Factors Influencing Infarct Size Following Experimental Coronary Artery Occlusions

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SUMMARY
The purpose of this study was the determination of whether hemodynamic and pharmacologic factors influence the extent and severity of myocardial necrosis produced by coronary occlusion. In 48 dogs, 10 to 14 epicardial leads were recorded on the anterior surface of the left ventricle in the distribution and vicinity of the site of occlusion of a branch of the left anterior descending coronary artery. The average S-T segment elevation for each animal was determined at 5-min intervals after occlusion. This elevation was used as an index of the presence and severity of myocardial ischemic injury. The number of sites showing this elevation provided an additional measure of the size of the injured area. Occlusion alone raised the average S-T segment elevation from 0.22 ± 0.04 to 3.32 ± 0.37 mv (SEM). Isoproterenol, ouabain, glucagon, bretylum, and tachycardia given prior to a repeated occlusion increased the severity and extent of ischemic injury, while propranolol decreased it. Elevation of arterial pressure with methoxamine reduced the occlusion-induced S-T segment elevation, and lowering of the mean arterial pressure by hemorrhage had the opposite effect. In 19 additional experiments, propranolol, isoproterenol, and alterations in arterial pressure produced similar alterations in S-T segment elevation when these interventions were applied as long as 3 hr after ligation. In a third group of dogs, myocardial creatine phosphokinase (CPK) activity was determined 24 hr after occlusion at the same sites at which epicardial electrocardiograms were taken. Depression of myocardial CPK activity in injured portions of the left ventricle 24 hr after coronary artery ligation correlated well with S-T segment elevation in the same sites 15 min after ligation. Moreover, isoproterenol increased and propranolol decreased the area of depression of myocardial CPK activity. We conclude that the hemodynamic status and neurohumoral background at the time of occlusion and for up to 3 hr thereafter can alter the extent and severity of myocardial ischemic injury and myocardial necrosis.

Additional Indexing Words:
Coronary occlusion S-T segment elevation Myocardial necrosis
Epicardial electrocardiogram Myocardial oxygen consumption
Myocardial creatine phosphokinase

THE CLASSICAL treatment of power failure of the heart consequent upon myocardial infarction is relatively ineffective. Considering that the infarct’s size is an important determinant of power failure, a possible therapeutic approach to this syndrome would be an attempt to limit the size of the infarction. Accordingly, the purpose of the present investigation was the examination of the hemodynamic conditions and pharmaco-

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logic interventions that might alter the size of an infarct by changing the balance between oxygen supply and demand in the area of myocardium supplied by the occluded vessel.

Despite the important clinical implications of limitation of infarct size, it has been difficult to assess quantitatively the extent of tissue damage immediately after coronary artery occlusion. This difficulty was circumvented in these studies by examination of the extent and magnitude of epicardial S-T segment elevation in areas surrounding the occluded coronary artery. This technique allowed rapid and reproducible determination of ischemic cellular injury in the same animal, permitting the use of each dog as its own control. However, epicardial electrocardiographic changes occur very early during the course of ischemic damage, and it has not yet been established to what extent S-T segment elevation presages the later development of cellular damage. As it has been shown recently that the reduction in myocardial creatine phosphokinase (CPK, activity is a quantitative indicator of the extent of cell death 24 hr after coronary artery occlusion,1 in the present investigation the magnitude and extent of S-T segment elevation measured with epicardial electrodes at specific myocardial sites were compared with CPK activity in myocardial specimens from these sites obtained 24 hr after coronary artery occlusion.

Methods

Studies were carried out in 85 dogs weighing between 15 and 27 kg and anesthetized with sodium thiamylal (25 mg/kg). Respiration was maintained with a Harvard respirator, and the heart was exposed through a left thoracotomy and suspended in a pericardial cradle. A branch of the left anterior descending coronary artery, usually the apical branch, or the main left anterior descending coronary artery itself, was dissected from the adjacent tissues and intermittently occluded with two Schwartz intracranial arterial clamps. Arterial pressure was monitored with a Statham pressure transducer (model P23Db). All variables, including limb leads and the epicardial electrocardiogram, were recorded on an oscillographic recorder.

Ten to 14 sites on the anterior surface of the left ventricle were selected for epicardial electrocardiography. The limb leads were connected in the usual fashion, and the precordial lead was terminated in a special hollow metallic cylinder 4 mm in diameter held at a right angle to the epicardium and connected to the standard precordial electrode. This exploring electrode was

Figure 1

(Right panel) schematic representation of the anterior surface of the heart. The coronary arteries and branches, and sites of the epicardial electrocardiograms are marked. LAD = left anterior descending coronary artery; LA = left atrial appendage; LC = left circumflex coronary artery.

