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PRINCIPAL INVESTIGATOR: Peter Hambright, Ph.D.

CONTRACTING ORGANIZATION: Howard University
Department of Chemistry
Washington, D.C. 20059

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This report describes our efforts to develop anti-cyanide drugs using simple, water soluble metalloporphyrin molecules. Vanadium(4), chromium(3) molybdenum(5), Mnaganese(3), iron(3), nickel(2), copper(2), silver(3), zinc, indium(3), aluminum, platinum (2), palladium(2),and gold(3) porphyrins had no affinity for cyanide at pH 7.2. In vitro work showed cobalt(3), rhodium(3) and ruthenium(2) porphyrins bind cyanide in both the di and tri-valent states, from kinetic, equilibrium and electrochemical studies. Nickel(2) central N-alkyl porphyrins liberate nickel to complex with cyanide and are otherwise stable to nickel loss. Sterically hindered porphyrins in the iron(III) form rapidly complex cyanide. The LD-50s of thirteen porphyrins and phthalocyanines are in the 100-600 mg/kg range in rats. None of the cobalt, iron, rhodium or silver porphyrins or phthalocyanines protect mice in a prophylactic fashion against 2 LD-50s of cyanide.

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Anti-Cyanide Drugs

Annual Report

Peter Hambright

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SUMMARY

This is the fifth and final year in of a project to identify compounds that act in a prophylactic fashion against the CW agent, cyanide. Cyanide inhibits certain cytochrome systems in the body that contain iron porphyrins. In addition, several treatments for cyanide intoxication also involve porphyrins. For example, the nitrate/thiosulfate method produces large amounts of the cyanide scavenger, methemoglobin (an iron porphyrin), and in France, massive doses of hydroxo-cobalamin (a cobalt(III) porphyrin-like natural product of Vitamin B-12) are administered in cases of cyanide poisoning. We thus sought simple water soluble metalloporphyrins that would combine rapidly with cyanide at the physiologic pH, and explored in some detail the kinetics and mechanisms of cyanide reactions with these macrocyclic compounds. The best molecules were submitted to WRAIR for examination in an anti-cyanide pretreatment screen. This report describes in one place the results of our efforts over the years.

Using a dedicated autoanalyzer or cyanide electrode at pH 7.2, we found that oxy-vanadium(IV), chromium(III), molybdenum(V), manganese(III), iron(III), nickel(II), copper(II), silver(III), zinc(II), indium(III), aluminum(III), platinum(II), palladium(II) and gold(III) water soluble porphyrin having a variety of substituent groups at the porphyrin periphery had little cyanide binding ability at this pH. The best cyanide scavengers were cobalt(III), rhodium(III) and ruthenium(II) porphyrins, and full kinetic and equilibrium studies were done to understand the mechanism of cyanide uptake. Cobalt(III) and rhodium(III) derivatives react with both the cyanide anion and undissociated HCN, while carbonyl ruthenium(II) binds only cyanide. The latter forms cyanide complexes in both the reduced (+2) and oxidized (+3) states. In terms of rates and equilibrium constants, the cobalt(III) porphyrins were superior in vitro agents. Electrochemical and pulse radiolysis studies indicated that the tetranegative sulfonated cobalt(III) compounds were reduced at the metal, whereas the tetrapositive N-methylated pyridyl derivatives added electrons to the porphyrin ring. The cobalt porphyrin retained cyanide at the +2 oxidation level, and lost cyanide affinity in the +1 state.

Cobalt(III) derivatives of positive and negatively charged porphyrins, silver(II), chromium(III), palladium(II), iron(III) and rhodium(III) porphyrins, as well as a cobalt(II) tetrasulfonated phthalocyanine were submitted the the screen. The LD-50s of these water soluble derivatives were low, ranging from 650 to 24 mg/kg. At doses of 1/4, 1/16, 1/64 and 1/256 of the LD-50, and when administered both 15 and 60 minutes ip before a challenge of 2 LD-50s of cyanide, none of the thirteen compounds gave adequate prophylactic protection against cyanide.

In vitro experiments were done with nickel(II) and cobalt(II) centrally N-methylated water soluble porphyrins. The Ni(II) derivative was stable at pH 7.2 in the absence of cyanide, and reacts with four moles of cyanide ultimately forming $Ni(CN)_4^{2-}$, where the metal ion is released from the porphyrin as a cyanide scavenger, only in the presence of cyanide. Preliminary synthetic work was done on sterically hindered porphyrins, which inhibit Fe-O-Fe dimerization at the physiologic pH, and allow such new iron(III) porphyrin complexes to radidly interact with cyanide.

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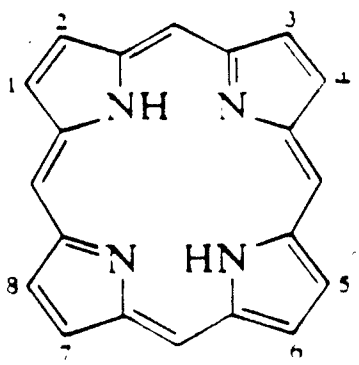
GENERAL REPORT

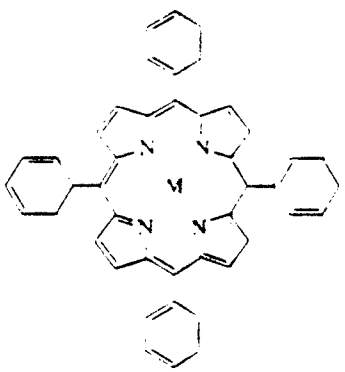
The goal of this project was to find a safe compound that could be taken orally in a prophylactic fashion to counteract the rapid acting CW agent, cyanide. To this end, chemicals were sought that would bind cyanide reasonably rapidly at the physiologic pH of 7.4. Such molecules considered were aldehydes, ketones, metal ions and metal complexes, alkylidenes, and water soluble metalloporphyrins and metallophthalocyanines. The better compounds were submitted to WRAIR for further testing. The present report will focus on metallo-porphyrins as anti-cyanide agents.

