Lognormal Distribution of Intercapillary Distance in Normal and Hypertrophic Rat Heart as Estimated by the Method of Concentric Circles: Its Effect on Tissue Oxygenation*

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Abstract. The inhomogeneity of the capillary net in the cardiac muscle was estimated using our morphometric measurements in normal and hypertrophic rats hearts. As entry data we used the distribution of tissue at different distances from the nearest capillary as measured by the method of concentric circles and the mean intercapillary distance independently calculated from the capillary density. The derived distribution of intercapillary distances was approximated by lognormal distribution in which the spread can be characterized by a single parameter, namely the log standard deviation.

The effect of the log standard deviation on tissue oxygenation was evaluated in normal and hypertrophic hearts, at normoxia and at hypoxia. The mean tissue $P_{O_2}$ and the percentage of anoxic tissue at the venous end of the tissue cylinder were calculated using Krogh's model. Two boundary situations were considered: A) the end-capillary $P_{O_2}$ was assumed to be equal in all capillaries due to compensatory adjustment in blood flow; B) the same flow in all capillaries was assumed resulting in varying end-capillary $P_{O_2}$. The real situation is expected to be between situations A and B. Increased variability of intercapillary distance proved to impair considerably the tissue oxygenation, especially when the results were expressed as a percentage of anoxic tissue. The percentage of anoxic tissue turned out to be a better index of tissue oxygenation than the mean $P_{O_2}$ particularly at hypoxia. The results suggest the presence of at least a partial adjustment of blood flow with respect to the width of tissue cylinder. Without such adjustment, a large part of tissue would become anoxic already in normal hearts at normoxia and this would be further aggravated by hypertrophy and/or hypoxia.

Key words: Capillaries — Hypertrophy — Myocardial tissue $P_{O_2}$ — Lognormal distribution — Tissue oxygenation

Introduction

Diffusion of $O_2$ from capillaries to tissue depends on the intercapillary distance, capillary radius, $O_2$ consumption, diffusion coefficient, arterial $O_2$ pressure and content, and on the flow through the capillaries. Intercapillary distance can be estimated from counting capillaries in histological sections, a method frequently used, especially in the skeletal and heart muscle. However, this approach yields only the mean value of intercapillary distance which can be used for calculation of $O_2$ partial pressure in the tissue. A basic deficiency inherent to such a calculation is that a uniform distribution of capillaries is assumed. It has been shown by a microcinematographic method that this is not the case on the heart surface (Bourdeau-Martini et al. 1974). Until recently, there was no feasible method available to study the pattern of the distribution of the capillaries in histological sections.

Loats and co-workers (1978) proposed for skeletal muscle a new method that yields an estimate of the distribution of tissue at different distances from the capillary. In this method, a system of concentric circles of known radii is randomly applied to the tissue. For each centerpoint in the above array, the largest circle around it which contains no capillary is recorded. In this fashion, the percentage of the total points corresponding to the percentage of the tissue which is within a certain distance from the nearest capillary can be determined. This method was also applied to cardiac muscle (Rakušan et al. 1980). Even though the results obtained by this method are expected to be linked with the non-uniformity of the capillary net, their exact relationship to the distribution of intercapillary distance and consequently to oxygen transport was not clear.

The purpose of this paper is twofold: first, to clarify the link between the results obtained by the method of concentric circles and the variability of the intercapillary distance; second, to demonstrate the influence of this variability on $O_2$ transport to the tissue.

Methods

Quantitative Morphology

Experimental results used as the basis for the calculations were our measurements of the distribution of tissue in different distances from the capillary in normal and hypertrophic rat hearts (Rakušan et al. 1980). Photomicrographs were taken from subendocardial and midwall regions of the left ventricle cut perpendicularly. First, the number of capillaries per unit area were counted and the average intercapillary distance was computed. Then, the distribution of tissue in different distances from the nearest capillary was estimated, using the method of concentric circles (Loats et al. 1978). An array of four concentric circles of known radii was superimposed on the photomicrographs in a systematic random sampling. For each centerpoint the largest circle around it which contained no capillary was recorded. The centerpoints lying within the cross-section

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of the capillaries were discarded. Four radii \( r_1 \) to \( r_4 \) of the concentric circles corresponding to 2.23, 4.47, 6.70 and 8.93 \( \mu \)m were used. In such a way, five classes of distances of points lying beyond or within a certain distance from the nearest capillary could be obtained: \( \leq 2.23, \leq 4.47, \leq 6.70, \leq 8.93 \text{ and } > 8.93 \), and (cumulative) frequency could be plotted (for details see Rakusan et al. 1980).