(Left panel) electrocardiograms from sites 1 and 7 before and 15 min after occlusion.
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held in such a way as to minimize pressure when it was applied to the surface of the heart. Some studies were done with the use of a cotton wick soaked in saline and impacted in the hollow cylinder. Epicardial recordings were obtained at one-tenth the normal electrocardiographic sensitivity (1 mv/mm recording).

Sites for recording were selected within the area supplied by the coronary branch that was occluded, in areas immediately adjacent to this zone, as well as portions of the left ventricle remote from this area which were presumed to be adequately perfused. Since the recordings obtained from each specified epicardial site had to be repeated several times, sites were selected near the bifurcation of arteries or veins so that the recording probe could be easily repositioned at the same location. Epicardial electrocardiograms were obtained from each site before each occlusion and 5, 10, 15, and 20 min after occlusion. In 60 of the 85 dogs the occlusion was released afterward, and further coronary occlusions were not performed until the epicardial S-T segments had returned to normal, a period of approximately 45 min.

The S-T segment elevation at each site was used as an index of the severity of local myocardial injury (fig. 1). The sites at which myocardial ischemic injury was considered to exist were those at which the S-T segment elevation exceeded 2 mv when recorded 15 min after ligation. Thus, for example, in figure 2 myocardial ischemic injury was considered to be present at sites 6, 7, 8, and 9. In each animal, the S-T segment elevations from all recording sites were added; this value, $\Sigma S-T$, was then used as an overall index of the intensity of myocardial injury in any given animal. The mean S-T segment

**Figure 2**

*Effects of occlusion alone and occlusion after the infusion of isoproterenol (0.25 $\mu$g/kg/min). (Right panel) abbreviations as in figure 1. Cross-hatched area: area of injury after 15 min of occlusion. Stippled area: increase of area of injury when the occlusion was performed under the influence of isoproterenol. Lined area: area that showed no ST segment elevation under any circumstances. (Left panel) $\Sigma S-T$ in the same experiment after two simple occlusions and after two occlusions under the influence of isoproterenol. Time = minutes after occlusion.*

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elevation in any dog (S-T) was calculated as the quotient of ∑S-T and the total number of sites, and changes in the average S-T for a group of animals analyzed by the paired t-test technique were used to determine the effects of a variety of interventions on the severity of myocardial ischemic injury.

The animals were divided into three groups. In the first group (48 dogs), the effects of acute interventions were studied. In all groups the experimental protocol was similar. A control occlusion was carried out with epicardial recordings just before, and 5, 10, 15, and 20 min after occlusion. After release of the occlusion and return of the aortic pressure, heart rate, and epicardial electrocardiogram to control values, one of eight interventions was employed:
1. An infusion of isoproterenol (0.25–0.50 μg/kg/min) was begun 5 min before occlusion and maintained during the occlusion (nine experiments).
2. Heart rate was increased by an average of 62 beats/min by right atrial stimulation (three experiments).
3. Propranolol (0.5, 1.0 or 2.0 mg/kg) was administered intravenously 5 min before occlusion (eight experiments).
4. Methoxamine (0.01–0.05 mg/kg/min) was infused for augmentation of the mean arterial pressure; the infusion was begun 5 min before occlusion and maintained during occlusion (eight experiments).
5. Arterial bleeding was carried out before occlusion so that mean arterial pressure might be reduced (six experiments).
6. Glucagon (5–20 μg/kg) was administered intravenously 5 min before occlusion (four experiments).
7. Bretylium tosylate (5 mg/kg) was administered intravenously 30 min before occlusion (two experiments).
8. Ouabain (0.03 mg/kg) was administered intravenously 5 min before occlusion (six experiments).

In the second group (19 dogs), coronary occlusion was maintained for 3 hr, and the effects of isoproterenol (two experiments), propranolol (six experiments), and methoxamine or phenylephrine HCl (11 experiments) on the epicardial electrocardiogram were then determined while the occlusion was maintained.

In the third group (18 dogs), the correlation between S-T segment elevation 15 min after occlusion and CPK depletion 24 hr later was studied. In the first subgroup (six dogs), which was not subjected to any pharmacologic interventions, epicardial electrocardiograms were recorded at 5-min intervals after coronary occlusion; the thorax was closed and the animal was allowed to recover. Twenty-four hr later, another thoracotomy was performed, and the heart was quickly removed. Full wall thickness myocardial specimens (approximately 500 mg) were obtained as previously described from the same sites from which epicardial recordings had been obtained 24 hr previously.

