Branching Patterns in the Porcine Coronary Arterial Tree
Estimation of Flow Heterogeneity
E. VanBavel and J.A.E. Spaan

The aim of this study is to quantify the porcine coronary arterial branching pattern and to use this quantification for the interpretation of flow heterogeneity. Two casts of the coronary arterial tree were made at diastolic arrest and maximal dilation. The relation between length and diameter of arterial segments was quantified, as well as the area expansion ratio and diameter symmetry of vascular nodes. These relations were used to construct computer models of the coronary arterial tree, covering diameters between 10 and 500 μm. Topology of these simulated trees was analyzed using Strahler ordering: Bifurcation ratio, diameter ratio, and length ratio were constant along orders 2–8 and equal to 3.30, 1.51, and 1.63, respectively. In each order, the number of segments per Strahler vessel was almost geometrically distributed. For the lowest orders, these predictions were confirmed by direct observations. From the network model, local pressure and flow were also predicted: Pressure fell from 90 to 32 mm Hg at the 10-μm level. The coefficient of variation (CV) of flow in individual segments was dependent on the number of perfused terminal segments (Nt) according to the fractal relation CV(Nt) = Nt(1-D), where D is the fractal dimension (1.20). CV of flow in 1-g tissue units was predicted to be 18%. This study shows that the structure of the coronary arterial bed is an important determinant of the fractal nature of local flow heterogeneity. (Circulation Research 1992;71:1200–1212)

KEY WORDS • coronary circulation • fractals • flow heterogeneity • topology • Strahler ordering

A central issue in the current research on coronary physiology is the high spatial heterogeneity of coronary blood flow and flow reserve as established by microsphere deposition,1–5 subepicardial NADH fluorescence patterns,6 and interpretation of tracer dilution curves.7–9 The branching network of arterial vessels forms an important determinant of flow distribution, and the large variability of branching, shown by qualitative studies on vascular casts,10–13 may be related to flow heterogeneity. However, a better understanding of these relations requires detailed quantitative knowledge on the structure of the coronary network.

The aim of this study was to develop a network description of the porcine coronary arterial tree, based on direct observations of arterial branching patterns, and to use this network to predict flow heterogeneity. Although some quantitative studies on coronary branching, based on vascular casts, are available,14–16 these studies do not provide sufficient information for network analysis. We performed an extensive set of measurements on two vascular casts made at maximal dilation and diastolic arrest. From these measurements, “branching rules” were derived that describe the stochastic relation between diameters of parent and daughter segments at arterial nodes, as well as the relation between the unbranched length and diameter of segments. Subsequently, computer constructions of coronary arterial networks were made by repetitive application of these branching rules. These networks were analyzed with respect to both topology, applying Strahler ordering,17–19 and local pressure and flow heterogeneity.

This study demonstrates that the irregular structure of the coronary arterial tree may be a major determinant of flow heterogeneity and its fractal nature.

Materials and Methods
Experimental Procedure
Data from two hearts obtained from male pigs were used in this study. The animals weighed 13.5 and 14.5 kg. They were anesthetized by intramuscular injection of 5.3 mg/kg azaperone and intraperitoneal injection of 13.3 mg/kg metomidate (both obtained from Janssen, Beerse, Belgium). Additional medication consisted of intramuscular injection of 0.03 mg/kg atropine sulfate (Centrafarm, Etten-Leur, The Netherlands) and intravenous injection of 0.27 mg/kg pancuronium bromide (Organon, Oss, The Netherlands). The animals were intubated and artificially ventilated (O2–N2O at 1:2). A midsternal thoracotomy was performed, and the pericardium was opened. Subsequently, the animals were heparinized by intravenous injection of 670 IU/kg Thromboliquine (Organon). The hearts were fibrillated,
removed, and stored in cold (4°C) bicarbonate-buffered Ringer’s solution (BR; millimolar composition: NaCl 115, KCl 4.7, KH₂PO₄ 1.2, MgSO₄ 1.2, NaHCO₃ 24, CaCl₂ 2.5, and glucose 10; equilibrated with 5% CO₂). The aortas were cannulated, and the hearts were mounted in a Langendorff setup.

The subsequent procedures varied somewhat between the hearts. The coronary bed of heart 1 was perfused with BR at 37°C, and this heart started beating again. Perfusion pressure was kept constant at 70 mm Hg. The bed was dilated by 10 μM adenosine (Sigma Chemical Co., St. Louis, Mo.). Full dilation was confirmed by the absence of reactive hyperemia. The heart was arrested by increasing the potassium concentration from 6 to 15 mM. Two minutes after cardiac arrest, the perfusion medium was changed to BR containing 2% glutaraldehyde. Heart 2 was perfused with BR at 4°C at a pressure of 90 mm Hg. Because of the low temperature, the heart was in diastolic arrest, and the vascular bed was maximally dilated. This heart was also fixated by glutaraldehyde within 2 minutes after the start of the perfusion. After several minutes of perfusion with the fixation solution, the casting material (Baton’s No. 17 corrosion cast, Polysciences Inc., Warrington, Pa.; 50 ml base + 16 ml catalyst + six drops promoter + 5% red color) was injected at 50 (heart 1) and 65 (heart 2) mm Hg. The injection pressure was maintained until the plastic had hardened. After fixation, the hearts weighed 90 and 107 g. Tissue surrounding the casts was removed by incubation in 20% NaOH at 50°C for 2 days.

**Measurements on the Vascular Casts**

Diameter and length of the smaller vessels in the arterial cast were measured on an inverted microscope (model IM2, Olympus Optical Corp., Tokyo), which was equipped with a drawing attachment. This drawing mirror was placed in such a way that a computer screen could be seen through the eyepieces, together with the microscopical preparation. Long distance objectives ×4 to ×40 were used. Small parts of the arterial tree were selected randomly and placed on the microscope. x and y coordinates of parts of the tree were measured by displacement of cursors on the screen. z coordinates were measured by a 10-turn potentiometer connected to the fine focusing adjustment of the microscope. This potentiometer produced a voltage proportional to the distance between the objective and microscope table. This voltage was converted to a digital level by a 12-bit analog-to-digital converter. Resolution of the position measurements were 0.5 μm for the x and y coordinates at the highest magnification and 1.5 μm for the z coordinate. The length of each vessel segment was calculated from the cartesian distances between successive points along the segment. Care was taken to place the tree in such a way that most of the segments were orientated in the x-y plane. Vessel segments were assumed to be round, and diameter was calculated from the distance in the x-y plane between cursors placed on each side of the segment. In general, the error in the position measurements caused by the finite resolution was less than the uncertainty caused by variation of diameter along a segment and interpretation of the starting and ending points of segments.

