

# Use of corrosion casting techniques to evaluate coronary collateral vessels and anastomoses in hearts of canine cadavers

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**Objective**—To study and investigate branching patterns of the canine coronary arteries and collateral circulation by use of corrosion casting techniques.

**Sample Population**—31 hearts obtained from cadavers of clinically normal dogs of various ages and breeds and of either sex.

**Procedure**—3-dimensional reproduction of coronary arteries was achieved by postmortem injection and perfusion with casting materials into the aortic sinus via the ascending aorta. Perfused hearts were macerated and carefully irrigated; the air-dried specimens were examined macroscopically and with a magnifying headset.

**Results**—Collateral arteries and inter- and intra-arterial anastomoses were successfully detected in 8 corrosion cast specimens. In total, 9 coronary collateral arteries and 3 interarterial anastomoses were found.

**Conclusions and Clinical Relevance**—Our finding of coronary collateral arteries in canine hearts is in agreement with recent findings in coronary flow study. On the basis of our results, vasodilation treatment to improve collateral vessel remodeling in dogs with myocardial dysfunction may be warranted. (*Am J Vet Res* 2005;66:1724–1728)

Controversy has existed for decades about the presence and true functional importance of the coronary collateral system.<sup>1</sup> Postmortem morphologic studies on anatomy, distribution, and anastomoses of coronary arteries in normal and pathologic human hearts have been pursued for hundreds of years.<sup>1</sup> Dogs have been used in recent years to study the development of coronary collateral arteries after progressive coronary artery obstruction; the latest findings suggest that dogs have an intermediate density of preexisting or native collateral arteries that can deliver, on average, 5% to 10% of basal flow before occlusion.<sup>2-5</sup>

The concept of coronary artery collateral circulation has not been widely accepted in veterinary medicine.<sup>6</sup> In a previous study<sup>7</sup> on the myocardial microvasculature in dogs, 2 casting materials (ie, an acrylic resin [AR]<sup>a</sup> and a lower-viscosity acrylic resin

[LVAR]<sup>b</sup>) have been used. Both materials belong to the family of cold-setting, all-acrylic cements consisting of 2 components, a resin and a catalyst. Characteristics of both systems are excellent permeability and infiltration properties, chemical resistance to maceration solvents, low viscosity, minimal shrinkage, and the ability to cure at room temperature (approx 25°C). One of the advantages in the use of LVAR, compared with AR, is the ability of LVAR to pass through smaller blood vessels (ie, vessels with diameters of 5 µm).

By definition, anastomoses are interconnections between vessels from 2 territories of blood supply,<sup>8</sup> for example, interconnections between the left coronary artery (LCA) and right coronary artery (RCA). Collateral vessels are defined as interconnections within a territory of blood supply, for example, collateral channels between the circumflex and left cranial descending branches of the LCA.<sup>8</sup>

In the literature,<sup>9-11</sup> anastomoses and collateral vessels are often used as synonyms. Other terms and synonyms for anastomoses are intercoronary anastomoses,<sup>12,13</sup> intercoronary collateral channels,<sup>14</sup> intercoronary interarterial anastomoses,<sup>15</sup> and interarterial anastomoses.<sup>16</sup> Alternative terms for collateral vessels are the following: collateral anastomoses or homocoronary anastomoses<sup>13</sup>, coronary collaterals, collateral vessels, collateral connections, or collateral channels<sup>12</sup>, and intra-arterial anastomoses.<sup>6</sup>

Collateral vessels were further differentiated into preexisting (or native) and mature collateral vessels in coronary collateral angiogenesis and flow studies.<sup>4,17</sup> The native collateral network in the canine heart is predominantly epicardial in origin, although endocardial connections are known to exist.<sup>11,17,18</sup> Schaper<sup>17</sup> described these native vessels as small arterioles, averaging 40 µm in diameter, thin-walled, and having only 1 or 2 smooth muscle layers.