In the second subgroup (12 dogs) the effect of drug interventions was studied. A control occlusion was performed in each animal for 20 min and epicardial electrocardiograms were recorded. After release and return of the electrocardiogram to control values, a second occlusion was carried out. Commencing 5 min before the second occlusion, which was maintained for 24 hr, isoproterenol or propranolol was administered intravenously to six dogs each. The dosage regimen for isoproterenol was 0.25–0.5 μg/kg/min for 3–5 hr. Propranolol was given in a dose of 0.5–2.0 mg/kg initially, followed by 0.5 mg/kg at 4-hr intervals for a total of 12 hr. The electrocardiograms were taken in the usual manner, and the chest was closed; the procedures followed were as in the first subgroup.

Chemical Procedures

All chemicals were of the highest grade commercially available, and were prepared for use in doubly distilled deionized water. CPK standard was obtained from Worthington Biochemical Corporation. Creatine phosphate, glucose-6-phosphate dehydrogenase, nicotinamide dinucleotide phosphate, sodium adenine diphosphate, sodium adenosine monophosphate, and hexokinase were obtained from Calbiochem. Cysteine hydrochloride and bovine serum albumin were obtained from Sigma Chemical Company. Ventricular myocardium was homogenized as previously described and CPK activity was assayed in the 16,000 × g supernatant fraction spectrophotometrically according to the method described by Rosalki. Diluents and assay conditions were exactly the same as those described previously. CPK activity was expressed as International Units (IU) (μmoles of substrate converted per min) per mg of supernatant fraction protein. Reaction rates were linear for at least 15 min after an equilibration period of 5 min; activity was proportional to the quantity of supernatant fraction protein added to the assay system; enzyme activity was acid and heat labile; and results of duplicate determinations of enzyme activity agreed within 3%.

Results

The Effects of Coronary Occlusion

After coronary occlusion, an area of the left ventricle that was cyanotic and bulged during systole could be observed in most experiments.
The correlation between this area and the area of myocardial ischemic injury as reflected in the S-T segment elevation was excellent. Also, the interventions, which altered the area of severe myocardial ischemic injury, affected the area of visible cyanosis in a parallel fashion.

Figure 1 shows the results of an occlusion of a branch of the left anterior descending coronary artery. Site 1, which was remote from the site of occlusion, showed no evidence of S-T segment elevation before or during occlusion, while site 7, well within the area of distribution of the occluded vessel, showed a 7-mv elevation of the S-T segment 15 min after occlusion. In the experiment illustrated in figure 2, epicardial recordings were obtained at 14 sites. In Run no. 3 SS-T increased from a control value of 7 mv before occlusion to 24 mv 15 min after ligation. In the 48 dogs studied, the average S-T segment elevation increased from 0.22 ± 0.4 mv (SEM) before occlusion to 3.22 ± 0.37 mv (P < 0.001) 15 min after occlusion, while the average number of sites showing S-T segment elevation exceeding 2 mv rose from 0.0 to 3.6 ± 0.4 (P < 0.001).

The reproducibility of the S-T segment elevation was studied in nine experiments in which two 20-min periods of simple occlusion were carried out in the same dog with an intervening period of 1 hr during which the occlusion was released. The epicardial sites at which ischemic injury was present 15 min after occlusion were identical, and the average S-T segments for the group were 4.2 ± 1.0 and 3.9 ± 1.0 mv during the first and second occlusions, respectively (P > 0.3, fig. 3). Moreover, after recovery from an intervention where repeated “control” occlusions were performed (fig. 2, runs 1 and 3), the magnitudes of the S-T segment elevations were similar.

**Effects of Isoproterenol**

The infusion of isoproterenol (0.25–0.50 μg/kg/min) elevated heart rate from 136 ± 7 to 179 ± 12 beats/min and lowered mean arterial pressure from 116 ± 8 to 99 ± 9 mm Hg. After a stable hemodynamic state was achieved and while the isoproterenol infusion was continued, coronary occlusion was repeated. In each dog, the number of sites of severe myocardial ischemia increased and the extent of the S-T segment elevation at any particular affected site was augmented. Moreover, the effects of isoproterenol were reproducible, resulting in similar increases in S-T segment elevation when separate infusions and coronary occlusions were done (fig. 2, runs 2 and 4). With 20-min periods of coronary artery occlusion, isoproterenol did not appear to produce irreversible damage; thus, the S-T segments became isoelectric after release of the ligation and cessation of isoproterenol; reocclusion then produced S-T segments similar to those during the first control occlusion (fig. 2, runs 1 and 3).

