

Coronary Reperfusion in Primates

SERIAL ELECTROCARDIOGRAPHIC AND HISTOLOGIC ASSESSMENT

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ABSTRACT After acute coronary occlusion in primates, the time period during which reperfusion results in significant salvage of reversibly injured myocardium was investigated. In 23 monkeys, the left anterior descending coronary artery was occluded from 1 to 6 h; and in 5 others, occlusion was maintained for the 1-wk study. Unipolar epicardial electrocardiograms were monitored from mapping points on the anterior and lateral left ventricle. S-T segment elevation (S-T \uparrow) and R + S wave amplitude (RS) were measured before occlusion and at regular intervals during occlusion and reperfusion. Summated S-T \uparrow (Σ S-T \uparrow) and summated RS (Σ RS), computed for mapping points demonstrating greater than 2 mV S-T \uparrow , were used as serial measures of electrical injury. Σ S-T \uparrow peaked within 2-h postocclusion and then gradually declined throughout the period of occlusion suggesting the progress of infarction within the area of injury. After reperfusion Σ S-T \uparrow rapidly declined to near control values indicating the extent of reversible injury. During the period of occlusion, the magnitude of voltage loss in Σ S-T \uparrow as a percent of maximum Σ S-T \uparrow was proportional to the duration of occlusion, though the rate of loss decreased with increasing time of occlusion. Reperfusion after 6 h of occlusion resulted in reversal of only a small remaining component of the maximum current of injury. The voltage decrease in Σ RS (from control values) was proportional to the duration of occlusion, though the decrease was accelerated during the first 2-h postocclusion. Whereas reperfusion interrupted the decline in Σ RS, a consistent increase in Σ RS postreperfusion was observed only after occlusion of 1 h. With respect to reperfusion groups, significance in Σ S-T \uparrow voltage loss as a percent of maximum Σ S-T \uparrow was demonstrated between 2-h and 4-h, 4- and 6-h, and 6-h and chronically ligated animals. Significance in Σ RS voltage loss as a

percent of control Σ RS was demonstrated between 2- and 4-h, and 4- and 6-h reperfusion groups. Hearts were excised at 7 days for histological assessment of infarct size. Planimetric determination of left ventricular areas and areas of necrosis using slides made from 10 serial cross sections were used in estimating the percent of left ventricle infarcted. A significant reduction in infarct size was demonstrated between reperfused animals at 2 h and the 4- and 6-h reperfusion groups. A trend was noted suggesting increasing infarct size up to 6 h after experimental occlusion.

INTRODUCTION

Experimental and clinical data have increasingly supported the concept of immediate reperfusion surgery for acute coronary occlusion with myocardial infarction (1-4). Clinically, however, immediate reperfusion is often not possible, and the time interval between the onset of progressive infarction and potentially beneficial surgery has not been well defined.

Previous studies have indicated that a large area of myocardium remains viable after 3 h of experimental occlusion. It has also been indicated that the electrocardiographic changes progress for up to 6 h after occlusion. These studies have not however, been in general agreement with regard to the amount of acutely injured myocardium which they suggest may be salvaged after reperfusion. It is the intent of the present investigation to elucidate the temporal progression of electrical injury and necrosis utilizing reperfusion from 1 to 6 h after coronary occlusion. Reperfused animals were additionally compared with animals with permanent coronary occlusion. It is hoped that the present model in assessing the time constraints for significant salvage of reversibly injured myocardium may clarify the results of other investigators addressing the problem. The experimental model provides a quantitative and dynamic characterization of electrical injury and necrosis during the process