#### Calculation of the Variability of Intercapillary Distances

It was assumed that there is a mathematically defined distribution of the radii of Krogh's tissue cylinders. For reasons explained in the discussion, the logarithmic-normal (lognormal) distribution was adopted. The lognormal distribution is a continuous distribution, its probability density function is defined by:

\[
\phi(x) = \frac{1}{x \sigma_{\ln x} \sqrt{2\pi}} e^{-0.5 \left( \frac{\ln x - \mu_{\ln x}}{\sigma_{\ln x}} \right)^2} \tag{1}
\]

where \( x \) is the variable (here identical to the radius of Krogh's cylinder \( R_0 \)), \( \mu_{\ln x} \) and \( \sigma_{\ln x} \) are the mean and standard deviation on the logarithmic scale. The lognormal distribution is asymmetrical and has the following characteristics:

mean = \( \text{antilog} \left( \mu_{\ln x} + 1.513 \sigma_{\ln x} \right) \tag{2} \)

and median = \( \text{antilog} \mu_{\ln x} \tag{3} \).

(Diem and Lentner 1970).

We knew the mean value \( (R_0) \) of the radius (half of the mean intercapillary distance) and we assumed several values of \( \sigma_{\ln x} \) as a fraction \( (F) \) of the mean on the logarithmic scale (\( \mu_{\ln x} \)):

\[
\sigma_{\ln x} = F \cdot \mu_{\ln x} \tag{4}
\]

Mean on the logarithmic scale is a function of both the mean on the linear scale and \( \sigma_{\ln x} \), it was calculated from eqs. (2) and (4) which yield after rearrangement:

\[
1.1513 F^2 \mu_{\ln x}^2 + 3 \mu_{\ln x} - \log R_{\text{m, x}} = 0 \tag{5}
\]

This quadratic equation was solved, giving only one realistic solution for \( \mu_{\ln x} \) in our situation. Then, \( \sigma_{\ln x} \) was obtained from (4).

In the next step, a value of outer capillary radius \( r \) was assumed. This value gives the smallest possible radius of Krogh's cylinder. As the distribution is symmetrical on the logarithmic scale, the maximal radius of Krogh's cylinder \( (R_{\text{max}}) \) is:

\[
R_{\text{max}} = \text{antilog} \left( \mu_{\ln x} + (1.513 \sigma_{\ln x} - \log r) \right) \tag{6}
\]

The subsequent procedure was to calculate in arbitrary units the total surface cross-section of circles (after subtracting the surface area of the capillaries) that would result from cutting perpendicularly a tissue consisting of a population of Krogh's cylinders. Integration was performed from \( r \) to \( R_{\text{m, x}} \) as follows. The surface of the annulus \( \left[\pi (R^2 - r^2)\right] \) was taken as being anoxic without any further calculations. The entry data were as follows.

\[
P_\text{av} = 13.3 \text{ or } 5.3 \text{ kPa} \text{ (100 or 40 mm Hg)}, \quad M = 0.4 \text{ ml/g} \cdot \text{min} \text{ (Honig and Bourdeau-Martini 1974)}, \quad F = 4 \text{ ml/g} \cdot \text{min} \text{ (Rakušan and Blahitka 1974)}, \quad K = 26.5 \times 10^{-6} \text{ ml/s} \cdot \text{cm} \cdot \text{mm Hg} \text{ at 38°C (Krogh 1919)}.
\]

\[
F = 4 \text{ ml/g} \cdot \text{min} \text{ (Rakušan and Blahitka 1974)}, \quad K = 26.5 \times 10^{-6} \text{ ml/s} \cdot \text{cm} \cdot \text{mm Hg} \text{ at 38°C (Krogh 1919)}.
\]

**Mean Tissue \( P_{O_2} \)**

For a calculation of the mean tissue \( P_{O_2} \) (\( P \)) for any \( R \), the formula of Kety (1957) was used:

\[
\hat{P} = P_c + \frac{M}{K} \left[ \frac{3R^2 - r^2}{8} - \frac{R^4}{2(R^2 - r^2) \ln \frac{R}{r} - \frac{R^4}{4}} \right] \tag{7}
\]

where \( M = O_2 \) consumption per gram of tissue and \( K \) is the diffusion coefficient. The end-capillary \( P_{O_2} \) was used as \( P_c \).