METALLOPORPHYRINS IN NATURE Porphyrin molecules are cyclic conjugated tetrapyrrole pigments, of the fundamental structure shown in Figure 1. The porphyrin nucleus can have various substituents in the beta-pyrrole positions 1-8, and in the remaining four meso carbon positions (see tetraphenylporphyrin, Fig 1), and many thousands of porphyrins are known. Hans Fischer (1881-1945) received the Nobel prize in Chemistry in 1930 for the first rational synthesis of such a large macrocyclic pigment (hemin, iron(III)-protoporphyrin-IX) and related derivatives, and Fischer and Orth's book in two volumes "Die Chemie des Pyrrols", Akademische Verlang, Leipzig, 1937, 1940 is still of major importance. The two central hydrogen atoms in the porphin structure can be replaced by almost any metal ion in the periodic table to form metalloporphyrins, and such metal derivatives are widely distributed in Nature. The iron complex of protoporphyrin-IX is the prosthetic group in the red hemoglobins or myoglobins, which transport and store oxygen in the blood and muscles in the iron(II) oxidation state. Slightly modified porphyrins constitute the cytochromes, the catalases and peroxidases, all of which function in oxidation/reduction and electron transfer reactions. For example, cytochrome P-450 adds an oxygen atom to certain substrates, and this could involve [Fe(IV)=O] ferryl porphyrins.

FIGURE 1



PORPHIN



TETRAPHENYLPORPHYRIN

The magnesium containing chlorophylls, mainly chlorophyll a and b, are the major green coloring matter in plants, and chlorophyll c is present in certain marine sources. These porphyrin-like pigments act as the initial traps for solar energy in photosynthetic reaction centers, where under the action of light a special Mg(II)-chlorophyll dimer is oxidized on the ring to a Mg(II)-chlorophyll^{+} radical cation species, beginning the process of the oxidation of water to oxygen, and the production of the reduced carbohydrates and sugars from carbon dioxide. Purple photosynthetic bacteria produce bacterio-chlorophylls. Metal free porphyrin derivatives are rare in Nature, and mainly occur due to malfunctions in normal porphyrin metabolism. Open chain bile pigments are formed by the in vivo degradation of hemes, where methene carbon atom leaves as carbon monoxide, and two O= fragments are bound in place of this meso carbon group. Due to various oxidations and reductions, numerous open chain bilirubinoids are known.

While it contains no formal pyrrole type rings, Vitamin B-12 (cyanocobalamin) is considered in the porphyrin family, and contains cobalt as the central atom. The form in which this corrin is commonly isolated from sewer bacteria is in the mono-cyano state. Finally, nickel is present in porphyrins in factor F430 of the methyl coenzyme M reductase, an enzyme which catalyzes the final step of methane production in certain biological systems. This enzyme possibly involves the unusual Ni(I) state, which can form a Ni-C bond upon reaction with organic molecules. Extensive monographs are available on porphyrins and metalloporphyrins [1,2].

METALLOPORPHYRINS AND CYANIDE: It is worth mentioning at this stage that hydrocyanic acid, HCN is a weak acid with a dissociation constant [3] of 9.2. Thus at pH 7.2, the ratio of HCN to CN^{-} is ~100:1. The stable cyano complexes have M-CN bonds, and many cases [4] are known where HCN reacts with a metal to form M-NCH adducts, which loose a proton to produce the isocyanates (M-NC), which can then rearrange in an intramolecular fashion to M-CN. While cyanide (or HCN) can inactivate many enzymes in the body, the main toxic site of action is considered to be cytochrome c oxidases, a terminal member of the cytochrome respiratory chain [5]. This enzyme contains two iron porphyrins and two moles of copper, all of which can be in oxidized or reduced states. Presumably, cyanide binds to an Fe(II) porphyrin, and upon oxidation, a stable inactive Fe(III)-CN species is formed which can no longer transport electrons, and thus oxygen cannot be reduced to water. This leads to cytotoxic hypoxia, a shift to anaerobic metabolism, the accumulation of lactic acid, and decreased blood pH and bicarbonate levels.

The aim of most scavengers is to reduce the amount of free cyanide in the body, to levels that natural cyanide enzymes (which add a sulfur atom to cyanide to form the less toxic thiocyanate) can handle, while at the same time allowing the reversible reactivation of the cytochrome oxidase. Interestingly, porphyrins are involved in cyanide toxicity, and porphyrins are also the basis of several of the therapeutic methods to scavenge cyanide. Iron(II) porphyrins in myoglobin and hemoglobin have a low affinity for the negatively charged cyanide, and prefer uncharged O2 or CO as ligands [2].The met (M+) or iron(III) states of these large proteins form extremely stable cyanide complexes. This probably is a reflection of a

requirement for electroneutrality in the iron-protein pocket. From kinetic [6] measurements of the $M^+ + CN^- = M\text{-}CN$ reaction for ferric horse metmyoglobin at pH 7, the forward rate constant is 170 $M^{-1}s^{-1}$, the dissociation rate constant is $3.0 \times 10^{-3}\ s^{-1}$, giving an equilibrium constant of $5.7 \times 10^4\ M^{-1}$, in terms of free cyanide. In contrast, the corresponding constant for Fe(II) myoglobin is ~ 1 M^{-1}, indicating that Fe(III) has 57,000 times the affinity for cyanide as the Fe(II) form. The forward rate constants for ligands (cyanide, azide, thiocyanate, nitrate, fluoride, imidazole) binding to this metmyoglobin are all around $10^2\ M^{-1}s^{-1}$, indicating that the mechanism is dissociative in character, involving the slow rate determining loss of water from the Fe(III)~H_2O myoglobin, followed by a more rapid ligation of the bare Fe(III) ion. In America, the anti-cyanide therapy of choice [7] involves injection of large doses of sodium nitrite, which transforms a certain fraction of the Fe(II) hemoglobin (a porphyrin) in the blood into the more active cyanide binding Fe(III) hemoglobin, which then combines with the cyanide present. This is followed by an injection of sodium thiosulfate, $Na_2S\text{-}SO_3$, which serves as a massive sulfane sulfur source for the rhodanese enzymes, which catalyze the addition of sulfur to cyanide (LD-50~5 mg/kg) to form the more benign thiocyanate SCN^- (LD-50 ~ 1 gm/kg), which is excreted via the kidneys. This procedure, while not prophylactic (70 kg doses of thiosulfate may be required), is usually a highly effective regimen. In the recent methyl isocyanate explosion in Bophal, many patients given thiosulfate recovered, even though it was initially thought that cyanide was not one of the products of the explosion.

