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COBALT COMPOUNDS AS ANTIDOTES FOR HYDROCYANIC ACID

BY

C. LOVATT EVANS

From the Chemical Defence Experimental Establishment, Porton Down, Salisbury, Wilts.

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The antidotal potency of a cobalt salt (acetate), of dicobalt edetate, of hydroxocobalamin and of cobinamide against hydrocyanic acid was examined mainly on mice and rabbits. All the compounds were active antidotes for up to twice the LD50; under some conditions for larger doses. The most successful was cobalt acetate for rabbits (5 × LD50), which was effective at a molar cyanide/cobalt (CN/Co) ratio of 5, but had as a side-effect intense purgation. Hydroxocobalamin was irregular in action, but on the whole was most effective for mice (4.5 × LD50 at a molar ratio of 1), and had no apparent side effects. Dicobalt edetate, at molar ratios of up to 2, was more effective for rabbits (3 × LD50) than for mice (2 × LD50), but had fewer side effects than cobalt acetate. The effect of thiosulphate was to augment the efficacy of dicobalt edetate and, in mice, that of hydroxocobalamin ; but, apparently, in rabbits, to reduce that of hydroxocobalamin. Cobinamide, at a molar ratio of 1, was slightly more effective than hydroxocobalamin on rabbits and also less irregular in its action. Cobalt acetate by mouth was effective against orally administered hydrocyanic acid. The oxygen uptake of the body, reduced by cyanide, is rapidly reinstated when one of the cobalt antidotes has been successfully administered.

Hydrocyanic acid acts as a poison because it combines with, and immobilizes the function of, the iron atom in cytochrome oxidase (derived from cytochrome a_3) (Keilin & Hartree, 1939), so blocking the electron transfer through the cytochrome system, and checking the final oxidations involving oxygen uptake of the tricarboxylic acid cycle. It also inhibits many other enzymes, for example, decarboxylases (Blaschko, 1942) ; these last effects have no recognizable relation to the toxic action.

Attempts to find an antidote for cyanide have usually been in the direction of breaking down the cyanide-cytochrome a_3 complex, and have looked for substances with a stronger affinity for cyanide ions than has the oxidase. One such method is to convert some of the blood haemoglobin into methaemoglobin, for example, by injection of a nitrite (Paulet, 1959) ; the methaemoglobin combines with the cyanide ion to form the very stable cyanmethaemoglobin. This has the drawback that it involves the loss of oxygen-carrying power of the blood, so that, for example, to antidote twice the LD50 of cyanide would be equivalent to the loss of about 10% (or in an adult man 450 ml.) of the blood.

Since cobalt salts form stable complexes with cyanide, the cobaltocyanides, $M_4Co(CN)_6$, and the cobalticyanides, $M_3Co(CN)_6$, they have been shown long ago

(Antal, 1894 ; Lang, 1895 ; Meurice, 1900 ; Evans & Watt, 1942, unpublished ; and Mercker & Bastian, 1959a, b) to be efficient antidotes. *In vitro* each mole of cobalt can fix up to 6 moles of cyanide, and if that holds for the conditions *in vivo*, relatively small amounts of cobalt salts would be needed for the neutralization of several lethal doses of cyanide.

Another substance which combines firmly with cyanide is hydroxocobalamin, or vitamin B_{12}a (Conn, Norman & Wartman, 1951), cyanocobalamin or vitamin B_{12} being formed thereby, and this is stable in the dark, but is decomposed again, yielding the hydroxo-compound in light. Braekkan, Njaa & Utne (1957) concluded that the liver stores the hydroxocobalamin in preference to the cyanocobalamin. In the body, small amounts of hydrocyanic acid present in the blood convert the hydroxocobalamin into vitamin B_{12} and Wokes & Picard (1955) believe that this reaction plays an important part in endogenous cyanide metabolism. Vitamin B_{12} functions in the body in the form of a coenzyme (a5 ;6-dimethylbenzimidazole-cobamide), in which an extra sugar and an extra adenine form an attached nucleoside (Lenhert, 1962).

The antidotal action of hydroxocobalamin against cyanide has been studied by Mushett, Kelly, Boxer & Rickards (1952), Mercker & Bastian (1959b), Delga, Mizoule, Veverka & Bon (1961), Delga, Mizoule & Veverka (1961) and Paulet, Bernard & Olivier (1963), and the compound was found to be effective. Various other cobalt complexes have also been tried, notably dicobalt edetate (the chelate of two cobalt atoms with ethylenediamine tetracetic acid), of cobalt with histidine, and also the gluconate and glutamate of cobalt (Paulet, 1957, 1958, 1960 ; Mercker & Bastian, 1959a, b ; Bartelheimer, 1962b ; Tauberger & Klimmer, 1963).

This paper describes a study and comparison of the antidotal actions of cobalt salts, hydroxocobalamin, the hydrolysis product cobinamide, and dicobalt edetate, with a view to providing information that may be of use in cases of poisoning with hydrocyanic acid or cyanides in industry, which now uses these substances in very large quantities.

