Coronary Collateral Development in Swine After Coronary Artery Occlusion

F.C. White, S.M. Carroll, A. Magnet, and C.M. Bloor

We have quantified the development of the coronary collateral circulation in the pig. The collateral circulation was induced to grow by placing an ameroid occluder on the left circumflex coronary artery. Two to 16 weeks after ameroid placement, the coronary collateral circulation was identified after the injection of several colors of a silicone polymer into the coronary arteries and the aorta. We identified intercoronary and extracardiac collaterals and quantified their number, location, size, and wall thickness. Intercoronary collaterals grew to a level that represents a 14-fold increase in normal collateral blood flow under resting conditions compared with the values in an animal not subjected to coronary artery occlusion. Extracardiac collaterals could potentially supply approximately 30% of resting flow. The sources of the extracardiac collaterals were the bronchial and internal mammary arteries. Coronary collateral morphometry and DNA synthesis in the pig heart also were examined. Coronary collaterals had significantly less smooth muscle than did normal arterioles. This may account, in part, for the reduced response of the coronary collaterals to vasodilators. We observed intense DNA synthesis in endothelial and smooth muscle cells in the first 2 or 3 weeks of ischemia. However, DNA synthesis rapidly ceased after this time, coincident with coronary collateral reserve values (ischemic/nonischemic regional blood flow ratios during maximal vasodilatation) reaching their maximum level. This suggests that failure of the vessels to continue proliferating accounts for the occurrence of the plateau in blood flow levels. (Circulation Research 1992;71:1490–1500)

KEY WORDS • endothelial cells • smooth muscle cells • DNA synthesis

The fate of the ischemic myocardium ultimately depends on the restoration of the blood supply to the ischemic region. Therefore, the coronary collateral circulation is critical to the survival of the heart when the blood supply is inadequate to meet the minimal metabolic demands.

The innate coronary collateral circulation and the ability to synthesize or open new coronary collaterals after coronary artery occlusion vary widely from species to species. In dogs, there is an abundance of innate epicardial collateral vessels and a sparsity of collateral vessels in the deeper layers of the myocardium.1 These innate vessels supply a significant level of collateral blood flow after sudden coronary artery occlusion.2 In addition, an intense and rapid proliferation of collateral vessels occurs when stimulated by slow coronary artery occlusion. Slow coronary artery occlusion can be modeled by use of an ameroid occluder, a device that gradually occludes the coronary artery of experimental animals.3 Dogs with these devices show rapid collateral development, which results in a return of normal levels of blood flow to the region at risk and therefore little infarction of this region.4 During pharmacological or physiological vasodilatation, blood flow to this region increases above resting levels, indicating a significant coronary collateral reserve (ischemic/nonischemic regional blood flow ratio during maximal vasodilatation) available for recruitment.5–7 Recent studies in dogs by Heusch et al8 have shown that animals with coronary stenosis and well-developed epicardial collateral vessels that are treated with a Ca2+ channel blocker have significantly increased blood flow during exercise-induced ischemia. However, their model does not distinguish between the extent of increased blood flow occurring through the stenosis and that occurring through the collaterals. In contrast, White et al9 observed no demonstrable reserve during exercise-induced ischemia in pigs with an occluded left circumflex coronary artery (LCx), even after treatment of the pigs with a Ca2+ blocker or adenosine. The response of the human coronary collateral vessels to vasodilators is highly variable. Some patients respond to treatment, whereas other patients exhibit little ability to augment collateral reserve.8,9 Similar to the results in pigs, the collateral circulation in humans is sparse in the normal heart, but the heart does respond to an ischemic stimulus by a proliferation of blood vessels.10–12 However, collateral vessels do not develop to a level at which normal blood flow is completely restored. New collateral vessels are limited in number and also contain less smooth muscle than do other arterial vessels. This lack of development may be, in part, responsible for the protracted angina often exhibited in patients with coronary artery disease.

The parallel between the response of swine and humans to an ischemic stimulus prompted us to examine the morphometry of the developing collateral circula-
tion in swine. Early reports by De Brabander et al.12 and Schaper et al.13 showed that the pig coronary collateral vessels were endomural and had subendocardial plexuses of anastomoses with a notable absence of epicardial collaterals. De Brabander et al, using tritiated thymidine labeling, described DNA synthesis occurring in mesenchymal cells, cardiac myocytes, and endothelial cells in and adjacent to the region of infarct. Their model of left anterior descending coronary artery (LAD) occlusion produced infarcts of varying degree. Thus, although they described regenerative changes in their model, the variable infarct sizes and the absence of correlation with functional collateral development left some unanswered questions.

Over the past several years, we have reported extensively on the swine ameroid model developed in our laboratory.3,7,14-16 This model consists of gradual occlusion of the LCx, which results in small uniform infarcts with minimal contractile dysfunction. In this model, we noted many pathophysiological responses of the chronically ischemic myocardium that suggest an abrupt cessation of collateral development early after ameroid placement3 and a lack of coronary collateral reserve.7 In this report, we present a comprehensive morphometric and functional study of coronary collaterals in swine, both in the native state and after ameroid coronary occlusion. The results suggest that the relative sparsity of collaterals, the lack of smooth muscle development in their walls, and their deep myocardial distribution may account for the diminished coronary collateral reserve observed after coronary artery occlusion.

