Arterial Bifurcations in the Cardiovascular System of a Rat

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ABSTRACT   Arterial bifurcations in the cardiovascular system of a rat were studied, using a resin cast of the entire arterial tree. At each bifurcation, measurements were made of the diameters of the three vessels involved, the two branching angles, and the angle δ, which the parent artery makes with the plane containing the two branches. The results were found to be consistent with those reported previously in man and monkey. In addition, measurements of δ in the present study indicate that arterial bifurcations are mostly two dimensional.

INTRODUCTION

Arterial branching is a dominant structural feature in the cardiovascular system. The most common mode of branching is that in which a single stream of blood divides into two separate streams. The two daughter streams may differ greatly in size and direction, but in all cases the branching site may be conveniently referred to as an "arterial bifurcation." Defined in this rather general way, an arterial bifurcation becomes the characteristic structural unit of the arterial tree. It has been estimated by Green (1950) from data by Mall (1906) that the number of capillaries in an arterial tree is of the order of one billion (10^9), from which it follows that the number of arterial bifurcations involved in the same tree is of the same order. The function of the cardiovascular system as a fluid-conducting network is therefore highly dependent on the efficiency of arterial bifurcations as stream-dividing units.

Surprisingly little study of arterial branching has been made so far, and very little is known about the morphological design of arterial bifurcations. The pioneering observations of Thoma (1896) provided an important beginning, and the well-known study of Mall (1906) provided the first quantitative information on the number and diameters of blood vessels. The first attempts to understand branching angles were reviewed by Thompson (1917), but a thorough analysis of this aspect of arterial branching was not achieved until Murray (1926 a, b). Several authors have since then expanded Murray's original ideas (Kamiya and Togawa, 1972; Kamiya et al., 1974; Zamir, 1976 a, b; Uylings, 1977). The related problem of branching angles in the bronchial

tree has been studied extensively by Horsfield and Cumming (1967). Present understanding suggests that the angles that the two branches make with the parent artery at an arterial bifurcation are such as to minimize the volume of blood that the bifurcation region must contain and, at the same time, minimize such fluid dynamic factors as the pumping power required to drive blood through the bifurcation and the shear force between moving blood and endothelial tissue. Although the first of these favorable conditions is in conflict with the second and third, there is for each branch an "optimum" branching angle (Zamir, 1978) that would produce a compromise.

Measurements of branching angles and branch diameters, whether from man or animals, are not available in adequate quantity. The terminal bifurcation of the abdominal aorta into the two common iliac arteries was frequently chosen in the past as a source of data (see, for example, Thompson, 1917). It would be unreasonable to treat such data as typical of the entire cardiovascular system, however, since this bifurcation is hardly typical of all others in the system as it may well be affected by gross anatomical factors. Also, it is very likely that no single bifurcation can be taken as typical of the entire system. In an attempt to survey a reasonably large number of bifurcations, Zamir et al. (1979) and Zamir and Medeiros (1982) used enlarged photographs of the vascular bed of the retina. Although these studies provided a good set of data on branching angles and branch diameters, two limitations were still inherent there and called for further study. First, the data related exclusively to a very small part of the cardiovascular system, namely the retina. Second, all measurements were derived from two-dimensional pictures of the vascular bed and were hence subject to three-dimensional viewing errors (Zamir, 1981). The purpose of the present study is to provide a new set of data that is free from these two limitations.

We present the results of a series of measurements of branching angles and branch diameters derived from resin casts of entire arterial trees from the cardiovascular systems of laboratory white rats. The measurements were taken from bifurcations in all parts of the tree and hence they are far more representative of the system than those derived from the retina only. Also, measurements were taken directly from the casts with special viewing equipment; hence the three-dimensionality of each bifurcation could be examined and a proper orientation of the bifurcation could be used to give a correct view of the branching angles. Indeed, the degree of three-dimensionality of each bifurcation was actually measured for the first time, in terms of the angle δ, which the parent artery makes with the plane containing the two branches. When δ is zero, the bifurcation is two-dimensional in the sense that the three vessels forming the bifurcation all lie in the same plane. When δ is different from zero, the bifurcation is three-dimensional. Finally, the present data, being derived from a different species, should supplement previous data from man and monkey to provide a wider range of evidence on arterial branching.

