of donor and acceptor, and how well these electronic states couple with the intervening DNA. It is not clear that nature has taken advantage of the DNA polymer as a mediator of electron transfer, but in fact oligonucleotides and polynucleotides should serve well as vehicles that may be readily manipulated to examine systematically the effects of medium, orientation, and separation distance on electron transfer in a polymeric system.

REFERENCES AND NOTES

1. D. Devault, *Q. Rev. Biophys.* **13**, 387 (1980).
2. S. L. Mayo, W. R. Ellis, R. J. Crutchley, H. B. Gray, *Science* **233**, 948 (1986); S. S. Isied, *Prog. Inorg. Chem.* **32**, 443 (1984).
3. J. L. McGourty, W. V. Blough, B. M. Hoffman, *J. Am. Chem. Soc.* **105**, 4472 (1983); N. Liang, C. H. Kang, P. S. Ho, E. Margoliash, B. M. Hoffman, *ibid.* **108**, 4665 (1986); K. P. Simolo, G. L. McLendon, M. R. Mauk, A. G. Mauk, *ibid.* **106**, 5012 (1984); G. L. McLendon and J. R. Miller, *ibid.* **107**, 7811 (1985).
4. J. J. Hopfield, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3640 (1974); M. Redi and J. J. Hopfield, *J. Chem. Phys.* **72**, 6651 (1980).
5. R. A. Marcus, *Annu. Rev. Phys. Chem.* **15**, 155 (1964); *J. Phys. Chem.* **72**, 891 (1968); R. A. Marcus and N. Sutin, *Biochim. Biophys. Acta* **811**, 265 (1985).
6. G. L. Closs, L. T. Calcaterra, N. J. Green, K. W. Penfield, J. R. Miller, *J. Phys. Chem.* **90**, 3673 (1986).
7. A. D. Joran, B. A. Leland, G. G. Geller, J. J. Hopfield, P. B. Dervan, *J. Am. Chem. Soc.* **106**, 6090 (1984); A. D. Joran *et al.*, *Nature* **327**, 508 (1986).
8. J. K. Barton, C. V. Kumar, N. J. Turro, *J. Am. Chem. Soc.* **108**, 6391 (1986).
9. P. Fromherz and B. Rieger, *ibid.*, p. 5361.
10. J. K. Barton, A. T. Danishefsky, J. M. Goldberg, *ibid.* **106**, 2172 (1984).
11. C. V. Kumar, J. K. Barton, N. J. Turro, *ibid.* **107**, 5518 (1985); J. K. Barton, J. M. Goldberg, C. V. Kumar, N. J. Turro, *ibid.* **108**, 2081 (1986); J. Rehmann, thesis, Columbia University, New York (1988).
12. J. K. Barton, *Science* **233**, 727 (1986).
13. N. J. Turro, *Modern Molecular Photochemistry* (Benjamin-Cummings, Menlo Park, CA, 1978), p. 246.
14. R. B. Macgregor, R. M. Clegg, T. M. Jovin, *Biochemistry* **26**, 4008 (1987); V. I. Ivanov, L. E. Minchenkova, A. Schyolkina, A. I. Poletayev, *Biopolymers* **12**, 89 (1973). The ratio of intercalation to surface binding of $Ru(phen)_3^{2+}$ does increase appreciably with glycerol present in solution. Thus it is likely that $Co(phen)_3^{3+}$ exists also predominantly in the intercalated form under these conditions.
15. T. Guarr, M. Mcguire, S. Strauch, G. McLendon, *J. Am. Chem. Soc.* **105**, 616 (1983). Under these conditions, the bulk viscosity is estimated to be 1.2×10^4 cP, and the calculated diffusion distance for the metal complexes not bound to DNA would be approximately 4 Å during the lifetime of the donor excited state.
16. I. Fujita and H. Kobayashi, *Inorg. Chem.* **12**, 2758 (1973).
17. The absence of self-quenching of the ruthenium excited state further supports this assumption.
18. M. Inokuti and F. Hirayama, *J. Chem. Phys.* **43**, 1978 (1965).
19. The distribution of separation distances was calculated with the nearest-neighbor distance distribution for a "hard rod gas" distributed randomly over a one-dimensional lattice