Unbranched length and diameter of successive segments were measured. Diameter measurements were performed at the middle of vascular segments. The tree was followed until either the most distal segment was reached or the diameter of a segment was smaller than a chosen threshold value. This threshold was chosen in advance to limit the number of segments to be measured. To relocate positions within the actual tree, the already measured part could be drawn and redrawn at will on the computer screen and projected on the microscope image by the drawing mirror.

Measurements on the large epicardial vessels were made in a different way. From cast 1, a large number of macrophotographs of the epicardial arterial cast were taken at angles that were, judged by eye, perpendicular to the epicardial surface. Lengths of the individual segments were measured on the photographs where the segment was central. The error introduced by a nonperpendicular angle of photography was estimated to be less than 5% on the basis of an upper estimate of a 20° deviation of the angle. For the estimation of diameter in these epicardial segments, measurements from multiple photographs were averaged. From cast 2, diameters of the large vessels were measured under a binocular microscope. This method did not allow measurement of segmental lengths, since z coordinates of starting and ending points could not be measured.

Collateral vessels were extremely rare in the casts; only about 10 were observed. We ignored the nodes to these collaterals and the vessels themselves.

Vascular nodes connect three segments per definition. Below, we will use the terms “mother segment” and “daughter segments” to denote the segments immediately proximal and distal, respectively, to a node.

**Results**

**Coronary Arterial Branching Pattern**

The left panel of Figure 1 shows a plot of unbranched length versus diameter of 2,366 vascular segments from the two hearts. As is clear from this figure, the lengths of segments of a certain diameter can vary about 100-fold. A linear regression line fitting the logarithm of length (L) as a function of the logarithm of the diameter (d) has been included in this plot. This line is described by \( \log_{10}(L) = 1.01 + 0.72 \cdot \log_{10}(d) \) (or, \( L = 10.2 \cdot d^{0.72} \)). The squared correlation coefficient (\( r^2 \)) was 0.45 and was highly significant (\( p < 0.0001 \)). For the individual hearts, this regression line equals \( \log_{10}(L) = 0.96 + 0.73 \cdot \log_{10}(d) \) (heart 1, \( n = 685 \)) and \( \log_{10}(L) = 1.00 + 0.73 \cdot \log_{10}(d) \) (heart 2, \( n = 1,681 \)). These data indicate that at a given diameter, segments were on average about 10% longer in heart 2 compared with heart 1. This interindividual difference is negligible compared with the variability within each heart. The right panel of Figure 1 shows the distribution of the ratio between observed lengths and their least-squares estimates. For practical purposes, a negative gamma distribution\(^{29} \) was fitted through these deviations, with density function

\[
 f(x) = (-x - y)^{(e-1)} \cdot e^{\frac{y}{x}} / \Gamma(\alpha) \cdot [\beta \alpha \cdot \Gamma(\alpha)]
\]

where \( x \) is the logarithm of the ratio of observed and expected length, and \( \Gamma(\alpha) \) is the gamma function. An optimal (least-squares) fit was obtained for \( \alpha = 6.0, \beta = 0.15, \) and \( y = -0.90 \) (see the right panel of Figure 1).
The distribution of observed deviations was not significantly different from this curve (χ² test, p>0.05).

From a total of 1,663 nodes, the diameters of the mother segment (d₀) and both the larger (d₄) and smaller (d₆) branches were measured. Figure 2 shows logarithmic plots of d₄ and d₆ versus d₀. Note that d₄ is always of the same order of magnitude as d₀ and, in most (but not all) cases, smaller than d₆. The variation in d₆ is much larger: for any given d₀, d₆ varies approximately 10-fold.

Figure 3 illustrates the relation between the diameters of sister segments. In this plot, these diameters are shown relative to that of the mother segment. The apparent negative correlation in Figure 3 illustrates that if a segment is thick, its sister segment is expected to be thin compared with the mother segment.

The relation between mother and daughter diameters at a node can be quantified by the area expansion ratio (A) and symmetry (S), which we defined as follows:

\[ A = (d₄^2 + d₆^2)/d₀^2 \]

\[ S = d₆/d₄ \]

In nodes where A is smaller than 1, the total available cross-sectional area decreases, and as a result, blood velocity increases. In nodes where A is larger than 1, the total cross-sectional area increases. The arc in Figure 3 depicts the relation between relative diameters corresponding to absence of area expansion (A=1). Nodes where S is 1 are perfectly symmetric; unity is the maximum value of S. In Figure 3, these nodes are situated at the identity line. The minimum value of S at a certain d₄ is determined by the lower limit of observed vessel diameters.

For each node measured, both A and S were calculated. Area expansion ratio A averaged 1.118±0.302 (mean±SD, n=1,663). For the individual hearts, A averaged 1.132±0.268 (heart 1, n=685) and 1.108±0.323 (heart 2, n=978). The left panel of Figure 4 shows A versus the mother diameter. The line shown in this figure is the linear regression line fitting A as a function of log(d₀): \[ A = 1.279 - 0.086 \cdot \log(d₀) \]. where d₀ is expressed in micrometers. \[ r^2 \] was 0.024, indicating that only a very limited (2.4%) but significant (p<0.001) part of the variation in A was accounted for by its dependence on the mother diameter. This relation was significant for both hearts. The right panel of Figure 4 depicts a histogram of the deviations of A from the regression line. Estimated standard deviation of this distribution is 0.298. This intrinsic variability therefore is much larger than the difference in A between both hearts. For comparison, a
normal distribution with the same standard deviation has also been drawn in the right panel of Figure 4. The distribution of the observed data was significantly different from this normal distribution (\(\chi^2\) test, \(p<0.001\)), but for modeling purposes, this difference will be neglected below.

