Effect of Glucose-Insulin-Potassium Infusion on Myocardial Infarction following Experimental Coronary Artery Occlusion

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SUMMARY
The effects of glucose-insulin-potassium (GIK) infusion and glucose (G) infusion started 30 min after experimental coronary occlusion and the combination of GIK and propranolol (P) started 3 hours after coronary occlusion on the development of myocardial infarction were studied in 37 dogs. Fifteen minutes after the coronary occlusion, epicardial electrocardiograms were recorded at 10–15 sites; 24 hours later transmural specimens were obtained from the same sites for determination of myocardial creatine phosphokinase (CPK) activity and the evaluation of morphologic changes. In the control group (normal saline infusion) the relationship between S-T-segment elevation (mv) 15 min after occlusion and CPK activity (IU/mg of protein) 24 hours later was: \[ \log \text{CPK} = -0.064 \times \text{S-T} + 1.24; \ r = 0.81. \] In the GIK group, the infusion was begun 15 min following epicardial mapping, and sites with the same S-T-segment elevations showed less CPK depression than did the control group: \[ \log \text{CPK} = -0.022 \times \text{S-T} + 1.25. \] The G group also showed less CPK depletion than the control group but to a somewhat lesser extent than the GIK group (log CPK = -0.030 S-T + 1.20). The group receiving GIK and P 3 hours after occlusion also showed less CPK depression than did the control group (log CPK = -0.034 S-T + 1.26). Histologic analysis in 24-hour specimens showed that sites which exhibited S-T-segment elevation 15 min after occlusion showed normal histology in 3% of specimens obtained from control dogs, while the other 97% showed early signs of myocardial infarction. However, in the GIK group, 36% of the specimens with S-T-segment elevation prior to the infusion were histologically normal 24 hours later, while in the G group 30% were normal, and in the GIK and P group 17% were normal. Electron microscopy confirmed the morphologic changes observed by light microscopy. Thus, in the presence of experimental coronary occlusion, GIK exerts a protective effect against myocardial ischemia and reduces the extent of myocardial necrosis. G alone acts similarly but to a lesser degree, while a beneficial effect can also be demonstrated when GIK and P are started 3 hours after the onset of coronary occlusion.

Additional Indexing Words:
Myocardial creatine phosphokinase
Anaerobic metabolism
S-T-segment elevation
Reversible myocardial injury
Histologic signs of myocardial necrosis
Ultrastructural changes in myocardial necrosis
Beta-adrenergic blockade

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TWO SERIOUS consequences result from occlusion of the coronary arteries and the resultant myocardial infarction: (1) the development of primary arrhythmias; and (2) the failure of the cardiac pump mechanism, manifesting itself in the acute stage either as acute pulmonary edema or cardiogenic shock or later as chronic congestive heart failure. Successful treatment and prophylaxis of the first group of complications is possible and is dependent on their early recognition, but management of pump failure has been far more difficult. Patients who develop one of the forms of this common complication generally have large areas of myocardium which have become infarcted as a consequence of the development of coronary occlusion. Therefore, measures which reduce the extent of infarction following occlusion are likely to prove beneficial in diminishing the incidence and severity of pump failure. In earlier studies performed in our laboratory it was shown that the extent of damaged myocardium following coronary occlusion, as assessed by epicardially recorded currents of injury and by reduction of myocardial creatine phosphokinase (CPK) concentration, can be modified by changes in the balance between the availability and requirements for oxygen by the myocardium.1–3

Normally, the heart derives essentially all of its energy from the oxidation of various substrates within the Krebs cycle. However, the myocardium possesses the capacity to derive significant amounts of energy from anaerobic glycolysis in the absence of oxygen.4,5 In the present investigation we wished to determine whether anaerobic glycolysis could provide sufficient energy to limit the extent of myocardial necrosis following coronary occlusion. It was reasoned that if the size of a myocardial infarct is dependent on the balance between the availability of and requirement for the various compounds involved in energy production, then the anatomie and functional integrity of cardiac muscle might be preserved by increasing anaerobic glycolysis. The specific goal of this investigation was to examine the effects of the infusion of glucose-insulin-potassium (GIK), glucose alone (G), and the combination of infusion of glucose-insulin-potassium and propranolol (GIK + P) on the extent of myocardial necrosis following acute coronary occlusion.

