The Electrochemistry of Vitamin B$_{12}$

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The various derivatives of vitamin B$_{12}$ belonging to the series of *cobalamins* are cobalt tetraazamacrocyclic complexes having the structure shown on Figure 1. The central cobalt atom is coordinatively bound to the four nitrogens of the *corrin* ring, one of these bearing a negative charge. Attached to a carbon of the corrin ring is a nucleotide side chain terminated by a 5,6-dimethylbenzimidazole group (Bzm), which may act as an axial ligand toward the cobalt atom. The side chain bears an additional negative charge on the phosphate group down to low pH values (~1). Cobalamin complexes in which the Bzm is bound to the cobalt atom are usually named *base-on* forms, whereas if such a coordination is absent or replaced by an exogeneous ligand the designation *base-off* is used. *Cobinamides* are obtained by hydrolytic cleavage of the nucleotide side chain at the phosphate group. The side chain, thus reduced to d-CH$_2$CH$_2$CONHCH$_2$C(CH$_3$)OH$_2$, has then lost its potential liganding ability toward the cobalt atom. Comparison between cobalamins and cobinamides is of particular interest for evaluating the role of Bzm in the reactions of cobalamins. Complexes in which the side chain still possesses the α-D-ribofuranose 3-phosphate residue but not necessarily the terminal 5,6-dimethylbenzimidazole, which may be replaced by other imidazolic structures, are named *cobamides*. The various cobamides, cobalamins, and cobinamides differ one from the other by the nature of the axial ligands in α (lower) and β (upper) positions: water, OH⁻, CN⁻, alkyl carbanions, giving rise respectively to aquo (B$_{12a}$), hydroxo, cyano (B$_{12}$), alkyl cobalamins.

Alkylcobamides are of particular interest since the prosthetic groups of the two main series of B$_{12}$ enzymes belong to this class of compounds. In "B$_{12}$ coenzyme" (adenosylcobalamin) the β-axial ligand is the 5′-deoxyadenosyl carbanion. The corresponding enzymes catalyze a series of rearrangement reactions involving the transfer of an hydrogen atom from one carbon atom to the adjacent carbon atom in exchange for another group that migrates in the opposite direction:[1]
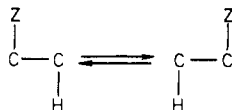
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Methylcobamides are involved in enzymatic catalysis of methyl-transfer reactions.[2] in N⁵-methyltetrahydrofolate homocysteine methyl transferase, methylcobalamin catalyzes the transfer of a methyl group from the former to the latter compound, yielding methionine. Methylcobamides are also involved in acetate biosynthesis starting from carbon dioxide.

Parallel to the biochemical studies, the chemistry and physical chemistry of vitamin B$_{12}$ derivatives has received considerable attention.[3,4] While primarily aiming at a better understanding of the enzymatic reactions, the chemical and physicochemical investigations have exceeded this primary objective and have become a contribution to the general field of coordination and organometallic chemistry.[3a]

The cobalt atom in cobalamins and cobinamides can exist under three main formal oxidation states, Co(III), Co(II), Co(I),[5] which display quite different chemical properties. Roughly speaking, Co(III) appears as an electrophile, Co(II) as a radical, and Co(I) as a nucleophile. Oxidoreductive conversions between the three oxidation states are thus of key importance in the chemistry of vitamin B$_{12}$. Being able to produce these conversions under controlled conditions, electrochemistry appears as a particularly valuable source of information for investigating the redox chemistry of vitamin B$_{12}$ derivatives. Early work in this field mostly used polarography as a technique and concerned mainly cyano- and aquocobalamins.[6-13] Polarographic data were also gathered concerning deoxyadenosyl-, methyl-, and other alkylcobalamins[13-16] and -cobinamides,[13,15]

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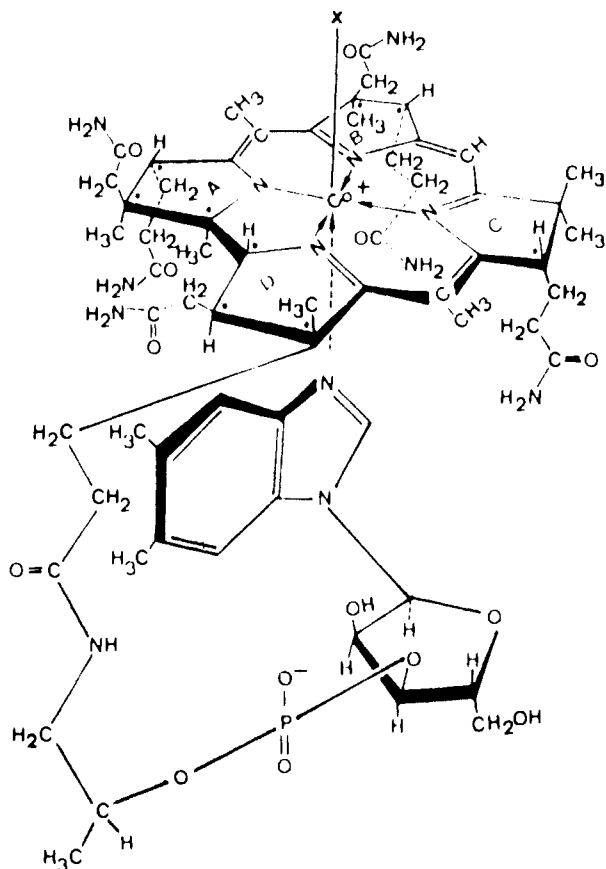(15) Bernhauer, K.; Muller, O.; Wagner, F. *Adv. Enzymol.* 1964, 26, 233.

Figure 1. Vitamin B_{12} derivatives.

hydroxocobalamin,[13] sulfitocobalamin,[15,16] and vitamin B_{12s}.[17] The work of Hogenkamp and Holmes[13] is particularly worthy of notice, giving a survey of the polarographic characteristics of a broad series of cobalamins and cobinamides. More recently, cyclic voltammetry (CV) has been used to describe the electrochemical behavior of B_{12},[18,19] B_{12a},[19,20] B_{12r},[18,20] B_{12s},[18] methyland (trifluoromethyl)cobalamins,[19] methyl and Co(II) cobinamides,[19] and several model complexes.[15] Potentiometry has been used to determine the B_{12r}-B_{12s} standard potential.[20,21] Controlled potential electrolysis and coulometry have also been used to prepare and characterize the reduced states of cyano- and aquocobalamin.[17,22-24]

When the formal oxidation state of the cobalt atom decreases, its coordination number tends to decrease accordingly. In the thermodynamically predominating forms, the coordination number is generally 6 (two axial ligands) for Co(III), 5 (one axial ligand) for Co(II), and 4 (no axial ligand) for Co(I). In B_{12} electrochemistry,

electron transfer is therefore expected not to be a simple outer-sphere process. It is generally accompanied by the expulsion of one ligand when going to a lower oxidation state and by the formation of a coordination bond upon increasing the oxidation state. On the other hand, according to the presence of potential axial ligands in the reaction medium, several forms of a given oxidation state may coexist and compete in reduction or oxidation processes. The electrochemistry of B_{12} derivatives under both its thermodynamic and kinetic aspects is thus anticipated to depend heavily upon trans-ligandation factors.

