JOURNAL OF CATALYSIS 9, 217–224 (1967)

Catalysis of the Autoxidation of Carbon Monoxide by Cobalt Corrins

J. H. BAYSTON AND M. E. WINFIELD

From the Division of Applied Chemistry, Commonwealth Scientific and Industrial Research Organization, Fishermen's Bend Victoria, Australia

Received June 5, 1967; revised August 29, 1967

It is shown that the oxidation of CO by O_2 in aqueous solution can be catalyzed by aquocobalamin. Ligands which displace the aquo group, e.g., cyanide ion or benzimidazole, inhibit the catalysis. Removal of the dimethylbenzimidazole ligand of aquocobalamin stimulates activity.

In the reductive half of the catalytic cycle the metal ion is reduced from the cobaltic to the cobaltous state. The reduction is first order in both CO and cobalamin although two molecules of cobalamin are reduced per molecule of CO consumed; the apparent activation energy is 12 kcal per mole of CO. Evidence is provided to support the following mechanism: An aquo ligand of the catalyst is replaced by CO, which thereupon combines with water to give an acyl complex that is rapidly attacked by aquocobalamin, yielding carbonic acid and two molecules of reduced catalyst. The overall rate of aquocobalamin reduction reaches maxima at pH 5.7, 5.1, and <1.1, the first due to protonation of hydroxocobalamin and the third to replacement of the benzimidazole ligand by an aquo group.

Although it reacts with reduced pentacyanocobaltate, CO will not combine with either of the reduced forms of aquocobalamin. No reaction is detected between H_2 and aquocobalamin or its cobaltous form. Uncatalyzed reduction of hydroxocobalamin by tank hydrogen is attributed to CO.

INTRODUCTION

In common with pentacyanocobaltate (*1*, *2*), cobalamins have the unusual property of coordinating to alkyl groups so strongly that the carbon-to-metal bond can be formed in aqueous solution (*3*, *4*). Resemblances in the catalytic activity of the two classes of cobalt complex are largely due to this property, as are the principal known biological catalyses involving corrinoid coenzymes (*5*). To fit the cobalt atom for its biological role, the extreme reactivity of the cobaltous state, as displayed in $[Co^{II}(CN)_5]^{3-}$, must be suppressed so that it does not overshadow the activity of the more reduced state in which the cobalt atom reacts with alkylating agents. Some of the resulting differences in the behavior of the cobalt ion in cyanocobaltates and cobalamins can be demonstrated by studying their reactions with CO and with H_2.

Abbreviations which will be used are as follows: $B_{12}^{III}CN$ = cyanocobalamin, i.e., 5,6-dimethylbenzimidazolylcobamide cyanide (the cobalt atom is coordinated to four coplanar nitrogen atoms of the corrin nucleus as well as to a cyanide ion and to a nitrogen atom of a dimethylbenzimidazolyl group which is attached via a sugar phosphate to a side chain of the corrin); $B_{12}^{III}OH$ = hydroxocobalamin; $B_{12}^{III}OH_2$ = aquocobalamin; $B_{12}^{III}CH_3$ = methylcobalamin; B_{12}^{II} = cobalamin in which the metal has a formal valency of two as a result of a one-equivalent reduction of $B_{12}^{III}OH_2$; $B_{12}^{III}H$ = hydridocobalamin or alternatively cobalamin in which the metal has a formal valency of one rather than having a hydrido ligand; ESR = electron spin resonance.

Diaquocobinamide is a hydrolysis product of $B_{12}^{III}OH_2$ in which the dimethylbenzimidazolyl–sugar phosphate has been detached from the corrin as well as from the metal atom and its coordination position filled by a water molecule.

METHODS

After recrystallizing $B_{12}^{III}OH_2$ (Glaxo) from aqueous acetone it was washed with dry acetone and stored in a desiccator. Its molecular weight was taken to be 1580, a value based on its absorbancy (in aqueous solution) at 351 mμ and the relation between absorbancy and concentration given by Smith *et al.* (*6*). $B_{12}^{III}CN$ and cyanoaquocobinamide (both Glaxo) were used without further purification. Paper electrophoresis on Whatman 3 mm paper with a potential gradient of 7 V/cm, in phosphate duffer at pH 6.5, and also in 0.5 *m* acetic acid, indicated negligible impurity in the $B_{12}^{III}CN$ and cyanoaquocobinamide and a few percent of an uncharged molecular species in the $B_{12}^{III}OH_2$. Diaquocobinamide was prepared in a crude state by hydrolysis of $B_{12}^{III}OH_2$ with perchloric acid by the method of Armitage *et al.* (*7*); B_{12}^{II} and $B_{12}^{III}H$ used in the preliminary experiments were prepared by reduction of $B_{12}^{III}OH_2$ with $NaBH_4$.

