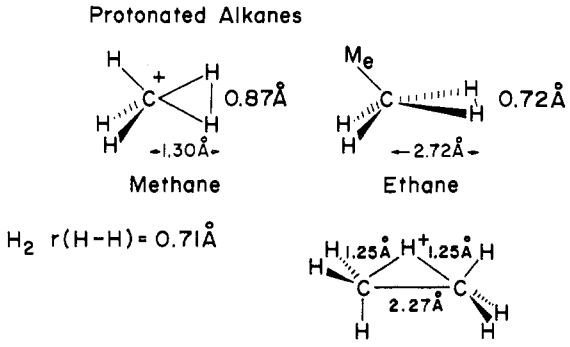
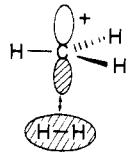


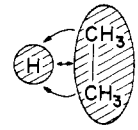
Figure 8. STO-3G geometries of protonated methane and ethane.

theoretical (STO-3G) structure for CH_5^+[8a,9a] looks very much like a loose complex between the methyl cation and H_2 (Figure 8). More extensive calcula-



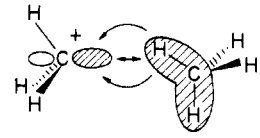
tions, including those in which partial account is taken for valence-shell correlation energy, favor a like structure.[29] One of the possible structures for protonated ethane[8a,b] shows even a more marked resemblance to an intermolecular addition complex involving the hydrogen molecule, in this situation the open ethyl cation providing the acceptor site.

The theory's choice for the ground-state structure of $C_2H_7^+$ (11 kcal/mol more stable than the hydrogen–ethyl cation complex at the STO-3G level)[8a,9b] may be considered to result from the interaction of a bare proton and the carbon–carbon σ-bonding orbital of ethane. Electron donation from σ to the proton



should lead to a significant lengthening of the C–C linkage, as indeed is clearly depicted by the theoretical geometrical structure of the ion[30] (Figure 8).

In principle, CH bonds might also be asked to assume the role of electron donors, leading to the suggestion of a possible asymmetric structure for protonated ethane (corresponding to an interaction between methane and the methyl cation).[31] While no



such geometrical minimum has been located on the $C_2H_7^+$ potential surface, one structure found for protonated propane does indeed correspond to complex formation involving the bonding σ orbital of a carbon–hydrogen linkage.

The variety of examples which we have presented have been directed at demonstrating the utility of simple arguments, based on the perturbation treatment of interacting orbitals, in anticipating the detailed findings of the quantitative ab initio calculations. Together, these two techniques provide a powerful, and yet intelligent, means of elucidating the structure and conformation of reactive carbocation intermediates.

(29) V. Dyczmons and W. Kutzelnigg, *Theor. Chim. Acta*, **33**, 239 (1974).

(30) At the extended basis 4-31G level this structure appears to relapse to a higher symmetry D_{3d} geometry (the same symmetry as ethane itself), corresponding to protonation at the center of the carbon–carbon bond. See ref 9b for a discussion.

(31) L. Radom, L. A. Curtiss, J. A. Pople, and P. v. R. Schleyer, to be submitted.

Mechanism of Cobalamin-Dependent Rearrangements

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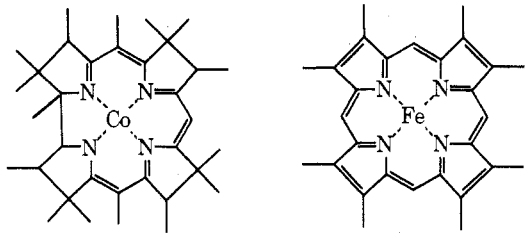
The corrins are an extensive family of compounds among which are included a unique group of cobalt-containing organometallic reagents of great biological significance. These compounds were found at the end of a long search for the agent that cured pernicious anemia, a disease which, before the discovery of corrins, was almost always fatal.

Bernard M. Babior received an M.D. from the University of California School of Medicine, San Francisco, in 1959, and a Ph.D. in biochemistry from Harvard University in 1965. After postdoctoral training he joined the faculty of Harvard Medical School. In 1972 he moved to Tufts Medical School where he is currently Associate Professor of Medicine. He studies the biochemistry of corrinoids and the physiology and biochemistry of white blood cells.

In 1922, Minot and Murphy found that pernicious anemia could be cured by liver fed in very large quantities.[1] Twenty-six years later, the isolation of the therapeutic principle in liver was announced independently by Smith[2] and by Folkers.[3] The substance they isolated was a beautiful red crystalline compound that prevented pernicious anemia at doses of only 1–2 μg per day. Over the next several years partial structures of this compound, which was given the names vitamin B_{12} and cobalamin, were de-

(1) G. R. Minot and W. P. Murphy, *J. Am. Med. Assoc.*, **87**, 470 (1926).
(2) E. L. Smith and L. F. J. Parker, *Biochem. J.*, **43**, VIII (1948).
(3) E. L. Rickes, N. G. Brink, F. R. Koniuszy, T. R. Wood, and K. Folkers, *Science*, **107**, 396 (1948).



Corrin Porphyrin

Figure 1. Corrin and porphyrin. The lines extending from the periphery of the macrocycles represent alkyl or acyl substituent groups. Strictly speaking, the terms "porphyrin" and "corrin" refer to the metal-free macrocycles. For purposes of emphasis, however, the structures are shown containing metals usually associated with them under biological conditions.

scribed, and cobalt was identified as a constituent. The complete structure was not unravelled until 1958, however, when X-ray crystallography in Hodgkin's laboratory[4] revealed the compound to be a tetrapyrrole macrocycle structurally related to the then well-known porphyrins. The cobalt atom lay in the center of the macrocycle, coordinated to the four pyrrole nitrogens.

While the early chemical studies were being carried out, investigations were simultaneously under way to find a specific biological function for the corrinoids. Such a function was first demonstrated by Barker and his associates, who found a corrinoid requirement for an enzyme-catalyzed interconversion of glutamic and β-methylaspartic acids.[5] This reaction turned out to be the prototype for the cobalamin-dependent rearrangements, which are the subject of this Account.

At the time of Barker's discovery, structural studies had indicated that cyanide was coordinated to the corrinoid cobalt atom in one of the two positions not occupied by the pyrrole nitrogens of the macrocycle. Barker's investigations, however, revealed that in the biologically active corrinoid the cyanide was replaced by an adenine nucleoside fragment. X-Ray crystallography, again performed in Hodgkin's laboratory, showed this fragment to be the 5′-deoxyadenosyl residue, attached to the corrinoid by a σ bond between its 5′-carbon atom and the cobalt.[6]

Co-Adenosylcorrinoids serve as cofactors in two types of reactions: rearrangements of a characteristic type, and the reduction of ribonucleotides to deoxyribonucleotides by certain single-celled organisms.[7] The discussion to follow will be concerned primarily with the mechanism of action of adenosylcorrinoids, as illustrated by their role in the corrinoid-dependent rearrangements. In the discussion, adenosylcobalamin will be chosen as representative, since most of the biochemical studies have been carried out with this compound.