METHODS

The LD50 of hydrocyanic acid, by various routes of administration, was determined for mice and rabbits. Alkali cyanides were not used as such, as the solutions are so strongly hydrolysed as to be very alkaline (for example 0.02 M solium cyanide has a *p*H of 10.7 and is 98% ionized), and to cause pain on injection, and subsequent tissue damage. Instead, a solution of sodium cyanide was neutralized with acetic acid to about *p*H 7, the content of hydrocyanic acid was estimated by silver titration, and the subsequent dilution to the required concentration was made with 0.9% saline. At *p*H 7 the hydrocyanic acid is about 1% ionized. In some experiments pure hydrocyanic acid was used, but this had no advantage and was more difficult to handle. Owing to the volatility of hydrocyanic acid, the solutions were kept in well-stoppered vessels and, when any doubt existed as to the final concentration, were again titrated at the end of the day's experiments. It was usually found that after 4 hr the concentration had fallen, often by 2 to 5%, and sometimes in hot weather by as much as 10%.

For the solution of cobalt salts the acetate (recrystallized) was chosen, as its solution in water is less acid than that of the chloride or nitrate. The *p*H of 0.1 mM solutions were for acetate 7.42; for chloride 5.78, and for nitrate 6.11.

Intravenous injections were made into the tail vein in mice and an ear vein in rabbits ; intramuscular injections were into the thigh muscles. Doses are expressed usually in terms of μmoles/kg of body weight, and for hydrocyanic acid sometimes also in terms of LD50.

Owing to the rapidity of action of hydrocyanic acid when large doses are given, and to the difficulty of giving intravenous injections when the animal is convulsing, it was more usual to give the antidote first, intravenously, and then to follow this in a matter of seconds by the hydrocyanic acid, usually by intraperitoneal or intramuscular injection, but in some experiments that order was reversed; the antidotal effects were at least as great, and often greater, when that was done, provided the antidote was given soon enough.

In order to indicate the molar relationship between the hydrocyanic acid and the antidote in each experiment, the ratio between them was usually expressed (μmoles of hydrocyanic acid per kg divided by μmoles of cobalt compound per kg) and is called the cyanide/cobalt ratio.

RESULTS

Toxicity of hydrocyanic acid

By intravenous injection. The LD50 for mice was found to be about 40 μmoles/kg, and for rabbits 30 μmoles/kg. Mice given two- to three-times the LD50 convulse in a few seconds and die in 1 to 2 min.

By intraperitoneal injection. As expected, the results showed a rather large spread, the mean LD50 for rabbits being 58 μmoles/kg, and for mice 111 μmoles/kg, so that by this route mice were only about half as sensitive as rabbits. This is probably due to a more rapid detoxification by the mouse liver.

By other routes. It would be expected that toxicity would be maximal when the cyanide is given quickly into a vein which drains into the inferior vena cava, so that it is quickly delivered to the central nervous system, less toxic when it enters more slowly, as by intramuscular injection, and least toxic when it enters the circulation slowly, as when given intraperitoneally, subcutaneously or orally. And, in fact, the descending order of toxicity proved to be intravenous—intramuscular—intraperitoneal—subcutaneous—oral, as shown in Table 1, which also includes some results by other authors. The results of inhalation are more vague, but are probably comparable with those by intravenous injection.

A method of administration often used (Paulet, 1955a, b, 1960 ; Delga *et al.*, 1961a, b), but not employed in the present investigation, is to anaesthetize the animal and to inject the cyanide intravenously at a constant slow rate of about 0.1 mg/kg/min. The results so obtained do not seem to differ greatly from those of the usual procedure for intravenous injection. When the first period of apnoea occurs the dose received is lethal, that is, if the infusion is stopped then, the animal will nevertheless die. These results refer to dogs. Mice do not restart respiration as a rule, after apnoea.

The toxicity of cobalt salts

The cobaltous ion is known to be toxic to some micro-organisms, to depress metabolism in tissue slices (Burk, 1946) and, under certain conditions, *metallic* cobalt can be carcinogenic (Heath, 1960), though this last action has not been demonstrated for the cobalt ion.

TABLE 1

TOXICITY OF HYDROCYANIC ACID

I.v., intravenous ; i.m. intramuscular ; i.p., intraperitoneal ; s.c. subcutaneous.
* At 0·1 mg/kg/min

Species	Route of administration	LD50 (μmoles/kg)	95% Fiducial limits (μmoles/kg)	LD50 (mg/kg)	Reference
Rabbit	I.v.	c.30	—	0·82	This paper
	I.v.*	c.27	—	0·7	Delga *et al.* (1961a, b)
	I.m.	40·7	30·3–52	1·10	This paper
	I.m.	55	—	1·5	Meurice (1900)
	I.p.	58·0	49·0–70	1·57	This paper
	S.c.	c.93·0	—	2·5	This paper, Lang (1895)
Mouse	I.v.	40·0	—	1·1	This paper
	I.v.	67·0	—	1·75	Paulet (1955a, b, 1960)
	I.m.	100·0	—	2·7	This paper
	I.p.	111·0	94–124	2·99	This paper
	Oral	155·0	—	4·17	This paper
	Oral	140	—	3·8	Delga *et al.* (1961a, b)
Rat	S.c.	137	—	3·7	Bartelheimer (1962 b)

In acute experiments, cobalt salts given intravenously cause a fall, followed by a rise and a later fall, of arterial pressure, an increase in breathing, cramps, vomiting and acute diarrhoea with intestinal inflammation (Stuart, 1884 ; Le Goff, 1930 ; Bartelheimer, 1962a ; Tauberger & Klimmer, 1963). These effects have been analysed by Bartelheimer (1962a), and by Tauberger & Klimmer (1963), and shown to be partly due to central action and partly to peripheral effects. Death is due to respiratory failure when the administration is rapid, to cardiac failure when more slowly given (Bartelheimer, 1962a).