The left panel of Figure 5 depicts the symmetry \(S\) as a function of the mother diameter. As is clear from this semilogarithmic plot, the symmetry of arterial nodes proved to be very variable. Furthermore, a lower bound of \(S\) exists, which is strongly dependent on \(d_m\). This is because arterial segments with diameters less than the capillary diameter do not exist. Assuming a smallest diameter of 5 \(\mu m\), this lower bound is indicated by the dashed line in the left panel of Figure 5. The nature of the distribution of symmetry is illustrated in the right panel of Figure 5 for values of \(d_m\) larger than 50 \(\mu m\). Mean values of \(S\) in this range are 0.511±0.243 (both hearts, \(n=912\), 0.473±0.244 (heart 1, \(n=388\)), and 0.539±0.238 (heart 2, \(n=524\)). Thus, also in this respect, the differences between the two hearts are minor compared with the high variability within each heart. The distribution of \(S\) is further documented in Table 1, which shows median and quartile values of \(S\) in six arbitrarily chosen classes of \(d_m\).

As discussed above, area expansion ratio \(A\) and symmetry \(S\) depend on the diameter of the mother segment. When corrected for this diameter dependency, these variables do not correlate \((r^2=0.001, n=1,652)\). Hence, area expansion and symmetry, corrected for diameter, can be considered to be independent variables.

The vascular tree may be organized by a dependence of properties of sequential segments and nodes. We studied such a connectivity by correlating length of sequential segments and area expansion ratio and symmetry of sequential nodes. After correction of the variables for their dependence on the mother diameter, we found neither length of sequential segments nor area expansion ratio of sequential nodes to be correlated:

\[ r^2=0.000, \quad n=2,786; \quad r^2=0.003, \quad n=1,561. \]

Also, lengths of sister segments did not correlate: \(r^2=0.001, n=1,393\). A test for connectivity of symmetry is more complicated, since distribution of this variable is considerably nonnormal, and no least-squares fit of symmetry versus the mother diameter was made. This problem was approached by defining two classes of symmetry, determined by the median in the appropriate diameter class (see Table 1). Each node was classified as being less symmetric or more symmetric than the median value. For the 1,561 available pairs of sequential nodes, we tested for a correlation between the classification of the symmetry in the proximal node and that in the distal node. In 783 cases, the symmetry of the proximal node was larger than the median. In 411 (54\%) of these cases, the symmetry of the distal node was also larger than the median. Also, in the 778 cases where the symmetry of the proximal node was smaller than the median, the symmetry of 53\% of the distal nodes was also smaller than the median. These numbers reveal a slight but significant \((\chi^2\) test, \(p<0.005\)) clustering of symmetry within the coronary arterial tree.

**Computer Construction of the Arterial Bed**

Based on the experimentally found distributions of segmental length, nodal area expansion, and symmetry as functions of the mother diameter, computer constructions of parts of the coronary arterial bed were made. Each construction started with one segment 500

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

**Figure 3.** Plot showing the relation between the diameters of sister segments \((d_1\) and \(d_2\)). For each observed node, these diameters have been normalized to the diameter of the mother segment \((d_m)\). The curve represents nodes where total cross-sectional area remains constant. The dashed line is the identity line and reflects the upper limit for the data points.

![Figure 4](http://circres.ahajournals.org/)

**Figure 4.** Left panel: Plot showing the area expansion ratio of vascular nodes as a function of the diameter of the mother segment, with the estimated regression line. Right panel: Histogram of the deviations of the data points in the left panel from the regression line. \(A\), area ratio. The line shows the fit of a normal distribution through this histogram.
μm in diameter. Based on this diameter, a length was chosen. For this choice, we used the fit through the experimental data of segmental length versus diameter (left panel of Figure 1), as well as the variability of segmental length, as expressed by the gamma distribution in the right panel of Figure 1. Subsequently, a node and two daughter branches were generated in the following way: Based again on the diameter of the mother segment, a value for the area ratio A was chosen. For this choice we used the fit of A versus the mother diameter (left panel of Figure 4), as well as the approximation of the variability in area ratio by a normal distribution (right panel of Figure 4). To choose a value for symmetry, the following procedure was used: The observations of symmetry were grouped in six classes based on the mother segment diameter (see Table 1 for the diameter intervals). These data were normalized with respect to the range between the lower bound shown in the left panel of Figure 5 and S=1. This way, six sets of observations of normalized symmetry were available with values ranging from 0 (dashed line, \(d_s=5 \, \mu m\)) to 1 (both daughter segments having equal diameter). Generation of \(S\) consisted of taking a random sample from the appropriate set of observations, and recalculating \(S\) from its normalized value and the value of the mother segment diameter. The diameters of the two daughter segments were calculated from the values of both A and S. Lengths of these segments were again generated on the basis of their diameter, and distal to each of these segments, two new segments were generated. The process was continued until segments were generated with diameters between 10 and 5 μm.

All values of length, area ratio A, and symmetry S were generated independent of each other and of previously generated values. Because variability of the experimental data is included, the model tree is stochastic in nature. To determine variability of the model predictions, 30 model trees were generated. The correctness of the computer programming was checked by comparing generated properties of nodes and segments with the experimentally determined properties.

**Prediction of Coronary Arterial Topology**

Strahler ordering17-19 was applied to the generated networks. This ordering algorithm is shown schematically in Figure 6. The precapillary segments are assigned order 1. Any other segment is given the highest of the orders of the two daughter segments, if these are unequal. If the segments distal to a node have an equal order, then the mother segment is given one order higher than that of its branches. A series of segments of equal order forms a “vessel,” with length equal to the sum of the lengths of the individual segments and diameter chosen equal to the arithmetic average of the diameters of the segments. The terminal segments in

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**Figure 5.** Left panel: Plot showing the symmetry of vascular nodes as a function of the diameter of the mother segment \(d_m\), \(d_s\) and \(d_l\) represent the diameters of the small and large branches, respectively. The dashed line indicates the minimal symmetry that is expected (see text). Right panel: Histogram of symmetry in nodes where \(d_m\) was larger than 50 μm.