Chemistry of Cobalamin

Structure. The basic structural unit of the corrins is the tetrapyrrole macrocycle. In this respect corrins

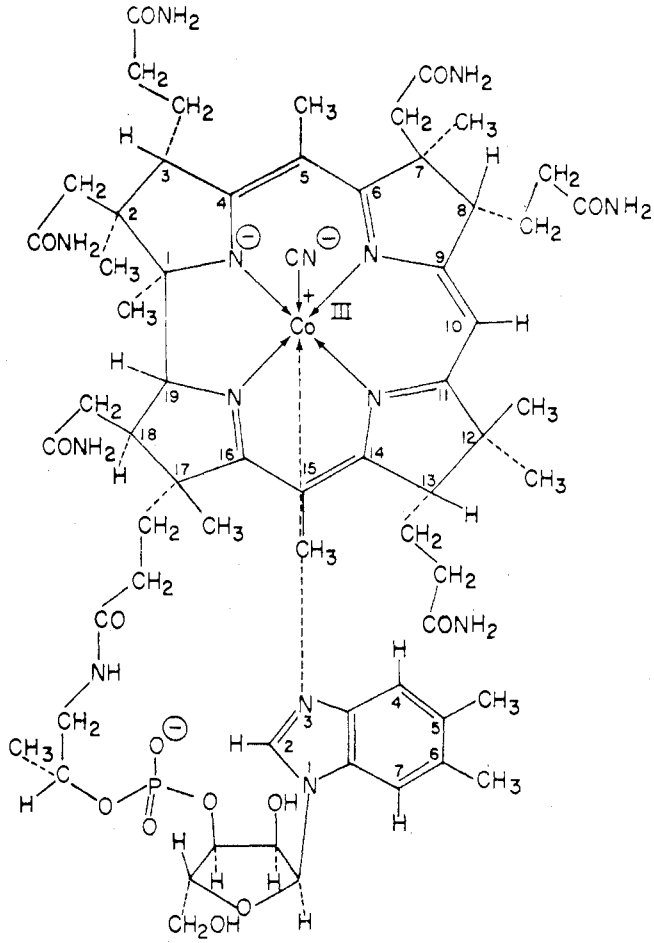


Figure 2. Cyanocobalamin (from J. M. Pratt, "Inorganic Chemistry of Vitamin B_{12}" Academic Press, New York, N.Y., 1972, p 2; reproduced with permission of the author and Academic Press).

resemble porphyrins. A closer look, however, reveals that the differences between corrins and porphyrins are as important as the similarities (Figure 1). (a) The biologically important porphyrins contain iron or magnesium; the corrins, cobalt. (b) The porphyrins possess four methine (methylidyne) bridges, one between each adjacent pair of pyrrole rings. In the corrins, one of the methine bridges is missing, so that two of the pyrrole rings are joined directly by a bond between their α carbons. (c) The porphyrins are fully unsaturated and aromatic, while the corrins are extensively reduced. The unsaturated portion of the corrins consists of a chain of six double bonds involving the nitrogens and α carbons of the pyrrole rings which partially encircles the cobalt.

What distinguishes cobalamins from other corrins[8] is the pattern of substitution of the carboxyl groups at the periphery of the ring (Figure 2). All seven are amidated, six with NH_3 and the seventh with (*R*)-2-aminopropanol. Attached to the propanolamine through a phosphodiester linkage is an unusual ribonucleoside which in cobalamins contains 5,6-dimethylbenzimidazole as the heterocyclic base. At neutral pH this base is coordinated to the α position of the cobalt through its free nitrogen atom. Under sufficiently acid conditions, however, the base will protonate and leave the cobalt coordination sphere, to be replaced by water or another suitable ligand.

(4) D. C. Hodgkin, *Science*, **150**, 979 (1965).
(5) H. A. Barker, H. Weissbach, and R. D. Smyth, *Proc. Natl. Acad. Sci. U.S.A.*, **44**, 1093 (1958).
(6) P. G. Lenhert and D. C. Hodgkin, *Nature (London)*, **192**, 937 (1961).
(7) F. K. Gleason and H. P. C. Hogenkamp, *Biochim. Biophys. Acta*, **277**, 466 (1972).
(8) IUPAC–IUB Commission on Biochemical Nomenclature, *Biochemistry*, **13**, 1555 (1974).

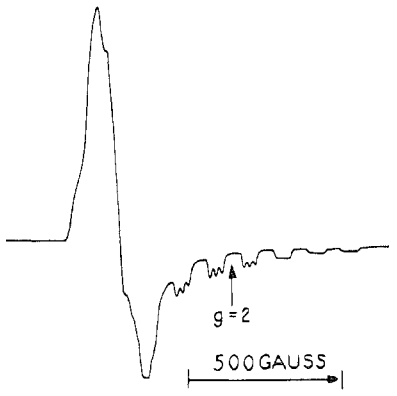


Figure 3. ESR spectrum of cob(II)alamin[12] (reproduced with permission of the author and The American Society of Biological Chemists, Inc.)

The cobalamins themselves vary in the ligand coordinated to the cobalt in the β position. This ligand may be a nucleophile, or it may be an alkyl group connected to cobalamin by a carbon–cobalt σ bond. Though a great many cobalamins with varying Coβ ligands have been prepared and studied, only four are regularly isolated from living systems: hydroxocobalamin,[9] cyanocobalamin, and the *Co*-alkyl derivatives, methyl- and adenosylcobalamin. The two naturally occurring alkyl derivatives are both active as coenzymes, though the reactions for which methylcobalamin is required are different than those requiring adenosylcobalamin; neither hydroxocobalamin nor cyanocobalamin have any cofactor activity.

Reactions. The portion of the cobalamin molecule of major biochemical interest is the cobalt atom and its β ligand. The following brief discussion will deal with aspects of the chemistry of this region of the molecule that are particularly pertinent to the role of the cofactor in the catalysis of adenosylcobalamin-dependent rearrangements.