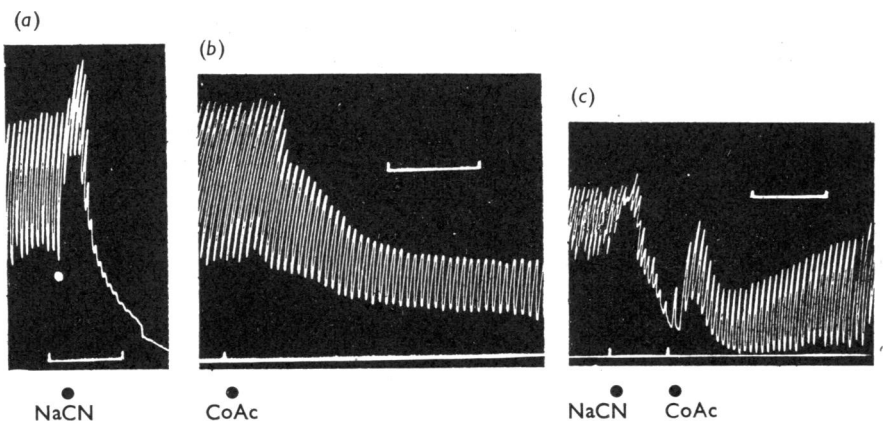


Fig. 1. Rabbit ileum preparation in oxygenated Tyrode solution, in organ bath of 25 ml. (*a*) addition of 1 mg (20·5 μmoles) of sodium cyanide (NaCN) ; (*b*) after washing addition of 1 mg (4 μmoles) of cobalt acetate (Co Ac); (*c*), after washing addition of 1 mg of sodium cyanide then 1 mg of cobalt acetate. Cyanide/cobalt molar ratio = 5·2. Time scale, 1 min.

In more chronic administrations the principal effects are polycythaemia, porphyrinuria, increase in the size of the adrenals, and goitre (Gairdner, Marks & Roscoe, 1954; Saikkonen, 1959). Cobalt salts have been given orally to human subjects in doses up to 150 mg of cobaltous chloride daily without clearly pronounced harmful effects (Davis & Fields, 1955). Orally administered cobalt is very slightly absorbed, and mainly excreted in the faeces; injected intravenously it is mainly, and rapidly, excreted in the urine (Taylor, 1962); according to experiments with radioactive ^{60}Co (Cuthbertson, Free & Thornton, 1950) what remains in the body is to be found mainly in liver, kidneys, pancreas and spleen.

In the present experiments, no histochemical evidence could be found that cobalt was present in any tissues or organs 12 hr after intravenous injection. *Post mortem* examination showed intense congestion of the mucosa of the intestinal tract.

On the rabbit isolated ileum preparation the effect was relaxation, with diminution of the rhythmic contractions (Fig. 1); on the rectum *in situ* intravenous injection caused powerful rhythmic contractions (Fig. 2), and these probably account for the colic and diarrhoea which often follow administration of cobalt salts.

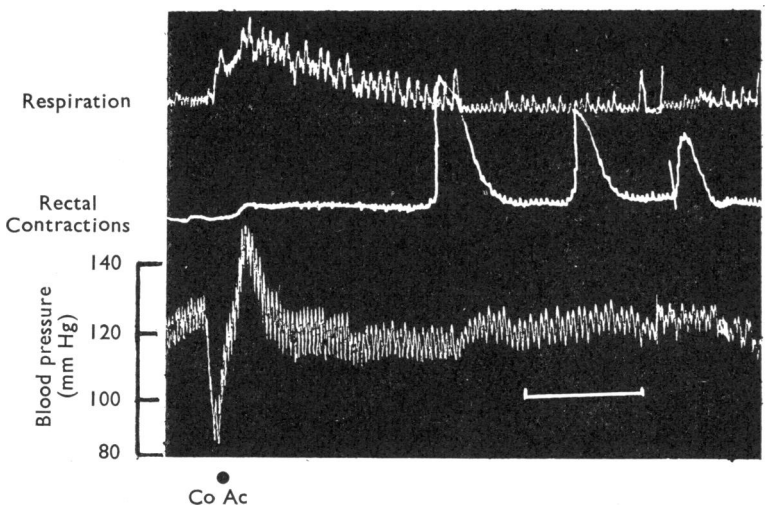


Fig. 2. Cat, anaesthetized with pentobarbitone sodium. Uppermost tracing, respiration; middle tracing, contractions of rectum; lowest tracing, arterial blood pressure. At the mark 20 mg/kg (μmoles/kg) of cobalt acetate (Co Ac) was injected intravenously. Time scale, 1 min.