Besides evaluating reversibility,[18-20] the work cited above has provided characteristic potentials for the oxidoreduction of various B_{12} derivatives. Although the knowledge of these potentials is certainly useful from a semiquantitative point of view, their exact meaning was open to question for several reasons. They may well include, besides the thermodynamic characteristics of the electron transfer, thermodynamic factors featuring the interference of ligand-exchange reactions. They may also partly reflect the kinetics of the electron transfers and accompanying axial ligand displacements. On the other hand, most of the above cited work was carried out with mercury as the working electrode material. Specific adsorption may then interfere and influence the characteristic potentials. This has been shown to be the case for B_{12r} in water with alkali-metal salts as supporting electrolyte.[25-28]

Our own work on the electrochemistry of vitamin B_{12} derivatives[25,29,31-42] was an attempt to unravel the complexities deriving from the interplay between electron transfer and changes in axial ligandation in the electrochemical reactions of the main vitamin B_{12} derivatives. Besides thermodynamic characterizations, the kinetics of the electrochemical reactions was investigated systematically in the aim of determining the reaction mechanisms and estimating the rate constants of the key steps. This also helped to understand the exact meaning of the thermodynamic data. Particular attention was devoted to minimizing reactant adsorp-

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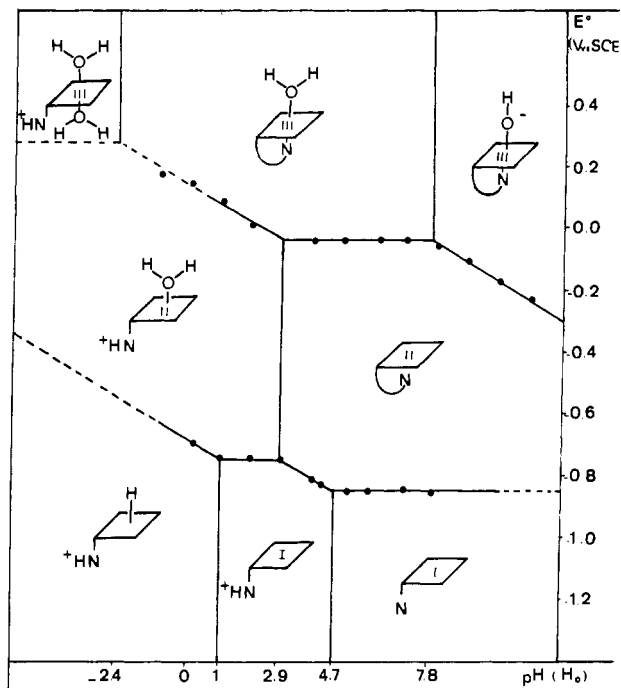
Figure 2. $E°$–pH diagram of the B_{12a}–B_{12r}–B_{12s} (Co(III)–Co(II)–Co(I)) in water at 22 °C. Potentials are referred to the saturated calomel electrode.

tion at the electrode surface in the purpose of obtaining data characterizing intrinsically the molecules under study. In this respect, the question of the use of mercury as electrode material is particularly important. On one hand, the use of mercury is advantageous as compared to that of solid electrode materials such as platinum, gold, and glassy carbon, allowing better reproducibility and the investigation of a more extended time range. On the other hand, specific adsorption on mercury, especially with the Co(II) complexes, may affect the electrochemical behavior.[25-28] Also mercury oxidation prevents its use in too positive potential ranges.[29,30] Our strategy was thus to use mercury as far as we could in the suitable potential ranges, eventually modifying the reaction medium so as to suppress the adsorption of Co(II) complexes and to use solid electrodes in the other cases. Comparison between the responses obtained with different electrode material was made in a number of cases in order to check that the observed behaviors do not depend upon the nature of the electrode.

Thermodynamics of the Co(III)–Co(II)–Co(I) System[25,29,31,36]

The thermodynamics of the B_{12a}–B_{12r}–B_{12s} system in water is summarized in Figure 2 under the form of a standard potential vs. pH diagram. The data for the Co(III)–Co(II) couple were obtained from thin-cell spectroelectrochemical experiments carried out on a platinum grid electrode with electrolysis times long enough for allowing equilibration.[29] The Co(III)–Co(II) kinetics is indeed rather slow (vide infra). Low sweep rate cyclic voltammetry was used for the Co(II)–Co(I) couple. The sweep rate has indeed to be low enough for allowing the equilibrium between base-off and base-on B_{12r} to be established in the whole diffusion layer.

There are two series of pH-independent standard potentials for the Co(III)–Co(II)–Co(I) system: –0.04,

–0.85 V vs. SCE, featuring the passage from base-on aquocob(III)alamin to base-on cob(II)alamin and to base-off cob(I)alamin successively, and +0.27, –0.74 V vs. SCE, featuring the passage from base-off diaquocob(III)alamin to base-off cob(II)alamin and to base-off cob(I)alamin successively (Bzm is protonated in all three complexes). The same values as in the latter series are found when starting from diaquocob(III)-amide.[35] Coordination by Bzm instead of water thus renders the reductions more difficult as anticipated from the greater electron-donating character of Bzm as compared to that of water. The difference in reducibility is larger at the Co(III) than at the Co(II) level, which parallels the fact that the replacement of water by Bzm is much easier at Co(III) ($K \simeq 10^7$) than at Co(II) ($K \simeq 60$).

