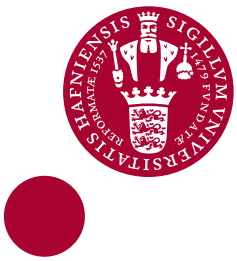

Study of tissue oxygen gradients by single and multiple indicators.

Quistorff, Bjørn; Chance, Britton

Published in:
Advances in Experimental Medicine and Biology

Publication date:
1977

Document version
Publisher's PDF, also known as Version of record

Reprinted from: OXYGEN TRANSPORT TO TISSUE - III (1978)
Edited by I. A. Silver, M. Erecińska,
and H. I. Bicher
Book available from: Plenum Publishing Corporation
227 West 17th Street, New York, N.Y. 10011

STUDY OF TISSUE OXYGEN GRADIENTS BY SINGLE AND MULTIPLE INDICATORS

Britton Chance and Bjørn Quistorff*

Johnson Research Foundation, School of Medicine
University of Pennsylvania, Philadelphia, Pa 19194 USA
*Department of Biochemistry A, University of Copenhagen
Blegdamsvej 3, 2200 Copenhagen, Denmark

There are two types of naturally occurring oxygen indicators. The first includes myoglobin and some soluble enzyme systems and may be uniformly distributed in low concentration over the cytosol: the second is localized and concentrated either in discrete organelles about a micron in diameter such as the mitochondria or the peroxisomes (Weibel 1969), or in more extended organelles such as the endoplasmic reticulum (Claude et al. 1947). In both cases, differing resolutions and sensitivities are possible.

The measurement of gradients of oxygen concentration requires determinations at two points sufficiently close together that they are on a gradient of oxygen concentration (a "two-point method". See e.g. Millikan 1936), or two closely spaced indicators that respond to different PO_2's or oxygen concentrations (a "two-indicator method. See e.g. Chance 1965, Oshino et al. 1975, Sies 1977a, b). The two methods have in common the requirements for distance resolution and oxygen sensitivity: either the two points or the two indicators must be separated by an appropriate small distance. Granted that these requirements can be fulfilled, the two methods would seem to give identical information, but this paper suggests that the two-indicator method may be more suitable for providing average tissue oxygen gradients over a large tissue volume. Furthermore, the two-point method is technically more difficult to execute since spatial resolution of the oxygen gradient itself may require apertures as small as a few microns. In fact, apertures of 1.5 microns (Thorell & Chance 1960) provide readily measurable optical signals with the mitochondrion which contains localized high concentrations of oxygen indicators (Thorell & Chance 1960). The two-indicator method, on the other hand, demands that the indicators be close enough to register PO_2's within the oxygen gradient (Weibel 1969);

however, in this case, larger signals are obtained since they are derived from all indicators in the tissue volume.

Heterogeneity of the tissue, particularly liver (Pette and Brandan 1966), can make interpretation of the results difficult. For example, the two points of the two-point method may not be in a homogeneous diffusion gradient need to be measured in order to obtain appropriately averaged values. However, the two-indicator method automatically averages all gradients within the span of the two indicators; the only basic assumption is that the two indicators exist at an appropriate average spacing in all cells in which gradients are to be measured. Such gradients may, however, vary in steepness due to tissue heterogeneity and a corresponding distribution will be obtained.

RESULTS

The results presented below decribe some chosen examples of application of the two-point and two-indicator methods for determination of oxygen gradients in cells and tissues.

Two-Point Method

Three-dimensional studies of oxygen gradients in a model cardiac infarct. In a Langendorf perfusion, a small branch of a coronary artery of a rat heart was ligated to produce an ischemic area in the wall of the left ventricle, 3 to 4 mm^2. The heart was gently freeze-clamped, and the ischemic area located on the surface of the frozen tissue - the general location can be determined by UV illumination and visual observation. Following the transfer of the sample to the low temperature redox scanner, a series of eight consecutive scans was recorded from the myocardium at depths between 100 μ and 1100 μ. In each scan, the fluorescence intensity of reduced pyridine nucleotide (PN) and oxidized flavoprotein (Fp) was measured at 3600 single points over an area of 36 mm^2. All eight scans were vertically aligned and it was therefore possible to obtain a three-dimensional representation of the ischemic area in the myocardium by combining the data from the eight two-dimensional scans.

