Creation of Anastomoses Between an Extracardiac Artery and the Coronary Circulation

Proof That Myocardial Angiogenesis Occurs and Can Provide Nutritional Blood Flow to the Myocardium

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The purpose of this investigation was to determine whether blood vessels could develop de novo between an extracardiac artery and a collateral-dependent zone of the heart and to quantify the nutritive blood flow afforded by the new vessels. We also adapted the preparation so that angiogenically active agents could be chronically administered directly to the site of neovascularization in subsequent studies. To induce neovascularization between a systemic artery and the coronary circulation, the left internal mammary artery (IMA) was implanted in an intramyocardial tunnel in proximity to the left anterior descending coronary artery (LAD). A tube situated in the distal IMA connected to an implanted pump provided for continuous intra-arterial infusion at the site of angiogenesis. During the same procedure, an ameroid constrictor was placed on the proximal LAD, rendering its perfusion territory collateral dependent during a 2–3 week period. After 8 weeks, the functional capacity of the anastomoses established between the implanted IMA and the LAD territory was assessed by determining regional myocardial blood flow under basal conditions, during adenosine-induced vasodilatation, and during differential occlusions of the IMA and left circumflex coronary artery (LCCA). For all dogs, IMA occlusion decreased maximal LAD territory flow from 1.31±0.11 to 1.16±0.10 ml/min/g (p<0.005). Occlusion of the LCCA decreased LAD zone flow to 0.73±0.12 ml/min/g, whereas occlusion of the IMA in addition to the LCCA further decreased LAD zone flow to 0.42±0.11 ml/min/g (p<0.02). The IMA provided measurable nutritive blood flow in seven of 12 dogs, and in these dogs, the artery provided 30.0±2.5% of total LAD zone collateral conductance under conditions of maximal vasodilatation (range, 23–42%). We conclude that angiogenesis can occur between an implanted internal mammary artery and the native coronary circulation in dogs, providing modest nutritive blood flow to a collateral-dependent region. Further studies will be necessary to determine whether direct, local infusion of angiogenically active factors can enhance neovascularization and whether sufficient flow can be reliably supplied to make some variant of this approach clinically applicable. (Circulation 1990;82:1449–1466)

One of the major problems facing cardiology today is how to more effectively treat the patient with occlusive coronary disease who has incapacitating and refractory symptoms and who has the type of extensive coronary disease that precludes currently available revascularization procedures. As major advances have been made in sequencing and cloning agents involved in normal angiogenic processes during the last several years,1–5 we have been interested in the possible therapeutic application of these angiogenesis factors to facilitate myocardial revascularization. In harnessing angiogenic factors for this purpose, two approaches are feasible: enhancement of intercoronary collateral formation and establishment of vascular connections between an independent, unobstructed source of blood flow and the myocardium. In using the former technique, delivery of collateral flow would be limited both by the resistance of the newly formed vessels and by the resistance of the donor artery itself, which would likely be a significant factor in
patients with obstructive coronary artery disease. Using the latter technique, the flow of blood would be restricted only by the resistance of the newly formed collaterals themselves.

Thus, the goals of the present investigation were to determine whether blood vessels could develop de novo between an extracardiac artery and a collateral-dependent zone of the heart, to quantify the nutritive blood flow afforded by the new vessels, and to determine its importance relative to that provided by collaterals from native coronary arteries. We also attempted to adapt the preparation so that in subsequent studies, angiogenically active agents could be administered directly to the site of neovascularization over an extended period of time.

In developing such a preparation, we thought that the procedure pioneered by Vineberg would provide a reasonable starting point. In this operation, the internal mammary artery (IMA) is tunneled directly into the myocardium with the supposition that anastomoses would form between it and the coronary circulation. By implanting the IMA into the territory of the LAD, and gradually occluding the latter with an ameroid constrictor, an independent artery could be brought into contact with the coronary circulation with the potential, by the process of angiogenesis, to provide nutritional blood flow to a collateral-dependent zone. We devised a method to quantify the collateral blood flow to the territory of the occluded LAD, and determine the relative contributions of the IMA, the left circumflex coronary artery (LCCA) and, by exclusion, all other sources as well. Through a technique based on electrical circuit analyses, we were able to calculate the respective collateral resistances and determine the relations among them.

Methods

This study was undertaken in accordance with the standards set forth by NIH manual 3040-2, “Animal Care and Use in the Intramural Program,” and the NIH “Guide for the Care and Use of Laboratory Animals,” 1985 edition. It is part of a larger study designed to test the efficacy of a potential angiogenic intervention, which will be presented in a separate communication.

Animals

Thirty-four foxhounds of either sex, age 7–18 months, weight 20–40 kg, were used for the studies. Twenty-three animals made up the IMA implanted group (group 1), and eleven dogs constituted the no IMA implanted group (group 2). Group 1 was the control group for a larger investigation designed to study the effects of pharmacological intervention, which will be reported separately.

Surgical Preparation

Prophylactic penicillin (45,000 units/kg i.m.) and gentamicin (4.3 mg/kg i.m.) were given preoperatively. Atropine (0.04 mg/kg i.m.) was administered, and general anesthesia was induced with acepro-

mazine (0.2 mg/kg i.m.), thiamyl sodium (15 mg/kg i.v.), and 0.5–2% inhaled halothane. Dogs underwent thoracotomy through the left sixth intercostal space, and a pericardial cradle was fashioned. The origins of the LAD and LCCA were carefully dissected. A 3-mm ameroid constrictor was placed on the proximal LAD coronary artery, just distal to the first septal perforator. A small polyethylene snare was positioned around the origin of the LCCA.

For group 1 dogs, the left IMA was isolated as a pedicle with its accompanying vein and overlying transversus thoracis muscle, from its origin at the subclavian artery to its bifurcation into the musculophrenic and superior epigastric arteries. After the distal portion of the vessel was doubly ligated and transected, the distal 2–3 cm of the artery was isolated from the pedicle with three or four intercostal branches, which were tied with silk. The vessel was cleaned to the adventitia and cannulated with a Silastic tube, i.d. 0.4 mm, o.d. 2.3 mm (Intermedics Infusaid Corp., Norwood, Mass.). A small hemostat was used to create a 25–30-mm tunnel in the anterior left ventricular wall, in the distribution of the LAD coronary artery. The free end of the tube was pulled through the tunnel, and the proximal artery was temporarily occluded. The intercostal arteries were cut flush with the surface of the IMA, and a retrograde saline injection through the tube ensured unobstructed flow from all branches. The artery was then pulled into the tunnel and allowed to bleed freely into the myocardium. A 2–3-mm portion of the Silastic tube was allowed to remain in an intramyocardial position in the distal tunnel, which was closed with a purse string suture around the tube. The free end of the tube was connected to an infusion pump (Intermedics Infusaid Corp.), which was implanted subcutaneously in the lateral abdominal wall through a separate incision. Pumps were refilled percutaneously with 0.9% saline at regular intervals, providing continuous flow throughout the duration of the experiment. Models 500, 200, and 400 were used, with reservoirs of 25, 35, and 50 ml, respectively. Depending on the particular pump, flow rate ranged from 0.88 to 2.73 ml/day.

Group 2 dogs (no implanted IMA) underwent placement of an ameroid on the LAD, but the IMA was left in situ on the chest wall and was not inserted into the myocardium. A pump was not implanted in the abdominal wall.

