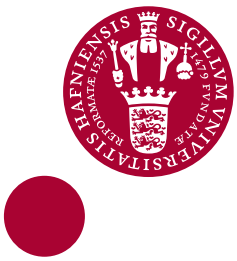

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AN EXPERIMENTAL MODEL OF THE KROGH TISSUE CYLINDER: TWO DIMENSIONAL QUANTITATION OF THE OXYGEN GRADIENT

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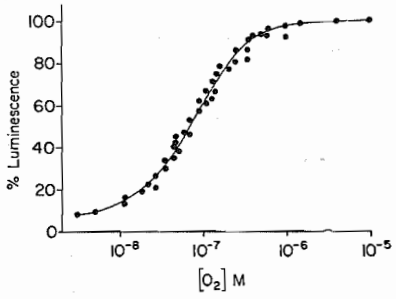
We have observed very steep regional redox ratio transitions In Vivo in various tissues (1,2,3,4). The most obvious example beeing the transition across the borderline of an ischemic area as first described by *Barlow and Chance* for an artificially produced infarct in a rat heart (5). We believe that the steep redox state transition may reflect similarly steep tissue oxygen gradients (6) and in order to probe that we have devised a simple model in which the ischemic borderzone in terms of oxygen gradient can be reproduced. The model is essentially a *Krogh Tissue Cylinder* (7) in macro scale, approximately 1:200, where the oxygen concentration profile perpendicular to the capillary may be recorded photographically in two dimensions. The model allows for independent variation of capillary oxygen tension, tissue oxygen consumption rate and facilitation of oxygen diffusion.

As oxygen indicator we have used Photobacterium Phosphoreum[x)]. Suspended in a hyperosmotic medium these bacteria will emit a green luminescence when oxygen is present as shown in Fig. 1 which is taken from *Sugano, Oshino and Chance* (8). The figure gives the relative luminescence intensity versus oxygen concentration in the medium. Saturation is achieved at rather low oxygen concentrations, approximately 1 μM which corresponds to 0.75 Torr. 10% luminescence intensity is reached at an oxygen concentration about two orders of magnitude lower. Thus, the photobacteria is an excellent

x) The bacteria was kindly provided by Dr. Y. Nakase.



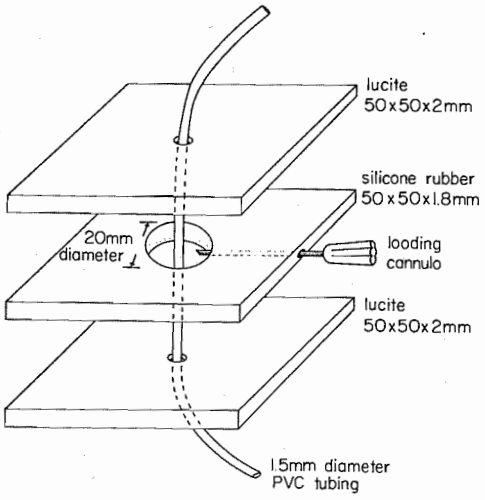


Fig. 1 (left). Relation between luminescence intensity of Photobacterium Phosphoreum and oxygen concentration. *Taken from T. Sugano, N. Oshino and B.Chance* (8).

Fig. 2 (right). Schematic diagram of the macro model of *The Krogh tissue Cylinder* (1). See text for further details.

indicator of oxygen concentration in the region of Km for oxygen for isolated mitochondria (8-10) Luminescence intensity which may be recorded photographically is therefore a convenient measure of respiratory activity in response to oxygen concentration.

Our model of the *Krogh Cylinder* is shown schematically in Fig 2. Two plexiglas plates spaced by a silicone rubber membrane in which a circular hole has been punched out from the chamber. An axially located PVC tube runs through the chamber. The suspension of the Photobacteria containing 4% gelatine is introduced into the chamber via the syringe needles and allowed to form a gel. When the gel is stable, which it becomes after 10-15 min in the refrigerator, the PVC tube may be pulled out leaving a perfect channel in the gel. This channel constitutes the capillary of the macro *Krogh Cylinder* through which oxygen is supplied as a gas. Mitochondria and suitable substrates as well as myoglobin may be added to the gelatine suspension prior to injection into the chamber in order to vary oxygen consumption rate or facilitate oxygen

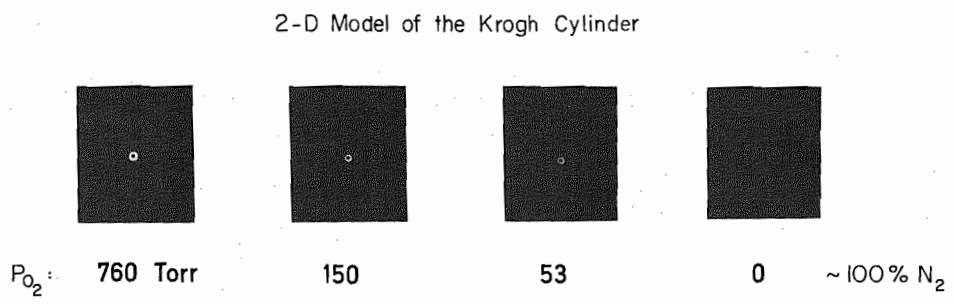


Fig. 3. Photographic recording of luminescence intensity of Photobacterium Phosphoreum in the chamber shown in Fig. 2. Bacteria (4 mg wet weight/ml) was suspended in hyperosmotic medium (0.5 M NaCl, 0.1 M Na_2HPO_4, 10 mM glucose) with 4% gelatine. Polaroid film (3000 Type 107) exposed for 2.5 min at f-stop 4.5. Image: object ratio was 0.9:1.

