A Mononuclear Hydroperoxo Complex and Its Significance in Catalytic Oxidation Mechanisms

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Evidence for formation of a mononuclear complex containing peroxide during autoxidation of $[(CN)_5Co^{III}H]^{3-}$ has been completed by preparation and analysis of $K_3[(CN)_5Co^{III}OOH]$.

Three steps have been distinguished in the hydrolysis of $[(CN)_5Co^{III}-O-O-Co^{III}(CN)_5]^{6-}$ to $[(CN)_5Co^{III}OH_2]^{2-}$, the second step yielding $[(CN)_5Co^{III}OOH]^{3-}$. The acid dissociation pK for $[(CN)_5Co^{III}-O-OH-Co^{III}(CN)_5]^{5-}$ is approximately 12; the pH required for rapid hydrolysis of $[(CN)_5Co^{III}OOH]^{3-}$ is 4–5 pH units less than that for the dinuclear complex.

A discussion is given of the role of hydroperoxo complexes, and their ease of hydrolysis compared with their reduction, in mechanisms of O_2 uptake and H_2O_2 decomposition. An example is provided by the formation of $[(CN)_5Co^{III}OOH]^{3-}$ during the oxidation of H_2 by O_2, catalyzed by $[(CN)_5Co^{III}H]^{3-}$.

INTRODUCTION

Intermediates of the type M–OOH have appeared in many of the mechanisms which have been proposed for the catalysis of H_2O_2 decomposition (*1–12*) and in a few of the mechanisms for autoxidation in the presence of metal ions (*6, 9, 13*). M represents the ion of a transition metal such as iron or cobalt, coordinated to water or other ligands. A literature search back to 1905 failed to find an example in which a mononuclear hydroperoxo complex of a transition metal has been isolated or its structure established. When, therefore, we found evidence for the formation of such a compound in the autoxidation of $[(CN)_5Co^{III}H]^{3-}$ (*14*) it seemed important to attempt its isolation and analysis. We had shown that the complex can be formed alternatively by hydrolysis of $[(CN)_5Co^{III}-O-O-Co^{III}(CN)_5]^{6-}$, which can be prepared in a high state of purity and is therefore a convenient starting material.

EXPERIMENTAL

A brief study of the hydrolysis of $[(CN)_5Co^{III}-O-O-Co^{III}(CN)_5]^{6-}$ is necessary to determine suitable conditions for prepara-tion of the mononuclear complex, which is itself subject to hydrolysis.

Hydrolysis of the Dinuclear Peroxo Complex

If pure $K_6[(CN)_5Co^{III}-O-O-Co^{III}(CN)_5]$, prepared by the method of Bayston et al. (*14*), is dissolved in water to form a 0.07M solution (at 0.4°C in an argon atmosphere) the pH is at first 12.98. [The high pH electrodes were standardized at 0.4°C at pH 13.43 in $Ca(OH)_2$ solution prepared by the method of Bates, Bower, and Smith (*15*)]. Addition of 0.5 N aqueous HCl results in the titration curve of Fig. 1. During titration the characteristic absorption band of $[(CN)_5Co^{III}-O-O-Co^{III}(CN)_5]^{6-}$ at 327 mμ (*14*) is replaced by one at 300 mμ; the solution changes from reddish brown to pale yellow.

If KOH is now added, the 300 mμ complex is immediately converted to $[(CN)_5Co^{III}-O-O-Co^{III}(CN)_5]^{6-}$. If on the other hand it is allowed to stand for about 20 min at 0°C it is converted irreversibly* to two

* Evidence for the irreversibility is given in a later section.

compounds,† one absorbing at 272 mμ and the second at 380 mμ. On addition of more acid the 272 mμ complex is hydrolyzed irreversibly* to $[(CN)_5Co^{III}OH_2]^{2-}$, identified by its absorption at 380 and 235 mμ (16, 17).

From Fig. 1A it is deduced that the pK for dissociation of a proton from the 300 mμ complex is close to 12. We are not able to detect reversibility on rapid addition of

for the mononuclear complex than for the dinuclear.

