for a group of Minnesotans. However, in another study (*8*), an average value of 0.058 ppm was reported for Minnesotans. Guy (*3*) reported a mean of 0.015 ppm F^- in inhabitants of New York State, and later showed the concentration of F^- in plasma to be dependent on the level of F^- in the drinking water. Therefore, the slightly higher concentrations of F^- in the Chinese may be due to fluoride in their food and drinking water.

It is difficult to compare reported fluorine values due to the variety of analytical procedures used. Negative factors (such as volatility and incomplete sample decomposition), positive factors [such as contamination with F^- and Freons (*3*)], and the method itself (*10, 11*) influence the reported values. Due to the paucity of values determined with the oxygen bomb method, it is necessary to use results obtained by ashing for comparing levels of organic fluorine in serum.

Many compounds containing organic fluorine have useful industrial and medical applications (*12*); the wide use of these compounds implies widespread exposure to them. Reviews have been written on the role of organic fluorine in biochemistry (*13*), psychiatry (*14*), and toxicology (*15, 16*). The fluoroorganic compounds methoxyflurane and halothane are anesthetics (*17*), and artificial blood contains perfluorocarbons (*18, 19*). Several natural sources have also been suggested (*3*).

In a recent study on the exposure of industrial workers to fluorochemicals (*20*), elevated concentrations of organic fluorine (1 to 71 ppm) were found in the serum of chemical employees handling a specific fluorochemical (ammonium salt of perfluorooctanoic acid, $C_7F_{15}CO_2^-NH_4^+$). It was also found that this fluorochemical is slowly eliminated from the body. Therefore, it appears that blood levels of organic fluorine are dependent on the frequency of exposure to specific fluorochemicals.

If man (*20*), rat, or monkey (*21*) is exposed to ammonium perfluorooctanoate, the compound is subsequently found in the blood serum. This is not surprising when one considers the results of a study on the binding of perfluorooctanoic acid to human serum (*6*): more than 99 percent of this added organic fluorine was bound to serum constituents.

It is clear that nearly everyone (> 98 percent) has both forms of fluorine in his blood and that the reported values are somewhat dependent on the method of analysis. The value for F^- depends on diet and drinking water while the value for organic fluorine could be influenced by exposure to certain fluorine-containing compounds from both natural and synthetic sources.

While it was originally suggested (*2*) that the prevalence of organic fluorine in human plasma is due to commercial sources, there now is evidence that the concentrations have been decreasing over the past 15 years (*2*)—although the trend may be due to the methods used to analyze the blood samples. As yet, we find no conclusive evidence to indicate that the prevalence of trace amounts of organic fluorine in human blood is primarily the result of industrial fluorochemicals. Rather, the main source may be some naturally occurring organic fluorine.

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Niacin Reduces Paraquat Toxicity in Rats

Abstract. *Rats poisoned with paraquat benefited from daily niacin therapy. Niacin-treated rats showed delayed and reduced dyspnea. Deaths began approximately 30 hours later. The time required for niacin-treated rats to reach 50 percent mortality increased from 60 to 120 hours, and the death rate was reduced from 75 to 55 percent. The benefit by niacin is consistent with the demonstrated role of niacin in preventing cellular decreases of nicotinamide adenine dinucleotide during poisoning of bacteria by paraquat and by hyperbaric oxygen.*

Paraquat [1,1′-dimethyl-4,4′-bipyridinium (cation) dichloride] is a nonselective postemergence herbicide and defoliant used on a wide variety of crops. Although paraquat is one of the safer herbicides as applied agriculturally, it has caused over 400 human deaths from accidental and suicidal ingestions (*1*). Paraquat also has allegedly caused lung damage to drug users who smoked marijuana obtained from Mexico (*2*). Human fatalities generally are caused by pulmonary impairment, regardless of the method of contact. We have found that niacin is beneficial to rats poisoned by paraquat.

This finding developed from earlier research which had disclosed common sites of damage at the enzyme level in bacteria poisoned by paraquat and by hyperbaric oxygen (*3–8*). The evidence included the discovery that niacin and thiamine were beneficial for the growth of *Escherichia coli* poisoned by hyperbaric oxygen (*5*) or by paraquat (*8*). The mechanism of thiamine protection remains unknown, but there is evidence (*6–8*) that niacin protects *E. coli* because it circumvents the consequences of the poisoning of quinolinate phosphoribosyltransferase. This enzyme is universally required for the de novo synthesis of nicotinamide adenine dinucleotide (NAD); therefore, there is reason to believe that the results may apply to higher life forms.