Figure 1 shows a two dimensional display of the scans, i.e. the ratio between Fp and PN fluorescence is displayed as a gray-scale image employing suitable clipping functions. Reduced (low Fp/PN areas) appear black, and oxidized areas appear light. The same gray scale was used for all scans. The shape of the infarct changes with depth, reflecting the perfusion pattern of the ligated artery.

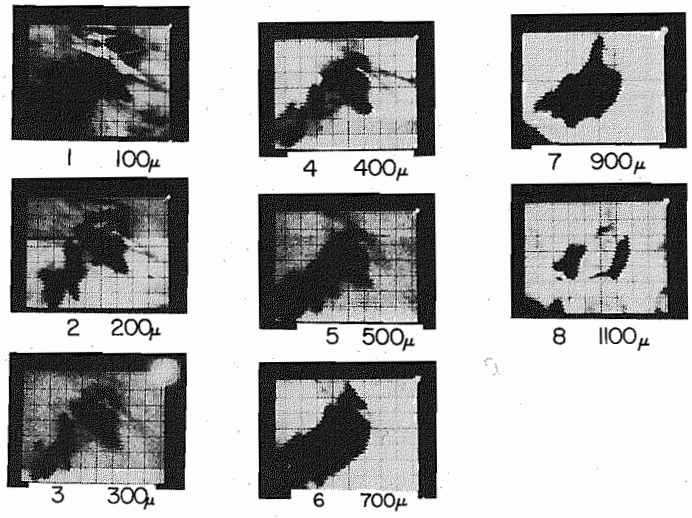

Figure 1: 2-D redox scans of freeze-trapped rat heart with model infarct. Sections are cut away every 100 μ.

At a depth of 1100 μ, the ischemic area starts to disappear, indicating that the endocardium has been reached. A striking feature of these images is the very sharp transition between oxidized and reduced tissue, which may be observed in all layers of the myocardium. As shown in Fig. 2, the transition involves a change in the Fp/PN ratio by a factor of five to ten which is achieved in most locations within 60 to 100 μ, i.e. the oxygen tension decreases sufficiently rapidly within 100 μ to cause a complete reduction of the mitochondria (see Quistorff, this volume). In isolated pigeon heart mitochondria, Sugano et al. (1974) found that a similar transition required a decrease in oxygen concentration from 5×10^{-7} M to less than 10^{-8} M, a difference of about 0.1 torr, or a gradient of at least 0.1 torr/100μ.

Two-Indicator Method

Cardiac tissue. In the studies of Fig.3, the two indicators are the mitochondria and the cytosolic myoglobin. In vitro, the experimental use of a suspension of mitochondria and myoglobin gives a clear-cut difference between the two indicators, as shown by the concavity of the cytochrome response vs. the myoglobin response. If the experiment is now repeated in the perfused heart, the concavity disappears and no difference in the responses of the two indicators can be detected; no observable amount of deoxymoglobin or oxidized cytochrome exists in the in vivo system. Thus, the gradient is so steep that it cannot be measured by these two

