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 μm). 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 μm 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. 1-3 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 1-8, 10, 11 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 ventricle7-9 and 1/4 the capillaries in left ventricle 10 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. Cannulae 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 (pHa) within normal limits. Po₂ of inspired gas (Pio₂) was adjusted to maintain PaO₂ between 100 and 250 mm Hg. In this range, a change in PaO₂ 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 photog-
graphic 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 epicar-
dium 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 perpen-
dicular 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 analy-
ized with two types of least squares calculations,14’15 “robust” regression,16 and analysis of variance,14 with sub-
stantially 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.

### Results

GENERAL CHARACTERISTICS OF CONTROL AND EXPERIMENTAL ANIMALS

No evidence of congestive heart failure was observed during life or at autopsy. Tolerance of control and experimen-
tal rats to surgery and filming was about the same; in both groups, a stable physiological state existed for 1-2
hours after thoracotomy. If either blood pressure or heart rate changed significantly and failed to respond to donor
blood or change in body position, the experiment was terminated. Mean values for some salient parameters are
shown in Table 1. Heart weight and left ventricular weight were about 1/3 larger in the experimental rats, even though
body weight was the same as in litermate controls. Never-
theless, mean arterial pressure in the experimental rats before the chest was opened was within normal limits and
was not significantly higher than in controls. Systolic pres-
sure and pulse pressure, however, were much greater in the experimental rats, and the rate of change of pressure
was also greater. Control and experimental rats were not
significantly different with respect to heart rate and pHₐ.

Mean arterial pressure, pHₐ, and Paco₂ do not compi-
icate interpretation of results in any event, because they
have no significant effect on coronary ICD.12

### EXTENT OF HYPERTROPHY

The relations among age, body weight, and ventricular weight for normal rats are estimated by the regressions in
Figure 1. The correlation coefficient indicates that 80% of the variability in right ventricular weight and more than
90% of the variability in left ventricular weight is ex-
plained by the regression. After adjusting for the differ-
ence in intercepts, the slope of the regression for left
ventricle is about twice that of the right (P < 0.001). In 8
of 10 rats subjected to salt loading and nephrectomy, right
ventricular weight fell within the confidence limits. In
contrast, all 10 left ventricles were significantly larger than
normal. Mean left ventricular weight in the experimental
rats was 840 ± 18 (SEM) mg, and mean body weight was
279 ± 7 (SEM) g. The predicted mean left ventricular
weight for a 279-g rat is 588 ± 35 mg. Thus the experi-
mental interventions caused an average extra growth of
252 mg. This corresponds to 30% of the observed weight
of the hypertrophic left ventricles and 43% of predicted
normal left ventricular weight. Older (hence larger) nor-
mal rats have left ventricles substantially larger than the
hypertrophied ones (see Fig. 1). The regression indicates
that the mean left ventricular weight observed in hyperto-

### Table 1

<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) (wall and septum)</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.

______________________________________________

FIGURE 2  Values outside the square are weighted mean differences in intercapillary distances (ICD) ± SE in microns. A 95% confidence interval for each difference is equal to the particular difference ±2 times its SE. Numbers inside the square indicate differences in calculated capillary densities. Differences are expressed as hypertrophy minus control (horizontal arrows), or functional ICD minus minimum ICD (vertical arrows).

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,
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 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.
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\(^17\) and, hence, minimum ICD is 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 discrepancy is highly significant; the dashed regression line lies outside a 99% confidence interval for the distribution obtained for a 290-g rat whose ventricle had hypertrophied to 831 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.

**Figure 4** Representative frequency distributions of individual values of ICD in a large and a small normal rat and in a rat with hypertrophy.

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 \(O_2\) transport.\(^5\)-\(^10\)

**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.\(^18\) 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,\(^2\),\(^8\)-\(^12\) 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.\(^19\) 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...
INTERCAPILLARY DISTANCE IN CARDIAC HYPERTROPHY/Henquell et al.

observe only the most superficial regions of the wall. In normal rats, mean ICD during diastole is shorter in subendocardium than in subepicardium. 18 This difference in ICD tends to minimize the transmural gradient in tissue Po2. 20 In hypertrophy, however, the abundance of capillaries appears to be uniform across the wall. 21 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 22-23 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. 1 Furthermore, our results pertain only to pathological hypertrophy; physiological hypertrophy produces quite different changes in coronary capillary circulation. 24 Finally, the reserve of diffusion distance is much greater in large animals, such as dog and man, than it is in the rat. 9

FREQUENCY DISTRIBUTIONS OF ICD AND FIBER DIAMETER

The frequency distribution of muscle-fiber diameter in our animal model 17 is remarkably similar to distributions in human hypertrophy secondary to chronic hypertension. 25 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, 1-2 fiber diameter increases and capillary density decreases in experimental hypertrophy in rodents and in various forms of cardiac hypertrophy in man. 1 Wearn et al. 1-2 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. 6 They and others conclude, 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, 4 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. 2 Our data, based on a technique free of histological artifacts, indicate that minimum ICD is unmistakably 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 littermates. 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 VO2/g is lower in the normal heart. Normal growth in the rat is accompanied by decreased whole body VO2, heart rate, cardiac output, and cardiac work/g 10 all of which are significant determinants of cardiac VO2/g. In addition, multiple regression analysis indicates that cardiac VO2 decreases with age, per se. 26 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 VO2/g of ventricle by at least 25%. Consequently, tissue PO2 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 O2 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. 27 Apart from the influence of Linzbach, 4 the chief reason diffusion distance is deprecated is that its effect on O2 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 \( \mu m \) had there been no compensation by recruitment. If all capillaries had been utilized, ICD would have been 15 \( \mu m \). We therefore selected 17 and 15 \( \mu m \) 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 \( \mu m \) in subepicardium and about 3 \( \mu m \) within the wall.\(^{13}\) The minimum diameter of a perfused capillary in subendocardium is 1.8 \( \mu m \).\(^{13}\)

