Limitation of Experimental Infarct Size by an Angiotensin-converting Enzyme Inhibitor

GEORG ERTL, M.D., ROBERT A. KLONER, M.D., PH.D., R. WAYNE ALEXANDER, M.D., PH.D., AND EUGENE BRAUNWALD, M.D.

SUMMARY The effects of angiotensin-converting enzyme inhibitor (CEI) SQI4225 on infarct size and regional myocardial blood flow were studied in 21 anesthetized dogs subjected to 6 hours of coronary occlusion. An area of myocardium at risk of necrosis was determined in vivo after 15 minutes of coronary occlusion but before CEI treatment (AR1) using an autoradiographic technique and after treatment with 6 hours of coronary occlusion (AR2) using a fluorescent dye technique. An in vitro area at risk (AR3), which measures coronary bed size, was determined by injecting Monastral dye postmortem. Infarct size was determined by planimetry of unstained myocardium after incubating heart slices in triphenyltetrazolium chloride. Regional myocardial blood flow (RMBF) was measured by injecting tracer microspheres simultaneously with measurements of AR1 and AR2. In 11 saline-treated control dogs (group A), infarct size averaged 93 ± 8% of AR1, 96 ± 2% of AR2, and 75 ± 6% of AR3, respectively. In 10 dogs treated with CEI (0.25 mg/kg/hour) between 30 minutes and 6 hours after coronary occlusion (group B), infarct size was smaller and averaged 68 ± 5% of AR1 (p < 0.01), 79 ± 5% of AR2 (p < 0.005), and 57 ± 7% of AR3 (p < 0.05). RMBF in the ischemic zone remained constant in group A but increased by 62 ± 26% in group B (p < 0.025). In group B, mean arterial pressure decreased from 115 ± 6 to 103 ± 7 mm Hg (p < 0.025) between 30 minutes and 6 hours after coronary occlusion and left atrial pressure decreased from 7.0 ± 1.8 to 5.7 ± 0.8 mm Hg (p < 0.0025). These measurements did not change in group A. Thus, CEI is potent in reducing infarct size in the dog after coronary occlusion. It may act by increasing collateral flow to the ischemic zone and reducing afterload.

MYOCARDIAL ISCHEMIA and, ultimately, infarction result from an imbalance between oxygen supply and oxygen demand. Coronary occlusion may induce hypotension, which results in baroreceptor activation and then systemic reflex vasoconstriction.1,2 Systemic vasoconstriction may worsen the imbalance between myocardial oxygen supply and demand. Renal nerve activity,3 a determinant of renin release,4 may be increased as well. Moreover, hypotension may activate intrarenal vascular pressoreceptors and may thus increase renin release directly.5 Because angiotensin II is a potent systemic and coronary vasoconstrictor, activation of the renin-angiotensin system may increase myocardial oxygen demand and decrease myocardial oxygen supply. Because interference with angiotensin II production might exert a beneficial effect in myocardial ischemia and myocardial infarction, we studied the effects of the angiotensin-converting enzyme inhibitor (CEI) captopril (SQI4225) on infarct size after experimental coronary occlusion. To elucidate the mechanism of the salutary action of the drug, we also determined the effect of CEI on regional myocardial blood flow (RMBF) and measured plasma renin activity (PRA).

Methods
Twenty-one mongrel dogs of either sex weighing 16–28 kg that had been maintained on normal laboratory chow and had free access to water were anesthetized with i.v. thiamylal sodium (10 mg/kg), intubated and ventilated with room air using a Harvard respirator pump. Anesthesia was maintained by additional thiamylal sodium when needed. Lead aVf of the ECG was monitored. Saline was infused at a rate of 2 ml/min throughout the experiment to minimize hypovolemia and renin release. The chest was opened in the fifth left intercostal space and the heart was suspended in a pericardial cradle. The left anterior descending coronary artery was freed up approximately 2 cm from its origin just distal to the first major diagonal branch. Polyethylene catheters were placed in the femoral artery and vein and in the left atrial appendage. Arterial and left atrial pressures were measured by Statham P23Db pressure transducers.