In the 12 experiments performed in nine animals, the average S-T 15 min after occlusion increased from 1.6 ± 0.4 mv during the control occlusions to 6.8 ± 1.1 mv after ligation during isoproterenol infusion (P < 0.01) (fig. 3), and the number of sites of ischemic injury increased from an average of 2.2 ± 0.9 to 7.0 ± 1.1 mv (P < 0.01).

When an infusion of isoproterenol (0.25 μg/kg/min) was begun 3 hr after coronary occlusion, in two dogs the S-T rose from 0.4 to 3.3 mv and from 1.7 to 8.3 mv, respectively.

**Figure 3**

*Summary of results. Influence of repeated occlusions under the influence of isoproterenol, ouabain, glucagon, propranolol, hypotension, and hypertension on the average ST segment elevation (ST) after an occlusion. Bars represent standard errors of the mean. Figures below columns indicate number of experiments.*
Effects of Simple Tachycardia

In order to identify the specific contribution of the tachycardia induced by isoproterenol on the effects produced by this drug in three separate experiments, we elevated the heart rate by electrical stimulation by an average of...
62 beats/min to a level similar to that which had been previously produced by isoproterenol infusion, and the effects of coronary occlusion were again studied. In each instance pacing-induced tachycardia augmented the S-T segment elevation produced by occlusion (fig. 4A; compare runs 1 and 3), but did so to a lesser extent than when similar levels of heart rate had been achieved with isoproterenol (fig. 4A; compare runs 2 and 3).

**Effects of Ouabain**

Ouabain (0.03 mg/kg) administered 5 min before occlusion, did not alter the arterial pressure and heart rate existing 15 min after occlusion. Generally, ouabain depressed the S-T segments at sites remote from the occluded vessel but despite this effect of the drug, the average S-T segment elevation increased from 2.2 ± 0.8 to 4.1 ± 1.2 mv (P < 0.01), and the number of sites with S-T segment elevations exceeding 2 mv increased from an average of 5.0 ± 1.7 to 7.2 ± 1.4 (P < 0.025) (fig. 3).

**Effects of Propranolol**

Propranolol (0.5-2.0 mg/kg), administered to eight animals, reduced heart rate from an average control level of 130 ± 6 to 111 ± 4 beats/min; systemic arterial blood pressure was not significantly altered. Coronary occlusion carried out in the presence of propranolol resulted in a decrease in the average S-T segment elevation from control levels of 3.9 ± 0.9 mv after simple occlusion to 1.6 ± 0.8 mv after occlusion carried out following the administration of propranolol (P < 0.01) (figs. 3 and 4B). The number of sites with S-T segment elevations exceeding 2 mv declined from 5.4 ± 0.6 to 1.1 ± 0.5 (P < 0.01). In two additional experiments, heart rate was maintained constant by electrical stimulation, and propranolol was found to reduce the S-T segment elevation 15 min after coronary occlusion to 33% and 40% of the levels observed after the control occlusions.

In the six experiments in which propranolol (1.0 mg/kg) was infused 3 hr after coronary occlusion, the average S-T segment elevation decreased from 7.6 ± 1.8 to 4.9 ± 1.3 mv (P < 0.01).

**Effects of Glucagon**

Glucagon (5-20 μg/kg), administered to four animals increased S-S-T segment elevation as well as the area of ischemic injury. Fifteen min after occlusion the average S-T segment was increased from a control value of 3.4 ± 1.8 to 8.0 ± 4.1 mv (fig. 3), and the number of sites of ischemic injury rose from an average of 3.5 ± 1.0 to 6.0 ± 0.4. Moreover, when heart rate was maintained constant by atrial stimulation, glucagon still increased the S-S-T and the number of sites showing an S-T segment elevation of more than 2 mv.

**Effects of Bretylium**

After a control occlusion in two dogs, bretylium tosylate* (5 mg/kg) was given slowly intravenously, and 30 min later coronary occlusion was repeated. The average S-T segment elevation increased from 1.4 to 3.0 mv, while the number of sites of myocardial ischemic injury rose from 2.5 to 8.0.

**Effects of Varying Arterial Pressure**

Arterial hypertension was produced in eight experiments by the infusion of methoxamine (0.01-0.05 mg/kg/min) (fig. 5B and D). Mean arterial pressure was elevated from 116 ± 8 mm Hg during the control occlusion to 189 ± 6 mm Hg 15 min after coronary occlusion during methoxamine infusion, while the corresponding heart rate fell from 123 ± 1 to 109 ± 8 beats/min. The average S-T 15 min after occlusion decreased from 4.9 ± 1.3 mv during the control occlusion to 1.6 ± 0.6 mv (P < 0.01) 15 min after occlusions carried out during methoxamine infusion (fig. 3), while the number of epicardial sites showing severe ischemic injury declined from 4.4 ± 0.9 to 2.1 ± 0.9 (P < 0.025).