Consequently, we studied the effects of niacin on paraquat-poisoned rats. The paraquat was given intraperitoneally in two doses of 30 mg per kilogram of body weight, 24 hours apart. Rats that received only paraquat began to die after approximately 30 hours, and 50 percent of the animals were dead by 60 hours (Fig. 1). The group of rats that also received intraperitoneal injections of 500 mg of niacin per kilogram of body weight every 24 hours for 5 days, beginning with

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the first administration of paraquat, began to die almost 36 hours later and did not reach 50 percent mortality until approximately 120 hours (Fig. 1). These differences were significant at $P \leq .05$. The mortality pattern for rats receiving, in addition to niacin, 100 mg of thiamine per kilogram of body weight was not significantly different ($P \leq .05$) from that of those receiving only paraquat and niacin (Fig. 1).

Survivors from all groups at day 7 were recovering from respiratory distress, had begun to consume food and water, and were gaining weight. Animals in all treatment groups showed respiratory distress, but the distress was delayed in the groups receiving niacin or niacin and thiamine. Within all treatment groups the onset of dyspnea for individual animals occurred over a considerable and varied time span and generally preceded death by several hours. Autopsy revealed hemorrhagic, fluid-filled lungs in rats that had received paraquat.

The vitamin treatment resulted in smaller weight losses. Initially, the average rat weight was 199 ± 12 g. Control rats which received neither paraquat nor vitamin therapy gained 9.8 ± 1.7 percent by day 2 and 13 ± 2.1 percent by day 3. Rats treated with paraquat lost 22 ± 3 percent by day 2 and 27 ± 3 percent by day 3. Paraquat-poisoned rats receiving either niacin or both vitamins showed weight losses that were not significantly different and thus were averaged (15 ± 2 percent on day 2 and 16 ± 6 percent on day 3). These losses were significantly different ($P \leq .05$) from those of rats receiving paraquat alone. These data were obtained from ten rats per group initially and at day 2, and from the survivors on day 3, which included ten rats in the control group, four rats in the group receiving paraquat only, and 16 in the group receiving paraquat and vitamin treatment.

Determinations of liver NAD and niacin in paraquat-poisoned rats (Table 1) showed that niacin therapy maintained liver niacin and NAD, that both NAD and niacin were significantly lowered in animals that died from paraquat poisoning, but that deaths also occurred in some animals whose liver NAD was maintained by niacin therapy. The latter is to be expected since other mechanisms of paraquat toxicity most probably exist.

The benefits of niacin observed in this study for paraquat-poisoned rats are supported by biochemical evidence of several kinds. Protection by niacin has been reported for *E. coli* poisoned by hyperbaric oxygen (*5*, *6*) and by paraquat (*8*); the literature also includes specific evidence of other common damage sites at the enzyme level for these two chemicals (*3*, *7*, *8*). Evidence specifically indicates that reduced specific activity of the enzyme quinolinate phosphoribosyltransferase occurs in oxygen-poisoned *E. coli* (*6*). This leads to a decreased NAD concentration which is prevented by niacin, an intermediate beyond the poisoned enzyme, but not by quinolinate which is a substrate for the poisoned enzyme (*7*). Paraquat has been shown to produce superoxide from cyclic reductions caused by electrons, probably provided by the reduced form of nicotinamide adenine dinucleotide phosphate (*9*). This suggests, but does not prove, that superoxide is an intermediate in common in the mechanisms of toxicity of paraquat and hyperbaric oxygen (*10*). It is well established that de novo NAD biosynthesis from quinolinate onward is the same in *E. coli*, rat, and man (*11*).

Clearly, the niacin therapy did not altogether prevent respiratory symptoms in paraquat-poisoned rats, neither did it entirely prevent weight loss or eliminate all deaths. However, benefits accrued in all three aspects of paraquat poisoning from therapy with a medically safe, therapeutic vitamin administration. It is to be anticipated that damage by other mechanisms also occurs in the lung and is not affected by niacin.