Final Study

After 8 weeks, animals in group 1 underwent a terminal study to ascertain the maximal functional capacity of the implanted internal mammary artery with its associated microvasculature. Group 2 dogs underwent a similar study, to determine the maximal collateral flow to the LAD zone. Dogs were anesthetized with morphine sulfate (3.0 mg/kg i.m.) and α-chloralose (80 mg/kg i.v. bolus with 0.25–0.35 mg/kg/min maintenance), and mechanically venti-
lated with room air and supplemental oxygen (1–2 l/min) to maintain arterial blood gases in the physiolog-
ical range. The lead II electrocardiogram was continuously monitored. Fluid-filled catheters were
connected to Statham P23Db transducers and inserted into the ascending aorta and left ventricle
through the left carotid and left femoral arteries, respectively, for continuous monitoring of aortic and
left ventricular pressures. Mean aortic blood pressure was obtained by electronic filtering. The chest
was opened in the fourth left intercostal space, and a dual lumen catheter was inserted in the left atrial
appendage for infusion of adenosine and tracer microspheres. The proximal portion of the IMA and
the LCCA snare were isolated for subsequent occlusions. A catheter was placed in the right femoral
artery for withdrawal of microsphere reference blood samples. The dog was anticoagulated with heparin,
5,000 units i.v. followed by a 500 units/hr maintenance drip. Prophylactic lidocaine, 1 mg/kg i.v. was
given before the first microsphere injection, followed by a 2-mg/min continuous infusion. An additional 1
mg/kg i.v. dose was administered before occlusions of the LCCA.

Tracer microspheres, 15 μm in diameter, sus-
pended in 10% dextrose with 0.01% Tween-80 were
used for the determination of regional myocardial
blood flow. Five of six gamma emitting radionuclides were randomly used: 125I, 85Sr (3M Company), 141Ce,
113Sn, 99Nb, and 46Sc (New England Nuclear). Vials
were vortex agitated for 3 minutes, and approxi-
mately 1 million microspheres in a volume of 0.4–0.7
ml were diluted with saline to a volume of 2 ml and
injected into the left atrial appendage over a 5–10-
second period, followed by a 10-ml saline flush.
Arterial occlusions were performed 15 seconds before isotope injection. Reference samples were
withdrawn from the abdominal aorta at a rate of 7.75
ml/min.

Regional myocardial blood flow was first quanti-
ﬁed under basal conditions with a microsphere injec-
tion. Adenosine was then infused continuously into
the left atrium at 0.16–0.70 μmol/kg/min to effect a
15–20% fall in mean arterial pressure or a 50% in-
crease in heart rate. When a steady state was
reached, flow and pressure determinations were
made under three circumstances: 1) with the IMA
and the LCCA patent, 2) during IMA occlusion, and
3) during LCCA occlusion. In later experiments, an
additional microsphere injection was made during
simultaneous occlusion of the IMA and LCCA. If
signiﬁcant cardiac arrhythmias occurred during a
microsphere injection, measurements were repeated
with a second isotope.

After all microsphere blood flow measurements
were completed, a square-wave electromagnetic flow
probe (model 501, Carolina Medical Electronics,
Inc., King, N.C.) was applied to the IMA just prox-
mal to the heart. Basal IMA blood flow was deter-
mined after the adenosine infusion was stopped and
the heart rate and blood pressure had returned to
baseline. The adenosine infusion was resumed, and
maximal IMA blood flow was recorded.

In group 2 dogs, microspheres were injected under
basal conditions, during adenosine infusion and dur-
ing adenosine with occlusion of the LCCA.

Animals were killed with an overdose of sodium
pentobarbital and potassium chloride. Hearts were
fixed for 48–72 hours in 10% buffered formalin,
weighed, and cut with a template into seven or eight
7-mm slices parallel to the atrioventricular groove.
From the middle four slices, the right ventricle,
papillary muscles, and epicardial fat were removed.
These were divided into 12 wedges (average mass,
1.34 grams), which were subdivided into endocardial
and epicardial portions. Samples were weighed and
counted together with the reference blood samples in
a well-type NaI multichannel scintillation counter
(Packard Autogamma Spectrometer). Counts in the
windows of interest corresponding to the peak emis-
sions of each isotope were corrected for background,
interchannel crossover, and decay. Myocardial blood
flow was calculated according to standard methods.7

Calculation of Collateral Conductance

As a consequence of LAD coronary artery occlu-
sion, several sources of collateral flow potentially
develop to the LAD territory: collaterals from the
IMA, the LCCA, and other sources, including sepal
branches of the LAD coronary artery proximal to the
am eroid device, branches of the right coronary
artery, and endomural vessels from the left ventric-
ular cavity. We thought it essential to calculate each
of these vascular resistances speciﬁcally because
small, but deﬁnite, flow contributions from the IMA
could be overshadowed by abundant intercoronary
anastomoses from the LCCA or other vessels.

By considering the myocardial vascular network as
an electrical circuit (Figures 2A and B), the mini-
imum vascular resistances between each collateral
source and the LAD coronary artery bed could be
calculated. Quantification of microsphere flow dis-
tributed to the LAD and LCCA perfusion zones
during conditions of adenosine-induced vasodilata-
tion, with concomitant measurement of arterial blood
pressure during the four situations described above
(IMA and LCCA patent, IMA occluded and LCCA
patent, IMA patent and LCCA occluded, IMA and
LCCA occluded) generated four sets of equations
that could be solved simultaneously for ﬁve resis-
tances of interest: 1) the resistance of IMA related
vessels to the LAD perfusion territory, designated
RIMA-LAD, 2) the resistance between the LCCA and
the LAD territory, termed R LCCA-LAD, 3) the resis-
tance of sources of perfusion to the LAD territory
independent of the IMA and the LCCA (i.e., collat-
ers from the proximal LAD coronary artery, right
coronary artery), designated RTH-LAD, 4) the resis-
tance of the pathway between the IMA implant and
the LCCA, RIMA-LCCA, and 5) the resistance of the
arteriolar bed of the control zone, RBL (see appendix
for details). In some dogs, data were not obtained
during simultaneous occlusion of the IMA and LCCA. In these cases, it was not possible to distinguish perfusion through the LCCA from perfusion through sources independent of both the IMA and LCCA. Thus, the resistance between all coronary collateral sources and the LAD zone, independent of the IMA, was designated $R_{\text{COR-LAD}}$, encompassing the resistances $R_{\text{LCCA-LAD}}$ and $R_{\text{OTH-LAD}}$ in parallel.

In group 2 animals, measurement of microsphere blood flow in the LAD and the control zones before and during LCCA occlusion generated a set of equations that could be solved to determine four resistances: 1) the LCCA to LAD collateral resistance, termed $R_{\text{LCCA-LAD}}$, 2) the resistance of all other sources to the LAD territory, independent of the LCCA, defined $R_{\text{OTH-LAD}}$, 3) the resistance of perfusion sources to the control area independent of both the LAD and LCCA, termed $R_{\text{IND}}$, and 4) the control zone arteriolar bed resistance, again defined $R_{\text{NL}}$.