Many ailments are associated with a change in regional blood supply, be it an increased supply to a cancerous tumor or a decreased supply to a cardiac muscle. An understanding of the laws and mechanisms of arterial branching

may provide a better understanding of how such changes are brought about. Also, certain arterial diseases (intracranial saccular aneurysms and atherosclerosis) are associated directly with arterial bifurcations (Roach, 1977). A good knowledge of the structure of arterial bifurcations may help determine the causes of that association.

MATERIALS AND METHODS

Vascular casts of the arterial systems of adult, male Sprague-Dawley rats weighing ~300 g were made with Batson's 17 corrosion casting compound (Clay Adams, Parsippany, NJ), a partially polymerized methylmethacrylate plastic. In general, the casting procedures followed those of Gannon (1978). Rats were anaesthetized with Metofane (methoxyflurane) by inhalation before exposing a carotid artery. For anticoagulation, heparin was injected into this artery; then several minutes later the rats were killed with a lethal, intraarterial infusion of sodium pentobarbital. The heart and major arterial trunks were exposed by a midline sternotomy and the aortic root was cannulated with a short length of PE 90 tubing (Polysciences, Inc., Warrington, PA). The inferior vena cava was nicked with scissors to facilitate perfusion with casting compound. After a brief infusion of Tyrode's solution, the aortic cannula was connected to a 25-cm^3 container of freshly mixed casting compound. The air trapped above the casting compound was coupled to a constant pressure source held at 110 mmHg so that the casting compound was perfused into the arterial system and set under physiological pressure. With the mixture of Batson's compounds we used (20 ml of compound A, 4 ml of compound B, 3 drops of compound C), the cast set within 15–30 min.

After the casts had set, the rats were immersed in 25% potassium hydroxide to digest tissue from the plastic. With frequent replacement of the KOH (every 1–2 d), the casts were cleared of tissue in 3–14 d.

Batson's casting compound was chosen because it reliably preserves vessel geometry. The cast is rigid, so that branching angles are preserved, and shrinkage during setting is reportedly <1% (Reidy and Levesque, 1977). In addition, the casting of the arterial system is relatively complete. In some beds the casting compound perfused the complete vasculature and exuded from the veins. In others, the casting compound did not pass through the capillaries but, in all beds, vessels down to the level of arterioles (radius <50 μm) are filled; thus, a complete representation of arterial vessels could be produced.

Measurements

Several casts were produced, some more complete than others. An example of a reasonably good cast is shown in Fig. 1. Two good casts, henceforth to be referred to as casts A and B, were selected for measurements. Basically, cast A was used to test the measurement procedures and cast B was used to produce the final results. Approximately 150 bifurcations were measured in cast A and 350 in B. At each bifurcation, measurements were made of the diameter of each of the three vessels involved, the branching angle of each of the two daughter vessels, and the angle δ, which the parent artery makes with the plane containing the two branches. In cast B every bifurcation was measured twice by two different operators, and the average of two measurements was taken in each case.