Preparation and Analysis of the Hydroperoxo Complex

Air is removed by a stream of argon from a closed reaction vessel which contains 30 ml of water at 0.4°C and is equipped with elec-
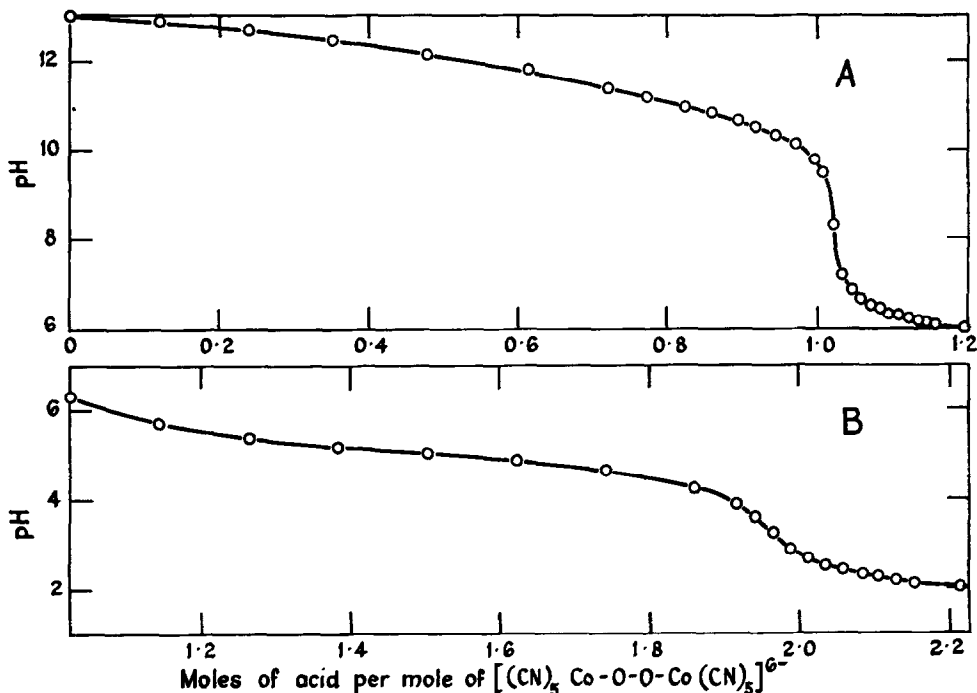

FIG. 1. Titration of $K_6[(CN)_5Co^{III}-O-O-Co^{III}(CN)_5]$ with dilute HCl at 0.4°C in an atmosphere of argon. Spectrophotometric measurements show that at the first point of inflection (Curve A) the conjugate acid $[(CN)_5Co^{III}-O-OH-Co^{III}(CN)_5]^{5-}$ has been hydrolyzed to $[(CN)_5Co^{III}-OOH]^{3-}$. At the second point of inflection (Curve B) $[(CN)_5Co^{III}-OOH]^{3-}$ has been hydrolyzed to $[(CN)_5Co^{III}-OH_2]^{2-}$. Curve A: First pH measured 3 min after adding $K_6[(CN)_5Co^{III}-O-O-Co^{III}(CN)_5]$ to water; addition of 1 equivalent of acid per mole of complex completed 18 min later. Curve B: First pH measured 1 min after adding $K_6[(CN)_5Co^{III}-O-O-Co^{III}(CN)_5]$ to water containing 1.02 equivalents of acid per mole of complex; addition of 2 equivalents of acid completed 11 min later.

acid, followed by alkali, to the 272 mμ complex, and therefore cannot give a corresponding pK nor show that the complex is hydrolyzed in two steps.

By observing the rate of hydrolysis at different pH values, it can be shown that the pH at which it would be practicable to detect peroxide coordinated to cobalt as an intermediate in autoxidation is 4–5 units lower

† By making use of the molar absorbancies given previously (14) it can be shown that the amounts of the two products are approximately equimolar.

trodes for pH measurement at high alkalinity. A minute after adding 1.5 g of $K_6[(CN)_5Co^{III}-O-O-Co^{III}(CN)_5]4H_2O$, deoxygenated precooled 0.4 M acetic acid is admitted dropwise until the pH is 9.95 (5 ml of acid in 9 min). At this time 150 ml of cold methanol are admitted, followed by enough acetone (100 ml) to just begin precipitation of the 272 mμ complex. The mixture is placed in the deep freeze (−19°C) for 5 min; 25 ml of acetone are added and 6 min later another 50 ml. After standing for

10 min the mixture is filtered rapidly under an argon shield, washed thoroughly with acetone, freed from acetone by passing a stream of argon through the stirred crystals and finally stored under argon in the deep freeze. (The role of argon in the later stages of the preparation is to exclude water vapor rather than oxygen.)