To prevent the potentially confounding effects of autoregulation, maximal coronary vasodilatation was induced with an intra-atrial infusion of adenosine, ensuring a linear relation between coronary perfusion pressure and flow. All resistances were, therefore, determined at a minimum value dictated by anatomical structure, avoiding potential autoregulatory alterations in arterial resistance and coronary blood flow which might occur in response to LCCA and IMA occlusions.

**Reporting of Data**

In situations where no flow occurred between two points despite a pressure gradient, resistance was infinite. From a mathematical and statistical standpoint, it was inconvenient to manipulate resistances of infinity, whereas matters were greatly simplified by using conductances in computations. Thus, for reasons of practicality and convenience, data were expressed as the reciprocal of resistance, that is, conductance, with units of ml$\cdot$min$^{-1}\cdot$100g$^{-1}\cdot$mm Hg$^{-1}$. Thus, $R_{\text{IMA-LAD}}$ was expressed as $C_{\text{IMA-LAD}}$, $R_{\text{LCCA-LAD}}$ as $C_{\text{LCCA-LAD}}$, $R_{\text{OTH-LAD}}$ as $C_{\text{OTH-LAD}}$, $R_{\text{IMA-LCCAL}}$ as $C_{\text{IMA-LCCA}}$, $R_{\text{NL}}$ as $C_{\text{NL}}$, $R_{\text{COR-LAD}}$ as $C_{\text{COR-LAD}}$ and $R_{\text{IND}}$ as $C_{\text{IND}}$.

**Sample Selection and Calculation of Regional Myocardial Blood Flow**

Of the 48 original wedges, eight wedges, each consisting of an endocardial and epicardial portion, were preliminarily selected to represent the control zone. This selection was based on functional criteria: the decrement in flow that occurred as a result of LCCA occlusion. Samples representative of the LCCA territory maintained normal vasodilator reserve, exhibiting fourfold to fivefold flow increases in response to adenosine while displaying extremely low flows during LCCA occlusion. Thus, during adenosine stimulation, the ratio of flow during LCCA occlusion to flow with the LCCA (and IMA) patent

**FIGURE 1.** Model of ischemia and revascularization procedure. See text for full description.

**FIGURE 2.** Circuit analysis of situation when IMA and LCCA are patent. See text for definitions and explanation.

\[ P = \text{mean arterial pressure}, \quad R = R_{\text{NL}} = 1/C_{\text{NL}}, \quad X = R_{\text{IMA-LAD}} = 1/C_{\text{IMA-LAD}}, \quad Y = R_{\text{IMA-LCCA}} = 1/C_{\text{IMA-LCCA}}, \quad C = R_{\text{LCCA-LAD}} = 1/C_{\text{LCCA-LAD}}, \quad Z = R_{\text{OTH-LAD}} = 1/C_{\text{OTH-LAD}}. \]
was calculated for each of the 48 wedges according to the formula:

\[
\text{Flow ratio} = \frac{\text{Inner flow} \times \text{Inner mass} + \text{Outer flow} \times \text{Outer mass}}{\text{Inner mass} + \text{Outer mass}}
\]

The eight wedges with the lowest flow ratios were selected to represent the control zone. Then, the mean and standard deviation of the eight flow ratios were calculated, and wedges with flow ratios greater than two standard deviations from the mean of the group were rejected.

For the LAD perfusion territory, we sought to select transmural samples from the central ischemic zone and to reject samples with evidence of subendocardial infarction/fibrosis. Inadvertent inclusion of small portions of normally perfused tissue in purportedly ischemic zone samples due to interdigitating boundaries has been shown to constitute a major source of error in the determination of collateral flow during adenosine-induced vasodilation. For this reason, blood flow criteria, rather than anatomic criteria, were used to select homogeneous sample groups representative of the central ischemic zone. Because the LAD coronary artery was occluded, wedges in its perfusion territory exhibited the most compromised vasodilator reserve. Therefore, the LAD zone was preliminarily defined as the group of eight wedges with the lowest flow during adenosine stimulation. Because the IMA could potentially contribute flow to LAD zone samples, the selection was made from flows determined during IMA occlusion. The transmural flow of each wedge was calculated as the weighted average flow of the endocardial and epicardial portions

\[
\text{Flow (Wedge)} = \frac{\text{Inner flow} \times \text{Inner mass} + \text{Outer flow} \times \text{Outer mass}}{\text{Inner mass} + \text{Outer mass}}
\]

and the eight wedges with the lowest flow were then preliminarily selected. To exclude wedges with subendocardial fibrosis, the endocardial/epicardial (endo/epi) flow ratio was calculated for each of the eight wedges under basal conditions and compared with the mean endo/epi flow ratio of the group of previously selected control zone wedges, also under basal conditions. ischemic zone wedges with endo/epi flow ratios greater than two standard deviations from the mean of the control zone flow ratios were rejected.

Once ischemic zone (IZ) and normal zone (N Z) samples were selected, the average regional flows during each tracer microsphere injection were computed from the inner and outer portions of all of the selected wedges together by the formula:

\[
\text{Mean flow (ml/min/g)} = \frac{\sum (\text{Mass (g)} \times \text{Flow (ml/min/g)})}{\sum \text{Mass (g)}}
\]

Thus, both the mean IZ and NZ flows represented the composite average flow of approximately six to eight transmural wedges, or 12 to 16 tissue samples, weighted for the mass of each sample.

IZ/NZ Blood Flow Ratios

NZ blood flow in the maximally vasodilated state is directly and linearly related to mean arterial pressure (MAP) and should be unaffected by IMA occlusion, yet NZ blood flows obtained before and during IMA occlusion differed by as much as 15%. This variation could be accounted for by differences in MAP prevailing at the time of the two microsphere injections, fluctuations in MAP that occurred during microsphere injections, or errors in arterial reference sample collection. To increase our ability to detect small contributions in collateral flow by the IMA, these variables were minimized by using IZ/NZ flow ratios to determine the impact of IMA occlusion on IZ blood flow. The IZ/NZ flow ratios are equivalent to counts per unit mass in the IZ divided by counts per unit mass in the NZ, independent of the activity of the arterial reference sample, because the blood flows in both regions are a function of the same reference sample:

\[
\text{IZ/NZ flow ratio} = \frac{\text{Flow}_{iz}}{\text{Flow}_{nz}} = \frac{\text{Counts}_{iz}/\text{Mass}_{iz}}{\text{Counts}_{nz}/\text{Mass}_{nz}}
\]

Because the ratio is determined during adenosine-induced maximal coronary vasodilatation, it is also independent of MAP. By dividing the IZ/NZ flow ratio obtained during IMA occlusion by that obtained with the IMA patent, the importance of the IMA to IZ collateral blood flow can be determined in individual dogs. This quantity we defined as the IMA flow ratio:

\[
\text{IMA flow ratio} = \frac{\text{Counts}_{iz}/\text{Mass}_{iz}}{\text{Counts}_{nz}/\text{Mass}_{nz}}
\]

Because the ratio is determined during adenosine-induced maximal coronary vasodilatation, it is also independent of MAP. By dividing the IZ/NZ flow ratio obtained during IMA occlusion by that obtained in the IMA patent, the importance of the IMA to IZ collateral blood flow can be determined in individual dogs. This quantity we defined as the IMA flow ratio:

\[
\text{IMA flow ratio} = \frac{\text{Counts}_{iz}/\text{Mass}_{iz}}{\text{Counts}_{nz}/\text{Mass}_{nz}}
\]

and

\[
\text{IMA flow ratio} = \frac{\text{Counts}_{iz}/\text{Mass}_{iz}}{\text{Counts}_{nz}/\text{Mass}_{nz}}
\]
Thus, the IMA flow ratio is independent of arterial reference sample collection, MAP, and tissue mass. In use, an IMA flow ratio of 0.80 corresponds to a 20% reduction in maximal IZ collateral flow during IMA occlusion, relative to maximal NZ flow. As the contribution of the IMA progressively decreases, the IMA flow ratio approaches unity.