Methods

Studies were carried out in 37 mongrel dogs weighing between 17 and 27 kg, anesthetized with sodium thiamylal (25 mg/kg) with respiration maintained with a Harvard respirator. The heart was exposed through a left thoracotomy and suspended in a pericardial cradle. A branch of the left anterior descending coronary artery (LAD), generally the apical branch, or the LAD itself was dissected from the adjacent tissues and ligated with a Mersilene tie. Epicardial unipolar electrocardiograms were obtained from 10 to 15 sites on the anterior surface of the left ventricle as described in detail previously.1 The epicardial sites chosen were anatomically easily recognizable and were located in the central area supplied by the occluded artery, in zones of the left ventricle remote from the occluded artery which presumably were adequately perfused, and in intermediate zones. The epicardial electrocardiograms were recorded prior to and 5, 10, 15, and 20 min following coronary occlusion. The chest was then closed in layers and the pleural cavity drained with an underwater self-retaining catheter. The dogs were allowed to awaken, but were sedated with additional small doses of sodium thiamylal throughout the following 24 hours; during this period their systemic arterial pressure (Statham pressure transducer, model 23Db) and electrocardiogram (lead aV) were continuously monitored. Blood samples for potassium, glucose, and hematocrit determinations were obtained before the start of the infusions and 6, 12, and 24 hours thereafter. Serum potassium was monitored during the experiment so that the potassium content of the infusate could be readjusted to prevent hyperkalemia. The animals were reanesthetized 24 hours after occlusion, placed on artificial respiration, their chests reopened, and the heart excised. Transmural specimens for the analysis of myocardial creatine phosphokinase (CPK) concentration and histologic examination were obtained from the sites at which epicardial electrocardiograms had been previously recorded. Biopsies for electron microscopy specimens were obtained from the beating heart in situ, using a pressurized biopsy drill, and were immediately fixed in glutaraldehyde.

The animals were divided into 4 groups. An intravenous infusion of 40 ml/kg/24 hours (0.028 ml/kg/min) was administered to all dogs. In groups 1, 2, and 3, it commenced 30 min

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following the occlusion. In group 4, it was initiated 3 hours following occlusion.

**Group 1—Control:** In the 11 dogs in this group the infusate was normal saline.

**Group 2—GIK:** In the 14 dogs in this group every liter of infusate contained 500 g glucose, 210 mEq KCl, and 102 units of regular insulin. It provided $14 \times 10^{-3}$ g glucose, $5.8 \times 10^{-3}$ mEq KCl, and $2.9 \times 10^{-3}$ units of insulin/kg/min. If the potassium blood levels rose above 5 mEq/liter at any time during the experiment the concentration of potassium in the infusion fluid was reduced to one third of the value indicated above, i.e., to 1.9 mEq KCl/liter, and if plasma potassium exceeded 7 mEq/liter a potassium-free infusion was substituted while insulin and glucose concentration and the rate of infusion remained constant.

**Group 3—G:** In six dogs the infusate was 500 g glucose/liter.

**Group 4—GIK + P:** In six dogs the infusate was a solution of GIK of the same composition and administered at the same rate as in group 2, but it was not begun until 3 hours after the occlusion; in addition, at the same time, 1 mg/kg of propranolol (P) was administered. Additional doses of propranolol (0.25 mg/kg) were subsequently administered by bolus intravenous injections, 9, 15, and 21 hours after occlusion.

**Chemical Procedures**

Myocardial CPK analysis was carried out as previously described¹ by spectrophotometric assay of CPK activity⁷ and was expressed as international units (IU = $\mu$moles of substrate converted per minute per milligram of supernatant fraction protein) per milligram of protein. Reaction rates were linear for at least 15 min after an equilibrium period of 5 min; activity was proportional to the quantity of supernatant fraction protein added to the assay system; the enzymatic activity was acid and thermostable; and the results of duplicate determinations of enzyme activity agreed within 3%.

The possible influence of insulin on the CPK assay system and/or myocardial CPK activity was assessed in duplicate experiments utilizing tissue slices incubated for 6 hours, dog heart CPK purified according to Noda,⁸ and rabbit skeletal muscle CPK standard. CPK activity in control slices and those exposed to insulin ($1 \times 10^{-3}$ units/ml) were indistinguishable. Dog heart and rabbit skeletal muscle CPK activity assayed in the presence of insulin ($1 \times 10^{-3}$ units/ml final concentration) did not differ from corresponding controls.

Plasma potassium concentrations were measured in duplicate by flame photometry⁹ and plasma glucose by Hoffman’s method.¹⁰

**Histologic Procedures**

Transmural sections were obtained from 267 sites from 23 animals and were fixed in 10% neutral formalin, sectioned, and stained with hematoxylin and eosin, periodic acid Schiff (PAS), and oil red 0. Other sections fixed in absolute alcohol were stained in Best’s carmine stain. All sections were coded so that their origins were unknown at the time the microscopic examination was carried out by an independent observer. The sections were classified either as normal or as abnormal, i.e., demonstrating histologic changes compatible with myocardial necrosis. Criteria for the latter included loss of cross striations in myocardial fibers, a deeper than normal eosinophilic appearance of the fibers, pyknotic nuclei, karyorrhexis, karyolysis, fragmentation of myocardial fibers, or a polymorphonuclear cell infiltration. For a section to be classified as abnormal at least two of the aforementioned criteria had to be present in at least 20% of the fields. The sections were also analyzed for glycogen depletion and the appearance of fat droplets within myocardial fibers.¹¹⁻¹³ Such changes had to be present within 20% or more of the myocardial fibers in order for the section to be classified as abnormal.