Reagents other than corrinoids were AR grade, used without further purification. Carbon monoxide, hydrogen, and argon were freed from interfering impurities by passage through a 15% pyrogallol solution in 50% aqueous KOH (two scrubbers in series, containing strips of filter paper to increase the surface area of liquid exposed to the gas), through a solution containing 5×10^{-3} *m* $B_{12}^{III}OH_2$ and B_{12}^{II} in phosphate buffer of pH 8, through a trap cooled in liquid oxygen, and finally through distilled water.

Manometric measurements were carried out by conventional Warburg techniques. Until deoxygenation of the reaction vessel had been completed, the corrinoid reactant, in crystalline form, was contained in a breakable ampoule lying in 2 ml of 0.1 *m* buffer solution in the main compartment of the vessel. The center well contained 0.2 ml of 0.1 *m* NaOH (with the usual wick of filter paper of a type which in NaOH solution does not evolve gas). Buffers used were sodium phosphate (pH 6–8), sodium phosphate–citrate (pH 4–6), and sodium sulfate (pH 1–4), all at a final concentration of 0.1 *m*. In most experiments the buffer solution contained ethylenediaminetetracetic acid to 0.02 *m*.

When measuring H_2 uptake at 35°C by solutions containing B_{12}^{II}, $B_{12}^{III}OH_2$ was reduced by shaking 2.0 ml of a 4.75×10^{-3} *M* solution with CO at 35°C and pH 6.0, the CO and CO_2 flushed out with argon at 20°C, then H_2 added at 0°C while adjusting the pH to 8 or 10 by addition of aqueous NaOH. In some experiments 0.2 ml of 0.04 *m* methyl viologen solution was added to the cobalamin.

Where B_{12}^{II} was formed in the presence of methyl iodide the technique was as follows: 4 ml of a 4.12×10^{-5} *m* aqueous solution of $B_{12}^{III}OH_2$ buffered at pH 7.99, 6.02, or 4.04 was pipetted into the main compartment of an optical quartz Thunberg tube of 1-cm light path. The second compartment contained 0.1 ml of methyl iodide beneath 0.5 ml of distilled water, cooled by an ice bath. Degassing of the liquids was accomplished by passing argon through two polythene capillary tubes, sufficiently fine and flexible that they could be insinuated into one of the compartments via the small sidearm of the Thunberg tube. After tipping the methyl iodide into the $B_{12}^{III}OH_2$ solution, CO was bubbled through the mixture at 20°C. At intervals, the optical density of the reaction mixture was determined at 311 and 475 mμ (absorption bands of B_{12}^{II}) and at 339, 370, and 514 mμ (absorption bands of methylcobalamin).

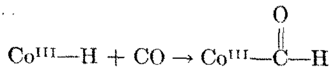
Optical absorption was measured with a Beckman DK2 spectrophotometer; ESR absorption with a Varian V4501 spectrometer, using a copper sulfate solution as standard when determining spin concentration.

RESULTS

Reaction with Carbon Monoxide

Usually it is the lower oxidation states of the transition metals which favor coordination to carbon monoxide. We were therefore not surprised to find that negligible change could be detected when CO was allowed to stand over an aqueous solution of $K_2[Co^{III}$

$(CN)_5OH_2]$ in the dark (*8*); nor that carbon monoxide allowed to stand over reduced pentacyanocobaltate was hydrogenated to a variety of organic compounds (*9*). It was reasonable to expect that reduced cobalamins would hydrogenate CO; also, that by analogy to the combination of acetylene with $B_{12}^{III}H$ (*10*) carbon monoxide would undergo the following reaction (in which only one of the ligands of the cobalt ion is shown):



$$Co^{III}—H + CO \rightarrow Co^{III}—\overset{\overset{O}{\|}}{C}—H$$

But a comprehensive set of manometric and spectrophotometric experiments at a number of pH values in the range 5.76 to 9.1 failed to demonstrate any reaction whatsoever between CO and $B_{12}^{III}H$, or B_{12}^{II}.