In six experiments arterial hypotension was produced by acute arterial hemorrhage (fig. 5A and C). Mean arterial pressure fell from an average of 140 ± 11 to 63 ± 10 mm Hg, while heart rate decreased from an average of 146 ± 10 to 123 ± 11 beats/min. The average

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*Kindly supplied by Burroughs-Wellcome Laboratories.
**Figure 5**

Influence of arterial blood pressure. (Panel A) effects of hypotension on ΣST segment elevation after coronary occlusion. (Panel B) effects of hypertension on ΣST segment elevation after coronary occlusion. (Panel C) summary of results in all experiments in which hypotension was induced. Each line connects two points that represent ΣST at 15 min after occlusion, when performed before and after bleeding in the same dog. (Panel D) summary of results in all experiments in which hypertension was induced. Each line represents the ΣST at 15 min after occlusion when performed before and after methoxamine infusion in the same dog.

ΣT 15 min after coronary occlusion increased from 2.8 ± 1.2 mv during the control occlusion to 9.0 ± 3.4 mv during hemorrhagic hypotension (P < 0.01) (fig. 3), and the number of sites exhibiting ischemic injury increased from 1.8 ± 0.7 to 6.3 ± 1.1 (P < 0.01).

In four experiments the effects of three or four levels of arterial pressure on the ΣS-T 15 min after coronary ligation were examined. An inverse relation between the arterial pressure and ΣS-T segment elevation was noted in each experiment (fig. 6A). The regression equation for all of the experiments in which arterial pressure was altered was
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Figure 6

(Panel A) relationship between arterial pressure and myocardial injury. \( \Sigma ST \) 15 min after an occlusion at three to four different levels of mean arterial pressure. Each line represents one animal.

Panel B) influence on myocardial injury of methoxamine administered 3 hr after occlusion. Black bar represents infusion of methoxamine (0.015 mg/kg).

\[
\Sigma S-T \text{ (mv)} = 0.36 \text{ MAP} + 75.4
\]

where MAP = mean arterial pressure in mm Hg \((r = 0.69; P < 0.01)\).

In 11 experiments in which arterial blood pressure was elevated from 112 ± 4 to 168 ± 5 mm Hg by methoxamine or phentolamine HCl 3 hr after coronary ligation, the average S-T decreased from 4.3 ± 0.4 to 2.2 ± 0.5 mv \((P < 0.01)\) (fig. 6B).

Correlation of Epicardial S-T Segment Elevations and Myocardial CPK Depletion

In the normal animal, the variation of CPK activity in multiple transmural specimens from divergent sites in the same heart averaged 6%. CPK activity was measured in samples from the myocardial wall, divided into an inner portion and a corresponding outer portion. Values obtained were similar: 20.1 ± 1.8 and 19.7 ± 1.4 IU/mg, respectively \((\text{mean} \pm \text{SEM}; N = 8; \text{samples from eight separate dogs})\).

A representative experiment demonstrating the correlation between S-T segment elevation and myocardial CPK activity depression is illustrated in figure 7. Near the border of the infarcted area, both S-T segment elevation and CPK depression 24 hr later were smaller than those in the center of the infarcted area, where S-T segment elevation and CPK activity were maximally altered. The relationship between the increase in S-T segment elevation 15 min after ligation and the decrease in myocardial CPK activity 24 hr later was studied in multiple corresponding specimens from six dogs. S-T segment elevations greater than 2 mv were invariably associated with a decrease in myocardial CPK activity 24 hr later. The inverse relationship

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between the two approximated an exponential curve:

\[
\log \text{CPK} = 1.27 - 0.065 \text{ S-T} \\
(\text{r} = -0.87; \quad P < 0.01) \quad (\text{fig. 8A})
\]

In myocardium clearly not supplied by the ligated coronary artery, S-T segment elevation was consistently less than 2 mV, and CPK activity in corresponding sites measured in six dogs was 18.2 ± 0.5 IU/mg protein (N = 27), not differing significantly from that in myocardium from four unoperated control animals (18.2 ± 0.9 IU/mg protein; N = 20).