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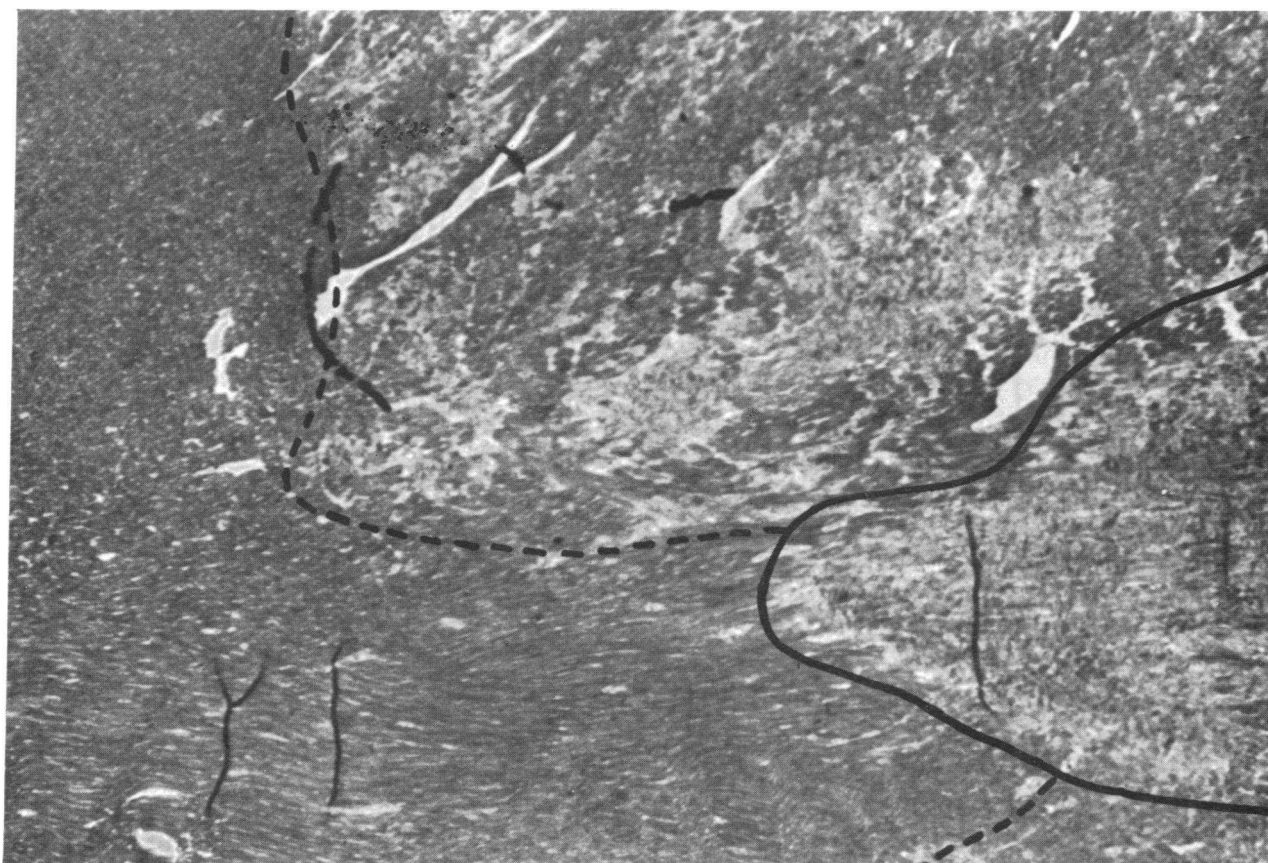


FIGURE 1 Areas of central necrosis (solid line), patchy necrosis (dashed line), and normal myocardium as outlined on a representative section of left ventricle.

of infarction and may be applicable in assessing the benefit of a number of interventions involved in increasing myocardial viability.

METHODS

29 monkeys (*Macaca cynmologous*), 3–7 kg, were anesthetized with phencyclidine hydrochloride (0.8–1 mg/kg) and maintained with sodium thiopental. They were intubated and connected to a volume respirator (Harvard Apparatus Co., Inc., Millis, Mass.) maintaining normal blood gases. Femoral arterial pressure and limb lead electrocardiogram (ECG) were monitored. A left anterolateral thoracotomy was performed and the heart was suspended in a pericardial cradle. A tourniquet was placed around the left anterior descending coronary artery just distal to the main diagonal branch. The animals were divided into groups (ligation groups) according to duration of coronary occlusion. Coronary arteries were ligated for 1 h (six animals), 2 h (five animals), 4 h (six animals), 6 h (six animals) or chronically (five animals). In one control animal the tourniquet was not tightened during the procedure. Unipolar epicardial ECG were monitored from 10 anatomically reproducible mapping points on the anterior and lateral surface of the left ventricle. The probe electrode was fashioned from a flexible 6-in 24-gauge stainless steel wire terminated with a small ball of silver solder. ECG were monitored

with a Gould, Brush, Mark 260 recorder system with ECG coupler (model 11430101, Gould, Inc., Cleveland, Ohio). The surface ECG were recorded (at a sensitivity of 1 mV/mm) before occlusion, at 10 min, then hourly after occlusion and after ligature release at 20 min, 1, and 2 h.