The toxicity of cobalt compounds is related to the ease with which they yield cobalt ions, and hence is greatest in salts of cobalt. Cobalt acetate yields ions readily; Siddhanta & Banerjee (1958) give the dissociation constant for

$$Co.O.CO.CH_3^+ \rightleftharpoons Co^{++} + O.CO.CH_3^-$$

as 30×10^{-3}, or $pK = 1.8$, and the compound would be expected to be somewhat toxic. The chelate compounds of cobalt, which will be referred to later, do not yield ions so readily, if at all.

The lethal doses of various cobalt compounds are given in Table 2.

TABLE 2
LETHAL DOSES OF COBALT COMPOUNDS
EDTA=editic acid ; i.v., intravenous ; i.p. intraperitoneal

Species	Compound	Route	LD50 (μ moles/kg)	LD50 (mg/kg)	Reference
Mouse	Co acetate	I.v.	125	31	This paper
Rabbit	Co acetate	I.v.	100	25	This paper
Rat	$CoCl_2$	I.v.	83	20	Tauberger & Klimmer (1963)
Cat	$Co\ Cl_2$	I.v.	120	27	Bartelheimer (1962a, b)
Mouse	$CoNa_2EDTA$	I.p.	6,700	1,948	Eybl *et al.* (1959)
Mouse	Co_2 EDTA	I.v.	122	50	Paulet (1955a, b, 1961)
Mouse	Co_2 EDTA	I.v.	175	71	This paper
Rat	Co_2 EDTA	I.v.	106	43	Tauberger & Klimmer (1963)
Rat	Co_2 EDTA	I.p.	100	41	Bartelheimer (1962a, b)
Rat	Co-histidine	I.v.	287	104	Tauberger & Klimmer (1963)
Rat	Co-histidine	I.p.	370	134	Bartelheimer (1962a, b)
Cat	Co-histidine	I.v.	136	50	Tauberger & Klimmer (1963)

Cobalt salts as antidotes

Exactly what occurs when cobalt and cyanide ions are introduced into the body is uncertain ; initially the cobaltocyanide ion ($Co(CN)_6^{----}$) would be formed, but the ultimate product is probably cobalticyanide ($Co(CN)_6^{---}$) which has been shown by Bartelheimer, Friedberg & Lendle (1962) to have a log stability constant (log K_S) of 19, so it is very stable, while being at the same time only slightly toxic (LD50 = 1,000 mg/kg). Cobalt ions can reach the brain, that is they can penetrate the blood-brain barrier (Smith, 1962 ; Bartelheimer, 1962a), have no precipitating action on proteins, and, when introduced into the blood stream, are eliminated by the kidney, which is not damaged.

The antagonism between cobalt and cyanide ions can be illustrated by experiments with isolated tissues *in vitro*. For example, as shown in Fig. 1, on the isolated small intestine the action of 20.5 μmoles of cyanide (giving a concentration of 0.82 μmoles/ml.) was eliminated by 4 μmoles of cobalt acetate, the cyanide/cobalt ratio being 5.2.

Some experiments were made to find whether cobalt ions were able *in vitro* to reverse the inhibition of the cytochrome oxidase system produced by cyanide, using *p*-phenylenediamine as the oxidation substrate. Only about 10% of the initial oxidase activity was restored, however, probably on account of the facts that phosphate buffers were used and the insolubility of cobalt phosphate, and these experiments were not pursued further.

Since the lethal dose of cobalt salts is around 100 μmoles/kg, the amount given as an antidote has mostly been lower than that ; but that dose should be able to antidote 600 μmoles of hydrocyanic acid, if the theoretical ratio holds good, and the mutual antidotal action should in theory reduce any risk of cobalt poisoning. This would mean that, for a safe dose of a cobalt salt, some 10 × LD50 of hydrocyanic acid intraperitoneally for rabbits, and 5 × LD50 intraperitoneally for mice, should be the maximal amounts that could be antidoted. In experiments done in 1942 (Evans & Watt, unpublished) the maximal dose antidoted was 10 × LD50, in a goat. The present series, using mice and rabbits, gave results which are satis-

TABLE 3

THE EFFECTS ON RABBITS OF COBALT ACETATE AND HYDROCYANIC ACID

The cobalt acetate was given intravenously ; the hydrocyanic acid intravenously (i.v.) or intraperitoneally (i.p.). The hydrocyanic acid was given first, except for asterisked results, when the cobalt acetate was given first. Figures in brackets after the number of rabbits died give the numbers of rabbits tested. * Indicates the first to be administered

Hydrocyanic acid			Cobalt acetate	Cyanide/ cobalt	Number died	
Dose (μmoles/kg)	Route	Multiple of LD50	(μmoles/kg)	Ratio	Total	%
178	I.v.	6·0	31*	5·8	0 (1)	0
170	I.v.	5·7	84	2·0	0 (1)	0
167	I.v.	5·6	100	1·7	0 (2)	0
148	I.v.	5·0	30	5·0	0 (1)	0
133	I.v.	4·4	100	1·3	0 (1)	0
119	I.p.	2·0	10*	12·0	1 (1)	100
116	I.v.	3·8	14	8·3	4 (4)	100
116	I.v.	3·8	23	5·1	0 (2)	0
85	I.v.	2·8	84	1·0	0 (5)	0
74	I.p.	1·3	42	1·8	0 (1)	0
60	I.v.	2·0	9	6·6	0 (1)	0
60	I.v.	2·0	19	3·2	1 (1)	100
54	I.v.	1·8	17*	3·2	0 (1)	0

factory for rabbits, but for mice fall short of the theoretical predictions mentioned above, as is shown in Tables 3 and 4.