Normalization of Blood Flows

As noted above, NZ blood flows with the IMA patent and the IMA occluded differed by as much as 15%. Because maximal IZ blood flow is a function of the same MAP and arterial reference sample as the NZ blood flow, we deduced that the IZ blood flows obtained with the IMA patent and occluded were subject to the same variation as the NZ blood flows (as much as 15%) and that the variation in IZ blood flow paralleled that in the NZ. Because the contribution of the IMA to IZ collateral flow was within this range of variation, it was necessary to correct the raw IZ blood flows using the NZ blood flow as a reference. This process of normalization is explained as follows: The maximal NZ conductance was computed by dividing the maximal NZ blood flow by arterial pressure, and the values obtained with the IMA both patent and occluded (which should theoretically be equivalent) were averaged. Thus, given an NZ flow of Q1 at a mean pressure of P1 with the IMA patent, and an NZ flow of Q2 at a mean pressure of P2 during IMA occlusion, the respective conductances (before and during IMA occlusion) are Q1/P1 and Q2/P2. The mean conductance is, therefore, (Q1/P1 + Q2/P2)/2, representing the best estimate of NZ conductance. To normalize the NZ blood flows, the mean conductance (which is theoretically unchanged by IMA occlusion) was multiplied by MAP during both of the microsphere injections. Thus, normalized flow with the IMA patent = P1 × (Q1/P1 + Q2/P2)/2, and normalized flow with the IMA occluded = P2 × (Q1/P1 + Q2/P2)/2. For example, with the IMA patent, a dog with an NZ flow of 3.8 at a mean pressure of 100 mm Hg has an NZ conductance of 0.038. In the same dog during IMA occlusion, an NZ flow of 4.40 obtained at a mean pressure of 110 mm Hg represents an NZ conductance of 0.040. The best estimate of the conductance is the mean of the two values, or 0.039. Normalized NZ blood flows would be obtained by multiplying 0.039 by the MAP obtained before and during IMA occlusion: normalized NZ blood flow (IMA patent) = 0.039 × 100 = 3.90, normalized NZ blood flow (IMA occluded) = 0.039 × 110 = 4.29.

Ischemic zone blood flows were also corrected; the same factors that were used to correct NZ blood flows were used to correct IZ blood flows, preserving the IZ/NZ blood flow ratio in each case. For a given isotopic injection, the IZ/NZ blood flow ratio is immune to errors in arterial reference sample collection and real or perceived differences in MAP (as discussed above) and, therefore, is of primary importance in determining the impact of IMA occlusion on IZ blood flow. Thus, the raw IZ flows were normalized by multiplying by the normalized NZ flow and dividing by the unnormalized NZ flow. To continue with the above example, normalized IZ blood flow with the IMA patent would be obtained by multiplying the raw IZ blood flow by the factor 3.90/3.80 (since normalized NZ flow with the IMA patent was increased by this factor). Likewise, normalized IZ blood flow with the IMA occluded would be obtained by multiplying the raw IZ blood flow by the factor 4.29/4.40 (since normalized NZ flow with the IMA occluded was decreased by this factor). In both cases, the IZ/NZ ratio is left unchanged. Unless otherwise stated, the transmural blood flows reported during adenosine infusion before and during IMA occlusion are normalized, and conductance calculations were based on normalized flows. Because LCCA occlusion altered blood flows in both the IZ and NZ, blood flows obtained during LCCA could not be normalized, and the raw blood flow data are reported.

Graphic Representation of Regional Blood Flow

For all dogs, the transmural blood flow for each of the 48 individual wedges was computed during each intervention. The average blood flow per unit myocardial mass was calculated (Equation 2), and graphs of transmural myocardial blood flow versus position were constructed for each of the four slices (Figures 3A and B). These graphs were helpful in visually depicting the locations of the ischemic and control zones, gauging the regional flow contributions of the IMA and LCCA, and assessing the consequences of IMA occlusion, with and without concomitant LCCA occlusion. In addition, three-dimensional graphs depicting the location and extent of the IMA flow contribution were generated for each dog (Figures 4A-M). For all 48 transmural wedges (96 tissue samples), the blood flows obtained during adenosine stimulation with the IMA occluded were subtracted from the flows obtained with the IMA patent. Subtraction of the two matrices produced a third matrix that provided quantitative spatial information regarding the role of the IMA in individual dogs.

Statistical Analysis

All values are expressed as mean±SEM. Comparisons between two groups were made using two-tailed Student’s t tests for paired or unpaired observations, as appropriate. The correlation between IMA blood flow (measured by flowmeter) and the IMA flow ratio was assessed by linear regression analysis with a least-squares method. A p value<0.05 was used to define statistical significance in all cases.

Success of Revascularization

In each dog, the contribution of the IMA to LAD zone perfusion was evaluated by assessing the impact of IMA occlusion on LAD zone flow. Thus, for each of the selected LAD zone wedges, the maximal conductance during adenosine infusion (transmural blood flow per wedge divided by mean arterial pres-
Mortality

Eleven of the 34 original dogs died before final study. Nine dogs died within 48 hours of initial surgery and were considered technical failures. Late death occurred in two group 1 dogs, 7 and 17 days after operation. There were no late deaths in the non-IMA implanted dogs (group 2). One group 1 dog developed ventricular fibrillation at the time of final study, and another group 1 dog received an inadequate dose of adenosine and was excluded. Data from the 21 remaining animals constitute the basis for this report: 12 group 1 dogs and nine group 2 dogs.

Hemodynamics

Resting heart rate and mean arterial pressure were similar in both groups (Table 1). Adenosine infusion increased heart rate and decreased blood pressure significantly in both groups, and there were no differences in heart rate or mean arterial pressure during adenosine infusion between the two groups. In group 1 dogs, IMA occlusion did not cause any significant change in either heart rate or mean arterial pressure. Occlusion of the LCCA reduced heart rate and mean arterial pressure in both groups, reaching statistical significance only in group 2. Heart rate and mean arterial pressure obtained during simultaneous IMA and LCCA occlusion in group 1 were not different from measurements obtained during occlusion of the LCCA alone.