S-T segment (J point) elevation (S-T \uparrow),¹ R+S wave amplitude (RS), and Q-wave amplitude were measured for mapping points which showed greater than 2 mV S-T elevation over base-line values during the 1st h of occlusion. Each parameter was summated over mapping points for each recording time, yielding Σ S-T \uparrow , Σ RS, and Σ Q. These served as serial measures of the progress of electrical injury during the periods of occlusion and reperfusion. The mean and SEM of these measures were computed over animals in each ligation group to examine electrocardiographic changes with respect to duration of occlusion and reperfusion.

Tetracycline (250 mg) was injected intraperitoneally 1 h before tourniquet release and fluorescent cardiac photographs, with the use of an ultraviolet filter, were obtained immediately before release of the tourniquet. Tetracycline

¹Abbreviations used in this paper: RS, R+S wave amplitude; S-T \uparrow , S-T segment elevation; Σ RS, summated RS; Σ S-T \uparrow , summated S-T \uparrow ; A_c, summated areas of central necrosis; A_p, summated areas of patchy necrosis; A_v, summated areas of total left ventricle.

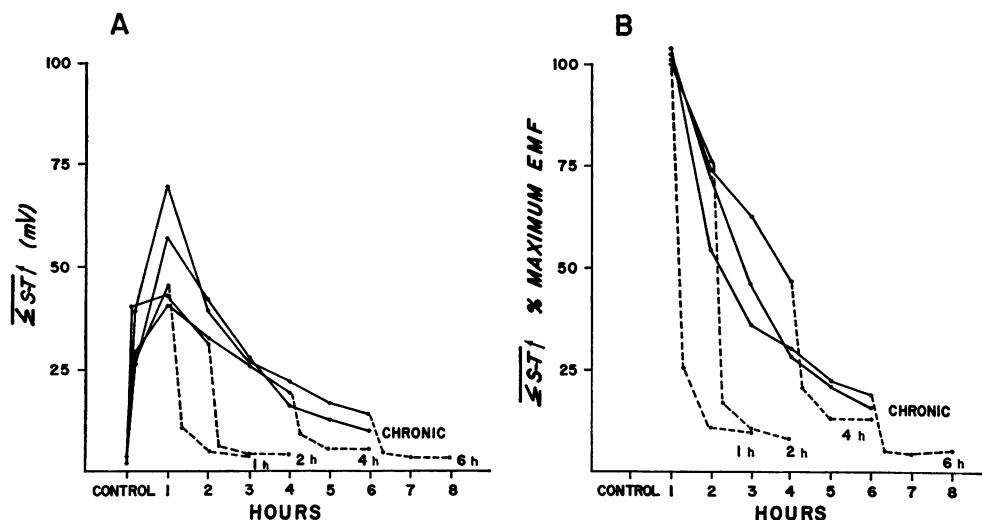


FIGURE 2 Mean $\overline{ST-T}$ changes over animals in each ligation group. Duration of occlusion in hours given for each group. (A) Actual observed voltages. (B) As a percent of peak voltage (at 1 h). EMF represents electromotive force.

was again injected 1 h after tourniquet release and photography was repeated 2 h after release.