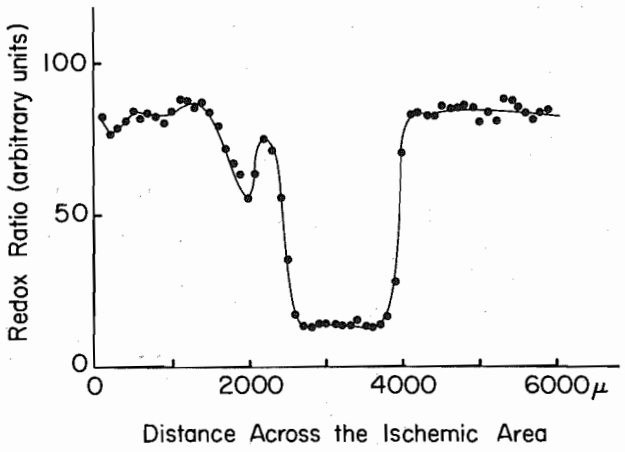

Figure 2: Typical redox profile across a model infarct.

indicators, whose spacing and P_{50}'s are too close together to be useful at normal respiration rates of perfused cardiac tissue. The difference of P_{50}'s is small, 1 torr, because of the high affinity of myoglobin for oxygen. The dimensions of the gradient are also small, possibly of the order of the intermitochondrial distance of 1μ. Therefore, the gradient may be greater than 1 torr/μ. Clearly, the myoglobin-cytochrome couple would be more appropriate for a tissue such as resting skeletal muscle or antimycin A-inhibited cardiac tissue, where the respiration rate is slower than in the perfused heart.

Mitochondria and peroxisomes. A variety of experiments are now vailable on rat liver in which the first indicator is the peroxisome and the second is the mitochondrion. The data considered for analysis are from Oshino et al. (1975) presented in their original form in Fig. 4. The two traces represent the responses of mitochondrial cytochrome c as measured by direct spectrophotometry (solid circles), and H_2O_2 generation (open circles), which was measured by peroxisomal catalase H_2O_2 and computed as the rate of production of H_2O_2 by the methanol titration method (Oshino et al. 1975). This approach depends upon equations that show that the H_2O_2 generation rate and the free H_2O_2 concentration can be precisely calculated from the molar concentration of an alcohol (methanol) which causes the concentration of the catalase H_2O_2 compound to decrease to half its maximal value. The H_2O_2 generation is thus sharply localized in the peroxisomes. In this experiment, H_2O_2 was generated by glycollate oxidase, which is also largely localized in the peroxisomes.

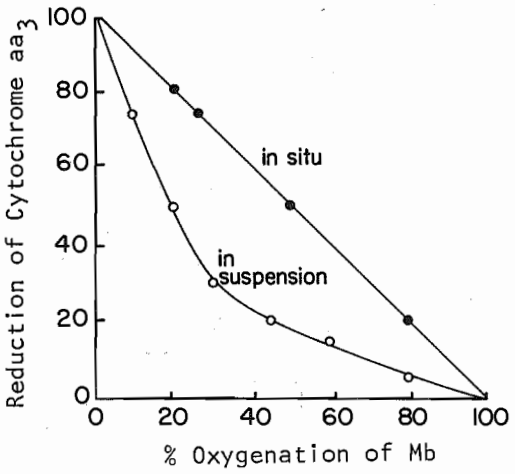

Figure 3: A plot of the relative response to oxygen of cardiac myoglobin and cardiac mitochondria in suspension and in situ.

The profiles A and B represent titrations for two rates of mitochondrial respiration: A, uninhibited, and B, antimcyin A-blocked. It can be seen directly from the graphs that the difference between the titration curves for peroxisomal H_2O_2 and cytochrome c oxidation is small, and maximal in the region of 50% oxygen in the perfusate. The amplitude of the difference is somewhat truncated, due to the fact that even before the peroxisomes achieve the reduced state, P_R, some of the mitochondria do likewise (M_R). Thus, Panel A corresponds to a steep tissue oxygen gradient, where the roughly 200 torr difference of K_M of the two systems occurs within the average separation of peroxisomes and mitochondria, so the gradient is of the order of several hundred torr per micron.

In Panel B, the oxygen gradient is decreased by slowing the respiration of the mitochondria and leaving that of the peroxisomes more or less constant. The extent of the decrease of the tissue oxygen gradient will have to be determined by decreasing the flux of the glycollate oxidase.