$$P(r)dr = \frac{N}{[L(1 - N\sigma/L)]} \times \left[1 - \frac{(r - \sigma)}{[L(1 - N\sigma/L)]}\right]^{N-1} dr,\ r \geq \sigma$$

where N is the number of bound particles with an exclusion length σ bound to a lattice of length L. In our case, L is 200 base pairs, σ is 4 base pairs per bound molecule, and N is the sum of donor and acceptors. The mean distance, $\langle r \rangle$, is

$$\langle r \rangle = \Sigma r P(r) / \Sigma P(r)$$

See L. Tonks, *Phys. Rev.* **50**, 955 (1936).
20. Driving forces were calculated based upon data reported in the following references: B. Brunschwig and N. Sutin, *J. Am. Chem. Soc.* **100**, 7568 (1978); S.-F. Chen *et al.*, *ibid.* **103**, 369 (1981); E. Paglia and C. Sironi, *Gazz. Chim. Ital.* **87**, 1125 (1957).
21. In buffer at 293 K in the absence of DNA, the rates with either Cr or Co as acceptor appear to be diffusion controlled based upon measurements at different ionic strengths, whereas the reaction with Rh, having a substantially smaller free energy change, is activation limited.
22. Results that vary in this fashion with driving force may be interpreted in terms of donor-acceptor pairs in the Marcus inverted region. For these experiments, however, the DNA would still be involved in mediating such transfer. In a model system, the possible role of virtual states of an intervening π-electron system has been discussed. See H. Heitele and M. E. Michel-Beyerle, *J. Am. Chem. Soc.* **107**, 8286 (1985).
23. G. Adams and M. Delbruck, in *Structural Chemistry and Molecular Biology*, A. Rich and N. Davidson, Eds. (Freeman, San Francisco, 1968), p. 198; P. Richter and M. Eigen, *Biophys. Chem.* **2**, 255 (1974).
24. O. G. Berg, R. B. Winter, P. H. von Hippel, *Biochemistry* **20**, 6929 (1981).
25. B. J. Terry, W. E. Jack, P. Modrich, *J. Biol. Chem.* **260**, 13130 (1985).
26. Theoretical studies have actually noted that some charge transfer from the ribose units along the DNA backbone to the bases may occur, creating what is essentially a substantial hole framework on the DNA backbone. See P. Otto, E. Clementi, and J. Ladik [*J. Chem. Phys.* **78**, 454 (1983)] and E. Clementi and G. Corongiu [*Int. J. Quant. Chem. Quant. Biol. Symp.* **9**, 213 (1982)].
27. Replacing the NaCl in our buffer with 30 m*M* $MgCl_2$, which may perturb the solvent structure around the helix, causes a decrease for k_q^s to $7 \times 10^9 M^{-1}\ s^{-1}$, with k_q^i remaining constant.

28. We thank B. Berne for stimulating discussions regarding the near-neighbor distance distributions. Supported by National Science Foundation and the Army Office of Research.

14 April 1988; accepted 26 July 1988

Imaging of Phosphorescence: A Novel Method for Measuring Oxygen Distribution in Perfused Tissue

WILLIAM L. RUMSEY,* JANE M. VANDERKOOI, DAVID F. WILSON

The imaging of phosphorescence provides a method for monitoring oxygen distribution within the vascular system of intact tissues. Isolated rat livers were perfused through the portal vein with media containing palladium coproporphyrin, which phosphoresced and was used to image the liver at various perfusion rates. Because oxygen is a powerful quenching agent for phosphors, the transition from well-perfused liver to anoxia (no flow of oxygen) resulted in large increases of phosphorescence. During stepwise restoration of oxygen flow, the phosphorescence images showed marked heterogeneous patterns of tissue reoxygenation, which indicated that there were regional inequalities in oxygen delivery.

IN MAMMALIAN TISSUES, OXYGEN MUST be continuously supplied to maintain cellular homeostasis. Even moderate reductions of blood flow compromise physiological function. In order to investigate the nature of oxygen supply to tissue in detail, a method is needed for the accurate measurement of the concentration of oxygen at its site of transfer from the vascular bed to parenchymal cells. Methods that have been used to assess tissue oxygenation not only have individual technical problems but in general are weakened by their inflexibility. For example, oxygen microelectrodes are not always suitable because (i) they are restricted to specific locations within the tissue, thereby preventing evaluation of a large area, and (ii) they must be inserted into the tissue, which disturbs its local environment.