**Electron Microscopy**

Transmural specimens were obtained from the beating heart in situ 24 hours after coronary occlusion with a biopsy drill and were placed immediately into precooled 5% gluteraldehyde buffered with 0.1 M cacodylate buffer. The epicardial and endocardial fourths of each specimen were removed. The sides of the cylinder-shaped specimens were removed to minimize torsion artifact. The central portion was cut into small pieces, 1 × 2 mm, and fixed for 3 hours at 4°C. The tissue was washed in 0.1 M cacodylate buffer (pH 7.4) for an hour, postfixed in 1% buffered osmium tetroxide for 1 hr, washed in lactated Ringer’s solution, dehydrated in graded ethanol and propylene oxide, and embedded in Araldite Epoxy Resin 502. Thick sections (1μ) were cut on a Sorvall MT2B Porter-Blum ultramicrotome and stained with toluidine blue for orientation. Thin sections were stained with uranyl acetate and lead citrate and examined with a Zeiss 9A electron microscope. Five grids from each of five blocks were studied so that a total of 25 grids were examined from each site. Eighteen biopsy sites from two control animals and 18 biopsy sites from two treated animals were studied. As was the case for the histologic examination, the biopsy sites were examined by an observer who did not have prior knowledge of their origin.

The criteria for grading the specimens were based upon the ultrastructural alteration which
occur with ischemia.\textsuperscript{14, 15} The following classification was employed: normal—no disturbance in the usual myocardial ultrastructure; minimal changes—depletion of glycogen granules, mild mitochondrial swelling, slight separation of sarcomeres, and/or slight margination of nuclear chromatin; severe changes—depletion of glycogen granules, marked swelling of mitochondria with disorganization and dilatation of the cristae, development of flocculant mitochondrial granules, disruption of mitochondrial membranes, margination of nuclear chromatin, karyorrhexis, and hyperextension of sarcomeres.

Data Analysis

Epicardial S-T-segment elevation in each site was measured in millivolts (1 mv = 1 mm S-T-segment elevation). Sites with S-T-segment elevations exceeding 2 mv were considered abnormal, showing myocardial ischemic injury;\textsuperscript{1} in no instance were elevations observed prior to coronary ligations. The data obtained from group 1 (occlusion alone) were used to establish the relationship between the level of the S-T segment 15 min following occlusion and CPK activities 24 hours later in the same sites,\textsuperscript{1} and this relationship was compared to that obtained in groups 2, 3, and 4, in which interventions were carried out. A significantly higher concentration of CPK 24 hours after occlusion, for any level of S-T-segment elevation than observed in the control group, was taken to reflect a protective effect of the intervention.

The histologic findings were analyzed by determining the relation between S-T-segment elevation 15 min after occlusion and the presence or absence of light-microscopic evidence of myocardial infarction. Again, a comparison of the relation in groups 2, 3, and 4 was made with respect to that in the control group. A similar approach was used to analyze the data in respect to normal or abnormal glycogen content, fat deposition, and electron microscopic appearance. The results were tested for significance by the chi-square test for goodness of fit.

Since the interventions employed are well known to affect the S-T segment in a nonspecific manner, even in the absence of myocardial ischemia, it is emphasized that the reference electrocardiographic measurement, i.e., the S-T segment 15 min following occlusion, was always made preceding the infusion of GIK or G; this measurement was used as a predictor of cellular damage 24 hours later.\textsuperscript{1}

Results

Group 1 (Control)

In the 11 dogs in which coronary occlusion was performed with no intervention, other than the infusion of normal saline, arterial pressure had declined significantly below preocclusion values 12 hours later. Similarly, ventricular rate had increased significantly 6 hours postocclusion and remained elevated thereafter (table 1). Six hours after occlusion all the dogs exhibited frequent tachyarrhythmias, as commonly observed in dogs after coronary ligation.\textsuperscript{16} These were not treated with electrical countershock or antiarrhythmic drugs. Serum K and plasma glucose showed little variation while the hematocrit rose significantly from a preocclusion value of 43% to 50% 6 hours later, and it then remained stable for the remainder of the 24-hour observation period.

In sites with normal S-T segments (<2 mv), the CPK values averaged 18.5 ± 0.5 (SEM) IU/mg protein, levels similar to those in the normal heart as previously reported.\textsuperscript{1} In sites with abnormal S-T-segment elevations, variable degrees of CPK depression were noted depending upon the degree of elevation (figs. 1 and 2). The relationship between S-T-segment elevation 15 min following occlusion and myocardial CPK values from the same sites 24 hours later was log CPK = −0.064 S-T + 1.24 (r = −0.81; 10 dogs; 72 biopsies; fig. 3).

Histologic studies (H&E, glycogen, and fat stains) were carried out in five dogs (58 biopsies). Ninety-five percent (18 of 19) of the sites with normal S-T segments exhibited normal histology, while 97% (38 of 39) of sites with abnormal S-T-segment elevations showed features compatible with early myocardial infarction (figs. 1 and 4; table 2). There was good agreement between the H&E sections and glycogen and fat stains. Thus, in 97% (56 of 58) of the sites examined, all three methods agreed on whether the tissue was normal or abnormal.

Electronmicroscopic study of 18 biopsies in two dogs revealed that in all five of the sites in which there had been no S-T-segment elevation the ultrastructure was normal (fig. 5, left upper), whereas in all 13 sites with abnormal S-T segments there were ultrastructural alterations consistent with ischemia. In one of these
Effects of Various Interventions on Hemodynamics, Hematocrit, Potassium, and Glucose at Various Times following Coronary Occlusion

<table>
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<td>15 min</td>
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<tr>
<td>1</td>
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<td>116±8</td>
</tr>
<tr>
<td>2</td>
<td>116±10</td>
<td>110±8</td>
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<tr>
<td>3</td>
<td>115±12</td>
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<tr>
<td>4</td>
<td>124±10</td>
<td>125±11</td>
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</table>

*P < 0.05.
†P < 0.01 in comparison to same group at 0 time.
§P < 0.05.
§P < 0.01 in comparison to group 1 at identical time.

