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Antidotal Efficacy of Vitamin B_{12a} (Hydroxo-Cobalamin) in Experimental Cyanide Poisoning. (19831)

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(Introduced by Hans Molitor.)

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It has been shown that vit. B_{12} (cyanocobalamin) contains one cyano group bound coordinatively to the cobalt atom, whereas in vit. B_{12a} (hydroxo-cobalamin) a hydroxo group is present instead of the cyano group(1). The addition of cyanide ions to a solution of vit. B_{12a} results in the formation of vit. B_{12} (2). That the cyano group is tightly bound within the coordination complex of vit. B_{12} is indicated by the observation that dose levels of vit. B_{12} up to 1600 mg/kg both intraperitoneally and intravenously are nontoxic to mice(3). Calculation reveals that a dose of 1600 mg/kg of vit. B_{12} contains the equivalent of about 32 mg/kg of cyanide ion, or about 8 times the LD_{100} dose.

The ease with which vit. B_{12a} reacts with cyanide to yield vit B_{12} and the apparent irreversibility of this reaction in animals suggested that vit. B_{12a} might influence beneficially the course of cyanide poisoning in a manner analogous to that by which B.A.L. counteracts arsenic poisoning(4). The present communication demonstrates that vit. B_{12a} is capable both of preventing and of reversing the toxic effects of cyanide in mice.

Material and Methods. Male mice of the Harpaul and Barckmann stocks, weighing 20-28 g, were employed in these experiments. Freshly prepared aqueous solutions of potassium cyanide were injected intraperitoneally, preceded or followed by the intravenous administration of vit. B_{12a} or physiologic saline. The volume of intravenously injected solutions was kept constant at 0.2 ml/20 g body weight. Exp. 1, 2, and 3 (Table I) were controlled by using a saline-treated group of mice at the beginning and again at the end of the test. In all other experiments alternate (pair-weighed) mice were injected with vit. B_{12a} or saline. The vit. B_{12a} was prepared by irradiation and aeration from crystalline vit. B_{12}(5) and was recrystallized from acetone.

Results. Data in Table I show that the prophylactic injection of vit. B_{12a} intravenously, approximately 20 seconds before the intraperitoneal administration of potassium cyanide, was effective in reducing the mortality due to the latter substance. Respiratory distress and convulsions were prevented completely or were minimized. Doses of 50 or 250 mg/kg of vit. B_{12a} gave adequate protection against doses of 5.5-8.0 mg/kg of potassium cyanide, whereas doses of 25 mg/kg of vit. B_{12a} or less did not. No prophylactic effect against potassium cyanide toxicity (7-8 mg/kg, intraperitoneally) could be shown for vit. B_{12} (cyano-cobalamin) given intravenously in doses of 50-250 mg/kg. The encouraging results obtained by pretreatment with vit. B_{12a} prompted a study designed to determine whether this compound would effect recovery after the toxic manifestations of potassium cyanide were evident.

Table II shows that an intravenous dose of 250 mg/kg of vit. B_{12a} administered within

TABLE I.
Protective Influence of Vitamin B_{12a} Against Lethal Action of Potassium Cyanide in Mice.

Exp.	Stock of mouse	No. of mice	IP dose KCN, mg/kg	IV dose B_{12a},* mg/kg	No. of deaths	% mortality
1	Harpaul	6	5.5	—	4	67
				50	1	17
				5	5	83
				—	4	67
2	"	6	7.2	—	2	33
				50	0	0
				5	4	67
				—	3	50
3	Barckmann	6	7.7	—	6	100
		6	7.7	250	0	0
		5	7.7	—	3	60
4	"	20	8	—	10	50
				(.5, 5, 25) †	12	60
5	"	12	8	—	10	83
				50	1	8
6	"	12	8	—	9	75
				50	1	8
Totals	Harpaul and Barckmann	42	5.5–8	50–250	3	7
		32	5.5–8	.5–25	21	66
		79	5.5–8	—	51	65

* B_{12a} inj. IV about 20 sec before KCN was administered IP.
† Data on 3 dose levels combined since all were ineffective.