The 60-mV linear dependence of the standard potential observed in various pH ranges reflects a change in axial ligandation or in another part of the molecule. Between 2.9 and –2.4 the nucleotide side chain opens, Bzm being replaced by water at Co(III) in the α-position. The same is true for B_{12r} between 4.7 and 2.9. The uncoordinated Bzm in B_{12s} undergoes protonation at 4.7. Above pH 7.8 the water molecule in the β-position of B_{12a} is converted into OH⁻. B_{12s} undergoes a second protonation at pH 1. This does not reflect the neutralization of the phosphate group since it occurs at the same pH for B_{12r}. The resulting complex slowly decomposes with an evolution of hydrogen, giving rise to a catalytic wave.[25,31] A further catalytic hydrogen reaction is observed at more negative potentials. A similar behavior is observed with cob(I)inamide.[35] This is compatible with the formation of a cobalt hydride by analogy with other cobalt complexes such as (CN)₅Co-(I).[43] There is not, however, formal structural proof that the proton is attached to the cobalt atom rather than to another point of the molecule. In any case, B_{12s}, which is known as a powerful nucleophile,[2] appears as a very weak Brønsted base.

As is shown in Figure 2, the disproportionation reaction

$$2\mathrm{Co(II)} \rightleftarrows \mathrm{Co(III)} + \mathrm{Co(I)}$$

is very much in favor of the left-hand side in the whole pH range. Disproportionation increases upon raising the pH above 7.8. However, even at pH 12 the disproportionation equilibrium constant remains extremely small ($\sim 10^{-10}$).

Role of the Base-Off/Base-On Reactions and of Other Ligand-Exchange Reactions

A typical example of the role of the base-off/base-on reaction in the redox chemistry of vitamin B_{12} derivatives is provided by the reduction of B_{12r} to B_{12s}. Figure 3a shows the cyclic voltammograms obtained in Me₂SO as a function of the sweep rate.[40] Mercury can be employed as the electrode material since Me₂SO adequately suppresses the adsorption of B_{12r}. The observed behavior reflects the reduction mechanism shown in Scheme I. The base-off form of B_{12r} is easier to reduce than the base-on form. Both of them give rise, upon one-electron transfer with concomittant expulsion of the axial ligand, Me₂SO or Bzm, to base-off B_{12s}. At the reduction potential of the base-off form, the current has

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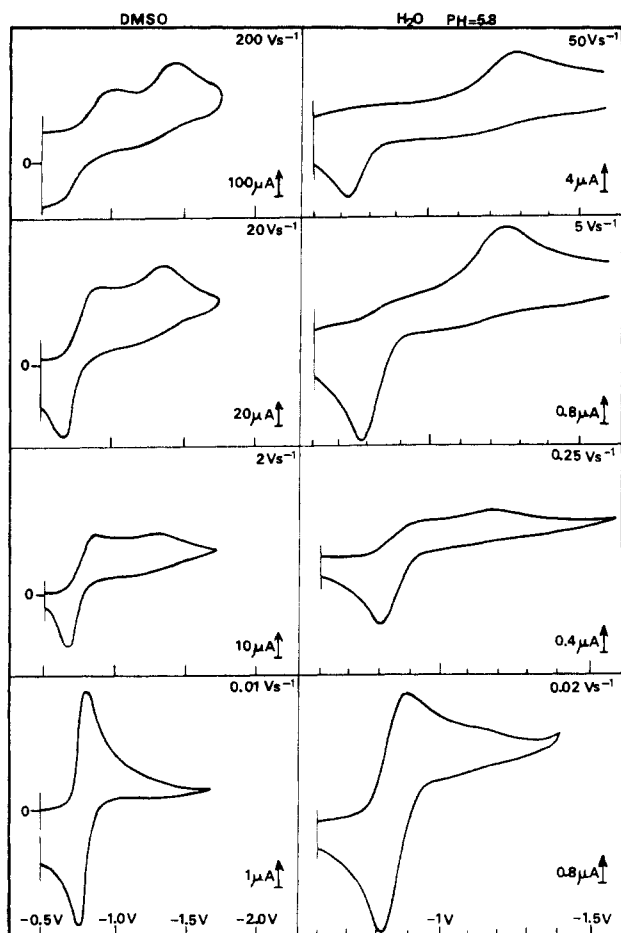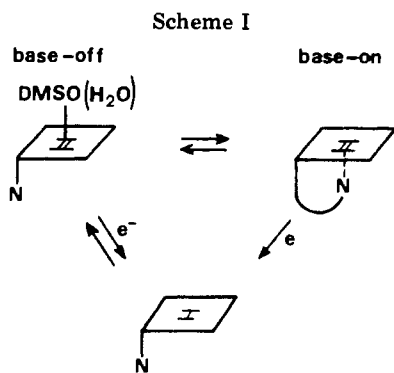
Figure 3. Cyclic voltammetry of the B_{12r}–B_{12s} couple: (a) in Me$_2$SO (+0.1 M NBu$_4$BF$_4$), (b) in water (+0.7 M Bu$_4$NTos), pH 5.8.

Scheme I

a first component corresponding to the equilibrium amount of this form. There is, however, an additional component resulting from the dynamic conversion of the base-on form into the more easily reducible base-off form. The importance of the second component depends upon the rate of the base-on/base-off conversion, relative to the sweep rate, v. At high sweep rates ($v >$ 100 V s^{-1}), two successive cathodic peaks are observed. The ratio of their heights is independent of v, reflecting the ratio of the base-off and base-on form concentrations at equilibrium. Upon decrease of the sweep rate, the first peak increases at the expense of the second, reflecting the growing kinetic interference of the base-off/base-on reaction. At very low sweep rates ($v <$ 0.1 V s^{-1}) the second peak disappears, the reduction of the base-on form occurring entirely through prior conversion into the base-off form. Application of the appro-

priate theoretical relationships[44] leads to the determination of the equilibrium and rate constants of the base-off/base-on reaction:[40]

solvent	$K_{off/on}$	k_{on}, s^{-1}	k_{off}, s^{-1}
Me$_2$SO	0.6	6.3×10^2	3.7×10^2
H$_2$O (above pH 5)	1.7×10^{-2}	10^4	1.7×10^2

In water,[25] the reduction mechanism follows qualitatively the same pattern. However, above pH 5 the base-off/base-on equilibrium constant is much smaller than in Me$_2$SO. At high sweep rates, the base-off wave thus completely disappears, while a kinetic wave builds up as the sweep rate decreases (Figure 3b). At low sweep rate a single wave is again observed, corresponding to complete mobility of the base-on/base-off reaction toward diffusion. Upon decrease of the pH, the equilibrium shifts toward the base-off form. Its wave thus increases, at a given sweep rate, as the pH decreases. Below pH 2.9, the base-off form completely predominates at equilibrium and a single reversible wave is observed whatever the sweep rate. Thermodynamic and kinetic characterization of the base-off/base-on reaction can again be derived from the analysis of the cyclic voltammograms as a function of the sweep rate.