Ventricular \( \dot{V}O_2 \) in a young adult (250-g) rat is about \( 6.5 \times 10^{-3} \) ml/g per sec.\(^{7,8}\) We use this figure for both hypertrophied hearts and littermate controls.\(^{28}\) Ventricular \( \dot{V}O_2 \)/g is assumed to be 25% less in normal animals whose hearts have grown to the weight observed in hypertrophy.\(^{10}\) \( O_2 \) extraction remains constant with normal growth, so mean capillary Po2 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 \( O_2 \) extraction is increased (\( P < 0.01 \)). We therefore computed \( O_2 \) 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 Po2 equal to venous Po2. One should recognize, however, that a frequency distribution of end-capillary Po2 exists with many values less than half the mean.\(^{29}\) Mean end-capillary Po2 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 \( \dot{V}O_2/g \) is constant,\(^{28}\) end-capillary Po2 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 \( O_2 \) 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 \( \mu m \). Mitochondria can respire at maximum rate until intracellular Po2 falls below 0.1 mm Hg.\(^{31}\) Consequently the partial pressure of \( O_2 \) in the tissue (\( P_{t,O2} \)) should be adequate to support aerobic metabolism for mean ICD if \( O_2 \) tension in the capillary is 25 mm Hg (curves 1–3). If subendocardial flow in hypertrophy were restricted and mean end-capillary Po2 were 15 mm Hg, up to \( \frac{1}{2} \) 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 \( \mu m \) in subendocardium,\(^{13}\) anoxia there would be extensive, even for mean capillary spacing.

\( O_2 \) 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
extensive around the venous ends of narrow capillaries even in the subepicardium (curves 2 and 3) and around all capillaries in the subendocardium (curves 4–6). The situation may be worse than shown, for we have ignored the frequency distribution end-capillary PO2.

The effect of recruiting all capillaries is shown in Figure 5C. Though this reduces ICD by only 2 μm, it greatly improves O2 transport. Even if ICD were 25% greater than the 15-μm mean, anoxia would exist only around the narrowest capillaries in subepicardium (see Fig. 5D, curves 1–3). The situation would be less favorable in the subendocardium (Fig. 5D, curves 4–6).

Had we waited until the 12th week, nephrectomized animals would have been in failure. Since the capillary reserve was fully utilized at about 9 weeks, ICD would have increased thereafter according to the solid line in Figure 3. At about 12 weeks, ICD would have reached 17 μm, and the gradients in Figure 5A and 5B would apply. Is the amount of anoxic tissue present under these conditions sufficient to explain the development of congestive failure? To answer this question we determined the fraction of tissue-cylinder cross-section which would be anoxic at the venous ends of 3 μm capillaries for multiples of the 17-μm mean spacing (see Fig. 6). In the normal heart, ICD must be 27% greater than the mean before any anoxia appears and 36% greater before half the tissue-cylinder is anoxic. Normally, less than 10% of capillary spacings exceed the mean by 36%. In contrast, in the subendocardium of the hypertrophied heart, anoxia appears when ICD is less than the mean, and 50% of the tissue-cylinder cross-section would be anoxic when ICD exceeds the mean by only 3%! About half the spacings are at least that long. We conclude that, when the capillary reserve is exhausted, focal anoxia is indeed sufficient to account for the development of cardiac failure. This anoxia also could underlie some of the biochemical and ultrastructural changes recently identified in hypertrophy.24 Finally, focal anoxia provides the best explanation for patchy necrosis and fibrosis, especially in the subendocardium, in the terminal stage of hypertrophy in animals and in man.22

**References**

13. Henquell L, Honig CR: Capillary diameter in rat heart in situ; relation to erythrocyte deformability, O2 transport and transmural O2 gra-
metrics 16:523-531, 1974
17. Chanarin A, Barksdale EE: Experimental renal insufficiency pro-
duced by partial nephrectomy. Arch. Intern. Med. 52:739-751, 1933
30. Henquell L, Honig CR: Capillary diameter in rat heart in situ; relation to erythrocyte deformability, O2 transport and transmural O2 gra-
33. Andrews DP: A robust method for multiple linear regression. Techno-
metrics 16:523-531, 1974
34. Chanarin A, Barksdale EE: Experimental renal insufficiency pro-
duced by partial nephrectomy. Arch. Intern. Med. 52:739-751, 1933

**Figure 6** Fraction of tissue-cylinder which is anoxic at venous end of 3 μm capillary for various multiples of 17 μm mean ICD. Curve 1 = normal rat, curves 2 and 3 = subepicardium and subendocardium, respectively, in hypertrophy.
heart muscle during development. Physiol Bohemoslov 12: 220-227, 1963
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 in the left ventricular enlargement of simple hypertension. Med. J. Aust 1: 467-469, 1948
Intercapillary distance and capillary reserve in hypertrophied rat hearts beating in situ.
L Henquell, C L Odoroff and C R Honig

Circ Res. 1977;41:400-408
doi: 10.1161/01.RES.41.3.400

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/41/3/400