Infarct size after coronary occlusion depends on both the quantity of myocardium perfused by the occluded coronary vessel and the quantity of collateral flow to the jeopardized tissue.6 7 The variations in infarct size after coronary occlusion can be limited when these factors are taken into account by determining the area of myocardium at risk of developing necrosis and expressing infarct size as a percentage of the area at risk. In the present study, three areas at risk were determined in each dog. Shortly after coronary occlusion, an area at risk was determined in vivo by determining the poorly perfused portion of the left ventricle. Because treatment might change this area, a second estimate was made after therapy. In addition, the size of the occluded coronary bed was determined by injecting Monastral dyes simultaneously and under physiologic pressure down the occluded and unoccluded coronary beds postmortem. This in vitro tech-
nique differs from the in vivo methods in that perfusion of myocardium by collaterals in the distribution of the occluded vessel is avoided. Therefore, it defines a larger area at risk than the areas determined in vivo.7, 8

**Area at Risk Before Treatment**

The area of myocardium at risk before treatment (AR) was determined by an autoradiographic technique described previously.7, 8 Human albumin microspheres 10–35 μ in diameter (3M Company) were labeled with technetium-99m (half-life 6 hours) immediately before use. Approximately 600,000 microspheres with a total radioactivity of 0.5 mCi/kg body weight were ultrasonicated for 10 minutes and agitated manually for another 10 minutes. After coronary occlusion, but before treatment, the microspheres were injected slowly into the left atrial line. Human albumin microspheres have no significant hemodynamic effect7 and should not be confused with the commercially available plastic microspheres usually used for measuring RMBF. Human albumin microspheres are biodegradable, are labeled with much higher levels of radioactivity and are larger in diameter (10–35 μ) than plastic microspheres (7–10 μ). Because the biologic half-life of human albumin microspheres exceeds 12 hours, they remained stable within the myocardium during the experiment.7

Autoradiographs were made as follows. After the experiment, the heart was sliced as described below, and the heart slices were exposed to high-speed x-ray film for 18 hours at 4°C and the film was then developed. Areas of low blood flow at the time of albumin microsphere injection (AR) appeared as “cold spots” on the film. Tracings of each heart slice were superimposed on the radiograph and the boundary of the ischemic area was drawn at the edge of the zone of decreased radiographic density.

**Area at Risk After Treatment (AR)**

The area of myocardium at risk of developing necrosis after 6 hours of coronary occlusion (AR) was also determined, because the intervention might have changed the perfusion of the ischemic zone and therefore the area at risk. As described previously,10 thioflavin S (0.5 ml/kg; 4% in normal saline solution) (Pfaltz and Bauer), a fluorescent dye that stains endothelial cells, was injected into the left atrium. Hearts were arrested by saturated KCl solution and excised 60 seconds after the injection of thioflavin S.10 Myocardium perfused by the thioflavin S fluoresces brightly and uniformly yellow-green under ultraviolet light; the absence of fluorescence indicates low or no flow.10

**Myocardium in the Distribution of the Occluded Vessel**

The area that does not take into account collateral flow into the ischemic zone was determined in vitro by simultaneously perfusing the occluded and nonoccluded beds under equal pressure. A, 8, 11 The proximal left coronary artery was carefully dissected to identify the left main, left anterior descending and large septal coronary arteries. Plastic cannulas were sutured into the left main coronary artery and the left anterior descending coronary artery just distal to the site of the previous occlusion. The location of the catheter tip in the left main coronary artery was always proximal to the takeoff of the first septal artery. Perfusion pressures were measured 5 cm proximal to the cannulas and were set at the level of the in vivo mean arterial pressure of the dog before coronary occlusion by the height of the infusion bottles. The left anterior descending coronary artery was perfused with 6% dextran-70 in a 5% dextrose solution (Macrodex); the left main coronary artery was perfused simultaneously with 0.5% Monastral blue (Dupont) in Macrodex. One hundred fifty to 250 ml were infused for 4–7 minutes into each coronary bed. The occluded coronary bed remained unstained, while the non-occluded bed stained dark blue.