The variations of the characteristics of the base-off/base-on reaction when passing from water to Me$_2$SO essentially reflects a better ability of the latter molecule to bind to the Co(II) center. This is anticipated from the fact that Me$_2$SO is a softer base than water, underscoring the soft-acid character of Co(II) in B_{12r}.

A rather complex reduction pattern is observed in pyridine–Me$_2$SO mixtures.[40] Three Co(II) complexes now interfere, involving Me$_2$SO, pyridine, and Bzm, respectively, as axial ligand. The ease of reducibility lies in the same order. Part of the Bzm complex is thus reduced through prior conversion into the pyridine complex, which is itself partly reduced through prior conversion into the Me$_2$SO complex. The respective contribution of the three complexes to the overall reduction of the B_{12r} molecule is a function of the sweep rate and the pyridine concentration.

Cyanide is also able to replace Bzm in B_{12r}. The corresponding binding constant has been determined from equilibrium spectroelectrochemical experiments involving the reduction of vitamin B_{12} in the presence of CN$^-$.[34] This system, however, exhibits a direct 2e reduction of Co(III) to Co(I) in cyclic voltammetry as described and discussed below. By comparison of the strength of the various ligands toward Co(II), the following order was found (Figure 4): H$_2$O < Me$_2$SO < pyridine < Bzm < CN$^-$.

During the reoxidation of B_{12s} in Me$_2$SO it was found that base-off B_{12r} is formed first and then converts into the thermodynamically more stable base-on form. The more stable form is not obtained directly for kinetic reasons, the overall electron-transfer process being slower the stronger the axial ligand[41] (see the next section). This is also observed with other axial ligands stronger than Me$_2$SO such as pyridine and CN$^-$.

The base-off/base-on reaction plays a much less important role in the reduction of Co(III). In the case of B_{12a},[33,36] down to pH 1, the equilibrium is too much in favor of the base-on form ($\sim 10^7$) and the base-on/
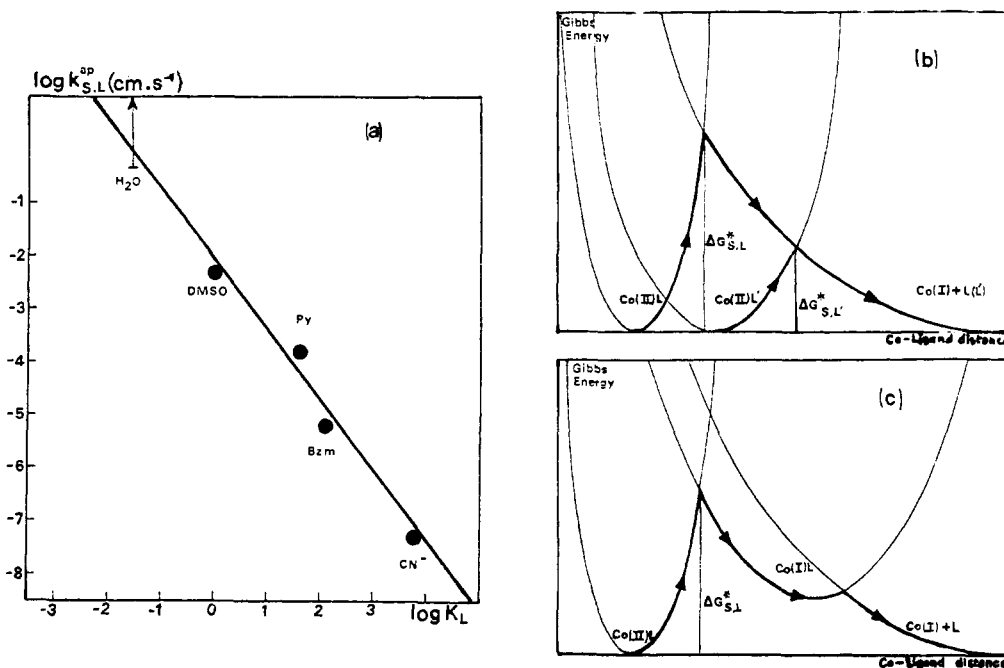
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Figure 4. Trans effects in the B_{12r}–B_{12s} oxido reduction kinetics. (a) Correlation between the rate of electron transfer and the strength of Co(II) β-axial coordination. (b) Gibbs energy diagram for concerted electron transfer and bond breaking and formation. (c) Gibbs energy diagram for electron transfer followed by a bond-breaking or a bond-formation step.

base-off conversion too slow for any significant reduction through the base-off form to occur. This appears only at pH 0 and below. The reduction of cyanocob-(III)alamin similarly does not involve the base-off form in neutral and basic media[34] at room temperature despite the fact that equilibrium is more in favor of the base-off form than in the preceding case ($\simeq 10^{-2}$). This underscores the slowness of the base-on/base-off reaction relative to diffusion in the context of cyclic voltammetry. A similar result is found for other ligand-exchange reactions involving the upper axial position of cob(III)alamin. For example, the addition of small amounts of CN^- to cyanocob(III)alamin results in the appearance of two waves corresponding to the reduction of base-on cyanocob(III)alamin and of dicyanocobalamin successively.[34] The rate of interconversion between the two complexes, however, is too small for any kinetic contribution of the conversion of the dicyano complex into the monocyano complex to be observed at the reduction potential of the latter. The same is true for the chloro- and acetetocob(III)alamins in Me_2SO.[42] Note in this connection that Cl^- and $CH_3CO_2^-$ replace the solvent molecule in the upper axial position much more easily in Me_2SO than in water.[42]

A rather different situation is found with the interference of the aquohydroxocob(III)alamin equilibrium in the reduction of B_{12a}.[33,36] The aquo complex is easier to reduce than the hydroxo complex for both thermodynamic and kinetic reasons. Now, however, a kinetic current involving the dynamic conversion of the hydroxo into the aquo complex is found at pH values above the interconversion pH of 7.8. The conversion of the hydroxo form into the aquo form is, however, likely to involve protonation of the OH^- ligand rather than ligand exchange at the cobalt center.

