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Structure-Activity Relationships for Ulcerogenic and Adrenocorticolytic Effects of Alkyl Nitriles, Amines, and Thiols

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In rats, a single administration of acrylonitrile (vinyl cyanide) produces a rapidly occurring bilateral adrenal apoplexy. Structure-activity studies have shown that a close derivative, propionitrile (ethyl cyanide), causes duodenal ulcer without markedly affecting the adrenal glands. Prolonging the two-carbon chain of propionitrile by a methyl group (*n*-butyronitrile) enhances, replacing the methyl by bromide or nitrile decreases, while substitution by an amino group abolistes the ulcerogenic potency and variably affects the adrenocorticolytic action. On assaying a large number of nonnitrile compounds as well for ulcerogenic effect, such as thiols and amines, this effect was found to be related to a two-carbon structure bearing electronegative radicals on one or both ends of the chain.

Alkyl nitriles (e.g., acrylonitrile, propionitrile) are used extensively in the manufacture of acrylic fibers, plastics, and surface coatings and as intermediates in the synthesis of antioxidants, pharmaceuticals, and dyes. Many people are exposed to these compounds, since 5–30% of acrylonitrile may remain as monomers in various synthetic products or may become liberated with the aging of polymers (*1*). Thus, health implications of these compounds are of considerable interest.

Previous Studies

While studying the effect of hormonal and nonhormonal steroids as well as various hepatic drug-metabolizing enzyme inducers on the toxicity of alkyl nitriles, we recently found (*2*) that in rats acrylonitrile produces bilateral adrenal apoplexy. Hemorrhage and necrosis develop rapidly (1–2 hr) and occur mostly in the outer layers of the adrenal cortex. Frequently, subcapsular bleeding is seen as well (Fig. 1). Pretreatment of rats with ACTH (*3*) or phenobarbital, unlike PCN (pregnenolone-16 α–carbonitrile) or spironolactone (*4*) offers complete protection against the adrenal lesions and mortality induced by acrylonitrile.

Experiments on structure–activity relationships of similar compounds revealed that propionitrile (ethyl cyanide) had no adrenocorticolytic action but produced solitary, often perforating duodenal ulcers in rats (*5*). These lesions occurred 3–5 mm from the pylorus of the stomach, mostly on the antimesenteric site of the duodenum and usually penetrated into the liver (Fig. 2). Microscopic examination revealed erosion of the mucosa or superficial or deep necrosis of the duodenal wall infiltrated by varying numbers of polymorphonuclear leukocytes (Fig. 3). The duodenal ulcer invariably developed 24–48 hr after the initial administration of propionitrile and was not accompanied by gastric ulcers.

Cysteamine also produced duodenal ulcers (*6*). Similarities in structure or biologic action between acrylonitrile, propionitrile, and cysteamine were not apparent. Hence, the ulcerogenic