Redox Properties of Cobalamin. The naturally occurring cobalamins discussed above all contain trivalent cobalt.[10] Under appropriate conditions the metal can be reduced to a lower valence state. Reduction by one electron produces cob(II)alamin, a paramagnetic compound containing low-spin divalent cobalt.[11] Cob(II)alamin gives a characteristic ESR signal[12] showing hyperfine coupling between the unpaired electron and the cobalt nucleus ($I = 7/2$) (Figure 3). It is indefinitely stable in aqueous solution under anaerobic conditions, but oxidizes slowly back to hydroxocobalamin in air.[13]

A further one-electron reduction yields cob(I)alamin, containing monovalent cobalt.[11a] Unlike cob(II)alamin, which is relatively stable, cob(I)alamin is extremely reactive. It is readily oxidized by agents as weak as H^+, so that it is unstable in aqueous solution,[14] giving up an electron to form cob(II)alamin with the evolution of H_2 gas. It is also extremely nucleophilic,[15] a property that is illustrated by its use in the synthesis of alkyl cobalamins from alkyl halides:

$$\text{cob(I)alamin} + CH_3I \longrightarrow \text{methylcobalamin} + I^-$$

Alkylcobalamins. Alkylcobalamins are compounds containing an alkyl group covalently attached to the metal by a carbon–cobalt σ bond. They are usually prepared by reacting cob(I)alamin with an organic compound substituted with a nucleophilic leaving group. The formation of methylcobalamin from cob(I)alamin and methyl iodide, illustrated above, is an example. Co(II)- and Co(III)-containing corrinoids have also been used on occasion for the alkylation reaction.[16,17]

The outstanding chemical property of alkylcobalamins is their susceptibility to photolysis. Illumination of a solution of alkylcobalamin leads to homolysis of the carbon–cobalt bond[18] (but see ref 19). Subsequent reactions of the immediate products of photolysis with other components of the reaction mixture determine the identity of the final products, which consequently vary from case to case.

$$\text{alkylcobalamin} \xrightarrow{h\nu} R\cdot + \text{cob(II)alamin} \longrightarrow \text{products}$$

In general, alkylcobalamins are stable to air and water. Certain of them, however, are susceptible to acid- or base-catalyzed heterolysis of the carbon–cobalt bond. In particular, adenosylcobalamin is cleaved by both acid and base.[20] In acid, the products are hydroxocobalamin, adenine, and 2,3-dihydroxy-4-pentenal,[20a] while base heterolysis yields cob(I)alamin and 4′,5′-anhydroadenosine.[20b]

Although the heterolytic reactions of adenosylcobalamin have been well characterized in chemical systems, the evidence obtained to date in enzymatic systems points to homolytic cleavage as the biologically significant process.

Adenosylcobalamin-Dependent Rearrangements

The rearrangements catalyzed by adenosylcobalamin-dependent enzymes all involve the migration of a hydrogen from one carbon atom to an adjacent one in exchange for a group X that migrates in the opposite direction. Nine such rearrangements have so far been discovered[21] (Table I). In three of these rear-

(9) Known as aquacobalamin when the Coβ-hydroxo group is protonated. In this paper, hydroxocobalamin will be used to refer to both forms.

(10) G. Boehm, A. Faessler, and G. Rittmayer, *Z. Naturforsch., Teil B*, **9**, 509 (1954).

(11) (a) B. Jaselkis and H. Diehl, *J. Am. Chem. Soc.*, **76**, 4354 (1954); (b) S. A. Cockle, H. A. O. Hill, J. M. Pratt, and R. J. P. Williams, *Biochim. Biophys. Acta*, **177**, 686 (1969).

(12) J. H. Bayston, F. D. Looney, L. R. Pilbrow, and M. E. Winfield, *Biochemistry*, **9**, 2164 (1970).

(13) J. M. Pratt, *J. Chem. Soc.*, 5154 (1964).

(14) S. L. Tackett, J. W. Collat, and J. C. Abbott, *Biochemistry*, **2**, 919 (1963).

(15) (a) A. W. Johnson, L. Mervyn, N. Shaw, and E. L. Smith, *J. Chem. Soc.*, 4146 (1963); (b) G. N. Schrauzer and E. J. Deutsch, *J. Am. Chem. Soc.*, **91**, 3341 (1969).

(16) G. N. Schrauzer, J. W. Sibert, and R. J. Windgassen, *J. Am. Chem. Soc.*, **90**, 6681 (1968).

(17) F. Wagner and K. Bernhauer, *Ann. N.Y. Acad. Sci.*, **112**, 580 (1964).

(18) (a) R. T. Taylor, L. Smucker, M. L. Hanna, and J. Gill, *Arch. Biochem. Biophys.*, **156**, 521 (1973); (b) H. P. C. Hogenkamp, *Biochemistry*, **5**, 417 (1966); (c) K. N. Joblin, A. W. Johnson, M. F. Lappert, and B. K. Nicholson, *J. Chem. Soc., Chem. Commun.*, 441 (1975).

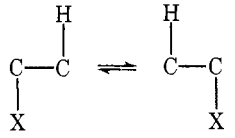
(19) Under certain conditions, corrins (and related cobalt complexes) in which the metal is alkylated with a group bearing a hydrogen on the β carbon will photolyze to cob(I)alamin and a terminal olefin. This process has been explained in terms of concerted photoelimination (K. N. V. Duong, A. Ahond, C. Merienne, and A. Gaudemer, *J. Organomet. Chem.*, **55**, 375 (1973)), but other work suggests a two-step mechanism in which homolysis by light is followed by the transfer of a β hydrogen from the alkyl radical to cob(II)alamin to yield the final products (R. H. Yamada, S. Shimizu, and S. Fukui, *Biochim. Biophys. Acta*, **124**, 197 (1966)).

(20) (a) H. P. C. Hogenkamp and H. A. Barker, *J. Biol. Chem.*, **236**, 3079 (1961); (b) G. N. Schrauzer and J. W. Sibert, *J. Am. Chem. Soc.*, **92**, 1022 (1970).

(21) (a) H. A. Barker, V. Rooze, F. Suzuki, and A. A. Iodice, *J. Biol. Chem.*, **239**, 3260 (1964); (b) H. G. Wood, R. W. Kellermeyer, R. Stjernholm,

Table I
Adenosylcobalamin-Dependent Rearrangements

Enzyme	Reaction	Group X
Glutamate mutase[21a]	L-Glutamate ⇌ L-*threo*-β-methylaspartate	$-CH(NH_2)COOH$
Methylmalonyl-CoA mutase[21b]	L-Methylmalonyl-CoA ⇌ Succinyl-CoA	$-COSCoA$
2-Methyleneglutarate mutase[21c]	2-Methyleneglutarate ⇌ Methyl itaconate	$-C(=CH_2)COOH$
Diol dehydrase[21d]	Ethylene glycol ⟶ acetaldehyde 1,2-Propanediol ⟶ propionaldehyde	$-OH$
Glycerol dehydrase[21e]	Glycerol ⟶ β-hydroxypropionaldehyde	$-OH$
Ethanolamine ammonia-lyase[21f,g]	Ethanolamine ⟶ acetaldehyde + NH_4^+ 2-Aminopropanol ⇌ propionaldehyde + NH_4^+	$-NH_2$
β-Lysine mutase[21h]	L-β-Lysine (L-3,6-diaminohexanoic acid) ⇌ 3,5-diaminohexanoic acid	$-NH_2$
α-Lysine mutase[21i]	D-Lysine ⇌ 2,5-diaminohexanoic acid	$-NH_2$
Ornithine mutase[21j]	D-Ornithine ⇌ 2,4-diaminopentanoic acid	$-NH_2$



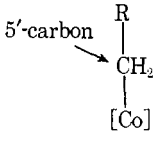
rangements, the migrating group is a bulky alkyl or acyl residue. In the remaining six, the reaction involves the migration of an electronegative group, either OH or NH_2.