Kinetics of Electron Transfer. Trans Effects

Electron transfer in the series Co(III)–Co(II)–Co(I) involves the expulsion of one axial ligand and the re-

coordination of one axial ligand in the opposite direction. Intermediates such as Co(III) with five coordinations, Co(II) with six or four coordinations, and Co(I) with five coordinations could not be detected by electrochemical methods. This means that, if they exist, their lifetime is less than $\sim 10^{-3}$ s. Bond breaking or bond formation is thus either a reaction concerted with the electron transfer or a rapid associated step. In the following, "electron transfer" designates the overall processes:

$$Co(III)LL' + 1e \rightleftharpoons Co(II)L' + L$$

$$Co(II)L' + 1e \rightleftharpoons Co(I) + L'$$
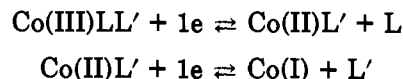
In water, electron transfer in the base-off B_{12r}/B_{12s} couple is fast; a reversible cyclic voltammogram is observed up to 600 V s^{-1} at pH values below 2.9 where protonation of the Bzm has converted all of the B_{12r} into its base-off form.[25] However, as soon as water is replaced by a slightly stronger β-axial ligand, Me_2SO, electron transfer slows down sufficiently and its kinetics become measurable.[41] Passing to stronger and stronger β-axial ligands, pyridine, Bzm, CN^-, makes the electron transfer slower and slower.[45] There is thus a correlation between the equilibrium constant for axial ligandation and the standard rate constant of electron transfer. As shown in Figure 4a, the correlation is approximately linear.

This can be rationalized in the framework of a model regarding the stretching of the cobalt–axial ligand bond as the main parameter of the kinetics of the overall electron-transfer reaction. Solvation is indeed likely to play only a minor role since the Co(II) complexes are uncharged and the Co(I) complex, being itself a nucleophile, is not anticipated to interact strongly with Me_2SO. Under these conditions, if electron transfer and

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(45) It was found possible to suppress the adsorption of B_{12r} on mercury by introducing large amounts (~ 0.7 M) of tetrabutylammonium p-toluenesulfonate in the solution.[25]

the decoordination are concerted processes (Figure 4b) the parallelism between K_L and $k_{S,L}^{ap}$ is readily understood: the stronger the axial coordination of Co(II), the shorter the equilibrium Co–L distance, the stronger the force constant, and therefore the larger the activation energy as shown on the Gibbs energy diagram.

Such a model predicts that the transfer coefficient at the standard potential be larger than 0.5 and be increasing with the strength of the ligand. This apparently contradicts the experimental observation that α does not vary significantly from one complex to the other, remaining close to 0.5. It is, however, noted that the potential ranges in which the peak potential determinations are made lie far from the standard potential; the farther they lie, the stronger the complex. The free energy of activation is then much lower than the standard free energy of activation shown on Figure 4b, which results in a lower value of the experimental transfer coefficient as compared to its value at the standard potential. The approximate constancy of the α value may well result from the compensation of the two effects.

The observation of a linear correlation between the standard rate constant and the association constant of B_{12r} does not, however, exclude a two-step mechanism as pictured in Figure 4c. It is indeed conceivable that the influence of the ligand in stabilizing or destabilizing the Co(I)L complex could vary in the same order as for the Co(II)L complex, thus subtracting or adding a linearly correlated term to that featuring the Co(II)L complex. The observed linear correlation actually represents a free energy relationship of the type of Hammet relation in aromatic organic chemistry: the standard free energies of activation or equilibrium of two different reactions, overall electron-transfer reaction and axial complexation of B_{12r} correlate when the axial ligand varies, similar to what occurs with a number of quite different reactions for which linear correlations involving kinetic and thermodynamics data are observed as the substituent varies in a Hammet series. In both cases this does not imply a simple and straightforward relationship between the intimate mechanisms of the correlating reactions.

This provides a useful mean of predicting, at least semiquantitatively, the kinetics of electron transfer to B_{12r} with a given axial ligand on the basis of its complexation constant. As far as the rate of the forward electron transfer, as indicated by the location of the peak potential at a given sweep rate, is concerned, the above observation implies that "the kinetics amplifies the thermodynamics." In other words, going to stronger and stronger ligands, the standard potential shifts negatively and, at the same time, the reduction becomes slower and slower. These two effects add up, resulting in a quite rapid shift of the reduction potential as the ligand strength increases.

The observation that "the stronger the ligand the slower the electron transfer" is in apparent contrast with the previous finding that, for the Fe(III)/Fe(II) tetraphenylporphyrin couple, the electron transfer becomes faster and faster as the Brønsted basicity of the axial ligand increases in a series of nitrogen bases.[46] A linear free energy relationship is also found in this case. Actually both this example and that of the B_{12r}–B_{12s} couple

(46) Constant, L. A.; Davis, D. G. *J. Electroanal. Chem.* **1976**, *74*, 85.

illustrate a more general rule: the more different the ligandation and solvation strength from one member of the redox couple to the other, the slower the electron transfer. In the case of the Fe(III)/Fe(II) porphyrin system, the nitrogen bases are very good ligands of the Fe(II) complex, while the strength of ligandation of the Fe(III) complex decreases with the basicity of the nitrogen base. The stretching contribution to the activation energy thus increases as the Brønsted basicity decreases. In the B_{12r}–B_{12s} case, the strength of ligandation is much larger at the Co(II) than at the Co(I) level. Its contribution to the activation energy thus increases as the ligand becomes stronger and stronger toward Co(II).

The same trends are also observed for the Co(III)/Co(II) couple although the kinetic determinations are less accurate due to the necessity of using solid electrodes.[33,42,47]

Stepwise One-Electron Transfers vs. Direct Two-Electron Transfer. Thermodynamic and Kinetic Aspects

A typical example of stepwise one-electron transfers to Co(III) leading successively to Co(II) and Co(I) is given by the reduction of B_{12a} in water below pH 7.8[29,33,36] or in neutral ethanol or Me_2SO.[42] There is then a large separation between the two successive waves. The distance between the two successive reduction potentials under dynamic conditions is not exactly the same as the difference between the corresponding standard potentials, but the modification is not such as to lead to a merging of the two waves. The situation is different when the pH increases above 7.8, involving the conversion of aquocob(III)alamin into hydroxocob(III)alamin. When the pH is high enough, there is no more contribution of the aquo complex, the reduction process involving only the hydroxo complex. Under these conditions, e.g., at pH 12, a single two-electron wave is observed, featuring the direct conversion of Co(III) into Co(I) without the formation of an intermediary Co(II) complex.

