Intercapillary Distance and Capillary Reserve in Hypertrophied Rat Hearts Beating in Situ

Louis Henquell, Charles L. Odoroff, and Carl R. Honig

SUMMARY Functional intercapillary distance (ICD) was measured in stop-motion photomicrographs of hypertrophied, normally compensated, well-oxygenated rat hearts beating in situ. Left ventricular hypertrophy was produced by salt loading and unilateral nephrectomy. Minimum ICD (when all capillaries are open) also was measured. Ventricular weight increased by 30-40% within 8-9 weeks after nephrectomy. To compare the effect of normal and pathological growth, ICD was also measured in normal rats. In normal animals, minimum ICD and functional ICD increased linearly and proportionately with left ventricular weight. Consequently, the extent to which capillary recruitment could decrease ICD was the same in large and small normal hearts (about 2 mm). In the hypertrophied hearts, capillary recruitment could have maintained ICD within normal limits at rest for several weeks. After 8-9 weeks, however, the capillary reserve in hypertrophy was fully utilized at rest, and mean functional ICD was 1.5-2.0 mm greater than normal for the age of the animal. An analysis of O2 transport indicates that anoxic foci would exist throughout the hypertrophied heart and particularly in subendocardium when the capillary reserve is exhausted. The calculated amount of anoxic tissue appears sufficient to account for the focal necrosis and fibrosis observed in hypertrophy and for the development of circulatory failure.

WEARN et al.1, 2 were the first to report that myocardial capillary circulation is compromised by the hypertrophy of disease. They concluded that neither the fibers nor the capillaries proliferate, and that the distance between capillaries increases because fiber diameter increases. This view has been challenged by recent studies based on different histological methods which purport to show that capillaries proliferate in pathological hypertrophy.3-5 However, it seems likely that capillary growth is not proportional to hypertrophy of the muscle fibers.1-6-8 All such studies to date have been performed on dead hearts, and have been designed to determine the total number of capillaries present. Previous reports from this laboratory have shown that, in the rat, about 1/2 the capillaries in right ventricle and 1/4 the capillaries in left ventricle are not perfused at rest. These unperfused capillaries constitute a functional reserve, available for adaptation to stress or disease. Consequently, diffusion distances could be maintained at or near the normal value at rest despite hypertrophy, as long as enough capillaries are available in the reserve. The study to be described was undertaken to: (1) evaluate the capillary reserve in hypertrophied hearts, (2) determine the effect of hypertrophy on the uniformity of diffusion distance, and (3) estimate the effect of altered capillary circulation on O2 transport.

Methods

GENERAL

We studied female Sprague-Dawley rats. Eight pairs of littermates were fed a standard laboratory diet until they reached 160 g at about 45 days. One member of each pair then was subjected to unilateral nephrectomy. In addition, a 60-mg pellet of deoxycorticosterone was implanted under the skin of the back. One week later, 1% NaCl solution was added to the drinking water. Control rats were not nephrectomized, did not receive deoxycorticosterone, and drank only tap water. On the foregoing regimen, the increase in heart weight relative to control is maximal in about 8 weeks.11 Cardiac failure appears at 10-12 weeks (J. Cohen, unpublished observations). Distances between perfused capillaries were measured at 8-9 weeks. Most experimental rats gained weight at the same rate as controls. If the rate of gain declined, food for the paired control littermate was restricted, so that body weight was the same for both members of the pair. In addition to the littermates described above, 17 female Sprague-Dawley rats weighing 130-580 g were studied to define the effect of normal growth.

To measure distances between capillaries, the rats were anesthetized with sodium pentobarbital, 5 mg/100 g body weight administered intraperitoneally. Cannulas were placed in the trachea, carotid artery, and jugular vein. Body temperature and the cardiac surface (see below) were maintained at 37°C. The rats were ventilated with an O2-N2 mixture saturated with water vapor by means of a Harvard rodent respirator at a rate which kept arterial pH (pH A) within normal limits. P O2 of inspired gas (Pins) was adjusted to maintain P ao2 between 100 and 250 mm Hg. In this range, a change in P ao2 has no significant effect on coronary ICD.10 Phasic and mean arterial blood pressure, rate of change of pressure, and heart rate were monitored with an Electronics for Medicine polygraph.