OCCLUSION + GIK SOLUTION

EXP 298

AREA OF ST SEGMENT ELEVATION

SITE OF BIOPSY

<table>
<thead>
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<th>SITE</th>
<th>ST (mv)</th>
<th>CPK I.U./mg prot</th>
<th>HISTOLOGY</th>
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<tr>
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<td>3</td>
<td>15.6</td>
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<tr>
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<td>F</td>
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<td>NORMAL</td>
</tr>
<tr>
<td>H</td>
<td>4</td>
<td>16.2</td>
<td>NORMAL</td>
</tr>
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Figure 1

Relationship between S-T-segment elevation 15 min after occlusion with CPK activity and histologic changes 24 hours later in an experiment in group 1 (control). (Left) Schematic representation of the anterior surface of the heart. L.A. = left atrial appendage; L.A.D. = left anterior descending coronary artery. The shaded area represents the area of S-T-segment elevation after occlusion. The circles represent sites from which specimens were obtained. (Right) Comparison between S-T-segment elevation with CPK activities and histologic analysis 24 hours later in the same sites.

sites the changes were minimal, while in the other 12 they were severe (fig. 5, right upper).

Group 2 (GIK)

In the 14 dogs that received GIK infusion for 24 hours commencing 30 min after occlusion, the heart rate and mean arterial pressure were not statistically different from those in the control group at any time following the occlusion (table 1); also there was no reduction in the incidence of arrhythmias. Glucose and potassium levels were significantly higher than in the control group.
while the changes in hematocrit were similar to those observed in the control group (table 1).

The infusion of GIK solution by itself did not change the CPK activity in dogs without coronary occlusion. In 24 biopsies taken from the hearts of three dogs without coronary occlusion in which GIK was infused at a rate identical to that used in group 2, the CPK values were 19.0 ± 0.5 IU/mg protein. Moreover, in animals in group 2, at sites remote from the coronary occlusion, which had normal S-T segments following occlusion, the CPK activity (18.9 ± 0.5; N = 35) was not significantly different from that observed in similar sites with normal S-T segments in dogs from group 1 (18.5 ± 0.5; N = 36) (fig. 2). In sites within the distribution of the occluded vessel, which showed S-T-segment elevation

<table>
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<th>Hematocrit (%)</th>
<th>Potassium (mEq/liter)</th>
<th>Glucose (mg %)</th>
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<td>0 6 hr 12 hr 24 hr</td>
<td>0 6 hr 12 hr 24 hr</td>
</tr>
<tr>
<td>13 ± 3 50 ± 2* 49 ± 2* 50 ± 2*</td>
<td>2.9 ± 0.2 3.1 ± 0.2 3.2 ± 0.1</td>
<td>3.1 ± 0.3 80 ± 4 83 ± 5 81 ± 8</td>
</tr>
<tr>
<td>12 ± 2 48 ± 2 55 ± 2 55 ± 3†</td>
<td>2.6 ± 0.1 4.1 ± 0.2† 4.6 ± 0.3†§</td>
<td>5.0 ± 0.6†§ 78 ± 5 153 ± 15†§ 148 ± 15†§ 196 ± 39†§</td>
</tr>
<tr>
<td>35 ± 2† 48 ± 3† 45 ± 3† 46 ± 2†</td>
<td>3.2 ± 0.2 3.3 ± 0.3 3.6 ± 0.3</td>
<td>3.1 ± 0.2 90 ± 4 183 ± 14†§ 217 ± 31†§ 260 ± 50†§</td>
</tr>
<tr>
<td>44 ± 2 47 ± 3 50 ± 3 52 ± 4*</td>
<td>4.0 ± 0.5†§ 5.0 ± 0.7†§</td>
<td>5.2 ± 0.7†§ 90 ± 8 170 ± 16†§ 201 ± 44†§ 233 ± 40†‡</td>
</tr>
</tbody>
</table>

**Figure 3**

Relationship between S-T-segment elevation 15 min after occlusion and log CPK values from specimens obtained in the same sites 24 hours later. (A) Group 1 (occlusion alone) log CPK = −0.064 ST + 1.24; r = −.081; 10 dogs = 72 biopsies. (B) Group 2 (GIK infusion 30 min after occlusion) log CPK = −0.022 ST + 1.25; r = −.61; 13 dogs = 96 biopsies. (C) Group 3 (glucose 50%) log CPK = −0.030 ST + 1.20; r = −.61; 6 dogs = 46 biopsies. (D) Group 4 (GIK and propanolol 3 hours following occlusion) log CPK = −0.034 ST + 1.26; r = −.72; 6 dogs = 48 biopsies (E) Propanolol alone, log CPK = −0.035 ST + 1.30; r = −.53. This line is given for comparison of the magnitude of effects. Data were obtained in previous investigation using identical technic.

*P < 0.05; **P < 0.01.