Regional Myocardial Blood Flow

Under basal conditions, regional myocardial blood flow was comparable in the two groups, both in the control and LAD zones (Table 2). In both groups,
MYOCARDIAL BLOOD FLOW (ml/min/g)

![Graph showing myocardial blood flow](image)

**Figure 4.** Three-dimensional representation of regional myocardial blood flow, and demonstration of the location and extent of IMA-derived collateral perfusion. Transmural myocardial blood flow (ml · min⁻¹ · g⁻¹) is plotted on the Z axis, and the slices and wedges are shown on the X and Y axes, respectively. Slice 1 is toward the apex; slice 4 is toward the base. Wedges 1 and 12 are contiguous. Panel A: Regional myocardial blood flow in a single representative dog during adenosine with both the IMA and LCCA patent. Vasodilator reserve is limited in the LAD territory (wedges 4 and 5 of all slices) because of the presence of the ameroid constrictor on the LAD. Panels B–M: For the 12 group 1 dogs, the mean blood flow of each of the 48 wedges (96 total endocardial and epicardial samples) is computed with the IMA occluded and subtracted from the blood flow with the IMA patent (both during adenosine). Plots reveal the distribution and magnitude of the IMA-derived collateral blood flow for each dog. IMA, internal mammary artery; LAD, left anterior descending coronary artery; LCCA, left circumflex coronary artery.

LAD zone flow was significantly lower than normal zone flow under basal conditions. During adenosine infusion, similar increases in regional myocardial blood flow occurred in both groups: adenosine increased normal zone flow by approximately 470% in both groups, while increasing LAD zone flow by 240% in both groups. Thus, vasodilator reserve was comparable in the two groups, both in the collateral dependent zone and in the normal zone.

**Effect of IMA Occlusion on Regional Blood Flow**

IMA occlusion decreased maximal LAD territory flow from 1.31±0.11 to 1.16±0.10 ml/min/g (p<
TABLE 1. Hemodynamic Data

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<tr>
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<th>Baseline</th>
<th>Adenosine IMA occluded</th>
<th>Adenosine LCCA occluded</th>
<th>Adenosine IMA+LCCA occluded</th>
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<td>Group 1, IMA implanted (n=12)</td>
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<tr>
<td>HR (beats/min)</td>
<td>83±15</td>
<td>139±8*</td>
<td>128±9</td>
<td>134±9</td>
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<td>MAP (mm Hg)</td>
<td>96±5</td>
<td>80±3*</td>
<td>76±4</td>
<td>78±2</td>
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<td>Group 2, IMA not implanted (n=9)</td>
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<tr>
<td>HR (beats/min)</td>
<td>83±2</td>
<td>144±9*</td>
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</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>105±6</td>
<td>83±5*</td>
<td>68±4</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SEM.
IMA, internal mammary artery; LCCA, left circumflex coronary artery; HR, heart rate; MAP, mean arterial pressure.
*Value is significantly different from baseline, p<0.05. †Value is significantly different from adenosine with the LCCA patent, p<0.05.

In dogs where simultaneous occlusion of the IMA and LCCA was performed, occlusion of the IMA in addition to the LCCA occlusion decreased LAD zone flow by 42%, from 0.73±0.12 to 0.42±0.11 ml/min/g (p<0.02, Figure 5B).

TABLE 2. Regional Myocardial Blood Flow (ml/min/g) and IMA Flow Ratio

<table>
<thead>
<tr>
<th>Dog</th>
<th>LAD zone</th>
<th>Control zone</th>
<th>IMA flow ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BL</td>
<td>ADO</td>
<td>IMA</td>
</tr>
<tr>
<td>GROUP 1, IMA implanted (n=12)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.45</td>
<td>0.94</td>
<td>0.92</td>
</tr>
<tr>
<td>2</td>
<td>0.62</td>
<td>1.27</td>
<td>1.13</td>
</tr>
<tr>
<td>3</td>
<td>0.94</td>
<td>0.84</td>
<td>0.89</td>
</tr>
<tr>
<td>4</td>
<td>0.37</td>
<td>0.92</td>
<td>0.73</td>
</tr>
<tr>
<td>5</td>
<td>0.31</td>
<td>2.08</td>
<td>1.61</td>
</tr>
<tr>
<td>6</td>
<td>0.42</td>
<td>0.93</td>
<td>0.83</td>
</tr>
<tr>
<td>7</td>
<td>0.44</td>
<td>1.13</td>
<td>1.07</td>
</tr>
<tr>
<td>8</td>
<td>0.35</td>
<td>1.74</td>
<td>1.80</td>
</tr>
<tr>
<td>9</td>
<td>0.77</td>
<td>1.49</td>
<td>1.25</td>
</tr>
<tr>
<td>10</td>
<td>0.33</td>
<td>1.27</td>
<td>1.02</td>
</tr>
<tr>
<td>11</td>
<td>0.92</td>
<td>1.79</td>
<td>1.53</td>
</tr>
<tr>
<td>12</td>
<td>0.57</td>
<td>1.33</td>
<td>1.09</td>
</tr>
<tr>
<td>Mean</td>
<td>0.54*</td>
<td>1.31*</td>
<td>1.16*</td>
</tr>
<tr>
<td>SEM</td>
<td>0.07</td>
<td>0.11</td>
<td>0.10</td>
</tr>
</tbody>
</table>

| GROUP 2, IMA not implanted (n=9) | | | | | | | | | |
| S1  | 0.85 | 1.41 | 0.36 | 0.83 | 3.71 | 0.23 |      |      |      |      |          |
| S2  | 0.61 | 1.27 | 0.36 | 1.08 | 5.07 | 0.05 |      |      |      |      |          |
| S3  | 0.68 | 1.39 | 0.80 | 0.81 | 2.30 | 0.09 |      |      |      |      |          |
| S4  | 0.45 | 0.68 | 0.21 | 0.68 | 1.81 | 0.19 |      |      |      |      |          |
| S5  | 0.46 | 2.13 | 0.85 | 0.62 | 4.10 | 0.50 |      |      |      |      |          |
| S6  | 0.33 | 0.64 | 0.19 | 0.37 | 1.36 | 0.06 |      |      |      |      |          |
| S7  | 0.65 | 1.30 | 0.15 | 0.60 | 3.04 | 0.03 |      |      |      |      |          |
| S8  | 0.48 | 1.07 | 0.25 | 0.49 | 2.47 | 0.04 |      |      |      |      |          |
| S9  | 0.32 | 1.87 | 0.62 | 0.84 | 5.99 | 0.04 |      |      |      |      |          |
| Mean| 0.54*| 1.31*| 0.42*| 0.70 | 3.32 | 0.14 |      |      |      |      |          |
| SEM | 0.06 | 0.16 | 0.09 | 0.07 | 0.51 | 0.05 |      |      |      |      |          |

*Significantly different from control zone flow.
Mean values are reported as ml/min/g wet mass.
LAD, left anterior descending coronary artery; BL, baseline flow; ADO, adenosine flow; IMA, flow during adenosine with internal mammary artery occluded; LCCA, flow during adenosine with left circumflex coronary artery occluded; Dual, flow during adenosine with internal mammary and left circumflex coronary artery occluded.

IMA Flow Ratio

The mean IMA flow ratio for the group of dogs with IMA implants was 0.90±0.03 (range, 0.79–1.08). Thus, on average, the IMA provided 10% of maximal IZ collateral flow, relative to NZ blood flow.
IMA Blood Flow (Flowmeter Method)

IMA blood flow could not be quantified in dog 1 because of technical difficulties. Under basal conditions, mean blood flow was 7.8 ± 1.1 ml/min in dogs 2–12 (range, 3–15 ml/min, Table 3). During adenosine infusion, blood flow increased to a mean of 14.4 ± 2.7 ml/min (range, 3–30 ml/min), despite a 9% drop in mean arterial pressure. By dividing mean arterial pressure by the maximal blood flow, the minimum vascular resistance could be calculated for each dog (Table 3, right). Minimum vascular resistance, based on flowmeter blood flows, correlated well with IMA flow ratio, derived from microsphere flow measurements (r=0.86, p<0.001; Figure 6).