Table 1. Effects of niacin therapy on liver NAD and niacin in paraquat-poisoned rats. Male Sprague-Dawley rats, approximately 200 g each, were individually caged and given unlimited amounts of food and water. The numbers in parentheses indicate the number of animals in each group whose livers were analyzed. All injected solutions of paraquat and niacin (concentrations are given in the text) were prepared in sterile, nonpyrogenic distilled water for injection, U.S.P. (McGaw Laboratories) and filter-sterilized through 0.45-μm membranes (Millipore Corporation). We examined the lability of NAD in rat liver after death by killing rats with ether and removing the livers immediately or at 5, 10, 15, and 60 minutes after breathing stopped. The analyzed values for NAD were 99, 98, 80, and 56 percent, respectively, of the values for controls. Livers of the killed animals were removed immediately and frozen in liquid nitrogen. Animals indicated as having died were observed to die; their livers were removed immediately or within 5 to 15 minutes of death, as determined by interval observations. We measured the NAD by a polarographic recycling method (*12*) after extraction as described by Klingenberg (*13*). Niacin was measured by a microbiological assay after extraction of niacin from livers frozen in liquid nitrogen (*14*).

Treatment	NAD (μg/g of liver)	Niacin (μg/g of liver)
Control, killed	484 ± 72 (6)	146 ± 29 (4)
Paraquat		
Killed, 24 hours	519 ± 96 (6)	134 ± 2 (3)
Killed, 48 hours	467 ± 74 (2)	71 ± 66 (2)*
Killed, 44 to 89 hours	125 ± 109 (2)*	63 ± 4 (6)*
Paraquat + niacin		
Killed, 48 hours	428 ± 101 (6)	208 ± 10 (5)*
Died, 81 to 123 hours	371 ± 4 (5)	132 ± 4 (6)

*Significantly different ($P \leq .05$) from controls.

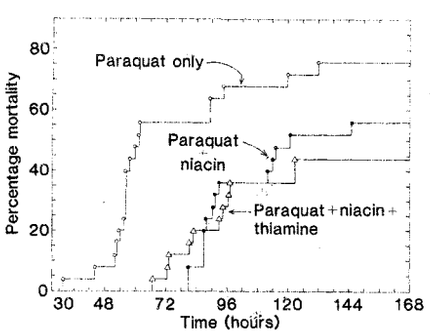

Fig. 1. Effects of niacin and thiamine supplementation on the survival of paraquat-poisoned rats. Experimental conditions were as described in Table 1. Combined results from two experiments are shown with a total of 25 rats per group, including controls (not shown) which received neither paraquat nor vitamin therapy and for which no deaths occurred.

Clearly, the niacin therapy did not altogether prevent respiratory symptoms in paraquat-poisoned rats, neither did it entirely prevent weight loss or eliminate all deaths. However, benefits accrued in all three aspects of paraquat poisoning from therapy with a medically safe, therapeutic vitamin administration. It is to be anticipated that damage by other mechanisms also occurs in the lung and is not affected by niacin.

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Lophotoxin: A Novel Neuromuscular Toxin from Pacific Sea Whips of the Genus *Lophogorgia*

Abstract. *A new neuromuscular toxin, lophotoxin, has been isolated from several Pacific gorgonians of the genus* Lophogorgia. *The structure of lophotoxin was deduced by combined spectrochemical methods, and belongs to the well-known cembrene class of diterpenoid molecules. Lophotoxin contains furanoaldehyde and α,β-epoxy-γ-lactone functional groups, in sharp contrast to the cationic ammonium functional groups of the established neurotoxins.*

As part of a program to explore the chemical defense adaptations of marine organisms, we have investigated numerous representatives of the horny corals or gorgonians (sea fans and whips; phylum Cnidaria, order Gorgonaceae), found mainly in tropical and subtropical waters. In contrast to the hard corals, gorgonians possess no skeletal protection, and hence their survival appears dependent on alternative strategies based certainly, in part, on the production of noxious and toxic chemicals (*1*).

During May and June 1978, a collaborative chemical and pharmacological investigation of the abundant gorgonian fauna of Pacific Mexico was undertaken onboard the research vessel *Alpha Helix*. On-site bioassays indicated that alcohol extracts of several gorgonians from this locale possessed considerable cytotoxic, ichthyotoxic, and antibacterial activity (*2*). In particular, extracts of the colorful sea whips of the genus *Lophogorgia* showed consistent ichthyotoxicity, and in nature these animals showed little evidence of predation. Subsequent chemical and pharmacological studies have now resulted in the isolation and structure assignment of a potent new neuromuscular toxin, lophotoxin (**1**), from four species of *Lophogorgia*. Reported herein are the structural chemistry and fundamental pharmocological properties of this toxin.