From a strictly thermodynamic point of view the two waves should be largely separated since the difference in standard potentials is 0.55 V. The merging of the two waves thus arises from kinetic reasons. According to the discussion in the preceding section, electron transfer to hydroxocob(III)alamin is expected to be slow in view of the strong affinity of OH⁻ toward Co(III). Its reduction potential is indeed found to be approximately the same as that of base-on B_{12r},[33] i.e., about 900 mV negative to the standard potential. At the same time OH⁻ does not displace Bzm in B_{12r} and therefore does not contribute to slow down the electron transfer to Co(II). The fact that hydroxocob(III)alamin undergoes a direct two-electron reduction yielding cob(I)alamin thus results from the slowness of the first electron transfer due itself to the strong affinity of OH⁻ toward Co(III).

An even more striking example of the interplay of thermodynamic and kinetic factors in determining the stepwise character of the Co(III)–Co(II)–Co(I) oxidoreduction is offered by the behavior of vitamin B_{12} in the presence of cyanide ions.[34,39] The $E°$–log [CN⁻] diagram in Figure 5 shows the zones of thermodynamic stability of the various forms of vitamin B_{12} under the three oxidation states as a function of the cyanide ion con-
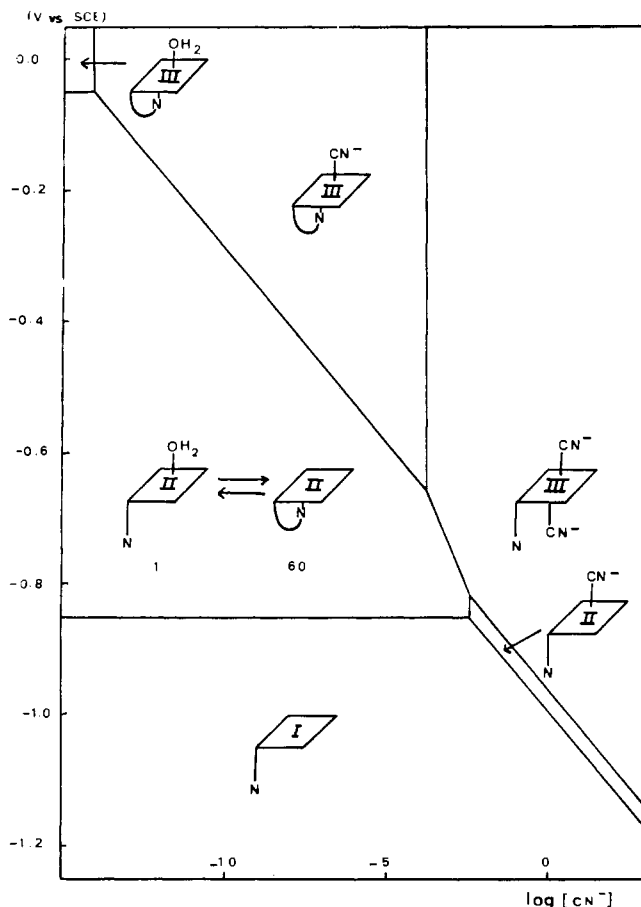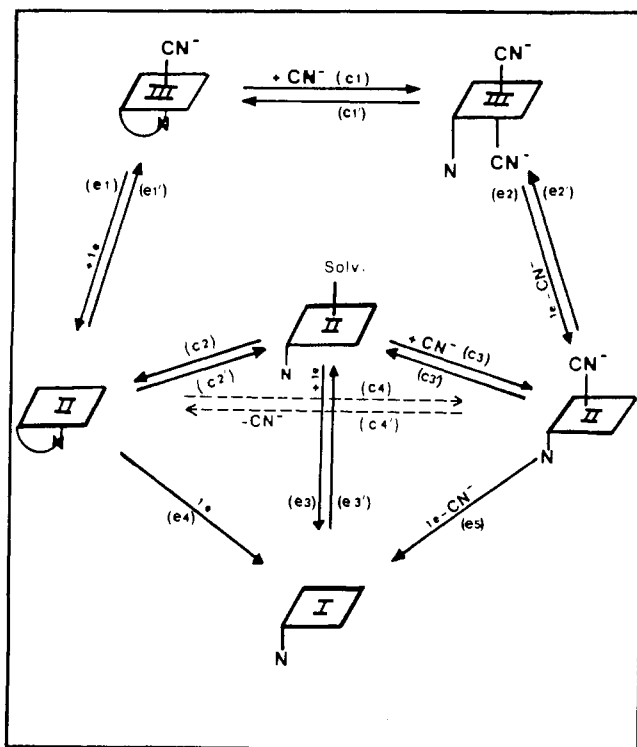
Figure 5. Redox thermodynamics of cyanocobalamins in water at 22 °C. Potentials are referred to the SCE.

Scheme II

of CN⁻ larger than 5.10^{-3} M, almost complete merging of the two waves should be observed. In the latter case, the starting complex is dicyanocob(III)alamin and the zone of stability of cyanocob(II)alamin is extremely narrow corresponding to a disproportionation equilibrium constant of 0.28. The reduction under dynamic
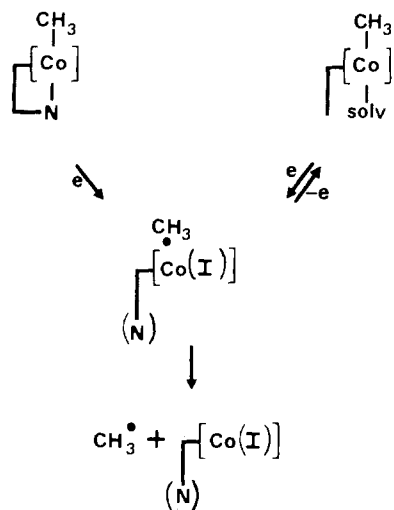
$$2\text{Co(II)CN}^- \rightleftarrows \text{Co(III)(CN)}_2^- + \text{Co(I)}$$
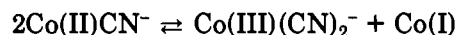
conditions is quite different, almost the opposite, as revealed by cyclic voltammetry. With monocyanocob(III)alamin in the absence of added CN⁻, a two-electron wave is observed at room temperature, i.e., the opposite of what is predicted on thermodynamic grounds. This corresponds to the $(e_1 + e_4)$ and/or $(e_1 + c_2' + e_3)$ pathways in Scheme II.

The situation is thus very similar to what happens with hydroxocob(III)alamin. Strong β-axial coordination of Co(III) by CN⁻ renders the first electron transfer slow for the reasons discussed in the preceding section. The reduction potential is thus shifted in a region where B_{12r} is itself reduced. The latter complex may exist then under its base-on and base-off forms but not as cyanocob(II)alamin since the CN⁻ concentration in the medium is too small for the CN⁻ to bind to the Co(II). When now CN⁻ is added into the solution at concentrations larger than 5.10^{-3} M, the starting complex is dicyanocob(III)alamin and the intermediate cyanocob(II)alamin is thermodynamically rather unstable.