When the molecular ratios of cyanide and cobalt are calculated, it is seen that, in rabbits, the ratios are spread over the range 1 to 12. If it is assumed that each mole of cobalt can deal with six of cyanide, then in all cases except two, where the cyanide/cobalt ratio exceeded 6, there should be no hydrocyanic acid left free. In the two instances where an excess would be left it would be only a fraction of an LD50, and this should have been nonlethal, but in one instance (cyanide/cobalt ratio of 12) was not, which indicates that the case is not so simple as it first seemed.

The results in Tables 3 and 4 show that the cobalt salt was more effective as an antidote for rabbits than for mice. For rabbits, provided the molar cyanide/cobalt ratio does not exceed 6, it seems that at least 6 × LD50 can be neutralized, while at a ratio of 12 even twice the LD50 is not antidoted. It should be noted,

TABLE 4

EFFECTS ON MICE OF COBALT ACETATE FOLLOWED BY HYDROCYANIC ACID

Cobalt acetate was given intravenously,and hydrocyanic acid intraperitoneally. Figures in brackets after the numbers of mice died give the total numbers of mice tested

Hydrocyanic acid		Cobalt acetate	Cyanide/ cobalt	Number died	
Dose (μmoles/kg)	Multiple of LD50	(μmoles/kg)	Ratio	Total	%
450 to 900	4·1 to 8·2	75 to 225	2·0 to 6·0	20 (20)	100
300	2·7	62	4·8	10 (11)	91
250	2·25	50	5·0	5 (5)	100
225	2·0	75	3·0	8 (9)	89
225	2·0	46	4·9	20 (32)	63
222	2·0	25	9·0	5 (5)	100
200	1·8	50	4·0	0 (9)	0
200	1·8	33	6·0	3 (4)	75
150	1·35	50	3·0	0 (8)	0

however, that, in the rabbit series, hydrocyanic acid was usually given intravenously, whether before or after the cobalt.

With mice the picture is much less satisfactory ; they are less sensitive to hydrocyanic acid, and about as sensitive to cobalt, as are rabbits, and the results are less regular. It appears from the results in Table 4 that, under the most favourable conditions, only upwards of twice the LD50 can be antidoted, and then only when the cyanide/cobalt ratio is below 5. The hydrocyanic acid was given intraperitoneally and the cobalt solution intravenously in this species, which might have affected the results.

Dicobalt edetate as antidote

Experiments were carried out with pure dicobalt edetate, and also with the solution sold under the name of Kélocyanor (Laboratories Laroche Navarron, 63 Rue Chaptal, Levallois, Paris, Seine, France) which is provided in 20 ml. ampoules, each containing a 1.5% solution in 20% glucose. The solution has a *p*H of 4.42. The dicobalt edetate and the cyanide ion have been shown by Paulet (1960) to be mutual antidotes, the antagonizing amounts found by him being 0.1 mg of cyanide : 0.8 mg of dicobalt edetate (or 3.9 μmoles : 1.96 μmoles, or 2 : 1, and not 6 : 1 as might have been expected if the second cobalt atom of the compound were fully ionized).

The toxicity of the compound, given in either form, was found to be about 175 μmoles/kg for mice (Paulet, 1960, found about 122 μmoles/kg) so, in spite of a probable mutual antidotal action, the amount given was usually kept below that dose. It was tried with and without simultaneously intravenously administered thiosulphate, and both before and after the cyanide. Results are given in Table 5.

Thiosulphate alone had antidotal action which varied ; for mice the value was around 1.5 to 2 × LD50 at the best, but was irregular, and in rabbits it was also around twice the LD50. The results are rather irregular, but show that, for mice, some 2 to 3 × LD50 can be antidoted when the cobalt compound is given in the form of Kélocyanor, that is, with glucose, and when thiosulphate is also given. The pure dicobalt edetate was less successful. Some of the cyanide/cobalt ratios were very high, as much as 19, but, as thiosulphate was also given, much of the beneficial effect must have been due to that. For rabbits the results were similar, but were also improved by giving thiosulphate.

For both species the edetate works adequately up to 2 or 3 × LD50 at cyanide/cobalt ratios greater than unity, as with ordinary cobalt salts, and provided that the dose of dicobalt edetate is not high enough to exert its own toxic effects. There is a suggestion in these findings that the best results are obtained when the dicobalt edetate is given after the hydrocyanic acid, and there was a similar inference to be drawn from all the compounds so far.