Materials and Methods

We used Yucatan minipigs in these studies. Group 1 consisted of pigs having ameroid occluders implanted on the LCx or sham-operated control pigs. These animals were used for blood flow measurements and for morphometric analysis. There were 17 pigs in this group, including nine males and eight females, weighing 36±2 (mean±SEM) kg. These animals were approximately 7 months old. Each subgroup consisted of three animals. The subgroups are as follows: sham group, thoracotomy performed without placement of an ameroid on the LCx; 3-week group, ameroid placement for 3 weeks; 8-week group, ameroid placement for 8 weeks; 16-week group, ameroid placement for 16 weeks. Five pigs experienced fatal arrhythmias; 12 pigs survived and were used in this study group. Group 2 animals were instrumented with ameroid occluders and were used for 

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labeling of endothelial cells and smooth muscle cells in the developing coronary collaterals. This group consisted of 20 pigs, including 11 males and nine females at approximately 4 months of age. These pigs were smaller (12±1 kg) than group 1 pigs to avoid excessive use of radiolabeled thymidine. We note that the different ages or sizes of the animals in group 1 and group 2 did not influence these studies. In a recently published study involving 47 pigs in different age groups, we showed that the development of the collateral circulation is similar in pigs between the ages of 4 months and 3 years.17 The parameters assessed in this comprehensive study included heart rate, blood pressure, endocardial and epicardial flow under resting and vasodilated conditions, and the effects of exercise on collateral develop-
cardiac total resting collateral flow equals (endocardial + epicardial flows in Table 4)÷2. Total resting collateral flow equals (LCx endocardial + LCx epicardial flows in Table 3)÷2. Coronary and collateral resistances were calculated as mean blood pressure + regional blood flow and are expressed as millimeters of mercury per milliliter per minute per gram. We did not include measurements of atrial pressures in this calculation, since they were similar in all animals and did not differ between the experimental groups.

Morphometric Studies

Tissue preparation. After the completion of the physiological measurements on group 1 animals, we anesthetized the animals with pentobarbital (25 mg/kg i.v.) and completed the open-chest experiments; then the hearts were stopped by an intra-atrial bolus of saturated KCl. We perfused the heart with a Krebs’ 2% glutaraldehyde solution retrogradely from the aorta at the level of the diaphragm. Perfusion pressure was 120–150 mm Hg. Papaverine (200 mg/l) was added to the Krebs’ solution to help open the vessels. After fixation of the heart, the coronary arteries and the aorta were injected with colored gelatin or Microfil as previously described.20 Each coronary artery and the aorta were injected with a different colored injectate to aid in identification of the collaterals. The hearts were then cut from base to apex into 5-mm-thick slices, dehydrated in alcohol, and cleared with oil of wintergreen. Using a dissecting scope, we identified the coronary collaterals at the confluence of two different colors of injectate found in the borders of the bed at risk of the LCx. The transmural distribution and numbers of the collaterals were recorded on planimetric images. Each collateral or groups of collaterals were dissected from the region, oriented in cross section, and embedded in plastic tissue blocks. Histological sections, 5 μm thick, were made of each collateral and cut serially to obtain the minimum diameter near the confluence of the two colors of injectate. Many of the plastic sections contained more than one collateral.

Morphometric Image Analysis

We measured coronary collateral external and internal diameters and wall thickness on each identified collateral directly from microscope slides by use of an automated image analysis system. Our system used a Hitachi model KP 140 high-resolution CCTV black and white video camera attached to a model 100 Image Analysis System (Analytical Imaging Concepts, Irvine, Calif.). This system is interfaced with an AT/286 computer for data storage, calculations, and analysis using LOTUS 1-2-3 (Lotus Development Corp., Cambridge, Mass.). This imaging system consists of an image capture and display board with $512 \times 512 \times 8$-bit resolution and 256 gray levels. Wall thickness was calculated as the thickness of the whole wall from the method of Barrett21: wall thickness = radius + [radius$^2$ + (area/π)]$^{1/2}$. The medial wall thickness was not used, since it requires visualization of the internal elastic lamina. This was either not readily identifiable or not intact in the collateral vessels. The ratio of wall thickness to the radius of each collateral was calculated from measurements described above.

Identification of Extracardiac Coronary Collaterals

We identified extracardiac collateral arterioles in the group 1 animals by aortic injections of colored gelatin or thickened Microfil.22 Thickened Microfil was used, since it rarely goes into venules. Morphometric measurements were carried out on vessels identified in cleared sections of the aorta and the basal 10-mm-thick epicardial section of the heart. These sections included all three coronary arteries and sections of the LCx with the ameroid occluder. Coronary collaterals that had systemic artery origins could be visualized when they anastomosed to the LCx. Cross-sectional morphometry was carried out on the narrow portion of the collaterals as they connected to the LCx. Most of these vessels anastomosed to the LCx epicardially. Some vessels arose from the proximal LAD and LCx and encircled the ameroid occluder. To decide the functional significance of the extracardiac coronary collaterals, we measured blood flow in the LCx bed during RC and LAD occlusions of 3-minute duration. This protocol allows the distinction between the flow potential of extracardiac coronary collaterals and the measured flow of the intercoronary collateral arteries.