Compared with preexisting (or native) collateral vessels, mature collateral vessels have well-developed media, have much clearer evidence of vasomotion, have considerably more blood flow, and develop as a result from chronic or recurrent ischemia.<sup>1,4,5</sup> The transformation of native collateral vessels into mature collateral vessels has been described as vascular remodeling or angiogenesis (ie, development of new vessels).<sup>5,19</sup>

The purpose of the study reported here was to find and investigate coronary collateral arteries and anastomoses in the canine heart by use of postmortem injection techniques and subsequent maceration of the vascular casts. This technique allows 3-dimensional models of the coronary arteries and their collateral vessels to be produced. For the purpose of our investiga-

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tion on coronary vessels, the use of AR appeared to be as satisfactory as that of LVAR. Because we did not study the coronary capillary network (with a vessel diameter range of 5 to 70  $\mu\text{m}$ ), the use of AR was justified. Another reason that AR was used more often in our study than LVAR was because of the higher cost of LVAR.

## Materials and Methods

**Specimens**—Hearts were used for postmortem vascular injections from 31 cadaver dogs of unknown age and weight.<sup>d</sup> They included 13 mixed-breed dogs (7 female and 5 male), 7 Beagles (3 females and 4 males), 5 German Shepherd Dogs (3 females and 2 males), 2 Standard Poodles (1 female and 1 male), 2 Dobermans (1 female and 1 male), 1 male Bull Terrier, and 1 male Dachshund. Cadaver dogs had died of natural causes or had been euthanized prior to receiving them. No evidence or signs of heart disease were reported for any dogs.

**Casting procedure**—Hearts were quickly removed en bloc with the aorta and major vessels and were flushed and rinsed with physiologic saline (0.9% NaCl) solution and tap water until the fluid ran clear. Of the 31 hearts, 19 were perfused with AR<sup>a</sup> and 12 were perfused with LVAR.<sup>b</sup> Prior to injection of AR, the perfusate was mixed with a 2% catalyst solution (ie, benzoyl peroxide and dibutyl phthalate).<sup>20</sup> In the case of LVAR, the resin-to-catalyst ratio in grams was 20:1.5, which allowed for a usable time of 2 to 3 minutes and a hardening time of 6 to 8 minutes. Because clear casts are macroscopically difficult to work with, Sudan red dye was added to the AR perfusate. The LVAR was a blue liquid, so the addition of dye was not required.

Injections were made under hand pressure into the aortic sinus via the ascending aorta. In 4 hearts, injections into the coronary sinus were performed to demonstrate the branching pattern and collateral vessels of the coronary veins. After a 24- to 48-hour period in a cool room at 8°C, the perfused hearts were macerated in a solution of 40% NaOH. Finally, the specimens were carefully irrigated with tap water for removal of soft tissue and then air-dried. The casts were

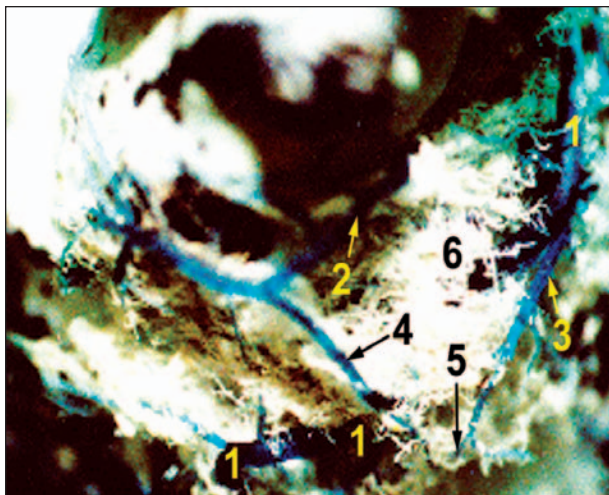


Figure 1—Left lateral photomacrographic view of a corrosion cast of lower-viscosity acrylic resin (LVAR)<sup>b</sup>-injected coronary arteries in a heart from a female Beagle. Notice the collateral artery (4) originating from a ventral branch (3) and a caudodorsal branch (2) of the caudal interventricular septal branch (1) of the left coronary artery (LCA; 1). Another collateral artery (5) connecting a ventral branch (3) with the caudal interventricular septal branch (1) of the LCA is depicted distally from the first collateral artery (4). Notice the capillary network (6).