7 days after the experimental procedure, the animals were anesthetized and hearts were excised and fixed in 10% formalin. 10 transverse sections of equal thickness were made along the major axis of the left ventricle. Slides from each section were stained with hematoxylin-eosin and Gomori's tri-chrome stain. Morphological changes associated with ischemia and necrosis and their temporal character-

ization have been extensively described previously (5-7). The infarcts generally appeared with a central area of uninterrupted fibrosis and marked degenerative changes bordered by patchy areas of necrosis intervened with normal appearing muscle fibers. The area of infarction was marked with a Rapidograph pen while the slides were viewed on a microscopic slide projector. The central area and area of patchy necrosis were marked separately. Areas of central and patchy necrosis and normal myocardium as marked on

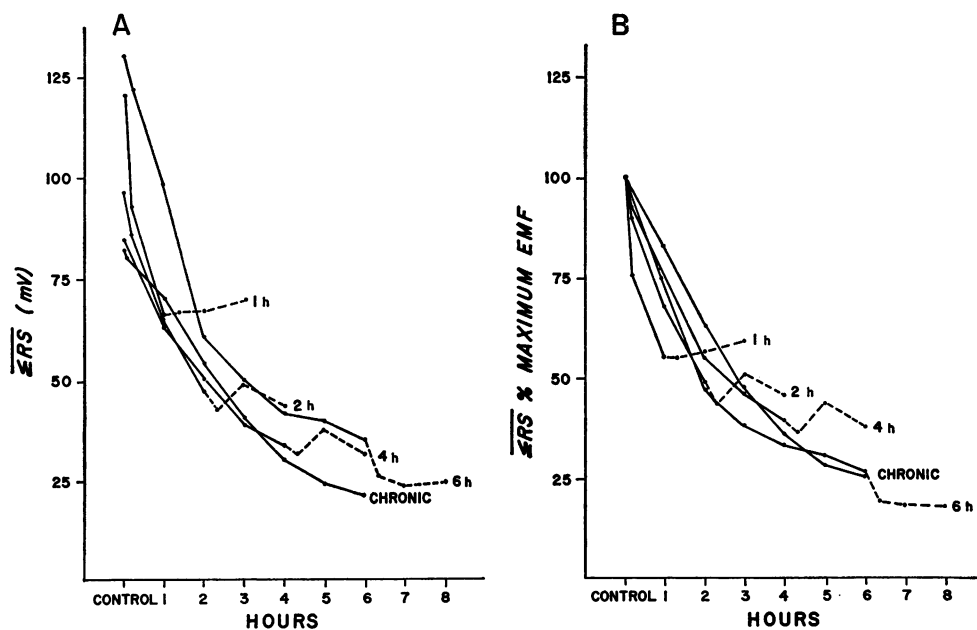


FIGURE 3 Mean \overline{RS} changes over animals in each ligation group. Duration of occlusion in hours given for each group. (A) Actual observed voltages. (B) As a percent of peak voltage (control). EMF represents electromotive force.