The chest was opened through a midline incision. Donor blood (1 ml) was given to compensate for the effect of thoracotomy on venous return. The rat was fixed to a carrier fitted to a Leitz Ortholux microscope. By means of a pivot, the rat could be rotated on its long axis to expose the right or left ventricle. Blood gases and pH were mea-
INTERCAPILLARY DISTANCE IN CARDIAC HYPERTROPHY/Henquell et al. 401

sured with an Instrumentation Laboratories system before and after each ventricle was filmed. Optical and photographic techniques for stop-motion micrography of the beating heart have been described in detail in several previous reports.2-10

Diffusion distances are smallest when all capillaries present are open and perfused. To estimate this minimum distance, we forced precapillary sphincters to relax. To do this, we induced asphyxia by turning off the respirator.10,12 The heart stopped beating in 3-4 minutes, and the epicardium was filmed at 15 minutes.

At the end of each experiment, the heart was removed and the atria were trimmed away. The right ventricular free wall was excised and the left ventricle and septum were laid open. Both specimens were blotted and weighed to the nearest milligram on a Mettler analytical balance.

DATA ANALYSIS AND STATISTICS

Photomicrographs in which the capillaries were in sharp focus were projected to a final magnification of exactly 500×. Vessels up to 7 μm in diameter were regarded as capillaries. Center-to-center distances between capillaries in the projected images were 1-2 cm. Films were coded, randomized, and read "blind" by a single observer (L.H.). Cardiac capillaries are, for the most part, long parallel tubes.7,13 For each field, a single line was drawn perpendicular to the long axis of the capillaries. All measurements of ICD were made on this line. This measurement procedure is illustrated in Martini and Honig7 and representative photomicrographs appear in Martini and Honig7 and Henquell and Honig.13 The method ensures that deviations from a parallel array do not bias our results and that each capillary is considered only once.

The distance measurements and other variables were entered into an IBM 360/65 computer data file. To check for possible error in the computations, results were analyzed with two types of least squares calculations,14,15 "robust" regression,16 and analysis of variance,14 with substantially the same result. Lack of sensitivity to choice of mathematical model is strong evidence that the analyses are valid descriptions of the data.

In previous reports, results were expressed as ICD, the distance from the wall of one capillary to the wall of the next. It represents the extra-capillary portion of the diffusion path. Mean capillary diameter with respect to time during the cardiac cycle is 4.4 μm in rat epicardium.13 We therefore subtracted 4.4 μm from mean center-to-center distances to obtain the values of mean ICD reported in this paper.

### Table 1

General Characteristics of Experimental Rats and Littermate Controls

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Experimental</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt (g)</td>
<td>275 ± 21</td>
<td>279 ± 12</td>
<td>&lt;0.4</td>
</tr>
<tr>
<td>Heart wt (g)</td>
<td>772 ± 53</td>
<td>1057 ± 76</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Right ventricular wt (g)</td>
<td>149 ± 36</td>
<td>159 ± 20</td>
<td>&lt;0.5</td>
</tr>
<tr>
<td>Left ventricular wt (g)</td>
<td>595 ± 39</td>
<td>840 ± 52</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>106 ± 31</td>
<td>118 ± 31</td>
<td>&lt;0.25</td>
</tr>
<tr>
<td>Pulse pressure (mm Hg)</td>
<td>38 ± 6.4</td>
<td>52 ± 6.4</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>378 ± 55</td>
<td>331 ± 59</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Arterial pH</td>
<td>7.43</td>
<td>7.47</td>
<td>&lt;0.25</td>
</tr>
</tbody>
</table>

Results are given in mean ±SD.
FIGURE 1 Filled circles and corresponding regression lines indicate relation between ventricular weight and body weight for normal rats. Dashed lines indicate 95% confidence intervals for prediction of an individual value. Open triangles and squares represent values in hypertrophy.

