Transmural Cellular Damage and Blood Flow Distribution in Early Ischemia in Pig Hearts

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SUMMARY. Transmural histological changes were determined morphometrically in the left ventricular free wall of 20 pigs after ligation of distal left anterior descending coronary artery for 10, 20, 40, and 120 minutes. Hemodynamics were recorded and regional blood flow distribution was measured in the ischemic zone. Coronary occlusion produced regional transmural ischemia without producing significant systemic hemodynamic change. The microsphere blood flow technique revealed that blood flow was less than 0.05 ml/min per g in all layers of the ischemic zone, i.e., inner, middle, and outer thirds. Ischemic cellular damage was classified and quantified from grade 0 to grade 5 (0 being normal and 5 being the most severe damage) with light microscopy and confirmed by electron microscopy. Layers of 200 µm immediately beneath the endocardium and epicardium showed minimal ischemic damage of less than grade 1.4 regardless of duration of ischemia in all hearts. In the ischemic left ventricular wall, except for the above layers, a definite transmural gradient of cellular damage existed from the inner third (grade 2.3 ± 0.1) to the outer third (grade 1.3 ± 0.2) at 20 minutes of ischemia and at 40 minutes of ischemia (grades 3.6 ± 0.1 and 1.9 ± 0.3, respectively). The transmural ischemic damage gradient disappeared at 120 minutes of ischemia, where the inner and outer third ischemic grades were both 5.0 ± 0.1. The data suggest that the limited ischemic damage which occurs in the few cell layers beneath endocardium and epicardium may be explained by regional collateral blood flow. An early ischemic damage wavefront phenomenon does exist in the pig myocardium and is independent of myocardial blood flow and its distribution. The transmural cell damage gradient may be the result of transmural gradients of wall stress and intramyocardial pressure in vivo. Therefore, it appears that factors other than blood flow are the major determinants of ischemic cellular damage in the left ventricular wall of hearts lacking a collateral blood supply. (Circ Res 51: 683-693, 1982)
minute occlusion. They were sedated with ketamine (2 mg/kg), anesthetized with sodium thiamylal (10 mg/kg), intravenously, and maintained throughout the experimental procedures on Nembutal (25 mg/kg). Antiarrhythmic agents were not given during the experiment. Three animals developed ventricular fibrillation and were accordingly excluded from the analysis. Therefore, this study is based on the 20 pigs that lived.

Tygon catheters (Formulation S-54-HL, 0.040" × 0.070" U.S. Stoneware) were inserted in the femoral vein and artery with their tips located at the level of the diaphragm. Through a median sternotomy, the heart was exposed and suspended in a pericardial cradle. A Tygon catheter was inserted into the left atrium. A solid state pressure transducer catheter (Millar Instruments, model P-1350) was inserted in the left ventricle through an apical stab wound and secured by purse string suture. The apical stab wound and purse string suture were located either outside or at the margin of the ischemic area. In all cases, the tissue sections for histology and blood flow studies were taken at least 1 cm from this site. Simultaneous and continuous recording of arterial (Statham Model P23Db) and left ventricular pressures and lead 2 of the ECG was also made on a rectilinear oscillograph (Brush Gould, model 2800). Thirty minutes after completion of instrumentation and acquisition of baseline hemodynamic variables, the distal one-third of the left anterior descending coronary artery was completely ligated with a Vesseloops® (Med General) rubber band. The chest then was closed to prevent epicardial surface dehydration and hypothermia during ischemia.

Regional Myocardial Blood Flow

Blood flow distribution was determined quantitatively by radionuclide-labeled microsphere technique (Heymann et al., 1977) and qualitatively by injection of blue violet dye (Randolph et al., 1964; Warltier et al., 1981). Two million 15 μm microspheres, labeled with either 141Ce or 103Ru, were injected into the left atrium during 20 seconds while a simultaneous timed blood collection of 15 ml was made into the arterial catheter over a 3-minute period beginning 15 seconds before the isotope injection. The first isotope label was injected 5 minutes before coronary artery ligation. The second microsphere injection was made 5 minutes before the end of the occlusion period. Hearts were excised immediately after injection of patent blue violet dye into the left atrium.