diffusion in the gel respectively. Oxygen will diffuse from the capillary into the gel and at steady state the bright luminescence zone will indicate the area around the capillary with oxygen tension above approximately 0.5 Torr. The luminescence zone is recorded photographically through one of the plexiglass plates with the optical axis of the camera aligned with the capillary.

Steady state recordings of the luminescence zone at 4 different values of capillary oxygen tension are shown in Fig. 3. The composition of the bacteria suspension is given in the figure legend. The oxygen/nitrogen gas mixtures were flushed through the capillary at a rate of 1 l/min, there was therefore no intra capillary oxygen gradient present. Oxygen consumption rate was calculated to 0.03 µmol/min x ml based on the time period elapsing from an instantaneous oxygen/nitrogen transition in the capillary until complete disappearance of the luminescence. This estimate will of course represent a maximum value because some oxygen will escape consumption in the gel by diffusing back into the capillary. There are two interesting observations to be made from Fig 3 . First, the borderzone transitions appear to be as sharp as the redox ratio changes we observe In Vivo, even with the much lower oxygen consumption rate of the model experiments. Second, a non-linear relationship exists between capillary oxygen tension and width of the luminescence zone as also shown in Fig. 4.

On this basis we decided to calculate the exact location and steepness of the luminescence transition. The distance required

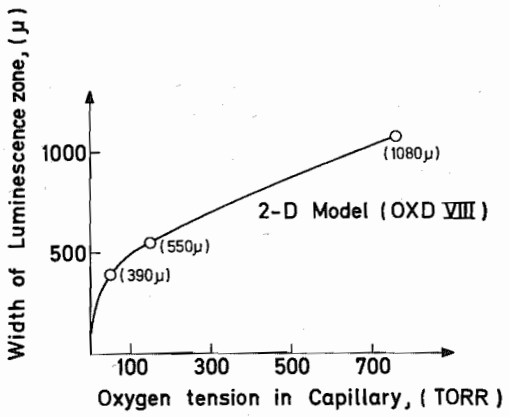


Fig. 4. Relation between width of luminescence zone and capillary oxygen tension. The width of the luminescence zone was recorded microdensitometrically with an accuracy of ±50 μ. Points of 50% intensity (≡ I_{50}) in the transition from luminescence to non-luminescence zone were taken as border lines in estimating the width.

for a decrease of luminescence intensity from 80% to 20% is taken as a measure of the steepness of the transition - in terms of isolated mitochondria that would be approximately the distance over which a 'full function/no function' transition would occur. The following equation describes oxygen diffusion for the circular geometry of our model:

$$\frac{\partial C}{\partial t} = D\left[\frac{\partial^2 C}{\partial r^2} + \frac{1}{r}\frac{\partial C}{\partial r}\right] - k_1 \frac{k_2 C^n}{k_3^n + C^n} \qquad (I)$$

and at steady state

$$\frac{\partial^2 C}{\partial r^2} + \frac{1}{r}\frac{\partial C}{\partial r} = \frac{k_1 k_2}{D} \frac{C^n}{k_3^n + C^n} \equiv K \frac{C^n}{k_3^n + C^n} \qquad (II)$$

D is diffusion constant for oxygen, k_1 is oxygen consumption rate, k_2 is the proportionality constant between oxygen consumption rate and luminescence intensity for a particular concentration of bacteria, k_3^n is K_m for oxygen for luminescence emission, n is the Hill coefficient, C is oxygen concentration and r the variable radius in the *Krogh Cylinder*. Values of 0.067 Torr and 1.5 may be obtained from Fig. 1 for k_3^n and n respectively.