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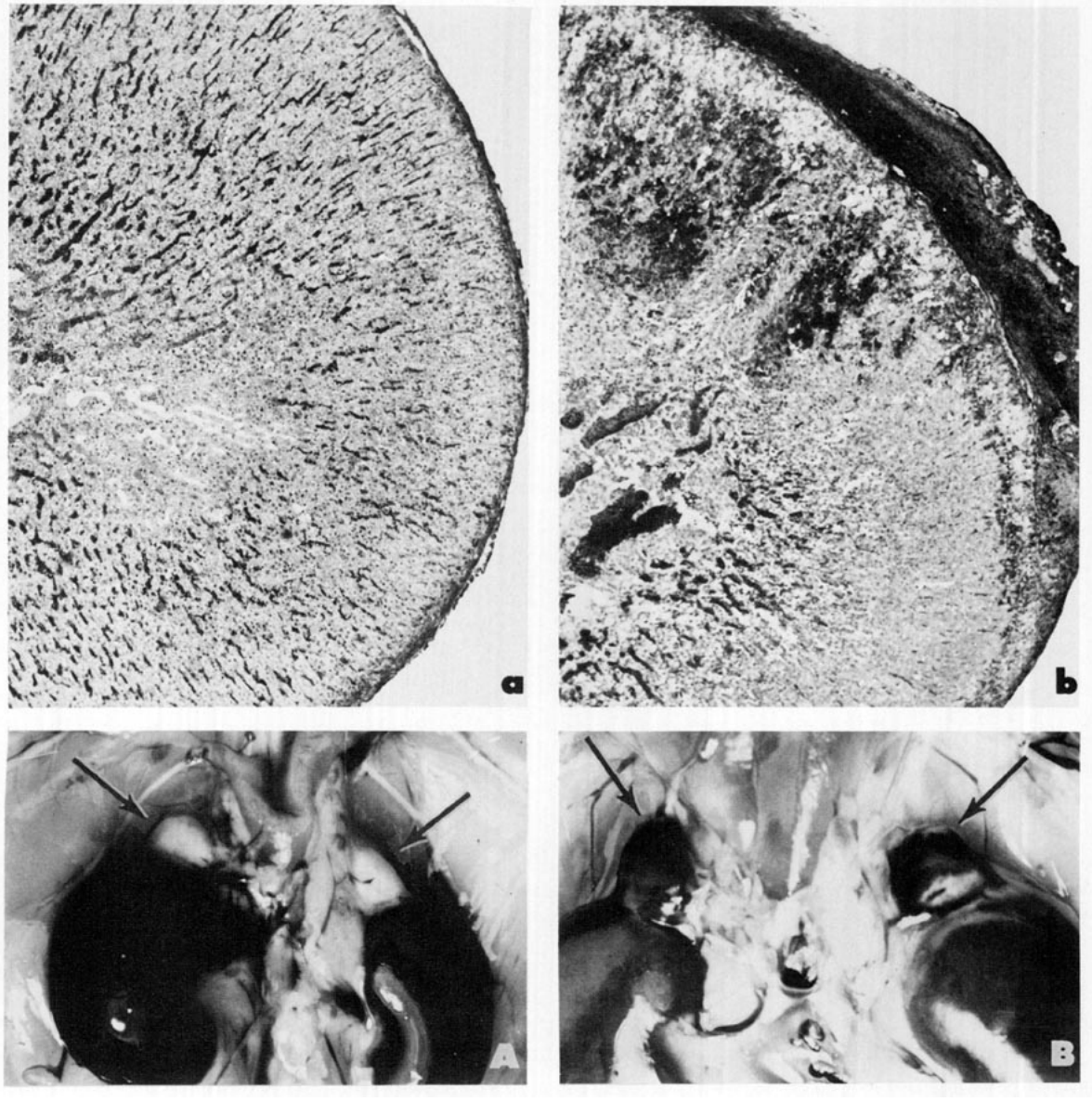

FIGURE 1. Gross and microscopic appearance of adrenals of two rats both given acrylonitrile: (*A*,*a*) pretreatment with ACTH protects against adrenal necrosis, gland appears normal; (*A*) gross, adrenal at arrow (×2); (*a*) microscopic (×49); (*B*,*b*) massive cortical and subcapsular hemorrhagic necrosis in animals not protected by ACTH, (*B*) gross, adrenal is hemorrhagic at arrow (×2.5); (*b*) micro, extensive hemorrhagic necrosis in outer layers of adrenal cortex and in subcapsular area (×49).

effect could not be ascribed to a common property (Table 1). However, more recently, these drugs were noted to have a common "two-carbon" or vicinal carbon (2-C) skeleton, bearing in some cases an electron-negative, high bond energy and/or reactive radical(s).

Based on these assumptions, we have now tested numerous compounds having a two carbon atom skeleton for possible ulcer-producing effects. Preliminary results have been reported elsewhere (*7*).

Current Research

Throughout the experiments, female Sprague-Dawley rats with an initial body weight of

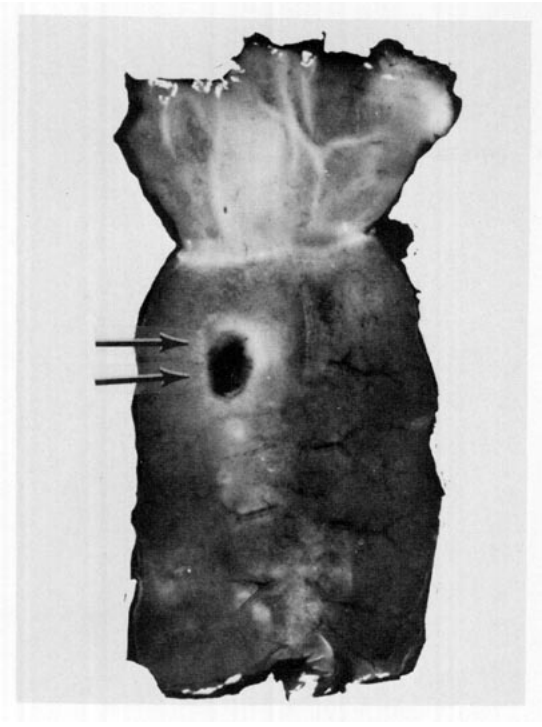

FIGURE 2. Perforated duodenal ulcer produced by propionitrile (mucosal surface): stomach at top, duodenum at bottom; ulcer at arrows, ×45.