Chloroform extracts of three Mexican *Lophogorgia* species, *L. alba* (Duch. and Mich.), *L. cuspidata* (Verrill), and *L. rigida* (Verrill), were each recognized to contain various concentrations of lophotoxin. The California gorgonian, *Lophogorgia chilensis* Verrill (*L. panamensis*), has subsequently also been found to produce lophotoxin. Therefore, the distribution of toxin-producing gorgonians extends from Panama Bay northward to Point Conception, California (*3*).

To isolate the toxin for structure analysis, an extract of *L. rigida* was chromatographed over Florisil and eluted with increasing amounts of ethyl acetate in isooctane. Pure ethyl acetate eluted the toxin in a mixture which was further purified by high performance liquid chromatography (μ-Porasil column; with 60 percent ethyl acetate and isooctane) (*4*). Lophotoxin crystallized from one liquid-chromatography fraction as white needles [melting point, 164° to 166°C; $[\alpha]_D^{27}$ +14.2°C (c = 1.7, $CHCl_3$) (0.2 percent dry weight)].

The structure of lophotoxin was determined by spectral and chemical methods. Along with lophotoxin, we isolated a minor amount of the cembrenolide, pukalide (**2**), a metabolite earlier described by Scheuer and co-workers from the Hawaiian alcyonacean soft coral *Sinularia abrupta* (*5*). The similarity in structure and in spectral features of lophotoxin and pukalide strongly supported our initial structural assignment. A molecular formula of $C_{22}H_{24}O_8$ was established for **1** by mass spectral analysis and by interpretation of ^{13}C nuclear magnetic resonance (NMR) data (Table 1), and this composition indicated 11 degrees of unsaturation. Infrared absorptions at 2850 and 1689 cm^{-1}, coupled with ^{13}C NMR bands at 184.5 (d), 161.8 (s), 149.8 (s), 123.0 (s), and 105.6 (d) allowed the formulation of a furanoaldehyde group. An additional absorption at 1792 cm^{-1} initially suggested the presence of a saturated γ-lactone functional group. However, after recognizing that two trisubstituted epoxides were present by analysis of ^{13}C NMR data [64.1 (d), 61.3 (s), 56.1 (s), and 55.3 (d)] the γ-

Table 1. ^1H and ^{13}C nuclear magnetic resonance data for lophotoxin (**1**); recorded in $CDCl_3$ solution at 220 MHz (^1H) and 20 MHz (^{13}C), with internal trimethylsilyl as standards. Abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; bs, broad singlet; gem, geminal; and J, coupling constant.

Assignments*	^{13}C chemical shift	J_r+	Proton chemical shift
18	184.5 (d)	38.0	9.87 (s)
20	170.2 (s)		
21 (CH_3COO)	168.1 (s)		
3	161.8 (s)		
6	149.8 (s)		
15	148.3 (s)		
4	123.0 (s)		
16	111.0 (t)	23.9	4.93 (bs) 4.91 (bs)
5	105.6 (d)	31.6	6.57 (s)
10	76.7 (d)	23.4	4.81 (dd,$J_{10,9\beta}$=4, $J_{10,9\alpha}$=3)
13	70.3 (d)	22.2	4.99 (d,$J_{13,14\beta}$=7)
11	64.1 (d)	28.1	4.17 (s)
12	61.3 (s)		
8	56.1 (s)		
7	55.3 (d)	26.1	4.09 (s)
9	39.0 (t)	15.4	2.49 (dd,J_{gem}=−16, $J_{9\beta,10}$=4) 2.05 (dd,J_{gem}=−16, $J_{9\alpha,10}$=3)
1	36.5 (d)	16.4	3.99 (ddd,$J_{1,2\beta}$=11, $J_{1,14\beta}$=11,$J_{1,2\alpha}$=4)
2	32.9 (t)	16.6	3.07 (dd,J_{gem}=−18, $J_{2\alpha,1}$=4) 2.95 (dd,J_{gem}=−18, $J_{2\beta,1}$=11)
14	31.7 (t)	14.3	2.50 (m,J_{gem}=−15, $J_{14\beta,1}$=11, $J_{14\beta,13}$=7) 1.70 (d,J_{gem}=−15)
17	21.1 (q)	14.4	1.90 (s)
22 (CH_3COO)	20.4 (q)	14.9	2.05 (s)
19	20.2 (q)	12.4	1.14 (s)

*Assignments are made based on spin-decoupling measurements (^1H) and proton single frequency decoupling experiments (^{13}C). Residual coupling constants (J_r) (*13*) became useful in interrelating proton and carbon resonances.

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