Hydroxocobalamin as antidote

In doses up to 400 μmoles/kg intravenously, no toxic effects of hydroxocobalamin were seen with mice. The substance was rapidly excreted in the urine, which

TABLE 5

DICOBALT EDETATE AS AN ANTIDOTE TO HYDROCYANIC ACID

I.p. intraperitoneal ; i.v. intravenous ; i.m. intramuscular ; numbers in brackets after the numbers of animals died give the numbers of animals tested

Hydrocyanic acid		Dicobalt edetate	Cyanide/cobalt ratio	Thiosulphate	Number died	
Dose (μmoles/kg)	Multiple of LD50	(μmoles/kg)		(g/kg)	Total	%
Mice : hydrocyanic acid (i.p.) followed immediately by dicobalt edetate (Kélocyanor, i.v.) with and without thiosulphate (i.v.)						
240	2·2	25	9·6	0·0	13 (13)	100
240	2·2	50	4·8	0·0	6 (8)	75
240	2·2	100	2·4	0·0	2 (2)	100
240	2·2	240	1·0	0·0	2 (2)	100
480	4·3	25	19·0	0·25	2 (7)	29
360	3·2	25	14·5	0·15	0 (5)	0
240	2·2	25	9·6	0·4	0 (6)	0
240	2·2	50	4·8	0·4	5 (6)	84
Mice : pure dicobalt edetate (i.v.) followed immediately by hydrocyanic acid (i.m.)						
600	6·0	75	8·0	0·0	3 (3)	100
500	5·0	75	6·7	0·0	2 (2)	100
400	4·0	75	5·4	0·0	6 (6)	100
300	3·0	75	4·0	0·0	2 (2)	100
200	2·0	75	2·7	0·0	5 (13	38
400	4·0	75	5·4	0·2	4 (4)	100
400	4·0	75	5·4	0·5	3 (4)	75
300	3·0	75	4·0	0·5	4 (4)	100
250	2·5	75	3·4	0·25	4 (4)	100
200	2·0	75	2·7	0·25	3 (4)	75
200	2·0	75	2·7	0·5	1 (2)	50
Rabbits : dicobalt acetate (Kélocyanor, i.v.) followed by hydrocyanic acid (i.p.)						
220	3·8	240	0·92	0·0	1 (1)	100
220	3·8	107	2·06	0·0	1 (1)	100
220	3·8	120	1·84	0·0	1 (2)	50
220	3·8	60	3·7	0·0	1 (2)	50
220	3·8	40	5·5	0·0	1 (1)	100
165	2·8	60	2·75	0·0	4 (4)	100
110	1·9	50	2·2	0·0	0 (2)	0
67	1·1	50	1·34	0·0	0 (2)	0
220	3·8	25	8·7	0·25	1 (3)	33
165	2·8	25	6·6	0·25	0 (2)	0
165	2·8	50	3·3	0·5	0 (2)	0
Rabbits : hydrocyanic acid (i.p.) followed by pure dicobalt edetate and thiosulphate (i.v.)						
165	2·8	25	6·6	0·25	1 (3)	33
165	2·8	50	3·3	0·25	1 (4)	25

became deep red in a few minutes. In these experiments, when mice were used, the theoretical expectations were as a rule approximately borne out. In most of the experiments the hydroxocobalamin was given immediately before the hydrocyanic acid, this being technically easier, and in these it was found that when the molar ratios of cyanide/cobalt were below unity and the dose of hydrocyanic acid less than 450 μg/kg (about 4 × LD50) recovery was the rule, though the results were rather irregular (Table 6). When the hydroxocobalamin was given after the hydrocyanic acid, and after respiration had failed, the results were similar (Table 7), recovery being effected after 500 μg of hydrocyanic acid (4.5 × LD50) provided

TABLE 6

EFFECTS ON MICE OF HYDROXOCOBALAMIN FOLLOWED BY HYDROCYANIC ACID

Hydroxocobalamin was given intravenously, and hydrocyanic acid intraperitoneally. Numbers in brackets after the numbers of mice died give the numbers of mice tested

Hydrocyanic acid		Hydroxo-cobalamin	Cyanide/ hydroxo-cobalamin	Number died	
Dose (μmoles/kg)	Multiple of LD50	(μmoles/kg)	ratio	Total	%
600	5·4	600	1·0	4 (4)	100
500	4·5	600	0·83	0 (7)	0
500	4·5	500	1·0	3 (4)	75
450	4·0	600	0·75	2 (8)	25
450	4·0	450	1·0	3 (4)	75
400	3·6	400	1·0	0 (4)	0
400	3·6	600	0·66	0 (2)	0
300	2·7	600	0·5	0 (4)	0
300	2·7	450	0·66	1 (5)	25
300	2·7	300	1·0	0 (3)	0
300	2·7	150	2·0	0 (3)	0
200	1·8	225	0·9	0 (5)	0
200	1·8	33	6·0	7 (7)	100
150	1·4	167	0·9	0 (10)	0
150	1·4	104	1·45	0 (2)	0
150	1·4	87	1·73	0 (3)	0
150	1·4	36	4·2	0 (2)	0

that an approximately equimolar amount of hydroxocobalamin was given. When smaller quantities of hydroxocobalamin were given, so that, theoretically, one or more LD50 of cyanide was unneutralized, death always occurred. When thiosulphate was also administered (0.42 g/kg) the results were improved (Table 8).