On the other hand when a little O_2 was admitted to a vessel containing CO which was being shaken with a solution of B_{12}^{II}, gas uptake commenced and proved to be greater in amount than could be accounted for by autoxidation of B_{12}^{II} to $B_{12}^{III}OH_2$. A gas was produced, and by mass spectrometry it was found to be CO_2. No other products were detected in the gas phase. Thus the overall reaction appeared to be the autoxidation of CO to CO_2, catalyzed by a cobalamin

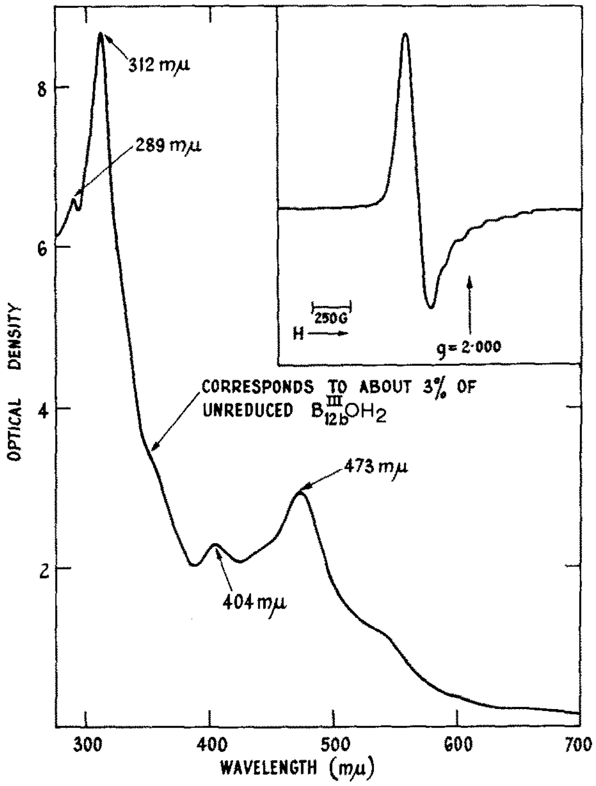


FIG. 1. Optical and ESR absorption spectra of solutions obtained by reducing $B_{12}^{III}OH_2$ with carbon monoxide: cobalamin concentration 4×10^{-6} *M*; pH 7.3 buffer, 0.05 *M* in phosphate and 0.02 *M* in EDTA; optical spectra measured at 35°C, ESR at −100°C.

$$2CO + O_2 \rightarrow 2CO_2 \qquad (1)$$

That the first step in the catalysis was a reaction between CO and the cobaltic form of the cobalamin was demonstrated by the following result:

Purified carbon monoxide was shaken with a 4.75×10^{-3} *M* solution of $B_{12}^{III}OH_2$ at 35°C and pH 7.3 in the strict absence of O_2 in a Warburg vessel containing NaOH in the center well. The gas pressure fell until it corresponded to an uptake of 0.48 moles of

CO per mole of $B_{12}^{III}OH_2 + OH$ (where $B_{12}^{III}OH_2 + OH$ represents aquocobalamin plus hydroxocobalamin). A diluted aliquot of the resulting solution, examined under anaerobic conditions, had the optical absorption spectrum shown in Fig. 1, which fitted the absorption characteristics of B_{12}^{II} given by Tackett, Collat, and Abbott (*11*). Failure of 3% of the $B_{12}^{III}OH_2 + OH$ to react would account for the weak bands at 350 and 530 mμ. No complexes other than $B_{12}^{III}OH_2$, $B_{12}^{III}OH$, and B_{12}^{II} were detected at any time during reduction. The figure also shows the eight-line ESR signal of a second aliquot of the solution, a signal strongly resembling that given by Hogenkamp, Barker, and Mason (*12*) for B_{12}^{II} and confirming that the metal ion has been reduced to an odd-electron state. In magnitude, the signal corresponded to 0.8 spins per cobalt atom if all were assumed to have been reduced. No free radicals were detected during reaction.