Several groups [8,9] have used stroma free methemoglobin itself as a cyanide scavenger. However, this molecule clears the body too rapidly to give adequate long term protection. In West Germany, 4-dimethylaminophenol (~3.5 mg/kg) is the antidote of choice [10]. DMAP acts in an indirect fashion to form methemoglobin much more rapidly than does sodium nitrite. Other workers favor para-aminopropiophenone (PAPP), which has a longer lasting effect, is less nephrotoxic and mutagenic, as a rapid methemoglobin former [11].

In England, dicobalt-ethylenediaminetetraacetic acid is the active ingredient of the cyanide scavenger Kelocyanor, which contains 300 mg of the dicobalt(II), 4.0 gms glucose and 20 ml water [12]. The cobalt(II) rapidly binds a number of cyanide ions, and upon oxidation, the cyanide is presumably trapped in the substitution inert cobalt(III) coordination sphere. Unfortunately, the excess cobalt(II) ions produce cardiac problems at later times. To overcome this difficulty of free metal ions, the hydroxo form of vitamin B12, hydroxocobalamin (LD-50~2 gms/kg) is also administered in cyanide intoxication cases in France [13]. This molecule has a formula weight of 1357 grams/mole, and 1.36 gms of this species can bind 26 mg of cyanide in a 1:1 fashion. The monocyano B-12 is a porphyrin like molecule where one cyanide is bound to the coordinated cobalt(III) ion in a very irreversible fashion, and a second cyanide is less tightly bound. It has been shown that administration of the hydroxy (but not the monocyano) B-12 was effective IV against cyanide in rats [14]. One problem is the massive amounts of the drug that must be administered to bind rather small amounts of cyanide, and it is not clear that this B-12 derivative can be

administered in a prophylactic fashion.

It is seen that a porphyrin like molecule (hemoglobin, cytochrome, vitamin B12) and a metal ion (either iron or cobalt) are active sites in cyanide toxicity and cyanide therapies. It was thought that simpler water soluble porphyrin or phthalocyanine molecules themselves, in combination with various metal ions might have the properties desirable in in-vivo cyanide scavengers, and as such, is the basis of the results to be described.

AUTOANALYZER CYANIDE BINDING RESULTS: A automated cyanide detection apparatus was used for the initial screening studies of cyanide binding to metalloporphyrins [15]. Basically, increasing concentrations of the water soluble porphyrins and related scavengers were equilibrated for several minutes with a constant amount of cyanide at pH 7.5 in a phosphate buffer, and then the solution were dialyzed through a membrane such that only free cyanide and not the scavenger ions passed through this barrier. This cyanide was determined by a fluorescence technique, where cyanide reacted with a non-fluorescent palladium-ligand complex, and the cyanide binding to the palladium displaced the then fluorescent ligand, and the amount of ligand displaced was proportional to the amount of cyanide bound. Plots of the percent cyanide bound vs. scavenger concentration were used to determine the parameter BC-50, which is the amount of scavenger needed to bind 50% of the cyanide present. Since all experiments were done with the same cyanide concentration (~ 5×10^{-4} M), the lower the BC-50, the better the cyanide binding ability of the drug. We found that oxy-vanadium(IV), chromium(III), molybdenum(V), manganese(III), iron(III), nickel(II), copper(II), silver(III), zinc(II), indium(III), aluminum(III) and gold(III) porphyrins of various types at this pH had little cyanide binding ability, as their BC-50 values were > 10^{-3} M. A chromium(III) tetrasulfonated phthalocyanine and a cobalt(III) myoglobin were also in this category. While noted above that Fe(III) hemoglobin and myoglobins have a high cyanide binding ability, we see here that simple Fe(III) porphyrins lack such affinity. This is possibly because Fe(III) porphyrins at this near neutral pH are in oxy-bridged dimeric forms [16,17], P-Fe(III)-O-Fe(III)-P, and the Fe-O-Fe bridge must be broken before cyanide can attach to the iron center. Cobalt(III) myoglobin has the nitrogen atoms of two imidazole ligands attached to the cobalt center, and a slow ligand dissociation must occur before reaction with cyanide [18]. Chromium(III) porphyrins might react too slowly, or have equilibrium constants too small to be of use in this study.

A number of porphyrins had BC-50s in the range 1×10^{-4} to 8×10^{-4} M, and were considered reasonable cyanide scavengers. These included cobalt(III), silver(II), palladium(II), platinum(II) and rhodium(III) complexes of a number of negatively charged carboxylic acid and sulfonic acid porphyrins, such as hemato, proto, copro and uroporphyrin, tetra(4-carboxyphenyl)porphyrin and tetra(4-sulfonatophenyl)-porphyrin. A cobalt(II) tetrasulfonated phthalocyanine and hydroxocobalamin were also in this class. The best cyanide scavengers with BC-50s in the range 1×10^{-5} to 8×10^{-5} were in general cobalt(III) porphyrins, where the porphyrin had positive charges on the periphery, due to (N-methyl-4(3 or 2)pyridyl), (N-methyl-3(or

2)quinoline) or (4-N,N,N-trimethylanalinium) functions. Iron(III) myoglobin and hemoglobin were, as expected, also in this superior scavenger category. Since cyanide is a negative ligand, we would expect that cyanide would complex better with positively charged, as opposed to negatively charged porphyrins, as the results indicated.