**Figure 6.** Illustration of the Strahler ordering algorithm. Numbers indicate the orders. Note the important discrimination between segments and vessels.
FIGURE 7. Results from the network simulations. The number of vessels (left panel), their mean diameters (middle panel), and their mean lengths (right panel) are plotted as functions of their Strahler order on a semilogarithmic scale. The results are shown for ordering procedure 1 (filled circles) and 2 (open circles). Linear regression lines for the results of ordering procedure 1, fitted through the points at Strahler orders 2–8, have also been drawn. Error bars in the left panel indicate standard deviations of the number of vessels between the 30 networks. Error bars in the middle and right panels indicate standard deviations of the diameter and length in each order and were calculated from the pooled results. All error bars are only drawn for ordering procedure 1. Their asymmetric appearance is due to the logarithmic scaling.

the generated beds have diameters between 5 and 10 μm, and it was assumed that these represent the precapillary arterioles. The impact of this assumption on the resulting Strahler ordering was tested as follows: In ordering procedure 1 all end segments were assigned order 1, whereas in ordering procedure 2, end segments larger than 7.5 μm were assigned order 2, and smaller end segments were assigned order 1. The topology of the generated beds was described by the bifurcation ratio\(^9\) (RB), defined as

\[
RB(i) = \frac{N(i)}{N(i+1)}
\]

where \(i\) is the order number and \(N(i)\) is the number of vessels having Strahler order \(i\). Note that in this context a vessel consists of one or more segments. RB in a symmetric tree is 2; asymmetric trees lead to higher values. Similarly, diameter ratio (RD) and length ratio (RL) were defined as

\[
RD(i) = \frac{D(i+1)}{D(i)}
\]

\[
RL(i) = \frac{L(i+1)}{L(i)}
\]

where \(L\) and \(D\) refer to the total length and the mean diameter, respectively, of a vessel. Both RD and RL will generally be larger than 1.

The starting segments of 500 μm were found to have order 9, 10, or 11 (6, 19, and 5 cases, respectively) in ordering procedure 1 and 9, 10, 11, or 12 (1, 10, 17, and 2 cases, respectively) in ordering procedure 2. Figure 7 shows the logarithmics of the number of vessels, their mean diameter, and their mean length as functions of the order number for both ordering procedures. As can be seen, these properties are reasonable linear functions of the Strahler order. Note the large variation in the lengths of the vessels. In all three plots, the choice of order numbers for the most distally generated segments influences the vertical position of the curves but not the slopes. Table 2 shows RB, RD, and RL, calculated from the slopes of regression lines through the data points at orders 2–8, as well as the ratios at order 1, as calculated using Equations 4–6.

All order-1 vessels necessarily consist of one segment only. Vessels of higher orders can contain one or more segments. Figure 8 depicts for orders 2 and 7, frequency distributions of the number of segments that together formed complete vessels in the network. These results were only calculated for ordering procedure 1 (all terminal segments order 1). As can be seen in Figure 8, the number of segments per vessel is very variable, and its distribution is highly skewed: One-segment vessels were most frequently present in the construction, and the occurrence of vessels consisting of more segments decreased with the number of segments. This decrease was far more rapid in order 2 than in order 7. Intermediate orders had intermediate distributions. For each of the orders 2–7, the mean number of segments per vessel has been indicated in Table 3. This number was found to increase gradually with order number. The shapes of the histograms in Figure 8 indicate geometric distributions\(^{21}\) of the number of segments per vessel. These distributions are described by

\[
N(k) = N_{tot} \cdot q \cdot (1-q)^{k-1}
\]

in which \(N(k)\) depicts the frequency of vessels consisting of \(k\) segments, \(N_{tot}\) is the total number of vessels, and \(q\) is the parameter of the distribution. Table 3 summarizes values of \(q\), as estimated from curves as in Figure 8.

| Table 2. Simulation Results for Bifurcation, Diameter, and Length Ratios for Ordering Procedures 1 and 2 |
|---|---|---|---|---|---|---|
| Order | RB Proc 1 | RB Proc 2 | RD Proc 1 | RD Proc 2 | RL Proc 1 | RL Proc 2 |
| 1 | 2.99±0.01 | ... | 1.49±0.00 | 1.43±0.00 | 2.40±0.02 | 1.65±0.00 |
| 2–8 | 3.30±0.06 | 3.24±0.04 | 1.51±0.02 | 1.49±0.01 | 1.63±0.04 | 1.69±0.01 |

RB, bifurcation ratio; RD, diameter ratio; RL, length ratio; Proc 1 and Proc 2, ordering procedures 1 and 2, respectively. Values are mean±SD of 30 generated networks; SD indicates the variability of the ratios between the networks.

Results for order 1 were directly calculated according to Equations 4–6. Results for orders 2–8 were calculated from linear regression lines through the points in Figure 7.
We could not attribute Strahler orders to the trees that were measured on the cast, since these were highly incomplete. To be able to test the model with respect to its predictions of topology, we performed an extra set of measurements on heart 2, this time concentrating on topology and (for practical reasons) skipping diameter and length measurements, except for diameter of the entrance segment. Eighteen trees were found in which all terminal segments were less than 10 μm in diameter. Entrance segments of these trees branched off much thicker vessels and were clearly the start of a new Strahler vessel. Diameters of these entrance segments ranged from 19 to 35 μm. The number of segments in the trees ranged from 29 to 73. Strahler ordering was applied to each of these trees. Order of the entrance vessel was either 3 (n=1), 4 (n=15), or 5 (n=2). Mean diameter of the order-4 entrance segments was 28.5±1.2 μm (mean±SEM). Model predictions (middle panel of Figure 7) were 24.4 μm for order 4 and 37.2 μm for order 5. These numbers indicate that order 4 was assigned to the same group of diameters in both the model and the data.