DNA Synthesis With Tritium-Labeled Thymidine

At 2, 3, 4, 6, and 8 weeks after ameroid placement, we infused each animal in group 2 with 0.3 mCi/kg $^3$H-T

### Table 1. Morphology of Intercorony Collateral Vessels

<table>
<thead>
<tr>
<th>Internal diameter (μm)</th>
<th>Wall thickness (μm)</th>
<th>Vessel diameter (μm)</th>
<th>No. of vessels</th>
<th>Wall thickness (μm)</th>
<th>Vessel diameter (μm)</th>
<th>No. of vessels</th>
<th>Wall thickness (μm)</th>
<th>Vessel diameter (μm)</th>
<th>No. of vessels</th>
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<tr>
<td>Control arterioles</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>20–40</td>
<td>4.5±0.2</td>
<td>30.1±0.6</td>
<td>70±0</td>
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<td>25.9±2.0</td>
<td>83±12</td>
<td>2.0±0.1*</td>
<td>30.9±2.6</td>
<td>122±26*</td>
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<td>6.0±0.4</td>
<td>49.2±2.4</td>
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<td>2.0±0.3*</td>
<td>50.4±2.7</td>
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<td>46.4±1.1</td>
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<td>NF</td>
<td>NF</td>
<td>2.4±0.1*</td>
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<td>NF</td>
<td>NF</td>
<td>3.3±0.3*</td>
<td>90.8±1.8</td>
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<td>NF</td>
<td>NF</td>
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<td>122.3±4.0</td>
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<td>&gt;140–180</td>
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<td>NF</td>
<td>NF</td>
<td>7.8±0.3*</td>
<td>158.0±6.4</td>
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</tbody>
</table>

NF, none found. Values are mean±SEM (n=3 pigs).

Control arterioles were evaluated in untreated pigs. Collaterals were evaluated in sham-operated pigs at 8 weeks after surgery; 3-, 8-, and 16-week pigs were examined at their respective times after placement of the ameroid occluder.

*p<0.05 compared with control arteriole wall thickness.

*p<0.05 compared with the number of sham collaterals.

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TABLE 1. Continued

<table>
<thead>
<tr>
<th>8-Week collaterals</th>
<th>16-Week collaterals</th>
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</thead>
<tbody>
<tr>
<td>Wall thickness (μm)</td>
<td>Vessel diameter (μm)</td>
</tr>
<tr>
<td>2.5±0.1*</td>
<td>32.9±2.4</td>
</tr>
<tr>
<td>2.6±0.2*</td>
<td>49.1±0.4</td>
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<td>2.8±0.7*</td>
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</tr>
<tr>
<td>4.3±0.2*</td>
<td>95.1±2.6</td>
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<tr>
<td>7.1±0.7*</td>
<td>129.0±5.4</td>
</tr>
<tr>
<td>8.4±0.4*</td>
<td>150.3±4.0</td>
</tr>
</tbody>
</table>

(3.7 Ci/mmol, New England Nuclear) three times at 1, 16, and 21 hours before euthanasia. Each animal was injected with label at three separate times in order to label the maximum number of dividing cells, since not all of the cells would be synthesizing DNA at the same time. Sham-operated animals were labeled similarly at 2 and 8 weeks. This pulse-labeling technique was used so that the maximum number of mitotic cells would be labeled during the 22-hour mitotic cycle. In these hearts, we injected Microfil (Canton Bio-Medical Products Inc., Boulder, Colo.) at 150 mm Hg into the RC, LAD, and LCx and into the thoracic portion of the aorta. The LCx was injected distal to the aortic occluder. We used different colors of Microfil in these injections to help identify coronary collateral vessels at the borders of the LCx bed. Three-micrometer-thick sections of the identified collaterals were cut in cross sections, autoradiographed by preparing the slides in diluted Ilford emulsion (3:2 with distilled water), and exposed for 70 days in a light-tight box at −20°C. After development in Dektol, the slides were lightly stained with hematoxylin and cosin, and coverslips were applied. Silver grains superimposed on the visible nuclei were counted on labeled mitotic cells.6 Visualization of the grains was enhanced by the use of ultraviolet reflected light. The labeling index data were accrued while viewing the slides at ×400 and ×1,000 using both transmitted light and epifluorescence. Nuclei with four or more grains were considered labeled. Background grain counts were less than 1 grain per nuclear area. A labeling index was calculated as follows: number of endothelial or smooth muscle cells labeled/total number of endothelial or smooth muscle cells examined × 100. Cells were defined as endothelial or smooth muscle cells by the criteria of their position within the vessel wall and their nuclear shapes. Sham animals were labeled and counted to account for naturally occurring DNA synthesis. Counts of endothelial and smooth muscle cells were based on the absolute counts of these cells in representative vessels of each size times the total number of collateral vessels observed. At least 110 collateral vessels were examined in each heart to determine this mitotic index. The total numbers of endothelial cells and smooth muscle cells observed for this index were approximately 3,500 and 4,500, respectively, for each animal. Approximately 1 cell per 10,000 cells was labeled in the sham animals.

Histological Studies

Transmural tissue samples from the ischemic regions of group 1 and group 2 animals were examined. We stained 5-μm-thick sections with Masson’s trichrome stain. Using point counting, we quantified infarct size in the outer third, middle third, and inner third of the transmural samples. Four samples were examined from each heart. The region at risk was quantified from each heart by planimetry of the cross-sectional slices before we dissected the collaterals. The LCx was examined at the site of the aortic occluder in each experimental animal to decide if closure had occurred. A white or red occlusive thrombus was found in all arteries but one. This one open artery was present in an animal from the 2-week group that was labeled with 3H-T.