Thus, the studies with the autoanalyzer indicated that cobalt(III), rhodium(III), silver(II), palladium(II) and platinum(II) porphyrins might be valuable as cyanide binding ligands. It was never clear to me that the membranes removed all of the metalloporphyrin before the cyanide determination, and thus another method was sought to rank the cyanide affinities of the metalloporphyrins. In addition, palladium and platinum(II) porphyrins show no affinity for simple nitrogenous bases (such as pyridine) in solution, and it was surprising that the automated method gave positive results for these compounds.

CYANIDE ELECTRODE STUDIES: A cyanide specific electrode in a 0.1 M phosphate buffer at pH 7.2 was used to determine the cyanide binding characteristics of various metalloporphyrins and related compounds. Four measured portions of standardized sodium cyanide (to a final cyanide level of 1 mM) was added to fifty ml of buffer solution, and the potential was recorded after each addition. The potentials stabilized very quickly in this rapidly stirred solution, and a pH electrode was present such that the pH could be returned to 7.2 by adding small amounts of acid or base. A plot of electrode potential vs. log(CN) was linear between 10^{-5} and 10^{-3} M total added cyanide. With such a calibration curve for each experiment, various concentrations of dissolved cyanide scavenger were added to this initially 1 mM cyanide solution, and the potentials were recorded after each addition. Knowing the calibration curve, a graph of the millimoles of free cyanide remaining vs. the millimoles of scavenger added could be constructed. If the curve was linear, with a sharp break when little free cyanide remained in solution, the slope of such a curve gives the number of cyanide ligands bound per mole of scavenger drug added, and corresponds to a stochiometric binding of cyanide by added scavenger. If the curve was not linear, it indicated an equilibrium addition of cyanide to the scavenger, and the initial slope corresponds to the moles of cyanide bound per mole of scavenger, when the cyanide was in excess. The lack of cyanide uptake was readily noted by the lack of change of potential with time. In addition, it was easy to determine how rapidly the scavenger bound cyanide, by noting the measured potentials as a function of time.

With the cyanide electrode, we determined that two moles of cyanide were rapidly and stochiometrically bound by one mole of cobalt(III)-tetra(4-sulfonatophenyl)porphyrin or cobalt(III) tetra(N-Methyl-4-pyridyl)porphyrin, which are negative and positively charged porphyrins, respectively. Hydroxocobalamin, the hydroxy cobalt(III)-B12 derivative also bound two moles of cyanide. Platinum(II), silver(II) and palladium(II) porphyrins showed no interactions with cyanide, in contrast to the positive results found with the autoanalyzer. In addition, rhodium(III) tetra(4-sulfonatophenyl)porphyrin bound one mole of cyanide slowly, and might have bound more at longer times. The iron(III) porphyrins tested showed little rapid cyanide uptake at this pH. The favorable behavior of the cobalt(III) porphyrins led to a detailed kinetic and

equilibrium examination of cyanide interactions with water soluble cobalt(III) porphyrins.

CYANIDE / COBALT(III) PORPHYRIN KINETICS: The interaction of cobalt(III)-tetra(4-sulfonatophenyl)porphyrin with cyanide and hydrocyanic acid were studied at an ionic strength of 0.1 $NaNO_3$ at 25°C. At pH 7.4, this cobalt porphyrin bound 1.9 ± 0.1 moles of cyanide. The di-cyano complex $CoP(CN)_2$ was formed at pH 7, and when the pH was lowered to 2.0, only the monocyano $CoP(CN)(H_2O)$ was present two days later, indicating a dissociation constant (eq 1) of $K < 10^{-12}$ for removal of the last, very tightly bound cyanide.

$$CoP(CN)_2 + H_2O = CoP(CN)(H_2O) + CN^- \quad K \qquad (1)$$

$$CoP(CN)(H_2O) + CN^- = CoP(CN)_2 + H_2O \quad K_2 \qquad (2)$$

The formation constant (K_2, eq 2) of $CoP(CN)_2$ from $CoP(CN)(H_2O)$ was 3.5×10^6. From pH 4 to 11, the kinetics of monocyano porphyrin formation were first order in metal and total cyanide concentration. Figure 2 shows the dependence of the specific rate constants upon pH. The kinetic results for the reactions of the various forms of the several cobalt(III) porphyrins and B-12 with cyanide are listed in Table 1.