Recently we developed a method for measuring oxygen that is based on its ability to quench phosphorescence of selected lumiphores (*1, 2*). This method is accurate and provides precise serial measurements of oxygen in the physiological range, from $10^{-3}M$ to less than $10^{-8}M$. To date, however, this methodology has been restricted to the in vitro study of biological samples that can be placed in a cuvette, such as suspensions of isolated mitochondria (*3*) and cells (*4*). We have shown that phosphorescence measurements can be used to obtain images of oxygen distribution in tissue without invasion of that tissue. Inhomogeneities of oxygen delivery to tissue of isolated perfused rat liver have been demonstrated with a video camera, which detects the phosphorescence of palladium (Pd)-coproporphyrin in the tissue perfusate.

When the tissue is illuminated, some light is absorbed by the Pd-coproporphyrin, which excites it to the triplet state. The

Department of Biochemistry and Biophysics, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.

*To whom correspondence should be addressed.

triplet state returns to the ground state either by light emission or by energy transfer to other molecules (quenching). In biological materials, the principal quenching agent is oxygen and this quenching is diffusion limited. The effect on phosphorescence is described by the Stern-Volmer relation:

$$I_0/I = \tau_0/\tau = 1 + k_q\tau_0[O_2]$$

where I_0 and τ_0 are the phosphorescence intensity and lifetime, respectively, in the absence of oxygen; I and τ are the phosphorescence intensity and lifetime, respectively, at a given oxygen concentration $[O_2]$; and k_q is a constant related to the rates of diffusion of oxygen and the selected lumiphore. The Stern-Volmer relation is accurately obeyed for the quenching of the phosphorescence of Pd-coproporphyrin by oxygen (*2*). This relation can be used to calculate the concentration of oxygen from the intensity of the emitted light or from the lifetime of the triplet state.

After fasted (24 hours) male Sprague-Dawley rats (275 to 325 g) had been anesthetized, the livers were perfused in situ via the portal vein with Krebs-Henseleit buffer supplemented with 5 m*M* glucose (*5*). The buffer was maintained at 37°C and equilibrated with 95% O_2:5% CO_2 (v:v) by mild bubbling. In addition, the influent was passed through gas-permeable Silastic tubing (60 cm long, 2 mm in internal diameter) placed within Tygon tubing (3.5 mm in internal diameter) through which the same gas mixture flowed. During this single-pass perfusion period, the liver was surgically removed from the body and placed in a clear plastic petri dish positioned on a tripod from which the tissue was illuminated from below (*6*). A xenon arc lamp was placed about 12 cm from a mirror positioned under the liver. The collimated light was reflected upward by the mirror to the surface of the liver where it illuminated a circular area 3 to 4 cm in diameter. Phosphorescence emission (>665 nm, with the use of a Schott cutoff filter) from the illuminated area was reflected to a silicon intensified target (SIT) video camera (Dage MTI model 66, Michigan City, Indiana) by a second mirror positioned beneath the liver. The video camera, placed approximately 0.25 m from the latter mirror, imaged the liver through a 90-mm lens. The camera output was continuously displayed on a monitor and recorded by a video cassette recorder (*7*).

The Pd-coproporphyrin (1 μ*M*) was added to Krebs-Henseleit buffer containing 1% bovine serum albumin (Fraction V, ICN Immunobiologicals, Lisle, Illinois) and recirculated for 5 to 10 min before data acquisition and then for the remainder of the experiment. Pd-coproporphyrin was chosen as the oxygen probe because it remained in the perfusate and was not taken up by the tissue, as evidenced by the complete disappearance of the image within 2 min after the tissue was returned to the perfusion medium without the probe. The data were digitized and displayed on an analog monitor (Sony PVM-1271Q). Photographs were made of the monitor image with a single-lens reflex camera (Yashica FX-3) equipped with a 35- to 70-mm macro lens. To improve the overall contrast of the images, we subtracted a constant amount of background intensity, just enough to delete light from the areas external to the liver and the perfusate pool immediately surrounding the liver. The remaining intensity was linearly expanded to make the maximal pixel value for the anaerobic liver equal to 250. All of the images were subjected to the same quantitative procedure, retaining the relative luminescence intensities.