A feature of the profile of Panel B is that the glycollate oxidase profile shows an extension towards lower PO_2's in the presence of antimycin A than in its absence, which is attributed to a decrease of the oxygen gradient. This allows faithful reproduction of the in vitro profile for glycollate oxidase at the lower tissue oxygen gradient. In fact, the K_m for glycollate oxidase in vitro and in the tissue is approximately the same (300 μM or 190 torr).

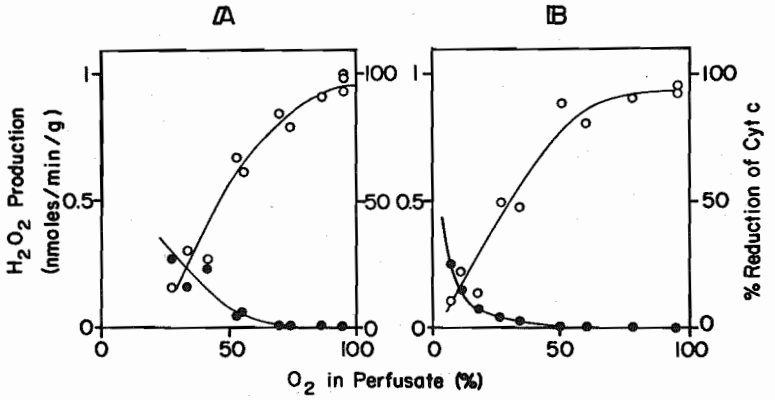

Figure 4: Rates of H_2O_2 production with various O_2 concentrations measured from methanol titrations (o) plotted vs. the extent of reduction of cytochrome c (●) with 1mM glycollate as substrate. A, in the absence of Antimycin A; B, with 8 μM antimycin A (Oshino et al. 1975, reproduced with permission).

Experiments with samples similar to those of Fig.4 employed urate as substrate. The estimated value of the gradient for urate oxidation is somewhat steeper, but still readily detectable by the two-indicator method.

If a peroxisomal substrate is omitted, there is a small residual H_2O_2 generation from the mitochondria, whose P_{50} must match that of cytochrome c. The maximal rate of this H_2O_2 generation is only 4% of that obtained from the peroxisomes with glycollate as substrate, so it is not a quantitative factor in the determination of the H_2O_2 gradient.

In a similar series of experiments, Sies has measured the actual consumption of urate in a flow-through liver perfusion (Sies 1977b), instead of the peroxisomal H_2O_2 generation. PaO_2 values are not available. Fig.5 shows a plot of the peroxisomal signal vs. the mitochondrial signal for Sies's experiment (solid triangles and compares it with data on the perfused organ under nearly identical conditions with glycollate as the peroxisomal substrate (open squares). In addition, data for hepatocytes (open circles) are included (Jones et al. 1977). The averaging problem is more severe with the flow-through studies, since the flow-through averages the H_2O_2 generation in all lobes of the liver, while the spectroscopic method for cytochrome measures its response in a particular part of the lobe under observation. Also, Sies used cytochrome a instead of cytochrome c. While it would seem that the two methods should have given the same result, they are in fact rather different (solid triangles); no difference

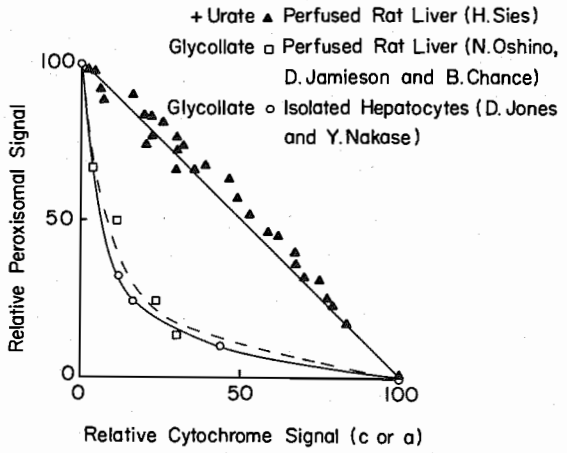

Figure 5: A plot of the peroxisomal signal (ordinate) vs. the mitochondrial signal (abscissa) for a variation of PaO_2 in urate-perfused liver by Sies (1977b) (▲); glycollate-perfused liver by Oshino et al. (1975) (■); and in a suspension of hepatocytes with glycollate as substrate by Jones et al. (1977 (o).