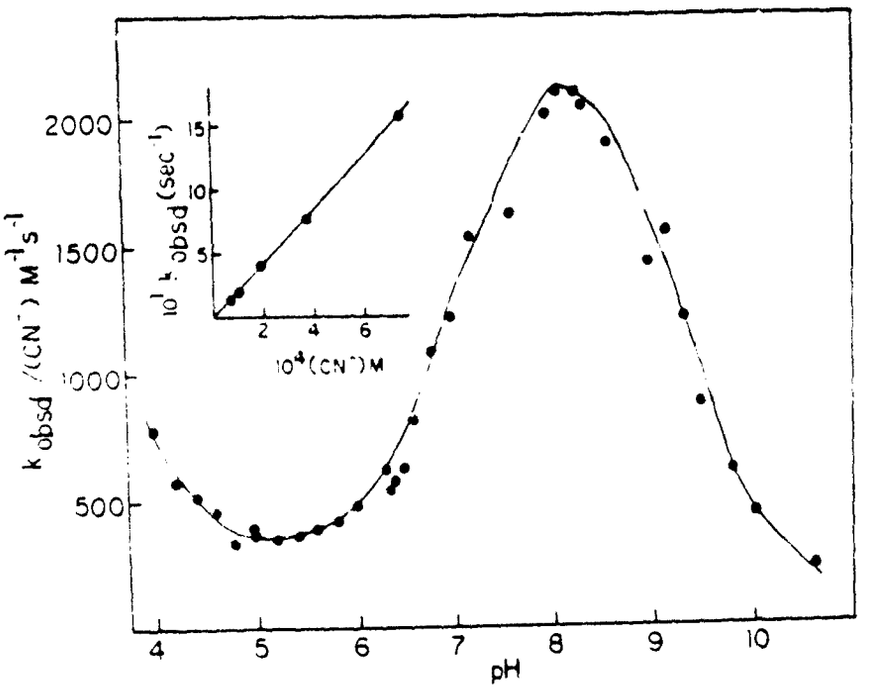


FIGURE 2. pH profile of the kinetics of cyanide addition to Co(III)-TPPS. I = 0.1 ($NaNO_3$).

Table 1. Rate Constant Comparisons, units of M^{-1} s^{-1}

Reaction[a]	M(III)-Porphyrin$(H_2O)_2$	M(III)-Porphyrin$(H_2O)(OH)$
Co(III)-TPPS/CN^-	3.1×10^2	2.4×10^3
Co(III)-TPPS/SCN^-	3.2×10^2	1.4×10^3
Co(III)-TMPyP/CN^-		1.6 x 103
Co(III)-TAP		2.2 x 103
Co(III)-TPPS/I^-	1.2×10^2	
Co(III)-TPPS/HCN	3.1×10^{-3}	
Co(III)-TPPS$(H_2O)(CN)$	3.1×10^4	
H_2O-B-12/CN^-	2.5×10^2	<<1.0
H_2O-B-12/HCN	8.0×10^1	
NC-B-12/CN^-	8.0×10^4	
Rh(III)-TPPS/CN^-	5.0	
Rh(III)-TPPS/HCN	2.3×10^{-3}	
Cr(III)-TMPyP/CN^-	7.9×10^{-1}	
CO-Ru(II)-URO/CN^-	2.3	

a. TPPS is tetrakis(4-sulfonatophenyl)porphyrin, TMPyP is tetrakis(N-methyl-4-pyridyl) porphyrin, TAP is tetrakis(4-N,N,N-trimethylanilinium)porphyrin, B-12 is hydroxo-cobalamin, URO is uroporphyrin-I.

Several important facts can be noted from Table 1. Water soluble cobalt(III) porphyrins scavenge cyanide as effectively as does B-12. Both types of molecules essentially irreversibly bind one cyanide molecule, and strongly bind a second near

the physiologic pH. For B-12, the cobalt must be reduced to the Co(II) oxidation state in order to release the tightly bound cyanide ligand. It is noted that the specific rates of ligand addition (cyanide, thiocyanate, iodide) to these cobalt(III) porphyrins are much the same, indicating that the slow step in the process is dissociation of the H_2O~Co(III)-P bond, followed by rapid, non-specific addition of the incoming ligand. In addition, the cyanide adds more rapidly to the HO-CoP(H_2O) than the(H_2O)$_2$-CoP form, showing that the electron density of the coordinated hydroxide is transferred through the cobalt atom, and thus labilizing the water molecule trans to it, allowing it to dissociate faster, and then rapidly react with cyanide. This is an important feature, as the extremely reactive hydroxy form predominates near neutral pH, and accounts for the rapid cyanide reactivity in this range (see Fig 2). The order of trans labilization is $CN^- > OH^- > H_2O$ in the ratio 200:20:1. Thus, a second cyanide adds to the porphyrin faster when a cyanide ligand is already present, making first cyanide addition rate-determining. In addition, the Co(III) porphyrins (and B-12) react with both CN- and HCN. Unfortunately, the HCN reacts with the di-aquo porphyrin rather than with the monohydroxy species, the latter being in high concentration near pH 7.2. From an in-vitro stand point, these cobalt(III) porphyrins are potent cyanide scavengers. Under cobalt in the periodic table is rhodium, and we looked briefly at the cyanide affinity of a rhodium(III) porphyrin.

CYANIDE / RHODIUM(III) PORPHYRIN KINETICS: The interactions of cyanide with rhodium(III)-tetrakis(4-sulfonatophenyl)porphyrin were investigated by kinetic and equilibrium methods [20]. The equilibrium constant for first cyanide addition to Rh-TPPS was 5.4 x 10^5 M^{-1} at pH 7.4, as compared to 1.2 x 10^2 M^{-1} found at pH 10.5. The reaction kinetics were first order in porphyrin and cyanide concentration, and a plot of the observed specific rates vs. cyanide pH are shown in Fig 3.

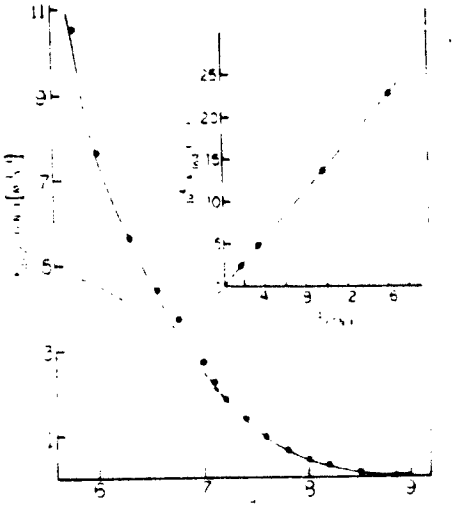


Figure 3. pH profile of the specific rates of rhodium(III)-tetrakis(4-sulfonatophenyl)-porphyrin reactions with cyanide. The dashed line shows the reactivity if HCN were not a reactant.