Table 4 compares the 15 experimentally found trees starting at order 4 and the model predictions with respect to RBs and mean number of segments per vessel. In the model, vessels of order 3 and lower occasionally sprout off orders 5 and higher. Such vessels are not counted in the data, since the entrance order was 4. In Table 5, the model predictions for RB are lower than the values mentioned in Table 2, since we considered subtrees starting at order 4 only. Unlike RB, the number of segments per vessel is independent of network size, and this quantity can be compared directly. No differences of these quantities between the data and model prediction could be demonstrated (t tests). Figure 9 compares the distribution of the number of segments per vessel with the model predictions. The latter were already described by Figure 8 for orders 2 and 7. As can be seen, in both orders, the data show the highly asymmetric distribution that was predicted by the model. Although, in comparison to the model prediction, the data tend to be less dispersed, for neither order could a significant difference between model and experiment be demonstrated (χ² test).

**Prediction of Local Pressure and Flow**

The generated networks were used to predict local pressure and flow in the dilated coronary arterial bed. For this, we needed to make a number of assumptions: Resistance of each segment was assumed to be constant and determined by the Poiseuille relation, with viscosity equal to that of Ringer’s solution, which was the perfusion medium before construction of the casts and of which flow was measured at a known pressure. Furthermore, extra hindrance by nodes was neglected. Last, precapillary pressure was assumed to be homogeneous; its level was estimated as indicated below.

We calculated precapillary pressure (Pc) from the experimentally determined coronary input pressure (Pp, 90 mm Hg) and coronary flow (Q, 526 ml/min) in heart 2 and an estimation of total coronary conductance (Gtot) based on the cast of this heart according to the relation

\[ P_c = P_p - Q/G_{tot} \]  

(8)

The determination of the total conductance of heart 2 would require the measurement of all vessels between the large entrance arteries and the capillary bed. This could not be done. Instead, the large arteries in the cast of this heart were followed down to their branches having a diameter smaller than 500 μm. These branches varied in diameter between 30 and 500 μm according to the symmetry distribution recorded. In this way, a complete set of 455 small arteries could be identified as being the origin of subtrees, together perfusing the whole myocardium. The experimentally determined diameter of each of these 455 arteries was taken as the
starting diameter for model generation of a subtree according to the rules described. All these subtrees in parallel form the model of the coronary circulation used to estimate total conductance, assuming that the resistance of the large arteries proximal to these 455 subtrees is negligible. Under this assumption, \( G_{\text{tot}} \), of the coronary arterial tree equals the sum of the conductances of the 455 subtrees starting at each of the segments. From seven repetitions of generation of all 455 subtrees, \( G_{\text{tot}} \) was estimated to be 9.06±0.31 ml/min \( \cdot \) mm Hg (mean±SD). Using Equation 8, \( P_c \), was estimated to be 31.9±1.1 mm Hg (mean±SD). This estimate was used to calculate the pressure profile in the 30 simulated trees started at 500 \( \mu \)m. Figure 10 shows this predicted pressure profile. From this profile, we estimate 14% of the total coronary resistance to be located proximal to 200-\( \mu \)m arteries, 23% proximal to 100-\( \mu \)m vessels, and 37% proximal to 50-\( \mu \)m arterioles.

Table 5 documents the effect of excluding variability of length, area ratio, or symmetry during network generation on the pressure profile. Excluding the variability of any of these quantities leads to somewhat higher and less dispersed local pressures, with the effect being the largest for exclusion of variability in area ratio.

Local blood flow, as measured by micrometer deposition, has been shown to be heterogeneous, with the level of heterogeneity being dependent on the volume of the tissue samples. We tested whether these relations are predicted by the model: The number of terminal segments distal to any segment in the generated trees (\( N_t \)) was taken as an index of the tissue volume perfused by that segment. Classes of \( N_t \) were defined with \( N_t \) increasing twofold for each class. Such a class was assigned to each segment in the model, and heterogeneity of estimated flow within each class was calculated. Because of the asymmetry of the network, two or more sequential segments may easily belong to the same class. Whenever this happened, only flow in the most proximal of these segments was included in the estimation of flow heterogeneity. Figure 11 plots the estimated coefficient of variation (CV, defined as the standard deviation divided by the mean) of flow per terminal segment in the various classes (pooled results from 30 simulations). The straight line indicates the fit of the model predictions to the fractal relation

\[
CV(N_t) = CV_1 \cdot N_t^{(1-D)}
\]  

where \( CV(N_t) \) is the coefficient of variation of flow per terminal segment through subtrees that consist of \( N_t \) terminal segments, \( CV_1 \) is the extrapolated value of this quantity in single end segments, and \( D \) is the fractal dimension. \( D = 1 \) would indicate a homogeneous distribution of flow, whereas \( D = 1.5 \) indicates the absence of correlation of flow in connected segments. The linear regression estimates of these quantities for the pooled results are \( CV_1 = 140\% \) and \( D = 1.189 \). Also, each of the 30 individual simulation results followed the above fractal relation over a wide range of subtree sizes. From the calculation of the 30 individual regression curves, \( CV_1 \) was found to be 149±17\% (mean±SD); \( D \) was 1.201±0.039. Excluding variability in either length, area

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**Table 5. Effect of Excluding Variability of Branching Variables on Physiological Predictions**

<table>
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<tr>
<th></th>
<th>( n )</th>
<th>( P_c ) (mm Hg)</th>
<th>( P_{50} ) (mm Hg)</th>
<th>( P_{100} ) (mm Hg)</th>
<th>CV (_1)</th>
<th>D</th>
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<td>31.9</td>
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<td>69.5±11.6</td>
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<td>1.20±0.04</td>
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<tr>
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<td>29.8±2.6</td>
<td>59.6±11.6</td>
<td>75.6±5.6</td>
<td>1.09±0.09</td>
<td>1.18±0.04</td>
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<tr>
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<td>41.4±0.6</td>
<td>67.1±5.9</td>
<td>76.5±4.4</td>
<td>1.06±0.04</td>
<td>1.28±0.02</td>
</tr>
<tr>
<td>No symmetry variability</td>
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<td>65.0±9.3</td>
<td>74.3±7.2</td>
<td>0.82±0.07</td>
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</tbody>
</table>

\( n \), Number of simulations; \( P_c \), precapillary pressure; \( P_{50} \) and \( P_{100} \), pressures at the 50- and 100-\( \mu \)m level, respectively; \( CV_1 \), extrapolated coefficient of variation of flow in single terminal segments; D, fractal dimension of flow heterogeneity. Values are mean±SD; SD values of pressures reflect variability within the networks, and SD values of \( CV_1 \) and D reflect variability between the networks.