For the purpose of measurement the complete casts were broken into small pieces, ~2–3 cm in length. In cast B the pieces were produced mostly by breaking branches off the aorta, whereas in cast A the aorta itself was cut to pieces. As a result of this,
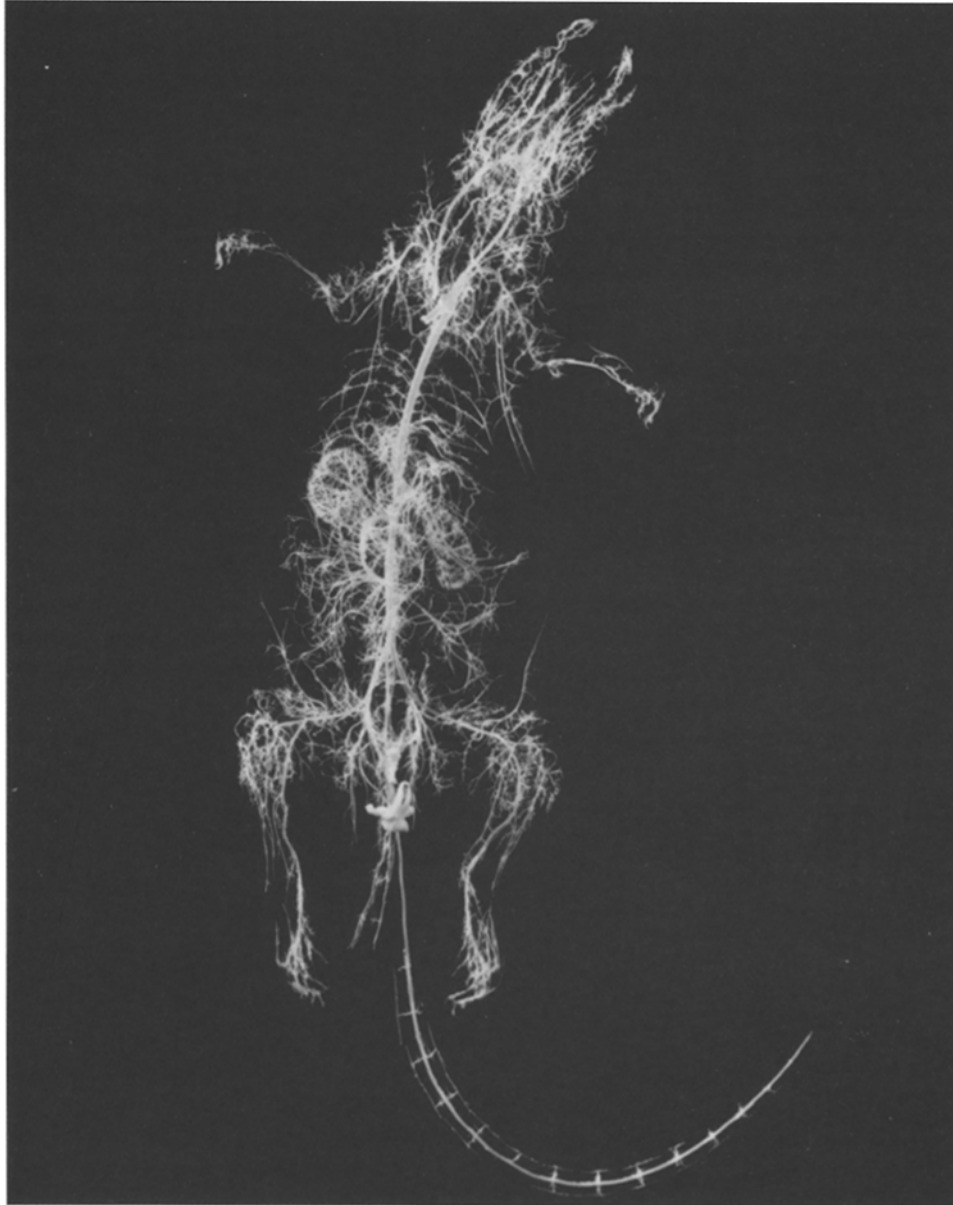
FIGURE 1.   Resin cast of the arterial tree of a rat, ~30 cm long. For the purpose
of measurements, the cast was broken into small pieces of ~2–3 cm long. A
number of arterial bifurcations were then identified and labeled in each piece
and, at each bifurcation, measurements were made of the diameters of the three
vessels involved, the branching angles of the two branches, and the angle δ,
which the parent artery makes with the plane containing the two branches. A
total of ~350 bifurcations were considered.

bifurcations along the aorta were included in A but not in B. Otherwise, data ranging from first- to seventh-order branches were included in the analysis. The various pieces were sketched or photographed and then catalogued according to their place in the arterial tree. Bifurcations on each piece were then numbered so that each measurement could be referred back to the bifurcation from which it originated.

Measurements were taken directly from the casts, using a Mitutoyo PJ-250c profile projector (Mitutoyo Manufacturing Company of Japan; MTI Corp., Palamus, NJ). The instrument consists of a magnifying system to ×10, ×20, and ×50, a screen on which the magnified image is projected, and a stage with provisions for rotation and translation that are measured with the aid of micrometers and a digital readout. A piece of cast was held by a gimbal device which was then placed on the stage of the profile projector. This provided six degrees of freedom for maneuvering the cast (three translations and three rotations), so that each bifurcation could be aligned with the proper orientation to give the correct view of the branching angles (see inset of Fig. 5).