examined macroscopically and with a headset at magnifications of 1.8X, 2.3X, 3.7X, and 4.8X. In addition, a stereomicroscope<sup>c</sup> at 10X magnification was used to further investigate areas of special interest. Photomicrographs were made and labeled. Measurements were done by use of a sliding caliper. Estimated shrinkage with the use of AR is between 12% and 15%,<sup>20</sup> and estimated shrinkage with the use of LVAR is 8%.<sup>d</sup>

## Results

**Collateral vessels**—In 7 vascular cast specimens, collateral vessels were demonstrated. In the proximal portion of the septum of 2 specimens, a collateral vessel, measuring approximately 90  $\mu\text{m}$  in diameter and located between a caudal interventricular septal branch and the circumflex branch (prior to its continuation as the caudal subsinuosal interventricular branch) of the LCA, was found. Because this interconnection is with-

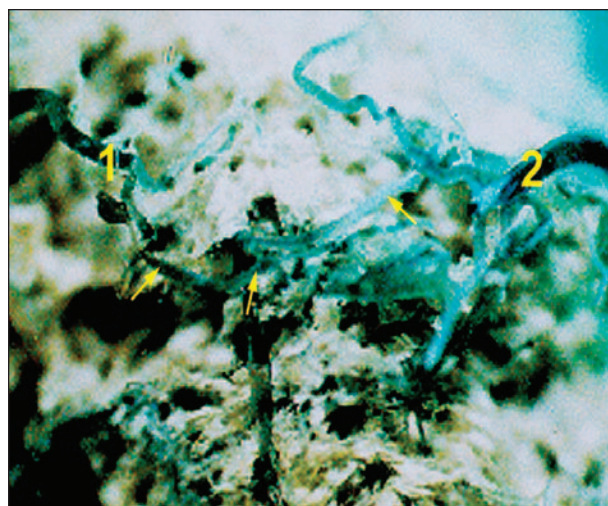


Figure 2—Ventral photomacrographic view of a corrosion cast of LVAR-injected coronary arteries in a heart from a male Beagle. Notice the collateral connection (yellow arrows) between the paraconal interventricular branch (1) and subsinuosal interventricular branch (2) of the LCA.

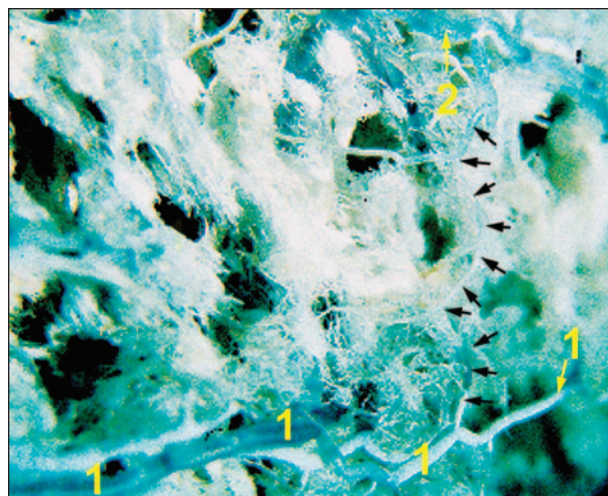


Figure 3—Left lateral photomacrographic view of a corrosion cast of LVAR-injected coronary arteries in the same heart as in Figure 2 from a male Beagle. Notice the collateral connection (black arrows) between the proximal collateral branch (1) and left proximal ventricular branch (2) of the LCA.