MINIMUM ICD

Mean values for ICD in experimental rats and paired littermate controls are shown in Table 2. Values at 0 PaO₂ indicate minimum ICD, provided: (1) asphyxia caused all precapillary sphincters to relax and (2) all capillaries contained enough erythrocytes to be recognized. For analysis of these assumptions, see Discussion. In each of 6 pairs, the apparent minimum diffusion distance was significantly greater in the hypertrophied heart. Mean minimum ICD was 12.89 μm in normal animals and 15.33 μm in hypertrophy. Analysis of histograms of ICD indicates that the higher mean minimum ICD in experimental rats is not attributable to a few very large values. Assuming a square capillary array, the apparent mean minimum ICD corresponds to about 3345 capillaries/mm² in the controls and 2570/mm² in hypertrophy.

MEAN ICD IN WORKING HEARTS (FUNCTIONAL ICD)

Results of nine comparisons in six pairs of rats are summarized in Table 2; see entries for which PaO₂ > 0. In eight of these comparisons, functional ICD was significantly greater, at the 2% level or higher, in the hypertrophied heart than in the paired control. A parabolic relation exists between PaO₂ and ICD; so ICD is short when PaO₂ is either less than 100 mm Hg or greater than 300 mm Hg. Intermediate values are associated with long ICD. Note in Table 2 that, in two comparisons, PaO₂ in the control and experimental animals differed by 10 mm Hg or less. In all other cases, the difference in PaO₂ is in the direction which would tend to decrease the observed difference in ICD. Mean functional ICD was 14.21 μm in controls and 15.97 μm in hypertrophy. These values correspond to about 2890 and 2410 capillaries/mm², respectively.

ANALYSIS OF MEAN DIFFERENCES IN ICD

The mean values cited above from data in Table 2 are not adequate for quantitative evaluation of differences in mean ICD in the population of rats. This is true because they are, in fact, means of mean ICD in individual rats. These latter are based on different numbers of observations in each experiment and, therefore, cannot be weighted equally. We therefore used the individual ICD measurements observed in each animal in a weighted, paired, two-way analysis of variance. Littermates, or the same ventricle when normally oxygenated and anoxic, were paired. The weights were proportional to the number of observations. Interactions were not statistically significant for any of the comparisons, indicating that the mathematical model is valid as applied to the data.

Results are summarized in Figure 2. In the normal rats,
INTERCAPILLARY DISTANCE IN CARDIAC HYPERTROPHY/Henquell et al. 403

Table 2  Mean Values of Minimum ICD (PaO₂ = 0) and Functional ICD (PaO₂ > 0)

<table>
<thead>
<tr>
<th>Identification no.</th>
<th>Control</th>
<th>PaO₂ (mm Hg)</th>
<th>n</th>
<th>ICD (μm)</th>
<th>SD (μm)</th>
<th>CAP/mm²</th>
<th>Hypertrophy</th>
<th>PaO₂ (mm Hg)</th>
<th>n</th>
<th>ICD (μm)</th>
<th>SD (μm)</th>
<th>CAP/mm²</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>31/32</td>
<td>a 1</td>
<td>60</td>
<td>92</td>
<td>13.65</td>
<td>3.37</td>
<td>3086</td>
<td></td>
<td>b 2</td>
<td>77</td>
<td>15.88</td>
<td>3.93</td>
<td>2429</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>33/34</td>
<td>a 2</td>
<td>77</td>
<td>97</td>
<td>15.65</td>
<td>3.97</td>
<td>2488</td>
<td></td>
<td>b 3</td>
<td>55</td>
<td>18.29</td>
<td>4.30</td>
<td>1942</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>36/37</td>
<td>a 3</td>
<td>103</td>
<td>98</td>
<td>15.33</td>
<td>3.48</td>
<td>3111</td>
<td></td>
<td>b 4</td>
<td>65</td>
<td>16.78</td>
<td>5.11</td>
<td>2229</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>39/40</td>
<td>a 5</td>
<td>137</td>
<td>72</td>
<td>17.77</td>
<td>4.53</td>
<td>2458</td>
<td></td>
<td>b 6</td>
<td>130</td>
<td>15.92</td>
<td>4.14</td>
<td>2422</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>57/58</td>
<td>a 7</td>
<td>210</td>
<td>78</td>
<td>14.19</td>
<td>4.84</td>
<td>2894</td>
<td></td>
<td>b 8</td>
<td>159</td>
<td>15.92</td>
<td>4.14</td>
<td>2422</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>59/60</td>
<td>Mean</td>
<td></td>
<td></td>
<td>14.21</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>15.97</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