**The Influence of Isoproterenol and Propranolol on CPK Depression**

Administration of isoproterenol (0.25–0.5 μg/kg/min for 5 hr) did not lead to subsequent depression of myocardial CPK activity in nonischemic tissue from dogs with coronary artery occlusion. Activity in samples of nonischemic tissue from six animals treated with isoproterenol averaged 19.4 ± 0.5 IU/mg (N = 25 samples), compared to 18.2 ± 0.5 IU/mg (N = 27 samples) in nonischemic tissue from six animals receiving no drug (fig. 9). A representative experiment demonstrating the effects of isoproterenol is illustrated in figure 10A. In several sites with an S-T segment elevation of less than 2 mV during control occlusion, as indicated by the asterisks in figure 10A, there was a marked increase in S-T segment elevation during isoproterenol infusion. The increase in S-T segment elevation associated with administration of isoproterenol over and above that produced by

Figure 8

(Panel A) depression of myocardial CPK activity and epicardial ST segment elevation. Epicardial recordings were obtained 15 min after coronary artery occlusion from readily identifiable sites. CPK activity was measured in homogenates from full wall specimens obtained from the same sites 24 hr later, and is expressed on a logarithmic scale. Multiple samples were obtained from six dogs. Corresponding symbols represent samples from the same dog.

(Panel B) depression of myocardial CPK activity after administration of isoproterenol and propranolol in animals with coronary artery occlusion. Data from multiple samples from six animals given isoproterenol and from six animals given propranolol. Line A: regression line for control study (see panel A), (log CPK = 1.269 - 0.065 ST; r = -0.87). Line B: regression line relating ST segment elevation after coronary artery occlusion before isoproterenol was given, and log CPK from myocardial sites that showed increased ST segment elevation during isoproterenol infusion (log CPK = 1.080 - 0.076 ST; r = -0.80). Thus, in animals that had received isoproterenol, depression of myocardial CPK activity was greater than that which would be expected from ST segment elevation occurring prior to isoproterenol infusion. Line C: regression line for ST segment elevation after coronary artery occlusion before the propranolol was given and log CPK from corresponding sites 24 hr later (log CPK = 1.302 - 0.035 ST; r = -0.53). Thus, in animals that had received propranolol, depression of myocardial CPK activity was less than that which would be expected from ST segment elevation occurring prior to drug administration.

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Augmentation of the area of epicardial ST segment elevation and myocardial CPK depression after coronary artery occlusion accompanied by isoproterenol. Values represented are means ± SEM. (●) = values in nonischemic tissue from six dogs receiving no isoproterenol. (○) = values from sites without ST segment elevation in animals subjected to coronary artery occlusion and given isoproterenol. (■) = values in the same animals from sites with no ST segment elevation after coronary artery occlusion alone but with ST segment elevation after occlusion plus isoproterenol administration. The results demonstrate that myocardial CPK depression occurred in these sites within 24 hr.

Coronary artery ligation alone, is reflected by corresponding changes in myocardial CPK activity. It can be seen that CPK depression after administration of isoproterenol was significantly greater ($P < 0.05$) than that which would have been expected on the basis of S-T segment elevation after coronary artery ligation but before isoproterenol administration (fig. 8B). This point is demonstrated also by data represented in figure 9. Specimens from portions of the myocardium with normal S-T segments after coronary artery occlusion before isoproterenol was administered, but with elevated S-T segments during administration of the drug, clearly demonstrated a depression of CPK activity 24 hr later ($P < 0.001$).

Administration of propranolol after coronary artery occlusion led to a marked reduction in the extent and magnitude of S-T segment elevation compared with that resulting from coronary artery occlusion alone and to lesser depression of CPK activity (fig. 10B). With propranolol the extent of depression of myocardial CPK activity was significantly less than that anticipated from the magnitude of S-T segment elevation produced by coronary artery occlusion alone (fig. 8B). The regression line relating CPK depression to S-T segment elevation in control animals differed significantly ($P < 0.05$) from that relating CPK depression after propranolol to S-T segment elevation before administration of the drug. Thus, tissue damage after administration of propranolol was reduced compared to that anticipated from the S-T segment elevation after the control occlusion.

Discussion

There is some evidence to suggest that factors that influence myocardial oxygen demands may aggravate or alleviate symptoms of myocardial ischemia. For example, in patients with angina and hyperthyroidism treatment of the hypermetabolic state, and the associated reduction of myocardial oxygen needs, is often associated with relief of angina. Also, the reduction of myocardial oxygen needs produced by beta-adrenergic blockade or carotid sinus nerve stimulation reduces symptoms of myocardial ischemia. Conversely, treatment of hypothyroidism or the development of tachycardia, influences that increase myocardial oxygen needs, increases the frequency and severity of myocardial ischemia in patients with coronary artery disease. The importance of the decreased availability of oxygen is apparent in cases where arterial hypotension and acute anemia may cause infarction in the absence of coronary occlusion. These clinical observations suggested to us that hemodynamic and pharmacologic stimuli might alter the extent of myocardial ischemic injury and infarction after coronary occlusion, and the present investigation was designed for exploration of this possibility.
The epicardial electrocardiographic technique utilized in this study overcomes several difficulties inherent in other methods for study of this problem. Since observations are carried out in the same heart and at the same epicardial sites, the influence of variations of coronary arterial distribution among different animals is eliminated, and each dog can serve as its own control. The constant number of sites showing elevations of the S-T segment and the small variations in the magnitude of S-T segment elevation during repetitive occlusions attest to the reproducibility of this method.