The first half of the catalytic cycle summarized by reaction (1) can now be written as either

$$CO + 2B_{12}^{III}OH_2 \rightarrow CO_2 + 2\,B_{12}^{II} + H_2O \qquad (2)$$

or

$$CO + 2B_{12}^{III}OH \rightarrow CO_2 + 2B_{12}^{II} + H_2O + 2H^+ \qquad (3)$$

Since the pK for deprotonation of $B_{12}^{III}OH$ is about 7.8 (*13*), and since the pH optimum for CO uptake is 5.7 at 35°C (Fig. 2), reaction (2) is the more likely of the two alternatives.

Although two molecules of the cobalamin were required to oxidize one molecule of CO, the rate of reaction at a given pH seemed to be a linear function of $[B_{12}^{III}OH_2 + OH]$ (and therefore of $[B_{12}^{III}OH_2]$) as well as [CO]. If this were so, the rate of reaction could be described by

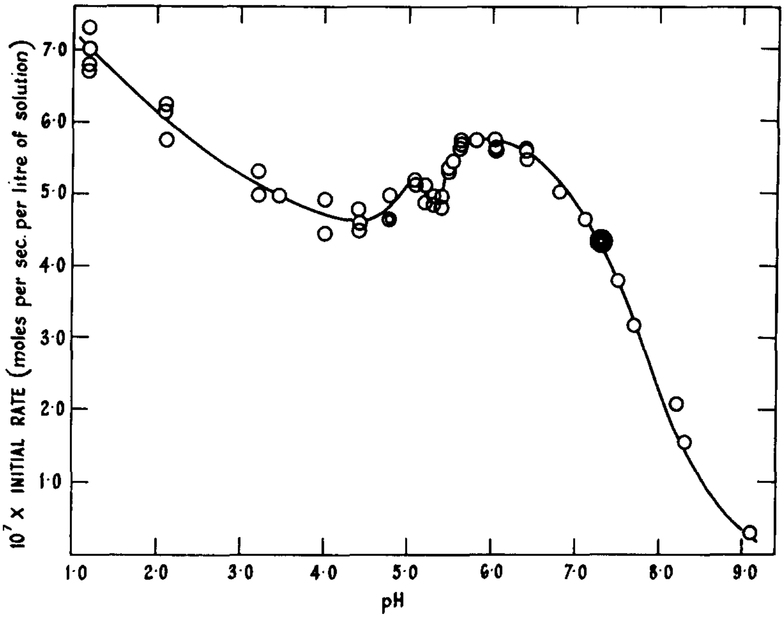


FIG. 2. Dependence on pH of the initial rate of carbon monoxide uptake by $B_{12}^{III}OH_2$ solutions. Cobalamin concentration 4.75×10^{-3} *M*; 0.1 *M* buffer, 0.02 *M* in EDTA; reaction temperature 35°C; CO pressure 0.904 atm.

$$dx/dt = k\,[B_{12}^{III}OH_2][CO] \qquad (4)$$

where *k* is the rate constant in liter $mole^{-1}$ sec^{-1}; *x*, the number of moles of CO consumed by 1 liter of reactant solution at time *t*; and *t* is the time in seconds after reaction commences. (In deriving *x* from changes in gas pressure, the molar volume of CO was taken to be 22.41 liter at 0°C and 1 atm.) Under our experimental conditions, there was negligible change in [CO] during the course of an experiment, compared with the changes in $[B_{12}^{III}OH_2]$, and the integrated form of Eq. (4) could be written

$$\ln[B_0/(B_0 - 2x)] = 2kKPt \qquad (5)$$

where B_0 is the initial concentration of $B_{12}^{III}OH_2$, P is the partial pressure of CO in atm, and K is the Henry's law constant for the assumed relationship between [CO] and P

$$[CO] = KP$$

When the experimental results were plotted according to Eq. (5), a family of straight lines through the origin was obtained. From their slopes, the initial rates of reaction could be calculated from the expression

$$\text{initial rate} = \text{slope}(B_0/2)$$

In Fig. 3 the initial rates at pH 7.3 and 35°, when P is 0.904, are plotted as a function of $[B_{12}^{III}OH_2 + OH]$. It is seen that the relationship is indeed linear.

A linear relation was also found between the initial rate and the partial pressure of CO, over the range investigated (P = 0.19 to 0.9).