n = number of observations; P based on paired t-test. Capillary densities are computed from ICD, assuming a square array.

The functional ICD was 1.69 μm larger than minimum ICD, because about 570 capillaries/mm² were not perfused. These values are different from 0 (P < 0.001). In contrast, the mean difference between minimum and functional ICD in hypertrophied hearts was 0.59 μm, corresponding to a mean capillary reserve of 145 capillaries/mm². A 95% confidence interval for these values includes 0, indicating that if a reserve of unperfused capillaries and of diffusion distance existed in the hypertrophied hearts, it was too small to be detected by our experiments.

Turning now to horizontal comparisons, both minimum ICD and functional ICD were significantly greater in hypertrophied hearts. Because the difference between normal and hypertrophy is significantly smaller for functional ICD than for minimum ICD (P < 0.001), recruitment did partly compensate for the effect of growth. This is, of course, expected, since the vertical comparisons indicate that virtually all capillaries in the hypertrophied hearts were being utilized.

COMPARISON OF MEAN ICD IN GROWTH AND HYPERTROPHY

The two regression lines in Figure 3 illustrate the effect of normal growth, as determined by a least-squares analysis performed on 3,483 individual observations of ICD in 30 rats. The normal rats in Table 2 were among those included in the analysis. The 10% difference in slopes of the two lines is not statistically significant, so the lines may be considered parallel. Thus, the maximum effect of capillary recruitment is the same at all ages and ventricular weights shown (about 2 μm). The fact that the open square lies precisely on the regression line indicates that the small sample of littermates used in our study of hypertrophy is representative.

The open triangle was positioned by adding the 1.69-μm difference between minimum and functional ICD obtained from Figure 2 to mean minimum ICD from Table 2. The dashed regression line falls within the 95% confidence interval computed for the 1.69-μm difference, indicating that functional ICD in the littermate controls was within the range expected for normal Sprague-Dawley rats.

The filled symbols in Figure 3 denoting values in hypertrophy were positioned by adding the appropriate differences from Figure 2 to the values represented by the open symbols in Figure 3. Note that minimum ICD in hypertrophy lies very close to the solid regression line. This means that the effect of hypertrophy on the total number of capillaries was limited. An analysis of variance performed on the hypertrophy values in Table 2 indicates that the maximum effect of capillary recruitment is the same at all ages and ventricular weights shown (about 2 μm).
capillaries is the same as if the ventricle had enlarged to the same weight more slowly through normal growth. Normal rats would be expected to have the same minimum ICD observed in hypertrophy only after growing for 2 additional months. At that time, their ventricular weight would have increased by about 45%, and mean minimum ICD would have increased by about 2.4 μm.