Hearts were placed in cold phosphate buffer solution and were sliced in 1-cm serial sections in a plane parallel to the atrioventricular groove (Fig. 1). The ischemic area was clearly demarcated with the absence of patent blue violet dye. Tissue samples for histology and measurement of regional blood flow were taken from the center of ischemic area in the anterolateral left ventricular wall. The posterior wall of the left ventricular wall was selected as the control region. Tissue color was observed at a magnification of four to 30 times by stereoscopy as a qualitative estimate of blood flow. Tissues for measurement of regional blood flow were first fixed in 10% formalin and then divided into the inner, middle, and outer thirds in ischemic and nonischemic tissues. Each sample weighed at least 400 mg and contained a minimum of 100 microspheres of each nuclide in nonischemic area and 0–15 microspheres in ischemic areas after occlusion. Radiation counts were made in a gamma well-counter (Packard, model A-5980). Regional blood flows were calculated based on established techniques (Buckberg et al., 1971) using a PDP-11/03 digital computer program.

Classification of Myocardial Cellular Damage

Light and electron microscopic findings were compared in adjacent sections of each tissue block. The cell-to-cell variation within any section was small at both light and electron microscopic levels. The criteria for assessing cell...
damage are listed below. The quantitative estimates of cell
damage reported in this study were based only on light
microscopic observations. Ultrastructural features of the
cellular injury were obtained by transmission electron mi-
croscopy to confirm the histological grading system em-
ployed.

**Light Microscopy**

The severity of ischemic injury was determined by the
following criteria (Fig. 2).

**Grade 0**

Major: no clumping or margination of nuclear chromatin;
minor: compact intermyofibrillar and perinuclear sub-
stances, compact myofibril with regular Z bands, no vacu-
ilization or granulation of cytoplasm.

**Grade 1**

Major: minimum clumping and margination of chroma-
tin; minor: decreased staining of intermyofibrillar and peri-
nuclear substances.

**Grade 2**

Major: mild clumping and margination of chromatin;
minor: markedly decreased staining of intermyofibrillar and perinuclear substances, occasional vacuolization in cyto-
plasm.

**Grade 3**

Major: moderate clumping and margination of chromat-
in; minor: no staining of intermyofibrillar and perinuclear substances, occasional vacuolization in cytoplasm, relaxation of myofibril (appearance of A band) with regular arrangement.

**Grade 4**

Major: marked clumping and margination of chromatin;
minor: arrangement of A band with irregular distance,
occasional granulation and increased vacuolization.

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**FIGURE 1.** Diagrammatic presentation of tissue sampling. Part A: The site of occlusion of distal one-third in the left ventricular descending coronary artery (LAD) is shown at “X,” and small ischemic zones not stained with patent blue violet dye are shown by white area. RV = right ventricle; LV = left ventricle; LCX = left circumflex coronary artery; LA = left atrium. Part B: Upper: Transmural tissue obtained from central zone in ischemic left ventricular wall is divided into inner, middle, and outer thirds. Four tissue samples from each of the three areas were cut, fixed and embedded. Lower: Oblique lines of these tissue samples show gross myocardial fiber orientation in inner, middle, and outer thirds of the left ventricular wall. Part C: Topography from layer I to layer V with corresponding actual tissue regions sampled from the LV wall. Layer I—a thin layer of approximately 200 μ immediately beneath endocardium, layer II—inner third except for layer I and B; layer III—middle third; layer IV—outer third except for layer V and B; layer V—a thin layer of approximately 200 μ immediately beneath epicardium. B = histological border layers between layers I and II, and between layers IV and V. Areas with dots show a transmural narrow layer where histological examination was done. V = endocardium; T = epicardium.
FIGURE 2. Light microscopic grading of cell damage. G-0, Normal nonischemic tissue showing evenly dispersed chromatin, compact intermyofibrillar and perinuclear substances (arrow), and regular arrangement of Z band (the photograph was taken from nonischemic area). G-1, grade 1 showing minimal margination of chromatin and slightly decreased staining of intermyofibrillar and perinuclear substances. (This photograph was taken from layer II in hearts with 10 minutes of occlusion.) G-2, grade 2 showing mild clumping of chromatin and marked decrease in staining of perinuclear and intermyofibrillar substances (the photograph was taken from layer IV in heart with 40 minutes of occlusion). G-3, grade 3 showing moderate clumping and margination of chromatin and occasional cytoplasmic vacuolization (the photograph was taken from layer III in heart with 40 minutes of occlusion). G-4, grade 4 showing marked clumping of chromatin, prominent cytoplasmic vacuolization and A bands with focally irregular arrangement (the photograph was taken from layer II in heart with occlusion of 40 minutes). G-5, grade 5 showing extreme clumping of chromatin with obscure nuclear membrane and increased number of vacuoles (the photograph was taken from layer II in hearts with occlusion of 120 minutes). Note that variation of cellular damage in the same picture is minimal. (All pictures are magnified at 600X and stained with toluidine blue.)