If a yellow oil is obtained after following the above directions for crystallization, the procedure should be repeated with vigorous rubbing of the walls of the reaction vessel with a glass rod. It is only when the purity of the product is high that crystallization can be induced. Every effort must be made to minimize oxidation of the dinuclear complex to $[(CN)_5Co^{III}-O-O-Co^{IV}(CN)_5]^{5-}$ (16, 14) or hydrolysis of the 272 mμ complex. Separation of the latter is dependent upon its smaller solubility than $K_2Co^{III}(CN)_5OH_2$ in methanol and in aqueous methanol, as well as on the greater difficulty in crystallizing the aquo complex. A cleaner separation can be achieved at pH values approaching neutrality than at the high pH needed to strongly inhibit hydrolysis of the 272 mμ complex. The pH of 9.7-9.9 chosen for the separation seems to be a good compromise between excessive hydrolysis and excessive coprecipitation of $K_2Co(CN)_5OH_2$.

Analysis for peroxide (14) shows that the crude product after vacuum drying at 0°C contains 8.8% $O_2{}^{2-}$. Two recrystallizations from 1 M KOH by methanol precipitation increases the figure to 9.0%. Since KOH increases the coprecipitation of $K_2Co^{III}(CN)_5OH_2$, we cannot hope to obtain pure $K_3Co^{III}(CN)_5OOH$ by crystallization from strongly alkaline solutions. Yet KOH must be added to suppress hydrolysis. By microanalysis the recrystallized material is found to contain 18.5% Co, 32.7% K, 18.0% C, 20.5% N; calculated values for $K_3Co(CN)_5OOH$ are 17.3% Co, 34.2% K, 17.6% C, 20.5% N, 9.4% $O_2{}^{2-}$. The discrepancies are believed due to the presence of about 4% of $K_2Co(CN)_5OH_2$ and to the inherent difficulties in microanalysis of cyano complexes.

Properties of $K_3Co(CN)_5OOH$

Unless thoroughly dried the compound may suffer appreciable decomposition within a few hours. In solution in 2 M aqueous KOH it is stable over long periods and has the absorption spectrum shown in Fig. 2. Since the molar absorbancy near 370 mμ is twice as great as that of $[(CN)_5Co^{III}OH]^{3-}$ (14), the principal band in the 360–380 mμ region
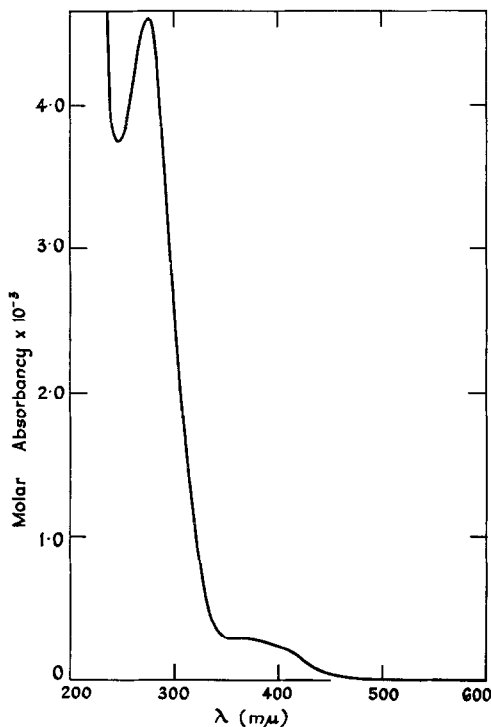
FIG. 2. Absorption spectrum of $K_3[(CN)_5Co^{III}-OOH]$ in aqueous 2 M KOH at 0°C. After correcting for 4% $K_2[(CN)_5Co^{III}-OH_2]$, formed by hydrolysis during recrystallization, the molar absorbancy is estimated to be 4440. [A provisional value of 4000 was given by Bayston et al. (14).]

cannot be attributed to the hydroxo complex, nor to the related aquo complex or its polymer (16). Nor is it due to traces of unhydrolyzed $[(CN)_5Co^{III}-O-O-Co^{III}(CN)_5]^{6-}$, which can be identified by its reaction with O_2 (16, 18). The bright yellow color of the hydroperoxo complex prepared as we have described is therefore assumed to be the color of the pure compound.