Conductance Data

Simultaneous IMA + LCCA occlusion was attempted only in the last nine of the 12 group 1 dogs studied, and technical problems precluded the use of the dual occlusion data in two dogs. Thus, Table 4 summarizes conductance data from the seven dogs in which dual IMA and LCCA occlusion was successful, and compares the conductances obtained with and without dual IMA and LCCA occlusion. In general, omission of the simultaneous occlusion yielded slightly lower values for CIMA-LAD. In all cases, the discrepancy between methods was between 8% and 18%. Conductances between the IMA and LCCA (CIMA-LCCA) were generally higher when calculated with the dual occlusion data.

### Table 3. Internal Mammary Blood Flow by Electromagnetic Flow Probe and Internal Mammary Artery Vascular Resistance

<table>
<thead>
<tr>
<th>Dog</th>
<th>Baseline flow (ml/min)</th>
<th>MAP (mm Hg)</th>
<th>Baseline resistance (mm Hg/ml/min)</th>
<th>Adenosine flow (ml/min)</th>
<th>MAP (mm Hg)</th>
<th>Minimum resistance (mm Hg/ml/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>91</td>
<td>13.0</td>
<td>6</td>
<td>70</td>
<td>11.7</td>
</tr>
<tr>
<td>3</td>
<td>4</td>
<td>76</td>
<td>19.0</td>
<td>6</td>
<td>80</td>
<td>13.3</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>96</td>
<td>19.2</td>
<td>18</td>
<td>92</td>
<td>5.1</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>105</td>
<td>13.1</td>
<td>22</td>
<td>100</td>
<td>4.5</td>
</tr>
<tr>
<td>6</td>
<td>3</td>
<td>60</td>
<td>20.0</td>
<td>3</td>
<td>40</td>
<td>13.3</td>
</tr>
<tr>
<td>7</td>
<td>9</td>
<td>98</td>
<td>10.9</td>
<td>12</td>
<td>85</td>
<td>7.1</td>
</tr>
<tr>
<td>8</td>
<td>6</td>
<td>100</td>
<td>16.7</td>
<td>5</td>
<td>90</td>
<td>18.0</td>
</tr>
<tr>
<td>9</td>
<td>7</td>
<td>110</td>
<td>15.7</td>
<td>15</td>
<td>97</td>
<td>6.5</td>
</tr>
<tr>
<td>10</td>
<td>8</td>
<td>93</td>
<td>11.6</td>
<td>16</td>
<td>85</td>
<td>5.3</td>
</tr>
<tr>
<td>11</td>
<td>15</td>
<td>112</td>
<td>7.5</td>
<td>25</td>
<td>120</td>
<td>4.8</td>
</tr>
<tr>
<td>12</td>
<td>14</td>
<td>90</td>
<td>6.4</td>
<td>30</td>
<td>80</td>
<td>2.7</td>
</tr>
<tr>
<td>Mean</td>
<td>7.8</td>
<td>93.7</td>
<td>13.9</td>
<td>14.4</td>
<td>85.4</td>
<td>8.4</td>
</tr>
<tr>
<td>SEM</td>
<td>1.1</td>
<td>4.5</td>
<td>1.4</td>
<td>2.7</td>
<td>6.0</td>
<td>1.5</td>
</tr>
</tbody>
</table>
For any number of resistances in parallel, the reciprocal of the net resistance equals the sum of the reciprocals of the individual resistances. Because conductance is the reciprocal of resistance, the total conductance of parallel sources can be obtained by simple addition. Therefore, total conductance to the ischemic zone = $\frac{1}{C_{\text{IMA-LAD}}} + \frac{1}{C_{\text{LCCA-LAD}}} + \frac{1}{C_{\text{OTH-LAD}}}$, and the proportion of flow arriving at the ischemic zone from each source is directly proportional to its term. For example, the IMA, LCCA, and other sources were of roughly equal importance in providing flow to the ischemic zone in dog 4 (Table 4, right). In dog 8, the LCCA and other sources each contributed approximately half of the flow to the LAD territory, whereas the IMA provided no flow. The IMA was singularly the most important source of collateral flow to the LAD zone in one of the seven dogs (dog 10). In two, the LCCA was of greatest significance, and in two others, sources independent of the IMA and LCCA were most important.

Table 5 summarizes conductance data from all 21 dogs, including group 2 dogs and group 1 dogs without simultaneous IMA and LCCA occlusion data. Conductance of the LCCA arteriolar bed ($C_{\text{NL}}$) was similar in both groups, with means of 3.88 and 3.91 ml · min⁻¹ · 100g⁻¹ · mm Hg⁻¹. In the non-IMA implanted dogs (group 2), the LCCA was the predominant source of LAD zone collateral flow (i.e., $C_{\text{LCCA-LAD}}>C_{\text{OTH-LAD}}$) in all dogs except dog S3, in which sources independent of the LCCA provided the majority of collateral flow to the LAD territory. During LCCA occlusion, collateral conductance to the LCCA territory was quite limited (i.e., $C_{\text{IND}}$ was generally insignificant).

In group 1 dogs, IMA occlusion did not bring about a statistically significant drop in the LAD zone flow/pressure ratio (i.e., LAD zone conductance), either with or without concomitant LCCA occlusion in five of 12 dogs. In these cases, $C_{\text{IMA-LAD}}$ was assigned a value of zero.

By comparing $C_{\text{IMA-LAD}}$ to $C_{\text{COR-LAD}}$, the importance of the IMA-derived collateral flow could be gauged relative to the magnitude of the collateral conductance arising from native intercoronary vessels. In the seven dogs with successful IMA to coronary collateral formation (Table 5), the artery provided a relatively constant 30.0±2.5% of total LAD zone collateral conductance under conditions of maximal vasodilatation (range, 23% to 42%).

**Discussion**

The recent purification and characterization of several polypeptides capable of inducing new blood vessel growth could potentially launch promising new modalities of treatment for patients with ischemic syndromes: angiogenic therapy. With our ultimate goal the utilization of angiogenic factors to promote myocardial revascularization, we first sought to prove...
that neovascularization could occur between an extracardiac artery and a collateral-dependent zone of the heart. If this process could be substantiated, then it could provide the basis for the exploitation of angiogenic agents for the amelioration of myocardial ischemia. The preparation we developed to test this hypothesis is based on the operation pioneered by Vineberg,6 in which a systemic artery is used to directly revascularize the coronary microcirculation. For this procedure to be successful, angiogenesis is requisite because there are no preexisting connections between the two arterial systems. Although Vineberg and subsequent investigators were able to demonstrate anatomic connections between the IMA and the coronary circulation,10–12 as well as flow through the IMA,13,14 the biological significance of these implants remained controversial.15–20 In previous studies, microspheres were not used to determine tissue blood flow.15–20 Thus, these investigators could not distinguish nutritive flow from arteriovenous shunt flow. With the advent of coronary artery bypass surgery, these studies, and the operation itself, were largely abandoned.