From an Arrhenius plot (Fig. 4) it was found that the apparent activation energy of reaction (2) was 12.3 kcal/mole of CO taken up. Account was taken of the temperature dependence of the solubility of CO in water, and of the vapor pressure of water in the Warburg vessels, but the effect of the buffer salts on the solubility of CO was neglected.

At pH 7.3, at temperatures from 25° to 65°C and CO pressures from 0.19 to 0.91 atm, the rate equation could be written

$$\text{rate of CO uptake} = (6 \times 10^7) \times e^{-12\,300/RT}[B_{12}^{III}OH_2 + OH][CO]$$
moles per liter of solution per sec

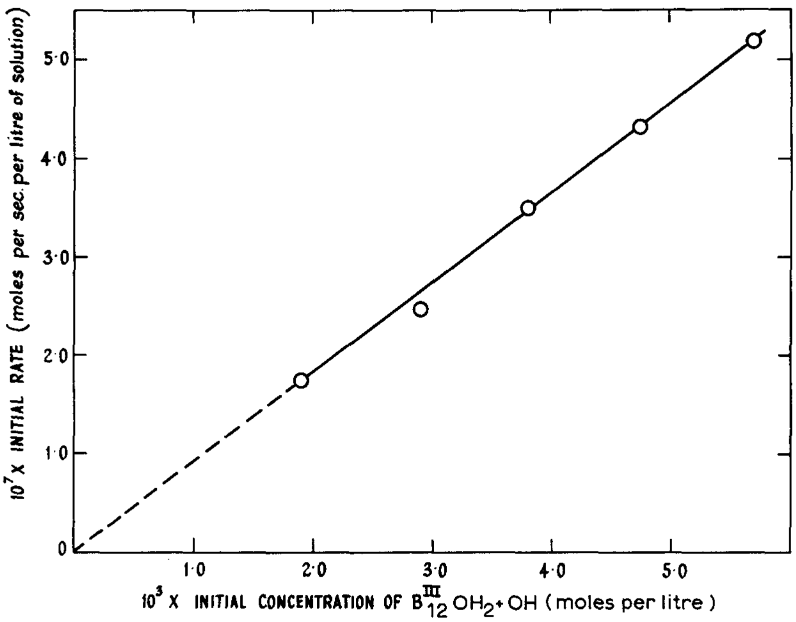


FIG. 3. Dependence of initial rates of carbon monoxide uptake on the total cobalamin concentration: pH 7.3 buffer; reaction temperature 35°C; CO pressure 0.904 atm.

In addition to clear maxima at pH 5.7 and at <1.0, the curve in Fig. 2, representing the dependence of the reaction rate on hydrogen ion concentration, has a small sharp peak at pH 5.1 which we believe to be significant. Change of buffer from citrate-phosphate to phosphate or to sulfate at a given pH did not alter the rate of CO uptake.

Cyanide ion and benzimidazole strongly inhibited CO uptake by $B_{12}^{III}OH_2$. As expected, $B_{12}^{III}CN$ and cyanoaquocobinamide had little reactivity towards carbon monoxide. But diaquocobinamide proved to be about twice as reactive as $B_{12}^{III}OH_2$, i.e., replacement of the nucleotide of the cobalamin by an aquo group enhanced the activity.

When methyl iodide was present during the reduction of $B_{12}^{III}OH_2$ by CO, the final product (other than CO_2) was a mixture of B_{12}^{II} and $B_{12}^{III}CH_3$. By plotting as a function of time the concentrations of both products, curves were obtained which suggested that the rate of $B_{12}^{III}CH_3$ formation was more closely related to the B_{12}^{II} concentration than to the concentration of an intermediate in the conversion of $B_{12}^{III}OH_2$ to B_{12}^{II}. Due to inaccuracies in the spectrophotometric determination of the components of the reaction mixture, we were unable to prove that the $B_{12}^{III}CH_3$ was formed from B_{12}^{II}. It is certain however that with our reagents and gassing techniques, methyl iodide which was added after formation of the B_{12}^{II} slowly converted the latter to $B_{12}^{III}CH_3$ in high yield at room temperature. The rate was comparable to the rate at which $B_{12}^{III}OH_2$ could be reduced by CO and was very much less than the rate of interaction of methyl iodide with $B_{12}^{III}H$. [Dolphin and Johnson (*14*) claim that B_{12}^{II} does not react with methyl iodide in the absence of sulfur-containing compounds.]