Branching angles were measured by aligning a cursor line with the longitudinal axis of the parent artery and then rotating it to align with the axis of each of the two branches, the angles of rotation being recorded in the process. Where a vessel was not straight, and many were not, the cursor line was aligned with the direction of the vessel at the bifurcation point. The angle δ between the parent artery and the plane containing the two branches was measured by turning the bifurcation on its side until the two branches were seen to coincide with each other, and then the cursor method was used again. Vessel diameters were measured by aligning the cursor line with one vessel wall and then with the other, the distance traversed in between being recorded in the process.

RESULTS AND DISCUSSION

Measurements of vessel diameters from bifurcations in cast B are shown in Fig. 2. The results are expressed in terms of the nondimensional ratios (d_1/d_0), (d_2/d_0), and (d_2/d_1) where d_0 is the diameter of the parent artery, d_1 is that of the larger branch, and d_2 is that of the smaller branch. The ratio (d_2/d_1), which we shall refer to as the bifurcation index, is a measure of the asymmetry of a bifurcation in terms of its branch diameters. When the two branch diameters are the same, the index has the value 1 and when one branch diameter is much larger than the other, the index value is near zero. The ratios (d_1/d_0) and (d_2/d_0) simply express the diameters of the two branches in terms of that of the parent artery. According to a theory put forward by Murray (1926 a, b), the diameter of a blood vessel is dictated by a compromise between the cost of blood volume, which calls for small diameters, and the cost of pumping power, which calls for large diameters. When this principle is applied at an arterial bifurcation, the following relations are obtained:

$$\frac{d_1}{d_0} = \frac{1}{[1 + (d_2/d_1)^3]^{1/3}}, \quad \frac{d_2}{d_0} = \frac{(d_2/d_1)}{[1 + (d_2/d_1)^3]^{1/3}},$$

which are represented by the curves in Fig. 2. Agreement of these curves with the data is remarkably good.

Measurements of branching angles from the same bifurcations are shown in Fig. 3, again in comparison with theoretical predictions. The formulas for the theoretical curves are given in details in Zamir (1978). The data points are
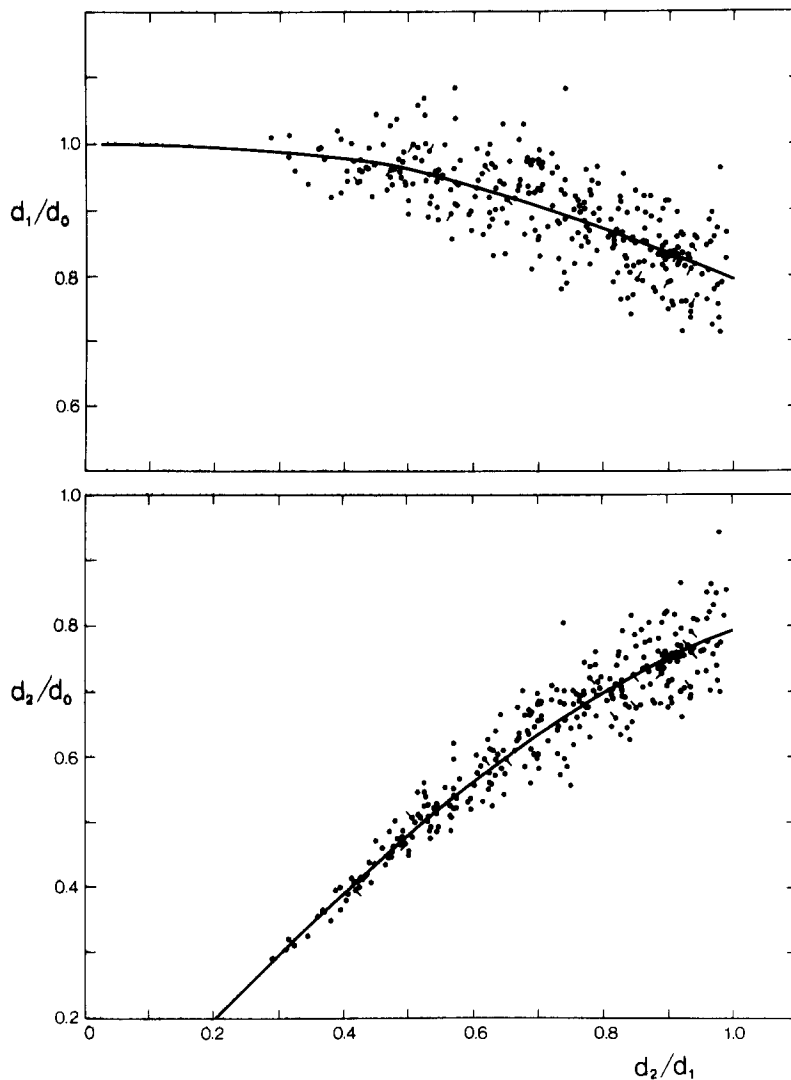
FIGURE 2. Measurements of branch diameters at arterial bifurcations in different parts of the arterial tree of a rat, where d_0 is the diameter of the parent artery, and d_1 and d_2 are those of the larger and smaller branches, respectively. A small dash on a data point indicates that there is another point in the same position. The curves represent diameter predictions (see text) based on a theory put forward by Murray (1926 a, b).

