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Recent Chemical Studies of Sodium Nitroprusside Relevant to its Hypotensive Action

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1 Introduction

Sodium nitroprusside was first prepared and investigated in the middle of the nineteenth century by the St Andrews chemist Playfair.[1] Its wide reactivity was early recognized[2] and comprehensive summaries of the earlier chemical investigations have been published.[3,4]

The hypotensive action of sodium nitroprusside was first demonstrated in 1929, when it was shown[5] to induce a significant reduction in the blood pressure of a severely hypertensive patient: no undesirable side effects were reported.

Despite a composition, $Na_2[Fe(CN)_5NO].2H_2O$, containing five cyanide ligands per anion, and despite its reported involvement in several cases of suicide,[6] sodium nitroprusside came into use some 30 years ago as a drug for the control of severe hypertension.[7] During the 1960s, sodium nitroprusside was introduced as an agent for the induction of hypotension during anaesthesia, both in North America[8] and in the UK.[9]

Since that time, a considerable research effort has attempted to establish the mode of action of nitroprusside and its metabolic fate; in particular, attention has centred on the fate of the cyano ligands, which if liberated *in vivo* as free cyanide, could have potentially disastrous consequences.

Sodium nitroprusside is thus a most unusual drug, whose introduction as a new drug today would probably not be sanctioned. It is the purpose of this review to outline its current usage, and to describe recent chemistry relevant to its use, mode of action, and metabolism.

2 The Clinical Use of Sodium Nitroprusside

Sodium nitroprusside is a potent vasodilator effective in the lowering of blood pressure. It is widely used to induce hypotension during surgery:[8—10] control of the rate of infusion of the sodium nitroprusside solution allows adjustment of the blood

[1] L. Playfair, *Annalen*, 1850, **74**, 317.
[2] F. Z. Roussin, *Ann. Chim. Phys.*, 1858, **52**, 30.
[3] V. N. Bernshtein and V. G. Belikov, *Russ. Chem. Rev.*, 1961, **30**, 227.
[4] J. H. Swinehart, *Coord. Chem. Rev.*, 1967, **2**, 385.
[5] C. C. Johnson, *Arch. Int. Pharmacodyn. Ther.*, 1929, **35**, 489.
[6] P. Lazarus-Barlow and B. M. Norman, *Br. Med. J.*, 1941, 407.
[7] R. W. Gifford, *Proc. Mayo Clinic*, 1959, **34**, 387; *Med. Clin. North Am.*, 1961, **45**, 441.
[8] P. P. Moraca, E. M. Bilte, D. E. Hale, C. E. Wasmuth, and E. F. Pontasse, *Anesthesiology*, 1962, **23**, 193.
[9] G. O. M. Jones and P. Cole, *Br. J. Anaesth.*, 1968, **40**, 804.
[10] J. H. Tinker and J. D. Michenfelder, *Anesthesiology*, 1976, **45**, 340.

pressure as required. A major advantage of sodium nitroprusside over many other hypotensive drugs is that on adjustment of the dose-rate there is very fast response, without overshoot. It has also been used, in a similar manner, in the treatment of chronic hypertension and in the management of myocardial infarction and other cardiac failure conditions.[11-13]

Sodium nitroprusside is thus an effective drug for the treatment of two conditions, high blood pressure and heart attack, which are extremely common and wide-spread. However, its usage has been severely restricted by reports[14-19] that, in the bloodstream after infusion, nitroprusside decomposes with liberation of most or all of the cyano ligands as free cyanide. In a number of cases administration of sodium nitroprusside has been followed by the symptoms of apparent cyanide poisoning. Several deaths following surgery have been attributed to this.[20-22]

3 The Problem of *in vitro* and *in vivo* Cyanide Release

Although there are many reports in the medical literature[23] which claim to demonstrate the ready liberation of four or five moles of free cyanide per mole of nitroprusside, very few, if any, of its chemical reactions[3,4] liberate any free cyanide. Indeed, the very high formation constants of cyanoferrate complexes $\{\beta_6 \simeq 10^{35}$ for $[Fe(CN)_6]^{4-}$ and $\simeq 10^{43}$ for $[Fe(CN)_6]^{3-}\}$[24] suggest that loss of CN^- is very unlikely unless some other powerful driving force for the reaction is provided. One such is protonation of CN^- to give HCN, which should occur at physiological pH, since pK_a for HCN is 9.21. On the other hand, $[Fe(CN)_5NO]^{2-}$ is substitution-inert, and exchanges its cyanide ligands with $[^{14}CN]^-$ extremely slowly ($t_{\frac{1}{2}} \sim 1000$ h even at pH 2.3).[25] Hence, the widely reported rapid liberation of cyanide from nitroprusside *in vivo* immediately poses problems to the inorganic chemist.

An essential prerequisite of any study, whether *in vivo* or *in vitro*, of cyanide release from sodium nitroprusside is an analytical technique which will distinguish reliably between free cyanide and bound cyanide. The most widely employed method for the analysis of cyanide in biological samples is that of Boxer and Rickards:[26] the sample is acidified (*e.g.* with sulphuric or trichloroacetic acid) and the resulting HCN removed on a stream of nitrogen gas from which it is trapped by

[11] D. Mukherjee, M. S. Feldman, and R. H. Helfant, *J. Am. Med. Assoc.*, 1976, **235**, 2406.
[12] V. Kottee, E. R. von Leitner, J. Wunderlich, and K. Schroder, *Br. Heart J.*, 1977, **39**, 116.
[13] K. Chatterjee, J. J. C. Swan, V. S. Kaushik, G. Jobin, P. Magnusson, and J. S. Forrester, *Circulation*, 1976, **53**, 797.
[14] C. J. Vesey, P. V. Cole, J. C. Linnell, and J. Wilson, *Br. Med. J.*, 1974, 140.
[15] C. J. Vesey, P. V. Cole, and P. J. Simpson, *Br. J. Anaesth.*, 1976, **48**, 651.
[16] H. E. Spiegel and V. Kucera, *Clin. Chem.*, 1977, **23**, 2329.
[17] C. J. Vesey, P. J. Simpson, L. Adams, and P. V. Cole, *Br. J. Anaesth.*, 1979, **51**, 89.
[18] C. J. Vesey, J. R. Krapez, and P. V. Cole, *J. Pharm. Pharmacol.*, 1980, **32**, 256.
[19] N. W. Lawson, A. B. Seifen, D. S. Thompson, and J. Gintautas, *Proc. West. Pharmacol. Soc.*, 1982, **25**, 281.
[20] D. W. Davies, D. Karar, D. J. Steward, and J. R. Munro, *Can. Anaesth. Soc. J.*, 1975, **22**, 547.
[21] A. J. Merrifield and M. D. Blundell, *Br. J. Anaesth.*, 1974, **46**, 324.
[22] R. D. Jack, *Br. J. Anaesth.*, 1974, **46**, 952.
[23] For a summary: V. Schulz, *Clin. Pharm.*, 1984, **9**, 239.
[24] G. D. Watt, J. J. Christensen, and R. M. Izatt, *Inorg. Chem.*, 1965, **4**, 220.
[25] A. G. MacDiarmid and N. F. Hall, *J. Am. Chem. Soc.*, 1954, **76**, 4222.
[26] G. E. Boxer and J. C. Rickards, *Arch. Biochem. Biophys.*, 1951, **30**, 372.

alkali. This trapping solution is then analysed either colorimetrically or potentiometrically. This method is excellent for cyanide alone but in the presence of cyanoferrate complexes it gives results which can be seriously in error, as it removes from the test solution not only free cyanide but also the cyano ligands in any substitution-labile cyanometallate complexes.

The early work by MacDiarmid and Hall showed[25] that although d^6 cyanometallates such as $[Fe(CN)_6]^{4-}$, $[Fe(CN)_5NO]^{2-}$, and $[Co(CN)_6]^{3-}$ exhibited extremely slow exchange rates with $[^{14}CN]^-$ in the dark (and thus are classic examples of substitution-inert complexes[27]), the exchange becomes very rapid upon illumination as the complexes become substitution-labile upon photo-excitation.

Consistent with these observations, we found[28] that while no cyanide was detectable using the Boxer and Rickards technique on solutions of sodium nitroprusside in the dark, similar solutions in the light gave quantitative liberation of cyanide. Use of a preformed sample of the primary photo-product[29] from nitroprusside, $[Fe(CN)_5H_2O]^{2-}$, gave essentially quantitative liberation of cyanide, independent of whether the sample was illuminated or not.