Excluding variability of length and area ratio was achieved by applying the linear regression lines in the left panels of Figures 1 and 4, respectively, and ignoring the distributions in the right panels of Figures 1 and 4. Symmetry was set constant at the median value found in the various diameter classes (see Table 1).

---

**Figure 9. Bar graphs comparing simulation and experimental results: distributions of the number of segments per Strahler vessel for orders 2 (left panel) and 3 (right panel). The simulation results have been normalized such that the total number of vessels equals that of the experimental results.**
Discussion

The major contributions of this study are the quantification of the very irregular branching pattern of the dilated coronary arterial bed and the use of a model representation of this pattern to predict local pressure and flow heterogeneity. This discussion will focus on the collection and description of the anatomic data, the topology of the bed, and the predictions for local pressure and flow values.

Anatomic Data

The pressures during injection of the casting material were below physiological levels, to prevent filling of the capillary bed. Since blood vessels are distensible, this might potentially bias the diameter measurements. Therefore, the hearts were perfusion-fixed before injection of the plastic. Previously,23 we showed that fixed coronary arterioles are virtually rigid. Thus, we believe that the injection pressure of the plastic is of no influence on the vessel diameters.

In the two coronary casts used for this study, only a very small fraction of the capillaries was filled. A higher degree of capillary filling was prevented to keep a clear vision on the arterioles. This induces a potential pitfall in the interpretation of the data, since very thin arterial branches might also have been unfilled. Nodes in which one of the daughter segments is unfilled would be missed, and the other branch would erroneously be considered part of the mother segment. This would lead to an overestimation of the lengths of especially the distally located segments. Moreover, the symmetry of nodes would be overestimated, since very thin daughter segments may have remained unfilled. However, the effect of incomplete filling of the bed may not be too large; one would expect that, in most cases, filling by the casting material does not stop at exactly the nodes but somewhere along the segments. Indeed, short stumps of thin segments were frequently observed, and the diameters of these stumps were included in the data. Obviously, the lengths of these stumps were not included. A sound estimation of the impact of the incomplete filling of the bed would require more knowledge on the complicated process of filling and hardening of the plastic. Therefore, an effort to quantify the branching in beds that have been filled up to the capillaries remains desired.

Data on segmental length versus diameter in the coronary circulation have previously been reported by Suwa and Takahashi24 for human hearts. These authors found the relation \( \log(L) = 0.58 + 1.05 \cdot \log(d) \), where \( L \) is the unbranched length and \( d \) is the diameter of arterial segments. Thus, in that case the expected lengths of the segments were almost proportional to their diameters, whereas in the current study the smaller vessels tended to be relatively longer. Like in our case, Suwa and Takahashi have reported a very large variability in the lengths of vessels of a given diameter. Because of this variability, care should be taken in interpreting these logarithmic fits: The fits give an estimate of \( \log(L) \) at any diameter. However, calculating \( L \) from this estimate will result in approximating the median rather than the mean value.24 In our data, this procedure would result in a 33% underestimation of the mean segment length. In our model, we included

Figure 10. Graph showing the simulated pressure profile in the dilated arterial bed. These results are pooled from 30 network simulations based on an experimentally determined input pressure of 90 mm Hg (dashed line) and an estimated precapillary pressure (see text) of 32 mm Hg. The solid line indicates the mean pressure. The shaded area reflects the standard deviation of the pressure within the networks. Circles represent data from Chilian22 in subepicardial (closed circle) and subendocardial (open circle) vessels (see "Discussion").

Figure 11. Graph showing flow heterogeneity as estimated from the pooled results of 30 network simulations. The abscissa indicates the number of terminal segments at various points within the trees as an index of perfused volume. The ordinate indicates the coefficient of variation (also called the relative dispersion) of the flow per terminal segment along the tree. The straight line in this double-logarithmic plot is a fit of the fractal relations in Equation 9.
the measured variation in log(L) before calculating L itself, bypassing this pitfall.

Zamir et al.15 and Zamir and Chee16 also quantified the area ratio and found it to average 1.06 for the rat coronary bed15 and 1.14 for the human coronary bed.16 These data are comparable to the value of 1.09 that we estimated for vessels of the same size. Also, variability of area ratio appears to be comparable.

An alternative description of area growth in vascular nodes can be made by the power relation

\[(d_b/d_0)^2+(d_l/d_0)^2=1\]  \hspace{1cm} (10)

Suwa and Takahashi14 found \(\tau\) to be 2.51 for human coronary arterial vessels larger than 100 \(\mu\m) and 2.82 for smaller vessels. The power relation has the convenient property that it can be compared with similar relations describing arteriolar density and flow as a function of diameter. Thus, Arts et al.25 used the power relation to describe the number of 400-\(\mu\m) arteries distal to arteries of a given diameter as a function of this diameter. They found \(\tau\) to be 2.55. Wieringa et al.26 estimated arteriolar density from the resistance increase on the injection of microspheres. The authors report \(\tau\) to be 2.81 for vessels between 15 and 37 \(\mu\m) in diameter. Mayrovitz and Rop27 determined flow as a function of diameter in the microcirculation of the rat cremaster muscle and found \(\tau\) equal to 3. We estimated \(\tau\) from our data by minimizing the shortest distance between the data points and the curve of Equation 10. This was done for three groups of nodes, classified by the mother diameter (\(d_b\)). \(\tau\) was found to be 2.82 for \(d_b\leq40 \mu\m\), 2.50 for \(40<d_b\leq200 \mu\m\), and 2.35 for \(d_b>200 \mu\m\). These numbers are in good agreement with the data cited above, obtained from quite different procedures.