considerably more scattered than those of branch diameters. This observation has been made before, in the studies of Zamir et al. (1979) and Zamir and Medeiros (1982), where it was suspected that at least part of the scatter is due to three-dimensional effects inherent in the *pictures* from which the measurements were derived there. In the present study this source of error has been
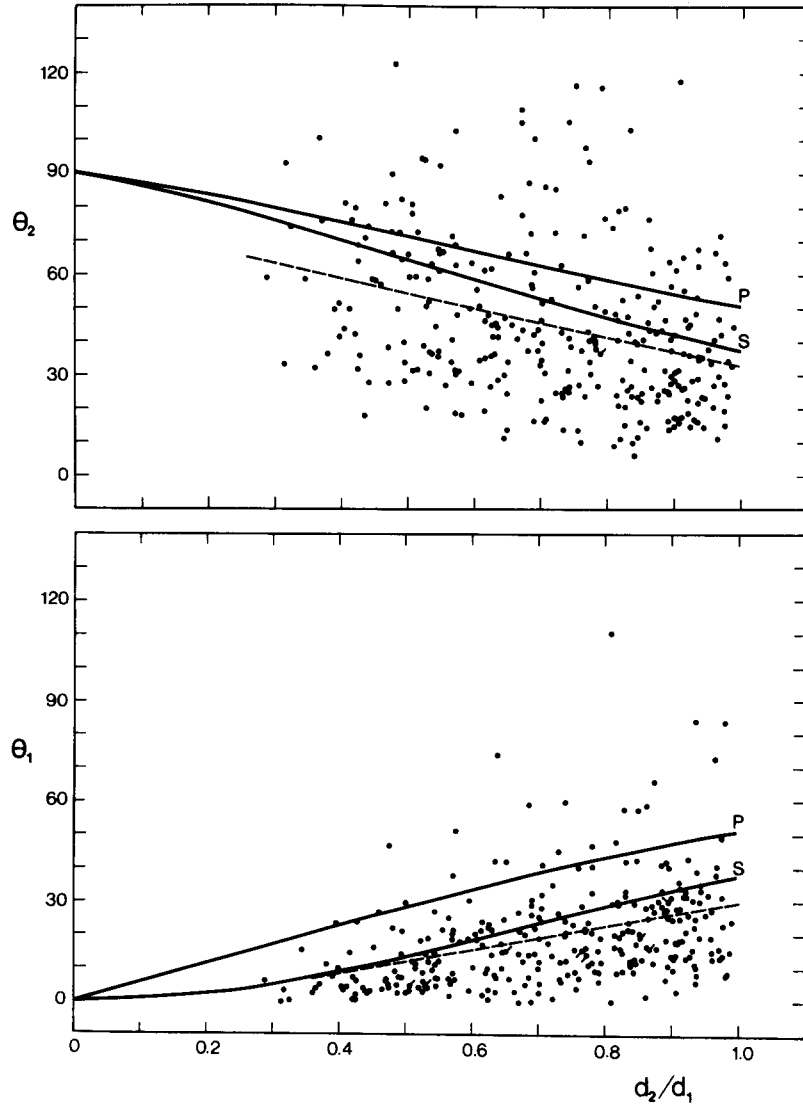
Figure 3. Measurements of branching angles from the arterial tree of a rat, where, at each arterial bifurcation, θ_1 is the angle made by the larger branch and θ_2 is that made by the smaller branch. The dashed lines represent the best fit of the data on a statistical basis (linear regression). The solid curves represent theoretical results based on the requirements (P) that the pumping power through the bifurcation be a minimum and (S) that the total shear force on endothelial tissue be a minimum. Formulas for the two curves are given in details in Zamir (1978). A small dash on a data point indicates that there is another point in the same position. A count of the data points shows that half the points are within $\pm 17°$ of the curve marked S for θ_2 and within $\pm 9°$ of that for θ_1.