Consequently, any Boxer and Rickards cyanide analysis undertaken on a sample which may contain nitroprusside will, unless rigorously protected from light throughout (the analyses take 2—3 hours), give a falsely high cyanide result, even if no free cyanide at all is initially present. On this basis we suggested[30] in 1981 that there was, at that time, no unambiguous evidence for the release of cyanide from nitroprusside *in vivo*. We further suggested that because of the extreme photo-lability of nitroprusside in solution (see below, Section 4D), it was possible that in some circumstances what was being delivered to the patient was not solely $[Fe(CN)_5NO]^{2-}$ but a mixture of $[Fe(CN)_5NO]^{2-}$ and the non-hypotensive but labile $[Fe(CN)_5H_2O]^{2-}$, which certainly liberates HCN at physiological pH. Hence there exists the possibility that the reported cases of *in vivo* or *in vitro* cyanide release are dependent on the artefactual photolysis of nitroprusside, either before or during infusion, or during analysis.

Although these views have proved controversial, subsequent work[31] using the wholly non-invasive technique of ^{13}C n.m.r. with 90% enriched $[Fe(^{13}CN)_5NO]^{2-}$ showed no trace either of CN^- or of HCN, when nitroprusside was incubated, in the dark, with whole blood.

The most widely held mechanism for cyanide liberation *in vivo* involves a reaction with haemoglobin[32,33] in which cyanmethaemoglobin is produced, accounting for one of the cyano ligands—the other four of which are liberated (Scheme 1).

[27] H. Taube, *Chem. Rev.*, 1952, **50**, 69; *Angew. Chem., Int. Ed. Engl.*, 1984, **23**, 329.
[28] W. I. K. Bisset, M. G. Burdon, A. R. Butler, C. Glidewell, and J. Reglinski, *J. Chem. Res.*, 1981, (S) 299, (M) 3501.
[29] S. K. Wolfe and J. E. Swinehart, *Inorg. Chem.*, 1973, **14**, 1049.
[30] W. I. K. Bisset, A. R. Butler, C. Glidewell, and J. Reglinski, *Br. J. Anaesth.*, 1981, **53**, 1015.
[31] A. R. Butler, C. Glidewell, J. McGinnis, and W. I. K. Bisset, *Clin. Chem.*, 1987, **33**, 490.
[32] R. P. Smith and H. Kruszyna, *J. Pharm. Exp. Ther.*, 1974, **191**, 557; *Fed. Proc.*, 1976, **35**, 69.
[33] O. R. Leeuwenkamp, W. P. Van Bennekom, E. J. Van der Mark, and A. Bult, *Pharm. Weekbl.*, 1984, **6**, 129.

$$[Fe(CN)_5NO]^{2-} + Hb \longrightarrow MetHbCN + 4CN^- + Fe^{2+} + NO$$

Scheme 1

The difficulty of this Scheme is the very low probability of finding any free CN^- alongside Fe^{2+} as reaction products, because of the immense formation constants for cyanoferrate complexes: our own work on the interaction of nitroprusside with haemoglobins (see below, Section 4E) does not substantiate the reaction in Scheme 1.

Investigators who have accepted the evidence for *in vivo* cyanide release from nitroprusside have experimented with cyanide antidotes to be administered concurrently with sodium nitroprusside. Both aquocobalamin (Vitamin B_{12a}),[34-37] which coordinates any free cyanide forming cyanocobalamin, and thiosulphate,[38-41] which converts cyanide into thiocyanate under the action of the enzyme rhodanese have been recommended for use as effective antidotes, (Scheme 2).

$$\underset{\text{Aquocobalamin}}{CN^- + H_2OCb} \longrightarrow \underset{\text{Cyanocobalamin}}{H_2O + NCCb}$$

$$CN^- + S_2O_3^{2-} \xrightarrow{\text{Rhodanese}} SO_3^{2-} + SCN^-$$

Scheme 2

4 Recent Chemistry of Sodium Nitroprusside Relevant to its Clinical Use

Amongst the questions to be answered concerning the physiological behaviour of sodium nitroprusside are the following:

(i) what is the chemical nature of the primary nitroprusside receptor?

(ii) what is the subsequent metabolism, and could CN^- be released?

(iii) how does nitroprusside induce vascular relaxation?

Much of the recent chemistry of sodium nitroprusside sheds light on these questions.

A. Reactions with Oxygen-, Nitrogen-, and Carbon-centred Nucleophiles.—In the early chemistry of sodium nitroprusside,[3,4] easily the most common reaction type encountered is that of nucleophilic attack at the nitrogen atom of the nitrosyl ligand, and these reactions have received further study in recent years from both the synthetic and the mechanistic standpoints. The nucleophile types of relevance in physiological conditions are those based upon oxygen, nitrogen, and thiol-type sulphur, all readily found for example in the side-chains of peptides and proteins; in addition, by analogy with co-enzymes such as thiamine pyrophosphate and

[34] M. A. Posner, R. E. Tobey, and H. McElroy, *Anesthesiology*, 1976, **44**, 157.
[35] M. A. Posner, F. L. Rodkey, and R. E. Tobey, *Anesthesiology*, 1976, **44**, 330.
[36] J. E. Cottrell, P. Casthely, and J. D. Brodie, *N. Engl. J. Med.*, 1978, **298**, 809.
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[39] J. R. Krapez, C. J. Vesey, L. Adams, and P. V. Cole, *Br. J. Anaesth.*, 1981, **53**, 793.
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[41] C. J. Vesey, J. R. Krapez, J. G. Varley, and P. V. Cole, *Anesthesiology*, 1985, **62**, 415.

pyridoxal phosphate, carbanionic nucleophiles are also relevant. A minimum requirement for any nucleophile to be regarded as plausible model for the primary nitroprusside receptor is that the rate of its reaction is high enough to mimic the very fast physiological response to nitroprusside.

Nitroprusside reacts with hydroxide to yield the nitrito complex (Scheme 3).[42]

$$[Fe(CN)_5NO]^{2-} + 2OH^- \overset{K}{\rightleftharpoons} H_2O + [Fe(CN)_5NO_2]^{4-}$$

$$[Fe(CN)_5NO]^{2-} \xrightarrow{OH^-} \left[Fe(CN)_5N\lessdot^{O}_{OH}\right]^{3-} \xrightarrow[\text{fast}]{+OH^-,\ -H_2O} [Fe(CN)_5NO_2]^{4-}$$

Scheme 3

The addition of hydroxide to the nitrosyl nitrogen atom is rate limiting with k = 0.55 $dm^3\ mol^{-1}\ s^{-1}$. Although the overall equilibrium constant K is *ca.* 10^6, the formation of $[Fe(CN)_5NO_2]^{4-}$ is negligibly slow at pH below 9.

Simple primary and secondary amines undergo nitrosation reactions with nitroprusside, but sterically hindered amines and tertiary amines do not react, (Scheme 4):[43–53] the amine complexes $[Fe(CN)_5NH_2R]^{3-}$ and $[Fe(CN)_5NHR_2]^{3-}$ are often accompanied by some $[Fe(CN)_5H_2O]^{3-}$.

Following formation of an adduct in a fast equilibrium, two pathways are observed. The dissociative pathway for which rate = k_1K[nitroprusside][amine] is followed by most simple primary amines[54,55] while the interchange pathway for which rate = k_2K[nitroprusside][amine]^2 is followed by most secondary amines.[50] Benzylamine,[50,54] morpholine,[50] and piperazine[50] follow both pathways.