Oxygenation of the liver was established by the flow of perfusate entering the portal vein, that is, the portal influent. The initial rate of perfusion of the liver was 28 to 32 ml/min (*8*). At this level of flow, the phosphorescence intensity from the surface of the liver was very low (Fig. 1A). The emitted light was diffuse and had a relatively homogeneous distribution, being equivalent in intensity or pixel value (60 to 64) throughout much of the illuminated area of the liver lobe. Phosphorescence intensity was nearly uniform (Fig. 1A), indicating that the tissue had a relatively even level of oxygenation. When we made the tissue anoxic by stopping completely the portal influent flow of oxygen (no flow), light intensity increased approximately fourfold (Fig. 1B). The illuminating light intensity was less at the periphery of the lobe on the right side of the photographs in Fig. 1, indicating the decreased intensity of phosphorescence in that region. A small amount of perfusate escaped the tissue, producing a low level of light emission at the perimeter of the tissue (the latter is most noticeable on the left side of Fig. 1, A and B).

The changes in phosphorescence intensity due to differences in the perfusion rate are best observed by subtraction of the phosphorescence from well-oxygenated tissue. Thus, the image of fully aerobic tissue was subtracted from the images of the tissue at lower rates of perfusion (Fig. 2, A through D). After a brief period (45 s) of zero influent, phosphorescence intensity reached a maximum value (Fig. 2A). Flow was then increased from 0 to 10 ml/min. Reperfusion caused a rapid decrease in phosphorescence intensity, which stabilized in 45 s (Fig. 2B). Regions of better oxygenated tissue appear as darker areas; regions with less oxygen appear as brighter areas. The image of the phosphorescence from the liver lobe has a mottled appearance, indicating a marked heterogeneity in the reperfusion and reoxygenation process. The differences can be seen as small peaks and troughs in the plot of intensity versus pixel number. The pixel

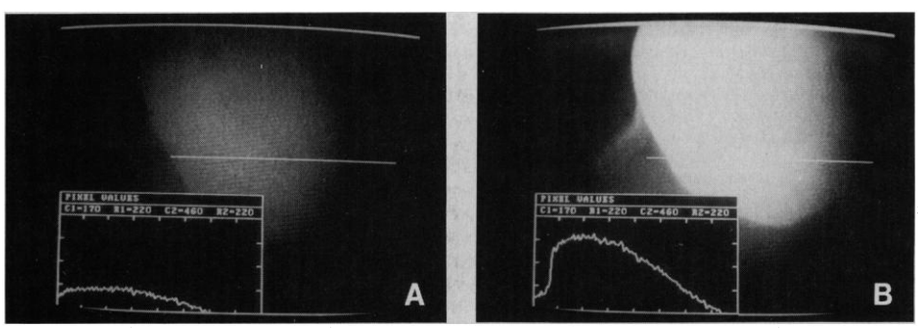

Fig. 1. Change in phosphorescence intensity in response to anoxia in perfused rat liver: (**A**) the underside of the right lobe under conditions of maximal oxygenation; (**B**) the same aspect of the liver after 45 s of zero flow (anoxia). During perfusion without Pd-coproporphyrin, illumination of the hepatic surface with a xenon arc lamp did not result in light emission when monitored with a SIT camera. The perfusate was then switched to an experimental medium containing Pd-coproporphyrin (1 μ*M*) and 1% bovine serum albumin. A typical experiment was completed within 2 to 3 hours in which several brief (30 to 45 s) pulses of anoxia were repeated. Anoxia was produced by stopping the flow of portal influent. The selected images are representative of eight individual experiments conducted over a 6-month period. Each photograph was prepared from an average of eight frames (1.5 s). The contrast was enhanced by subtracting a background intensity of approximately 40 gray levels and linearly stretching the remaining intensities to a full scale of 0 to 250. The white line appearing across the lobe was arbitrarily scribed for measurement of the values of phosphorescence intensity shown in the inset of each photograph. This line is 290 pixels long and represents 20 mm on the liver surface. The intensity value for each pixel along the line has a range of 0 to 256 gray levels. The letters C and R in the insets correspond to the column and row, respectively, at which the white line is positioned on the monitor.