**Infarct Size**

Myocardial necrosis was determined by a macroscopic enzyme technique.12 Heart slices were incubated in a 1% solution of 2,3,5-triphenyltetrazolium chloride (TTC) (Sigma Chemicals) in phosphate buffer for 15 minutes at 37°C. Incubation in TTC stains myocardium containing dehydrogenase enzymes (i.e., uninfarcted myocardium) brick red; infarcted myocardium remains unstained. The validity of this method in differentiating infarcted from uninfarcted myocardium has been established.13

**Regional Myocardial Blood Flow**

RMBF was measured by the radioactive microsphere technique.13 Plastic microspheres 7–10 μ in diameter (New England Nuclear) labeled with 90Co, 125Sn or 44Sc, were suspended in 50% sucrose with Tween-80 added. Volumes containing approximately 2 × 106 spheres were sonicated for 15 minutes, manually agitated and injected over 30 seconds into the left atrium. A reference sample was drawn from the femoral artery over a 90-second period at 15.3 ml/min. After excision of the heart, samples of tissue were cut from 3 slices of the apical half of the heart (which always included the infarct), excluding the apex itself (fig. 1). Samples from the normal zone (zone 1) (perfused by Monastral blue dye), from the center of infarct zone 4 (unstained by Monastral blue, unperfused by human albumin microspheres and thioflavin S and unstained by TTC), from the border of infarct zone 3 (containing tissue stained and unstained by TTC), and from ischemic but nonnecrotic tissue, zone 2 (unstained by Monastral blue but perfused by microspheres and thioflavin S and stained by TTC) were pooled from each of three slices to totals of about 1.5 g. RMBF was calculated as: Cm × R/Cm, where RMBF = flow in ml/min/g, R = reference blood flow in ml/min, Cm = counts per 1000s and g of heart, and Cm = reference counts per 1000s.10

**Plasma Renin Activity**

Blood samples for determination of PRA were drawn from the femoral vein catheter into iced
trol dogs (group A), a 0.9% solution of saline was infused at a rate of 30 ml/hour. In group B, 30 minutes after coronary occlusion, 0.25 mg/kg of CEI (Squibb) in 10 ml of saline solution (0.9%) was injected as an i.v. bolus and followed by an infusion of CEI, 0.25 mg/kg/hour, in 30 ml of saline solution. Six hours after coronary occlusion, immediately before the dog was sacrificed, thioflavin S was injected into the left atrium for subsequent determination of AR₂. To permit successful postmortem injections of dye into the coronary arteries, the dogs were anticoagulated with i.v. heparin (1000 U/kg). The hearts were arrested by injection of saturated KCl solution, quickly removed and washed in normal saline.

After determination of AR₃ as described above, the left ventricle was dissected free from the atria, great vessels, right ventricle and fat, cut into 5-mm slices parallel to the atrioventricular groove using a commercial meat slicer, and each slice was weighed. A clear acetate sheet was placed over the slices and the myocardium supplied by the occluded vessel (AR₃) was traced using 10 × magnification. AR₃ was similarly drawn by placing the slices under ultraviolet light. After incubation in TTC, the infarcted myocardium (TTC unstained) was also traced on clear acetate sheets under magnification and the heart slices were exposed to x-ray film for 18 hours. AR₃ was similarly drawn on acetate sheets. All areas were planimetered, the slices were corrected for weight, added, and the volumes of the three areas at risk and of the infarcts were expressed as percentage of the left ventricle. The actual tracing of heart slices and planimetry was performed by two of the investigators and one technician who did not know the origin of the heart slices. Finally, tissue samples were cut for determination of RMBF.

Statistical Methods
The significance of differences between the areas at risk and infarct size was calculated by paired t test. The paired t test was also used to assess the differences between postcoronary occlusion pretreatment values and values at 6 hours of coronary occlusion for RMBF, hemodynamic data, PRA, and hematocrit. Differences between groups were evaluated using an unpaired t test. Data are presented as mean ± SEM.