Mono-amino acids undergo similar nitrosation reactions to give either hydroxy acids or lactones according to the chain length, while di-amino acids such as ornithine and lysine yield cyclic imino acids (Scheme 5).[56,57]

Although no kinetic study has yet been made of the reactions of nitroprusside with amino acids, rather than simple amines, the rate constants for the amine

[42] J. H. Swinehart and P. A. Rock, *Inorg. Chem.*, 1966, **5**, 573.
[43] D. J. Kenney, T. P. Flynn, and J. B. Gallini, *J. Inorg. Nucl. Chem.*, 1961, **20**, 75.
[44] N. E. Katz, M. A. Blesa, J. A. Olabe, and P. J. Aymonino, *J. Inorg. Nucl. Chem.*, 1980, **42**, 581.
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[46] N. E. Katz, J. A. Olabe, and P. J. Aymonino, *J. Inorg. Nucl. Chem.*, 1977, **39**, 908.
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[53] G. J. McGarvey and M. Kimura, *J. Org. Chem.*, 1986, **51**, 3913.
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[55] L. Dózsa, V. Kormos, and M. T. Beck, *Inorg. Chim. Acta*, 1984, **82**, 69.
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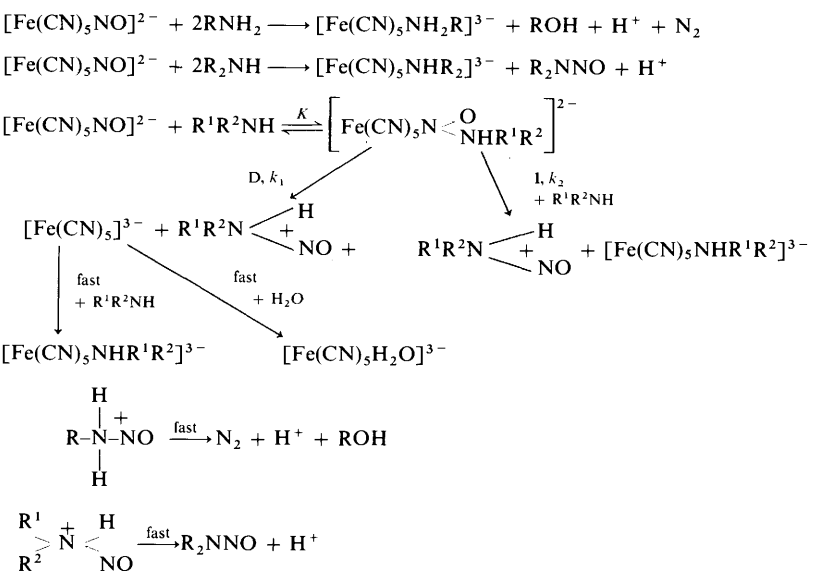


Scheme 4

reactions (Table 1) suggest that, as for hydroxide, the rates of reaction with the amino function are too low for the NH_2 group to provide a plausible model for a nitroprusside receptor.

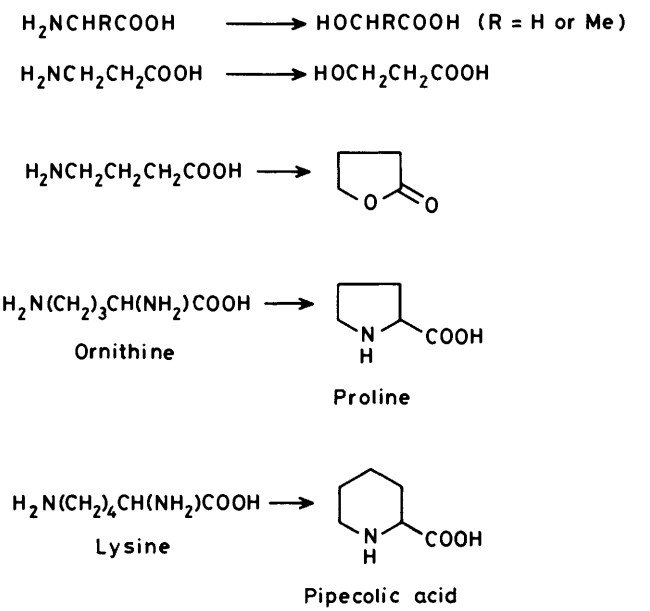
Simple acyclic ketones $R^1CH_2COR^2$ react with nitroprusside in aqueous alkali hydroxide solution to form red complexes $[Fe(CN)_5N(O)CR^1COR^2]^{4-}$ which decompose in the alkaline medium to yield $[Fe(CN)_5H_2O]^{3-}$ and the oxime $R^1C(=NOH)COR^2$ resulting from nitrosation.[58–61] Cyclic ketones give similar complexes,[62,63] which decompose in alkali to provide ω-(hydroxyimino)carboxylic acids and in acid to give ω-cyanocarboxylic acids.

The reactions proceed *via* the anion of the ketone[59,60] (Scheme 6), and the reaction with $CHEt(CN)_2$ likewise proceeds *via* the carbanion formed in a fast

Table 1 *Representative rate constants for amine reactions*[50,54]

Dissociative pathway k_1K/dm^3 mol^{-1} s^{-1}		*Interchange pathway* k_2K/dm^6 mol^{-2} s^{-1}	
$EtNH_2$	1.95×10^{-2}	Piperidine	1.4×10^{-3}
Pr^nNH_2	7.8×10^{-4}	Morpholine	0.21×10^{-3}
$PhCH_2NH_2$	1.3×10^{-3}	$PhCH_2NH_2$	2.0×10^{-3}

[58] K. W. Loach and T. A. Turney, *J. Inorg. Nuclear Chem.*, 1961, **18**, 179.
[59] J. H. Swinehart and W. G. Schmidt, *Inorg. Chem.*, 1967, **6**, 232.
[60] S. K. Wolfe and J. H. Swinehart, *Inorg. Chem.*, 1968, **7**, 1855.
[61] N. Y. M. Iha and H. E. Toma, *Inorg. Chim. Acta*, 1984, **81**, 181.
[62] A. Ishigaki, M. Oue, Y. Matsushita, I. Masuda, and T. Shono, *Bull. Chem. Soc. Jpn.*, 1977, **50**, 726.
[63] C. H. Brieskorn and W. Schwack, *Arch. Pharm. (Weinheim)*, 1982, **315**, 207.



Scheme 5

equilibrium step.[64] For acetone,[59] acetophenone,[60] and $CHEt(CN)_2$[64] respectively, the values of the product k_1K are, at 25 °C: 0.39, 6.6, and 220 $dm^6\ mol^{-2}\ s^{-1}$, and hence at physiological pH, the rates of such reactions will be extremely slow.

$$R^1CH_2COR^2 + OH^- \overset{K}{\rightleftharpoons} [R^1CHCOR^2]^- + H_2O$$

$$[Fe(CN)_5NO]^{2-} + [R^1CHCOR^2]^- \xrightarrow{k_1} \left[(NC)_5FeN{<}\begin{matrix}O\\CHR^1COR^2\end{matrix}\right]^{3-}$$

$$\left[(NC)_5FeN{<}\begin{matrix}O\\CHR^1COR^2\end{matrix}\right]^{3-} \xrightarrow[-H^+,\ \text{fast}]{} \left[(NC)_5FeN{<}\begin{matrix}O\\CR^1COR^2\end{matrix}\right]^{4-} \xrightarrow[+H_2O,\ +H^+]{D,\ k_2} [Fe(CN)_5H_2O]^{3-} + R^1C(=NOH)COR^2$$

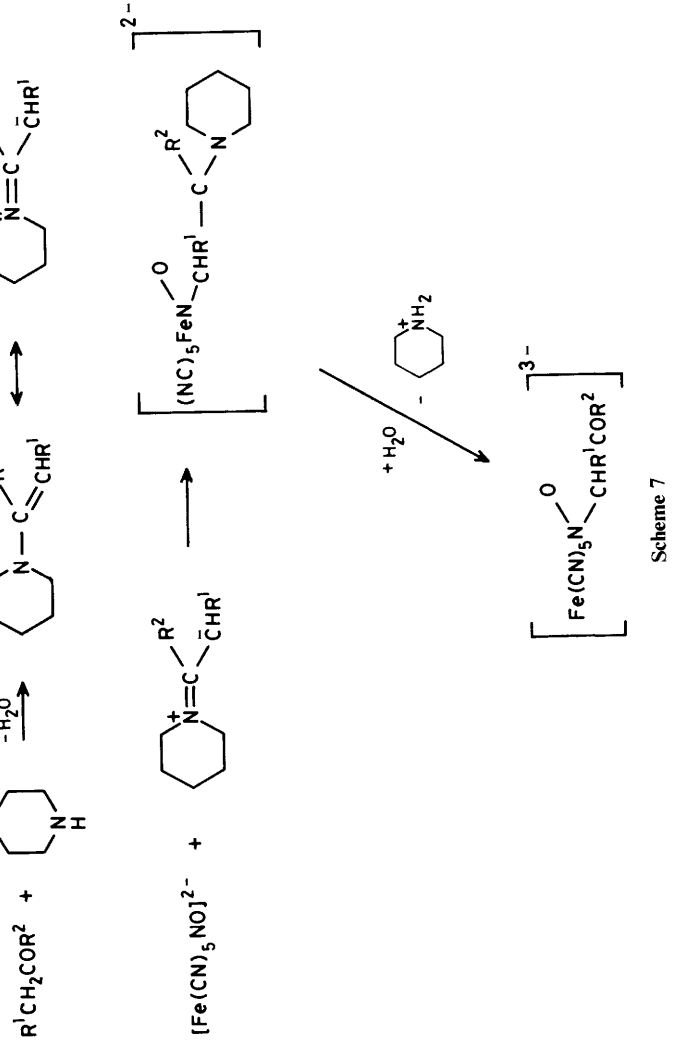
$$CHEt(CN)_2 + OH^- \overset{K}{\rightleftharpoons} [CEt(CN)_2]^- + H_2O$$