These reactions have been studied from a number of points of view. Particular attention has been paid to their stereochemical features and to the structures of the enzymes responsible for their catalysis. These topics are beyond the scope of the present Account, which will be restricted to a discussion of the mechanism of the rearrangements.

In considering the mechanism of adenosylcobalamin-dependent rearrangements, it is useful to separate the hydrogen-transfer step from the group X migration. In the following discussion, the mechanism of hydrogen transfer will be treated in some detail, since this step is reasonably well understood. Group X migration, about which much less is known, will be discussed primarily in terms of model reactions, since there is little pertinent enzymological data regarding this step.

Hydrogen Transfer. In the initial studies on the mechanism of hydrogen transfer, the fate of the migrating hydrogen atom was examined using deuterated substrate. These studies showed that all of the label appeared in the expected location in the product, a result taken to indicate that the migration of hydrogen was intramolecular. In fact, this was an erroneous conclusion, and the experiment that revealed the error provided the key to the mechanism of hydrogen transfer. This experiment,[22] performed in Abeles' laboratory, took advantage of the fact that diol dehydrase converts both ethylene glycol and propylene glycol to the respective aldehydes. When the enzyme was allowed to act on a mixture of unlabeled ethylene glycol and [1,1-^{2}H]propylene glycol, deuterated acetaldehyde was obtained. This could only have arisen by the transfer of a deuterium from one molecule to another. Thus, it was shown that hydrogen transfer was not necessarily *intra*molecular, as previously thought, but could occur by an *inter*molecular process as well.

From this observation, Abeles postulated that the enzyme–adenosylcobalamin complex serves as an intermediate hydrogen carrier, first accepting a hydrogen from the substrate and then, in a subsequent step, giving a hydrogen back to the product. Experiments with tritiated substrate confirmed this formulation, showing that the migrating hydrogen atom was transferred from the substrate to the Coβ-linked carbon atom of the cofactor[23] (the C-5′ atom of the cobalt-linked adenosyl group; henceforth this will be referred to as the C-5′ position). Subsequent investi-



gations showed that other adenosylcobalamin-dependent rearrangements involve a similar transfer of hydrogen.[24]

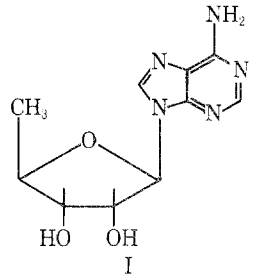
A remarkable feature of the transfer of hydrogen to and from adenosylcobalamin is that both of the C-5′ positions participate in this reaction.[23] This is surprising, because enzymes are ordinarily able to distinguish between the *pro-R* and *pro-S* positions of meso carbon atoms such as the C-5′ carbon of adenosylcobalamin. Considerable experimental evidence indicates that this unusual labeling pattern results from a process whereby the two C-5′ hydrogen atoms of the cofactor and the migrating hydrogen of the substrate all become equivalent. This process is postulated to involve cleavage of the carbon–cobalt bond of adenosylcobalamin followed by the reversible transfer of hydrogen from substrate to the C-5′ carbon to form 5′-deoxyadenosine (I). Rotation of the methyl group would lead almost instantly to equilibration of the three hydrogens.

and S. H. G. Allen, *Ann. N.Y. Acad. Sci.*, **112**, 661 (1964); (c) H. F. Kung, S. Cederbaum, L. Tsai, and T. C. Stadtman, *Proc. Natl. Acad. Sci. U.S.A.*, **65**, 978 (1970); (d) H. A. Lee, Jr., and R. H. Abeles, *J. Biol. Chem.*, **238**, 2367 (1963); (e) K. L. Smiley and M. Sobolov, *Ann. N.Y. Acad. Sci.*, **112**, 706 (1964); (f) B. H. Kaplan and E. R. Stadtman, *J. Biol. Chem.*, **243**, 1787 (1968); (g) T. J. Carty, B. M. Babior, and R. H. Abeles, *ibid.*, **249**, 1683 (1974); (h) E. E. Dekker and H. A. Barker, *ibid.*, **243**, 3232 (1968); (i) C. G. D. Morley and T. C. Stadtman, *Biochemistry*, **10**, 2325 (1971); (j) Y. Tsuda and H. C. Friedmann, *J. Biol. Chem.*, **245**, 5914 (1970).

(22) R. H. Abeles and B. Zagalak, *J. Biol. Chem.*, **241**, 1245 (1966).

(23) P. A. Frey, M. R. Essenberg, and R. H. Abeles, *J. Biol. Chem.*, **242**, 5369 (1967).

(24) (a) R. L. Switzer, B. G. Baltimore, and H. A. Barker, *J. Biol. Chem.*, **244**, 5268 (1969); (b) B. M. Babior, *Biochim. Biophys. Acta*, **167**, 456 (1968); (c) W. W. Miller and J. H. Richards, *J. Am. Chem. Soc.*, **91**, 1498 (1969); (d) H. F. Kung and L. Tsai, *J. Biol. Chem.*, **246**, 6436 (1971); (e) J. Rétey, F. Kunz, T. C. Stadtman, and D. Arigoni, *Experientia*, **25**, 801 (1969).



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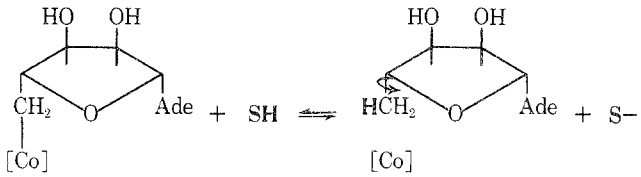
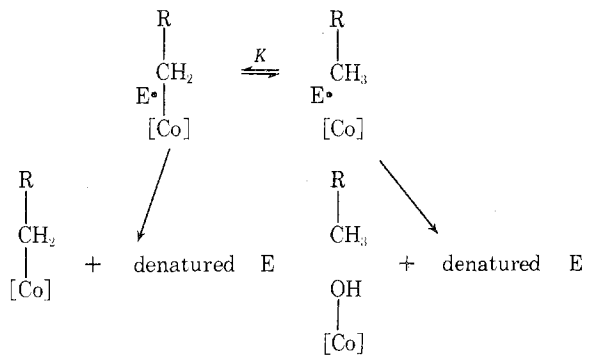


5′-Deoxyadenosine. The involvement of 5′-deoxyadenosine in adenosylcobalamin-dependent rearrangements was first proposed by Ingraham in 1964.[25] Its actual formation was shown 2 years later by Wagner et al.,[26] who found that, when diol dehydrase and adenosylcobalamin were incubated with glycolaldehyde, the cofactor was converted to 5′-deoxyadenosine and a cobalamin derivative that was not identified at the time. The extra hydrogen on 5′-deoxyadenosine was supplied by glycolaldehyde, which was oxidized to glyoxal in the reaction.