Results
Hemodynamic Data
After coronary occlusion, arterial pressures dropped, while left atrial pressures increased to a similar extent in both groups (figs. 2 and 3). The hemodynamic variables remained stable 15–30 minutes after coronary occlusion. In control dogs (group A), changes in arterial and left atrial pressures between 30 minutes and 6 hours of coronary occlusion were not significant (fig. 2), but heart rate increased by 27 ± 11 beats/min (p < 0.025).

In CEI-treated dogs (group B), precooclusion and postocclusion pretreatment arterial, left atrial pressures and heart rate were similar to values in group A (figs. 2 and 3). The diastolic and mean ar-
vascular pressures declined in group B ($p < 0.05$) between 30 minutes and 6 hours of coronary occlusion. After administration of CEI, left atrial pressure decreased ($p < 0.005$) and at 6 hours of coronary occlusion was $5.7 \pm 0.7$ mm Hg, a level similar to that before coronary occlusion. The change in left atrial pressure 30 minutes to 6 hours after occlusion ($-3.3 \pm 0.9$ mm Hg) differed significantly ($p < 0.0025$) from that in group A during the same period ($1.0 \pm 0.9$ mm Hg). Although there was a slight decrease in heart rate immediately after administration of CEI (fig. 3), the heart rate then increased. The differences in heart rate between groups A and B were not significant. Thus, hemodynamically, the major difference between control and CEI-treated dogs was a decrease in left atrial pressure after CEI therapy.

**Plasma Renin Activity and Hematocrit**

PRA was stable during the preocclusion period and did not differ between the two groups (table 1). PRA increased from an average of 2.0 to 2.9 ng/ml/hour 10 minutes after coronary occlusion ($p < 0.025$). PRA increased significantly in both groups between 10 minutes and 6 hours of coronary occlusion. PRA increased to $52 \pm 11$ ng/ml/hour in group B and to $5.1 \pm 1.2$ ng/ml/hour in group A. Thus, at 6 hours of coronary occlusion, PRA was higher in CEI-treated than in control dogs ($p < 0.005$). The hematocrit was $46 \pm 3\%$ at the beginning of the experiment and $44 \pm 4\%$ after 6 hours of coronary occlusion and did not differ in the two groups.

**Areas at Risk and Infarct Size**

Figure 4 shows the tracings of a typical heart slice from a control and a CEI-treated dog. The areas at risk determined by in vivo perfusion before (AR$_1$) and after (AR$_2$) treatment were similar in group A, but both were considerably smaller than the occluded coronary bed (AR$_3$). In group A, infarct size as a fraction of the area at risk was not significantly different

---

**FIGURE 2.** Hemodynamic data for control (saline-treated) dogs (group A). Asterisk indicates $p < 0.05$ compared with pretreatment value.

**FIGURE 3.** Hemodynamic data for dogs treated with converting enzyme inhibitor (CEI) (group B). Asterisk indicates $p < 0.05$ compared with pretreatment value.
for AR₁ and AR₃; 93 ± 8% of AR₁ and 96 ± 2% of AR₂ became necrotic. However, infarct size was considerably smaller than AR₃ (p < 0.01); only 75 ± 6% of AR₃ became necrotic (table 2).

In group B, both the volume of tissue unperfused by Monastral blue (AR₃) and the area at risk determined by in vivo perfusion before treatment (AR₁) were similar to the values in group A. AR₁ constituted 75 ± 6% of AR₃ (table 3). After CEI treatment, however, the in vivo area at risk (AR₂) declined to 82 ± 5% of AR₁ (p < 0.025) and to 67 ± 89% of AR₃ (table 3). Thus, because AR₂ was less than AR₁, CEI decreased the area of myocardium at risk of developing necrosis. Moreover, infarct size was reduced even further and averaged 68 ± 5% of AR₁, 79 ± 5% of AR₂ and 57 ± 7% of AR₃. Thus, compared with control dogs, CEI reduced both the area of myocardium at risk of developing necrosis and, within that area, the amount of necrosis that actually developed.