The filled triangle in Figure 3 lies well below the value of functional ICD predicted for a normal 840-mg ventricle. The discrepancy is highly significant; the dashed regression line lies outside a 99% confidence interval for functional ICD in hypertrophy. This means that the rats represented by the filled triangle do not belong to the population represented by the dashed regression line. The regression analysis therefore supports the conclusion from Figure 2 that the hypertrophied hearts utilized most or all of their capillary reserve, even under basal conditions. Despite this recruitment, functional ICD in hypertrophy is significantly longer than predicted for the age of the animal (P < 0.01); compare open and filled triangles. This is true because fiber diameter and, hence, minimum ICD are larger than predicted for the age; compare open and filled squares.

**FREQUENCY DISTRIBUTIONS**

Frequency distributions of individual measurements of functional ICD from each of three rats are compared as ogives in Figure 4. They are representative of ogives in other animals, and are similar in shape to ogives for minimum ICD. Two of the 3 rats were normal. One of these weighed 135 g, and its left ventricle weighed 323 mg. The other weighed 520 g and had a 1020-mg ventricle. The shape of the ogives for these rats is similar; the only difference is location on the abscissa. The frequency distribution obtained for a 290-g rat whose ventricle had hypertrophied to 831 mg is also shown. It is nearly superposable on the distribution for the large, normal rat. The filled triangle in Figure 3 lies well below the value of minimum ICD predicted for a normal 840-mg ventricle. The discrepancy is highly significant; the dashed regression line lies outside a 99% confidence interval for the number of available capillaries, and on the arrangement of capillaries, is the same as if the ventricle had attained the same weight through normal growth.

For all 3 rats, the frequency distributions are kurtotic, with attenuated upper and lower "tails." Only about 20% of spacings exceed the median by more than 25%. This high degree of uniformity of spacing greatly facilitates O₂ transport.

**Discussion**

The unique contribution of the present study is the measurement of ICD in the beating, hypertrophied heart, in situ. This permits us to evaluate the effect of hypertrophy on the coronary capillary reserve. The principal findings are: (1) Recruitment can keep diffusion distances nearly normal at rest, despite a 30-40% increase in ventricular weight. (2) In the model we studied, the effect of hypertrophy on the number of available capillaries, and on the arrangement of capillaries, is the same as if the ventricle had attained the same weight through normal growth. (3) The capillary reserve is exhausted in the final stage of experimental hypertrophy. The following discussion interprets these results.

**ROLE OF BLOOD PRESSURE**

It is remarkable that ventricular weight should increase 30-40% without significant change in mean arterial pressure. However, peak systolic pressure and rate of change of pressure were greatly increased in every rat. Recent demographic evidence indicates that systolic hypertension is at least as important a determinant of hypertrophy as diastolic hypertension. Our results provide experimental evidence consistent with this view.

**CRITICISM AND LIMITATIONS OF EXPERIMENTS**

We chose to measure ICD 1-2 weeks before congestive failure was expected. This permitted us to observe the largest possible change in the capillary circulation, but it resulted in the loss of four rats in which the disease progressed more rapidly than anticipated. Consequently, sample size was smaller and confidence intervals were larger than planned. This limits interpretation of our finding that the difference between minimum and functional ICD in hypertrophied hearts is indistinguishable from 0. In fact, a reserve of up to 0.6 μm could exist. However, our sample probably underestimates the effect of hypertrophy, because the reserve should have been smaller in those rats that died before measurements could be made.

The major measurement error in our experiments is in the estimation of minimum ICD. Although it is likely that all precapillary sphincters are relaxed by 15 minutes of asphyxia, capillaries are free to empty during this time. Since we could identify only those capillaries which contained erythrocytes, doubtless some capillaries were not recognized. To evaluate this error, in 2 experiments we identified capillaries by means of the optical properties of the endothelial cells. Minimum ICD so determined was less than 10% shorter than minimum ICD measured in the usual way. The foregoing error does not apply to functional ICD, for, in this case, capillaries that do not contain erythrocytes should, in fact, be omitted from consideration.