We obtained anatomic proof of neovascularization (Figures 7A, 7B, and 8), but rejected a formal stereological assessment of the extent of blood vessel formation because of the extreme difficulty involved in subjecting data regarding vessel size, number, location, and orientation to quantitative analysis. Instead we assessed the physiological correlate of angiogenesis, calculating the collateral conductance of the newly formed vessels. Based on tracer micro-

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**TABLE 5. Conductance Data, All Dogs**

*Dogs with IMA implants; data obtained without simultaneous internal mammary and left circumflex coronary artery occlusion (n=12)*

<table>
<thead>
<tr>
<th>Dog</th>
<th>CIMA-LAD</th>
<th>CIMA-LCCA</th>
<th>C_COR-LAD</th>
<th>C_NL</th>
<th>CIMA-LAD/C_COR-LAD</th>
<th>CIMA-LAD as % of total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.84</td>
<td>0.01</td>
<td>2.85</td>
<td>2.31</td>
<td>0.29</td>
<td>23</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2.35</td>
<td>5.64</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0.24</td>
<td>4.54</td>
<td>1.87</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0.63</td>
<td>0</td>
<td>1.66</td>
<td>2.90</td>
<td>0.38</td>
<td>28</td>
</tr>
<tr>
<td>5</td>
<td>1.69</td>
<td>0.38</td>
<td>2.99</td>
<td>6.41</td>
<td>0.57</td>
<td>36</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>0</td>
<td>1.49</td>
<td>3.52</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>0</td>
<td>0</td>
<td>2.53</td>
<td>2.95</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>0</td>
<td>0.03</td>
<td>4.57</td>
<td>4.99</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>0.59</td>
<td>0.06</td>
<td>1.64</td>
<td>3.55</td>
<td>0.36</td>
<td>26</td>
</tr>
<tr>
<td>10</td>
<td>1.09</td>
<td>0.70</td>
<td>1.53</td>
<td>4.14</td>
<td>0.71</td>
<td>42</td>
</tr>
<tr>
<td>11</td>
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<td>0.05</td>
<td>2.59</td>
<td>4.13</td>
<td>0.39</td>
<td>28</td>
</tr>
<tr>
<td>12</td>
<td>0.61</td>
<td>0.15</td>
<td>1.61</td>
<td>4.15</td>
<td>0.38</td>
<td>27</td>
</tr>
<tr>
<td>Mean</td>
<td>0.54</td>
<td>0.14</td>
<td>2.53</td>
<td>3.88</td>
<td>0.26</td>
<td>18</td>
</tr>
<tr>
<td>SEM</td>
<td>0.16</td>
<td>0.06</td>
<td>0.31</td>
<td>0.38</td>
<td>0.07</td>
<td>5</td>
</tr>
</tbody>
</table>

**Non-IMA implanted dogs (n=9)**

<table>
<thead>
<tr>
<th>Dog</th>
<th>COTH-LAD</th>
<th>C_IND</th>
<th>C_LCCA-LAD</th>
<th>C_NL</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>0.59</td>
<td>0.24</td>
<td>1.94</td>
<td>4.12</td>
</tr>
<tr>
<td>S2</td>
<td>0.76</td>
<td>0</td>
<td>1.25</td>
<td>6.04</td>
</tr>
<tr>
<td>S3</td>
<td>4.12</td>
<td>0</td>
<td>2.15</td>
<td>4.11</td>
</tr>
<tr>
<td>S4</td>
<td>0.42</td>
<td>0.33</td>
<td>0.96</td>
<td>2.33</td>
</tr>
<tr>
<td>S5</td>
<td>1.70</td>
<td>0.38</td>
<td>2.42</td>
<td>3.83</td>
</tr>
<tr>
<td>S6</td>
<td>0.61</td>
<td>0</td>
<td>0.90</td>
<td>1.70</td>
</tr>
<tr>
<td>S7</td>
<td>0.31</td>
<td>0</td>
<td>2.66</td>
<td>3.94</td>
</tr>
<tr>
<td>S8</td>
<td>0.61</td>
<td>0</td>
<td>1.76</td>
<td>3.09</td>
</tr>
<tr>
<td>S9</td>
<td>1.11</td>
<td>0</td>
<td>1.74</td>
<td>6.31</td>
</tr>
<tr>
<td>Mean</td>
<td>1.14</td>
<td>0.11</td>
<td>1.75</td>
<td>3.91</td>
</tr>
<tr>
<td>SEM</td>
<td>0.40</td>
<td>0.05</td>
<td>0.21</td>
<td>0.50</td>
</tr>
</tbody>
</table>

All values are mean±SEM with units ml·min⁻¹·100g⁻¹·mm Hg⁻¹. CIMA-LAD. Conductance from the internal mammary artery to the territory of the left anterior descending coronary artery; CIMA-LCCA. Conductance between the internal mammary artery and the left circumflex coronary artery; C_COR-LAD. Conductance of all collateral sources to the territory of the left anterior descending coronary artery, independent of the internal mammary artery; C_NL. Arteriolar conductance of the normal zone; COTH-LAD. Conductance of all other collateral sources to the territory of the left anterior descending coronary artery, independent of the left circumflex coronary artery; C_IND. Conductance of sources to the territory of the left circumflex coronary artery, independent of the left anterior descending coronary artery; C_LCCA-LAD. Conductance from left circumflex coronary artery to the territory of the left anterior descending coronary artery.
sphere flow measurements during adenosine-induced vasodilatation, the method permitted quantification of the maximal collateral conductance to the LAD coronary artery perfusion territory, and determination of the relative contributions from disparate collateral sources to total collateral flow. Thus, collateral conductance from the IMA, the LCCA, and all other sources of perfusion to the LAD zone (ischemic zone, IZ) could be determined independently, and their interrelationships explored. By assessing collateral conductance during adenosine infusion, we were able to test the maximal conductance of any newly formed vessels while minimizing autoregulatory influences that might confound the results.

We found that the anastomoses formed between the IMA and the LAD circulation after 8 weeks were capable of providing significant nutritive blood flow in 58% of cases. This success rate was similar to the rates of anatomic patency reported previously in the experimental and clinical literature.21 Given the many variables associated with IMA implantation (surgical preparation of the vessel, its anatomy and the configuration of its side branches, the creation of the tunnel, and the arrangement of the recipient vessels of the LAD territory), it is not surprising that the IMA often failed to provide substantive flow. In many of the dogs with failed implants, subintimal proliferation was noted in the implanted section of the IMA. Systematic, quantitative analysis of the histology at the anastomotic site was not performed, however, and it cannot be determined whether differences in histopathology occurred between grafts that functioned and those that failed. It is unclear whether subintimal proliferation constituted a cause or effect of graft failure. We speculate that the complete lack of neo-vascularization noted in some dogs resulted from technical factors, and not a failure of angiogenesis per se. This is not a point that can be proven, however, because technical errors can always be invoked in an animal with a failed implant.

For the entire group of dogs with IMA implants (group 1), IMA occlusion decreased maximal IZ flow from 1.31 to 1.16 ml/min/g (p < 0.005), and occlusion of the IMA in addition to the LCCA decreased IZ flow from 0.73 to 0.42 ml/min/g (p < 0.02). On average, occlusion of the IMA decreased the IZ/NZ blood flow ratio by 10%. Thus, IMA-derived collaterals supplied 10% of maximal IZ blood flow, relative to maximal NZ blood flow. Direct measurement of IMA blood flow by electromagnetic flow probe revealed a mean basal flow of 7.8 ml/min with a mean maximal blood flow of 14.4 ml/min.