100 g or, if so stated, 200 g were maintained on Purina Lab Chow and tap water *ad libitum*. The compounds were given by mouth (PO) and/or subcutaneously (SC) thrice daily for 4 days, unless otherwise stated at the dose level shown in Table 2. Autopsy was performed soon after the animal died or on the fifth day, when survivors were killed by chloroform. The criterion for "positive ulcer" was a lesion identifiable by gross examination.

For every compound, detailed dose-response and toxicity studies were performed (in some cases up to 50 rats were used per drug). Hence, only representative findings of a large series of investigations are reported.

It can be seen (Table 2) that close derivatives of propionitrile, e.g., *n*-butyronitrile, 3-bromopropionitrile, ethylene dicyanide, unlike 3-aminopropionitrile, showed prominent ulcerogenic effect. However, all of these compounds exerted slight adrenocorticolytic action.

Effects of alkylamines and thiols were compared with those of alkyl nitriles. Of the alkyl amines, ethylamine was the most active ulcerogenic compound and also produced adrenal necrosis. Methylamine and propylamine, having structures with one and three carbon atoms, respectively, exert weaker ulcerogenic effects; *n*-butylamine was completely devoid of ulcerogenic action. A hydroxyl attached to the β carbon of ethylamine (resulting in ethanolamine) abolished ulcerogenic potency.

Alkyl aminothiols, close derivatives of cysteamine (cystamine and 2-*n*-butylaminoethanethiol, containing the cysteamine molecule "hidden" in a long carbon chain) produced a high incidence of duodenal ulcer and adrenal necrosis.

In contrast, ethanethiol was not ulcerogenic. However, a derivative of this drug, 2-diethylaminoethanethiol, like 1,2-propanedithiol and less actively 1,3-propanedithiol, showed marked ulcerogenic activity. The four-carbon compound (1-butanethiol) was inactive, while 1,4-butanedithiol had very mild effect.

Discussion

The results presented here demonstrate that certain alkyl nitriles readily produce adrenal necrosis and/or duodenal ulcer in rats. The mechanism of action and the high specificity of localization of the lesion (adrenal and/or duodenum) are poorly understood. No clear structure–activity relationship can be established for the adrenocorticolytic action, although it is striking that the nonsaturated acrylonitrile (vinyl cyanide) causes adrenal apoplexy, the saturated derivative propionitrile (ethyl cyanide) produces duodenal ulcer, and cysteamine (β-mercaptoethylamine) is very active in affecting both of these organs.

There are other data on the ulcerogenic effect of these compounds. It was recently found that propionitrile or cysteamine stimulates gastric secretion and acid output (*8*). Duodenal ulcers following the administration of cysteamine and propionitrile can be prevented by antacids and ligation of the pylorus (*9*). Addition at the terminal carbon of propionitrile with a methyl group (*n*-butyronitrile) enhances the ulcerogenic effect. Bromine or nitrile addition decreases and an amino group completely abolishes the ulcerogenic action. Thus, in this case, the ulcerogenic potency of these radicals added to the terminal carbon of propionitrile may be presented as follows: $—CH_3 > —CN \geqq —Br >> —NH_2$.