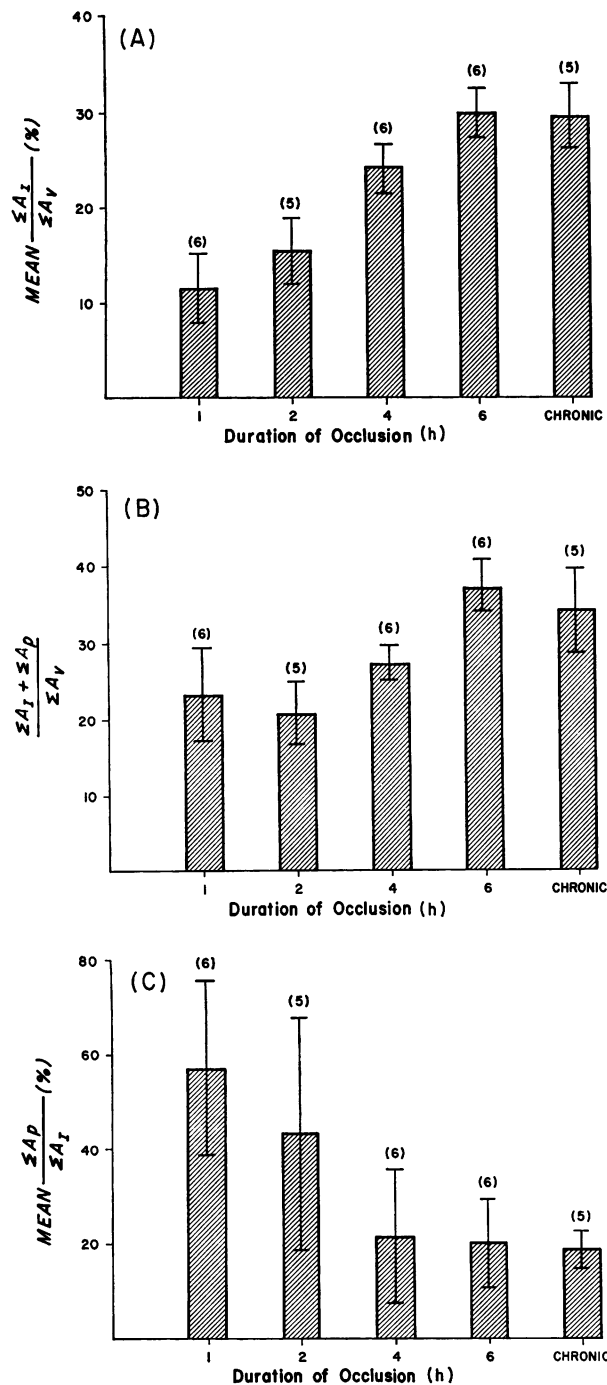


FIGURE 4 (A) Mean (\pm SEM) central infarct size as a percent of the left ventricle for animals in each ligation group. Number of animals in each group given in parentheses. (B) Mean (\pm SEM) combined area of central infarct plus patchy necrosis as a percent of the left ventricle. Number of animals in each group given in parentheses. (C) Mean (\pm SEM) area of patchy necrosis as a percent of the area of central infarct size. Number of animals in each group given in parentheses.

a representative section are shown in Fig. 1. The slides were placed in a photographic enlarger and at constant magnification the area of the left ventricle and areas of central and patchy necrosis were outlined. These outlined drawings were then planimetered. Planimetered areas were summated over the 10 serial slides for an individual animal. The ratio of summated areas of central necrosis (ΣA_I) to summated areas of total left ventricle (ΣA_V) estimated the percentage of infarcted left ventricle. The ratio of summated areas of patchy necrosis (ΣA_P) to ΣA_I was used as a measure of the progression of infarction into a more marginally ischemic area. The ratio $(\Sigma A_I + \Sigma A_P)/\Sigma A_V$ was also computed for individual animals. To examine the predictive validity of serial ECG measures to histological results, derived indices of $\Sigma S-T\uparrow$ and ΣRS were correlated with the percentage of the left ventricle infarcted, $\Sigma A_I/\Sigma A_V$.

RESULTS

The animal in which the tourniquet was not tightened showed no evidence of ECG change, uniform myocardial fluorescence after tetracycline injection, and no histologic evidence of infarction. After occlusion all animals showed signs of severe ischemia in the anterolateral left ventricle. This was grossly evidenced by paradoxical anterior wall motion, by a well-defined nonfluorescent region, and by significant early S-T \uparrow .

The S-T segment changes were characterized by a period of rapid rise which resulted in a maximum value of $\Sigma S-T\uparrow$ between 1 and 2 h after occlusion (Figs. 2A and 2B). Following this peak value, $\Sigma S-T\uparrow$ declined gradually throughout the period of occlusion. The ratio of voltage loss during the period of gradual decline to voltage at peak $\Sigma S-T\uparrow$ was proportional to the duration of occlusion, though the rate of voltage loss decreased with increasing time of occlusion. During the first 20 min after tourniquet release, the phase of gradual decline was interrupted by a rapid decline in $\Sigma S-T\uparrow$ to near control values.

ΣRS values (R + S wave amplitude as measured from the base line) decreased markedly for a period of (1–2 h after occlusion; after which, the rate of decline gradually decreased (Figs. 3A and 3B). The magnitude of ΣRS showed a high negative correlation with the magnitude of $\Sigma S-T\uparrow$ during the period of rapid S-T elevation. The decrease in ΣRS amplitude was proportional to the duration of occlusion, though as can be seen in Fig. 3A, voltage differences between groups decreased with increasing duration of occlusion. Measures of standard error for group data shown in Figs. 2A and 3A are given in Table I.