Reaction with Hydrogen

In an early paper on cobalamin chemistry, Jaselskis and Diehl (15) remarked in passing that they had observed an uncatalyzed reaction between H_2 and $B_{12}^{III}OH$. When attempting to reproduce their result we bubbled unpurified tank hydrogen through aqueous solutions of $B_{12}^{III}OH$ and $B_{12}^{III}OH_2$. Some reduction to B_{12}^{II} did indeed occur, but the reaction was eliminated when the hydrogen was first passed through a scrubber containing a strong solution of $B_{12}^{III}OH_2$. We estimate that 3 ppm of CO in the tank hydrogen would account for the reduction observed.

When solutions of B_{12}^{II} at pH 8 or 10 were shaken with H_2 at 35° for 24 hr, negligible gas uptake was observed. Addition of methyl viologen to act as hydrogen acceptor had little or no effect on the amount of H_2 consumed.

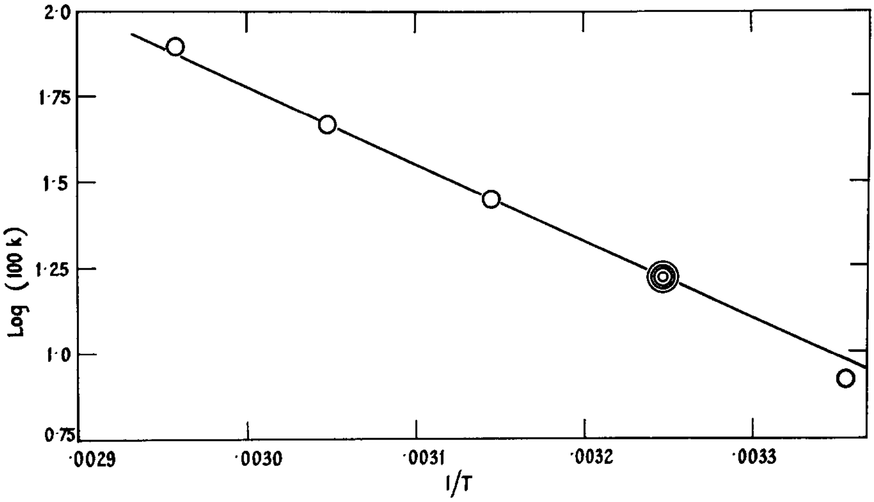


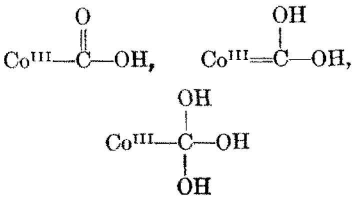
FIG. 4. Arrhenius plot showing temperature dependence of the initial rate of carbon monoxide uptake: cobalamin concentration 4.75 × 10^{-3} *M*; buffer pH 7.3; CO pressure 0.713 to 0.929 atm (all rates corrected to 0.904 atm).

DISCUSSION

Reaction with Carbon Monoxide

From the kinetic and stoichiometric information presented we conclude that the slowest step in reaction (2) involves one cobalamin molecule and is followed by a succession of more rapid reactions, one of which involves two cobalamin molecules and yields two molecules of B_{12}^{II}. Since the activity in alkaline solution tapers off towards high pH, in a manner expected for a hydrogen-ion-dependent process, we deduce that aquocobalamin reacts with CO but hydroxocobalamin does so either more slowly or not at all.

The conceivable intermediates between carboxycobalamin and carbonic acid are



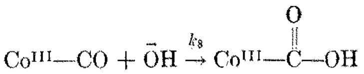
$$\mathrm{Co^{III}-\overset{O}{\overset{\|}{C}}-OH,\quad Co^{III}{=}\overset{OH}{\overset{|}{C}}-OH,\quad Co^{III}-\underset{OH}{\underset{|}{\overset{OH}{\overset{|}{C}}}}-OH}$$

or their deprotonated forms. Of these we prefer the first; the second, if produced, is likely to be an intermediate in the formation of the first; if the third proved to be the correct alternative it would not greatly alter the argument which follows. A mechanism can now be proposed to describe the kinetics of CO uptake in neutral and alkaline solution:

$$\mathrm{Co^{III}{-}OH_2 + H_2O \overset{K_6}{\rightleftharpoons} Co^{III}{-}OH + H_3O^+} \quad (6)$$