values in the left lateral aspect of the lobe range from 64 to 100 units and are about 40 to 60% of the intensity observed during anoxia. Phosphorescence emanating from the right central portion of the lobe, however, was not markedly affected. When the influent flow was increased to 18 ml/min (Fig. 2C), reoxygenation proceeded from the peripheral regions of the lobe, that is, those areas at the perimeter of the lobe, to the more central portions of the tissue. Pixel values at the periphery were now reduced to near 0, equivalent to those for maximal perfusion rates, whereas those in the central area of the lobe were about 64 to 100. After the flow was increased to 23 ml/min, there were marked regions of heterogeneity in the phosphorescence image, but the overall intensity approached that of fully perfused tissue (Fig. 2D).

These results demonstrate that phosphorescence is an effective indicator of tissue oxygenation levels. The increase in phosphorescence as a result of anoxia (no flow) was large (*9*), and the response time of phosphorescence intensity to changes in local oxygen concentration was a few milliseconds. Thus the rate of change in oxygen pressure can be measured with a temporal resolution limited only by the time between camera frames (60 s^{-1}). Phosphorescence intensity can be mapped (with appropriate software) across defined regions of tissue, providing two-dimensional images of oxygen pressure in the organ of interest. In these experiments, the illuminating light penetrated only a few millimeters into the tissue, thereby preventing the visualization of oxygen pressures at greater depths. On the other hand, the resolution was sufficient to identify differences in oxygen pressures at the level of individual pixels. In Figs. 1 and 2, a pixel represents about 0.07 mm of the liver surface. Higher resolution could be obtained by replacing the 90-mm lens used here with optics capable of greater magnification.

The pattern of reoxygenation initiated by reperfusion indicates that (i) there is marked heterogeneity of perfusion within a given region of tissue and that (ii) the peripheral aspects of the lobes are preferentially reoxygenated as compared with the central zones. The portal vasculature is a low-pressure system without valves. Alterations of oxygen pressure arising from the portal vein are thought to be rapidly transmitted to the peripheral branches of the portal system including the specialized capillary network of the liver, the sinusoids (*10*). The spatial resolution of the video system that we used was approximately 70 μm per pixel. This was not sufficient to determine differences in perfusion at the level of individual sinusoids. It is possible, however, that the small irregularly shaped areas of greater and lesser phosphorescence intensity shown in Fig. 2, B, C, and D, correspond to periportal (around the portal vein) and pericentral (around the central vein) patches of the liver. Thus oxygen delivery during reperfusion was quite different for adjacent areas of tissue (*11*).

Oxygen consumption has been reported to be greater in the periportal zones than in the pericentral regions (*12*). Differences in oxygen consumption could contribute to the heterogeneity in reoxygenation rate observed on reperfusion under our experimental conditions. In areas of tissue with greater metabolic rate, greater oxygen demand would require higher levels of perfusion to produce a decline in phosphorescence intensity upon reperfusion.