The histologic measure of percentage of the left ventricle infarcted for ligation groups, mean $\Sigma A_I/\Sigma A_V$, as shown in Fig. 4A, showed an increase through the 6-h occlusion. Similar results are seen in Fig. 4B, in which the summated areas of central and patchy necrosis as a percent of left ventricular areas, $(\Sigma A_I + \Sigma A_P)/\Sigma A_V$, are compared over ligation groups. With increasing dura-

TABLE I
Mean Grouped Data for ΣRS and $\Sigma S-T^\dagger$ Measures

Ligation group	n	Control	Postocclusion						Postreperfusion				
			10 min	1 h	2 h	3 h	4 h	5 h	6 h	20 min	1 h	2 h	
ΣRS													
1-h occlusion	6	121.0±21.5	92.4±13.3	67.0±6.7						67.6±9.0	68.9±9.5	71.5±8.4	
2-h occlusion	5	96.2±17.2	87.2±11.9	65.6±7.0	47.8±7.2					42.7±9.5	49.6±11.2	44.2±10.3	
4-h occlusion	6	84.9±10.2	80.0±15.9	64.7±13.8	50.7±15.2	39.2±13.7	33.17±11.10			31.4±11.0	37.9±4.9	32.3±5.5	
6-h occlusion	6	130.5±15.4	123.9±15.9	99.1±16.5	61.7±10.9	50.1±8.3	43.1±6.1	40.7±7.48	35.5±4.29	25.8±4.6	23.9±5.3	24.6±4.9	
Chronic	5	84.1±10.9	81.4±5.5	70.2±6.7	54.5±5.4	40.2±5.99	30.6±4.61	24.8±3.9	21.9±4.2				
ΣS-T†													
1-h occlusion	6	3.3±1.6	26.8±7.1	45.4±5.7						12.0±5.3	5.1±1.9	4.5±1.4	
2-h occlusion	5	1.6±1.4	40.9±12.0	41.0±7.2	31.4±8.5					7.1±4.5	4.4±2.7	3.4±2.2	
4-h occlusion	6	4.0±1.3	29.4±5.9	41.6±7.9	31.1±9.4	26.0±7.2	19.6±4.03			9.0±2.9	5.4±2.6	5.5±2.5	
6-h occlusion	6	4.9±1.0	41.7±10.9	71.9±11.9	39.2±6.3	26.5±2.7	22.0±3.9	16.7±1.6	13.9±2.3	4.1±0.9	3.6±0.9	3.7±0.9	
Chronic	5	2.4±1.1	22.0±3.1	57.3±8.1	41.6±5.0	26.0±3.24	16.5±3.48	12.6±1.9	9.7±1.9				

Mean \pm SEM (millivolts) for grouped data used in constructed Figs. 1A and 2A.
n equals the number of animals in each ligation group.

tion of occlusion up to 4 h the area of patchy necrosis is reduced in extent relative to the central infarct size, as can be seen from Fig. 4C.

Decrease in $\Sigma S-T\uparrow$ when expressed as a percent of maximum (Fig. 2B) demonstrated a significant difference between groups reperfused at 2 and 6 h ($P < 0.001$), between groups reperfused at 4 and 6 h ($P < 0.05$), and between the group reperfused at 6 h and permanently ligated animals ($P < 0.05$). Decrease in ΣRS expressed as a percent of the control voltage (Fig. 3B) demonstrated significance between groups reperfused at 2 and 6 h ($P < 0.05$) and 4 and 6 h ($P < 0.05$). A significant decrease in ΣRS between the 6-h group and permanently ligated animals was not shown.

ΣA_1 in permanently ligated animals was $29.5 \pm 4.1\%$ of ΣA_v . $\Sigma A_1/\Sigma A_v$ was $15.6 \pm 4.5\%$ with 2-h occlusions, 25.9 ± 2.1 with 4-h occlusions, and $30.5 \pm 2.3\%$ with 6-h occlusions. Significance was found in percent infarct size between groups reperfused at 2 and 4 h ($P < 0.05$) and between 2 and 6 h ($P < 0.01$). No significant difference was observed between permanently ligated animals and animals in the 4- or 6-h groups.

