At the present stage it appears that the principal, if not the only, role of coenzyme B_{12} is to serve as the precursor for an organic free radical which triggers the substrate rearrangement. This role implies and utilizes a very weak Co–alkyl bond, susceptible to homolytic cleavage under mild conditions, for which there has until recently been no convincing evidence or precedent.

We have developed the first reliable systematic methods for the determination of transition metal–alkyl bond dissociation energies (BDE) and have applied these to the determination of such BDE's in a variety of organocobalt compounds (notably containing dioxime or Schiff base ligands) related to coenzyme B_{12}. The Co–C BDE's yielded by such measurements are in the range 15–25 kcal/mol, *i.e.*, low enough to be consistent with the proposed role of coenzyme B_{12} depicted in Eqn. (1). The influences of electronic and steric factors on Co–C bond dissociation energies, revealed by these studies, are such as to suggest that coenzyme B_{12} is an ideal candidate for such a role and that steric factors probably are responsible for the enzyme-induced Co–C bond weakening and dissociation.

An analogy is developed between the role of coenzyme B_{12} in biological systems as a reversible 'free radical carrier' (*i.e.*, according to Eqn. (1)) and the role of hemes as reversible dioxygen carriers, *i.e.*,

$$[Fe^{III}–O_2^-] \rightleftarrows [Fe^{II}] + O_2 \quad (4)$$

A3

Atomic Structures of Oxymyoglobin and Oxyhaemoglobin and the Cooperative Mechanism of Oxygen Binding

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Until very recently, it had not been possible to determine the structures of oxymyoglobin and oxyhaemoglobin, because the crystals autoxidized. My colleagues S. E. V. Phillips and B. Shaanan have been able to overcome this problem. Phillips did an X-ray analysis of sperm whale oxymyoglobin at 1.6 Å resolution, followed by a neutron diffraction analysis at 1.7 Å resolution in collaboration with B. Schoenborn. Shaanan solved the structure of human oxyhaemoglobin at 2.1 Å resolution. These analyses have provided firm stereochemical data about the conformation of the haem and the geometry of the Fe–O_2 bond. They have also revealed the role of the distal histidine in discriminating between O_2 and CO.

Bent geometry for the Fe–O_2 bond with an Fe–O–O angle of 114° was predicted by Pauling. This prediction was confirmed by X-ray studies of J. P. Collman's oxygenated picket fence complexes, except that the Fe–O–O angle there was 130°. Intuitively, one would have expected that angle to be determined by the nature of the Fe–O_2 bond, and to remain the same in all oxygenated haem derivatives, but this is not true. The Fe–O–O angle is 115(±5)° in oxymyoglobin and 156(±10)° in the α and β oxyhaemoglobin. The difference is due to the different constraints imposed by the distal residues in the haem pocket (His E7, Val E11 and Phe CD1).

Collman's picket fence complexed with dimethylimidazole has a partition coefficient between O_2 and CO of 4280; the same complex with a covalently attached imidazole has a partition coefficient of 26,600. By contrast, the partition coefficients of myoglobin and haemoglobin are 150 and 250 respectively. If they were as high as in the picket fence complex, respiratory transport by haem proteins would not be possible, since CO is produced endogenously in the breakdown of porphyrin (one mol CO per mol porphyrin). How do the two proteins discriminate between O_2 and CO? The electronic structures of the two ligands ensure that O_2 binds preferentially in the bent conformation, while CO prefers to bind linearly with Fe–C–O on the haem axis. The distal pockets in the myoglobin and haemoglobin are tailored so as to fit the bent oxygen, but to oppose the binding of the linear CO which is forced off the haem axis by steric hindrance. This appears to be one of the discriminating devices. The other consists of hydrogen bonding by the distal histidine. Solvent effects and spectroscopic evidence suggested that the Fe–O_2 bond is polar, with transfer of negative charge from the iron to the oxygen, while the Fe–CO bond is purely covalent. Pauling first suggested that the distal histidine could form a hydrogen bond to the terminal oxygen which carries a formal negative charge in his view of the FeO_2 complex. Evidence suggesting such a bond comes from electron paramagnetic resonance and oxygen affinity data on cobalt-substituted haemoglobins and myoglobins. Phillips and Schoenborn have now proved the existence of that hydrogen bond by a neutron diffraction analysis of oxymyoglobin. In D_2O at pH 8.4 the distal histidine carries one exchangeable deuteron which can bind either to N_ϵ facing the bound haem ligand or to N_δ, facing the external solvent. The neutron maps showed that in oxymyoglobin the deuteron is on N_ϵ, forming a hydrogen bond with the bound oxygen, while in carbonmonoxymyoglobin it is on N_δ, facing the solvent, and the histidine is further removed from the ligand. This result confirms the polar character of the FeO_2 bond and the non-polar character of the Fe–CO bond. It also tells us that nature employs not one but two methods to discriminate between the two ligands.