Zamir et al.15 and Zamir and Chee16 demonstrated the existence of both symmetric and very asymmetric nodes in the coronary bed in a graphic way. As judged from their plots, the distribution of symmetry in the human coronary bed16 is roughly comparable to the current findings, whereas in the rat heart, the nodes seem to be more symmetrical.15

We considered the coronary arterial network to be a branching tree with no collateral connections. This is not quite realistic, since collaterals and arcades can be observed at both the epicardial and endocardial surface of porcine hearts. However, we could find only very few of these structures in the cast, and we feel that ignoring them in the model does not largely bias the predictions of local pressure and flow. If anything, one may expect collaterals to decrease the variability of these quantities, while average levels remain largely unchanged.

The current study shows that the length of sequential segments and the area ratio in sequential nodes are not correlated. Symmetry of sequential nodes was found to be only slightly correlated. Although more subtle patterns of connectivity may exist that were not tested for, these results indicate that the branching pattern of the coronary arterial bed can adequately be described by a few stochastic variables: length, area ratio, and symmetry. These variables determine over and over again the lengths and diameters of segments and therefore also the topology of the bed and the distribution of arterial resistance. As such, they form a concise way of describing the architecture of the coronary bed and allow for comparison between species or developmental conditions. The current network description is not a fractal per definition; length is not proportional to diameter, and both area ratio and symmetry decrease with increasing diameter. Yet, the simulation results do indicate fractal properties with respect to the Horton ratios and flow heterogeneity.

**Coronary Arterial Topology**

The topology of the generated bed was analyzed by Strahler ordering. The networks were characterized by their constant ratios of bifurcation, as well as diameter and length ratios, over many orders. In the computer generation of the bed, an arbitrary termination criterion was used. By applying two ordering algorithms, we showed that this criterion does not greatly affect the predicted values for the Horton ratios. Table 6 compares these predictions with the ratios reported by others based on direct observations on less orders and in other vascular beds. This table indicates that the currently found values fall within the range of values that has been reported. Also, a direct measurement in this study of the ratio of bifurcation in orders 1–3 revealed no differences with the predicted values. However, predictions of these ratios may not form a very critical test of the model. More information is obtained from the distribution of the number of segments that together form one vessel, according to the Strahler scheme. In the model, this number was approximately geometrically distributed in each of the orders (Figure 8). For orders 2 and 3, the distribution of this quantity in the data was not significantly different from the model predictions. Similar distributions have been reported for cat sartorius muscle.30 These matches sup-

### Table 6. Comparison of the Currently Reported Horton Ratios With Values From Literature

<table>
<thead>
<tr>
<th>Reference</th>
<th>Species</th>
<th>Tissue</th>
<th>Orders involved</th>
<th>RB</th>
<th>RD</th>
<th>RL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Present study</td>
<td>Pig</td>
<td>Heart</td>
<td>2–8</td>
<td>3.30</td>
<td>1.51</td>
<td>1.63</td>
</tr>
<tr>
<td>Horsfield28</td>
<td>Human</td>
<td>Lung</td>
<td>1–5</td>
<td>3.15</td>
<td>1.60</td>
<td>1.50</td>
</tr>
<tr>
<td>Fenton and Zweifach19</td>
<td>Rabbit</td>
<td>Omentum</td>
<td>1–3</td>
<td>3.12</td>
<td>1.30</td>
<td>1.61</td>
</tr>
<tr>
<td>Koller et al.30</td>
<td>Cat</td>
<td>Sartorius</td>
<td>1–4</td>
<td>3.28</td>
<td>1.24</td>
<td>2.13</td>
</tr>
<tr>
<td>Hudetz et al.39</td>
<td>Rat</td>
<td>Brain</td>
<td>1–3</td>
<td>4.14</td>
<td>1.5</td>
<td>...</td>
</tr>
</tbody>
</table>

RB, bifurcation ratio; RD, diameter ratio; RL, length ratio.

Order 1 from Reference 29 represents pial vessels of 50 \(\mu\m\) in diameter, which are not directly connected to the capillary bed.
port our view that the currently used procedure for network generation leads to realistic arterial trees.

The geometric nature of these distributions has an important implication: Consider, for instance, the node distal to an order-3 vessel and travel upstream along this vessel. Then, in the next node encountered, the vessel may either reach its proximal end (if the connecting segment is of order 3 or higher) or may continue (if the connecting segment has order 1 or 2). If the vessel continues, the same possibilities hold in the next proximal node, and so on. The resemblance to the geometric distribution now indicates that the probability that the vessel reaches its proximal end in a node is independent of its number of segments. This termination probability is equal to the parameter \( q \) in Equation 7 and was found to decrease from 63% in order 2 to 19% in order 7. The decrease of \( q \) with order number, despite the constant bifurcation ratio, reflects that jumps in order occur very frequently in the model. Thus, whereas an order-2 vessel typically has two or three order-1 branches, an order-7 vessel may be very long and have a large series of branches that are of order 1–6. The appearance of these order jumps is a direct consequence of the variability in nodal symmetry. Networks generated by completely random algorithms also show the above properties, and we suggest that modifications of such algorithms form an alternative way of generating quite realistic coronary arterial networks, with the advantage that insight may be gained with respect to the way the coronary tree develops.

**Pressure Profile**

It appears not to be feasible to derive mathematical expressions for the pressure profile directly from the values of the branching variables. As a rule of thumb, the profile is roughly determined by the balance between the strong fourth power increase in resistance and both the increment in arteriolar density and decrease in segmental length with smaller diameter. Yet, the influence of variability in the branching variables is hard to predict. For that reason, we generated computer models of the bed and calculated the profile in these models. According to Figure 10 and Table 5, the effect of variability in segmental length, nodal area expansion, and symmetry is both a considerable dispersion of local pressure and a distribution of vascular resistance over a larger diameter range.

Recently, Chilian\(^{22}\) reported measurements of local pressures in 100-\(\mu\)m porcine coronary arterioles and venules during maximal dilation and diastolic arrest. We interpolated his results for the perfusion pressure of 90 mm Hg, which was chosen in the present study. At this perfusion pressure, arteriolar pressure would have been 71 mm Hg in the subepicardial vessels and 56 mm Hg in the subendocardial vessels. These data have been indicated by the circles in Figure 10. This comparison indicates that at the 100-\(\mu\)m level our estimated mean pressure is close to the subepicardial pressure, while pressures close to the measured subendocardial pressure also occurred in the network. Pressure in 100-\(\mu\)m venules, calculated from Chilian’s data, would have been 15 mm Hg in the subepicardium and 25 mm Hg in the subendocardium. Our estimate of precapillary pressure (32 mm Hg) is higher and, therefore, not inconsistent with these experimental results.