For $C^n >> k_3^n$ (II) has the following solution, as found by *Erlang*

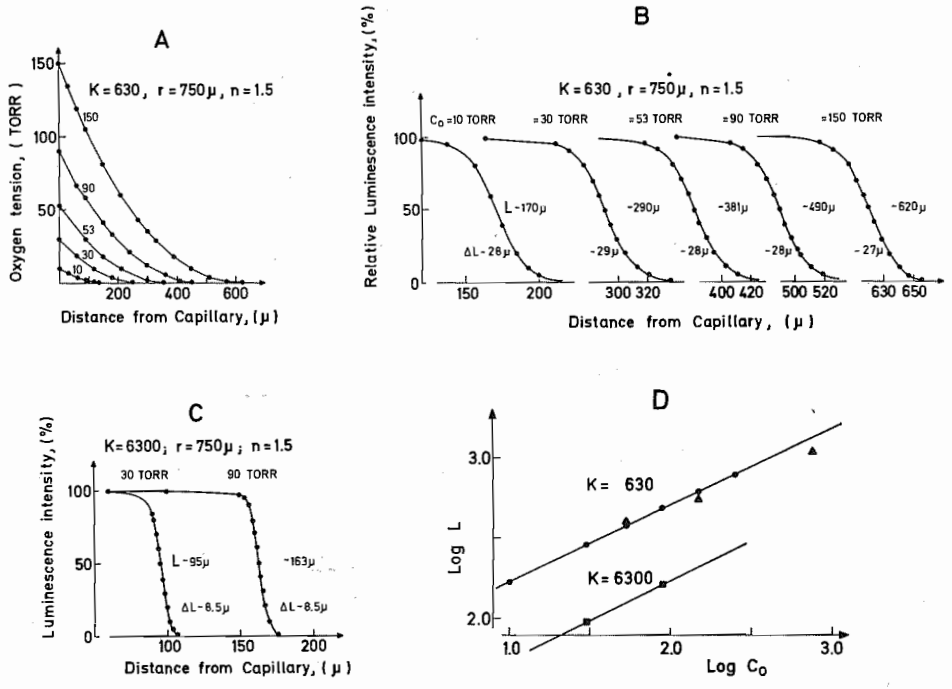


Fig. 5. A: Computer calculated oxygen tension profiles perpendicular to the capillary. B: Computer calculated luminescence intensity profiles showing the transition zone at 5 different capillary P_{O_2} at an oxygen consumption rate of 0.03 μmol/min x ml. C: Same as B but calculated for a 10 times higher oxygen consumption rate at capillary P_{O_2} of 30 and 90 Torr. D: The values for L and C_o plotted in a log/log diagram. ● is data from Fig. 5B, ■ from Fig. 5C and ▲ the experimentel data from Fig. 4. See text for further details.

$$C = C_0 - \alpha \ln \frac{r}{r_0} + \frac{K}{4} (r^2 - r_0^2) \qquad \text{(III)}$$

with r_o radius of the capillary. For $C = 0$, $\frac{dc}{dr} = 0$ and $r = R$ radius of the *Krogh Cylinder:* $\alpha = \frac{K}{2} R^2$. With L defined as the width of the luminescence zone, i.e., $L \equiv r - r_0$, (III) gives for $L \ll r_0$

$$L \simeq \sqrt{\frac{2C_0}{K}} \qquad \text{(IV)}$$

For $L \gg r_0$, (IV) is again obtained but with a correction factor

which reduces L slightly. For L/r_0 between 1 and 10, L is given by (IV) to within 25%, though. Equation (IV) is therefore taken as the exact relation between L and C_0 allowing for estimation of K = 630 in log L/log C_o plot of the experimental data from Fig. 4.

The equation (II) is then solved numerically under the boundary conditions:

$$c = c_0, \quad r = r_0$$

$$\frac{dc}{dr} \rightarrow 0, \quad c \rightarrow 0$$

for a number of different capillary P_{O_2}. The results are shown in Fig. 5.

The oxygen tension profiles perpendicular to the capillary for different capillary P_{O_2} & K values are given in Fig.5A whereas Fig.5B gives the relation between luminescence intensity and distance from the capillary. It appears that the 80%-20% transition (≡ ΔL) takes place over a distance of 28 μ at the present oxygen consumption rate and furthermore is independent of capillary oxygen tension. The effect of a 10 fold increase (i.e., K = 10 x 630) in oxygen consumption on L and ΔL is demonstrated in Fig.5C with capillary oxygen tension of 30 and 90 Torr as examples. L decreases to 95 μ and 163 μ for 30 Torr and 90 Torr respectively, whereas ΔL decreases to 8.5 μ. In Fig. 5D the calculated L values of Fig. 5B and 5C are plotted together with the experimental data in a log/log plot of L versus C_0. In agreement with equation (IV) a slope close to 0.5 as well as a reasonable fit to the experimental data is obtained. A decrease in capillary diameter by two orders of magnitude to physiological dimensions (from the experimental 1500 μ to 8 μ) does not cause any change in the calculated ΔL. However, as may be predicted from equation (III), the width of the luminescence zone decreases, (from 163 μ to 106 μ, K = 6300, C_0 = 90 Torr). Finally, with physiological values for both oxygen consumption rate, 3 μmol/min x ml, and capillary diameter, 8 μ, ΔL and L may be calculated to 2.7 μ and 33 μ respectively at a capillary oxygen tension of 90 Torr.

Even though the gelatine model is not directly comparable with the capillary unit In Vivo the present results indicate that anoxic zones may develop due to only a moderate decrease in arterial oxygen tension or to occlusion of single capillaries in a tissue with high oxygen consumption as for example brain where the average intercapillary distance is about 60 μ. Furthermore the results emphasize the point made by *Chance* (6) that the normoxic/anoxic transition in a tissue with high oxygen consumption can be expected to divide a single cell into a functional and a non-functional compartment.

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