Cyanate and Thiocyanate: Acute Toxicity[1] (37171)

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Thiocyanate (SCN^-) salts have been used in man to estimate extracellular fluid space and to control hypertension. Even when the therapeutic dose is adjusted on the basis of periodic determinations of the blood level, the drug possesses unpredictable toxic effects (1). Of particular relevance to this report are recorded incidents of miosis, toxic psychosis, hyper-reflexia and convulsions (1, 2). Most authorities agree that SCN^- is largely excreted unchanged. When injected into rats (1.2–1.9 mmole/kg), only 1–4.5% of the sulfur is excreted as sulfate (3).

It has been shown (4) that oxyhemoglobin (HbO_2) functioning as a peroxidase can oxidize SCN^- to sulfate and cyanide (CN^-). In this process methemoglobin (MetHb) is generated, and the CN^- is further converted to cyanate (OCN^-) and ammonium ion. It has long been suggested (5) that CN^- is in part responsible for the toxic effects of SCN^-. In intact animals, CN^- is detoxified largely via the enzyme, rhodanese, which converts the CN^- to SCN^-, thereby completing a cycle. The rhodanese reaction is greatly accelerated in animals by the injection of sodium thiosulfate which serves as a source for the required sulfur (*e.g.*, 5).

Cyanate salts are under active investigation for the management of sickle-cell anemia. By carbamylating the N-terminal valine residues on hemoglobin S, OCN^- prevents the sickling reaction (6, 7). In human subjects given moderate doses intravenously, a transient but extreme miosis is noted together with drowsiness and mild diuresis (8). Acute human poisoning by OCN^- has not been described; CN^- is not detectable in the tissues of animals given OCN^- (9), nor is OCN^- known to react with HbO_2 to generate MetHb. Since some OCN^- always exists in equilibrium with urea in aqueous solutions (8), it seems likely that OCN^- is a normal body constituent; SCN^- is well-known to exist in normal body fluids (5). Both OCN^- and SCN^- form weak complexes with MetHb, but nitrite-induced methemoglobinemia does not protect mice against death after the acute administration of either anion (10).

Materials and Methods. NaOCN (K & K Laboratories, Plainview, N. Y.) and NaSCN as freshly prepared aqueous solutions were injected intraperitoneally (10 ml/kg) into female mice (Charles River Breeding Labs, N. Wilmington, Mass.) which were then observed for at least 24 hr. Both 20–30 g virgins and breeder discards were used. Recrystallization of NaOCN from ethanol-water in more recent experiments produced no obvious changes in its biological activity. Groups of at least 10 mice were injected with the same dose of either salt and placed in community cages (45 × 23 × 15 cm) except for the experiment labeled "isolation" in Table II where the animals were housed individually in small wire cages. Blood samples for pigment analyses (11) were taken by cardiac puncture under ether anesthesia.

Results. The toxic syndrome induced by either NaOCN or by NaSCN was characterized by tremor, hyper-reactivity, extensor rigidity and tonic-clonic convulsions. Death appeared to be due to respiratory arrest during the tonic phase. At doses which produced equivalent mortality, hyper-reflexia was more pronounced with OCN^- than with SCN^-. Although animals appeared to be more depressed with SCN^-, loss of right-

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ing reflex did not occur except transiently with convulsive episodes. At the LD_{50} for NaOCN, death occurred at an average time of 2 hr whereas death was usually delayed for 4–8 hr with NaSCN. In both cases auditory and tactile stimuli triggered convulsive episodes. Recovery was usually complete by 24 hr.

Two hours after 6.3 mmole/kg of NaSCN, the HbO_2 and MetHb levels were determined in blood taken from surviving mice (Table I). One animal in this series died before 2 hr, and all showed severe symptoms. The MetHb levels were significantly elevated in mice given NaSCN (Table I) although such levels *per se* could not account for the toxic signs (12). It naturally follows that the HbO_2 levels in these same animals should be significantly reduced, but it was unexpected that the sums of the HbO_2 and MetHb levels were also significantly different (Table I). Thus, about 10% of the total blood pigment in mice given NaSCN cannot be accounted for after 2 hr.

The effect of various other treatments on mortality after NaOCN and NaSCN are summarized in Table II. A dose of phenobarbital which alone did not produce loss of righting reflex, significantly protected mice against death by either NaOCN or NaSCN. The combination of OCN^- and phenobarbital did produce loss of righting reflex for several hours although the combination of phenobarbital and SCN^- did not. A dose of morphine alone sufficient to produce the Straub tail reaction without obvious signs of central nervous depression strikingly potentiated mortality after either SCN^- or OCN^-. Morphine appeared to summate with OCN^- to produce a greater degree of motor activity and hyper-reflexia, but this effect was not so obvious with SCN^-.