$$[Fe(CN)_5NO]^{2-} + [CEt(CN)_2]^- \xrightarrow[\text{slow}]{k_1} \left[(NC)_5FeN{<}\begin{matrix}O\\CEt(CN)_2\end{matrix}\right]^{3-}$$

Scheme 6

In the presence of secondary amines *e.g.* piperidine acting as the base, the reactions of ketones take a different course, (Scheme 7)[65] proceeding *via* enamine

[64] A. R. Butler, C. Glidewell, V. Chaipanich, and J. McGinnis, *J. Chem. Soc., Perkin Trans. 2*, 1986, 7.
[65] W. Wiegrebe and M. Violbig, *Z. Naturforsch.*, 1981, **36b**, 1297; 1982, **37b**, 490.



Scheme 7

intermediates to the tri-negative complexes $\left[Fe(CN)_5N{<}\begin{matrix}O\\CHR^1COR^2\end{matrix}\right]^{3-}$, whereas with hydroxide as base tetra-negative complexes are formed (*cf.* Scheme 6). However, although such a mechanism probably represents a more plausible reaction pathway under physiological conditions than reaction of an unprotected carbanion, it does not appear to involve any significant rate acceleration.

A single example of a strong carbon acid $CH_2(CN)_2$ has been investigated;[64] in this instance, the active carbanion appears to be the doubly ionized species $[C(CN)_2]^{2-}$, (Scheme 8): the observed rate constant kK_1K_2 at 25 °C, 7.5×10^4 $dm^9\ mol^{-3}\ s^{-1}$, again indicates negligibly slow reaction at any pH near physiological.

$$CH_2(CN)_2 \underset{K_1}{\overset{+OH^-,\ -H_2O}{\rightleftharpoons}} [CH(CN)_2]^- \underset{K_2}{\overset{+OH^-,\ -H_2O}{\rightleftharpoons}} [C(CN)_2]^{2-}$$

$$[Fe(CN)_5NO]^{2-} + [C(CN)_2]^{2-} \xrightarrow{k} \left[(NC)_5FeN{<}\begin{matrix}O\\C(CN)_2\end{matrix}\right]^{4-}$$

Scheme 8

None of the very large number of nitrogen or carbon nucleophiles hitherto examined exhibits fast enough reaction with nitroprusside to justify serious consideration as a potential model for the primary nitroprusside receptor.

Since many of the important sulphur nucleophiles, those of type RSH, react with nitroprusside in reactions which involve redox processes, discussion of these will be deferred until after the discussion of the redox chemistry of nitroprusside itself.

B. Redox Chemistry.—Reduction of the nitroprusside ion yields a paramagnetic species characterized by $g = 2.024$ and A (^{14}N) $\sim$ 15 G. This species, which has been variously described as $[Fe(CN)_5NO]^{3-}$, $[Fe(CN)_4NO]^{2-}$, $[Fe(CN)_5NOH]^{2-}$, and $[Fe(CN)_5NO_2]^{5-}$, amongst other formulations, can be generated electrochemically,[66] or by chemical reduction employing borohydride, ascorbic acid, quinol, or dithionite,[66] or superoxide;[67] it is also formed in reactions of nitroprusside with thiols.[66,68] By the use of ^{13}C enriched nitroprusside, this species has been shown to be $[Fe(CN)_4NO]^{2-}$ since in $[Fe(^{13}CN)_4NO]^{2-}$ the triplet e.s.r. spectrum observed for $[Fe(^{12}CN)_4NO]^{2-}$ becomes a triplet of quintets.[69]

However, pulse radiolysis experiments in aqueous solution show[70] electron attachment to nitroprusside at rates approaching the diffusion limit to yield presumably $[Fe(CN)_5NO]^{3-}$, followed by unimolecular decay with a half life of a few milliseconds to give $[Fe(CN)_4NO]^{2-}$. If organic radicals R$^\bullet$ are present in the same solution they combine[71] with the initial $[Fe(CN)_5NO]^{3-}$, again at rates near the diffusion limit, to form complexes $[Fe(CN)_5N(O)R]^{3-}$; these are complexes of neutral *C*-nitroso compounds RNO, identical in type with those

[66] D. Mulvey and W. A. Waters, *J. Chem. Soc., Dalton Trans.*, 1975, 951.
[67] H. P. Misra, *J. Biol. Chem.*, 1984, **259**, 12678.
[68] A. R. Butler, A. M. Calsy-Harrison, C. Glidewell, and I. L. Johnson, to be published.
[69] C. Glidewell and I. L. Johnson, *Inorg. Chim. Acta*, 1987, **132**, 145.
[70] R. P. Cheney, M. G. Simic, M. Z. Hoffman, I. A. Taub, and K.-D. Asmus, *Inorg. Chem.*, 1977, **16**, 2187.
[71] R. P. Cheney, S. D. Pell, and M. Z. Hoffman, *J. Inorg. Nucl. Chem.*, 1979, **41**, 489.

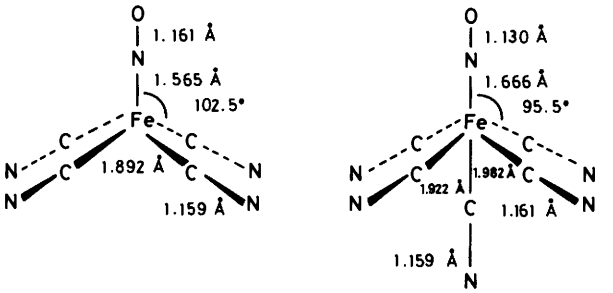


Figure 1 *Structures of* $[Fe(CN)_4NO]^{2-}$ *and* $[Fe(CN)_5NO]^{2-}$ (*Refs.* 73, 74)

formed by attack of enamines on nitroprusside,[65] or by direct substitution of RNO into $[Fe(CN)_5H_2O]^{3-}$.[72]

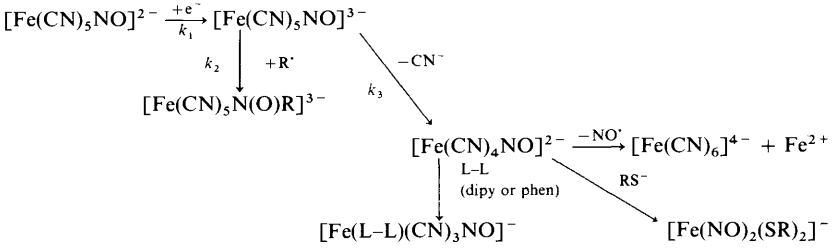
In the absence of organic radicals $[Fe(CN)_5NO]^{3-}$ decays to $[Fe(CN)_4NO]^{2-}$, whose lifetime in aqueous solution is of the order of minutes. Indeed, from liquid ammonia, salts of this ion can be crystallized, and the *X*-ray analysis of $(Ph_4P)_2[Fe(CN)_4NO]$ has demonstrated the square pyramidal geometry of the anion.[73]

This structure (Figure 1), and the e.s.r. parameters[69] support a formulation Fe^{I} $-NO^{+}$ for this anion, rather than $Fe^{II}-NO^{\bullet}$, despite the ease with which NO is lost. Not surprisingly therefore the anion $[Fe(CN)_4NO]^{2-}$ is substitution labile, giving with chelating di-amines L–L = dipy or phen, the 6-coordinate $[Fe(L\text{–}L)(CN)_3NO]^{-}$,[75] and with excess of MeS^{-} or HS^{-}, the dinuclear $Fe_2(SMe)_2(NO)_4$ {*via* $[Fe(NO)_2(SMe)_2]^{-}$} and the tetranuclear $[Fe_4S_3(NO)_7]^{-}$ {*via* $[Fe(NO)_2(SH)_2]^{-}$} respectively,[68] (Scheme 9).