Even if we could measure ICD with no error whatever, interpretation would be limited by the fact that we can
observe only the most superficial regions of the wall. In normal rats, mean ICD during diastole is shorter in subendocardium than in subepicardium. This difference in ICD tends to minimize the transmural gradient in tissue P_{02}. In hypertrophy, however, the abundance of capillaries appears to be uniform across the wall. The difference in ICD between normal and hypertrophied hearts should therefore be larger in subendocardium than subepicardium.

In considering the clinical relevance of our results, the reader should bear in mind that, in the rat, ventricular weight and ICD increase linearly throughout most of the rat's life, whereas, in normal human beings, ventricular growth virtually ceases after puberty, and minimum ICD remains nearly constant thereafter. Furthermore, our results pertain only to pathological hypertrophy; physiological hypertrophy produces quite different changes in coronary capillary circulation. Finally, the reserve of diffusion distance is much greater in large animals, such as dog and man, than it is in the rat.

FREQUENCY DISTRIBUTIONS OF ICD AND FIBER DIAMETER

The frequency distribution of muscle-fiber diameter in our animal model is remarkably similar to distributions in human hypertrophy secondary to chronic hypertension. The curves have broad flanks, are skewed toward large diameters, and, in most cases, exhibit a long upper tail. In the absence of capillary growth, frequency distributions of minimum ICD in hypertrophy should exhibit similar characteristics. However, this is not the case. Distributions of ICD in hypertrophy have narrow flanks and very short upper tails. The sharp difference between frequency distributions of fiber diameter and ICD is the best evidence that capillaries proliferate in pathological as well as physiological hypertrophy. Capillary growth appears to be greatest where fiber diameter is largest. Nevertheless, growth is not proportional to growth of fibers, because mean minimum ICD is significantly increased.

MINIMUM ICD

According to Wearn and associates, fiber diameter increases and capillary density decreases in experimental hypertrophy in rodents and in various forms of cardiac hypertrophy in man. Wearn et al. claim that the fiber/capillary ratio remains constant, indicating that neither the fibers nor the capillaries multiply. However, the fiber/capillary ratio in their experiments ranged from 0.85-1.65, so partially compensatory growth of capillaries might not have been recognized. A small but statistically significant decrease in the fiber/capillary ratio has in fact been reported by Rakusan and Poupa. They and others concludes, as do we, that capillary growth occurs, but is insufficient to prevent minimum diffusion distance from increasing.

The only investigator who dissents from the foregoing conclusion is Linzbach, who believes that replication of capillaries in pathological hypertrophy maintains diffusion distances within normal limits. However, his histological data have been criticized on technical grounds. Our data, based on a technique free of histological artifacts, indicate that minimum ICD is unmistakeably greater in hypertrophy. Since this has been observed in many forms of hypertrophy and in various species, including man, we conclude that a long diffusion path and small capillary reserve underlie the physiology of all forms of pathological hypertrophy.

FUNCTIONAL ICD AND THE CAPILLARY RESERVE

Our study provides the first measurements of functional ICD in hypertrophy. As shown in Figure 3, the rats could have maintained functional ICD within normal limits at rest for several weeks by drawing on the capillary reserve. This is a significant time, since 1 day in the life of a rat is roughly equivalent to 1 month of human life. The cost, of course, is capacity for adaptation to stress.

At the time we made our measurements, ICD was longer than predicted for age, even though virtually all capillaries were being utilized. Note in Figure 3 that minimum ICD in 100-day-old rats with hypertrophy was about the same as the functional ICD in normal litters. If the experimental rats had not been killed, their functional ICD should have increased at the rate predicted by the solid rather than the dashed regression line. Calculations indicate that this uncompensated increase in diffusion path would have been accompanied by sufficient anoxia to account for cardiac failure.

Recent observations of T.H. Marsicano, R.W. Anderson, and W.N. Duran (personal communication) are in accord with our results. They measured the permeability-surface area product (Na^{+}) in dogs with experimental left ventricular hypertrophy. They interpret their data to mean that a large capillary reserve exists in normal myocardium and that this reserve is indeed exhausted in the final stage of hypertrophy.