We did not aim to reveal differences between subendocardial and subepicardial branching patterns, nor did we try to include positions in space in the network generation. Yet we feel that the dispersion in local pressure may reflect endocardial and epicardial differences: The starting segment of 500 \(\mu\)m would generally be an epicardial segment, giving rise to a few transmural trees. Consequently, any 100-\(\mu\)m segment close to the root would also be located in the epicardial region. Such segments exist, as evidenced by the appearance of very asymmetric nodes. Pressure in such segments would be relatively high, since the entrance path is short. On the other hand, 100-\(\mu\)m segments that are located rather distally in one of the transmural trees would be situated more closely to the endocardium and have lower local pressure. Thus, to some extent the variability in Figure 10 may reflect transmural differences. This reasoning supports the view that transmural arterial resistance is not negligible.

**Flow Heterogeneity**

Flow in the simulated networks was very heterogeneous. Furthermore, the relation between the level of flow heterogeneity and the perfused volume, as expressed by the number of terminal segments in a subtree, obeyed the fractal relation suggested by Bassingthwaighte et al\(^{10}\) over a 1,000-fold variation in volume. Fractal dimension \( D \) was found to be 1.20. According to Table 5, the level of flow heterogeneity appears to be a consequence of dispersion in each of the branching variables: segmental length, area ratio, and symmetry. Without dispersion in any of them, flow is less heterogeneous, but this heterogeneity still follows a fractal pattern. Only dispersion in the area ratio appears to be critical for the value of \( D \). From experiments using microspheres and iododeisethyliimonipramine, Bassingthwaighte et al reported \( D \) to be 1.20, 1.16, and 1.22 for autoregulated baboon, sheep, and rabbit hearts, respectively. Using an estimate of the perfused volume per end segment, we found flow heterogeneity in subtrees that perfuse 1-g tissue pieces to be 18%. This value too is well within the range of 7% to 43% reported by Bassingthwaighte et al.\(^{12}\)

Some care must be taken in comparing our predictions with the data of Bassingthwaighte et al.\(^{4}\) Their data involve autoregulating hearts, whereas our network model was based on data from a maximally dilated tree. If coronary reserve were spatially homogeneous, this difference in physiological state would be of no importance in comparing these data. However, Austin et al\(^{5}\) show absence of correlation of resting and maximal flow and, consequently, heterogeneity of coronary flow reserve at the 0.1-g level in the dog heart. That study did not use fractal analysis but did show that flow during maximal dilation is spatially correlated. The authors reported CV during maximal dilation to be 30% in 0.10-g tissue pieces. In an earlier study, Sestier et al\(^{13}\) reported CV in the dilated canine coronary bed to be 31% in 0.74-g pieces. Our estimates of CV in tissue pieces of these weights would be 28% and 19%, respectively. Thus, our estimate of flow heterogeneity is in good agreement with the value reported by Austin et al, but it is lower than the result of Sestier et al.

A previous attempt to correlate flow heterogeneity with the structure of the coronary bed was presented by...
Van Beek et al. These authors derived hypothetic fractal networks that predict the reported flow heterogeneity. These networks were characterized by distribution of flow in vascular nodes rather than by diameter and length relations. A small asymmetry of flow in each node was sufficient to explain the high level of flow heterogeneity in tissue samples and its fractal nature. We did not analyze the level of nodal flow asymmetry in the generated networks and therefore cannot compare the two models in this respect. A fundamental difference is that the networks of Van Beek et al were topologically symmetrical, with an equal number of segment generations along all paths and a bifurcation ratio of 2, whereas in the current model, both very short and long paths between the root and the terminal segments exist.

To compare the level of flow heterogeneity for a given sample weight with heterogeneity in the tree, we had to estimate the average weight of the cardiac tissue that is perfused by each end vessel in the model. We found this weight to be 19.3 μg. As far as we know, no direct measurements of this quantity are available. From interpretation of the reduction in flow after partial embolization of the vasculature with microspheres, Wieringa et al report the density of 15-μm arterioles in the rat heart to be 162 mg⁻¹, which is equivalent with a perfused weight of 7.5 μg per 15-μm arteriole. Using a similar approach, Pelosi et al reported a value of 41.6 μg for 20-μm arterioles in the dog heart. In both these studies, as well as the present study, the procedure for estimation of this quantity is quite complex, and an estimation of perfused weight for various vessel sizes from direct observations remains highly desired.

In estimating heterogeneity of local tissue flow, we assumed that all terminal segments perfuse equal volumes of cardiac tissue. Without doubt, this assumption is not realistic. Thus, an unknown part of the 200% flow variation in the terminal segments may be compensated for by assuming that segments with relatively high flows also perfuse large volumes. Yet, if this uncertainty is not spatially correlated, it would rapidly decrease for larger scales of resolution. Extrapolating from 200% flow variation in single segments with a slope of -0.5 in Figure 11, indicating D=1.5 and thus absence of spatial correlation, we estimate that flow variation in 1-g tissue pieces would have been reduced by only 0.6% if proper assumptions regarding the size of terminal vessel perfusion volumes had been made.

It was further assumed that the sample pieces into which the heart would have been cut would correspond exactly with the area perfused by one single subtree. In reality, however, tissue pieces contain multiple subtrees, which result in less heterogeneity at any scale. This problem was discussed in detail by Van Beek et al. These authors suggest that this mismatch results in roughly a leftward shift of the relation between CV and sample size by a factor of 2. In our model, this would indicate that a measurement of CV in 1-g tissue pieces results in 15% heterogeneity in local tissue flow rather than the 18% that was calculated from flow in the branching tree. Therefore, this bias would not greatly affect the conclusion that the coronary branching pattern is a major determinant of coronary flow heterogeneity. However, we feel that a full understanding of the relation between local tissue flow and flow distribution in the branching network requires additional knowledge on the spatial organization of the coronary tree, and future work should be performed that adds this spatial information to the model.

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