from myocardium (CPK_D). If CPK_r/CPK_D increased, an extension of infarction might be inferred when one had not occurred. Conversely, if CPK_r/CPK_D decreased, apparent salvage might be inferred erroneously. In the animal experiments included in the present investigation and in those reported previously (8, 13–15) we have evaluated the ratio CPK_r/CPK_D and found it to vary by less than 10%. Unfortunately, this ratio cannot be evaluated in patients as we have pointed out previously (1). It should be noted that calculations of infarct size according to the methods described in the present investigation are independent of the rate of release of CPK from myocardium as long as the relationship of CPK_r/CPK_D remains constant.

In the present study, when infarct size was increased by administration of isoproterenol or reduced by administration of propranolol in conscious dogs with coronary artery occlusion, observed serum CPK values exceeded or fell below 95% confidence limits of projected values within several hours. Similarly, in patients with spontaneous extension of infarction observed serum CPK values deviated significantly from predicted values. In addition, reduction of afterload led to subsequent serum CPK values significantly lower than projected values indicative of at least temporary salvage of jeopardized myocardium. It is important to recognize that calculations of infarct size based on serial serum CPK changes reflect only the extent of damage that has occurred within the observation period. Interventions which slow the evolution of necrosis will not necessarily result in overall salvage of myocardium subsequently. Nevertheless, at any given time after the onset of an ischemic insult, the extent of damage which has already occurred can be assessed by the method proposed.

Estimation of infarct size from analysis of serial serum CPK changes is based on the concept that myocardial CPK depletion is related quantitatively to the extent of myocardial necrosis. Enzyme release from myocardium into serum has been observed to be associated with irreversible injury by others (19–26). The magnitude of myocardial CPK depletion has been correlated with the extent of ischemic injury assessed electrocardiographically (5, 11), sustained decrease in regional blood flow (9), abnormalities in function of

mitochondria isolated from the heart (27), prevalence of histologic changes indicative of necrosis (11), and the magnitude of functional impairment (11). Myocardial CPK depletion after sustained coronary occlusion appears to be directly related to infarct size estimated as a proportion of the left ventricle in animals of similar body weight measured grossly (9, 12). Thus, it appears that myocardial CPK depletion is a quantitative index of the extent of necrosis after sustained coronary artery occlusion.

Our results indicate that serial serum CPK changes after acute myocardial infarction conform closely to a log-normal function which can be defined within 7 h of the onset of initial serum CPK elevations in patients, and that extension of infarction and salvage of jeopardized myocardium can be recognized and quantified by comparison of observed to predicted infarct size. The approach described is of potential value in the assessment of therapeutic interventions designed to modify infarct size favorably in patients with acute myocardial infarction and in appropriate selection of patients for potentially therapeutic procedures with significant attendant risks.

ACKNOWLEDGMENTS

We gratefully acknowledge Dr. Robert I. Jennrich, University of California, Los Angeles, who provided valuable mathematical assistance.

This investigation was supported by U. S. Public Health Service MIRU Contract PH 43-68NH1-1332.

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