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Figure 4

Representative photomicrographs from hematoxylin and eosin sections (upper row, reduced from X 100) and oil red 0 fat-stained sections (lower row, reduced from X 160). Inserts in the panels on the upper row are epicardial ECG traces obtained 15 min after occlusion from the same site from which the section shown in the photomicrograph was obtained. Left-hand panels are H & E and oil red 0 stained sections obtained from a site without S-T-segment elevation. The myocardial fibers are intact with oval nuclei and contain numerous cross striations. No fat granules are present within the individual fibers. The middle panels are photomicrographs obtained from a site with S-T-segment elevation from group 1 (occlusion alone). There is extensive fragmentation of the myocardial fibers and loss of the cross striations. The myocardial nuclei are pyknotic and exhibit karyolysis, while an extensive polymorphonuclear cell infiltrate lies within the interstitial spaces. Oil red 0 fat stains demonstrate the presence of numerous, massive fat granules within the cytoplasm of most of the myocardial fibers undergoing ischemic necrosis. Right-hand panels are photomicrographs obtained from a site with S-T-segment elevation of the same magnitude as that in the middle column in a dog from group 2 (GIK infusion). The myocardial fibers are intact with normal cross striations present. There is no evidence of fragmentation of the fibers or any cellular infiltrate within the interstitial...
following occlusion, GIK infusion resulted in a higher CPK activity (i.e., less CPK depression) than that expected on the basis of observations in the control group (fig. 2). At every level of S-T-segment elevation the differences in CPK activity in groups 1 and 2 were significant. Several sites with S-T-segment elevations 15 min following occlusion actually exhibited CPK values within the normal range 24 hours later, while all sites showed less CPK depression than expected from the extent of S-T-segment elevation (fig. 6). The effects of GIK were reflected in the lower slope of the regression line between CPK and S-T (log CPK = -0.022 S-T + 1.25; \( r = -0.61 \); six dogs; 96 biopsies; fig. 3); this slope differed significantly from that in the control group (\( P < 0.001 \)).

Histologic examination showed that 32 of 35 (92%) sites with no S-T-segment elevation were normal, a finding similar to that obtained in group 1. However, only 38 of 59 (64%) sites with abnormal S-T-segment elevations (i.e., exceeding 2 mv) exhibited histologic evidence compatible with myocardial necrosis, as compared to the significantly higher percentage (97%; \( P < 0.005 \)) in group 1 (figs. 4 and 6). Thus, 36% of the sites which were expected to show histologic signs of necrosis did not, demonstrating that the development of necrosis was prevented by GIK infusion (table 2).

There was close agreement (87 of 94 specimens; 93%) between histologic evaluation and estimation of glycogen depletion. Thus, among the animals in group 2, 31 of 35 sites without S-T-segment elevation showed preservation of glycogen, while only 40 of 59 sites with S-T elevations greater than 2 mv showed glycogen depletion; this compares with the significantly (\( P < 0.005 \)) higher fraction, i.e., 56 of 58 sites in group 1 which exhibited S-T elevation and subsequent glycogen depletion.

Of the 18 sites in two dogs studied by means of electron microscopy, six had normal S-T segments and all of these revealed normal ultrastructure (fig. 5, bottom left). Twelve sites had abnormal S-T segments, ranging from 3 to 10 mv. In four of these the ultrastructure was completely normal; in five sites the ultrastructural alterations were considered minimal, while only three showed changes of severe ischemia. Thus, the frequency of ultrastructural alterations of severe ischemia were significantly less in group 2 (25%) than those seen in group 1 (97% \( P < 0.005 \)) at comparable levels of S-T-segment elevation (fig. 5, bottom right).

**Group 3 (G)**

In six animals in which 50% glucose without insulin or potassium, was administered for 24

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**Table 2**

*Effects of Various Interventions on Histologic Findings*

<table>
<thead>
<tr>
<th>Group</th>
<th>Normal S-T (0–2 mv)*</th>
<th>Abnormal S-T (≥3 mv)*</th>
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<tr>
<td>4</td>
<td>16 (4)</td>
<td>94</td>
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*S-T segment 15 min after coronary occlusion, i.e., prior to intervention.
†Histology 24 hours after coronary occlusion.
‡Significantly greater than in group 1 (\( P < 0.005 \)).

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spaces. Glycogen stains of this section demonstrated the presence of normal amounts of glycogen within the myocardial fibers. Oil red 0 stain resembles the section observed in the control animal (lower left-hand panel) although an occasional small fat granule is present within a myocardial fiber.
Electron microscopic observations. (Top left) Biopsy from a site with no S-T-segment elevation from group 1 (control). Normal myocardium reveals slightly relaxed sarcomeres. The mitochondria (M) are intact. Abundant glycogen granules (G) are seen in the perimitochondrial regions and within the sarcomeres (reduced from × 28,200). Insert shows epicardial ECG from this site. (Top right) Biopsy from a site with S-T-segment elevation (7 μv) from group 1. Severe ischemic changes are present. The sarcomeres are hyperextended and separated. Mitochondria (M) show marked swelling and contain flocculent precipitate (P). Many dilated tubules (T) represent ruptured mitochondria (reduced from × 28,500). Insert shows epicardial ECG from this site. (Bottom left) Biopsy from a site with no S-T-segment elevation from group 2 (occlusion + GIK infusion). Normal myocardium shows slight relaxation of sarcomeres. Mitochondria (M) are intact. Glycogen granules (G) are abundant (reduced from × 25,300). Insert shows epicardial ECG from this site. (Bottom right) Biopsy from a site with S-T-segment elevation (10 μv) from group 2. The ultrastructure is well preserved as compared to B. The sarcomeres are slightly relaxed. The mitochondria (M) are intact. Glycogen granules (G) are