If all capillaries are eventually utilized in hypertrophy, how do equally large normal hearts continue to function? Part of the explanation is that ventricular VO_{2}/g is lower in the normal heart. Normal growth in the rat is accompanied by decreased whole body VO_{2}, heart rate, cardiac output, and cardiac work/g, all of which are significant determinants of cardiac VO_{2}/g. In addition, multiple regression analysis indicates that cardiac VO_{2} decreases with age, per se. During the 2 months which would be required for the ventricle to grow normally to the size observed in hypertrophy, all the foregoing factors would be expected to lower VO_{2}/g of ventricle by at least 25%. Consequently, tissue PO_{2} can be maintained despite longer diffusion distances, and, hence, by a smaller fraction of the available capillaries than would be the case in hypertrophy.

EFFECT OF HYPERTROPHY ON O_{2} TRANSPORT

In recent years, investigators have sought ultrastructural and biochemical explanations for ventricular dysfunction in hypertrophy and have suggested that diffusion distance is unlikely to be of major importance. Apart from the influence of Linzbach, the chief reason diffusion distance is deprecated is that its effect on O_{2} transport in hypertrophy has not been quantified. In the following calculations based on the Krogh equation, we use measured values of capillary diameter, ICD, and the frequency distributions of these parameters, to illustrate the interaction of meta-
bolic and geometrical factors in the pathophysiology of hypertrophy.

Parameters were selected as follows. Mean functional ICD in hypertrophied hearts would have been about 17 \( \mu \text{m} \) had there been no compensation by recruitment. If all capillaries had been utilized, ICD would have been 15 \( \mu \text{m} \). We therefore selected 17 and 15 \( \mu \text{m} \) for our calculations. Obviously, these values also represent functional and minimum ICD in normal ventricles of the same size.

Capillary diameter and its frequency distribution are not significantly affected by hypertrophy (unpublished observations). Mean capillary diameter over the entire cardiac cycle is 4.4 \( \mu \text{m} \) in subepicardium and about 3 \( \mu \text{m} \) within the wall.13 The minimum diameter of a perfused capillary in subendocardium is 1.8 \( \mu \text{m} \).13

Ventricular \( \text{VO}_2 \) in a young adult (250-g) rat is about 6.5 \( \times 10^{-3} \text{ ml/g per sec} \).7 We use this figure for both hypertrophied hearts and littermate controls. 28 Ventricular \( \text{VO}_2/g \) is assumed to be 25\% less in normal animals whose hearts have grown to the weight observed in hypertrophy.10 O2 extraction remains constant with normal growth, so mean capillary \( \text{Po}_2 \) should also be constant in normals.10

The only study of left-ventricular hypertrophy due to pressure overload in which coronary flow was measured by means of a flowmeter appears to be that of Malik et al. 28 They report that flow/g is reduced by 25\% (\( P < 0.05 \)), and that O2 extraction is increased (\( P < 0.01 \)). We therefore computed O2 gradients for these conditions. Since their work lacks confirmation, gradients were also calculated assuming flow and extraction to be normal.

To obtain a "worst case" analysis, we set mean capillary \( \text{Po}_2 \) equal to venous \( \text{Po}_2 \). One should recognize, however, that a frequency distribution of end-capillary \( \text{Po}_2 \) exists with many values less than half the mean.29 Mean end-capillary \( \text{Po}_2 \) in the normal adult rat is 20–25 mm Hg,7 and should be the same in hypertrophy if flow per gram is normal. If flow per gram is reduced by 25\% in hypertrophy and \( \text{Vo}_2/g \) is constant,29 end-capillary \( \text{Po}_2 \) would fall to about 15 mm Hg. In hypertrophy, the reduction in flow may be largely confined to the deep regions of the wall.30 In this case, gradients computed for normal flow/g and normal extraction represent conditions in subepicardium, and gradients for reduced flow/g and increased extraction denote conditions in subendocardium.