Statistical Analysis

Data from morphometric analyses are expressed as mean±SEM. Statistical analysis was carried out using Student’s mean t test for intergroup comparisons with the Bonferroni correction23 or repeated-measures analysis of variance for intragroup comparisons using Rao’s r for the test statistic comparison. Blood flow data were analyzed by Student’s t test. Values were considered significantly different at p<0.05. Calculations were carried out using a STATS+ statistical package (Statsoft, Tulsa, Okla.).
**Table 2. Morphometry of Extracardiac Collateral Vessels**

<table>
<thead>
<tr>
<th>Internal diameter (µm)</th>
<th>Control arterioles</th>
<th>Sham collaterals</th>
<th>3-Week collaterals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Wall thickness (µm)</td>
<td>Vessel diameter (µm)</td>
<td>No. of vessels</td>
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</tr>
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<td>12.9±0.4</td>
<td>155.0±3.9</td>
<td>10±0</td>
</tr>
<tr>
<td>&gt;180</td>
<td>13.0±0.8</td>
<td>188.0±12.0</td>
<td>10±0</td>
</tr>
</tbody>
</table>

NF, none found. Values are mean±SEM (n=3 pigs).
Control arterioles were evaluated in untreated pigs. Sham collaterals were evaluated in sham-operated pigs at 8 weeks after surgery; 3-, 8-, and 16-week pigs were examined at their respective times after placement of the ameroid occluder.

*p<0.05 compared with control arteriole wall thickness.

Results

**Hemodynamics**

Heart rates at the time of microsphere injection were similar in all the experimental subgroups (Table 3). Heart rates ranged from 93±4 to 115±4 beats per minute. Blood pressures also were similar during resting conditions (Table 3). However, during adenosine infusion, mean aortic blood pressures significantly decreased to approximately 55 mm Hg (Table 3). When extracardiac collateral blood flows were measured during open-chest conditions, mean aortic blood pressures significantly decreased to 69±4 mm Hg (p<0.05).

**Morphometry and Transmural Distribution of Intercoronary Collateral Vessels**

Table 1 presents the morphometric features of the intercoronary collateral vessels. These vessels are defined as arterioles that interconnect two or more of the three major coronary arteries, i.e., RC, LAD, and LCx. In Table 1, coronary arteriolar and collateral vessel internal diameters, wall thicknesses, and the number of vessels per heart are listed. Coronary collateral blood vessels were divided into six groups based on internal diameters of 20–40, 40–60, 60–80, 80–100, 100–140, and 140–180 µm. It should be noted that coronary collaterals smaller than 20 µm in diameter could not be quantified, but they were numerous in the pigs with ameroids. However, coronary collaterals smaller than 20 µm in diameter are not considered functionally significant, since Poiseuille’s law indicates that flow through such small channels is miniscule compared with the vessels larger than 20 µm. Similar-sized arterioles were analyzed from sham-operated pigs to compare wall thicknesses. All collaterals from each diameter group had wall thicknesses that were significantly thinner than normal arterioles of the same diameter (p<0.05). However, as collaterals developed, they showed some increase in wall thickness. For example, the 16-week group had collaterals that were significantly thicker than the native collaterals in the sham group, but the 16-week group showed no indication of further growth when compared with the 8-week group. The number of collateral vessels occurring in diameter groups 20–40 µm and 40–60 µm increased significantly from the sham group up through the 8-week group. No further increase occurred in the 16-week group. No native collaterals of greater than approximately 60 µm were observed in the sham group. As the collaterals increased in number and wall thickness, there were increases in the maximum internal diameters, so that in the 3-week group there were a few collaterals as large as 150 µm in internal diameter. Larger collaterals are even more predominate in the 8- and 16-week groups. Collaterals in the two largest groups comprise 3.1%, 4.8%, and 5.8% of the total collateral population at 3, 8, and 16 weeks, respectively. This may have a significant effect on blood flow, since flow is proportionate to the radius of the vessel to the fourth power.

Figure 1 shows the transmural distribution of the intercoronary collaterals. Each dot near the border of the LCx bed represents three collaterals. In the sham collateral state, there are 115±11 collaterals per animal. Vessels were uniformly distributed in the midmyocardium, with a notable clustering in the region of the posterior papillary muscle. There was a notable absence of collaterals in the epicardial region. In the 8-week group, the number of vessels had increased to 270±33 collaterals per animal, but the regional distribution was similar to the native distribution in the sham group. The few epicardial collaterals seen were usually associated with extracardiac collateral vessels.