TABLE II. Therapeutic Action of Vitamin B_{12a} in Mice* Poisoned with Potassium Cyanide. IP dose KCN, 10 mg/kg.

Exp.	No. of mice	IV dose B_{12a}, mg/kg	Interval between inj. of KCN and B_{12a}, (min.)	No. of deaths	% mortality
7	6	250	1	0	0
			2	0	0
			4	1†	17
	2	"	6	2	100
	3	"	8	3	
	23‡	—	—	23	
8	6	100	1	0	0
	6	"	2	2	33
	12‡	—	—	12	100

* Barckmann stock.
† Of 5 mice which recovered, 3 had shown cessation of respiration at the time vit. B_{12a} was inj.
‡ Individual mice received saline IV at same time intervals after KCN as their respective pair-weighed partners treated with vit. B_{12a}.

one or two minutes, or 100 mg/kg within one minute, after the intraperitoneal injection of an otherwise lethal dose of potassium cyanide, 10 mg/kg, was capable of preventing death. The severe respiratory distress and convulsions, present at the time of B_{12a} injection, disappeared immediately. Vit. B_{12a}, at 250 mg/kg or 100 mg/kg, given 4 or 2 minutes respectively after the injection of potassium cyanide brought about recovery of most mice, even in several animals exhibiting complete respiratory failure. The administration of vit. B_{12a} 6 or 8 minutes after potassium cyanide treatment was ineffectual.

To study further the antidotal action of vit. B_{12a} it was decided to evaluate the efficacy of this compound in mice "apparently dead" of cyanide poisoning, *i.e.*, mice showing no respiration nor responsiveness to external stimuli, such as handling. The majority of mice, thus treated, reacted dramatically to the injection of vit. B_{12a}. Respiration frequently

TABLE III. Recovery Effected by Vit. B_{12a} in Mice* "Apparently Dead" from Cyanide Poisoning. IP dose KCN, 10 mg/kg.

Exp.	No. of mice	IV dose B_{12a}, mg/kg	Time of "apparent death"† (sec after inj.) Mean	Range	Final No. deaths	% mortality
9	5	250	176	(150-215)	1	20
		—	155	(135-195)	5	100
10	6	250	159	(135-210)	5	83
		—	164	(145-240)	6	100
11	7	250	135	(120-155)	1	14
		—	191	(120-520)	7	100
12	3	250	147	(130-175)	0	0
		—	132	(120-150)	3	100
Totals	21	250	153	(120-215)	7	33
		—	166	(120-520)	21	100

* Barckmann stock.

† By "apparent death" is meant the state in which all respiratory movement has ceased and the animal does not react to external stimuli.

TABLE IV. Urinary Excretion Products of Mice Injected with Vit. B_{12a} (Hydroxo-Cobalamin) and KCN. 2.5 hr urine collection.

Excretion products	Amount in μg: Untreated controls (6)	Mice inj. with B_{12a} & KCN (6)	As % of total amt of CN^- inj. (550 μg)
Total cobalamin	—	2950	
as CN^-	—	59	
Cyano-cobalamin (B_{12})	—	2625	
as CN^-	—	52.5	9.6
Free cyanide	—	3.7	.7
Thiocyanate	9.2	52.2	
as CN^-	4.1	23.4	3.5*

* 3.5% = 19.3 μg, difference between that found in urine of inj. (23.4 μg) and untreated (4.1 μg) mice.

returned even before the entire dose of vit. B_{12a} was injected and following completion of injection many of the mice were able immediately to walk. The reduction in mortality effected by vit. B_{12a} treatment is shown in Table III.