At pH 7 the principal absorption band is at 272 rather than 274 mμ (14), indicating that the absorption at 274 mμ is due to $[(CN)_5Co^{III}-O-O]^{4-}$. The pK for the dissociation is about 12. At neutral pH hydrolysis is too fast to permit accurate determina-

tion of the molar absorbancy at 272 mμ. At pH 5 hydrolysis is complete in about 2 min at 0°C at a cobalt concentration of $2 \times 10^{-4} M$. The aquo complex which results from hydrolysis is accompanied by its polymer in proportions which are probably determined by concentration, temperature, and pH. The irreversibility of the hydrolysis is indicated by the constancy of the absorption spectrum of a solution containing H_2O_2 (8 M), $[(CN)_5Co^{III}OH]^{3-}$ (3 $\times$ 10^{-4} M) and KOH (2 M) observed over 17 hr. (Similarly the irreversibility of the hydrolysis of the dinuclear peroxo complex can be demonstrated by prolonged spectrophotometric examination of an equimolar mixture of purified $K_3[(CN)_5Co^{III}OOH]$ and $K_2[(CN)_5Co^{III}OH_2]$ in 1 M KOH. No increase in absorption at 327 (or 485) mμ is detected, nor any significant change at 371 (14) or 274 mμ.)

The hydroperoxo complex is insoluble in organic solvents. It is not attacked by O_2, at least in alkaline solution. Like other pentacyanocobaltate(III) complexes it is reduced to $[(CN)_5Co^{III}H]^{3-}$ by NaBH$_4$.

Discussion

Hydrolysis

Haim and Wilmarth (16) have shown that the complete hydrolysis of the dinuclear peroxo complex requires two equivalents of acid per mole and yields one mole of H_2O_2 per mole of complex. They write the reaction

$$[(CN)_5Co^{III}-O-O-Co^{III}(CN)_5]^{6-} + 2H^+$$
$$+ 2H_2O \rightarrow H_2O_2 + 2[(CN)_5Co^{III}OH_2]^{2-} \quad (1)$$
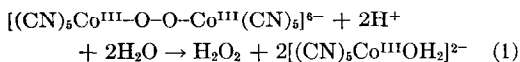
We have confirmed their statement that the conjugate acid of $[(CN)_5Co^{III}-O-O-Co^{III}-(CN)_5]^{6-}$ can be formed reversibly if the experiment is rapid and have proved that reaction (1) takes place via a hydroperoxo complex by showing that the substance which absorbs at 272 mμ contains, when crystallized as the potassium salt, one O_2^{2-}, five CN$^-$, and three K$^+$ per cobalt atom. We may therefore write:
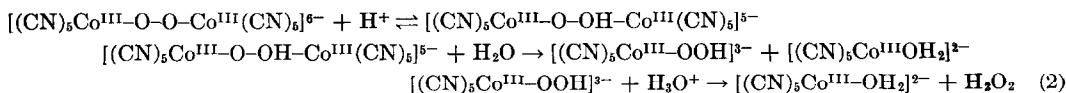
It is possible to distinguish the individual steps in the hydrolysis of $[(CN)_5Co^{III}-O-O-Co^{III}(CN)_5]^{6-}$ because the conjugate acid hydrolyzes at a convenient rate at pH 10, while at the same pH hydrolysis of the mononuclear hydroperoxo complex is slow. These are unlikely to be unique conditions—other ions of the type $L_5M^{III}-OOH$ may be found, in particular when L_5M^{II} or $L_5M^{II}-OH_2$ or L_5M^{II} is a complex which can react with O_2.

Elsewhere (14) we have shown that addition of O_2 to $[(CN)_5Co^{III}H]^{3-}$ yields the ion which absorbs at 272 mμ. Complexes of the type $L_5M^{III}H$ and L_5M^I should therefore be added to the above list. (M^I is a highly reduced state of a metal which tends to assume the formal valency state M^{III} when attached to the ligands L).

It appears to be far more difficult to form complexes of the type L_5M-OOH, in isolable amount, by addition of H_2O_2 to L_5M-OH_2. There may be complexes which are unable to react with O_2 when in the reduced form, yet in the oxidized state have a much higher affinity for $\bar{O}OH$ than does $[(CN)_5Co^{III}OH_2]^{2-}$. However the equilibrium concentration of the hydroperoxo complex will be very low in neutral or acid solution, significant only when there is a means of rapidly reducing the complexed peroxide. Thus any hypothesis for H_2O_2 decomposition in acid solution which proposes as the first step a reaction of the type

$$M + H_2O_2 \rightleftharpoons M - OOH + H^+ \quad (3)$$
$$\text{or}$$
$$M - HOOH$$
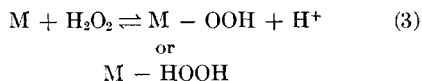
will be difficult to substantiate except by a kinetic demonstration of the reversibility of the step, such as that achieved by Chance (7).