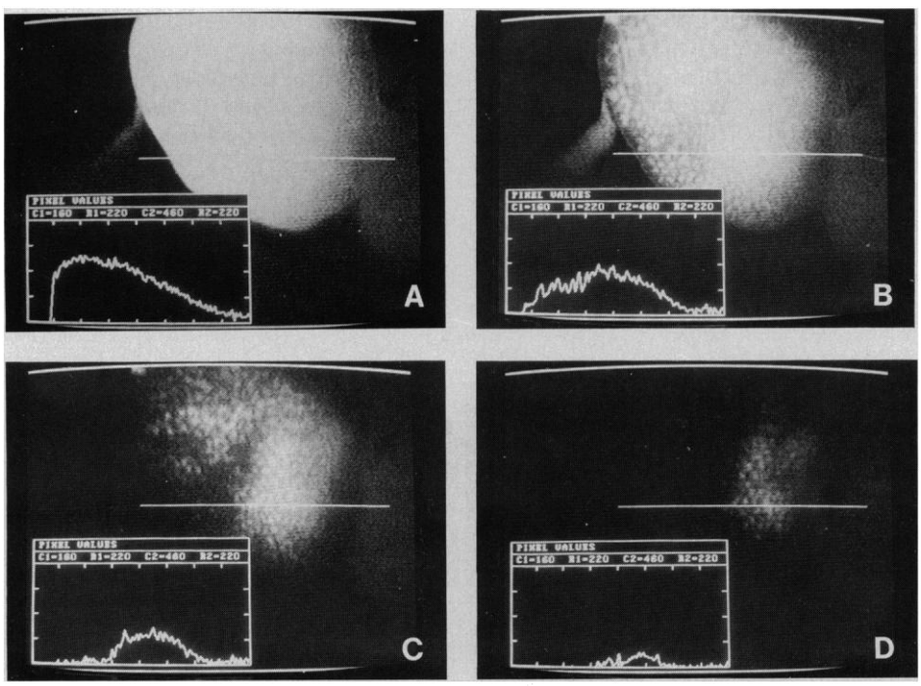

Fig. 2. Changes in oxygen distribution in response to reperfusion after anoxia. The images were obtained from the same animal as those in Fig. 1 with the experimental conditions essentially unchanged. In each case, the image derived from the fully oxygenated state was subtracted from those obtained during hypoxic conditions, thereby eliminating extraneous phosphorescence. In (**A**), the selected image represents the anoxic state, that is, the portal influent was stopped for 45 s. Reperfusion was then initiated at a rate of 10 ml/min (**B**), increased to 18 ml/min (**C**), and increased to 23 ml/min (**D**). Each image is produced from data taken 45 s after the increase in perfusion rate.

REFERENCES AND NOTES

1. J. M. Vanderkooi and D. F. Wilson, *Adv. Exp. Med. Biol.* **200**, 189 (1987); D. F. Wilson, J. M. Vanderkooi, G. Maniara, S. P. Defeo, D. C. Bloomgarden, *ibid.* **215**, 71 (1987).
2. J. M. Vanderkooi, G. Maniara, T. J. Green, D. F. Wilson, *J. Biol. Chem.* **262**, 5476 (1987).
3. D. F. Wilson, W. L. Rumsey, T. J. Green, J. M. Vanderkooi, *ibid.* **263**, 2712 (1988).
4. D. F. Wilson and W. L. Rumsey, *Adv. Exp. Med. Biol.* **220**, 121 (1988).
5. The animals were anesthetized with a single intraperitoneal injection of sodium pentobarbital (35 mg/kg). The animals were fasted to reduce the content of glycogen.
6. The light source was a xenon arc lamp filtered with a 1.5-cm thickness of a saturated solution of $CuSO_4$ in 0.5*M* H_2SO_4 and with a cutoff filter to remove near-ultraviolet light (50% block at 395 nm).
7. The video recorder (Realistic model 17, Tandy Corp., Fort Worth, TX) was interfaced with a computer (Spear 386, Northbrook, IL) for digitization (series 100 board from Imaging Technology Corporation, Woburn, MA) and processing (Imagelab by Werner Frei Associates, Santa Monica, CA) of the images.
8. We measured the flow by collecting the portal effluent for 30 s in a graduated cylinder.
9. When the perfusion pump was stopped, small volumes of perfusate continued to exit the vena cava, which led to a diminished total volume of perfusate within the liver. This effect may cause the phosphorescence intensity during anoxia to be somewhat (a few percent) less than would be the case had the volume of perfusate remained constant. This difference is, however, very small relative to the several-fold increase in phosphorescence due to the decrease in oxygen concentration in the perfusate.
10. J. C. Campra and T. B. Reynolds, in *The Liver: Biology and Pathobiology*, I. Arias, H. Popper, D. Schachter, D. A. Shafritz, Eds. (Raven, New York, 1982), p. 632.
11. In separate experiments, a cycle of anoxia and reoxygenation through the portal vein was followed by a cycle in which flow was reversed, that is, reperfusion occurred through the vena cava. Reperfusion through the vena cava resulted in a pattern of light emission opposite to that shown in Fig. 2, B, C, and D.
12. T. Matsumura, F. C. Kauffman, H. Meren, R. G. Thurman, *Am. J. Physiol.* **250**, G800 (1986); E. M. Kekonen, V. P. Jauhonen, I. Hassinen, *J. Cell. Physiol.* **133**, 119 (1987).

13. We thank S. Lahiri and W. Englander for comments on the manuscript. Supported by NIH grants GM 36393 and GM 21524.

28 March 1988; accepted 14 July 1988