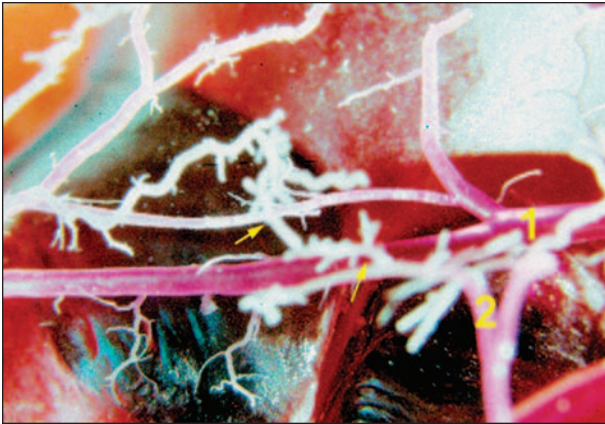


Figure 4—Right lateral photomicrographic view of a corrosion cast of acrylic resin (AR)-injected coronary arteries in a heart from a female German Shepherd Dog. Notice the collateral connection (yellow arrows) between the right proximal atrial branch (1) and right intermediate atrial branch (2) of the right coronary artery (RCA).

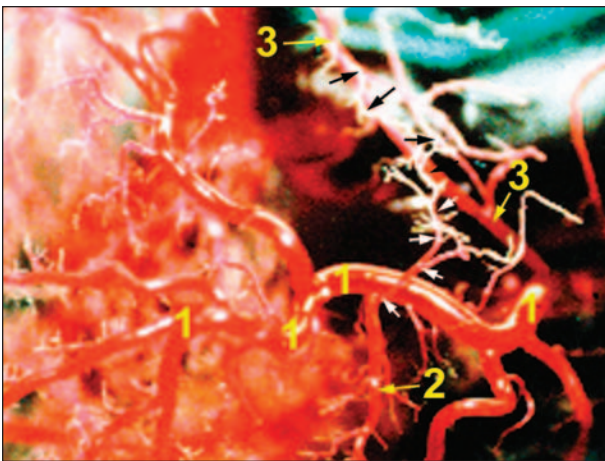


Figure 5—Right lateral photomicrographic view of a corrosion cast of AR-injected coronary arteries in a heart from a female Beagle. Notice the collateral artery (white and black arrows) between the right ventricular (descending) branch (2) and right proximal atrial branch (3) of the RCA.

in a territory of blood supply (ie, that of the LCA), it is described as a collateral vessel. A similar finding was shown in another vascular cast where 2 branches originating from the interventricular septal branch of the LCA anastomosed near the proximal portion of the septum (Figure 1).

Another collateral connection (approx 100  $\mu\text{m}$  in diameter) was found between the paraconal interventricular and subsinuosal interventricular branches of the LCA across the apex of 2 hearts (Figure 2). A collateral connection (approx 90  $\mu\text{m}$  in diameter) was found between the left proximal collateral branch of the paraconal interventricular artery and the left proximal ventricular branch of the circumflex artery (Figure 3).

Collateral vessels (approx 85  $\mu\text{m}$  in diameter) were also found among ascending atrial branches of the RCA. In 1 specimen, the right distal atrial branch anastomosed with the right proximal atrial branch, whereas in another heart, a collateral interconnection

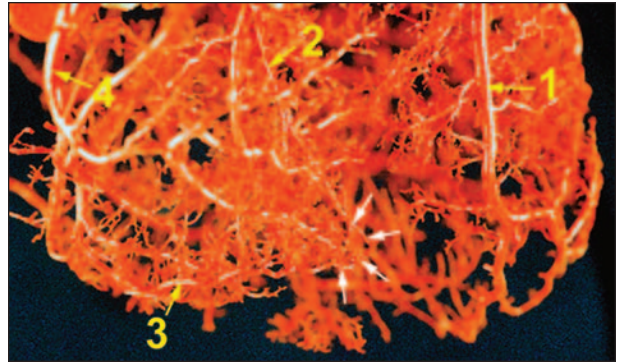


Figure 6—Left lateral photomicrographic view of a corrosion cast of AR-injected coronary arteries in a heart from a female German Shepherd Dog. Notice the intercoronary anastomosis (white arrows) between a cranial branch of an interventricular septal branch (2) of the LCA and right distal ventricular branch (3) of the RCA (4). Notice the paraconal interventricular branch (1) of the LCA.