M-OOH in Autoxidation Mechanisms

Since Vortman's preparation of $[(NH_3)_5Co^{III}-O-OH-Co^{III}(NH_3)_5](NO_3)_5$ in 1885 (19), a number of dinuclear peroxo complexes of cobalt, chromium, and rhenium

$$[(CN)_5Co^{III}-O-O-Co^{III}(CN)_5]^{6-} + H^+ \rightleftharpoons [(CN)_5Co^{III}-O-OH-Co^{III}(CN)_5]^{5-}$$
$$[(CN)_5Co^{III}-O-OH-Co^{III}(CN)_5]^{5-} + H_2O \rightarrow [(CN)_5Co^{III}-OOH]^{3-} + [(CN)_5Co^{III}OH_2]^{2-}$$
$$[(CN)_5Co^{III}-OOH]^{3-} + H_3O^+ \rightarrow [(CN)_5Co^{III}-OH_2]^{2-} + H_2O_2 \quad (2)$$

have been described, but in no case that we can find was a mononuclear peroxide established as an intermediate in either its formation or its subsequent hydrolysis. Calvin's (20) crystalline salicylaldehyde ethylenediiminocobalt(II), after oxygenation, probably contained O_2 attached at one end only to cobalt atoms, but there is no evidence as to whether the oxygen was present as peroxide.

L e m b e r g a n d Legge (6) suggested Fe^{III}–OOH as an intermediate in the autoxidation of ferrohemoglobin, while King and Winfield (9) wrote Fe^{II}–OOH and Fe^{III}–OOH as intermediates in the respiratory autoxidation catalyzed by cytochrome oxidase. Okunuki (21) has described experiments, on the reduction by ferrocyanide ion of the oxygenated form of cytochrome a, which we believe can be explained in terms of a hydroperoxo complex which is rapidly hydrolyzed:

$$Fe^{II}\text{–}OH_2 + O_2 \rightleftharpoons Fe^{II}\text{–}O\text{=}O$$
$$Fe^{II}\text{–}O\text{=}O + e \xrightarrow{H^+} Fe^{III}\text{–}OOH$$
$$Fe^{III}\text{–}OOH + H_2O \rightarrow Fe^{III}\text{–}OH + H_2O_2$$
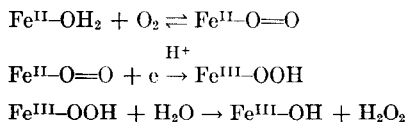
A mononuclear intermediate was proposed by Joyner and Wilmarth (13) as an intermediate in one of the reaction paths for autoxidation of Cr^{II} ion in an ammoniacal solution.
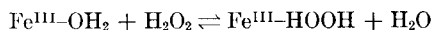
M–OOH in Mechanisms for H_2O_2 Decomposition

Coordinated hydroperoxide is a common feature in schemes to explain the catalysis of H_2O_2 decomposition by iron compounds.
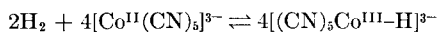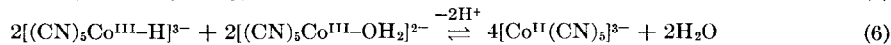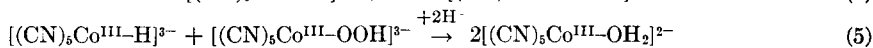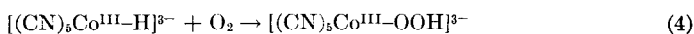
$$Fe^{III}\text{–}OH_2 + H_2O_2 \rightleftharpoons Fe^{III}\text{–}HOOH + H_2O$$

is reversible is the only substantial evidence for the reality of the hydroperoxide intermediate. As the authors point out, however, the reversibility may be explained alternatively in terms of a product in which H_2O_2 has combined with the porphyrin or protein in a way which retains the components of H_2O_2 within the complex,* rather than splitting off $H\dot{O}$ or $\bar{O}H$. Coordination of peroxide to the metal receives some support from an analysis of the optical and magnetic properties of the complex by Brill and Williams (10) who suggest that there are two components, one of which contains Fe^{III}–OOH or Fe^{III}–HOOH.