There is considerable evidence that the epicardial S-T segment elevation directly reflects myocardial cellular injury. Wégria and associates reported that the degree of S-T segment elevation is dependent on the severity of experimentally produced coronary constriction, thus indicating that this electrocardiographic finding reflects myocardial injury. A correlation between the magnitude of ischemic alteration of myocardial cellular membrane potentials and the height of the epicardial S-T segment has been demonstrated. Sayen and his collaborators found a poor correlation between S-T segment elevation and intramyocardial Po2 immediately after coronary occlusion, but reported that 5 min after occlusion the sites at which S-T segment elevation occurred correlated well with those at which Po2 were reduced.

Scheuer and Brachfeld reported a close correlation between S-T segment elevation and the development of anaerobic metabolism when coronary blood flow was reduced.

Results obtained in this investigation demonstrate a close correlation between the extent and magnitude of early elevation of S-T
segments in epicardial recordings after acute coronary artery occlusion and the later development of cellular damage, as evidenced by depression of myocardial CPK activity in specimens from the same sites. In contrast to many enzymes, CPK activity is found predominantly in skeletal and cardiac muscle cells. Accordingly, the activity of this enzyme in infarcted heart muscle is less likely to be influenced by other cellular components participating in the inflammatory reaction after coronary artery occlusion. This is supported by recent evidence showing that decrease of myocardial CPK activity correlates closely with the amount of cell death in rabbits subjected to coronary artery occlusion. All of these considerations, taken together, suggest strongly that epicardial S-T segment elevation is the result of ischemic cellular injury and that its persistence presages the ultimate development of myocardial necrosis.

There is substantial evidence, based on histological, electron microscopic, and histochemical studies, that irreversible injury does not occur within 20 min of coronary occlusion, and the total disappearance of epicardial S-T segment elevation after release of the occlusion in the present studies is consistent with these findings. Therefore, it was possible to compare the effects of several different interventions on the severity and extent of ischemic injury after repetitive occlusion of the same coronary artery.

A number of stimuli not necessarily associated with myocardial ischemic injury, such as changes in the ionic milieu of the myocardium and stimulation of specific areas within the central nervous system and of the stellate ganglia have been shown to produce alterations of the S-T segment. However, the S-T segment elevations produced in this study by inotropic drugs and hypotension were not of the nonischemic type, since they were not observed in portions of the left ventricle remote from that perfused by the occluded artery.

The present investigation indicates that factors that influence the balance between myocardial oxygen supply and demand can substantially alter the extent of myocardial ischemic injury when a coronary vessel is occluded. Myocardial oxygen consumption was increased by augmentation of cardiac contractility by the use of several stimuli, including the administration of isoproterenol, glucagon, bretlyum, and ouabain, and the induction of tachycardia. All of these influences increased the area of ischemic injury and the magnitude of S-T segment elevation. In contrast, an intervention that lowered myocardial oxygen consumption (i.e., the administration of propranolol) decreased the extent and magnitude of S-T segment elevation after coronary occlusion. Since these various interventions alter myocardial contractility by a variety of mechanisms, it is very likely that it is the change in oxygen consumption per se, rather than the specific interventions, that were responsible for the corresponding changes in the magnitude and extent of ischemic injury.

Isoproterenol in doses higher than those used in this study has been shown to produce myocardial necrosis directly, and the possibility must be considered that this agent might have produced electrocardiographic changes even in the absence of coronary occlusion. However, with the concentrations of isoproterenol employed, epicardial sites remote from those supplied by the occluded coronary artery did not show changes in the epicardial S-T segment, indicating that isoproterenol did not produce ischemic injury in normal tissue. Also, the studies of Raab al., in which catecholamine stimulation in the presence of a constant coronary flow produced epicardial S-T segment elevations, are consistent with our findings. Moreover, the dose of isoproterenol used in the present study did not influence CPK activity in nonischemic myocardium, although doses in this range do lead to increased lactate production in experimental infarction produced by microspheres.