These last reactions may be of especial significance for the reduction of nitroprusside in the presence of thiol-containing peptides, since cyanide-free iron complexes are formed. However, in the absence of any other ligands in the system, $[Fe(CN)_4NO]^{2-}$ loses NO and undergoes a ligand redistribution to yield the very stable $[Fe(CN)_6]^{4-}$.[68,69] In this connection it is of interest that the reduction of nitroprusside by borohydride in aqueous solution, known[66] to provide *via* $[Fe(CN)_4NO]^{2-}$, is reported[76] to yield Fe^{2+} and $[Fe(CN)_6]^{4-}$, as indicated in Scheme 9.

C. Reactions with Sulphur Nucleophiles.—The reactions of the nitroprusside ion with thiols and thiolate anions are very much more complex than the reactions with oxygen-, nitrogen-, or carbon-centred nucleophiles discussed above.

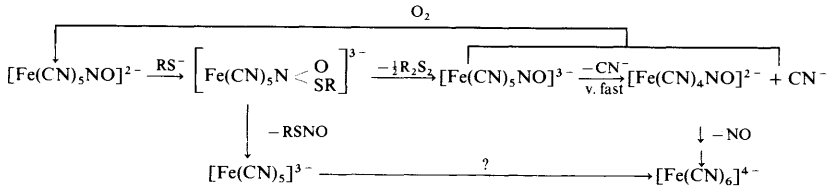
[72] W. A. Waters, *J. Chem. Soc., Perkin Trans. 2*, 1976, 732.
[73] J. Schmidt, H. Kühr, W. L. Dorn, and J. Kopf, *Inorg. Nucl. Chem. Lett.*, 1974, **10**, 55.
[74] M. Yu. Antipin, V. G. Tsirel'son, M. P. Flyugge, Yu. T. Struchkov, and R. P. Ozerov, *Koord. Khim.*, 1987, **13**, 121.
[75] J. Fiedler and J. Mašek, *Inorg. Chim. Acta*, 1984, **81**, 117.
[76] N. G. Giniyatullin and Yu. M. Kargin, *Izv. Vysch. Uchebn. Zaved., Khim. Khim. Tekhnol.*, 1976, **19**, 1668; *Chem. Abstr.*, 1977, **86**, 64862r.



$k_1 = 1.0 \times 10^{10}$ dm^3 mol^{-1} s^{-1} (ref. 70)
k_2 in range 0.6×10^9—12×10^9 dm^3 mol^{-1} s^{-1} (ref. 71)
$k_3 = 2.8 \times 10^2$ s^{-1} (ref. 70)

Scheme 9

The primary reaction step[66,77,78] is formation of the adduct $[Fe(CN)_5N(O)SR]^{3-}$, in which only RS^- is active. RSH does not react with nitroprusside prior to deprotonation.[78] Thereafter the dominant reaction pathway is loss of RS• (yielding R_2S_2 as an isolable product[66,77]) to form $[Fe(CN)_5NO]^{3-}$, which very rapidly (*cf.* Section 4B above) loses one cyanide ligand to form the paramagnetic complex $[Fe(CN)_4NO]^{2-}$, whose further decay gives $[Fe(CN)_6]^{4-}$ + NO•. However a second reaction pathway, observed[79] only in the pH range 6.5—8.5, involves loss of the thionitrite RSNO, which in turn decays[80] to NO and RSSR: the inorganic product from this pathway was not identified but is presumably $[Fe(CN)_5]^{3-}$, which may undergo a ligand redistribution to yield $[Fe(CN)_6]^{4-}$ and Fe^{2+} as the final products, (Scheme 10).



Scheme 10

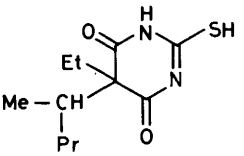
In the rigorous absence of air, a stoicheiometric oxidation of RSH by nitroprusside is observed;[77,81] in the presence of oxygen, nitroprusside is regenerated by oxidation either of $[Fe(CN)_5NO]^{3-}$ or of $[Fe(CN)_4NO]^{2-}$ and cyanide, and the oxidation of cysteine to cystine can be rendered catalytic in nitroprusside.[77]

[77] P. J. Morando, E. B. Borghi, L. M. de Schteingart, and M. A. Blesa, *J. Chem. Soc., Dalton Trans.*, 1981, 435.
[78] M. D. Johnson and R. G. Wilkins, *Inorg. Chem.*, 1984, **23**, 231.
[79] L. J. Ignarro, H. Lippton, J. C. Edwards, W. H. Baricos, A. L. Hyman, P. J. Kadowitz, and C. A. Gruetter, *J. Pharm. Exp. Ther.*, 1981, **218**, 739.
[80] S. Oae, Y. H. Kim, D. Fukushima, and K. Shinhama, *J. Chem. Soc., Perkin Trans. 1*, 1978, 913.
[81] A. R. Butler, A. M. Calsy-Harrison, C. Glidewell, I. L. Johnson, J. Reglinski, and W. E. Smith, *Inorg. Chim. Acta*, in press.

In the reaction forming the initial adduct $[Fe(CN)_5N(O)SR]^{3-}$, only RS^- is active; when RSH represents an amino-thiol such as cysteine or glutathione, the two forms protonated and unprotonated at nitrogen have similar rates of reaction.[78] For cysteine the rate constants for the formation of the adducts from the protonated and unprotonated forms are 4.8×10^4 dm^3 mol^{-1} s^{-1} and 2.6×10^4 dm^3 mol^{-1} s^{-1}, and the corresponding rate constants for the back reactions are 2.6×10^3 s^{-1} and 1.4×10^3 s^{-1}: the rate constants vary little with the substituent R, and all the reactions are fast at physiological pH because of the low pK_a values of thiol group (in the range 8.5—9.0 for thiols in amino acids and peptides).

When RSH represents cysteine, further kinetic study[77] of the reactions of $[Fe(CN)_5N(O)SR]^{3-}$ has been complicated not only by oxidation of part of the product back to nitroprusside,[77] but also by the very fast dissociation of $[Fe(CN)_5NO]^{3-}$ to $[Fe(CN)_4NO]^{2-}$ and cyanide, and by the reaction[82,83] of cyanide with the organic product cystine with the re-formation of the cysteinate anion (Scheme 11).

A similar 1:1 adduct of type $[Fe(CN)_5N(O)SR]^{3-}$ is formed[84] by the anaesthetic thiopental (1).

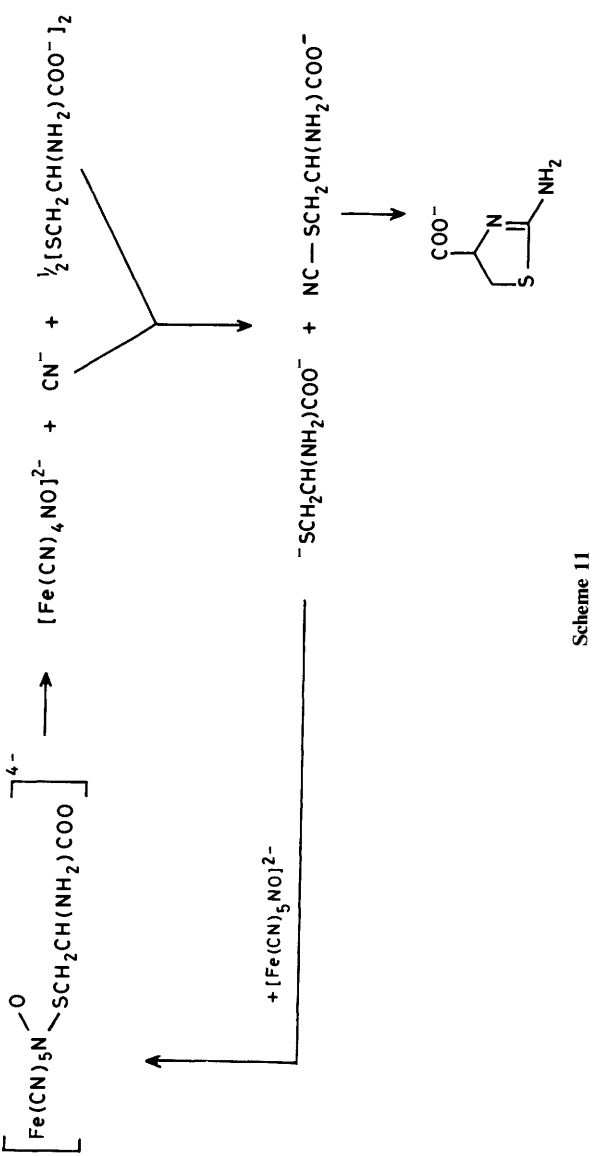


(1)