**Morphometry and Distribution of Extracardiac Collateral Vessels**

Table 2 presents the number of extracardiac collateral vessels per animal, their internal diameters, and their wall thicknesses. Extracardiac collateral blood vessels are derived in part from the vasa vasorum of the great vessels or other systemic arteries and serve as arteriolar bridges that connect branches of the coronary arteries with bronchial, mediastinal, and intercostal arteries. For the sake of simplicity, atrio coronary artery collaterals and retrocardiac vessels are included in this study as extracardiac collateral vessels. The extracardiac collaterals were predominantly located in the epicardial region. The same size groups as shown for the intercoronary collaterals plus an additional size category (diameter, >180 µm) are used to categorize the extracardiac vessels. These vessels are less numerous than the intercoronary collaterals, but they have...
larger diameters. Some extracardiac collateral vessels are as large as 240 μm in internal diameter in the 8-week and 16-week groups. The growth patterns are similar to those of the intercoronary collaterals. The number of vessels and their wall thicknesses increased up to 8 weeks and then plateaued, although a trend toward larger vessels was observed at 8 and 16 weeks. The wall thicknesses of the extracardiac collaterals were significantly less compared with normal arterioles, but they were thicker in almost all diameter classes when compared with intercoronary collaterals. Thus, smooth muscle mass of the wall of extracardiac coronary collaterals is intermediate between normal vessels and intercoronary collaterals. Figure 2 shows an artist’s rendition of the extracardiac and intercoronary collaterals observed in this study. Both types of collateral arterioles are shown anastomosed to the LCx. The intercoronary collaterals originated from the LAD and from the RC (not shown); the extracardiac collaterals originated from the bronchial, internal mammary, and retrocardiac arteries. The extracardiac vessels anastomosed to each other and to and through the pericardium. In other studies in which the pericardium was not closed after surgery, the left lung adhered to the heart and provided a direct access for the anastomoses of extracardiac collaterals.26

Wall Thickness to Radius Ratios

The calculated wall thickness/radius ratios21,27 were plotted against internal diameter (Figure 3). This measurement was used because it allows the comparison of arterioles of similar though not identical size. Wall thicknesses in all of the collaterals at all time points are significantly less than those of the arterioles (p<0.05) because of the relative lack of smooth muscle growth in the collaterals. These data show that there was little preferential growth to a particular size collateral; i.e., vessel growth was not specific for a particular size.

Coronary and Collateral Blood Flows

Table 3 presents coronary and collateral blood flows measured at rest and during adenosine infusion. Blood flows were measured in the epicardial and endocardial regions (Table 3) and in the midmyocardium (not shown). In addition, we measured the native collateral flow in the sham and ameroid groups at the time of surgery by acute occlusion of the LCx (data not shown) (see “Materials and Methods”). Under operative conditions, the preexisting collateral flows in all of the groups were very low; e.g., blood flows in the sham group were 0.06±0.01 and 0.07±0.01 ml·min⁻¹·g⁻¹ in the endocardium and epicardium, respectively. Nearly identical results were obtained in all of the ameroid groups. During adenosine infusion, the preexisting collateral flows did not increase total flow but did show a shift favoring the epicardium; for instance, in the sham group, blood flows with adenosine were 0.04±0.01 and 0.09±0.01 ml·min⁻¹·g⁻¹ in the endocardium and epicardium, respectively. Again, nearly identical results were obtained in all of the ameroid groups.

In both the sham and ameroid groups, the LAD blood flow (Table 3) represents the flow in the nonischemic region of the heart. In the sham group, blood flow in the LCx region (Table 3) represents the normal flow through the collateral-independent circulation. In contrast, in the ameroid group, this avenue for blood flow has been cut off; thus, the LCx flow is the result of an increase in the collateral-dependent circulation. Comparison of the preexisting collateral flow in the LCx region (0.06±0.01 ml·min⁻¹·g⁻¹; see above) with the collateral flow in the endocardium of the 3-week ameroid group (0.86±0.08 ml·min⁻¹·g⁻¹, Table 3) indicates that collateral-dependent flow has increased 14-fold in the ameroid animals. In addition, there were differences in the endocardial and epicardial blood flows between the sham and ameroid animals. As shown in Table 3, under resting conditions, flows in the sham and 16-week groups were slightly higher in the endocardium than in the epicardium. In contrast, blood flows in the endocardial region of the 3- and 8-week ameroid
groups were lower than blood flows in the epicardial region. The data in Table 3 indicate that the epicardium in the ameroid animals is not ischemic under these experimental conditions. However, several parameters indicate that only the 3-week group experienced a relative underperfusion of the endocardial region during non-vasodilated conditions. Endocardial blood flow decreased in the 3-week group compared with the sham group but then increased in the 8- and 16-week groups. This underperfusion in only the 3-week groups is also indicated by the significantly lower LCx/LAD endocardial flow ratio of 0.66±0.07. In contrast, inspection of the resistance values shows that the endocardium of all of the ameroid groups appears to be underperfused during vasodilated conditions. Resistances measured during adenosine infusion were higher in the endocardium of the ameroid animals compared with the sham animals, which is consistent with the lower endocardial blood flows measured in the ameroid groups and with

![Figure 3](http://circres.ahajournals.org/)

**Figure 3.** The ratio of wall thickness to coronary collateral radius is plotted versus diameter in normal arterioles and in collateral vessels from 3 to 16 weeks after ameroid implantation. There is significant thinning of the walls of the collateral vessels compared with normal arterioles of the same diameter, and there is little increase in wall thickness with time. Values are mean±SEM. *p<0.05 for all collaterals vs. normal arterioles.