In cyclic voltammetry, however, it is possible to obtain a decrease of the two-electron wave toward one electron and the simultaneous appearance of a more negative wave that increases up to one electron. This is observed, at a given CN⁻ concentration, by raising the sweep rate and, at a given sweep rate, by raising the CN⁻ concentration. This reflects the increased kinetic stability of cyanocob(II)alamin formed upon one-electron reduction of the $(\text{CN})_2\text{Co(III)}$ complex. As discussed in the preceding section, electron transfer to cyanocob(II)alamin is slow, which shifts its reduction potential beyond that of dicyanocob(III)alamin. This is the reason why the kinetic control results in a situation—two well-separated one-electron waves—quite different from what is observed under thermodynamic control where the two successive reductions are almost merged. The passage from two one-electron successive waves to one two-electron wave reflects the occurrence of an ece mechanism (Scheme II) in which the interposed chemical reaction is the conversion of the CNCo(II) complex into the base-off and base-on form of B_{12r}. The kinetic interference of this reaction increases as the sweep rate and the CN⁻ concentration decrease.

The oxidoreduction reactions of cyanocobalamins also raise the interesting question of the origin of the second electron in the two-electron reactions. Does it come from the electrode giving rise to an ece mechanism or from reducing species present in the solution—base-off and base-on B_{12r} in the present case—along a disproportionation mechanism? This ece-disproportionation problem has received active attention in the context of organic electrochemistry where the two-electron character of the wave results from thermodynamic reasons.[48]

centration. On purely thermodynamic grounds, starting from monocyanocob(III)alamin in the absence of added CN⁻, two successive Co(III)–Co(II), Co(II)–Co(I) waves are expected, while in the presence of concentrations

Scheme III
Electrochemical Reduction of Methylcobalamin
and Methylcobinamide

In the present case the situation is quite different: the thermodynamics is in favor of two separated one-electron waves and the two-electron character derives from the slowness of the first electron transfer. For the same reasons, i.e., changes in axial coordination, the homogeneous electron transfers are also slow.[34] Thus the reduction mechanism will involve a true ece pathway, the second electron being provided by the electrode. This is what has indeed been shown to occur with use of potential step chronoamperometry to gather the kinetic data.[39] This is the first proven example of an ece mechanism occurring in conditions where it can be discriminated from the corresponding disproportionation mechanism. It is anticipated that such mechanisms can occur in a number of other reactions of coordination electrochemistry involving slow heterogeneous and homogeneous electron transfers.

Alkylcobalamins and Cobinamides

The reduction of the methyl derivatives is of particular interest in view of their role in biological reactions occurring under reducing conditions.

Upon reduction, the C–Co bond of methylcobalamin is broken. Methane has been detected as one of the reduction products of controlled potential electrolysis[49] although more recent data[50] indicate the quantitative formation of ethane in water on a mercury-covered nickel electrode. Reduction of alkylcobalamins on mercury in the presence of ethanol leads to the formation of the corresponding alkylmercury compounds.[51] The reduction of methylcobalamin on mercury, glassy

carbon, platinum, gold involves the consumption of one electron per molecule.[52] These observations are compatible with the reaction given in Scheme III, involving a cleavage of the C–Co bond, which yields a methyl radical and the Co(I) complex. The further reduction of the $CH_3\cdot$ radical thus generated is apparently rather slow, thus leading to dimerization into ethane or to reaction with mercury.

The formation of the Co(I) complex upon reduction of alkylcobalamins has been used for devising a catalytic cycle for the electrochemical reduction of alkyl halides.[51,53] The rate-determining step is then the nucleophilic attack of Co(I) on the alkyl halide.[53] The catalytic system appears much more stable than with the related cobalt tetraphenylporphyrin for which degradation of the catalyst occurs after a few cycles.[53]

In following the detailed course of the reduction process, the intermediate complex formed upon attachment of one electron was detected by using high sweep rate cyclic voltammetry[54] on mercury in 1:1 DMF–1-propanol.[55a] Its half-life varies from 0.1 to 4.10^{-4} between –20 and 19 °C. The standard potential is –1.45 V vs SCE. The one-electron intermediate formed upon reduction of methylcobalamin[55a] in the same medium appears as more unstable. It gives rise to the base-off one-electron reduced intermediate that is reoxidized as in the case of methylcobinamide.[54] The appearance of a faint reoxidation wave at more a positive potential, possibly featuring the reoxidation of the base-on intermediate, is not fully understood. The standard potential, $\sim$–1.6 V vs. SCE, appears as more negative than with methylcobinamide as expected from the electron-donating character of Bzm being stronger than that of a solvent molecule. Similar results were obtained with Me_2SO[55a] at room temperature. It was shown, however, that the cleavage of the Co–C is slower in this solvent than in DMF–propanol mixtures.[52] No influence of the base-off/base-on reaction at the level of the starting complex could be detected in these two solvents as in water,[55b] even when the temperature was raised up to 80 °C.[56]

Further investigation of the electrochemical reduction of alkylcobalamins and cobinamides are clearly warranted. Several points indeed remain open to question: is it possible to modify the conditions of direct or indirect electrolysis in order to obtain methane rather than ethane as it is the case upon platinum-catalyzed reduction by hydrogen[57] and what is the role of the base-off/base-on reaction and that of five coordinated alkyl complexes?

It is noteworthy that alkylcobalamins and cobinamides behave differently from other B_{12}–Co(III) complexes bearing strong axial ligands such as OH^- and CN^-. The reduction potential is quite negative, while its kinetics appears as rather fast. Accordingly, the $Co(II)\text{-}R \leftrightarrow Co(I)\cdot R$ complex obtained upon attachment of one electron is easier to oxidize than any other among

(47) In this connection, the intrinsic character of the k_s^{ap} values thus determined may be questioned.[27] It is, however, emphasized that quite similar results were obtained with use of two different solid electrode materials, gold and glassy carbon[33] or platinum and glassy carbon.[42] Correlation between the heterogeneous and homogeneous electron-transfer rates[28] should be made with extreme caution. For simple outer-sphere processes the choice between the Hush and the Marcus correlation remains open to question. In addition, the electron-transfer processes in the B_{12} series are not simple outer-sphere reactions since electron transfer is accompanied by ligand–metal bond formation or bond breaking as emphasized earlier.