The quantitative relationship between electrocardiographic and histologic measures was examined for individual animals to determine the ability of serially monitored $\Sigma S-T\uparrow$ and ΣRS to predict the progress of infarction as assessed histologically. The voltage decrement in $\Sigma S-T\uparrow$ from its maximum value until immediately before tourniquet release was expressed as a percent of its maximum. This index, $\Sigma S-T\uparrow$ decline/ $\Sigma S-T\uparrow$ max (%), was correlated with the measure of percent left ventricle infarcted, $\Sigma A_1/\Sigma A_v$ (%), for individual animals. Similarly, the voltage decrement in ΣRS from its maximum value (control value) until tourniquet release was expressed as a percent of its maximum value, ΣRS decline/ ΣRS max (%), and correlated with percent left ventricle infarcted. The regression lines are shown in Fig. 5. The extent of the regression lines along the abscissa represents the observed range of percent of infarction of the left ventricle. There was a strong tendency for these electrocardiographic indices to predict the percent infarct size at 7 days; supporting their validity in profiling the progress of infarction. These indices, $\Sigma S-T\uparrow$ decline/ $\Sigma S-T\uparrow$ max and ΣRS decline/ ΣRS max, may be visualized for group data in Figs. 2B and 3B respectively.

Tetracycline studies demonstrated nonfluorescence in an area corresponding to but slightly smaller than the area of injury or infarction as determined by ECG mapping. After tourniquet release, repeated tetracycline injection demonstrated reperfusion (fluorescence) in the previously nonfluorescent region. Fluorescence after reperfusion reflected the serum level of tetracycline and was not associated with reversible myocardial injury.

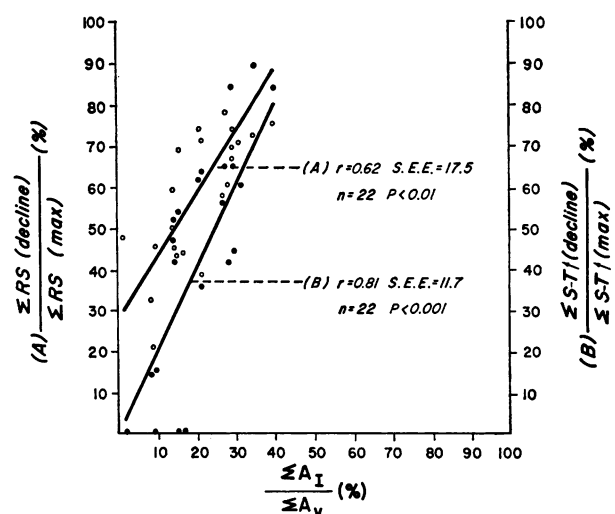


FIGURE 5 Regression lines comparing electrocardiographic indices of the progression of infarction to the histologic measure of central infarct size, as discussed in the text. Individual data points for S-T index (closed circles) and RS index (open circles) are also shown. S.E.E. represents the standard error of the estimate.

DISCUSSION

The rapid increase in S-T elevation within 1–2 h after occlusion implies a rapidly increasing area of injured muscle, though irreversible muscle necrosis is concurrent, beginning probably within 20–30 min after occlusion (2, 3). During this initial phase, however, the area of injury is increasing at a much faster rate than the area undergoing necrosis. The progressive decline in $\Sigma S-T$ elevation during the period of occlusion after peak $\Sigma S-T$ elevation suggests that the area of necrosis is at this time increasing at a faster rate, at the expense of the area of reversible muscle injury. This suggestion is supported by concomitant increasing ΣQ -wave depth, decreasing ΣRS amplitude and by the histologically revealed decrease in the area of patchy necrosis relative to the central infarct area with the increasing duration of occlusion. The rapid decline in $\Sigma S-T$ elevation after tourniquet release indicates the extent of the ischemic area that remains viable.

The form of the unipolar epicardial ECG associated with myocardial injury and infarction and the ability of this measure to outline discretely areas of underlying injury have been extensively investigated (8–12). Ginks et al. (13) have utilized epicardial mapping in demonstrating reduction of infarct size after reperfusion after 3 h of occlusion (compared with control animals) with histological assessment at 1 wk. In their study (in dogs), infarct size at 1 wk was expressed as a percentage of the volume of acute injury indicated by S-T mapping of the left ventricle at 15 min. This control technique is