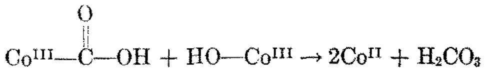
$$\mathrm{Co^{III}{-}OH_2 + CO \xrightarrow[\text{slow}]{k_7} Co^{III}{-}CO + H_2O} \quad (7)$$

followed by the faster reactions



$$\mathrm{Co^{III}{-}CO + \bar{O}H \xrightarrow{k_8} Co^{III}-\overset{O}{\overset{\|}{C}}-OH} \quad (8)$$

and



$$\mathrm{Co^{III}-\overset{O}{\overset{\|}{C}}-OH + HO{-}Co^{III} \rightarrow 2Co^{II} + H_2CO_3} \quad (9)$$

If we are correct in believing that steps (6) and (7) control the overall rate in the way indicated, we may write

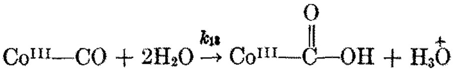
$$\text{Rate} = k_7[\mathrm{B_{12}^{III}OH_2}][\mathrm{CO}]$$
$$= k_7/[\mathrm{B_{12}^{III}OH}][\mathrm{H_3\overset{+}{O}}][\mathrm{CO}]/K_6 \quad (10)$$

which can be shown to describe the experimental results with reasonable accuracy at pH values as low as 5.6 when pK_6 is taken to be 7.8, the value determined by Hayward *et al.* (*13*).

In the pH range 5.6–5.4 the decline in rate suggests that $[\bar{O}H]$ is limiting, i.e., step (8) has been slowed sufficiently to influence the overall rate. Step (7) on the other hand has been accelerated by conversion of practically all of the $\mathrm{B_{12}^{III}OH}$ to $\mathrm{B_{12}^{III}OH_2}$ and we now write in place of reaction (7) the equilibrium*

$$\mathrm{Co^{III}{-}OH_2 + CO \overset{K_7}{\rightleftharpoons} Co^{III}{-}CO + H_2O} \quad (7a)$$

Below a pH of about 5.0 the rate of step (8) is taken to be negligible. There must be an alternative route to the carboxyl complex which is operative even when the solution is made strongly acid. It is assumed to be



$$\mathrm{Co^{III}{-}CO + 2H_2O \xrightarrow{k_{11}} Co^{III}-\overset{O}{\overset{\|}{C}}-OH + H_3\overset{+}{O}} \quad (11)$$

and to have a rate constant about 3×10^{10} times smaller than k_8. The rate equation at pH 5.6–5.4 then becomes

$$\text{Rate} = k_8[\mathrm{Co^{III}{-}CO}][\bar{O}H] + k_{11}[\mathrm{Co^{III}{-}CO}]$$
$$= K_7[\mathrm{B_{12}^{III}OH_2}][\mathrm{CO}](k_8[\bar{O}H] + k_{11}) \quad (12)$$

Below pH 5.4 the process (7a) + (11), followed by more rapid steps, is unable to describe the experimentally determined rate curve (Fig. 3) without contributions from other processes for carbon monoxide oxidation. As the hydrogen ion concentration is increased two supplementary mechanisms are introduced. The first, resulting in a maximum at pH 5.1, we shall not attempt to explain until further evidence is available. The second, resulting in a maximum at pH < 1.1, is with little doubt a consequence of breaking the bond between nucleotide and cobalt ion. It is known that in strong acid the nucleotide is detached by protonation (*16*), and we have shown that diaquocobinamide (in which the nucleotide has been completely removed from the cobalamin) has a greater activity for CO oxidation than does $\mathrm{B_{12}^{III}OH_2}$. Thus the first two steps in the low pH mechanism are the conversion of $\mathrm{B_{12}^{III}OH_2}$ to diaquocobalamin, followed by displacement of one of the aquo groups by CO.

---

[*] Careful spectrophotometric measurements over the range 250–600 mμ showed that the cobalamin is insensitive to hydrogen ion concentration in the neighborhood of pH 5. Thus the rate-limiting step in CO oxidation cannot be one involving $\mathrm{B_{12}^{III}OH_2}$ in the pH range 5.6–5.4.