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OCCLUSION ALONE

EXP. 305

<table>
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<th>SITE</th>
<th>ST (mv)</th>
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<td>H</td>
<td>5</td>
<td>4.5</td>
<td>ABNORMAL</td>
</tr>
</tbody>
</table>

Figure 6

Example of an experiment in group 2 (GIK infusion). (Left) Schematic representation of the heart. The shaded area represents the area of S-T-segment elevation 15 min after occlusion; circles represent the sites from which specimens were obtained. (Right) Comparison between S-T-segment elevation 15 min following coronary occlusion and CPK activity and histologic changes 24 hours later (GIK infusion was started 15 min after the ECG recordings). Note that sites B, D, G, and H exhibited S-T-segment elevations but only slightly reduced CPK activity and normal histology.

hours commencing 30 min after occlusion, the changes in heart rate, rhythm, arterial pressure, and hematocrit were similar to those observed in groups 1 and 2 (table 1). Plasma potassium did not vary and was similar to that in group 1, while blood glucose was significantly higher than that observed in group 2 (table 1).

The depletion of CPK activity in 46 samples was less than in group 1, but greater than in group 2: log CPK = -0.030 S-T + 1.20 (r = -0.61; six dogs; 46 biopsies; fig. 3); this slope differed significantly from that in the control group (P < 0.001), but not from that in groups 2 or 4. Sixty-nine samples were taken for histologic and histochemical analysis. As was the case in groups 1 and 2, 21 of 22 (95%) of sites without S-T elevations were normal, while 14 of 47, i.e. 30%, of sites in which S-T-segment elevation had been present did not exhibit signs of early infarction by microscopic examination (table 2). Similarly, in 21 of 22 (95%) of sites without S-T-segment elevation, normal glycogen granules and no fat deposits were seen. However, 13 of 47, i.e. 28%, of sites in which S-T-segment elevation had been present did not exhibit lack of glycogen granules or fat deposition.

Group 4 (GIK and P Commencing 3 Hours following Occlusion)

In six dogs in which this intervention was carried out, mean arterial pressure and hematocrit showed no significant differences

present but slightly reduced as compared to C (reduced from × 18,000). Insert shows epicardial ECG from this site.

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from those observed in group 1 (table 1). Heart rate was lower but the incidence of arrhythmias was the same. Blood glucose levels were slightly but not significantly higher than in group 2 (table 1). In sites with abnormal S-T-segment elevation there was less CPK depression after this treatment than expected from the results in group 1 (occlusion alone); the regression line relating CPK activity to S-T-segment elevation was less steep: log CPK = -0.034 S-T + 1.26; r = -0.53; six dogs; 48 biopsies; fig. 3); this slope differed significantly from that in the control group (P < 0.001) but not from those in groups 2 and 3.

Histologic studies showed that 15 of 16 (94%) of sites in which S-T-segment elevation had not been present did not exhibit signs of infarction, while five of 30, i.e., 17%, of sites in which S-T-segment elevation has been present did not exhibit signs of necrosis, glycogen depletion, or abnormal fat accumulation (table 2).

Discussion

The possibility of modifying the extent of tissue damage following coronary occlusion has recently come under serious consideration. It has been demonstrated that the electrocardiographic evidence of myocardial ischemic injury, as reflected in S-T-segment elevations 15 min following coronary occlusion, is useful in predicting the extent of tissue damage, as reflected in local myocardial CPK activity 24 hours later. Subsequently, it was shown that following coronary occlusion the extent of myocardial damage is influenced substantially by the oxygen demands of the myocardium. Interventions such as tachycardia, isoproterenol, glucagon, bretylium, and digitalis, which increase myocardial oxygen needs in the nonfailing heart, augmented the extent of myocardial injury, while propranolol and practolol in the nonfailing heart, and digitalis in the failing heart, interventions which decrease myocardial oxygen consumption, reduced the extent of ischemic injury and/or infarct size.1, 3, 17, 18 On the other hand, methoxamine, phenylephrine, and norepinephrine, which tend to increase the supply of oxygen to the tissue bordering the central ischemic zone by improving coronary perfusion, reduce the severity of ischemic damage, while hemorrhagic hypotension, which lowers coronary perfusion, results in extension of ischemic injury.1, 3 Moreover, intraaortic balloon counterpulsation, an intervention which both reduces myocardial oxygen needs and increases coronary flow, showed a substantial decrease in the extent of myocardial injury.2 In essence then, it appears that, as might be predicted, the size of the injured zone can be influenced in a decisive manner by alterations in the balance between oxygen availability and oxygen requirements.19