Eisenberger *et al.* used extended X-ray absorption fine structure (EXAFS) to measure the Fe–N distance in deoxyhaemoglobin and concluded that the irons lie only $0.2^{+0.1}_{0.2}$ Å from the plane of the porphyrin nitrogens and that the cooperative mechanism I had proposed is therefore invalid. We have now compared the EXAFS of deoxyhaemoglobin with that of the ferrous 'picket fence' 2-methylimidazole complex in which the displacement of the iron from the plane of the porphyrin nitrogens is known to be 0.399 ± 0.004 and 0.426 ± 0.004 Å from the mean porphyrin plane. The two EXAFS spectra are very similar, consistent with similar displacements of the irons. We find the same Fe–N distance of 2.06 ± 0.01 Å in deoxyhaemoglobin as Eisenberger *et al.*, but show that the displacement of the iron cannot be calculated from that distance.

A4

Electronic and Geometric Structure-Function Correlations of the Coupled Binuclear Copper Active Site

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A coupled binuclear copper active site exists in a variety of proteins and enzymes which perform different biological functions. These metalloproteins are listed in Table I. The hemocyanins are highly aggregated molecules whose sub-units exhibit significant intersite interactions, while the multicopper oxidases contain additional coppers which are involved in interactions with the coupled binuclear copper site. The unique spectral features associated with these binuclear copper active sites and their variation in the proteins of Table I provide an important opportunity for structure-function correlations in Bioinorganic Chemistry.

TABLE I. Coupled Binuclear Copper Proteins.

Hemocyanin	Arthropod	deoxy + $O_2 \rightleftarrows$ oxy
	Mollusc	deoxy + $O_2 \rightleftarrows$ oxy
		$2H_2O_2 \rightleftarrows O_2 + 2H_2O$
Tyrosinase		deoxy + $O_2 \rightleftarrows$ oxy
		$2H_2O_2 \rightleftarrows O_2 + 2H_2O$
		phenol + $2e^- + O_2 + 2H^+ \rightleftarrows$ *o*-diphenol + H_2O
		2*o*-diphenol + $O_2 \rightleftarrows$ 2*o*-quinone + $2H_2O$
Laccase, Ceruloplasmin, Ascorbic acid Oxidase		$4AH + O_2 \rightarrow 4A + 2H_2O$

A variety of different spectroscopic and magnetic techniques have been used to probe the unique electronic structure of the coupled binuclear copper active site. However, in order for results from these studies to be meaningful, the system must be subjected to perturbations and the spectral responses systematically investigated. In studying active sites in bioinorganic chemistry, not only can physical perturbations such as magnetic fields (Zeeman effect) or electric fields (Stark effect) be applied, but one also has the ability to chemically perturb the system. For the coupled binuclear copper active site, a series of protein derivatives has been prepared which allows the site to be systematically varied. These are summarized in Fig. 1.

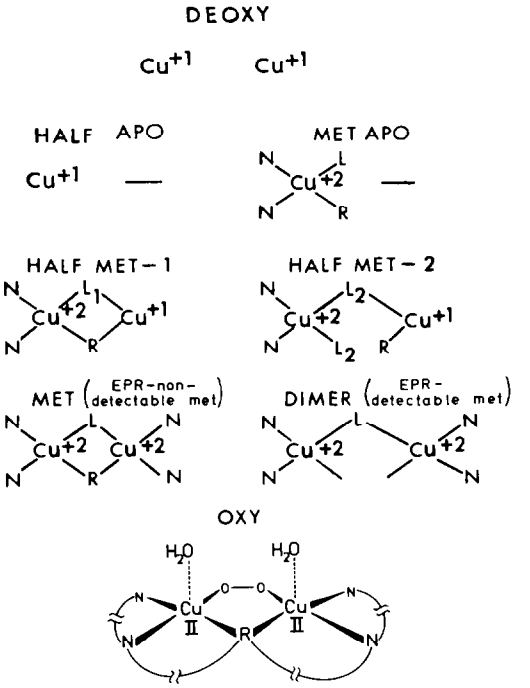

Fig. 1. Coupled binuclear copper active site derivatives.

Detailed chemical and spectroscopic studies of these derivatives of the hemocyanin site have led to the 'spectroscopically effective' model for the coupled binuclear copper active site shown at the bottom of Fig. 1. A comparison of the half met and met apo derivatives indicates the presence of class 2 mixed valence behavior which correlates with the exogenous bridging ligand providing the pathway for electron delocalization. The met and dimer derivatives both contain formally binuclear cupric active sites, yet met (like oxy) exhibits no EPR signal. This has been related to the presence of an endogenous protein bridge (RO^-) which provides a superexchange pathway for antiferromagnetic coupling between the coppers. When the endogenous bridge is broken, either by a group 2 ligand maintaining the coppers