Grade 5

Major: markedly clumped and marginated nuclear chromatin with obscure nuclear membranes; minor: increased granulation and vacuolization in cytoplasm, disappearance of architecture in cytoplasm.

Electron Microscopy (Fig. 3)

In samples with histological grade 0, sections revealed abundant glycogen, intact mitochondria, normal nucleus, and no I band. In samples with grade 1, nuclear chromatin was minimally clumped and marginated, the amount of glycogen was slightly decreased, and mitochondria were nearly normal. Histological grade 2 was characterized by mild clumping and margination of chromatin and markedly reduced glycogen. Mitochondria were mildly swollen and had a relatively clear matrix. The myofibrils generally had no I bands, and focal changes in the Z band or intercalated disc were rarely noted.

Electron microscopic features in histological grade 3 included moderately clumped and marginated chromatin, sparse glycogen, regular arrangement of myofibrils with short I bands, mild intermyofibrillar edema, moderate swelling of mitochondria, abnormally clear mitochondrial matrices, and the appearance of dense mitochondrial granules.

In samples with histological grade 4, cells had markedly clumped and marginated chromatin, disruption of myofibrils with wide I bands, increased myofibrillar edema, increased dense mitochondrial granules, abnormally clear matrices and marked swelling in mitochondria, occasional loss of mitochondrial cristae, sparse glycogen, and early signs of sarcolemmal disruption. In samples with histological grade 5, cells showed a very marked clumping and margination of nuclear chromatin and the nuclear outline was relatively blurred. The myofibrils had very wide I bands and were frequently broken at the Z bands and intercalated discs. Other changes included marked and abnormally clear intermyofibrillar spaces, marked swelling...
Statistical Analysis

Comparison of hemodynamic changes with time were made by paired t-test analysis with data obtained at each time point after occlusion contrasted with preocclusion baseline values. Regional blood flow data were analyzed by two-way ANOVA (analysis of variance) of occlusion vs. respective baseline values and between different layers of the myocardium at a fixed time. Histologically graded cell damage was also analyzed with two-way ANOVA. Statistical significance and rejection of the null hypothesis was achieved when $P < 0.05$.

Results

Hemodynamic Changes

All pigs had ventricular premature beats between 10 minutes and 35 minutes after ligation of the distal one-third of left anterior descending coronary artery. The arrhythmia in most cases decreased spontaneously.

Heart rate, aortic systolic and diastolic pressures, and left ventricular systolic and end-diastolic pressures showed no significant change before and during coronary arterial occlusion (Table 1).

Regional Blood Flow of Left Ventricular Wall

Microsphere techniques revealed that, before occlusion of the left anterior descending coronary artery, the anterior and posterior walls of the left ventricle had similar arterial flow and there were no significant transmural differences. After the occlusion, regional arterial blood flow in the center of ischemic area decreased markedly and uniformly to 0.05 ml/min per g in cell layers in all hearts (Table 2).