With rabbits, owing to the rather large amounts of hydroxocobalamin involved, fewer experiments were possible; the results were less regular than with mice. Here, although in one experiment 5.2 × LD50 was neutralized, most of the animals died at doses over 2 × LD50, even when the ratio of cyanide to hydroxocobalamin was unity. Addition of thiosulphate (0.25 g/kg) did not improve the results and, in fact, thiosulphate alone seemed to be more effective than when given with hydroxocobalamin. Thus, after thiosulphate (0.25 g/kg intravenously) followed by 110 μmoles/kg of hydrocyanic acid intraperitoneally (2 × LD50) three out of four rabbits recovered, though after 2.8 × LD50 (165 μmoles/kg) three out of three died. We may say, therefore, that thiosulphate alone, administered before the

TABLE 7

EFFECTS ON MICE OF HYDROCYANIC ACID AND HYDROXOCOBALAMIN WHEN RESPIRATION HAD FAILED

Hydrocyanic acid was given intraperitoneally and hydroxobalamin intravenously. Numbers in brackets after the numbers of mice died give the numbers of mice tested

Hydrocyanic acid		Hydroxo-cobalamin	Cyanide/ hydroxo-cobalamin	Number died	
Dose (μmoles/kg)	Multiple of LD50	(μmoles/kg)	ratio	Total	%
500	4·5	500	1·0	1 (4)	25
500	4·5	400	1·25	0 (4)	0
500	4·5	333	1·5	4 (4)	100
500	4·5	250	2·0	3 (3)	100
400	3·6	600	0·66	1 (4)	25
200	1·8	450	0·45	0 (4)	0

TABLE 8

EFFECTS ON MICE OF HYDROCYANIC ACID FOLLOWED BY HYDROXOCOBALAMIN AND THIOSULPHATE

The hydroxocobalamin and thiosulphate were given intravenously mixed together. Numbers in brackets after the number of mice died give the numbers of mice tested

Hydrocyanic acid		Hydroxo-cobalamin	Thio-sulphate	Cyanide/hydroxo-cobalamin	Number died	
Dose (μmoles/kg)	Multiple of LD50	(μmoles/kg)	(g/kg)	ratio	Total	%
0·0	0	0	0·42	0	0 (4)	0
240	2·2	0	0·42	0	2 (2)	100
240	2·2	240	·00	1·0	2 (2)	100
240	2·2	240	0·42	1·0	0 (3)	0
240	2·2	120	0·0	2·0	1 (1)	100
240	2·2	120	0·42	2·0	0 (5)	0
480	4·3	240	0·42	2·0	1 (4)	25
480	4·3	120	0·42	4·0	1 (3)	33
720	6·5	240	0·42	3·0	3 (3)	100

hydrocyanic acid, can in this species antidote about 2 × LD50 of hydrocyanic acid, but is less effective when used in conjunction with hydroxocobalamin. The reason for this may be that the hydroxocobalamin reacts with thiosulphate to form a new compound, a thiosulphato-cobalamin, which can no longer, or less firmly, fix a cyanide ion. If equimolar solutions of hydroxocobalamin and thiosulphate are mixed, the colour instantly changes from brown to purple, and the ultraviolet absorption spectrum also changes even more definitely than when cyanide is added. Before the addition, the ultraviolet spectrum shows maximal absorption at 350 mμ and 530 mμ ; after the addition of thiosulphate there is a plateau at 330 to 370 mμ, and a peak at 550 mμ. When we consider that thiosulphate, when given, was at dosages of 0.25 to 0.42 g/kg (1,000 to 1,680 μmoles/kg) whereas the hydroxocobalamin was added at levels of at most 240 μmoles/kg, there would be, if the

TABLE 9

EFFECTS ON RABBITS OF HYDROXOCOBALAMIN AND OF HYDROCYANIC ACID

Hydroxocobalamin and thiosulphate were given intravenously (i.v.) ; i.p. intraperitoneal; i.m., intramuscular. (i) hydrocyanic acid first ; (ii) second. * Thiosulphate, 0·25 g/kg. Numbers in brackets after the numbers of rabbits died give the numbers of rabbits tested

