of the enzymes involved in the breakdown of the ethanolamine phospholipids and this will be discussed.

This work has been supported in part by a grant from the Multiple Sclerosis Society.

- Ansell, G. B. & Hawthorne, J. N. (1964). *Phospholipids*, p. 318. Amsterdam: Elsevier Publishing Co.
 Ansell, G. B. & Spanner, S. (1965). *Biochem. J.* **94**, 252.
 Ansell, G. B. & Spanner, S. (1966). *Biochem. J.* **98**, 23P.

- Ansell, G. B. & Spanner, S. (1967). *J. Neurochem.* **14**, 873.
 Ansell, G. B. & Spanner, S. (1968). *Biochem. J.* **108**, 207.
 Dawson, R. M. C. (1955). *Biochem. J.* **59**, 5.
 Gallai-Hatchard, J., Magee, W. L., Thompson, R. H. S. & Webster, G. R. (1962). *J. Neurochem.* **9**, 545.
 Gatt, S. (1968). *Biochim. biophys. Acta*, **159**, 304.
 Knauff, H. G., Marx, D. & Mayer, G. (1961). *Hoppe-Seyler's Z. physiol. Chem.* **299**, 248.
 Leibovitz, Z. & Gatt, S. (1968). *Biochim. biophys. Acta*, **164**, 439.
 Strickland, K. P., Thompson, R. H. S. & Webster, G. R. (1956). *J. Neurol. Neurosurg. Psychiat.* **19**, 12.

MOLECULAR ENZYMOLOGY GROUP COLLOQUIUM ON 'MECHANISMS OF OXYGEN ABSORPTION AND UTILIZATION'

Free-Radical Intermediates in the Reduction of Oxygen

By R. C. BRAY. (*Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, Fulham Road, London S.W.3, U.K.*)

Free-radical species derived from oxygen have frequently been proposed as intermediates in biochemical reactions (e.g. Norman & Radda, 1962; Yamazaki & Piette, 1963; Staudinger & Ullrich, 1964; see also Guroff *et al.* 1967). It may therefore be useful to review some of the known properties of oxygen radicals in aqueous solution. We shall not be concerned with radicals co-ordinated to metal ions, though these may be of considerable biochemical importance.

Oxygen reduction can in principle occur in four single-electron steps:

The redox potentials at pH 7 for these reactions have been calculated as about -0.6, +1.1, +0.4 and +2.3V respectively (George, 1965; Mason, 1965). Thus quite strongly reducing conditions are required to add the first electron to oxygen, and the two free radicals, HO₂ and OH, are oxidizing agents, the latter a powerful one. There is also the possibility of oxygen atoms being formed in the reduction, by the O-O bond breaking in the second of the above reactions, to give water and O, instead of H₂O₂ (George, 1965).

Quite a number of oxygen free radicals have been known to physical chemists for some years past, and their formation and reactions have been quite extensively studied. Radiation techniques particularly with high-energy electrons have played a major role in this work. Both HO₂ and OH can undergo acid dissociations, giving O₂⁻ and O⁻

respectively, with pK values of 4.5 and 11.9 (Schested, Rasmussen & Fricke, 1968; Rabani & Matheson, 1964). Thus at pH values of biochemical interest O₂⁻ and OH might occur, HO₂ also being a possible species in the low pH range. There appears to be no conclusive evidence for the existence of the species OH⁺ (Samuel, 1968). Radiation work has given data on the lifetimes of the oxygen radicals. Though unstable, they do have measurable lifetimes in aqueous solution at room temperature. In the absence of anything else for them to react with, two free radicals react together. The least stable of the three radicals of interest is OH, which at moderate concentrations has a half-life in the region of a few tens of microseconds only (Schested *et al.* 1968). However, in the neutral pH region both O₂⁻ and HO₂ are distinctly more stable than this, with half-lives coming up to tens of milliseconds (Czapski & Dorfman, 1964). Further, it has recently come to light that in the alkaline pH range the stability of O₂⁻ is markedly increased, e.g. it has a half-life of about 200ms at pH 10 (R. Nilsson, F. M. Pick, R. C. Bray & E. M. Fielden, unpublished work; see also Knowles, Gibson, Pick & Bray, 1969).

In general, electron paramagnetic resonance is now the method of choice for detecting and identifying free-radical species, and the electron-paramagnetic-resonance parameters in frozen aqueous solution of HO₂, O₂⁻ and OH are all known (Wyard, Smith & Adrian, 1968; Knowles *et al.* 1969; Bravati, Symons, Tinling, Wardale & Williams, 1967). However, the usefulness of electron paramagnetic resonance in this field is severely limited both by instrument sensitivity, the minimum useful radical concentration being in the region of 10 μM, and by lack of time-resolution, since it usually takes several minutes to obtain an electron-paramagnetic-resonance spectrum.

It may not be surprising that so far, apparently, only the most stable oxygen radical, O₂⁻ on the alkaline side of neutrality, has conclusively been identified by electron paramagnetic resonance in a biochemical system. O₂⁻ was observed by Knowles

et al. (1969) during xanthine oxidase-catalysed oxidation of substrates by oxygen at pH 10. They employed a special method to freeze the reaction solution within a few milliseconds and to cool it to temperatures (below -80°C) at which the radical is stable. Work with less direct methods had earlier indicated that O_2^- might be formed by xanthine oxidase (e.g. Fridovich & Handler, 1958; Totter, Dugros & Riviero, 1960). From the results of Komai, Massey & Palmer, (1969) it seems clear that it is the flavin of the reduced enzyme molecule that reacts with oxygen in producing this radical. Indeed, this group has recently shown that reduced free flavins also give O_2^- (Ballou, Palmer & Massey, (1969). O_2^- has also been detected in the oxidation of reduced ferredoxin from *Clostridium*, but not of that from spinach (Nilsson, Pick & Bray, 1969; Orme-Johnson & Beinert, 1969). Finally there is now electron-paramagnetic-resonance evidence, both direct and indirect, for the participation of O_2^- in the oxidase action of peroxidase (Nilsson *et al.* 1969; see also Ohnishi, Yamazaki, Iyanagi, Nakamura & Yamazaki, 1969). The radical apparently arises here from reaction of a substrate radical with an oxygen molecule. It may be reasonable to generalize that biochemical generation of O_2^- depends on low redox potentials rather than on specific enzyme structures. O_2^- itself seems to be unreactive and its fate is probably generally disproportionation to H_2O_2 and O_2 (cf. Nilsson *et al.* 1969).