The mean end-capillary \( O_2 \) content \( (C_c) \) was derived from arterial \( P_{O_2} \) (\( P_{O_2} \)) with the use of appropriate \( O_2 \) dissociation curve (ODC), \( O_2 \) consumption (\( M \)) and flow in the capillaries (\( F \)) as

\[
C_c = \left( C_a - \frac{M}{F} \right) \tag{8}
\]

(\( C_a \) designates arterial \( O_2 \) content).

From \( C_c \) the end-capillary \( P_{O_2} \) was derived, again using the ODC. Adair's formula (1925) and the constants of the rat ODC (Turek et al. 1973) were used. A mean arteriovenous \( pH \) difference (\( \Delta pH \) of 0.04 (Doll et al. 1966) was assumed. Calculations were done for normoxic and hypoxic conditions.

It may be expected that the end-capillary \( P_{O_2} \) would probably not be the same in Krogh's cylinders of different width as the \( O_2 \) desaturation during the passage of blood through the capillary might be larger in wider cylinders. However, two boundary situations can be expected. A) Flow in the capillaries would be proportionally larger in wider cylinders and would compensate for the large \( O_2 \) consumption. Then, end-capillary \( P_{O_2} \) would be the same in all capillaries and equal to the mean \( P_c \) calculated from formula (8) using the mean \( F \). B) The other possibility is that flow would always be the same and therefore \( P_c \) would be higher in the thin and lower in the thick tissue cylinders. Both possibilities were examined, while expecting the real situation as being somewhere in between.

In the first situation (A), \( P, r \) and \( r \) remain constant and only \( R \) varies. Kety's formula (9) was incorporated within each integration step described before, the mean \( P_{O_2} \) was calculated, multiplied by the surface of the annulus \( \left[\pi (R^2 - r^2)\right] \), the frequency of \( R \) (eq. (1)) and integration interval. Finally, the sum of the products was divided by the total surface derived as explained in the preceding paragraph. This procedure was further expanded so that when the mean \( P_{O_2} \) calculated became negative it was always taken and summed as being equal to zero.

In the situation B it was necessary to calculate \( P \) for each integration step separately using formula (8). \( F \) was taken as equal to the mean value. \( M \) and also \( \Delta pH \) were assumed to vary proportionally to the mass of tissue cylinder (assuming the same length). ODC was used for interconversion between \( P_{O_2} \) and \( O_2 \) content.

**Percentage of Anoxic Tissue**

Krogh's formula was used for calculation of the percentage of anoxic tissue (Krogh 1919).

\[
P_a = \frac{P_c - \frac{M}{K} \left( \frac{R^2}{2} \ln \frac{R}{x} - \frac{x^2 - r^2}{4} \right)}{P_c} \tag{9}
\]

where \( P_c \) is the \( P_{O_2} \) at any distance \( x \) from the centre of the capillary; other symbols have been explained before.

First, \( P_{O_2} \) was calculated for \( x = R_1 \) and the following procedure was omitted if \( P_a \geq 0 \). At \( P_{O_2} \) lower than 0, \( x \) was calculated for \( P_a = 0 \) by trial and error method. This procedure was incorporated into each integration step described before. The product of \( \left[\pi (R^2 - x^2)\right] \), frequency (formula (1)) and integration interval gives an estimate of the percentage of anoxic tissue for each integration step. The sum of these products divided by the total surface cross-sections of Krogh’s “circles” yields the fraction of anoxic tissue. The percentage of anoxic tissue was calculated for situation A, i.e. assuming the same (mean) \( P_{O_2} \) in all capillaries and also for situation B assuming the same flow in the capillaries, as described above.

At \( P_a = 0 \) (which occurred only in situation B) the whole surface \( \left[\pi (R^2 - r^2)\right] \) was taken as being anoxic without any further calculations. The entry data of morphological measurements are summarized in Table 1, while the remaining entry data were as follows.

\[
P_{O_2} = 13.3 \text{ or } 5.3 \text{ kPa} \text{ (100 or 40 mm Hg)}, \quad M = 0.4 \text{ ml/g} \cdot \text{min} \text{ (Honig and Bourdeau-Martini 1974)}, \quad F = 4 \text{ ml/g} \cdot \text{min} \text{ (Rakušan and Blahitka 1974)}, \quad K = 26.5 \times 10^{-6} \text{ ml/s} \cdot \text{cm} \cdot \text{mm Hg} \text{ at 38°C (Krogh 1919)}. \]