Thiopental is commonly used[15,37,85] as an anaesthetic at the same time as sodium nitroprusside is employed as a hypotensive, but if the interaction of thiopental with nitroprusside proceeds along the same type of pathway observed for other RSH (Scheme 10) then this has the effect of destroying simultaneously both the anaesthetic drug and the hypotensive drug.

The reaction of SH^- with nitroprusside[86] is very much faster than the corresponding reaction[42] with OH^- with a rate constant for initial adduct formation (298 K) of 170 dm^3 mol^{-1} s^{-1}, compared with 0.55 dm^3 mol^{-1} s^{-1} for OH^-. However there is some doubt concerning the identity both of this product, described[86] as $[Fe(CN)_5N(O)SH]^{3-}$, since it has $\lambda_{max.}$ of 572 nm[66] whereas all other $[Fe(CN)_5N(O)SR]^{3-}$ have $\lambda_{max.}$ in the range 520—525 nm, and of the product to which it decays, described variously as $[Fe(CN)_5NOS]^{4-}$,[86] and as the dimeric $[Fe_2(CN)_{10}(N_2O_2S_2)]^{8-}$,[87] where the nature of the $N_2O_2S_2$ ligand was undetermined.

[82] O. Gawron and J. Fernando, *J. Am. Chem. Soc.*, 1961, **83**, 2906.
[83] K. Antal, I. Banyai, and M. T. Beck, *J. Chem. Soc., Dalton Trans.*, 1985, 1191.
[84] M. Jakševac-Mikša, V. Hankonyi, and V. Karas-Gašparec, *Z. Phys. Chem. (Leipzig)*, 1980, **261**, 1041.
[85] W. R. MacRae and M. Owen, *Br. J. Anaesth.*, 1974, **46**, 795.
[86] P. A. Rock and J. H. Swinehart, *Inorg. Chem.*, 1966, **5**, 1078.
[87] E. J. Baran and A. Müller, *Angew. Chem., Int. Ed. Engl.*, 1969, **8**, 890.


[Fe(CN)5NO]2-
[Fe(CN)4NO]2-
CN-
½[SCH2CH(NH2)COO-]2
-SCH2CH(NH2)COO-
NC—SCH2CH(NH2)COO-
COO-
NH2

Scheme 11

Sulphite ion, SO_3^{2-}, reacts rapidly with nitroprusside, $k = 470\ dm^3\ mol^{-1}\ s^{-1}$, to yield a product $[Fe(CN)_5(NOSO_3)]^{4-}$ of unknown constitution.[88] Neither of the sulphur nucleophiles thiocyanate and thiourea reacts with nitroprusside at neutral pH in the dark: however upon photolysis, or upon acidification of a basified solution, blue-green Fe^{III} complexes are formed (Scheme 12).[89,90]

$$[Fe^{II}(CN)_5NO]^{2-} \xrightarrow{h\nu} [Fe^{III}(CN)_5H_2O]^{3-} \xrightarrow{L} [Fe^{III}(CN)_5L]^{x-}$$

$$[Fe^{II}(CN)_5NO]^{2-} \xrightarrow{OH^-} [Fe^{II}(CN)_5NO_2]^{4-} \xrightarrow[-NO_2^-]{+L} [Fe^{II}(CN)_5L]^{(x+1)-}$$

$$[Fe^{II}(CN)_5NO_2]^{4-} \xrightarrow{+H^+} \text{oxidation by } HNO_2 \rightarrow [Fe^{III}(CN)_5L]^{x-}$$

$L = (NH_2)_2CS,\ x = 2;\ NCS^-,\ x = 3$

Scheme 12

D. Photochemistry.—The primary photochemical product from nitroprusside in aqueous solution, independent of both pH and photolysis wavelength over wide ranges, is the aqua-complex $[Fe(CN)_5H_2O]^{2-}$, together with NO gas.[29,91,92] Similar photolyses in other donor solvents L (L = alcohols, amides, pyridine, MeCN, or Me_2SO) give analogous solvo-complexes $[Fe(CN)_5L]^{2-}$.[93] The quantum yields increase with decreasing wavelengths,[94] *e.g.* in H_2O from 0.17 at 436 nm to 0.37 at 313 nm, and pressure studies (for L = H_2O, MeOH, and Me_2SO) show that the photolysis reaction is dissociative (Scheme 13).

$$[Fe(CN)_5NO]^{2-} \xrightarrow[D,\ -NO^{\bullet}]{h\nu} [Fe(CN)_5]^{2-} \xrightarrow{L} [Fe(CN)_5L]^{2-}$$

Scheme 13

The net reaction is a redox process oxidizing Fe^{II} to Fe^{III} and reducing NO^+ to $NO^{\bullet}$: thus the substitution inert $[Fe(CN)_5NO]^{2-}$ is converted in aqueous solution into the substitution-labile $[Fe(CN)_5H_2O]^{2-}$.

The high quantum yields mean that aqueous solutions of sodium nitroprusside are extremely vulnerable to light; the photolysis can readily be visualized by addition of thiocyanate (Scheme 12). Exposure of nitroprusside/thiocyanate solutions to diffuse daylight cause rapid formation of the blue complex $[Fe(CN)_5(SCN)]^{3-}$: at typical operating-theatre lighting levels, its formation is

[88] C. Andrade and J. H. Swinehart, *Inorg. Chem.*, 1972, **11**, 648.
[89] C. Andrade and J. H. Swinehart, *Inorg. Chim. Acta*, 1971, **5**, 207.
[90] P. A. Stoeri and D. X. West, *J. Inorg. Nucl. Chem.*, 1974, **36**, 2347, 3883.
[91] A. Lodzinska and R. Gogolin, *Rocz. Chem.*, 1973, **47**, 1101.
[92] A. B. Nikolski and A. M. Popov, *Dokl. Akad. Nauk S.S.S.R.*, 1980, **250**, 902.
[93] G. Stochel and Z. Stasicka, *Polyhedron*, 1985, **4**, 481.

almost immediate. When used medically any exposure to light of nitroprusside infusion solutions will therefore mean delivery to the patient not only of less nitroprusside than calculated but of non-hypotensive $[Fe(CN)_5H_2O]^{2-}$ also.

Continued photolysis of sodium nitroprusside solutions in a closed system leads to the eventual precipitation of Prussian Blue:[29] however under the conditions of the Boxer and Rickards analytical method,[26] photolysis of nitroprusside leads to complete removal of cyanide from the test solution leaving behind only aqueous Fe^{3+}.[28] Hence under normal conditions of patient ventilation and physiological pH, infusion of any $[Fe(CN)_5H_2O]^{2-}$ will lead to the export of HCN from the bloodstream not only to the lungs, but to most other organs also. Furthermore any Boxer and Rickards analysis of blood samples taken after nitroprusside infusion is likely to measure not only free cyanide, but any cyanide present in labile cyanoferrate complexes (*e.g.* from nitroprusside metabolism), plus cyanide arising from artefactual photolysis (during analysis) of hitherto intact nitroprusside.