of value in reducing the variability between animals with respect to the extent of acute injury and subsequent infarction. The effects of varying periods of occlusion on the progression of infarction may be more effectively assessed by accounting for this interanimal variability. In the present experiment, the quantitative electrocardiographic changes are derived from a routine epicardial map. The indices of $\Sigma S-T\uparrow$ decline/ $\Sigma S-T\uparrow$ max and ΣRS decline/ ΣRS max are not subject to the variability in extent of acute injury between animals if the epicardial mapping points remain comprehensive of the area of injury. These indices reflect the evolution of infarction quantitatively as a ratio of a control measure ($\Sigma S-T\uparrow$ max and ΣRS max). While the voltages $\Sigma S-T\uparrow$ decline, $\Sigma S-T\uparrow$ max, ΣRS decline, and ΣRS max are dependent on the size of the acutely injured area and the progressive infarction, the indices utilized are not. These indices may be expected, therefore, to correlate more closely with the ratio of area of infarct (assessed histologically) to acutely injured area rather than with the ratio of infarction to the total left ventricle. An estimate of the area of acute injury from epicardial S-T segment mapping, however, was not possible in the present experiment due to septal involvement of the infarcts and masking of this area by the right ventricle. The good correlation between ECG indices and the histologic area of infarct as a percentage of the total left ventricle, suggests that variability in the area of acute injury between animals, was minimized in the present experiment.

Cox, Daniel, and Boineau (14) have examined the time course of changes in the bipolar potential using epicardial and intramural electrodes placed within an area of acute ischemic injury. In a series of reperfusion experiments in dogs, the voltage loss of the bipolar potential was interrupted by reperfusion after up to 6 h of temporary occlusion. The rate of voltage loss decreased with increasing duration of occlusion, and little further voltage decrease occurred after 6 h of occlusion.

A disparity appears in comparison of the results of Ginks et al. (13) with those of Cox et al. (14). Ginks et al. have demonstrated salvage of 84% of acutely injured myocardium with reperfusion after 3 h when compared with control animals. Cox et al. utilizing intramural and epicardial electrode placement throughout the acutely injured area have indicated a mean voltage loss in this area in excess of 50% after 3 h of occlusion, and further that reperfusion halted but did not reverse this voltage loss. It is also indicated that electrodes showing a greater voltage loss correlated with histological zones exhibiting more pronounced necrosis. Interpreting the two studies is difficult as both employed the dog heart in the experimental model. The study of Cox et al. suggests salvage of a much smaller area of the acutely injured myocardium after 3 h of occlusion than the 84%

found by Ginks et al. if a proportionality may be assumed between ECG voltage loss and tissue necrosis. The present study in primates involving a serial study of epicardial ECG parameters as well as quantitative histological assessment generally provides support for the results of Cox et al. In support of their findings, voltage loss in $\Sigma S-T\uparrow$ and ΣRS from control values was on the order of 50% after occlusion for 3 h. Measures of infarct size further suggest that histological results correlate well with measures of electrical necrosis, as in the present study salvage of only 50% of acutely injured myocardium was accomplished with reperfusion after 2 h of occlusion. It is not readily apparent how methodological differences may have given rise to differences in findings of this magnitude. In the study of Ginks et al., variable septal injury following left anterior descending coronary artery occlusion cannot be evaluated utilizing discrete surface mapping of acute injury. In addition, histological infarct size in this study was derived from color photographs of gross tissue sections which may not have fully characterized zones with variable amounts of tissue necrosis. These comments, however, address sources of error whose impact has not been quantitated, which may not have been greatly confounding and which would not necessarily bias their results.

The present study attempts to provide a model which may be useful in longitudinally tracing the effects of a number of surgical or pharmacological interventions involving myocardial viability. It is based on electrocardiographic measures which have proven sensitive in quantitating myocardial injury and necrosis. Examination of the degree of voltage loss in $\Sigma S-T\uparrow$ and ΣRS , compared with control values, provides an effective way of demonstrating differences in ultimate infarct size. The unipolar complex allows electrical segregation of the current of injury and electrical death, whereas with the bipolar complex this is not possible. The present electrocardiographic model in noninvasive procedures is further applicable by the use of a precordial multilead electrode blanket or by the assessment of S-T and mean QRS vector changes using an electrically orthogonal vectorcardiographic system.

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