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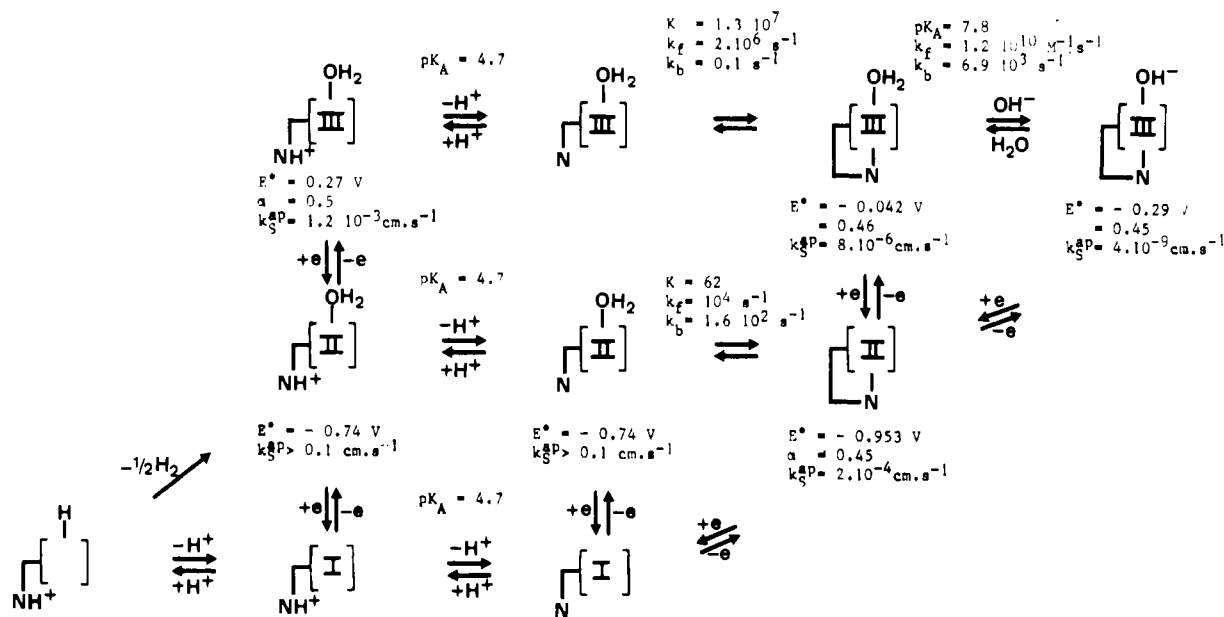(53) Lexa, D.; Savéant, J. M.; Soufflet, J. P. *J. Electroanal. Chem.* 1979, *100*, 159.

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(55) (a) DMF–propanol mixtures and Me_2SO minimize adsorption on mercury, allowing this electrode material to be used. (b) Glassy carbon was used as electrode material in this case in order to avoid adsorption, which appears as strong on mercury.

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Scheme IV
Thermodynamics and Kinetics of the
B_{12a}-B_{12r}-B_{12s} System

Scheme IV with redox/acid-base equilibria and rate/potential data:

$K = 1.3 \cdot 10^7$, $k_f = 2.10^6\ s^{-1}$, $k_b = 0.1\ s^{-1}$

$pK_A = 7.8$, $k_f = 1.2 \cdot 10^{10}\ M^{-1}s^{-1}$, $k_b = 6.9 \cdot 10^3\ s^{-1}$

$pK_A = 4.7$

$E^* = 0.27\ V$, $\alpha = 0.5$, $k_S^{ap} = 1.2 \cdot 10^{-3}\ cm.s^{-1}$

$E^* = -0.042\ V$, $= 0.46$, $k_S^{ap} = 8.10^{-6}\ cm.s^{-1}$

$E^* = -0.29\ V$, $= 0.45$, $k_S^{ap} = 4.10^{-9}\ cm.s^{-1}$

$K = 62$, $k_f = 10^4\ s^{-1}$, $k_b = 1.6 \cdot 10^2\ s^{-1}$

$E^* = -0.74\ V$, $k_S^{ap} > 0.1\ cm.s^{-1}$

$E^* = -0.74\ V$, $k_S^{ap} > 0.1\ cm.s^{-1}$

$E^* = -0.953\ V$, $\alpha = 0.45$, $k_S^{ap} = 2.10^{-4}\ cm.s^{-1}$

$-\tfrac{1}{2}H_2$

$pK_A = 4.7$

the $B_{12}Co(II)$ or even $Co(I)$ complexes.

Concluding Remarks

The $Co(II)/Co(I)$ standard potential in water, -0.85 V vs. SCE, lies slightly beyond the negative edge of the potential range of biological reductants. However, this does not seem to preclude the involvement of vitamin B_{12s} in biological processes. It is indeed conceivable that a slightly uphill reaction can be pulled by further exergonic processes involving the reactive intermediates thus initially formed. Note, however, that for kinetic reasons such a reaction is more likely to occur at the base-off than at the base-on B_{12r}. More generally, strong axial coordination of $Co(II)$ will render the generation of $Co(I)$ more difficult for thermodynamic and kinetic reasons. This even occurs with $B_{12}Co(III)$ complexes bearing a strong axial ligand for which very negative effective reduction potentials are found. The case of methylcobalamin is worthy of notice in this connection, its reduction potential being not less than -1.5 V vs. SCE. The reductive generation of $CH_3\cdot$ or CH_3^- by means of biological reductants therefore requires intimately assisted electron-transfer processes rather than the juxtaposition of a simple electron transfer pulled by a downhill followup reaction.

From a more chemical point of view, the oxidoreduction patterns of most B_{12} derivatives are now pretty well understood with the exception of alkylcobalamins. This is illustrated in Scheme IV, which shows that the thermodynamics and the kinetics of most of the steps involved in the redox pattern of the B_{12a}-B_{12r}-B_{12s} system are now quantitatively characterized. Further investigation of the temperature dependence of these reactions leading to the determination of the energetic and entropic contributions would, however, be of interest.

The electrochemistry of vitamin B_{12} derivatives offers a remarkable illustration of the essential role of axial coordination in redox coordination chemistry. The strength of axial coordination heavily controls the ease of electron transfer both on thermodynamic and kinetic grounds. The latter factors generally amplify the effect of the former. This is the basis for the involvement of ligand-exchange reactions that allow easier reduction processes through relay by a weaker ligand. The same factors also control occurrence of stepwise or direct two-electron-transfer processes.