eliminated by using a cast rather than a picture of the vascular bed. It appears, therefore, that the scatter of branching angles is not due to misaligned views of these angles. It is possible that branching angles are not as strictly governed by physiological considerations as branch diameters are, or that physiological factors not yet considered by the theoretical studies may be involved. But in spite of the scatter in Fig. 3, the data points follow the general trend of the theoretical curves, particularly the curves marked S, which are based on the requirement that the total shear force acting on endothelial tissue be a minimum (Zamir, 1976a). A count of the data points shows that half the points are within $\pm17°$ of the curve marked S in Fig. 3 for θ_2, and within $\pm9°$ in that for θ_1. The dashed lines represent the best lines that fit the data on a statistical basis (linear regression). Although the validity of these
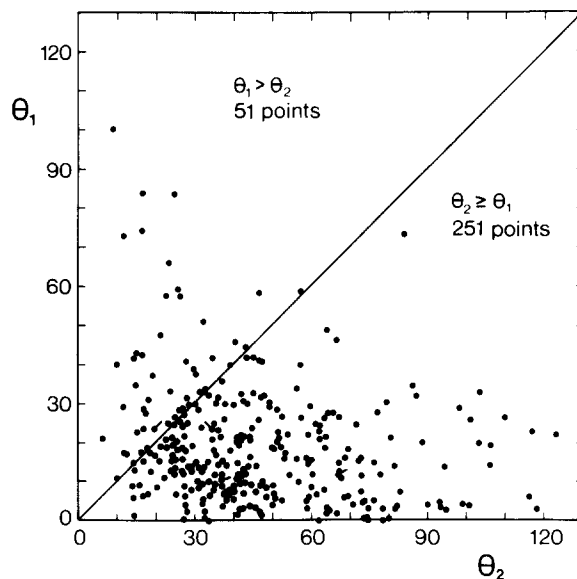

FIGURE 4. Comparison of the angle (θ_1) made by the larger branch at an arterial bifurcation with that made by the smaller branch (θ_2). Theoretical considerations suggest that θ_2 should always be larger than θ_1, the two angles being equal only when the two branch diameters are equal.

lines may be questioned because of the amount of scatter, they provide a useful guide for comparison of the data with the theoretical curves. Another orderly trend of branching angles is shown in Fig. 4, where the angle θ_1 made by the larger branch is plotted vs. the angle θ_2 made by the smaller branch in each bifurcation. Theoretical considerations predict that θ_2 should always be larger than θ_1, the two angles being equal only when the two branch diameters are equal. In Fig. 4 there are 251 bifurcations in which this rule is followed and only 51 bifurcations in which it is not.

The extent to which arterial bifurcations are two- or three-dimensional is not known. An arterial bifurcation is three dimensional when the parent artery makes a nonzero angle δ with the plane containing the two branches.