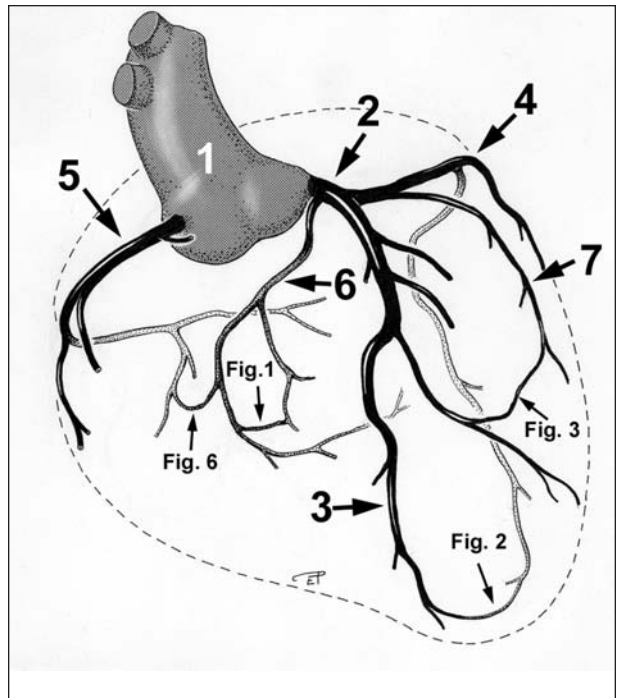


Figure 7—Drawing of the left lateral view of collateral arteries and anastomoses of the canine heart as found in the photomicrographs of Figures 1, 2, 3, and 6. Left lateral view. 1 = Aorta. 2 = LCA. 3 = Cranial paraconal interventricular branch of the LCA. 4 = Circumflex branch of the LCA. 5 = RCA. 6 = Interventricular septal branch of the LCA. 7 = Left proximal ventricular branch of the LCA.

(approx 85  $\mu\text{m}$  in diameter) was found between the right intermediate atrial branch and right proximal atrial branch of the RCA (Figure 4). A remarkable and novel finding was a collateral artery (approx 80  $\mu\text{m}$  in diameter) between the right marginal ventricular branch and the right proximal atrial branch (Figure 5). To our knowledge, a collateral vessel between a descending ventricular and ascending atrial branch of the RCA has not been described before. In total, 9 coronary collateral arteries and 3 interarterial anastomoses were found.

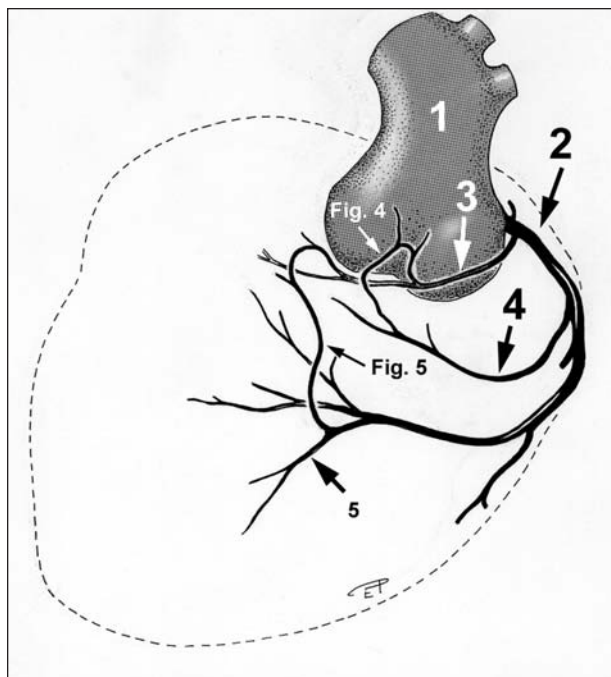


Figure 8—Drawing of the right lateral view of collateral arteries and anastomoses of the canine heart as found in the photomicrographs of Figures 4 and 5. 1 = Aorta. 2 = RCA. 3 = Right proximal atrial branch of the RCA. 4 = Right intermediate atrial branch of the RCA. 5 = Right ventricular (descending) branch of the RCA.