**Table 3.** Hemodynamics and Coronary and Collateral Blood Flow

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Experimental animal groups</th>
<th>3 Weeks</th>
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<td>HR (bpm)</td>
<td>Rest</td>
<td>106.0±4.0</td>
<td>115.0±4.0</td>
<td>108.0±5.0</td>
</tr>
<tr>
<td></td>
<td>Adenosine</td>
<td>109.2±4.0</td>
<td>116.5±4.0</td>
<td>109.0±3.0</td>
</tr>
<tr>
<td>BP (mm Hg)</td>
<td></td>
<td>95.0±3.0</td>
<td>98.0±4.0</td>
<td>104.0±4.0</td>
</tr>
<tr>
<td>LCx blood flow (ml·min⁻¹·g⁻¹)</td>
<td>Endo</td>
<td>1.07±0.21</td>
<td>0.96±0.08</td>
<td>1.34±0.18</td>
</tr>
<tr>
<td></td>
<td>Epi</td>
<td>0.94±0.21</td>
<td>1.06±0.18</td>
<td>1.27±0.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.07±0.21</td>
<td>2.69±0.17</td>
<td>1.15±0.09</td>
</tr>
<tr>
<td>LAD blood flow (ml·min⁻¹·g⁻¹)</td>
<td>Endo</td>
<td>1.07±0.26</td>
<td>1.34±0.18</td>
<td>1.44±0.12</td>
</tr>
<tr>
<td></td>
<td>Epi</td>
<td>0.97±0.21</td>
<td>2.66±0.07</td>
<td>1.01±0.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.02±0.07</td>
<td>0.98±0.03</td>
<td>0.66±0.07</td>
</tr>
<tr>
<td></td>
<td>LCx Endo/LAD Endo</td>
<td>0.98±0.02</td>
<td>1.01±0.04</td>
<td>0.98±0.01</td>
</tr>
<tr>
<td></td>
<td>LCx Epi/LAD Epi</td>
<td>0.94±0.19</td>
<td>34±3</td>
<td>115±15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>109±24</td>
<td>20±2</td>
<td>86±10</td>
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<tr>
<td></td>
<td></td>
<td>97±24</td>
<td>34±3</td>
<td>75±11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>107±26</td>
<td>20±1</td>
<td>85±11</td>
</tr>
<tr>
<td>Resistance (mm Hg·ml⁻¹·min⁻¹·g⁻¹)</td>
<td>LCx Endo</td>
<td>94±19</td>
<td>34±3</td>
<td>115±15</td>
</tr>
<tr>
<td></td>
<td>LCx Epi</td>
<td>109±24</td>
<td>20±2</td>
<td>86±10</td>
</tr>
<tr>
<td></td>
<td>LAD Endo</td>
<td>97±24</td>
<td>34±3</td>
<td>75±11</td>
</tr>
<tr>
<td></td>
<td>LAD Epi</td>
<td>107±26</td>
<td>20±1</td>
<td>85±11</td>
</tr>
</tbody>
</table>

HR, heart rate; bpm, beats per minute; BP, blood pressure; LCx, left circumflex coronary artery; Endo, endocardium; Epi, epicardium; LAD, left anterior descending coronary artery. Values are mean±SEM (n=3). See text and methods for description of blood flow measurements and areas. Blood flow was measured in pigs at 8 weeks after sham surgery and in the pigs with ameroid constrictors at the time indicated after ameroid placement. Resistance is calculated as mean BP/blood flow.

*tp<0.05 vs. corresponding BP value at rest.

†p<0.05 vs. corresponding values for sham group.
the observation that blood pressure was not significantly different among the ameroid and sham groups (Table 3). Also, the LCx/LAD endocardial resistance ratios during adenosine infusion were significantly higher for the ameroid groups (ratios are not shown but were calculated from the values presented in Table 3) and parallel the decrease in LCx/LAD endocardial flow ratios. This is consistent with our findings that adenosine-induced decreases in blood pressure were similar in all groups.

Ameroid closure occurs at approximately 3 weeks. Our results suggest that only the 3-week group experienced a relative underperfusion during resting conditions. Also, it occurred only in the endocardial region. By 8 weeks after ameroid placement, "normal" blood flow is present in the occluded bed. It is likely that the difference between the 3-week group and the 8- and 16-week groups in the nonvasodilated endocardial flow ratio values reflects the slightly greater number of large collateral vessels observed in the 8- and 16-week groups (see Table 2). However, it is noteworthy that under conditions of vasodilation all of the ameroid groups were underperfused, implying a lower than normal (i.e., sham) blood flow. This suggests that the bulk of collateral development already has occurred by 3 weeks and that further collateral growth after this time is minimal. This is consistent with the observation that the index of endothelial and smooth muscle cell labeling with $^3^H$-T falls dramatically 2 weeks after ameroid placement (Figure 4).

**Extracardiac Collateral Blood Flow**

Extracardiac collateral blood flow was measured in all groups while the LAD and RC were occluded during an acute open-chest procedure. This procedure allows only an estimate of the flow potential of these arteries; to accurately measure the extracardiac collateral flow, extensive surgical intervention and concomitant disruption of the circulatory architecture would have been required. Extracardiac collateral blood flows were extremely low in the sham animals and dramatically increased in the ameroid groups (Table 4). This suggests (although it does not directly prove) that the extracardiac collaterals are functional. In all of the ameroid groups, the extracardiac collateral blood flows were higher in the epicardium than in the endocardium (Table 4), in parallel with the results shown for total collateral blood flow, i.e., LCx endocardial blood flow (Table 3). We estimate that the fraction of the extracardiac collateral blood flow relative to total collateral