The present investigation amplifies the scope of these studies in two significant directions. First, the value of epicardial S-T-segment elevations 15 min following occlusion in predicting tissue damage 24 hours later is extended to include histologic and electron microscopic verification of tissue damage; and, secondly, the concept of the dependence of infarct size on myocardial energy balance is extended to encompass substances that are capable of augmenting energy production by stimulating anaerobic glycolysis. Thus, in this study, it was shown in control animals that epicardial S-T-segment elevation exceeding 2 mv not only correlates well with depletion of CPK activity 24 hours later,1 as had been demonstrated earlier, but also that it presages myocardial necrosis as determined histologically. This correlation is of special relevance since the diagnosis of myocardial infarction has classically been based on histologic rather than on biochemical findings.11–13

The present findings are also in accord with previous observations indicating that total depletion of CPK in rabbit myocardium is proportional to the size of experimentally produced myocardial infarcts defined by gross inspection.20 Furthermore, electron microscopic studies in control dogs showed that areas which exhibited S-T-segment elevations 15 min following occlusion showed a variety of changes 24 hours later. These included a reduction of glycogen granules, swelling and rupture of mitochondria, intramitochondrial

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granules, myofibrillar stretching, contracture bands, rupture of sarcolemma, and margination of nuclear chromatin, all of which are characteristic of ischemic necrosis. On the other hand, areas of epicardium which showed no S-T-segment elevations had a normal ultrastructure 24 hours later. In conclusion, in the absence of interventions, epicardial S-T-segment elevations 15 min following occlusion accurately predicted the morphologic and biochemical integrity of the tissue, as determined 24 hours later. These findings are in accord with earlier observations that electrocardiographic changes characteristic of ischemic injury correlate well with an increase in anaerobic myocardial metabolism.21, 22

The rationale for trying to enhance energy production in the ischemic myocardium with glucose-insulin-potassium infusion was based on several considerations. It has been shown by Morgan et al.23 that under aerobic conditions, in the presence of physiologic concentration of glucose, the heart derives only a negligible fraction of its energy from anaerobic glycolysis. However, this fraction increases progressively as: (1) the heart is rendered anoxic, (2) the perfusion medium is enriched with glucose, and (3) insulin is added. Weissler et al.24 have demonstrated that both the electrical and mechanical function of an anoxic isolated Langendorf heart preparation was improved and recovery made more rapid when glucose was included in the perfusate. Moreover, there is evidence that the glycogen content of the myocardial cell parallels its capability to withstand anoxia25-28 and that administration of glucose and insulin increases the cellular concentration of glycogen as well as muscular function in both normal and anoxic hearts.5, 23, 29-32

It is well recognized that under normal conditions the heart derives a substantial fraction of its energy from the oxidation of fatty acids. This process cannot continue in the absence of oxygen, and during ischemia the heart extracts less free fatty acids than glucose, and glucose in fact becomes the principal source of energy.33 The membrane barrier to glucose entry into the cell may be overcome by high extracellular concentration of glucose and insulin, and glucose metabolism is then limited only by the activity of the phosphorylating enzymes, especially phosphofructokinase and hexokinase.23, 34-36 Also, it has been demonstrated in the dog heart that oxidative phosphorylation as well as cardiac function are enhanced by glucose-insulin-potassium infusion. All of these studies24, 26, 29, 30, 37 have shown that when the oxygen available to the myocardial cell is reduced the stimulation of anaerobic metabolism is capable of improving the energy balance of the heart. Other beneficial effects have also been ascribed to glucose or the combination of glucose, insulin, and potassium. These included increases in contractility due to the hypomolar action of glucose, reduction in circulating free fatty acids, which may exert an arrhythmogenic effect, restoration of intracellular potassium, and stabilization of the membrane potential.41, 42

In the present investigation we have shown that glucose-insulin-potassium infusion strikingly reduced the degree of myocardial damage resulting from coronary occlusion in the dog; this conclusion is based on a comparison of the relation between electrocardiographic evidence of myocardial ischemic injury 15 min following coronary occlusion with biochemical, histologic, histochemical, and ultrastructural evidence of damage at the same sites 24 hours later in control dogs and glucose-insulin-potassium-treated groups. It was found that this treatment reduced the expected lowering of myocardial CPK activity; many sites, which on the basis of observations in control dogs were expected to show striking reductions of CPK activity, actually exhibited normal CPK activity. Similar findings were observed by histologic analysis. Areas of myocardium which exhibited S-T-segment elevations 15 min following occlusion and which on the basis of observations in control animals were expected to exhibit such signs of necrosis as lack of cross striations, pyknosis, karyorrhexis, fat granules, glycogen depletion, and inflammatory cell...
infiltration did not always exhibit these changes; actually, they were absent in 36% of the specimens obtained from the glucose-insulin-potassium-treated dogs in which they were expected. Since glycogen depletion is the first sign of myocardial infarction observed by light microscopy, and since preservation of glycogen stores could be responsible for the preservation of normal histology following GIK treatment, sections from the same specimens were stained for glycogen, and again 34% of the specimens which were expected to show glycogen depletion exhibited normal glycogen stores. Similarly, the appearance of fat granules in myocardial fibers, which is a later sign of cellular injury, was less evident in the treated animals. In almost every instance, there was excellent correspondence between preservation of glycogen stores and histologic appearance. Moreover, electron microscopic studies also showed a protective effect on the ultrastructure of the cell, conserving the normal morphology of mitochondria, myofibrils, membranes, and nuclei; the preservation of glycogen, histochemically demonstrated by light microscopy, was confirmed by electron microscopy.