In contrast to the cobalt(III) porphyrins, the mono and di-hydroxy forms of the Rh(III)-TPPS were unreactive with cyanide, and since the pK_a for the formation of the monohydroxy rhodium porphyrin is 6.9, this unreactive species is in high concentration at the physiologic pH. As shown in Table 1, both CN- and HCN react with the diaquo $(H_2O)_2$-Rh(III)-TPPS to form what appears to be only a monocyano adduct. The cyanide ion reacts over two thousand times faster with Rh-TPPS than does HCN. In comparison with the cobalt(III) derivatives, which show faster cyanide uptake rates and a more favorable reactivity pattern at pH 7.2, the rhodium porphyrin complexes appear less attractive as cyanide scavengers.

CYANIDE / RUTHENIUM(II)-PORPHYRIN INTERACTIONS: The high oxidation state Co(III) and Rh(III) ions are hard metal ion centers, and react with hard ligands such as SCN^- and OH^-. To extend this work, the interactions of cyanide with carbonyl-ruthenium(II)-uroporphyrin-I was investigated [21]. The lower oxidation state Ru(II) is soft, as can be seen from its bonding with CO, and in contrast to the Co(III) and Rh(III) porphyrins, the CO-Ru(II)-P does not show any reaction with SCN^- or OH^-. An equilibrium constant (which was pH independent from 6 to 12) of 1.1×10^4 M^{-1} was found for monocyano addition to this ruthenium porphyrin (eq 3),.

$$CN^- + \text{CO-Ru(II)-P} = \text{(CO, CN-)-Ru(II)-P} \qquad (3)$$

The specific rate constant for the first cyanide addition was rather slow, 2.3 M^{-1} s^{-1}. If allowed to stand under a nitrogen atmosphere overnight, the CO ligand was replaced by a second cyanide, forming $(NC)_2$-Ru(II)-P, and the rate of this second cyanide addition was independent of cyanide concentration. This implies that dissociation of the CO was rate limiting, followed by the rapid addition of the second cyanide to the carbonyl depleted species. When exposed to oxygen, the dicyano Ru(II) porphyrin was oxidized to the corresponding dicyano Ru(III) state. The slow addition of one cyanide, and the slower reaction with the second does not make this a particularly favorable scavenger agent from a kinetic standpoint. Nevertheless, cyanide addition takes place in the reduced state for this Ru(II) porphyrin, and in the oxidized forms for the Co(III) and Rh(III) derivatives. It may be possible that the trivalent cyanide bound porphyrins could be reduced to their divalent forms in the body, with loss of cyanide carrying capacity. This would not be possible for the ruthenium porphyrin, where both oxidized and reduced states carry two cyanide ligands.

REDUCTION OF COBALT(III)-DICYANO PORPHYRINS: The reductions of di-cyano-cobalt(III) porphyrins were studied by radiolytic and electrochemical techniques to indicate if cyanide remained bound to the porphyrins upon electron addition [22]. The cobalt(III) complexes of the tetrakis(4-sulfonatophenyl) (-TPPS) and tetrakis(4-N,N,N-trimethyl-anilinium) (TAP) porphyrins behaved in a similar fashion. The cyanide free porphyrins are reduced from the Co(III) states to the Co(II) and Co(I) states under pulse radiolysis conditions, where the reducing agents are hydrated electrons and

$(CH_3)_2COH$ radicals. The di-cyano forms are radiolytically reduced to Co(II) states that have spectra similar to, but not exactly the same as compounds without cyanide, indicating some cyanide retention in the coordination shells upon reduction. Electrochemical results showed that the presence of cyanide makes the trivalent compounds more stable towards reduction. The $E_{1/2}$ values (in volts) for the Co^{III}/Co^{II} couple move from +0.202, +0.047 to -0.842 as the ligands around the Co(III) are changed from H_2O to OH^- to CN^-. The Co(III) and Co(II) states retain cyanide, whereas the porphyrin in the Co(I) state looses the bound cyanide.

The results for Co(III)-TMPyP (tetrakis(N-methyl-4-pyridyl)porphyrin are rather different from the TAP and TPPS complexes. Again, without cyanide, the Co(III)-TMPyP can be reduced at the metal to the Co(II) and Co(I) forms. With two cyanides bound, however, electron addition is now at the porphyrin ring rather than at the metal ion, forming a dicyano-Co(III)-radical anion, $[(CN)_2Co^{III}TMPyP]^{2-}$, as in eq 4.

$$[(CN)_2Co^{III}TMPyP]^{-} + e^{-} = [(CN)_2Co^{III}TMPyP]^{2-} \quad (4).$$

This radical anion then disproportionates into the initial cyano Co(III) porphyrin, and the di-ring reduced porphyrin dianion, [(CN)2-CoIIITMPyP]3-, as in eq 5.

$$2\,[(CN)_2Co^{III}TMPyP]^{2-} = [(CN)_2Co^{III}TMPyP]^{-} + [(CN)_2Co^{III}TMPyP]^{3-} \quad (5)$$

As a consequence, we would expect that cyanide addition to Co(II)-TMPyP might result in the electron moving from the metal onto the ligand during cyanide binding, both reducing the ligand and oxidizing the metal. This process (eq 6) was in fact demonstrated, and the product radical anion then undergoes disproportionation, as shown in eq 5.

$$Co(II)\text{-}TMPyP + 2\,CN^- = [(CN)_2Co^{III}TMPyP]^{2-} \quad (6)$$

The Co-TMPyP system thus shows the novel effect of cyanide addition in both oxidation states, in where the cyanide is bound in both cases to the Co(III) center. The B-12 system shows similar reactivity. Addition of cyanide to Co(II)-B12 causes disproportionation to the Co(III) and Co(I) states:

$$2\,Co^{II}\text{-}B\text{-}12 + 2\,CN^- = (CN)_2Co^{III}B\text{-}12 + Co^{I}B\text{-}12 \quad (7)$$

In conclusion, all of the in-vitro experiments indicate favorable cyanide binding for cobalt(III) and in some cases cobalt(II) porphyrins. We had previously [23] looked at the kinetics of the reduction of a series of cyano cobalt(III) porphyrins by dithionite ($S_2O_4^{2-}$). The di-cyano adducts were reduced by the dissociation product SO_2^-,

while the mono-cyano derivatives preferred $S_2O_4^{2-}$.