Comparing groups 1 and 2, there was no net difference in either basal or maximal IZ flow. Thus, the increment in perfusion provided by the IMA in dogs with implants was offset by a decrease in collateral flow from coronary collaterals, because total flow was the same in both groups. These results are compatible with the hypothesis that regional ischemia provides a stimulus for collateral proliferation that continues until collateral flow is sufficient to meet the metabolic needs of the tissue, at which point the ischemic stimulus is removed and collateral growth ceases. If an independent artery (the IMA) is in contact with the ischemic zone and available as a perfusion source, then angiogenesis can occur, and anastomoses develop between the artery and the coronary circulation. However, in a canine model with single coronary artery occlusion, peak flow will not be improved.

Although the maximal IZ blood flows before and during IMA occlusion are by themselves useful, they may incompletely illustrate the importance of IMA-associated collaterals in an individual dog. For example, the formation of a low-resistance pathway between the IMA and the LCCA (Figure 2A, "Y") may falsely lower the perceived importance of IMA-derived IZ flow. The development of such a pathway could result from intrinsic IMA to LCCA collateral formation, or from interconnection of IMA-associated vessels with LCCA to LAD collaterals. When the IMA is occluded and the LCCA is patent, the LCCA provides collateral flow to the IZ not only across "C," but also through "Y" and "X" in series. As the resistance of "Y" decreases relative to "X," the impact of IMA occlusion on IZ flow decreases. Thus, the importance of the IMA is more appropriately gauged during LCCA occlusion, at which time the IMA contributes collateral flow both to the IZ and the NZ, through "X" and "Y," respectively. The conductance calculations provide an algebraic solution for the collateral resistances that uniquely predicts both the IZ and NZ blood flows during four separate conditions (differing combinations of perfusion sources and blood pressures, Tables 4 and 5), and thereby take into account the fact that occlusions affect blood flow in both the LAD and LCCA territories simultaneously. This is illustrated by the data of Table 4, which provide insight into the relative importance of the contributions of the IMA, the LCCA, and other sources in providing collateral flow to the LAD territory in individual dogs. The IMA provided a variable measure of IZ collateral conductance, providing none in several dogs, but contributing as much as the LCCA in one dog (4) and more than the LCCA in another (10). These examples illustrate the importance of this technique because this information could not have been derived from the blood flow data alone.

**Limitations of Study**

Theoretical problems were posed by the limited flow to the LCCA territory during LCCA occlusion. The delivery of greater than 400 microspheres to the LCCA area samples at all times was not practical; therefore, some errors may have occurred in these flow determinations.22 This problem was minimized by averaging the flow from several tissue samples. Thus, the ischemic and control zone flows were computed from the flow of generally eight transmural samples, comprising about 11 grams of tissue, and the standard errors were actually quite small.
FIGURE 7. Gross pathological cross-section of an IMA implantation site. Top panel: Short-axis heart slice revealing the internal mammary artery (IMA) in cross section. Fibrosis adjacent to the implantation site is typical. Bottom panel: Enlargement of the box of top panel. Two vessels cut longitudinally appear to be collaterals linking the IMA with epicardial vessels.
In calculating the contribution of the IMA to myocardial perfusion, samples from the central ischemic zone, that is the area with the most compromised collateral flow, were used to calculate LAD territory blood flow (i.e., wedges 4, 5, and 6; Figure 3A). Contributions by the IMA contiguous with its implantation site or at the periphery of the LAD territory (i.e., wedge 3; Figure 3A) were not considered. Therefore, there is the possibility that our data underestimate IMA-dependent, as well as overall, collateral flow.

In summary, this investigation offers direct proof that new vessels can develop between an extracardiac artery and the native coronary circulation, supplying modest but significant nutritive flow to the myocardium. Although neither basal collateral flow nor maximal vasodilator reserve were improved by arterial implantation in this preparation, and the overall failure rate was quite high (42%), it is possible that IMA implantation could add importantly to tissue perfusion if implanted in a region where flow supplied by the native coronary collateral system was inadequate to satisfy the metabolic demands of the myocardium.

Appendix

Resistance Calculations

In Figure 2A, the IMA and the LCCA are patent during adenosine infusion. The arteriolar resistances of both the LAD and LCCA arteriolar beds are minimized and assumed to be essentially equivalent, as discussed above. Therefore, they are both denoted “R”. Because there is no potential across Y, the circuit simplifies to Figure 2B, where X, Z, and C in parallel are represented by “B”

\[ 1/B = 1/X + 1/Z + 1/C \]

(1)

\[ P = JR = I(R + B) \]

Rearranging terms:

(3) \[ B = P/I - P/J \]

Temporarily occluding the IMA produces the circuit displayed in Figure 9. Q, K, and L represent the mean arterial pressure and regional myocardial blood flows in the LAD and LCCA zones, respectively. The circled resistance network “A” defines the series resistance Y plus X, in parallel with C and Z.

(4) \[ 1/A = 1/(X + Y) + 1/C + 1/Z \]

(5) \[ Q = LR = K(R + A) \]

Substitution yields:
(6) \( A = \frac{Q}{K} - \frac{Q}{L} \)

Occlusion of the LCCA produces the circuit seen in Figure 10. With \( N \) the mean arterial pressure during LCCA occlusion, \( S, T, I7, I8, \) and \( H \) represent flows as indicated. Applying Kirchhoff's rules to the circuit and defining \( V \) as the pressure (voltage) across \( C \):

- (7) \( V = HC = R(S - T) \)
- \( V \) can be expressed as:

\( (8) \ V = Y(T - V/C) - D(S + V/C) \)

Simultaneous occlusion of the IMA and LCCA produces the final circuit shown diagrammatically in Figure 11. \( M \) is the mean arterial pressure during the occlusions; \( F \) and \( G \) represent the flows to the LAD and LCCA beds, respectively. The resistance network of \( C \) in parallel with \( X \) and \( Y \) in series is defined as \( E \):

- (9) \( 1/E = 1/C + 1/(X + Y) \)

Applying Kirchhoff's rules and rearranging solves \( Z \):

- (10) \( Z = (M - FR)/(F + G) \)

The final task is to solve for \( X, Y, \) and \( C \) as functions of other, measurable, variables.

Subtracting Equation 1 from Equation 4 and defining \( W \):

- (11) \( W = 1/A - 1/B \)
- \( Y \) can be expressed as a function of \( X \):

- (12) \( Y = -WX^2/(1 + WX) \)

Rearrangement of Equation 1 yields \( C \) as a function of \( X \):

- (13) \( C = 1/(1/B - 1/Z - 1/X) \)

Substituting Equations 12 and 13 into Equation 8 and rearranging terms eventually produces a quadratic equation for final solution of \( X \):

- (14) \( V/B - T - V/Z \) \( X^2 + (-TZ + S - 2V)X + (-SZ/W - ZV/WB - ZV) = 0 \)

Once \( X \) is determined, \( Y \) and \( C \) can be solved from equations 12 and 13, respectively.

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