Qualitative analysis of regional blood flow by visual evaluation of the patent blue violet dye distribution revealed a deep blue violet staining in all nonischemic regions, as defined by microsphere distribution. The ischemic zone in the anterior and septal portions of the left ventricular wall showed no staining except that a slight blue color was seen stereoscopically in the endocardium and occasionally in the epicardium.

Figure 3. Cell damage at the electron microscopic level in sections where light microscopic quantitation was made. G-0, normal nonischemic tissue showing uniform dispersion of chromatin in the nucleus (N), rich glycogen (arrow), intact mitochondria (M). G-1, grade 1 showing slight thinning of chromatin of nucleus (N) and minimal disruption of mitochondrial cristae, mild decrease in the amount of glycogen (arrow). G-2, grade 2 showing mild clumping of chromatin of nucleus (N), moderate decrease in glycogen (arrow), and mild mitochondrial swelling. G-3, grade 3 showing moderate clumping of chromatin of nucleus (N), loss of glycogen, swollen mitochondria with electron dense deposits (arrow) and short I bands. G-4, grade 4 showing increased edema, swollen T-tubules and mitochondria with electron dense deposits, marked clumping of chromatin of nucleus (N), wide I band (arrow) and loss of glycogen. G-5, grade 5 showing very marked margination and clumping of nuclear chromatin (N). Note the indistinct nuclear outline, very wide I band, abnormally clear intermyofibrillar space. Other findings are similar to grade 4. ID, intercalated disc. The bars indicate 1 μm.

of mitochondria, loss of cristae, frequent dense mitochondrial granules, increased disruption of sarcolemma, and near absence of glycogen.
The staining with blue dye in these areas was variable. Upon close examination (at 30X), we were unable, visually, to detect blue dye stained vascular epithelium or myocytes in any section from layers II, III, or IV in two additional pigs with 40 minutes of occlusion.

Quantitative Estimate of Transmural Cell Damage of the Left Ventricular Wall

The quantitative evaluation of ischemic cellular damage of the left ventricular wall is summarized in Table 3. Thin layers immediately beneath both the endocardium and epicardium were least affected by ischemia and showed minimal changes regardless of the duration of occlusion. The width of these layers varied from 100 to 400 μm in different hearts and within different locations in the same heart and was about 200 μm in most hearts. The border layers, the width of which varied from 100 to 400 μm, had intermediate histological injury and were not included in analysis. Therefore, the transmural histological findings are described in five layers (Fig. 1), two layers of approximately 200 μm immediately beneath endocardium (layer I) and epicardium (layer V), respectively, inner endocardial third (layer II), and mid-myocardial third (layer III), and outer third (layer IV).

Layers I and V

Layers of approximately 200 μm immediately beneath endocardium and epicardium showed normal histology of grade 0 in the nonischemic control area. Cellular injury, evident after 10 minutes of occlusion, was 0.5 ± 0.1 for layer I and 0.4 ± 0.2 for layer V. Myocardial cell injury increased slightly after 20 minutes of occlusion and was 1.1 ± 0.1 for layer I and 1.0 ± 0.1 for layer V. With 40 minutes of occlusion, ischemic grades were 1.4 ± 0.2 for layer I and 1.2 ± 0.3 for layer V. After 120 minutes of occlusion, these least affected cell layers showed a similar degree of ischemic cellular damage to that seen at 40 minutes of occlusion. Contraction bands were occasionally seen in layers I and V and in the border layers of the left ventricular wall after 20, 40 and 120 minutes of occlusion (Fig. 4).
TABLE 3
Quantitative Estimate of Transmural Cellular Damage of the Left Ventricular Wall in Early Ischemia