Regional Myocardial Blood Flow

In control dogs, RMBF did not change significantly in any zone or any layer between 15 minutes and 6 hours of coronary occlusion (fig. 5). There was a gradient of flow from lateral to central zones of ischemia and in the tissue at the border of the necrotic zone (zone 3), from epicardium to endocardium. Although flow was subnormal in both epicardium and endocardium but not critically restricted in zone 2 where no necrosis appeared, it was critically depressed both in the epicardium (0.41 ± 0.05 ml/min/g) and in the endocardium (0.25 ± 0.06 ml/min/g) in zone 3 before treatment. Fifteen minutes after coronary occlusion, RMBF was similar in groups B and A in all four zones (figs. 5 and 6). However, in contrast to the findings in group A, blood flow to both the epicardium and endocardium increased in CEI-treated dogs between 15 minutes and 6 hours of coronary occlusion in the normal zone (zone 1) by 61 ± 17% (p < 0.005) and 56 ± 19% (p < 0.01), respectively. RMBF increased by 44 ± 19% in the epicardium (p < 0.005) and by 48 ± 30% in the endocardium (p < 0.005), respectively, in zone 2, and by 61 ± 21% (p < 0.025) and 62 ± 26% (p < 0.025), in zone 3, at the edge of the infarct. Flow changes in zone 4 were not statistically significant. The ratios of endocardial to epicardial flow were not influenced by CEI in any zone.

Discussion

The major finding of the present study was that CEI reduced infarct size after 6 hours of coronary occlusion. Reimer and Jennings* reported that after 6 hours of coronary occlusion in untreated dogs, about 95% of the tissue destined to become necrotic with a permanent coronary occlusion does so. To determine the area of myocardium at risk for developing necrosis, we used both an in vitro technique for determination of the myocardium in the distribution of the occluded vessel, i.e., the occluded coronary bed size, and two in vivo techniques for determination of areas at risk, and two in vivo techniques for determination of areas at risk, one before and one after treatment. Infarct size was significantly reduced using all three techniques.

The second major finding was that CEI reduced the area at risk as measured by thioflavin S 6 hours after coronary occlusion, compared with 15 minutes after coronary occlusion measured by human albumin microspheres, so that AR₃ constituted 82% of AR₁. This was associated with a marked increase in RMBF, not only to the normally perfused zone, but to the edge of the infarct as well (zone 3). Because blood flow to the center of the necrosis did not increase, one might ask if the increase in flow to zone 3 represented a real

**Table 1. Plasma Renin Activity Before and After Coronary Occlusion**

<table>
<thead>
<tr>
<th></th>
<th>Before occlusion</th>
<th>After occlusion</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20 min</td>
<td>1 min</td>
</tr>
<tr>
<td>Control group A (n = 7)</td>
<td>2.1 ± 0.7</td>
<td>2.1 ± 0.7</td>
</tr>
<tr>
<td>CEI group B (n = 5)</td>
<td>2.3 ± 2.0</td>
<td>2.0 ± 0.3</td>
</tr>
<tr>
<td>Groups A and B (n = 12)</td>
<td>2.0 ± 0.4</td>
<td>2.9 ± 0.7*</td>
</tr>
</tbody>
</table>

Values are given in ng/ml/hour.

*Higher than at 1 minute before occlusion (p < 0.025).
†Higher than plasma renin activity in group A at 6 hours (p < 0.005).

Abbreviation: CEI = converting enzyme inhibitor.