![Figure 4. Bar graph showing the DNA labeling index of smooth muscle and endothelial cells found in the identified coronary collaterals. The DNA labeling index is the percentage of cells labeled as a fraction of all of the cells observed in the same collaterals. The labeling index was highest at 2 weeks and was significantly lower at 3, 4, 6, and 8 weeks (*p<0.05) after coronary ameroid implantation. The DNA labeling indexes for the sham animals were the same at 2 and 8 weeks.](image-url)

**Table 4. Extracardiac Collateral Blood Flow**

<table>
<thead>
<tr>
<th>Experimental animal groups</th>
<th>Sham</th>
<th>3-Week</th>
<th>8-Week</th>
<th>16-Week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endocardial blood flow</td>
<td>0.01±0.01</td>
<td>0.23±0.08*</td>
<td>0.35±0.08*</td>
<td>0.39±0.08*</td>
</tr>
<tr>
<td>(ml·min(^{-1})·g(^{-1}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Epicardial blood flow</td>
<td>0.03±0.01</td>
<td>0.39±0.06*</td>
<td>0.51±0.08*</td>
<td>0.59±0.09*†</td>
</tr>
<tr>
<td>(ml·min(^{-1})·g(^{-1}))</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SEM (n=3).

See text for description of extracardiac collateral blood flow measurements. Blood flow was measured in sham pigs at 8 weeks after surgery and in the pigs with ameroid constrictors at the time indicated after ameroid placement.

*\(p<0.05\) compared with sham group.

†\(p<0.05\) compared with 3-week group.
blood flow is 30–40% (see “Materials and Methods” for calculation).

**Postmortem Findings, Infarct Size, and DNA Labeling**

Infarct sizes were not different among any of the ameroid groups, suggesting that the small degree of infarction occurs at the time of ameroid occlusion. The LCx bed at risk is defined as that region bordered by the convergence of dyes injected into the LAD and LCx. Infarct sizes, as percentages of the LCx bed at risk, were 9.7±0.9%, 2.9±0.08%, and 0.76±0.07% for the endocardial, midmyocardial, and epicardial regions, respectively. The sizes of the regions at risk also were not different among the animals in group 1. The regions at risk comprised approximately 22.2±2.3% (mean value) of the left ventricle.

In general, there is very little cell growth or division in the normal adult heart. To investigate directly the effects of an ischemic stimulus on cell division in the heart, the group 2 pigs with ameroid occluders were injected with ³H-T three times in a 24-hour period before euthanasia. The animals were euthanatized 2, 3, 4, 6, and 8 weeks after surgery. ³H-T is incorporated into the S phase nucleus, which can then be detected by autoradiography. Figure 4 shows the percentages of endothelial and smooth muscle cells in collateral vessels that were labeled with ³H-T (see “Materials and Methods” for description of calculations). Endothelial and smooth muscle cells in both sham animals had an average labeling index of 0.011%. In contrast, there is a 50–70-fold higher labeling index of both endothelial (0.7%) and smooth muscle (0.5%) cells 2–3 weeks after ameroid placement. This index begins to decrease after this time and returns to low levels by 8 weeks. The time of the decrease in the labeling index parallels the time at which the plateau of vasodilated collateral blood flow is reached. Although the labeling index is similar for endothelial cells and smooth muscle cells, this actually represents a decrease in the relative number of smooth muscle cells per blood vessel. Normally there are more smooth muscle cells than endothelial cells in a blood vessel. However, as previously shown, the ischemia-induced collaterals are considerably thinner than the standard arterioles.

Figure 5 shows photomicrographs of representative labeled endothelial and smooth muscle cells. We identified the ³H-T-labeled nuclei by epi-illumination.

**Discussion**

This report describes collateral vessels that exist in the normal pig heart and collateral vessels that develop in pig hearts subjected to LCx occlusion. The results indicate several important features about the composition, location, function, and growth rate of developing collateral vessels. This detailed understanding of the porcine coronary collateral system is important, since swine are considered to be a relevant model for human coronary collateral development in coronary artery disease.

In this study, we used a standard injection technique to identify collateral vessels. There are two reasons supporting our view that most of the collateral vessels with diameters greater than 20 μm were identified. First, similar numbers of collateral vessels were found in all of the animals in each experimental group. Second, collateral blood flow increased as the number of collateral vessels increased. Our determinations of collateral numbers did not include vessels smaller than 20 μm, since they could not be identified consistently. However, it is noteworthy that many small vessels were observed near larger collaterals in the DNA-labeling experiments.

Coronary collateral vessels can be divided into two major classes: those that anastomose to vessels originating outside of the heart (extracardiac) and those that anastomose to branches from the other coronary arteries (intercoronary). Our results indicate that, in swine, the extracardiac collateral vessels occur in the epicardial region. By 16 weeks after ameroid placement, the extracardiac collaterals exhibit a moderate level of smooth muscle development, as evidenced by their
medial wall thickness being 80% of that observed in control arterioles. In contrast, the intercoronary collaterals occur primarily in the midmyocardial and endocardial regions. The smooth muscle development of the intercoronary collaterals is less extensive than that seen in the extracardiac collaterals. Their medial wall thickness is only 50–70% of that observed in control arterioles. It is likely that the deep myocardial location of the intercoronary collaterals affects their function. Ventricular pressure and increased end-diastolic pressures are known to inhibit collateral flow, and intercoronary vessels that lie deep in the myocardium probably are affected more by variations in these forces than are the extracardiac vessels lying on the epicardial surface of the heart.