<table>
<thead>
<tr>
<th>Grades of ischemic cellular damage in layers I to V</th>
<th>Control</th>
<th>10-min occlusion</th>
<th>20-min occlusion</th>
<th>40-min occlusion</th>
<th>120-min occlusion</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of cases</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>0.0 ± 0.0</td>
<td>0.1 ± 0.1</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>III</td>
<td>0.5 ± 0.1*</td>
<td>0.7 ± 0.1*</td>
<td>0.5 ± 0.2*</td>
<td>0.4 ± 0.2*</td>
<td>0.4 ± 0.2*</td>
</tr>
<tr>
<td>IV</td>
<td>1.1 ± 0.1*</td>
<td>2.3 ± 0.1*</td>
<td>1.9 ± 0.3*</td>
<td>1.3 ± 0.2*</td>
<td>1.0 ± 0.1*</td>
</tr>
<tr>
<td>V</td>
<td>1.3 ± 0.2*</td>
<td>3.6 ± 0.1*</td>
<td>2.2 ± 0.3*</td>
<td>1.7 ± 0.4*</td>
<td>1.2 ± 0.3*</td>
</tr>
<tr>
<td>Results are expressed as mean ± SD.</td>
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</table>
| I = a layer of about 200 μm immediately beneath endocardium; II = inner third except for layer I and thin border layer between layers I and II; III = middle third; IV = outer third except for layer V and thin border layer between layers IV and V; V = a layer of about 200 μm immediately beneath epicardium. Ischemic cellular damage was classified from 0 to 5 based on light and electron microscopy. * P < 0.05 vs. same layer, control.

Inner Third Layer (Layer II)
The cells of the inner third of the myocardium had grade 0 and rarely grade 1 changes in nonischemic regions (average 0.1 ± 0.1). A slight increase in ischemic injury grade was observed after 10 minutes of occlusion (0.6 ± 0.1). Ischemic injury was increased further after 20 minutes of occlusion (2.3 ± 0.1) and even more after 40 minutes of occlusion (3.6 ± 0.1). Ischemic injury was most severe after 120 minutes of occlusion (5.0 ± 0.1).

Middle Third Layer (Layer III)
The myocardial cells of the middle third layer were devoid of injury in the control samples. After 10 minutes of occlusion, the injury was graded 0.5 ± 0.2. After 20 minutes of occlusion, the injury score was further elevated to 1.9 ± 0.3 and rose to 2.2 ± 0.3 at 40 minutes of occlusion. The ischemic score after 120 minutes of occlusion was again most severe at 5.0 ± 0.1.

Outer Third Layer (Layer IV)
Prior to coronary ligation, the cells in the outer myocardial third showed normal morphology. As in the middle and inner thirds of ischemic myocardium, the degree of cell injury progressively increased with ischemic duration and was 0.4 ± 0.2 at 10 minutes, 1.3 ± 0.2 at 20 minutes, 1.9 ± 0.3 at 40 minutes and 5.0 ± 0.1 at 120 minutes of occlusion.
A definite transmural gradient of ischemic cellular damage existed from the inner third (layer II) to the outer third (layer IV) of the myocardium after 20 minutes of occlusion (from 2.3 ± 0.1 to 1.3 ± 0.2) and 40 minutes of occlusion (from 3.6 ± 0.1 to 1.9 ± 0.3) (Table 2). This transmural gradient disappeared in hearts made ischemic for 120 minutes.

Although mildly swollen myocardial cells were frequently seen in the ischemic area, no marked swelling was observed in any of the tissue samples analyzed. Cell-to-cell variations in ischemic grade in any section were very small (Fig. 2), as they were in the different

FIGURE 4. Contraction bands (arrow) are seen in border layer between layers IV (lower half of A) and V (upper half of A) in a heart after 120 minutes of occlusion. Panel A: light microscopic findings (600X, toluidine blue stain); panel B: electron microscopic findings. The bar indicates 1 μm.
hearts with the same duration of ischemia (Table 3). For example, the transmural left ventricular wall had myocardial cells with mostly grade 0 and sometimes grade 1 after occlusion of 10 minutes. Layer II was comprised of cells with ischemic grades 2 and 3 after 20 minutes of occlusion, and was comprised of cells with ischemic grade 3 and 4 after 40 minutes of occlusion. Layers II, III, and IV were comprised of cells with mostly grade 5 and, rarely, grade 4 after 120 minutes of occlusion. In layers I and V, after 120 minutes of occlusion, most of the cell injury was grade 1 and sometimes was grade 0 or grade 2.