It is for these reasons, dependent upon the very ready photolysis of nitroprusside to give the labile aqua-complex that the analytical method of Boxer and Rickards is inappropriate for cyanide in the presence of nitroprusside or of any other cyanoferrate complex: as noted earlier, (Section 3) many substitution-inert cyanometallates become substitution-labile upon photo-excitation.[25] Thus all results of cyanide analyses by this method made after nitroprusside infusion are subject to uncertainty (in addition to the uncertainty introduced by the possibility of photolysis before infusion) and consequently must be interpreted with care and circumspection. It is remarkable that almost the whole literature on cyanide release from nitroprusside is based upon this inappropriate analytical technique and upon an equal disregard for the known photochemistry.[28,89–94]

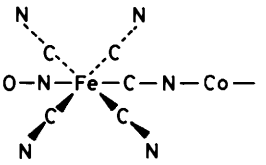
The by-product of nitroprusside photolysis is NO, which in the presence of oxygen will give with water a mixture of nitrite and nitrate in an acid solution (Scheme 14).

$$NO + \tfrac{1}{2}O_2 \longrightarrow NO_2$$
$$2NO_2 + H_2O \longrightarrow NO_2^- + NO_3^- + 2H^+$$

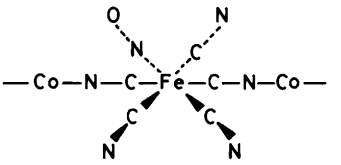
Scheme 14

E. Reactions with Electrophiles.—Aquocobalamin, Vitamin B_{12a}, H_2OCb, has been suggested as a possible antidote to cyanide liberated from nitroprusside.[34–37] We have found[95] that nitroprusside forms both dinuclear and trinuclear complexes with H_2OCb. In the dinuclear complex, the axial cyanide ligand of nitroprusside is coordinated to the cobalt(III) of the cobalamin, so forming a Fe–C–N–Co bridge (2), while in the trinuclear complex, a mutually *trans* pair of equatorial cyanide ligands are involved in forming two such bridges (3).

[94] G. Stochel, R. van Eldik, and Z. Stasicka, *Inorg. Chem.*, 1986, **25**, 3663.
[95] A. R. Butler, C. Glidewell, A. S. McIntosh, D. Reed, and I. H. Sadler, *Inorg. Chem.*, 1986, **25**, 970.

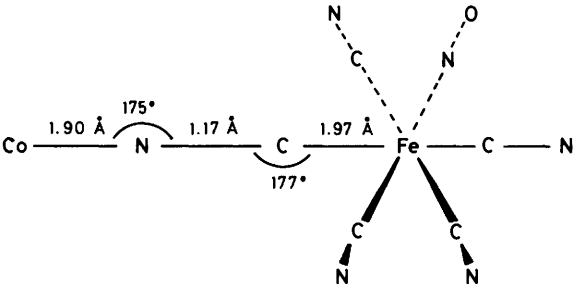


(2)



(3)

A simple structural model for such a bridge is found[96] in $[(NH_3)_5CoNC\text{-}Fe(CN)_4NO]^+$ (4), where an equatorial cyano ligand is preferred in the solid state, while the reaction[97] of nitroprusside with *trans*-$[Co(en)_2(SO_3)H_2O]^+$ to form $[(O_3S)(en)_2CoNCFe(CN)_4NO]^-$ provides a simple mechanistic model (Scheme 15)[98] for the formation of the Co–N–C–Fe bridge.



(4)

Rate-limiting dissociation of the aqua ligand from Co^{III} within an ion-paired intermediate is followed by fast reaction of the electrophile fragment $[Co(en)_2(SO_3)]^+$ with one of the cyano ligands of the nitroprusside.

$$[ONFe(CN)_4CN]^{2-} + [Co(en)_2(SO_3)(H_2O)]^+ \rightleftharpoons \underset{\text{Ion-pair I}}{\{[ONFe(CN)_4CN][Co(en)_2(SO_3)(H_2O)]\}^-}$$

$$\{[ONFe(CN)_4CN]\{Co(en)_2(SO_3)(H_2O)]\}^- \xrightarrow[\text{slow}]{-H_2O} \underset{\text{Ion-pair II}}{\{[ONFe(CN)_4CN][Co(en)_2(SO_3)]\}^-}$$

$$\{[ONFe(CN)_4CN][Co(en)_2(SO_3)]\}^- \xrightarrow{\text{fast}} \underset{\text{Product}}{[ONFe(CN)_4(\mu\text{-}CN)Co(en)_2(SO_3)]^-}$$

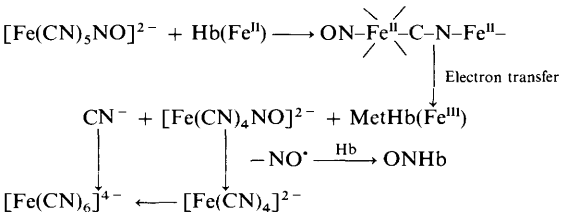
Scheme 15

[96] H. Ribas, J. M. Julia, X. Solans, M. Font-Albana, A. Isalgue, and X. Tejada, *Transition Met. Chem. (Weinheim)*, 1984, **9**, 57.
[97] K. L. Scott, R. S. Murray, W. C. E. Higginson, and S.-W. Foong, *J. Chem. Soc., Dalton Trans.*, 1973, 2335.
[98] K. L. Scott, R. S. Murray, and W. C. E. Higginson, *J. Chem. Soc., Dalton Trans.*, 1975, 1339.

Although the formation of cyanocobalamin from aquocobalamin and cyanide has a very high formation constant,[99] 1.2×10^{14}, the reaction is slow.[100,101] On the other hand, the formation of both 1:1 and 1:2 complexes between nitroprusside and aquocobalamin is very rapid, and is complete within minutes. The great ease of formation of such M–N–C–Fe bridges is demonstrated for example by the formation[102] of *cis*-$[CrF(L\text{–}L)_2NCFe(CN)_4NO]$ (L–L = 1,3-diaminopropane or 1,2-diaminocyclohexane) simply by gentle heating, in the solid state, of the salt *trans*-$[CrF(L\text{–}L)_2H_2O][Fe(CN)_5NO]$, and by the formation[103,104] of coordination polymers of type $M^{II}[Fe(CN)_5NO]$ in which all five cyano ligands are engaged in the formation of M–N–C–Fe bridges.

As well as reacting much faster with the drug nitroprusside, than with the toxic by-product cyanide, Vitamin B_{12a} has a second disadvantage as a potential antidote. The ready formation of complexes, which is limited only by the availability of aquocobalamin, causes a significant change in the pharmacology of nitroprusside:[105] complexes with aquocobalamin cause a significant increase of the hypotensive response-times in rats, both at the start of infusion and on the withdrawal of the drug. Thus complex formation lessens one of the major advantages of nitroprusside (fast response) over other vasodilators. Overall Vitamin B_{12a} cannot be regarded as a satisfactory antidote for potential cyanide liberation from nitroprusside.

When, in a bridged system M–N–C–Fe^{II}, the metal M is Cr^{III} or Co^{III}, no redox reactions occur across the bridge. However, if M is Fe^{II} as in haemoglobin, then following bridge formation an electron transfer reaction occurs, in a classic inner-sphere redox reaction, to give initially methaemoglobin and $[Fe(CN)_4]^{2-}$ (Scheme 16),[106] followed eventually by the formation of $[Fe(CN)_6]^{4-}$ and nitrosyl-haemoglobin.



Scheme 16

The Fe^{II}–N–C–Fe^{II} bridge in $Fe[Fe(CN)_5NO]$ has also been investigated.[107]

[99] D. Lexa, J. M. Savéant, and J. Zickler, *J. Am. Chem. Soc.*, 1980, **102**, 2654.
[100] G. C. Hayward, H. A. O. Hill, J. M. Pratt, N. J. Vanston, and R. J. P. Williams, *J. Chem. Soc.*, 1965, 6485.
[101] W. W. Reenstra and W. P. Jencks, *J. Am. Chem. Soc.*, 1979, **101**, 5780.
[102] M. Corbella, M. Monfort, and J. Ribas, *Z. Anorg. Allg. Chem.*, 1986, **543**, 233.
[103] A. N. Garg and P. S. Goel, *Inorg. Chem.*, 1971, **10**, 1344.
[104] H. Inoue, H. Iwase, and S. Yanagisawa, *Inorg. Chim. Acta*, 1973, **7**, 259.
[105] D. S. Hewick, A. R. Butler, C. Glidewell, and A. S. McIntosh, *J. Pharm. Pharmacol.*, 1987, **39**, 113.
[106] A. R. Butler, C. Glidewell, I. L. Johnson, and A. S. McIntosh, *Inorg. Chim. Acta*, 1987, **138**, 159.
[107] K. Yoshinori and T. Kamigaki, *Jpn. Pat.*, 61,231,527 (to Alps Electric Co. Ltd.); *Chem. Abstr.*, 1987, **106**, 147171x.