**Intercoronary anastomoses and arteriovenous connections**—In 3 specimens, an intercoronary anastomosis (each ranging from 90 to 100  $\mu\text{m}$  in diameter) was found. A cranial branch of an interventricular septal branch of the LCA anastomosed with a right distal ventricular branch of the RCA (Figure 6). Because the previously mentioned branches belong to arteries supplying 2 territories, the interconnection was called an anastomosis (Figures 7 and 8).

In another heart, an intercoronary anastomosis was found between the right distal atrial branch of the RCA and the left proximal atrial branch of the left circumflex branch of the LCA. In another specimen, a similar anastomotic connection was found between the right distal atrial branch of the RCA and left distal atrial branch of the circumflex artery. No arteriovenous-shunt vessels were found in the corrosion casts.

## Discussion

Despite a great deal of study, a consensus on the existence of end arteries in the coronary circulation of dogs has not been reached.<sup>11</sup> The network distribution of large epicardial vessels, as described in our study of the heart of clinically normal dogs, has been known for many years.<sup>11,17,18</sup> Yet, the functional importance of these vascular interconnections, which are between branches of the same artery and between branches of different arteries, is still questioned, and the physiologic term, end artery, is still used to characterize coronary vessels.<sup>6,11</sup>

It would seem unreasonable to deny function to a system such as that of the anastomosing vessels of the canine and human heart. In particular, findings in

recent coronary collateral blood flow and angiogenesis studies<sup>1,2,4,17,21</sup> have demonstrated the existence and protective capacity of this system in the event of prolonged ischemic stimuli.

In our study, we did not examine blood flow following the ligation of coronary arteries because research investigating this issue has been extensive.<sup>1,4,13,22</sup> Also, our main interest involved investigation of the morphologic and topographic characteristics of the various coronary branching patterns and collateral channels.

On postmortem examination, coronary collateral blood flow, resulting from peripheral coronary blood pressure and retrograde blood flow, might be high. Also on postmortem examination, collateral vessel dilatation could be potentially mediated by mechanical stretching caused by the filling pressure of the injected cast material.

The existence and finding of inter- as well as intracoronary collateral channels in 8 of our canine heart specimens are in agreement with findings in other anatomic studies.<sup>4,10,11,13,23,24</sup> Our findings and those of recent coronary collateral blood flow studies<sup>1,4,19,25</sup> and reperfusion studies<sup>4,19,22,26</sup> on the ischemic canine heart strongly suggest that the canine coronary collateral circulation is functional and that the existence of end arteries in the coronary circulation of dogs is not likely. Findings in all of these studies indicate that the recruitment of native collateral vessels or collateral vessel remodeling and transformation into mature collateral arteries are stimulated by chronic vessel occlusion or recurrent ischemia.

Findings in studies<sup>2,3,22,27,28</sup> on inadequate collateral circulation indicate that a protective effect or mechanism of coronary collateral vessel remodeling occurs in hearts of clinically normal individuals. In these studies,<sup>2,3,22,27,28</sup> individuals with adequate collateral circulation had lower end-diastolic pressures, a higher cardiac output fraction, and improved left ventricular function, compared with individuals with inadequate collateral circulation. Similar findings were demonstrated in reperfusion studies<sup>2,4,19,22,25,29</sup> in dogs in response to endothelial-dependent vasodilators, such as prostaglandins E1 and E2, adenosine, and epoxy-eicosatrienoic and dihydroxyepoxyeicosatrienoic acids, and in response to vascular endothelial growth factors, such as fibroblast growth factor and insulin growth factor. In conclusion, findings in our study support the existence of coronary collateral arteries as well as a mechanism for collateral vessel remodeling. The ability to improve left ventricular function in advanced myocardial dysfunction may be of clinical interest in the vasodilation treatment of dogs with heart disease.

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- b. Mercocx CL-2B, SPI Supplies Ing, Buro fur Prozesstechnik und Instrumentelle Analytik, Connollystrasse 29, D-80809 Munich, Germany.
- c. Leica Microsystems Ltd, Max-Schmidheiny-Str. 201, CH-9435 Heerbrugg, Switzerland.
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