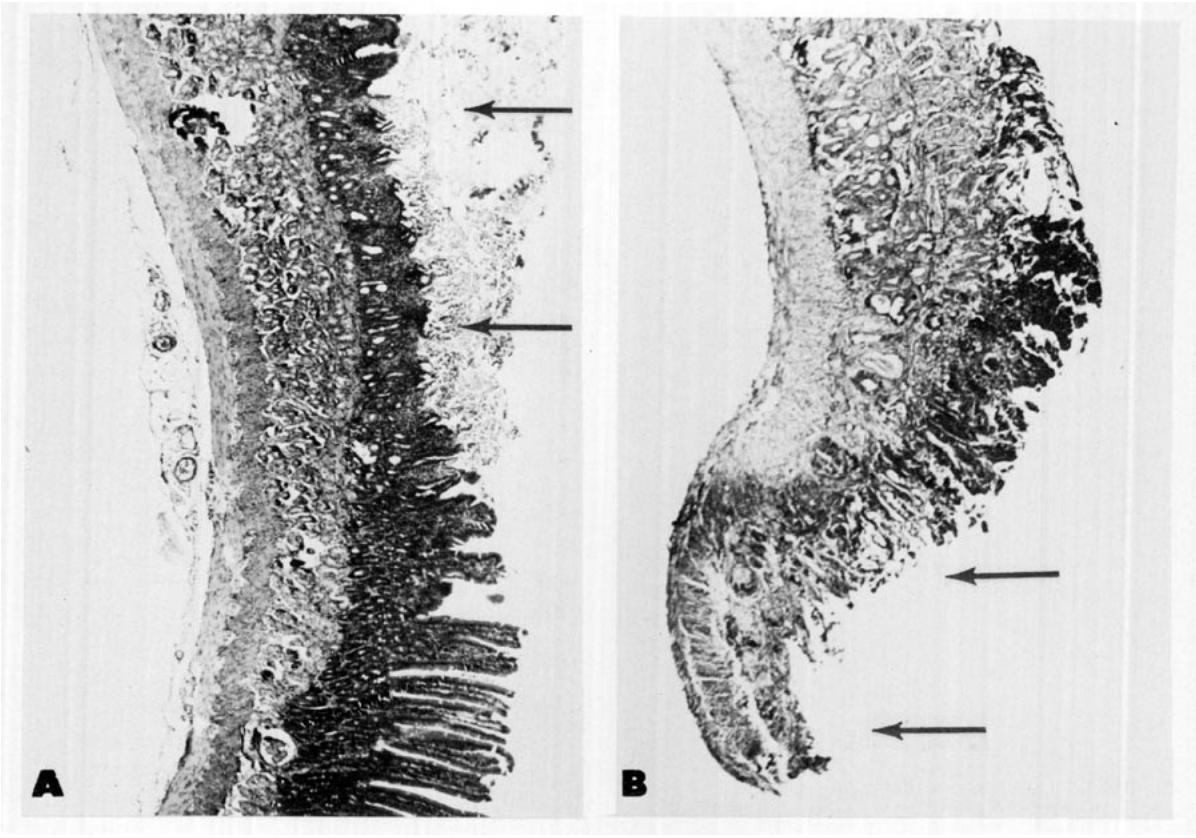

FIGURE 3. Stages of duodenal ulcer development (stomach at top, serosa on left, mucosa on right): (*A*) early, mucosal erosion in *n*-butyronitrile-trea ed rat (arrows) showing villous amputation (H&E, ×39); (*B*) transmural necrosis (arrows) and perforation (at bottom) of duodenum from rats given propionitrile (PAS, ×39).

Table 1. Effect of acrylonitrile, propionitrile, and cysteamine on duodenum and adrenal gland.

Compound	Duodenal ulcer[a]	Adrenal necrosis[a]	Mortality[b]	Dose, mg/100 g
Acrylonitrile $CH_2{=}CH{-}CN$	0/10	10/10	10/10	20, (IV, × 1)
Propionitrile $CH_3{-}CH_2{-}CN$	8/10	0/10	10/10	6 (1st day), 8 (2nd day), 10 (3rd, 4th day); SC×3
Cysteamine $H_2N{-}CH_2{-}CH_2{-}SH$	9/10	8/10	2/10	30, PO×3 or 100, PO×1

[a] Ratio positive/total.
[b] Ratio dead/total.

It can also be assumed that the ulcerogenic effect of drugs is mostly, but not exclusively, related to a two-carbon group bearing radical(s), e.g., —CH_3, —CN, —SH, —NH_2. One of the most important of these observations is the fact that ethylamine, a part of the histamine molecule 4-(2-ethylamine)imidazole, which has been known for a long time to be a potent stimulant of gastric secretion, easily produces duodenal ulcer. It has also been described that 24-hr infusion of histamine in rats results in duodenal ulcer (*10,11*). Our findings, however, demonstrate that the entire histamine molecule is not necessary for this action! The two-carbon

Table 2. Production of duodenal ulcer and adrenal necrosis by alkyl compounds.