OH is the active hydroxylating species in Fenton's reagent (Norman & Lindsay-Smith, 1965). However, it appears that any direct evidence on possible OH involvement in normal biological systems will have to wait for new methods to become available.

- Ballou, D., Palmer, G. & Massey, V. (1969). *Biochem. biophys. Res. Commun.* **36**, 898.
 Bravati, J. A., Symons, M. C. R., Tinling, D. J. A., Wardale, H. W. & Williams, D. O. (1967). *Trans. Faraday Soc.* **63**, 2112.
 Czapski, G. & Dorfman, L. M. (1964). *J. phys. Chem., Ithaca*, **68**, 1169.
 Fridovich, I. & Handler, P. (1958). *J. biol. Chem.* **233**, 1581.
 George, P. (1965). In *Oxidases and Related Redox Systems*, vol. 1, p. 3. Ed. by King, T. E., Mason, H. S. & Morrison, M. New York: John Wiley and Sons Inc.
 Guroff, G., Daly, J. W., Jerina, D. M., Renson, J., Witkop, B. & Udenfriend, S. (1967). *Science, N.Y.* **157**, 1524.
 Knowles, P. F., Gibson, J. F., Pick, F. M. & Bray, R. C. (1969). *Biochem. J.* **111**, 53.
 Komai, H., Massey, V. & Palmer, G. (1969). *J. biol. Chem.* **244**, 1692.
 Mason, H. S. (1965). *A. Rev. Biochem.* **34**, 595.
 Nilsson, R., Pick, F. M. & Bray, R. C. (1969). *Biochim. biophys. Acta*, **192**, 145.
 Norman, R. O. C. & Lindsay-Smith, J. R. (1965). In *Oxidases and Related Redox Systems*, vol. 1, p. 131. Ed. by King, T. E., Mason, H. S. & Morrison, M. New York: John Wiley and Sons Inc.

- Norman, R. O. C. & Radda, G. K. (1962). *Chem. Commun.* p. 138.
 Ohnishi, T., Yamazaki, H., Iyanagi, T., Nakamura, T. & Yamazaki, I. (1969). *Biochim. biophys. Acta*, **172**, 357.
 Orme-Johnson, W. H. & Beinert, H. (1969). *Biochem. biophys. Res. Commun.* **36**, 905.
 Rabani, J. & Matheson, M. S. (1964). *J. Am. chem. Soc.* **86**, 3175.
 Samuel, D. (1968). In *Biochimie des Sauerstoffs*, p. 6. Ed. by Hess, B. & Staudiner, H. Berlin: Springer-Verlag.
 Schested, K., Rasmussen, O. L. & Fricke, H. (1968). *J. phys. Chem., Ithaca*, **72**, 626.
 Staudinger, H. & Ullrich, V. (1964). *Z. Naturf.* **19B**, 877.
 Totter, J. R., Dugros, E. C. & Riviero, C. (1960). *J. biol. Chem.* **235**, 1839.
 Wyard, S. J., Smith, R. C. & Adrian, F. J. (1968). *J. chem. Phys.* **49**, 2780.
 Yamazaki, I. & Piette, L. H. (1963). *Biochim. biophys. Acta*, **77**, 47.

Initial Steps in Oxygen Interaction with Protein-Bound Metals

By R. J. P. WILLIAMS. (*Inorganic Chemistry Laboratory, University of Oxford, Oxford OX1 3QR, U.K.*)

In biological systems oxygen usually reacts with metal complexes. Either a transportable (storable) form of oxygen or a reactive intermediate is produced. I shall discuss the nature of the bound oxygen in such complexes, drawing heavily upon model reactions. In the first place two very different cases must be distinguished: one-metal centres and two-metal centres.

Single-metal centres. The obvious biological examples of this case are haemoglobin and certain oxidases. Before turning to them I wish to describe model systems that we have examined. The models are vitamin B_{12} and cobalt(II) porphyrin. In both cases Bayston, King, Looney & Winfield (1969) and ourselves observe that reaction with oxygen leads to the $\text{O}_2^{\cdot-}$ superoxide anion. This can be demonstrated by electron-paramagnetic-resonance measurements. The results show that the single unpaired electron is about 20% on the cobalt. Turning to the biological materials we cannot expect to see the $\text{O}_2^{\cdot-}$ by electron-paramagnetic resonance in the presence of iron(III). We must rely instead on absorption and Mössbauer spectra to give us the electron distribution. The absorption bands of the O_2 complex of myoglobin and haemoglobin are very like those of the CO, NO and RNC complexes of low-spin Fe(II) haem except for a new band at 1000 nm. This could well mean that the ground state is Fe(II)O_2 but that $\text{Fe(III)O}_2^{\cdot-}$ lies very close to it. The Mössbauer spectrum shows a very high quadrupole splitting, and such a spectrum could arise if these two states are mixed in the ground state. [The formation of $\text{M(III)O}_2^{\cdot-}$ from M(II)O_2 is energetically much