TABLE I. Methemoglobin and Oxyhemoglobin Levels Observed in Mice Surviving 2 Hr after NaSCN.

Treatment	*N*	% HbO_2	% MetHb
Saline, 10 ml/kg	4	98 ± 5	4 ± 1
NaSCN, 6.3 mmole/kg	5	74 ± 10[a]	15 ± 4[b]

[a] $P < 0.01$ relative to controls.

[b] $P < 0.001$ relative to controls. As compared separately, the individual sums of the % HbO_2 and the % MetHb were also significantly different ($P < 0.05$). Values are means ± SD.

A dose of L-arginine sufficient to protect animals against poisoning by hydrazine (13) or ammonium salts (14) protected mice against OCN^- (Table II) and against a low dose of SCN^- but not a higher dose. A dose of amphetamine which increases locomotor activity in grouped mice without increasing mortality (15), significantly increased mortality after SCN^- but not after OCN^-. The combination of amphetamine and either anion, however, dramatically increased motor activity and hyper-reflexia, and the average time to death was much shorter with the combination than with either anion alone.

Although not shown in Table II, pyridoxal phosphate (25 mg/kg subcutaneously at the same time as the challenge) in a dose which protects against some vitamin B_6 antagonists (16) had no effect on mortality after NaSCN. Similarly, strychnine sulfate (0.2 mg/kg subcutaneously), either when given together with the SCN^- or 90 min after it, did not influence mortality due to the anion. Because the mice were grouped in the experiments of Table II, it seemed possible that convulsing mice could trigger lethal seizures in animals that might otherwise have survived. However, as shown in Table II, "isolation" into individual cages did not protect against death at the dose of NaSCN tested. Sodium thiosulfate also had no effect on mortality after NaSCN.

In the experiments of Table II, control and treated animals were always injected on the same day and with the same solution of NaSCN or NaOCN, but some of these experiments were performed in 1965 and some in 1972. Despite this time interval, the administration of NaOCN resulted in a predictable and reproducible dose–mortality relationship (Fig. 1). Mortality after NaSCN, however, was highly unpredictable. The range of doses which included some deaths and some survivors in each injected group was 5.1 to 7.9 mmole/kg. On three separate occasions a negative slope was obtained in the dose range of 5.5–5.9 mmole/kg (Fig. 1). A similar phenomenon has been observed at least once before by others (17) on oral administration of a higher dose range of KSCN to rats. No remarkable differences in behav-

TABLE II. The Effect of Various Treatments on the Mortality of Mice after NaOCN or after NaSCN.

Treatment	NaOCN (mmole/kg)	No. dead/No. dosed		*P* value
		Control	Treated	
Phenobarbital sodium[a]	4.6	8/15	0/15	<0.001
L-Arginine[b]	4.2	11/15	1/15	<0.001
Morphine sulfate[c]	3.8	1/15	14/15	<0.001
Amphetamine sulfate[d]	4.3	3/10	7/10	<0.1
	NaSCN (mmole/kg)			
Phenobarbital sodium[a]	6.5	6/12	0/12	<0.01
L-Arginine[b]	6.5	6/12	6/12	NS
L-Arginine[b]	5.5	14/15	8/15	<0.02
Morphine sulfate[c]	4.6	0/12	5/13	<0.02
Amphetamine sulfate[d]	5.1	2/11	7/10	<0.02
Sodium thiosulfate[e]	6.3	9/11	5/11	<0.1
Isolation	7.0	9/10	10/10	NS

[a] Given intraperitoneally (50 mg/kg) 1 hr prior to challenge.
[b] Given subcutaneously (6 mmole/kg) together with challenge.
[c] Given subcutaneously (100 mg/kg) together with challenge.
[d] Given intraperitoneally (5 mg/kg) together with challenge.
[e] Given intraperitoneally (1 g/kg) together with challenge.

ior were noted at the various dose levels of NaSCN. Hyper-reactivity was more pronounced with OCN^- than with SCN^-, yet a linear dose–mortality relationship was obtained with the former (Fig. 1).