Compound	Duodenal ulcer	Adrenal necrosis	Mortality	Dose, mg/100 g
Nitriles				
Propionitrile $CH_3—CH_2—CN$	8/10	0/10	10/10	6 (1st day), 8 (2nd day), 10 (3rd, 4th day); SC (200 g)
n-Butyronitrile $CH_3—CH_2—CH_2—CN$	8/10	2/10	4/10	10 (1st, 2nd day), 20 (3rd, 4th day); SC (200 g)
3-Bromopropionitrile $Br—CH_2—CH_2—CN$	2/5	3/5	5/5	10; PO
Ethylenedicyanide $NC—CH_2—CH_2—CN$	2/5	2/5	2/5	4 (1st day), 8 (2nd day), 10 (3rd, 4th day); SC
3-Aminopropionitrile $H_2N—CH_2—CH_2—CN$	0/5	2/5	5/5	50; SC
Amines				
Methylamine $CH_3—NH_2$	1/5	0/5	5/5	20; PO
Ethylamine $CH_3—CH_2—NH_2$	4/8	3/8	8/8	120; SC (200 g)
Propylamine $CH_3—CH_2—CH_2—NH_2$	1/5	0/5	4/5	40; PO
Butylamine $CH_3—CH_2—CH_2—CH_2—NH_2$	0/5	0/5	5/5	30; PO
Ethanolamine $HO—CH_2—CH_2—NH_2$	0/5	0/5	1/5	50; PO or SC
Aminothiols				
Cystamine·2 HCl $(H_2N—CH_2—CH_2)_2S_2$	4/5	5/5	5/5	20; PO
2-*n*-Butylaminoethanethiol $CH_3—(CH_2)_3—NH—CH_2—CH_2—SH$	5/10	4/10	5/10	20; PO
Thiols				
Ethanethiol $CH_3—CH_2—SH$	0/5	0/5	0/5	20 (1st, 2nd day), 40 (3rd day); PO
2-Diethylaminoethanethiol $(CH_3—CH_2—NH)_2—CH—CH_2—SH$	2/5	0/5	4/5	25; PO
1,2-Propanedithiol $HS—CH_2—CH(SH)—CH_3$	4/5	0/5	2/5	0.5 (1st day), 2 (2nd day), 5 (3rd, 4th day); SC
1,3-Propanedithiol $HS—CH_2—CH_2—CH_2—SH$	2/5	0/5	5/5	4 (1st day), 8 (2nd day), 10 (3rd, 4th day); SC
1-Butanethiol $HS—CH_2—CH_2—CH_2—CH_3$	0/5	2/5	0/5	20 (1st, 2nd day), 40 (3rd, 4th day); PO
1,4-Butanedithiol $HS—CH_2—CH_2—CH_2—CH_2—SH$	1/5	2/5	5/5	15; PO

ethylamine (fragment of histamine) is capable of producing perforating duodenal ulcers.

Adrenal necrosis and duodenal ulcer produced by alkyl derivatives and some other compounds may be useful animal models to study the mechanism of development of these lesions. Until recently, only a few drugs, e.g., 7,12-dimethylbenzo[a]anthracene (*12*), hexadimethrine bromide (*13,14*), thioguanine (*15*), thioacetamide (*16*), and basic polyglutamic acids (*17*) were known to produce adrenal necrosis in rats. Among these compounds acrylonitrile acts the most rapidly; adrenal hemorrhage and necrosis (resembling the Waterhouse-Friderichsen syndrome in man) occur within 1–2 hr, and most of the animals die within 3–4 hr.

Similarly, there are no readily available models for study of duodenal ulcer. It has long been known that stress and various compounds cause gastric but not duodenal ulcers in rats (*18*). Only chronic deficiency of pantothenic acid in certain strains of rats (*19*) and infusion of histamine and other secretagogues (e.g., carbachol, pentagastrin) in fasted rats (*10,11*) are known to produce duodenal ulcers. Most of the compounds presented here cause similar lesion after a single SC or PO dose or after multiple injections at short intervals.

Although there is no overt evidence that these chemicals may in fact be responsible for the production of duodenal ulcers or adrenal apoplexy or atrophy in man, this possibility must be considered in the future.

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