A two-dimensional bifurcation provides the shortest route for blood flow from the parent artery to the branches. It would be expected, therefore, that although the arterial tree is highly three-dimensional, arterial bifurcations would in general be mostly two-dimensional. Measurements of δ are not easy to obtain and no data on this issue have been available so far. Fig. 5 shows measurements obtained in the present study. The results indicate indeed that arterial bifurcations are mostly two-dimensional. The deviations from zero δ in Fig. 5 are not unreasonable in a biological system.
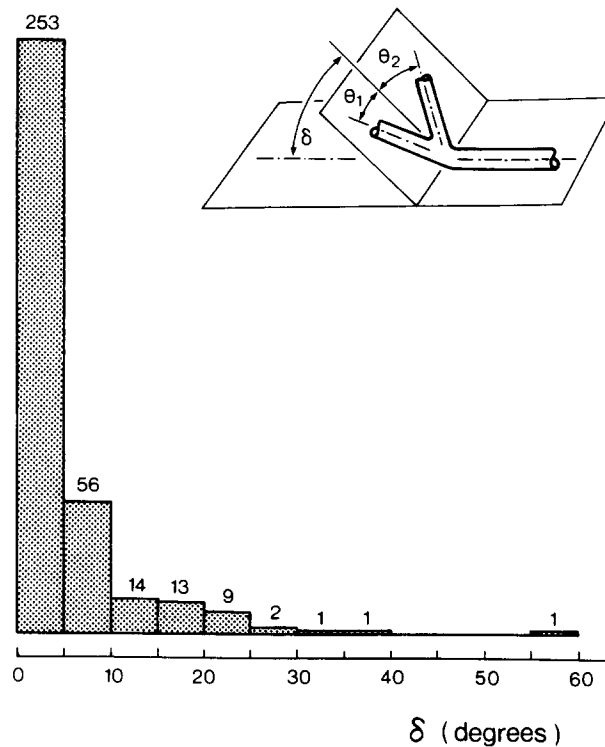

FIGURE 5. Measurements of the angle δ, which the parent artery at an arterial bifurcation makes with the plane containing the two branches. When δ is zero, the bifurcation is two dimensional in the sense that all three vessels then lie in the same plane. When δ is different from zero, it gives a measure of the extent to which the bifurcation is three dimensional. The histogram shows the number of bifurcations found for each range of values of δ, out of a total of 350 bifurcations measured. The results suggest that arterial bifurcations are in general two-dimensional.

The data discussed so far derive entirely from cast B, where all measurements were made by two different operators and an average of the two was taken. In cast A the measurements were made by a single operator, mainly to test the experimental procedure. When data from this cast were compared with those from cast B, however, very little difference was found in the final results. In fact the data were put in graphical form as in Figs. 2–5 and the

pattern of data points was found to be the same in all cases. We were led to conclude from this that the scatter associated with these results is not due to measurement errors. In fact, on the basis of this and previous studies it appears now that there is genuine scatter, particularly in the distribution of branching angles in the arterial tree. Data from cast A also differed in that they included bifurcations directly off the aorta, whereas in cast B these were not included. Again, this difference did not produce any difference in the final results.

CONCLUSIONS

Measurements from a large number of arterial bifurcations in the cardiovascular system of a rat confirm the results of earlier measurements from man and monkey, with the conclusion that branching angles and branch diameters at bifurcation sites are governed by certain physiological rules that aim to increase the efficiency of arterial bifurcations as flow dividing units, although the case of branching angles does not appear to be fully understood.

Branch diameters at bifurcation sites appear to follow very closely a pattern that represents a compromise between the requirements for minimum lumen (and hence blood) volume and minimum pumping power for driving blood through a bifurcation.

Branching angles at bifurcation sites appear to favor a pattern that, in addition to the above requirements, satisfies the requirement for minimum drag force exerted by the moving blood on endothelial tissue. Unlike branch diameters, however, the angles follow this pattern with considerable scatter.

Branching angles at bifurcation sites appear to follow the rule that at an arterial bifurcation the larger branch makes a smaller angle with the direction of the parent artery than does the smaller branch. Again, however, the rule is not followed rigidly. Of 302 bifurcations, 51 were found to be in violation of the rule, though most of them by only a small amount.

Of 350 bifurcations measured, the angle δ between the parent artery and the plane containing the two branches was found to be between 0 and 5° in 253 cases, and between 5 and 10° in 56 cases. Considering the difficulties involved in measuring this angle accurately, and allowing for a reasonable amount of biological scatter, we conclude from this that arterial bifurcations are in general mostly two-dimensional, i.e., the angle δ is either zero or very nearly so.

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