Discussion. A contribution of CN^- to the toxic signs observed in mice given NaSCN would appear to be ruled out by observations reported here. Induced methemoglobinemia does not protect against SCN^- poisoning (10); a dose of sodium thiosulfate sufficient to increase the LD_{50} of NaCN in mice more than 4-fold (18) had no significant effect on death after NaSCN (Table II); and, NaSCN itself significantly increases the circulating methemoglobin titer (Table I).

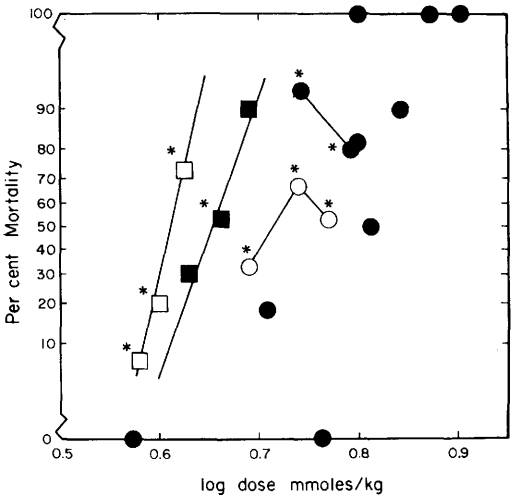

FIG. 1. Log dose–mortality relationships for NaOCN (squares) and NaSCN (circles). Closed symbols indicate 20–30 g virgin female mice; open symbols indicate breeder discard mice. Asterisks show data points collected in 1965.

Since CN^- is known to be generated *in vitro* in the HbO_2–SCN^- reaction (4), it is possible that the 10% of the total pigment unaccounted for in the experiments of Table I might exist as the complex, cyanmethemoglobin. An amount of CN^- sufficient to saturate a 10% level of methemoglobin in mice could arise from conversion of as little as 0.1% of the given dose of SCN^- (19). Irrespective of the nature of this inert pool of blood pigment, even a 25% compromise (15% MetHb + 10% unknown) of the oxygen-transport capability of mouse blood *in vivo* cannot account for the severity of the signs or death after NaSCN.

Other known products of the HbO_2–SCN^- reaction (4) would appear to be unlikely contributors to the toxic syndrome. Although OCN^- is one such product, a 60–90% con-

version of SCN^- to OCN^- would be required in order for the latter to account for death (Fig. 1). Such a degree of conversion is inconsistent with available data (3, 5). A final possibility suggested by the results with arginine (Table II) is intoxication by ammonium ion, a known metabolic product of SCN^- and a likely candidate for OCN^-. Some features of ammonium intoxication mimic signs described here except for coma (20) which was not seen after either SCN^- or OCN^-. Even though its toxicity can be potentiated dramatically by short periods of hypoxia, lethal doses of ammonium salts under ordinary circumstances are at least 1.5 times greater on a molar basis than lethal doses of OCN^-, and they are about the same order of magnitude as those reported here for SCN^- (20).

The structure of thiocyanic acid is known to be: H—S—C≡N, whereas controversy has surrounded the structure of cyanate. Most authorities agree that isocyanic acid, H—N=C=O, is the predominant form, but some samples are said to be contaminated with small amounts of cyanic acid: H—O—C≡N (21). No conclusions appear to have been drawn about possible tautomeric equilibrium between the two forms, yet apparently —S—C≡N is converted to —N=C=O by reaction with HbO_2 (4).

As noted here the toxic syndromes produced by SCN^- and OCN^- in mice have certain similarities, and the data in Table II suggest a similarity in their mechanisms of action. Indeed, these observations appear to be most consistent with a central neurotoxic mechanism of action as the primary effect of both SCN^- and OCN^-. As others have suggested (3, 8), these effects appear to be due to the anions themselves, and not to metabolic products of them. Doses of NaSCN smaller than those used here have been shown to increase transmission through monosynaptic and polysynaptic pathways in the neuronally isolated spinal cord of the cat (22). Although the unpredictable toxicity of SCN^- remains unexplained, it is notable that negative slopes and/or nonlinear dose–mortality relationships are well established features of acute amphetamine poisoning in laboratory rodents (15), and that an interaction between amphetamine and SCN^- is implicit in the data of Table II.

Summary. In toxic doses sodium cyanate and thiocyanate produce similar syndromes in mice: tremor, hyper-reactivity and tonic-clonic convulsions. A common pattern of interactions with central nervous system drugs suggests that these anions have similar mechanisms of action. Thiocyanate had highly unpredictable lethal effects, but its conversion to cyanide *in vivo* cannot account for its toxicity.

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