IN VIVO SCREEN RESULTS FOR METALLOPORPHYRINS: A number of chemically analyzed cobalt, palladium, iron chromium silver and rhodium porphyrins were submitted to WRAIR for testing as prophylactic anti-cyanide drugs, and the results are shown in Table 2.

Table 2. Screen Results for Metalloporphyrins and Phthalocyanines

Compound[a]	LD_{50}	DRUG/CN	HI DOSE--Survival(60,15)--LOW DOSE			
Co(II)-PHTH	325	1.7	0,2,	0,0	0,0	0,0
Co(III)-TMPyP(4)	260	0.5	1,1	1,0	0,0	0,0
Co(III)-TMPyP(3)	320	0.5	1,0	0,0	0,0	0,0
Co(III)-TAP	275	0.4	0,0	1,0	1,0	0,0
Co(III)-Hemato	500	3.0	0,0	1,3	1,0	0,1
Co(III)-TPPC	23.7	0.1	0,1	1,2	2,0	0,0
Co(III)-TPPS	650	1.1	0,2	1,1	1,0	0,0
Co(III)-Proto	500	3.2	0,0	0,0	2,1	0,0
Pd(II)-Hemato	500	3.0	1,0	2,1	1,0	1,3
Fe(III)-TMPyP(4)	100	0.2	0,1	0,0	0,0	0,1
Cr(III)-TPPS	650	1.1	1,0	3,0	0,0	0,0
Rh(III)-TPPS	245	1.4	1,3	0,0	0,1	2,1
Ag(II)-TPPS	403	3.0	0,1	0,0	2,0	0,0

a. PHTH is tetrasulfonated phthalocyanine, TMPyP(4,3) are the tetrakis(N-Methyl-4(or 3)pyridyl)porphyrins, TAP is tetrakis(4-N,N,N-trimethylanilinium)porphyrin, Hemato is hematoporphyrin-IX, Proto is Protoporphyrin-IX, TPPS is tetrakis(4-sulfonatophenyl)-porphyrin. The doses were 1/4, 1/16, 1/64 and 1/256 the LD_{50}.

The 24-hour mean lethal dose (LD-50s) was determined and reported in terms of mg/kg, when the scavenger drugs were given by the intraperitoneal route to mice. Pretreatment doses of 1/4, 1/16, 1/64 and 1/256 of the known LD-50 were administered to each of ten mice at 15 and at 60 minutes intervals before they were challenged with 2 LD-50s of NaCN. As a control experiment, 100 mg/kg of $Na_2S_2O_3$ and 100 mg/kg of $NaNO_2$ was administered to a separate group of animals, and the number alive after 24 hours in each set was noted. For the control mice, usually 9/10 or 10/10 survived. At least 4 out of 10 mice needed to survive for the scavenger drug to be considered useful. As noted in Table 4, none of the twelve metalloporphrins, or the one cobalt(II) tetrasulfonated phthalocyanine showed any prophylactic anti-cyanide activity.

It is noted that these macrocyclic compounds have rather low LD-50s, in the neighborhood of ~500 mg/kg, and drugs that have values above ~1000 would be more profitable. The low LD-50s means that relatively little compound was administered, with respect to the total amount of cyanide injected. Table 2 shows that in no case was the ratio (moles of scavenger injected/moles of cyanide injected) greater than ~3.2/1. If we assume that at least a 1/1 ratio is required to stochiometrically bind cyanide, and considering the probable loss of macrocycle to other body compartments, one might conclude that too little of the drug had been administered to be effective. In addition an iv rather than an ip administration of the scavengers might have led to more rapid drug distribution, and better apparent scavenging action. We submitted the B-12 compound hydroxocobalamin to the screen, but as yet the results are not available. It is now obvious that in vitro activity does not necessarily correlate with iv vivo results.

N-METHYL-METALLOPORPHYRINS AND CYANIDE: We found that the chloride salts of Ni(II) and Co(II) were excellent prophylactic anti-cyanide scavengers. For example, the LD-50 of $CoCl_2.6\ H_2O$ was 195.5 mg/kg, and 9/9 mice survived at 24 hours after a challenge of 2 LD-50s of cyanide, both after 15 and 60 minutes pretreatment with 48.9 mg/kg of the cobalt(II) scavenger. With $NiCl_2.6H_2O$, the LD-50 was 299 mg/kg, and 10/10 mice survived both the 15 and 60 minute pretreatment with 75 mg/kg of the nickel(II) salt. These ions are cardiotoxic themselves, and the Ni(II) and Co(II) porphyrins have little cyanide affinity. The idea was to synthesize Ni(II) and Co(II) water soluble centrally N-methylated porphyrins. These species have a central porphyrin proton replaced by an N-methyl group, and this group for steric reasons raises the metal ion above the porphyrin plane, where it should be more active towards nucleophiles such as cyanide. The notion was that one or two moles of cyanide would bind to the above-the-plane nickel ion, and begin the removal of the Ni(II) from the porphyrin. Since Ni(II) can form $Ni(CN)_4^{2-}$, two further cyanides would ultimately be bound to the Ni(II) center after it left the porphyrin. In essence, the metallated N-methyl porphyrin would keep free metal ions out of solution, and supply the metal scavenger agent only in the presence of, and upon demand by cyanide.