The pattern of coronary collateral development that we have documented in pigs is similar to the pattern observed in human hearts. Cohen reported that the human myocardium has an extensive network of functionally significant collaterals. Most of these collaterals are intercoronary and are located in the midmyocardial and endocardial regions. Epicardially located extracardiac collaterals are less commonly found, but they have been observed in postmortem studies and in angiograms. Also, it is generally agreed that coronary collaterals in humans lack extensive smooth muscle development even after years of coronary artery disease.

In contrast to the underdeveloped vessels that are found in swine and humans, the coronary collaterals of the dog are well developed, with a medial wall thickness that approaches that of normal arterioles. The rate of medial development in emerging collaterals during the first month after ameroid placement is similar in dogs and pigs. However, in subsequent months, medial wall maturation continues in the dog, whereas collateral vessels in the pig show little further development. After several months the dog’s collateral vessels have a normal amount of medial smooth muscle. This development is used as evidence of a “mature” collateral. According to these criteria, the pig does not develop mature intercoronary collateral vessels. The predicted consequences of this underdevelopment are a decrease in the vasoconstrictive and autoregulatory potential of these vessels. Peters et al. showed that vasopressin induces a relative coronary collateral constriction in dogs with mature coronary collaterals. It seems likely that pig collaterals would respond differently to vasopressin because of their lack of smooth muscle. In fact, our previous work suggests that the undeveloped coronary collaterals in pigs may not respond predictably to vasodilator therapy. We found that ameroid-occluded pigs, which exhibit normal exercise-induced hyperemia at moderate exercise levels, show ischemia at strenuous exercise levels. This suggests that pigs, in contrast to dogs, have a limited collateral reserve that does not increase with vasodilators.

During maximal vasodilation with adenosine infusion, mean aortic pressure decreased to 54–63 mm Hg in both sham-operated and ameroid-occluded animals. Previously, we reported that the pressure-flow relation is linear in the bed at risk in both control and ameroid-occluded animals at aortic perfusion pressures ranging from 25 to 125 mm Hg. Although one should be cautious in interpreting adenosine data when perfusion pressure decreases, the finding of Roth et al. suggests that vessel distensibility has a linear relation with pressure over the pressure ranges observed in our sham-operated and ameroid-occluded animals.

The vasomotor characteristics of developing collateral vessels partly depend on adrenergic receptors. In our previous studies, we showed that the adrenergic innervation of the region at risk and the collateral vessels is normal. Thus, we would expect that the α-adrenergic receptors in the pig coronary collaterals would be normal. Feldman et al. examined the binding characteristics of α-adrenergic receptors in the mature canine coronary collaterals and found them to be similar to those of normal vessels. Other studies by Harrison and Simanetti suggested that the role of the α-adrenergic receptors in collateral vessels is small. Thus, the dominant effect of sympathohumoral stimulation would be vasodilation.

In previous studies, we showed that extracardiac sources of blood flow were important in the perfusion of ischemic swine hearts. In this study, we found that extracardiac sources of collateral flow contribute approximately 30% of the total nonvasodilated collateral flow potential in the bed at risk. Extracardiac vessels are known to play a significant role in normal and pathological human hearts and are positively correlated with increasing age and coronary stenoses. Our results suggest that these vessels are equally as important in the swine model. Indeed, preliminary evidence indicates that extracardiac coronary collaterals may provide complete restoration of coronary blood flow to the ischemic region, particularly if the lung is allowed to adhere directly to the heart.

Our morphometric measurements of the number and sizes of blood vessels and physiological data on blood flow strongly suggest that collateral development plateaus at approximately 8 weeks after placement of the ameroid constrictor on the LCX. Direct evidence for this was obtained by analyzing the level of incorporation of H-T into cells as a function of time after placement of the occluder. The labeling index, a direct indicator of cell division was maximal 2–3 weeks after ameroid placement on the LCX and decreased rapidly in the succeeding weeks for both endothelial cells and smooth muscle cells. Other investigators reported similar results in canine models of collateral development in response to coronary occlusion. It is very likely that the pig and dog respond to similar signals that are generated by the ischemic heart and stimulate DNA synthesis. However, the absolute labeling index was three times greater in the dog.

This may reflect an inherent difference in the angiogenic potentials that exist in the dog and pig. The dog can provide almost complete restoration of blood flow to the ischemic region via newly synthesized or expanding collateral vessels, whereas neovascularization of the ischemic heart in the pig is far more limited. It will be of great interest to determine why blood vessel development ceases after a certain point in pigs and humans.

In conclusion, our findings are summarized as follows: First, intercoronary collateral vessels were characterized by a low level of smooth muscle development, whereas extracardiac collateral vessels contained levels of smooth muscle development that were intermediate between intercoronary collaterals and normal arteri-
oles. Second, intercoronary collateral vessels were prominent in the midmyocardial and endocardial regions and were notably absent from the epicardial region; in contrast, the extracardiac collaterals were located in the epicardial region. Third, extracardiac collaterals supply a significant amount of resting collateral flow (30%). Fourth, total collateral blood flow with adenosine infusion did not increase after 8 weeks of ameroid constriction of the LCx. Fifth, DNA labeling indexes were high in endothelial cells and smooth muscle cells at 2–3 weeks after ameroid placement on the LCx but declined rapidly in the following weeks. Taken together, these data provide an anatomic and biochemical basis for the limited development of coronary collateral circulation in the ischemic pig heart.

Acknowledgments

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