**Electron Microscopic Findings (Fig. 3)**

Electron microscopy was done only to establish the ultrastructural features of myocardium with various histological grades of injury. After 10 minutes of occlusion, the ischemic left ventricular wall was comprised transmurally of cells with grades 0 and 1 in electron microscopic classification described on page 685 and shown in Figure 3.

After occlusion for 20 minutes, ischemic grades of cells were mostly of grade 1 and sometimes of grades 0 or 2 in layers I, IV, and V. Layer II was comprised of cells with grades 2 and 3. Layer III was comprised of cells with grades 1, 2, and 3. After occlusion for 40 minutes, layers I and V had ischemic grades with grades 1 and 2, and layer II had cells with grades 3 and 4. Layers III and IV had cells with grades 1, 2, and 3. In qualitative analysis, using the electron microscope, we saw a definite transmural gradient of ischemic cellular damage in hearts with 20 and 40 minutes of occlusion.

After 120 minutes of occlusion, most myocardial cells were of grade 1 and rarely of grade 2 in layers I and V. Layers II, III, and IV had mostly cells with grade 5 and, rarely, cells with grade 4. No transmural gradient from layer II to layer IV was evident at the electron microscopic level after 20 minutes of occlusion.

**Discussion**

The pig has no significant collateral circulation (White and Bloor, 1981; Millard, 1982). For this anatomic reason, reproducible transmural ischemia results from coronary artery ligation and frequently produces a high incidence of ventricular fibrillation (Savage et al., 1981). However, by occlusion of the distal one-third of the left anterior descending coronary artery, we could produce stable preparations with small transmural infarction and a low incidence of fatal dysrhythmias without pharmacological intervention.

**Fixation Artifacts and Classification of Ischemic Cellular Damage**

Rapid and complete perfusion of the coronary arteries with fixative is the best fixation method. However, it is frequently difficult to perfuse within less than several minutes after sacrifice. Therefore, study of cell damage in early ischemia after periods as short as 10 minutes is limited by this technical constraint. In addition, it may be impossible to perfuse completely all ischemic areas of heart after 40 and 120 minutes of occlusion, because myocardium exposed to ischemic periods of this duration presents the so-called “no-reflow phenomenon” on reperfusion (Kloner et al., 1974; Beller et al., 1977). Accordingly, fixation was performed by rapid tissue sample immersion (<2 minutes) after excision of the heart. Because histological damage in early ischemia is not detected in usual paraffin-embedded sections, tissue samples processed for electron microscopy and stained with toluidine blue were used in light microscopic study.

Since ischemic myocardial cells become progressively hypertonic as ischemia proceeds, cellular swelling can occur artifically during fixation with isotonic solutions (312 mOsm/kg) (Tranum-Jensen et al., 1981). Although the precise rate of osmolarity increase in ischemic myocardial cells in vivo is unknown, the osmolarity of isolated ischemic porcine myocardium was 372 mOsm/kg after 60 minutes of occlusion (Tranum-Jensen et al., 1981). Accordingly, we used a mildly hypertonic phosphate buffer solution (0.2 M, 320 mOsm/kg) in 2.5% glutaraldehyde for all fixation perfusions.

To obtain the transmural histological sections, we separated the left ventricular wall into the inner, middle, and outer thirds. Large tissue samples, about 2 X 3 X 1 mm in size, were embedded. A dramatically different histology was seen between the center and the surface areas in all tissue samples taken from ischemic areas of the heart after 10, 20, and 40 minutes of occlusion. In the central area, cells with severe swelling and marked nuclear changes were frequently found, and an elevation in ischemic grade was distinctly evident. The myocardial cells of the surface areas were less swollen and showed fewer nuclear changes. The width of the surface areas was narrow and was within 0.1–0.2 mm from the surface of the tissue sample. The surface cells were found to have uniform cell histology and cells from the center of the tissue samples showed a wide variation in cell histology. Similar differences in samples with low-grade changes were noted in surface cuts from tissues fixed immediately after sacrifice with surfaces from the same tissue sample cut 15 or 20 minutes later in cold buffered glutaraldehyde solution.