We synthesized the N-methyl tetraphenylporphyrin, and sulfonated it to a water

soluble tetrasulfonated form using hot sulfuric acid. An excess of nickel chloride was added to the porphyrin, which led to the rapid formation of the nickel-porphyrin adduct, and this was passed through an ion exchange column in the Na^+ form to remove excess Ni(II). Using the cyanide electrode, exactly one mole of this nickel(II) porphyrin reacted with four moles of cyanide, and we could demonstrate spectrophotometrically that the Ni(II) had been removed from the porphyrin. The experiments with the Co(II) N-methylated water soluble porphyrin were more complicated to interpret, as addition of cyanide to the Co(II) causes at some stage oxidation to the cyano-cobalt(III) state. It was difficult to synthesize large amounts of the pure N-alkylated porphyrins, and so these derivatives were not submitted to the cyanide screen. They might be excellent agents.

Table 3. Equilibrium and Cyanide Addition Rate Constants

Compound[a]	K_{eq} (M^{-1})	k_f (M^{-1} s^{-1})
H_2O-Co^{III}-TPPS	$>10^{12}$	3.1×10^2
H_2O-Co^{III}-B-12	1.3×10^{14}	2.5×10^2
H_2O-Ru^{III}-TPPS	5.4×10^5	5.0
HO-Cr^{III}-TMPyP	1.5×10^3	7.9×10^{-1}
CO-Ru^{II}-URO	1.1×10^4	2.3
HO-Fe^{III}-MESO		7×10^3
HO-Fe^{III}-TPPS		1×10^2
H_2O-Fe^{II}-Mb	1.0	1.0 x 10-1
H_2O-FeIII-Mb	5.7×10^4	1.7×10^2
H_2O-Fe^{III}-Mp-8	3.6×10^7	6.0×10^5

a. TPPS is tetrakis(4-sulfonatophenyl)porphyrin, TMPyP is tetrakis(N-methyl-4-pyridyl)porphyrin, Mb is met-myoglobin, Mp-8 is a microperoxidase.

STERICALLY HINDERED IRON PORPHYRINS AND CYANIDE: Table 3 gives the equilibrium constants and formation rate constants of a number of porphyrin like species and cyanide. The iron(III) myoglobins and microperoxidase have high equilibrium constants for cyanide addition, and the latter has a favorably rapid cyanide complexation rate. In addition, iron would certainly be the scavenger of choice, in comparison to cobalt or nickel. The reason that iron porphyrins were not further investigated was that around pH 7, the iron porphyrins are oxo bridged dimers, PFe-O-FeP, and the oxybridge must be broken, usually by protonation, before cyanide can complex with the porphyrin. The protein chains in myoglobin, hemoglobin and the microperoxidase serve to keep one porphyrin away from another, not allowing oxy bridge dimer formation, and presenting a bare Fe, an $Fe\text{-}OH_2$ or Fe-OH iron environment favorable for cyanide complexation. Tetraphenyporphyrin has four phenyl groups around the porphyrin periphery (see Fig 1), and its iron(III) porphyrin dimerizes. However tetra(2,6-dichlorophenyl)-porphyrin in the Fe(III) form remains in a monomer at the physiologic pH, because the two large chloro groups sterically inhibit porphyrin approach, and hence Fe-O-Fe formation. We made a number of sterically substituted porphyrins, using 2,6-dichloro, 2,6-difluoro, 2,6-dimethoxy, and 2,4,6-trimethyl groups attached to the porphyrin phenyl rings. We sulfonated these porphyrins using fuming sulfuric acid, to render them water soluble. Reverse phase thin layer chromatography showed that numerous products were formed, all of which were water soluble. It seems that sulfonation occurs in the meta(3) position, and either one or two sulfonic acids can be on each phenyl ring, and each molecule has four phenyl rings If one sulfonic acid is on each ring, various atropisomers can also be present, which further complicates the problem. In any case, we spent much time trying to separate these various isomer forms, and characterize them by C,H,N,S analysis and NMR techniques. The final yields approached ~1%, and we have yet to develop a method that will give only one product. The corresponding para (4-position) phenyl substituted porphyrins were prepared for comparison purposes, and in general they were simply mono-sulfonates in the meta position.

Beers law studies were done on all derivatives over a wide concentration range to establish the nature of the species present. All of the 3-sulfonato-4-substituted phenyl porphyrins were dimers in solution in their metal free forms, with dimerization constants of 10^4 to 10^5 M^{-1}. In several cases, temperature jump relaxation studies were done to measure the dimer formation (~10^6 M^{-1} s^{-1}) and dissociation rate constants. In contrast, all of the ortho (2-phenyl) and di-ortho (2,6-diphenyl) substituted porphyrins were monomeric in solution, as evidenced by Beers law data, and the lack of T-jump relaxations. Thus, not only do these ortho substituted derivatives inhibit oxo bridged iron dimerization, they prevent pi-pi dimers from forming with non metallated species. These water soluble free base porphyrins, H_2-P can protonate in solution into H_3-P^+ and H_4-P^{2+} forms, and we measured the pK_a values for a number of compounds. The ortho derivatives protonate much less readily than do the corresponding para derivatives, and since the mono and di-cations are non-planar, the ortho porphyrins might inhibit such porphyrin ring deformation that could give rise to non-planar structures. It would be interesting to find out how the nature of

these new derivatives affects their LD-50s, as dimerization might play a large part in the observed toxicity.

The whole body magnetic resonance imaging machine in Howard Hospital was used to determine the relaxivity of a number of metal complexes of the new porphyrins. The ortho substituted iron(II) derivatives had substantially higher relaxivities than the para substituted compounds, indicating that the former were more highly paramagnetic at pH 7.2 than the latter, which feature antiferromagnetic Fe-O-Fe coupling. More importantly, preliminary work indicates that the ortho iron(III) porphyrins react rapidly with cyanide near the physiologic pH, much more so than is found in the non-hindered water soluble derivatives. We plan to continue this and related work on sterically hindered porphyrins and cyanide in the future.

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