Similar reactions have since been demonstrated with several enzymes, using a variety of substrate analogs[27] (Table II). These reactions have in common the features that the *Coβ*-adenosyl residue of the cofactor is converted to 5′-deoxyadenosine and that (where investigated) the third hydrogen of the methyl group is derived from the analog. It is clear that the ability to form 5′-deoxyadenosine from adenosylcobalamin is a rather general property of enzymes catalyzing adenosylcobalamin-dependent rearrangements. The difficulty in interpreting these results in terms of a catalytic role for 5′-deoxyadenosine is that all the reactions listed in Table II are irreversible: 5′-deoxyadenosine is produced at the cost of the destruction of the cofactor. Unless it were possible to show that 5′-deoxyadenosine formation is reversible, it could be argued that this compound is not a catalytic intermediate, but is merely the product of an abortive side reaction leading to the inactivation of the enzyme–cofactor complex. Attempts to demonstrate reversibility by showing exchange of 5′-deoxyadenosine into adenosylcobalamin have failed uniformly, indicating that a reversible process involving the formation *and release* of 5′-deoxyadenosine does not take place. The experiments described below, however, have demonstrated reversible formation of 5′-deoxyadenosine by ethanolamine ammonia-lyase without release from the enzyme.

When ethanolamine ammonia-lyase was denatured during incubations with substrates (i.e., while engaged in catalysis), some of the cofactor was released as 5′-deoxyadenosine.[28] This finding was similar to results obtained with substrate analogs. With true substrates, however, 5′-deoxyadenosine formation was reversible. The first evidence for this was the finding that the fraction of total cofactor recovered as 5′-deoxyadenosine varied with the denaturing agent (Table III). Such variability could be seen if a reversible interconversion were occurring between two forms of enzyme–cofactor complex, one form carrying the cofactor as adenosylcobalamin and the other as 5′-deoxyadenosine plus a corrin fragment.



During catalysis, the relative amounts of the two forms would depend on the equilibrium constant K. Upon denaturation, however, differences in the susceptibility of the two forms to a given denaturing agent could pull the equilibrium to the left or right during denaturation. If the relative susceptibilities varied from agent to agent, the extent of cleavage in

Table II
5′-Deoxyadenosine Formation in the Presence of Substrate Analogs

Enzyme	Analog promoting 5′-deoxyadenosine formation
Diol dehydrase	Glycolaldehyde[26] Chloroacetaldehyde[27a]
Methylmalonyl-CoA mutase	Malonyl-CoA[27b]
β-Lysine mutase	L-3,5-Diaminohexanoate[27c,a]
Ethanolamine ammonia-lyase	Ethylene glycol[27d] Acetaldehyde[27e,a]

[a] Product of the catalytic reaction.

Table III
Fraction of Cofactor in the Form of 5′-Deoxyadenosine as a Function of the Denaturing Agent

Denaturing agent	Fraction as 5′-deoxyadenosine, %
Ethanolamine as Substrate	
Hot 1-propanol	3.6 ± 0.4[a]
Tetrahydrofuran	1.3 ± 0.2
Propanolamine as Substrate	
Trichloroacetic acid	86.9 ± 1.3
Heat	89.3 ± 5.3
Ethanol	12.7 ± 3.2

[a] Mean ± standard error.

(25) L. L. Ingraham, *Ann. N.Y. Acad. Sci.*, **112**, 713 (1964).
(26) O. W. Wagner, H. A. Lee, Jr., P. A. Frey, and R. H. Abeles, *J. Biol. Chem.*, **241**, 1751 (1966).
(27) (a) T. H. Finlay, J. Valinsky, K. Sato, and R. H. Abeles, *J. Biol. Chem.*, **247**, 4197 (1972); (b) B. M. Babior, A. D. Woodams, and J. D. Brodie, *ibid.*, **248**, 1445 (1973); (c) J. J. Baker, C. Van der Drift, and T. C. Stadtman, *Biochemistry*, **12**, 1054 (1973); (d) B. M. Babior, *J. Biol. Chem.*, **245**, 1755 (1970); (e) T. J. Carty, B. M. Babior, and R. H. Abeles, *J. Biol. Chem.*, **246**, 6313 (1971).
(28) (a) B. M. Babior, *J. Biol. Chem.*, **245**, 6125 (1970); (b) B. M. Babior, T. J. Carty, and R. H. Abeles, *ibid.*, **249**, 1689 (1974).

Table IV
Reversible Formation of Adenosylcobalamin

Compound	Distribution of isotope, cpm × 10^{-4}			
	Propanolamine only		Propanolamine followed by ethanolamine	
	^{3}H	^{14}C	^{3}H	^{14}C
5′-Deoxyadenosine	19.4	3.0	0.7	0.4
Adenosylcobalamin	2.0	0.6	0.6	3.1
Acetaldehyde			19.1	
Total	21.4	3.6	21.4	3.5

an incubation terminated with one agent would differ from that seen in a similar incubation terminated with another.

Assuming the above explanation to be correct, the results presented in Table III suggest that there is much less 5′-deoxyadenosine at the active site during steady-state deamination of ethanolamine than during propanolamine deamination. The best evidence for reversibility was based on this observation and consists of the finding that the addition of ethanolamine to a reaction mixture originally containing enzyme, adenosylcobalamin, and propanolamine led to a reversal of the high 5′-deoxyadenosine/adenosylcobalamin ratio prevailing during propanolamine deamination.[28b]

In the experiment, [1-^{3}H]propanolamine was incubated with [^{14}C]adenosylcobalamin and enzyme. Analysis of ^{14}C distribution in a reaction terminated at 15 sec with trichloroacetic acid showed that 80% of the cofactor had been converted to 5′-deoxyadenosine (Table IV). Moreover, both 5′-deoxyadenosine and intact adenosylcobalamin were labeled with tritium, which had been transferred to the cofactor from the substrate. The ratio of tritium specific activities in 5′-deoxyadenosine and adenosylcobalamin was that expected if the two were in equilibrium.

In an otherwise identical reaction mixture to which unlabeled ethanolamine was added at 15 sec followed by trichloroacetic acid 1 min later, the isotope distribution was very different. Analysis of ^{14}C showed that 5′-deoxyadenosine present at the time the ethanolamine was added had been converted almost completely back to adenosylcobalamin. Moreover, the tritium that was present in both adenosylcobalamin and 5′-deoxyadenosine at 15 sec was now to be found in the acetaldehyde formed by the enzyme-catalyzed deamination of unlabelled ethanolamine. It is apparent that the 5′-deoxyadenosine present on the enzyme before the addition of ethanolamine must have been converted back to catalytically competent adenosylcobalamin.