F. Metabolism.—Apart from the question of cyanide liberation (Section 3 above) there is rather little secure evidence from which to deduce the metabolic rate of nitroprusside in whole organisms, although a wide range of essentially speculative suggestions exist.

Administration of sodium nitroprusside to dogs causes a rapid increase in plasma thiocyanate, which is similar to the increase caused by administration of potassium cyanide;[17] on the other hand the plasma thiocyanate concentration in human patients rises only after prolonged nitroprusside administration,[14,15,108,109] presumably because of the much slower conversion of cyanide into thiocyanate in humans compared with dogs.[23] The conversion relies upon transfer of sulphur from thiosulphate under the action of the enzyme rhodanese (thiosulphate:cyanide sulphur transferase, EC 2.8.1.1) (Scheme 2). Because of the efficacy of this detoxication process, sodium thiosulphate has been recommended for use alongside sodium nitroprusside in therapeutic use.[38–41] Unlike aquocobalamin, thiosulphate does not react with nitroprusside under physiological conditions and has no adverse affects on the rate or magnitude of the hypotensive response.[105] It reacts rapidly with free cyanide, and hence appears to be superior to aquocobalamin as an antidote to potential cyanide toxicity.[110,111]

It has been reported[112] that abdominal injection of sodium nitroprusside solutions into mice yields free $[Fe(NO)]^{x+}$ groups, detected by e.s.r. as $Fe(NO)(S_2CNEt_2)_2$ after complexation with $(Et_2NCS_2)^-$. However, it is very difficult to distinguish, even in fluid solution, between the e.s.r. spectra of $Fe(NO)(S_2CNEt_2)_2$ and of $[Fe(CN)_4NO]^{2-}$: furthermore it is known[113] that $(Et_2NCS_2)^-$ reacts with nitroprusside in acid media *in vitro* to form $Fe(NO)(S_2CNEt_2)_2$, so that this evidence for metabolic $[Fe(NO)]^{x+}$ formation is not unambiguous.

However, with organ homogenates or perfused organs (liver or heart) e.s.r. spectroscopy shows[112,114] the reduction of nitroprusside giving $[Fe(CN)_4NO]^{2-}$, followed by the formation of both nitrosyl haemoglobin (*cf.* Section 4E above) and of $g = 2.03$ complexes of type $[Fe(NO)_2(SR)_2]^-$.[115] The formation of both nitrosyl haemoglobin and $[Fe(NO)_2(SR)_2]^-$ from nitroprusside has been observed[68,106] *in vitro*, so that none of these reactions in organ preparations is necessarily enzymic.

5 Chemistry of the Hypotensive Action of Sodium Nitroprusside

Sodium nitroprusside is one of a number of NO-containing compounds [others include glyceryl trinitrate, amyl nitrite, sodium nitrite, *N*-methyl-*N'*-nitro-*N*-

[108] M. Bogusz, J. Moroz, J. Karski, J. Gierz, A. Regieli, R. Witkowska, and A. Golabek, *Clin. Chem.*, 1979, **25**, 60.
[109] C. J. Vesey and P. V. Cole, *Br. J. Anaesth.*, 1985, **57**, 148.
[110] A. D. Ivankovitch, B. Braverman, M. Shulman, and A. J. Klowden, *Anesth. Analg.*, 1982, **61**, 120.
[111] C. J. Vesey, J. R. Krapez, J. G. Varley, and P. V. Cole, *Anesthesiology*, 1985, **62**, 415.
[112] A. L. Kleshchev, P. I. Mordvintsev, M. M. Shabarchina, and A. F. Vanin, *Russ. J. Phys. Chem.*, 1985, **59**, 266.
[113] N. S. Garif'yanov and S. A. Luchkina, *Dokl. Acad. Nauk S.S.S.R.*, 1969, **189**, 779.
[114] D. I. Aliev and A. F. Vanin, *Russ. J. Phys. Chem.*, 1982, **56**, 2362.
[115] A. R. Butler, C. Glidewell, A. R. Hyde, and J. C. Walton, *Polyhedron*, 1985, **4**, 797.

nitrosoguanidine (MNNG) and NO itself] which both cause relaxation of smooth vascular muscle, and activate the enzyme guanylate cyclase [GTP pyrophosphate-lyase (cyclizing), EC 4.6.1.2] which converts GTP into cyclic-GMP. In addition both azide and hydroxylamine are smooth muscle relaxants, which activate guanylate cyclase. For the NO-containing compounds, as well as for azide and hydroxylamine, muscular relaxation and enhancement of cyclic-GMP levels are simultaneously inhibited by cyanide, suggesting that the two functions are related.[116] This is supported by the observation[117] that the phosphodiesterase inhibitor aminophylline simultaneously enhances cyclic-GMP levels and nitroprusside-induced hypotension in dogs.

Purified guanylate cyclase is only responsive to nitroso-vasodilators such as nitroprusside, nitrite, NO, or MNNG, in the presence of haem or haemoproteins; the most effective stimulant of the purified enzyme is nitrosyl haemoglobin.[118] Guanylate cyclase activity is not stimulated by sodium nitroprusside alone in haem-free preparations:[119] a reducing thiol such as cysteine stimulates the activity, although the evidence on the effectiveness of other reductants, such as ascorbate, is conflicting.[118–120]

The stimulation of guanylate cyclase activity by nitroprusside in the presence of thiols, together with the observations[121–123] that *S*-nitrosothiols, RSNO, derived from a range of thiols also stimulate the enzyme's activity as well as acting as potent vasodilators, has led to the suggestion that the vasodilator action of nitroprusside is at least partly attributed to the formation of RSNO as the active intermediate. Against this must be set the fact that in the reactions of thiols and thiolates with sodium nitroprusside, *S*-nitrosothiol formation is not usually a major pathway (Section 4C above), and the reported ability[118,120] of reducing agents other than thiols to simulate the activation of guanylate cyclase by nitroprusside.

Whatever the importance of *S*-nitroso compounds, there is no doubt that guanylate cyclase not only requires haem, which is required for the enzyme's activation by NO-containing compounds,[124,125] but also binds NO-haem groups in stable 1:1 haem:protein complexes.[126] In addition, guanylate cyclase can capture NO-haem groups from any of nitrosylhaemoglobin, nitrosylmyoglobin,

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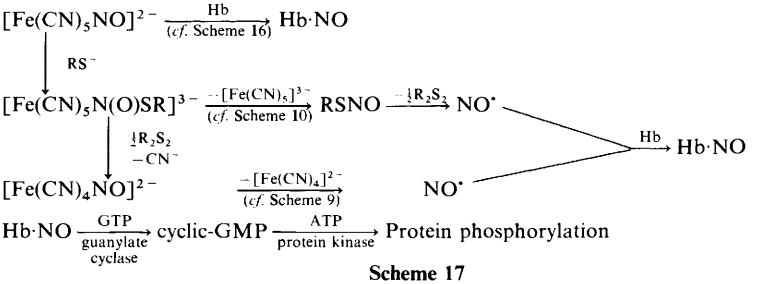
and nitrosylcatalase.[126] While the haem-free guanylate cyclase apoenzyme has only low activity, capture of an NO-haem group enhances the activity around 50-fold.

The vasodilator activity of NO-containing compounds of all types is therefore likely to be dependent upon their ability to generate NO-haem groups. For sodium nitroprusside, there is a direct reaction[106] with haemoglobin to form nitrosyl haemoglobin, as well as the reactions with thiols which liberate NO, and which can thus be precursors to nitrosylhaem compounds. It is possible that *S*-nitrosothiols also readily produce nitrosyl-haem compounds, either directly or by prior decomposition to give R_2S_2 and nitric oxide.

The role of cyclic-GMP, the product of guanylate cyclase activity, appears to be the mediation of protein phosphorylation/dephosphorylation, presumably by a cyclic-GMP dependent protein kinase, of the light chains of the muscle protein myosin.[127–129] Scheme 17 summarizes the possible mechanisms for the hypotensive active of the nitroprusside ion.



Scheme 17

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