The course of the oxidative half of the catalytic cycle, in which O_2 reacts with reduced catalyst, has been in part elucidated and will be the subject of a later paper.

The mechanisms outlined above should contribute to the understanding of the intramolecular methyl transfer reactions which are catalyzed by enzymes containing coenzyme B_{12}, since they involve the making and breaking of metal–alkyl and metal–acyl bonds and possibly the interconversion of acyl and keto groups (*17*).

As a method of preparing B_{12}^{II}, the reduction of $B_{12}^{III}OH_2$ by CO is proving to be particularly useful in spectrophotometric studies in which it is important to minimize the concentration of interfering reagents.

Reaction with Hydrogen

A hydrogen molecule can undergo a fast reaction with two pentacyanocobaltate (II) ions in aqueous solution at low temperatures to give two hydrido complex ions $[HCo^{III}(CN)_5]^{3-}$ (*1*). Our failure to detect an analogous reaction between H_2 and B_{12}^{II} can be attributed to a lower energy level of the unpaired electron in B_{12}^{II} or to steric hindrance by the acetamide groups, which prevent close approach of the metal ions of two colliding cobalamin molecules, according to D. C. Hodgkin [quoted in ref. (*16*)]. We are unwilling to accept the observation by Jaselskis and Diehl (*15*) that H_2 can reduce $B_{12}^{III}OH$ without the intervention of a catalyst. A possible explanation of their result is that the hydrogen used contained traces of CO.

ACKNOWLEDGMENTS

The ESR measurements were carried out by Mr. F. D. Looney, of this Division. We wish to thank Dr. J. A. Wunderlich and Mr. A. C. K. Triffett for mass spectrometric analyses, and Glaxo-Allenburys (Australia) Pty. Ltd. for a gift of cyanoaquocobinamide.

REFERENCES

1. KING, N. K., AND WINFIELD, M. E., *J. Am. Chem. Soc.* **83,** 3366 (1961).
2. GRIFFITH, W. P., AND WILKINSON, G., *J. Chem. Soc.,* p. 1629 (1959).
3. BARKER, H. A., WEISSBACH, H., AND SMYTH, R. D., *Proc. Natl. Acad. Sci. U.S.* **44,** 1093 (1958).
4. LENHERT, P. G., AND HODGKIN, D. C., *Nature* **192,** 937 (1961).
5. INGRAHAM, L. L., *Ann. New York Acad. Sci.* **112,** 713 (1964).
6. SMITH, E. L., MARTIN, J. L., GREGORY, R. J., AND SHAW, W. H. C., *Analyst* **87,** 183 (1962).
7. ARMITAGE, J. B., CANNON, J. R., JOHNSON, A. W., PARKER, L. F. J., SMITH, E. LESTER, STAFFORD, W. H., AND TODD, A. R., *J. Chem. Soc.,* p. 3849 (1953).
8. BAYSTON, J. H., BEALE, R. N., KING, N. K., AND WINFIELD, M. E., *Australian J. Chem.* **16,** 954 (1963).
9. WINFIELD, M. E., Annual General Meeting Australian Biochem. Soc., January 1965.
10. JOHNSON, A. W., MERVYN, L., SHAW, N., AND SMITH, E. L., *J. Chem. Soc.,* p. 4146 (1963).
11. TACKETT, S. L., COLLAT, J. W., AND ABBOTT, J. C., *Biochemistry* **2,** 919 (1963).
12. HOGENKAMP, H. P. C., BARKER, H. A., AND MASON, H. S., *Arch. Biochem. Biophys.* **100,** 353 (1963).
13. HAYWARD, G. C., HILL, H. A. O., PRATT, J. M., VANSTON, N. J., AND WILLIAMS, R. J. P., *J. Chem. Soc.,* p. 6485 (1965).
14. DOLPHIN, D. H., AND JOHNSON, A. W., *Proc. Chem. Soc.,* p. 311 (1963).
15. JASELSKIS, B., AND DIEHL, H., *J. Am. Chem. Soc.* **76,** 4345 (1954).
16. SMITH, E., LESTER, MERVYN, L., MUGGLETON, P. W., JOHNSON, A. W., AND SHAW, N., *Ann. New York Acad. Sci.* **112,** 565 (1964).
17. WHITLOCK, H. W., *Ann. New York Acad. Sci.* **112,** 721 (1964).