Glucose alone also limited myocardial damage following coronary occlusion, but, perhaps to a somewhat lesser extent than the GIK combination. In the absence of added insulin, but in the presence of added glucose, membrane transport may be the step which limits glucose utilization and may therefore limit the enhancement of energy production by means of anaerobic glycolysis. The action of insulin in stimulating the activity of the more active form of glycogen synthetase may also explain the tendency to a somewhat lesser protective effect on glycogen stores when glucose alone is infused as compared to GIK. The importance of a possible protective action of potassium in the GIK-treated animals cannot be excluded by these data. However, it is unlikely since we did not observe any reduction in cardiac arrhythmias in the GIK-treated dogs. Although they do not definitely exclude it, our results do not support the theory that the effect of glucose administration is simply through its osmolar action because GIK did not exert less of a protective effect than glucose alone, despite the lower blood glucose concentration in group 2 than in group 3 (table 2). Rather, the results support the hypothesis that glucose maintains cellular integrity by increasing energy production through enhanced glycolysis.

For the GIK solution to exert any effect it must reach the oxygen-deprived cells. The areas of myocardium totally spared were, in general, in the periphery of the zones exhibiting ischemic injury. Presumably the cells in this zone were perfused sufficiently by neighboring unoccluded vessels to allow them to be exposed to GIK, yet their \( \text{pO}_2 \) was sufficiently reduced that their viability was threatened, in the absence of enhanced glycolysis.

The results obtained in the dogs in group 4 which received GIK and propranolol commencing as late as 3 hours after occlusion demonstrated that even at this time the extent of myocardial damage may still be favorably influenced by this combination. This finding is in accordance with previous observations of reduced electrocardiographic evidence of ischemic injury in experimental animals and in patients several hours after coronary occlusion, by decreasing myocardial oxygen consumption or by increasing coronary perfusion pressure.

The combination of propranolol with GIK was employed because of our earlier finding that propranolol decreased the extent of ischemic injury and subsequent CPK reduction, probably by decreasing myocardial oxygen requirements. The beneficial metabolic effect of beta-adrenergic blockade can also be appreciated by the observed shift from anaerobic to aerobic metabolism. The effects of propranolol may be related also to redistribution of coronary flow. The relative contributions made by the beta-adrenergic blockade and by GIK in diminishing myocardial necrosis 3 hours after occlusion in the present study were not examined.
The time interval following coronary occlusion during which myocardial viability may be maintained has not been defined adequately, since there has been no method available previously to predict reliably the size of an infarct following coronary occlusion. It has been well documented that some cells may show irreversible changes commencing 18 min following occlusion.\textsuperscript{51} Oclusions maintained for more than 45 min in the dog generally result in infarcts.\textsuperscript{52-54} However, the contention that at 2 or 3 hours following occlusion almost all cells supplied by the occluded vessel are irreversibly damaged and that at this time the size of an infarct is fully and irrevocably established\textsuperscript{55} is not supported by our data which show a definite reduction in the degree of ischemic injury by histologic and biochemical analysis when the combination of GIK and propranolol was begun as late as 3 hours following coronary occlusion.

The extrapolation of the results of the present study to clinical application must be made with caution, because of the many differences between coronary occlusion induced experimentally in the healthy dog and that occurring in patients with diffuse coronary disease. Nevertheless, this investigation certainly suggests the possible usefulness of GIK treatment, perhaps combined with beta-adrenergic blockade, in patients without concomitant heart failure, in an attempt to reduce the extent of myocardial damage following coronary occlusion. Although a number of clinical studies have reported that GIK treatment may result in a reduction in mortality and arrhythmias following acute myocardial infarction,\textsuperscript{44, 56-59} these findings have not been confirmed.\textsuperscript{60, 61} These discrepancies may be due to several factors.\textsuperscript{59, 62} First, evaluation of effectiveness has been based primarily on mortality, an insensitive parameter for evaluation of the potential efficacy of any agent in reducing the extent of myocardial damage, especially when the cause of death is not associated with heart failure or cardiogenic shock. Second, differences between the results of various investigators\textsuperscript{56, 57} and between participating centers in a collaborative study\textsuperscript{61} suggest that they might be related to differences in the concentrations of insulin and glucose employed; they may also be related to the varying time intervals between the onset of the ischemic process and the onset of therapy. It is quite possible that patients whose treatment started within an hour after the onset of symptoms might be affected differently from those whose treatment is delayed for 48 hours.

In conclusion, GIK administration in the normal dog prevents some myocardial cells from undergoing ischemic necrosis following occlusion. Moreover, when it is employed in combination with propranolol, the extent of myocardial damage may be reduced even when the intervention is delayed for as long as 3 hours following coronary occlusion. These results suggest that further trial of this treatment in patients with acute myocardial infarction is warranted, perhaps using higher doses of glucose-insulin-potassium than those employed clinically heretofore; that treatment be begun just as soon after the onset of ischemia as possible; and that the results be evaluated not only by an examination of the effect on mortality but also with the use of methods capable of estimating infarct size.

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