![Figure 4](https://via.placeholder.com/150)

**Figure 4. Typical heart slices from a control dog and a dog treated with converting enzyme inhibitor (CEI). In the control dog AR₃ > AR₁ = AR₂ = infarct size; in the CEI-treated dog AR₃ > AR₁ > AR₂ > infarct size. Hatched areas indicate areas at risk; cross-hatched areas indicate infarct size. CEI = converting enzyme inhibitor.**
increase in collateral flow or an increase in flow to normal tissue that contaminated the ischemic samples. We believe the latter possibility is unlikely because this tissue was obtained from areas unperfused by Monastral blue in vitro (i.e., from AR₂), which is an excellent criterion for identifying the occluded coronary bed. Moreover, samples from zone 3 contained critically low flow and necrotic tissue.

**Mechanism by Which CEI Reduced Infarct Size**

One mechanism by which CEI reduced infarct size may be its improvement in RMBF. However, CEI must have exerted another mechanism of action, for the infarction was even smaller than AR₂. CEI may have reduced myocardial oxygen consumption by reducing preload (with a significant decrease in left atrial pressure). There was a trend toward afterload reduction after the initial infusion of CEI, which may also have had a beneficial effect on infarct size. Although the use of certain vasodilators has sometimes been considered unwarranted in the setting of acute myocardial infarction because of reflex tachycardia or a coronary steal phenomenon, these findings were not observed with CEI. In fact, Ader et al. reported a decrease in heart rate after CEI.

**Renin-Angiotensin System in Coronary Occlusion**

PRA increased after coronary occlusion, which suggests that the renin-angiotensin system is activated by coronary occlusion. Changes in PRA were observed despite high basal levels, which might be attributed to barbiturate anesthesia, and despite the fact that reflexes that might have triggered the renin release were already activated by anesthesia itself. The observed increase in PRA after infusion of CEI suggests that CEI did indeed block the generation of angiotensin II. What causes activation of the renin-angiotensin system after coronary occlusion is not clear. Activation of the sympathetic nervous system and elevation of plasma catecholamine concentrations occur after coronary occlusion, and both can result in release of renin. Local vascular renin might also be activated, particularly in the coronary vasculature, where catecholamines are released from the ischemic myocardium. In addition, reduction in renal perfusion pressure activates renal vascular stretch receptors, which may autonomously control the release of renin. Thus, interference with the vasconstrictive action of angiotensin II by blockade of its formation might be responsible for CEI vasodilator action. CEI may also act by inhibiting kininase II and the hydrolysis of bradykinin, and it may thereby activate prostaglandin production. Because bradykinin is released from ischemic myocardium, inhibition of its breakdown by CEI may be particularly effective after coronary occlusion. However, from the present study, it is not possible to determine whether limitation of myocardial infarct size results from changes in angiotensin II, bradykinin or both.

**Area-at-risk Technique**

Two types of area-at-risk techniques were used in this study to assess infarct size changes in relationship to the native coronary bed size (AR₂) and also in relationship to a zone of low flow that occurs during coronary artery occlusion (AR₅). The in vivo techniques of injecting albumin microspheres or dyes into the left atrium during coronary occlusion allow one to delineate an area of low flow under physiologic conditions, in which perfusion pressure in the occluded coronary artery is low. In previous studies with this technique we found that under control conditions, more than 90% of the in vivo area at risk becomes necrotic; thus, these zones are truly at risk for developing necrosis.

The cutoff for radioactivity on the autoradiograms and cut off for thioflavin S fluorescence were well de-

### Table 2. Effect of Converting Enzyme Inhibitor on Infarct Size

<table>
<thead>
<tr>
<th></th>
<th>Left ventricular mass (g)</th>
<th>Occluded coronary bed (AR₂)</th>
<th>Pretreatment area at risk (AR₁) (% of left ventricle)</th>
<th>Posttreatment area at risk (AR₂)</th>
<th>Infarct size Infarct size (of AR₂) Infarct size (of AR₅)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group A (n = 11)</td>
<td>114 ± 6</td>
<td>36 ± 4</td>
<td>28 ± 2*</td>
<td>28 ± 3*</td>
<td>26 ± 3*</td>
</tr>
<tr>
<td>CEI group B (n = 10)</td>
<td>117 ± 4</td>
<td>38 ± 2</td>
<td>29 ± 3*</td>
<td>25 ± 3*</td>
<td>19 ± 3†</td>
</tr>
</tbody>
</table>

*Smaller than AR₂ (p < 0.01).
†Different from control (p < 0.05).