signal from the two indicators was detected in the normally respiring liver. It seems highly unlikely that the titration for cytochrome c would have been altered by the change of procedure, and so a reasonable assumption is that the flow-through method has detected portions of the liver that are less well-perfused than those under direct spectroscopic observation of the cytochrome c and catalese in the work of Oshino et al. (1975). The agreement of the cell and tissue data support the idea of intracellular oxygen gradients in the hepatocyte.

In summary, it is preferable to measure the two indicators in the same portion of an organ than to rely upon the assumption that the total organ would be as well-perfused as the parts under observation.

DISCUSSION

These experimental observations and analyses suggest a new approach to the detection of tissue oxygen gradients by the two-indicator method. The method requires perfusion of the organ with a variety of PaO_2's and an adjustment of the K_m's of the indicators so that they appropriately scan the tissue oxygen gradient and thus give a maximal difference signal (cf Fig.4) or an appropriate curvature of the plot of peroxisomal vs. mitochondrial signals (Fig. 5). The latter indicates the presence of a steep

gradient by the approach of the plot towards linearity. In the case of Figs.4 and 5 a crude estimate of the portion of the equivalent two dimensional Krogh model occupied by the reduced peroxisomes and the oxidized mitochondria - a mixed redox state of the two indicators - can be obtained by simply assuming that this portion is given by the fractional deviation from linearity in Fig.5 or the fractional difference of the two traces in Fig. 4A. These values are 58 and 52% respectively, i.e., about half the equivalent Krogh model is occupied by "mixed" redox states of the two indicators. Simplifying the model from two to one dimensions, the linear dimension of the gradient becomes half the length of the model from arterial to venous end, or for most models, about half of 200μ or 100μ. The difference of the peroxisomal K_m (∿200 torr) and the mitochondrial K_m (0.1 torr) is 200 torr, and the gradient is ∿2 torr/μ. Obviously, a thorough mathematical analysis of the combined reaction and diffusion in the two-indicator system will lead to an accurate calculation of the tissue oxygen gradient.

ACKNOWLEDGEMENT

The assistance of Benson Margules, Eric Benshetler, Wolfgang Nadler and John Sorge is gratefully acknowledged as is the support of NIH HL-18708 and NS-10939,

REFERENCES

Chance, B. (1965) J. Genl. Physiol. 49, 163-188.

Claude, A., Porter, A.R. and Pickels, E.G. (1974) Cancer Res. 7, 421-430.

Jones, D.F., Mason, H.S. Nakase, Y. and Chance, B. (1977) Federation Proc. 36 (Abstr. 1555).

Millikan, G.A. (1936) Proc. Roy. Soc. London B-120, 366-388.

Oshino, N., Jamieson, D. and Chance, B. (1975) Biochem. J. 146, 53-60.

Pette, D. and Brandau, H. (1966) Enzym. Biol. Chim. 6, 79-87.

Sies, H. (1977a) In Tissue Hypoxia and Ischemia (M. Roberts, R. Coborn, S. Lahiri and B. Chance, Eds.) Adv. Expt. Med. 78, 51-66.

Sies, H. (1977b). This Volume, pp.

Sugano, T., Oshino, N. and Chance, B. (1974) Biochim. Biophys. Acta. 340-358.

Thorell, B. and Chance B. (1960) Exptl. Cell Res. 20, 43-45.

Weibel, E.R. (1969), J. Cell Biol. 42, 68-91.