Since all tissues were fixed by immersion in buffered glutaraldehyde, it is likely that the surface of the tissue is fixed better than the interior due to variable penetration of the fixative (Hopwood, 1969). In addition, severe acidosis seen in ischemic myocardium may have further decreased the power of fixation by glutaraldehyde (Hopwood, 1969). The histological findings in the tissue sample center are presumably not a direct result of the in situ ischemic stimulus. Therefore, a narrow surface area of the tissue sample, cut and fixed immediately after sacrifice, was used transmurally for histological evaluation of ischemic injury (Fig. 1).

The classification of myocardial cellular damage
based on light microscopic and electron microscopic findings confirm the general concept of ischemic cellular damage obtained previously from posterior papillary muscle of the dog left ventricle by immersion fixation (Jennings et al., 1965; Kloner et al., 1974b).

Transmural Distribution of Coronary Arterial Flow

It is generally agreed that the center of an ischemic zone lacks blood flow uniformly and transmurally (Harken et al., 1981; Savage et al., 1981). The microsphere blood flow technique has a few limitations. Buckberg et al. (1971) pointed out that blood flow quantification by this method was proportional to number of microspheres in the study sample and that error estimates increased significantly when samples held significantly less than 100 microspheres. In the present study, flow to the ischemic myocardium (<0.05 ml/min per g) was below the resolution of the microsphere blood flow technique. Injection of 15 μm microspheres used in the present study does not detect arterial blood flow throughout the collateral vessels with less size than 15 μm. However, a study using 9-μm microspheres revealed no blood flow in the center of ischemic zone in pigs (Savage et al., 1981).

However, if the left ventricular wall is divided into three layers and each of them are approximately 1 g, blood flow determinations may overlook localized blood flow inhomogeneities (e.g., collateral flow in thin or focal layers). To resolve this methodological problem in ischemic hearts of pigs, the injection of patent blue violet dye into the left atrium revealed that the center of the ischemic zone, where all sampling was done, was not stained by the dye. Although the relation of vital dye staining of vascular endothelium to blood flow has not been rigorously tested, we feel that absence of intramural vascular staining by circulating patent blue violet dye effectively eliminates the existence of a transmural blood flow gradient to explain our histological observations. Slight staining could be detected stereoscopically at the endocardium and epicardium surfaces. The blue violet dye may penetrate the endocardium and thereby infiltrate from the left ventricular cavity to the ischemic region. In addition to a potential epicardial collateral arterial route, staining in this layer may have resulted from venous drainage of adjacent normally perfused regions.

Previously, Eckstein (1954) revealed that in the young pig heart, minimal back flow occurs in the occluded coronary artery. Postmortem injection several days after coronary occlusion showed coronary interarterial anastomoses in the endocardium and in a thin layer of the subendocardium (Schaper et al., 1967). Thebesian vessels also exist in the subendocardium and offer a route for the dye (Moir, et al., 1964). In the present study, thin layers immediately beneath endocardium and epicardium showed minimal ischemic change by both light and electron microscopy, regardless of duration of ischemia. The depth of these layers from epicardial and endocardial surfaces varied from 100 to 600 μm in different pigs and in the different areas of the same heart. Contraction bands, which suggest the presence of reflow (Kloner et al., 1974a), were seen in these layers. The intensity of the blue color in the endocardium and epicardium after dye injection was variable. This suggests the presence of a small arterial flow in the endocardium, epicardium, and thin layers immediately beneath them in ischemic left ventricular wall in pigs. However, we cannot reject the possibility that O₂ diffusion from left ventricular cavity and the atmosphere may contribute to cellular protection seen in these thin layers.

“Wavefront Phenomenon” of Ischemic Cellular Damage

Our data reveal, at both light and electron microscopic levels, a transmural gradient of ischemic cell damage from inner third (layer II of Fig. 1) to outer third (layer IV of Fig. 1) of the left ventricular wall was present at 20 and 40 minutes of occlusion, and became uniform with 120 minutes of ischemia. The transmural gradient after 20 and 40 minutes of occlusion is independent of distribution of coronary arterial flow.