### Table 3. Effect of Converting Enzyme Inhibitor on the Area of Myocardium at Risk of Necrosis

<table>
<thead>
<tr>
<th></th>
<th>Pretreatment area at risk (AR₁) (% of the occluded coronary bed—AR₂)</th>
<th>6 hrs treatment area at risk (AR₁) (% of AR₂)</th>
<th>6 hrs treatment area at risk (AR₅) (% of AR₅)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control group A (n = 11)</td>
<td>81 ± 3</td>
<td>78 ± 6</td>
<td>97 ± 7</td>
</tr>
<tr>
<td>CEI group B (n = 10)</td>
<td>75 ± 6</td>
<td>67 ± 8*</td>
<td>82 ± 5†</td>
</tr>
</tbody>
</table>

*Smaller than AR₁ (p < 0.025).
†Smaller than control (p < 0.05).

Abbreviation: CEI = converting enzyme inhibitor.
in vitro techniques in which dyes or colored latex are injected postmortem into an occluded and an unoccluded coronary bed simultaneously under physiologic pressures.\(^9\)\(^{,\ }\)\(^{11}\) should define a different type of area at risk. The in vitro techniques show the native coronary beds when coronary perfusion pressures in the occluded and unoccluded bed are both normal and equal. Therefore, the effect of collateral flow from one bed to the other is negated. Hence, the area at risk defined by in vitro techniques in which the effect of collateral flow is negated will be larger than that determined by in vivo techniques in which the effect of collateral flow is accounted for. As a result, a smaller percentage of the in vitro area at risk than in vivo area at risk becomes necrotic.\(^8\)

**The Triphenyltetrazolium Chloride Technique**

TTC is a macroscopic enzyme stain that stains viable dehydrogenase-containing cells brick red but does not stain infarcted cells.\(^8\) Lie et al.\(^8\) showed that the infarct could be visualized 4 hours after coronary ligation. TTC has been used to determine infarct size in several studies in which coronary occlusion was maintained for 6 hours.\(^7\)\(^{,\ }\)\(^{30}\)\(^{,\ }\)\(^{31}\)\(^{,\ }\)\(^{36}\) Recently, Fishbein showed that 6-hour infarcts visualized by TTC correlated with histologic findings of giant whole-mount histologic sections.\(^36\) We recently examined ultrastructural aspects of tissue stained and unstained by TTC from dogs that underwent 6 hours of coronary artery occlusion. Tissue that was unstained by TTC always showed ultrastructural evidence of irreversible...
Injury with intramitochondrial amorphous dense bodies and sarcolemmal membrane disruption; tissue just outside of the infarct that was stained with TTC either appeared entirely normal or had mild loss of glycogen, as would be expected during the 10-minute incubation in TTC. A recent ultrastructural study showed similar results when TTC was used to examine collateral flow and reduce the extent of infarction. The way in which this border was assessed or the intervention used for salvage. For example, if one looks for a border zone of collateral flow using in vitro perfusion techniques only, it is unlikely that one will be found, as the effect of collateral flow is negated by equal perfusion pressures in both coronary beds. The mode of salvaging tissue may also be important. For example, we found no significant lateral zone of salvage when infarct size was reduced in conscious sodium depleted dogs. Effects on systemic and coronary hemodynamics. J Clin Invest 62: 874, 1978.


24. Kloner RA, Reimer KA, Jennings RB: The "wavefront phenomenon" of myocardial ischemic bed size (myocardium at risk) and collateral flow. Lab Invest 40: 81, 1976.


Limitation of experimental infarct size by an angiotensin-converting enzyme inhibitor.
G Ertl, R A Klomer, R W Alexander and E Braunwald

Circulation. 1982;65:40-48
doi: 10.1161/01.CIR.65.1.40

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1982 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/65/1/40.citation

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org/subscriptions/