These findings show that an adenosylcobalamin-dependent enzyme can catalyze the reversible cleavage of the cofactor at the carbon–cobalt bond and provide strong, though not absolutely conclusive, evidence that 5′-deoxyadenosine is one of the products of cleavage. The residue of uncertainty regarding this point relates to the possibility that the third hydrogen atom involved in the equilibrium between the two C-5′ hydrogens and the substrate hydrogen may not be connected to the C-5′ carbon in the active enzyme but may only attach to it during denaturation. This possibility notwithstanding, reversible formation of 5′-deoxyadenosine by carbon–cobalt bond cleavage and hydrogen abstraction remains the best explanation for hydrogen transfer in adenosylcobalamin-dependent rearrangements.

Species with Unpaired Electrons. The foregoing indicates that cleavage of the carbon–cobalt bond occurs during adenosylcobalamin-dependent rearrangements. This bond can be broken in four ways: heterolytically with carbonium ion formation; heterolytically with carbanion formation; heterolytically with olefin formation;[19,20b] and homolytically. Evidence obtained by ESR spectroscopy indicates that the mechanism of these rearrangements involves homolysis of the carbon–cobalt bond with the participation of species with unpaired electrons.

Of the enzymes listed in Table I, ESR signals have been reported with three: ethanolamine ammonia-lyase, glycerol dehydrase, and diol dehydrase.[29] With all three, the signals are seen when the enzyme–cofactor complexes are frozen while engaged in catalysis (Figure 4). With diol dehydrase, signals also appear when the enzyme is incubated with cofactor in the presence of analogs that promote cleavage of the carbon–cobalt bond.[29c] Signals are seen with ethanolamine ammonia-lyase plus cofactor even in the absence of substrate or a substrate analog.[29a]

In all cases, the ESR signals consist of two components: a broad low-field peak representing enzyme-bound cob(II)alamin; and one or two narrower peaks situated upfield from the cob(II)alamin peak. The species responsible for the upfield peaks have been the subject of controversy, but in two instances they have been unequivocally identified as radicals derived from substrate or a substrate analog.[29b,30] The species represented by the high-field signal appearing during the ethanolamine ammonia-lyase catalyzed deamination of propanolamine[29] (Figure 4) was identified by ESR spectrometry of isotopically labeled substrates (Figure 5). Spectra with unlabeled and [2-^{2}H]propanolamine are essentially the same. With [1,1-^{2}H]propanolamine, however, the signal is several gauss narrower than control, while substantial broadening is seen with [1-^{13}C]propanolamine. These findings identify the species in question as the 1-hydroxy-2-aminopropyl radical. Similarly, an enzyme-bound radical derived from the substrate analog was identified as one of the products of the reaction between diol dehydrase and chloroacetaldehyde.[30]

Are these ESR-absorbing species relevant to the catalytic mechanism? Two arguments have been offered supporting their participation in catalysis. The first is that the paramagnetic species account for a major fraction (5–65%, depending on the enzyme and

(29) (a) B. M. Babior, T. H. Moss, and D. C. Gould, *J. Biol. Chem.*, 247, 4389 (1972); (b) B. M. Babior, T. H. Moss, W. H. Orme-Johnson, and H. Beinert, *ibid.*, 249, 4537 (1974); (c) T.H. Finlay, J. Valinsky, A. S. Mildvan, and R. H. Abeles, *ibid.*, 248, 1285 (1973); (d) S. A. Cockle, H. A. O. Hill, R. J. P. Williams, S. P. Davies, and M. A. Foster, *J. Am. Chem. Soc.*, 94, 275 (1972).

(30) J. E. Valinsky, R. H. Abeles, and A. S. Mildvan, *J. Biol. Chem.*, 249, 2751 (1974).

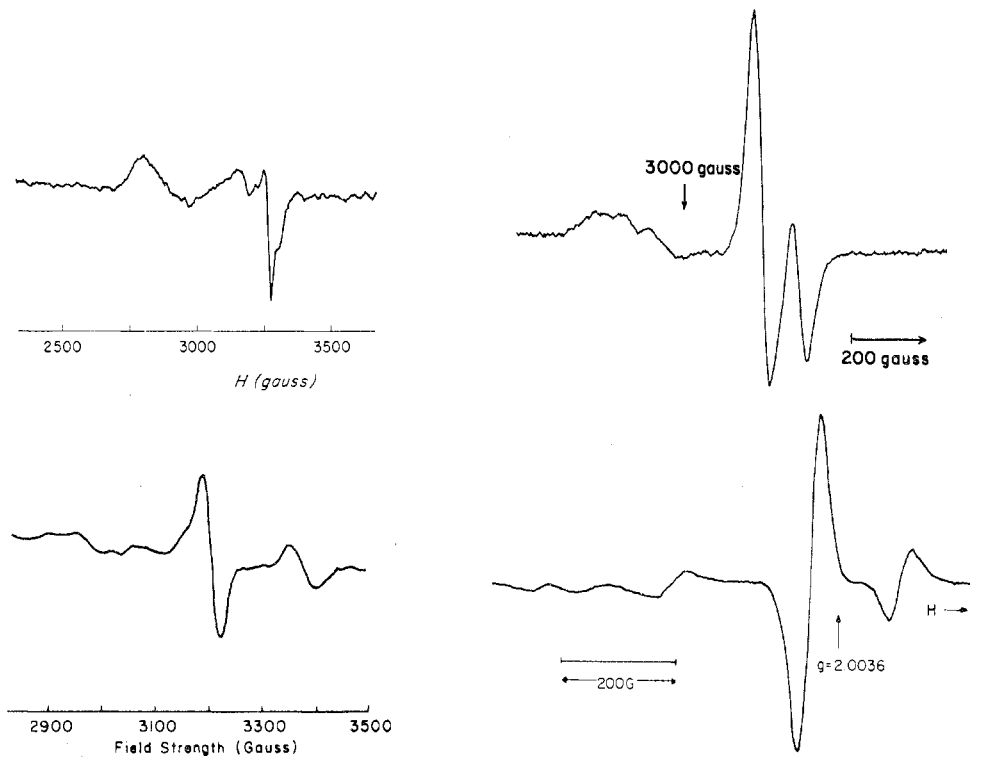


Figure 4. ESR spectra of enzymes frozen while engaged in catalysis. Top left: ethanolamine ammonia-lyase, ethanolamine.[29a] Top right: ethanolamine ammonia-lyase, propanolamine.[29b] Bottom left: diol dehydrase, propanediol.[29c] Bottom right: glycerol dehydrase, glycerol[29d] (reproduced with permission of the author and The American Society of Biological Chemists, Inc.).