Findings in the present study demonstrate further that the augmentation of S-T segment elevation following isoproterenol infusion after coronary artery ligation is associated with a more profound depression of myocardial

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CPK activity. In specimens from myocardial sites that had normal S-T segments after simple ligation but that exhibited S-T segment elevations after isoproterenol administration, the CPK activity was only two-thirds of that found in corresponding marginal sites in animals with coronary artery occlusion alone (fig. 9). Thus, it is apparent that under these conditions, infusion of isoproterenol not only alters the epicardial electrocardiogram but also results in an increment of cell death, evidenced by myocardial CPK depression.

It might be argued that myocardial CPK depression after isoproterenol infusion may reflect increased washout of the enzyme. However, this is unlikely since virtually no further depletion of CPK takes place after 24 hr. Although CPK depletion may be flow dependent soon after coronary occlusion, the linear relationship obtained between relative regional perfusion and CPK depletion 24 hr after coronary artery ligation suggests that washout is not a major factor influencing myocardial CPK levels after 24 hr.

Digitalis glycosides ordinarily depress the S-T segment, and indeed in this study, such changes were generally induced by ouabain in areas of the myocardium supplied by unoccluded vessels. In spite of this effect of the glycoside, it produced a significant increase in the area of ischemic injury and in the magnitude of the S-T segment elevation after coronary occlusion. Since glycosides normally tend to depress the S-T segment, the intensification of ischemic injury produced by the ouabain may actually have been underestimated by the technique employed in this study.

In contrast to the influence of positive inotropic agents, increase of arterial pressure, which also augments myocardial oxygen consumption, was found to decrease the extent of ischemic injury while arterial hypotension, which reduces the heart's oxygen needs, had the opposite effect. From these observations it appears that, in the presence of coronary occlusion, the changes of coronary perfusion pressure exert a greater and opposing influence on the extent of myocardial ischemia than do the changes in myocardial oxygen consumption resulting from the arterial pressure change. This finding is not surprising since, in studies on the effects of alterations in arterial pressure on the relation between myocardial oxygen consumption and coronary blood flow, it was shown that elevation of arterial pressure increases coronary flow proportionately more than myocardial oxygen consumption. The latter observation, as well as the dependence of the collateral flow upon coronary perfusion pressure, could explain the decrease in ischemic injury when arterial pressure was elevated in the presence of coronary occlusion. Conversely, coronary blood flow is known to fall abruptly when perfusion pressures decline below 60-70 mm Hg, and the fact that this decline is proportionately greater than the accompanying reduction in myocardial oxygen consumption could explain the observed extension of the area of ischemic injury associated with hypotension. Moreover, Corday et al. showed that elevation of arterial pressure in hemorrhagic hypotension in dogs augmented both anterograde and collateral coronary flow. These observations led to the treatment of cardiogenic shock in man with vasoconstrictor drugs.

Some comment concerning the extrapolation of the results of these studies to acute coronary occlusion in man are warranted. Obviously, the fact that in these experiments one coronary vessel is occluded while the remaining vessels are entirely normal differs from the clinical situation in which coronary vascular involvement is usually more extensive. While in the normal dog the number and size of coronary collateral vessels greatly exceed those occurring in normal man, the relatively abundant collateral vessels in the dog do resemble those occurring in patients with coronary artery disease, and in this respect the experimental observation on the effects of various stimuli on the extent of myocardial ischemic injury may have considerable clinical relevance.

The present studies suggest that tachycardia and/or hypotension occurring in a patient

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with acute coronary occlusion might extend the size of an ischemic zone and thereby further impair left ventricular function and result in a vicious cycle. These observations also point to the potentially deleterious effects of administration of positive inotropic agents such as isoproterenol, digitalis glycosides, or glucagon to patients with acute myocardial infarction. However, it is important to stress that these agents augment myocardial oxygen demands in the nonfailing heart but do not necessarily exert such an effect when administered to a patient with an enlarged, failing heart. Bretylium tosylate has recently been shown to be an effective antiarrhythmic agent, and its use in the treatment of patients with acute myocardial infarction has been suggested, since it also possesses positive inotropic properties. It should be pointed out, however, that under the conditions of these experiments this drug also increased the magnitude of ischemic injury in the presence of coronary occlusion.

Of greatest interest, from the clinical point of view, is the finding that the severity and extent of myocardial ischemic injury resulting from coronary occlusion could be radically altered not only by pretreatment of the animal but also by an appropriate intervention as late as 3 hr after the coronary occlusion. This suggests that measures designed for reduction of myocardial oxygen demands and improvement of coronary perfusion, when effected promptly after a patient has been brought to a hospital, might potentially reduce the ultimate size of the infarction.

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Factors Influencing Infarct Size Following Experimental Coronary Artery Occlusions

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