In an attempt to elucidate the mechanism by which vit. B_{12a} counteracts the toxic effects of cyanide, the following experiment was performed. A group of 6 mice was first injected intraperitoneally with 10 mg/kg of potassium cyanide and approximately one minute later was given 100 mg/kg of vit. B_{12a} intravenously. The animals were placed in a metabolism cage, with access to water, and urine was collected for a period of 2½ hours. The urine of 6 control mice was collected similarly. Results of analyses of the urines are presented in Table IV. The data show that, of a total of 550 mcg CN^- administered, 9.6% could be accounted for as cyano-cobalamin, 3.5% as thiocyanate and 0.7% as free cyanide.

Discussion. Cyanide ion exerts its acute toxic effect by combining with the metalloporphyrin containing enzyme systems concerned in tissue respiration. Most drugs used for the treatment of cyanide poisoning, such as sodium nitrite or methylene blue, act by virtue of converting hemoglobin to methemoglobin which in turn combines with cyanide to form cyanmethemoglobin. Subsequent treatment with thiosulfate brings about the formation of thiocyanate, a relatively nontoxic substance which is excreted, though somewhat slowly(6,7). The present experiments show that vit. B_{12a} is also effective in counteracting cyanide toxicity. It is evident that the comparatively large quantity of vit. B_{12} found in the urine of mice treated with vit. B_{12a} and potassium cyanide resulted from the reaction of these two compounds, but the precise site where this reaction took place is not known. Urinary excretion data for the first 2½ hours after injection indicate that some of the administered cyanide was detoxified by physiologic conversion into thiocyanate, but that a greater proportion of it was detoxified by combination with vit. B_{12a} to form vit. B_{12}.

The failure of vit. B_{12} to influence favorably the course of cyanide poisoning, despite its capability of binding 1 or 2 moles of cyanide in addition to the cyano group already present in its molecule(8) may be due in part to the lability of the resulting complex.

Summary and conclusions. 1. Vit. B_{12a} (hydroxo-cobalamin), but not vitamin B_{12} (cyano-cobalamin), has been found to be capable of preventing in mice the toxic symptoms and death due to cyanide administration. 2. When injected into mice exhibiting complete respiratory arrest and coma due to cyanide poisoning, vit. B_{12a} effected rapid recovery of most animals. 3. In mice injected with potassium cyanide followed by vit. B_{12a}, some of the cyanide appears in the urine as thiocyanate, but a greater percentage of the cyanide appears as vit. B_{12}, having formed this compound by reacting with the vit. B_{12a}.

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Crystalline Catalase, A Peroxidase. (19832)

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Catalase is generally classified as a non-oxidizing enzyme; its only function is said to be to destroy the toxic hydrogen peroxide. This classification was made because no other substrate has been found for this enzyme. Alkyl peroxides (which are not present in living organisms) are attacked only by concentrated catalase solutions(1). Keilin and Hartree(2) observed that a portion of the hydrogen peroxide, formed when certain oxidases aerobically oxidize their substrates, is utilized by catalase to oxidize ethyl alcohol to acetaldehyde. Oxidation by catalase and hydrogen peroxide was much slower. Theorell (3) and his school believe that catalases are a special group of peroxidases. Their deduction is based on studies concerning the mechanism of action of catalases and of peroxidases. No evidence is available, however, to indicate that catalase can oxidize any large or small molecule of biological importance, or any of the several types of the substances that are oxidized by peroxidases.

The experiments here described demonstrate a series of new functions of catalase. Since the reactions require the presence of hydrogen peroxide, they are peroxidatic in nature.

Experimental. Preparation of crystalline catalase. The crystalline cow liver catalase employed in these experiments was prepared according to the method of Tauber and Petit (4). The crystals which formed during dialysis were recrystallized by dissolving them in the least volume of 0.01 N sodium hydroxide. Without centrifuging, the solution was immediately adjusted to pH 5.8 by the addition of the calculated volume of 0.1 N acetic acid. A small quantity of insoluble matter was removed by centrifuging at room temperature. The clear supernatant was placed in a refrigerator at 4°. In about 3 hours the catalase began to crystallize. The crystals were dissolved in sodium chloride-phosphate buffer at pH 7.3(5). The activity of the crystals, as found by the method of von Euler and Joseph-