$[(CN)_5Co^{III}OOH]^{3-}$ as Intermediate in the Catalytic Autoxidation of Hydrogen

Proof of the structure of the cyanocobaltate complex which absorbs at 272 mμ confirms the hydride autoxidation reaction proposed earlier (14):

$$[(CN)_5Co^{III}\text{–}H]^{3-} + O_2 \rightarrow [(CN)_5Co^{III}\text{–}OOH]^{3-}$$

It can therefore be claimed with reasonable certainty that the hydroperoxo complex is an intermediate in one of the two principal cycles† responsible for the catalytic oxidation of H_2 in the following system: A solution containing $[Co^{II}(CN)_5]^{3-}$ is hydrogenated until most of the cobalt is present as the hydrido complex (17); O_2 is then admitted slowly. When the supply of O_2 is cut off, most of the cobalt gradually returns to the hydrido state, confirming the catalytic nature of the process, which we write,

$$[(CN)_5Co^{III}\text{–}H]^{3-} + O_2 \rightarrow [(CN)_5Co^{III}\text{–}OOH]^{3-} \tag{4}$$

$$[(CN)_5Co^{III}\text{–}H]^{3-} + [(CN)_5Co^{III}\text{–}OOH]^{3-} \xrightarrow{+2H^-} 2[(CN)_5Co^{III}\text{–}OH_2]^{2-} \tag{5}$$

$$2[(CN)_5Co^{III}\text{–}H]^{3-} + 2[(CN)_5Co^{III}\text{–}OH_2]^{2-} \underset{-2H^+}{\rightleftharpoons} 4[Co^{II}(CN)_5]^{3-} + 2H_2O \tag{6}$$

$$2H_2 + 4[Co^{II}(CN)_5]^{3-} \rightleftharpoons 4[(CN)_5Co^{III}\text{–}H]^{3-}$$

$$\overline{2H_2 + O_2 \qquad\qquad \rightarrow 2H_2O}$$

Examples are HSO_4–Fe–OOH (1), Fe^{III}–OOH (2–11), and Fe^{IV}–OOH (8, 9). Garten (12) has proposed a scheme which contains the dinuclear hydroperoxide $[(Fe_2(OH)_2^{4+}OO\text{–}H]$.
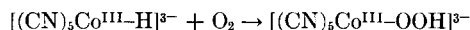
Chance and Schonbaum's (7) demonstration that the catalase reaction

The chemistry of the oxidation of $[(CN)_5Co^{III}\text{–}H]^{3-}$ and of $[Co^{II}(CN)_5]^{3-}$ (14, 16)

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* For example, a reversible hydroxylation of a C=C bond close to the iron atom (22).

† Both cycles, and the experimental conditions under which they have been studied, will be discussed in a later communication.

indicates that $[(CN)_5Co^{III}OH_2]^{2-}$, and to a much lesser extent its polymer, are the only conceivable end products of reaction (5). Direct experimental evidence for the course of the reaction will be presented elsewhere.* The disproportionation (6) is discussed by Kwiatek, Mador, and Seyler (23), by de Vries (24), and by Bayston et al. (14).

The Significance of pK

In the cycle given above, reaction (5) will be progressively replaced by

$[(CN)_5Co^{III}-OOH]^{3-}$
$\qquad + H_3O^+ \rightarrow [(CN)_5CoOH_2]^{2-} + H_2O_2$
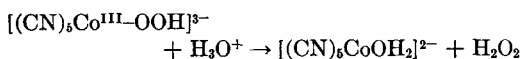
(and the subsequent reduction of H_2O_2) if the pH is lowered. It will be noted however that reaction (5) could predominate even at neutral pH, provided that a high concentration of $[(CN)_5Co^{III}-H]^{3-}$ is maintained. A much higher pH would be required if the intermediate were $[(CN)_5Co^{III}-O-O-Co^{III}(CN)_5]^{6-}$. Lowering the temperature can assist by slowing hydrolysis much more than reduction.

In general, in autoxidations via peroxide, we may suppose that the coordinated peroxide will be reduced to hydroxyl ions before it can be removed as H_2O_2 if electrons can be supplied quickly and efficiently and if the pH of the medium is not much less than the pK of the conjugate acid.

Simple considerations such as these underlie the discrepancies in the published literature on H_2O_2 production by oxygenases. Although many oxygenases catalyze the reaction of O_2 with an organic free radical, some react directly with O_2. Certain of the latter class liberate H_2O_2 during experiments *in vitro;* They do not necessarily do so *in vivo;* the formation of H_2O_2 may have been induced by the inefficiency of the H donor functional in the *in vitro* experiment.

ACKNOWLEDGMENTS

* See † footnote, p. 127.

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