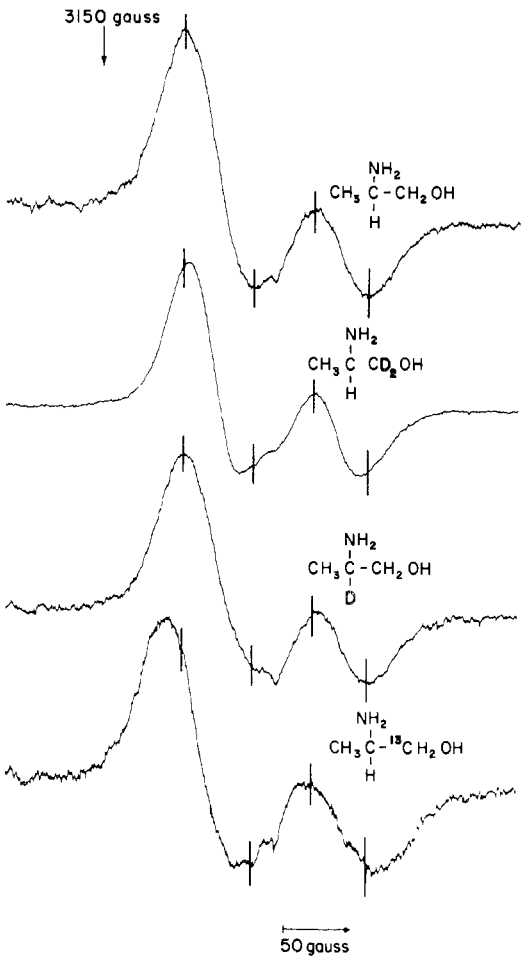
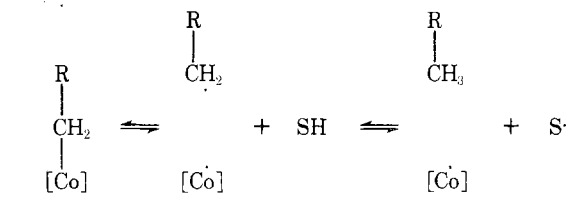


Figure 5. Identification of 1-hydroxy-2-aminopropyl as the free radical formed during the ethanolamine ammonia-lyase catalyzed deamination of propanolamine[29b] (reproduced with permission of the author and The American Society of Biological Chemists, Inc.).

substrate) of the active sites present in a reaction mixture.[29] It seems unreasonable to propose that species representing such a large proportion of the total enzyme are not involved in catalysis. Even stronger arguments are provided by experiments using rapid freezing techniques. For two reactions—the ethanolamine ammonia-lyase catalyzed deamination of L-2-aminopropanol[29b] and the dehydration of L-propanediol by diol dehydrase[31]—such experiments have shown that the rates of appearance of the ESR signals exceed the catalytic turnover numbers. The formation of the paramagnetic species in these reactions is thus a kinetically competent process, a result strongly suggesting a catalytic role for these species.

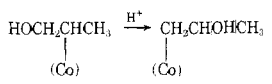
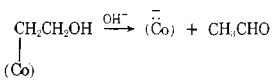
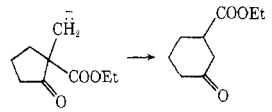
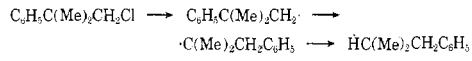
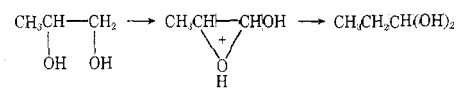
Most of the free-radical signals consist of two peaks, a feature which is not completely understood. Since the peaks do not separate when the microwave frequency is increased,[29b-d] they represent a single absorption split by an interaction with a nearby paramagnetic species with spin ½. One group has proposed that the nearby magnet is the unpaired electron of enzyme-bound cob(II)alamin, while another has proposed a hydrogen nucleus as the interacting species. The question is unresolved at present.

The current view of the mechanism of hydrogen transfer is



(31) J. E. Valinsky, R. H. Abeles, and J. A. Fee, *J. Am. Chem. Soc.*, **96**, 4709 (1974).

Table V
Model Reactions for Group X Migration

Reaction	Class	Ref
	σ ⇌ π rearrangement	35a
	Hydride shift (nucleophilic attack)	16
	Carbanion rearrangement	35b
	Radical rearrangement	35c
	Carbonium ion rearrangement	23

Homolysis of the carbon–cobalt bond of adenosylcobalamin is followed by abstraction of a substrate hydrogen by the resulting adenosyl radical, forming 5′-deoxyadenosine and the substrate radical. After rearrangement the process is reversed, the product radical removing a hydrogen atom from 5′-deoxyadenosine to re-form the adenosyl radical. This then rejoins the enzyme-bound cob(II)alamin to reconstitute the original cofactor.

An Alternative Mechanism. Based largely on model reactions and on studies of the effect of N_2O on enzyme-catalyzed reactions that are open to varying interpretations, Schrauzer has proposed a mechanism for adenosylcobalamin-dependent rearrangements involving heterolysis of the carbon–cobalt bond with the participation of cob(I)alamin and 4′,5′-anhydroadenosine as catalytic intermediates. This proposal, which has been the subject of some dispute among various groups working in the field, is discussed at length in papers from Schrauzer's[20b,32] and Abeles'[27e,33] laboratories. Interested readers are referred there for details.

Group X Migration. Unlike hydrogen transfer, the mechanism of group X migration is not understood at all. The enzymological experiments that have been performed with a view to understanding this process have consisted mostly of attempts to demonstrate enzyme-catalyzed exchange of postulated intermediates into substrate or product.[34] Like the previously discussed attempts to show exchange of 5′-deoxyadenosine into adenosylcobalamin, these have all failed. The only positive finding that might be related to the mechanism of group migration is the observation by Morley and Stadtman that α-D-lysine mutase, a pyridoxal phosphate and adenosylcobalamin requiring enzyme that converts α-D-lysine to 2,5-diaminohexanoic acid, catalyzes a pyridoxal phosphate dependent exchange of hydrogen between solvent and C-6 of the substrate.[21i] This suggests Schiff base formation between pyridoxal phosphate and the ω-amino group of D-lysine. How this relates to the rearrangement, though, is not clear.

Lacking enzymological data, investigators have attempted to explain the mechanism of group X migration in terms of model reactions. A partial list of these reactions is presented in Table V. This list, by no means exhaustive, contains representatives of several reaction types. Each of these reactions may be defended as the model that truly describes group X migration, since each involves a group migration, and in the absence of further enzymological data none can be ruled out. This proliferation of model reactions seems to suggest that, while the models are of considerable intrinsic interest and great heuristic value, elucidation of the mechanism of enzyme-catalyzed group X migration will require studies with enzymes.