Analysis of O2 gradients for normal hearts even larger than the hypertrophied ones in this study indicates that almost no anoxic tissue is present.19 Figure 5A illustrates the situation in hypertrophy if capillary recruitment did not or could not occur, and if mean ICD were 17 \( \mu \text{m} \). Mitochondria can respire at maximum rate until intracellular \( \text{Po}_2 \) falls below 0.1 mm Hg.31 Consequently the partial pressure of O2 in the tissue (\( \text{Pr}_2 \)) should be adequate to support aerobic metabolism for mean ICD if O2 tension in the capillary is 25 mm Hg (curves 1–3). If subendocardial flow in hypertrophy were restricted and mean end-capillary \( \text{Po}_2 \) were 15 mm Hg, up to 1/3 of the tissue-cylinder cross-section would be anoxic around narrow capillaries (curves 5 and 6). Since mean capillary diameter over the entire cardiac cycle is about 3 \( \mu \text{m} \) in subendocardium,13 anoxia there would be extensive, even for mean capillary spacing.

O2 profiles around capillaries spaced 25% more widely than in Figure 5A are shown in Figure 5B. About 20\% of capillaries are at least that far apart. Anoxia would be
Exercise hypertrophy differs from the hypertrophy of disease in that capillary growth maintains or even reduces minimum diffusion distances. The stimulus is unknown. Quite recently, a humoral factor that promotes growth of capillaries has been isolated from tumors and from ischmic kidneys. Perhaps in pathological hypertrophy there is insufficient production, excessive destruction, or inability to respond to a similar humoral stimulus. If so, therapy designed to shorten diffusion distance may become possible. Our results and analysis strongly suggest that such therapy would eliminate a root cause of cardiac failure in pathological hypertrophy.

Acknowledgments

We are indebted to Dr. Jules Cohen, Professor of Medicine, University of Rochester, for stimulating discussions, and for generously sharing his supply of DOCA pellets. We thank Janet Gough for help with data processing and James L. Frierson for expert technical assistance.

References

23. Rakusan K, Poupa O: Changes in the diffusion distance in the rat
heart muscle during development. Physiol Bohemoslov 12: 220-227, 1963
24. Tomanek RJ: Effects of age and exercise on the extent of the myocar-
25. Lowe TE, Bate EW: The diameter of cardiac muscle fibers: A study of
the diameter of muscle fibers in the left ventricle in normal hearts and
1: 467-469, 1948
26. Cohn AE, Steele JM: The metabolism of the isolated heart of dogs
27. Alpert NR (ed) Cardiac Hypertrophy. New York, Academic Press,
1971, pp 7 and 15
28. Mallik AB, Abe T, O'Kane H, and Geha AS: Cardiac function,
coronary flow, and oxygen consumption in stable left ventricular
29. Grunewald WA, Lubbers DW: Die Bestimmung der intracapillären
HbO2-Sättigung mit einer kryomikrofotometrischen Methode ange-
wandt am Myokard des Kaninchens. Pflügers Arch. 353: 255-273,
1975
30. Einzig S, Leonard JJ, Tripp MR, Burchell HB, Fox JJ: Regional
myocardial blood flow in closed-chest dogs with left ventricular hyper-
trophy (absr). Physiologist 18: 205, 1975
31. Chance B, Oshino N, Sugano T, Mayevsky A: Basic principles of
tissue oxygen determination from mitochondrial signals. Adv. Exp
Deut Gesellschaft Kreislaufoforsch. 16: 26-43, 1950
33. Folkman J, Merlen E, Abernathy C, Williams G: Isolation of a tumor
34. Cuttino JT Jr, Barrum RJ Jr, Hollenberg, NK and Abrams HL:
Collateral vessel formation: Isolation of a transferable factor promot-
ing a vascular response. Basic Res Cardiol. 70: 568-573, 1975