Perhaps the transmural gradient of ventricular tissue metabolites from subendocardium to subepicardium that has been reported after cessation of coronary blood flow for 15 and 30 seconds in dog heart (Dunn and Griggs, 1975) may account for the observed transmural cell injury gradient. As glycogen content and the activity of several glycolytic enzymes are highest in the subendocardium (Jedeikin, 1964), it is possible that loss of intrinsic substrate may contribute to the ischemic process. Rivas et al. (1976) reported, in a histological study of dogs, evidence of endocardial damage greater than that which might be accounted for on the basis of reduced blood flow alone. The existence of transmural gradients of wall stress (Yin, 1981) and intramyocardial pressure (Sabbah and Stein, 1982) from endocardium to epicardium of the left ventricular wall may contribute to the differences in ischemic cell injury. In the normal myocardium, dimensional changes (both thickening and shortening) during systole are greater in the subendocardium than in the subepicardium (Sabbah et al., 1981). However, until a gradient in systolic wall stress has been demonstrated in the ischemic myocardium, this factor as a cause of the cellular damage remains speculative.

The significance of I band width is not evaluated systematically, since most of the samples were immersion fixed. The wider I bands are seen in the ischemic myocardial cells with prolongation of the ischemic injury (Jennings, et al., 1965; Herdson et al., 1969; Kloner, et al., 1974b). Similarly, the length of the sarcomere increases significantly in the ischemic zone and is associated with bulging of the myocardium (Crozatier et al., 1977). In spite of the fact that our samples were fixed by immersion, the wider I bands first appeared in the inner third layer after 40 minutes of coronary occlusion and extended transmurally after 120 minutes of occlusion. However,
with perfusion fixation, all regions showed I bands. The absence of I bands may be an artifactual manifestation of tissue fixation by immersion. Therefore, the role of longer sarcomeres with wider I bands in the pathogenesis of cell injury remains to be determined.

Whereas we have made no direct measurements or calculations, we postulate that the observed flow independent “wavefront phenomenon” of cellular damage in early ischemia in pig results principally from a transmural gradient of wall stress and intramyocardial pressure. However, we cannot exclude the possibility that these effects are the product of a more intense acidosis developing in early ischemia in the inner third of the ventricular wall because of high glycogen content (Garlick et al., 1979). The appearance of a transmural gradient after 120 minutes of occlusion presumably indicates the termination of this “wavefront phenomenon,” regardless of cause, in myocardium with verified uniform transmural ischemia.

Generally, the reason for transmural gradient of ischemic change in canine hearts with rich collateral circulation is attributed to the presence of the transmural gradient in the distribution of collateral coronary blood flow (Griggs et al., 1972; Reimer et al., 1977, 1979; Rivas et al., 1976). It has been suggested that the transmural gradient of the blood flow distribution is due to the transmural differences of diastolic tissue pressure (Rouleau et al., 1979) because a close correlation between distal diastolic coronary pressure and ischemic endocardial-epicardial flow ratios has been detected (Rouleau et al., 1979; Gross and Warltier, 1981; Weintraub et al., 1981).

Therefore, we conclude that factors other than blood flow (e.g., wall stress) are important determinants of the rate of myocardial cell injury during ischemia. Our results indicate that a transmural morphological gradient does exist in early myocardial ischemia where blood flow is uniformly absent. From these observations, we would speculate that restoration of blood flow would result in restored myocardial cell viability in different regions of the transmurally ischemic ventricular wall in direct relation to the duration of ischemia and distribution and degree of cell injury at the time of reperfusion. Additionally, pharmacological interventions which are antecedent to ischemia may modify the rate of cell injury if such treatments alter the aforementioned determinants, including myocardial metabolism and/or wall stress.

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INDEX TERMS: Transmural cellular damage • Collateral blood flow • Early ischemia • Cell death • Glycogen • Wavefront phenomenon • Electron microscopy • Light microscopy
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