Conclusions

Adenosylcobalamin is one of a group of closely related biological organometallic reagents all of which are *Co*-alkyl derivatives of corrinoid compounds. It is required as the cofactor for certain enzyme-catalyzed rearrangements in which a hydrogen atom migrates from one carbon atom to an adjacent one in exchange for a group X that migrates in the opposite direction. The nature of group X is highly variable: it may be a complex alkyl (e.g., $-CH(NH_2)COOH$)) or acyl (–COSCoA) group, or a conventional nucleophilic leaving group (–OH or $-NH_2$).

The mechanism of hydrogen transfer in these reactions is reasonably well understood. An enzyme-catalyzed homolysis of the carbon–cobalt bond of the cofactor leads to the appearance at the active site of cob(II)alamin and a free radical, 5′-deoxyadenos-5′-

(32) (a) G. N. Schrauzer and R. J. Windgassen, *J. Am. Chem. Soc.*, **89**, 143 (1967); (b) G. N. Schrauzer, R. J. Holland, and J. A. Seck, *ibid.*, **93**, 1503 (1971); (c) G. N. Schrauzer, J. A. Seck, and R. J. Holland, *Z. Naturforsch., Teil C*, **28**, 1 (1973).

(33) (a) M. K. Essenberg, P. A. Frey, and R. H. Abeles, *J. Am. Chem. Soc.*, **93**, 1242 (1971); (b) P. A. Frey, M. K. Essenberg, R. H. Abeles, and S. S. Kerwar, *ibid.*, **92**, 4488 (1970).

(34) (a) H. A. Barker, R. D. Smyth, E. J. Wawszkiewicz, M. N. Lee, and R. M. Wilson, *Arch. Biochem. Biophys.*, **78**, 468 (1958); (b) R. Swick, *Proc. Natl. Acad. Sci. U.S.A.*, **48**, 288 (1962); (c) M. Sprecher, M. J. Clark, and D. B. Sprinson, *J. Biol. Chem.*, **241**, 872 (1966); (d) H. F. Kung and T. C. Stadtman, *ibid.*, **246**, 3378 (1971); (e) R. C. Bray and T. C. Stadtman, *ibid.*, **243**, 381 (1968).

(35) (a) K. L. Brown and L. L. Ingraham, *J. Am. Chem. Soc.*, **96**, 7681 (1974); (b) J. N. Lowe and L. L. Ingraham, *ibid.*, **93**, 3801 (1971); (c) H. Eggerer, P. Overath, F. Lynen, and E. R. Stadtman, *ibid.*, **82**, 2643 (1960).

yl. The radical then abstracts the migrating hydrogen atom from the enzyme-bound substrate molecule, forming 5′-deoxyadenosine and the substrate radical. After migration of group X, the rearranged radical retrieves a hydrogen from 5′-deoxyadenosine to form the product, with the reappearance of the 5′-deoxyadenos-5′-yl radical. This then recombines with cob(II)alamin to regenerate the cofactor and complete the catalytic cycle.

Though hydrogen transfer is understood, at least in outline, little is known about the mechanism of group X migration. Enzymological experiments have thus far provided almost no information concerning this process. Conversely, experiments with chemical systems have provided an abundance of results. These have been interpreted in terms of a large number of reaction mechanisms, each of which represents an attractive model for group X migration and among which the lack of biochemical data makes it impossible to choose. Future progress in adenosylcobalamin-dependent rearrangements will undoubtedly yield a clarification of the mechanism of the migration step. With the rapid advances that have taken place in recent years in enzyme mechanism studies, it is likely that a deeper understanding of these rearrangements, including an explanation for the group X migration step, is not too far away.

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Synthetic Oxygen Carriers of Biological Interest

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Certain metal–ligand combinations provide the delicate balance required to form a 1:1 dioxygen complex without the metal (M) and/or the ligand (L) being irreversibly oxidized. These systems, which are

$$M(L)_n + O_2 \rightleftharpoons M(L)_n(O_2) \quad (1)$$

called oxygen carriers, have long been recognized in vitally important biological processes, and as early as 1852 Fremy[1] reported that solid cobalt ammine salts absorb oxygen from air and release it again when dissolved in water. However, the significance of synthetic oxygen carriers was not apparent until 1938.

Since the initial discovery by Tsumaki[2] that Schiff base chelates of cobalt(II) are oxygen carriers, there has been a continued interest in this property of such metal complexes. Much of the research effort has been devoted toward the more fundamental aspects of the problem, such as the metal–ligand properties of a good oxygen carrier, the nature of the metal–dioxygen bonding, and the structure. However, considerable research has also been devoted to possible applications of these oxygen carriers. For example, Calvin and his students[3] extensively investigated the absorption–desorption of molecular oxygen on various solid cobalt(II)–Schiff-base chelates. These complexes were studied for the purpose of isolating pure oxygen from air, and oxygen so produced was used for several months aboard a destroyer tender for welding and cutting.[4a] Other applications of synthetic oxygen carriers include their use as catalysts for reactions of molecular oxygen.[4b] It is even possible that they may eventually find use in artificial blood.[5]

Twelve years ago, Vaska[6] reported that $IrCl(CO)(P(C_6H_5)_3)_2$ is an oxygen carrier, and the structure of its oxygen complex was determined.[7] Other low-valent transition-metal compounds of this type have since been found to be oxygen carriers and/or homogeneous catalysts for certain reactions of dioxygen.[8] Structural studies on these complexes have consistently revealed a triangular MO_2 arrangement with each oxygen atom equidistant from the metal and with the O–O bond lengthened over that in dioxygen. Because of structural differences[9] from

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(1) E. Fremy, *Justus Liebigs Ann. Chem.*, **83**, 227, 289 (1852).
(2) T. Tsumaki, *Bull. Chem. Soc. Jpn.*, **13**, 252 (1938).
(3) See, for example, A. E. Martell and M. Calvin, "Chemistry of the Metal Chelate Compounds", Prentice-Hall, Englewood Cliffs, N.J., 1952, pp 336–357.
(4) (a) R. F. Stewart, P. A. Estep, and J. J. S. Sebastian, *U.S. Bur. Mines, Inf. Circ.*, **No. 7906** (1959); (b) A. E. Martell and M. M. Taqui Khan, *Inorg. Biochem.*, **2**, 654 (1973).
(5) R. P. Geyer, *Bull. Parenter. Drug Assoc.*, **28** (2), 88 (1974); J. E. Baldwin, *Newsweek*, 91 (March 24, 1975).
(6) L. Vaska, *Science*, **140**, 809 (1963).
(7) J. A. Ibers and S. J. La Placa, *Science*, **145**, 920 (1964).
(8) J. P. Collman, *Acc. Chem. Res.*, **1**, 136 (1968); J. Valentine, *Chem. Rev.*, **73**, 235 (1973).
(9) J. L. Hoard, "Structural Chemistry and Molecular Biology", A. Rich and N. Davidson, Ed., W. H. Freeman, San Francisco, Calif., 1968, p 573.