Hydrocyanic acid			Hydroxo-cobalamin	Cyanamide/hydroxo-cobalamin	Number died	
Dose (μmoles/kg)	Route	Multiple of LD50	(μmoles/kg)	ratio	Total	%
300	I.p. (ii)	5·2	300	1·0	3 (3)	100
300	I.p. (i)	5·2	300	1·0	0 (1)	0
300	I.p. (ii)	5·2	150	2·0	1 (1)	100
200	I.p. (i)	3·5	200	1·0	3 (3)	100
135	I.m. (i)	3·3	135	1·0	2 (2)	100
120	I.m. (ii)*	2·9	120	1·0	2 (2)	100
120	I.p. (ii)	2·1	30	1·0	2 (2)	100
110	I.p. (ii)	1·9	110	1·0	2 (2)	100
110	I.p. (i)	1·9	110	1·0	0 (2)	0
110	I.p. (ii)	1·9	55	2·0	1 (2)	50
90	I.m. (ii)	2·2	90	1·0	3 (3)	100
82	I.m. (ii)*	2·0	82	1·0	6 (7)	85
68	I.m. (i)	1·7	68	1·0	0 (1)	0
62	I.m. (ii)*	1·5	62	1·0	5 (6)	83
60	I.p. (ii)	1·0	30	2·0	0 (1)	0
41	I.m. (ii)	1·0	41	1·0	1 (4)	25

two reacted mole for mole, from 760 to 1,440 μmoles/kg of thiosulphate left over, which might have some antidotal effect of its own, but which could be less than that of the hydroxocobalamin, the effect of which had been annulled or weakened.

A crucial test would be to compare the antidotal action of hydroxocobalamin against 1.5 × LD50 of hydrocyanic acid before and after the addition to it of an equimolar amount, instead of an excess, of thiosulphate. This test had to be carried out on mice, for which species excess of thiosulphate improved the result. This experiment gave the following results:

(i) Of mice given hydroxocobalamin, 166 μmoles/kg intravenously, followed by 1.5 × LD50 of hydrocyanic acid intraperitoneally (166 μmoles/kg), none of four (0%) died.

(ii) Of mice given hydroxocobalamin and thiosulphate, each 166 μmoles/kg intravenously, and then 166 μmoles/kg of hydrocyanic acid intraperitoneally, two of five (40%) died.

This result appeared to support the above suggestion; but when the hydrocyanic acid (1.5 × LD50) was given intramuscularly, equal numbers died in the two samples (five out of six, or 83%). It was often noticed that, as in this instance, the antidotal action was more effective in those instances in which the hydrocyanic acid had been given intraperitoneally than when given intramuscularly. Another interesting observation was that, by whichever route the hydrocyanic acid was given, the action of the antidote was greater when it followed the cyanide than when it preceded it by a few seconds:

(i) Of mice given hydrocyanic acid, 3 × LD50 intramuscularly, followed by hydroxocobalamin intravenously (ratio = 1), none of six (0%) died.

(ii) Of mice given hydroxocobalamin intravenously followed by hydrocyanic acid 3 × LD50 intramuscularly (ratio = 1), four of seven (57%) died.

Cobinamide as antidote

The hydrolysate of hydroxocobalamin, known as cobinamide or Factor B, is derived from it by removal of the nucleotide (Smith, 1963). Its molecular weight is about 1,049, and *in vitro* it can fix two cyanide ions. The aqueous solution of 0.1 M had a *p*H of 2.17. The results with rabbits are shown in Table 10, from which it appears that the compound is more regular in action and, up to twice the

TABLE 10

EFFECTS ON RABBITS OF COBINAMIDE FOLLOWED BY HYDROCYANIC ACID

Cobinamide was given intravenously, and hydrocyanic acid intramuscularly. Numbers in brackets after the numbers of rabbits died give the numbers of rabbits tested

Hydrocyanic acid					Number died	
Dose (μmoles/kg)	Multiple of LD50	Cobinamide (μmoles/kg)	Thio-sulphate (g/kg)	Cyanide/ cobinamide Ratio	Total	%
62	1·5	62	0	1·0	0 (4)	0
82	2·0	82	0	1·0	0 (2)	0
123	3·0	123	0	1·0	2 (4)	50
123	3·0	123	0·25	1·0	2 (2)	100
123	3·0	62	0	2·0	2 (2)	100

LD50, somewhat more effective in this species than is hydroxocobalamin, and that its action, as with hydroxocobalamin, is not reinforced by thiosulphate.

Previous mixing of hydrocyanic acid and antidote

The most favourable condition for the action of the cobalt antidotes would be expected to be if they were mixed with the hydrocyanic acid before administration. It would be expected that, if the resulting compound were not toxic and held the cyanide firmly enough in competition with cytochrome oxidase, the effect of the hydrocyanic acid would be entirely eliminated if enough antidote were present, and that, for instance, cobalt salts (including the edetate on the assumption that it had one ionizable cobalt atom) would be able to fix six molar equivalents of hydrocyanic acid, while hydroxocobalamin would be able to neutralize only on a mole for mole basis.

A simple experiment shows that the complex formed by cobalt salts *in vitro* though not innocuous is not very toxic. Solutions of cobalt acetate and sodium cyanide were mixed in such proportions that the mixture contained 256 μmoles of cyanide and 46 μmoles of cobalt salt per ml. (ratio 5.5). This was injected into an ear vein of a rabbit in a dose of 1 ml./kg. This would be, in terms of hydrocyanic acid, about 8.5-times the LD50. For some minutes after injection there was panting and convulsions, followed by paresis of the hind limbs, and loss of consciousness, as would have been the effects of, say, half a lethal dose of hydrocyanic acid ; but after 5 min, apart from a little panting, the animal was apparently normal and made an uninterrupted recovery, with no subsequent diarrhoea. We might suppose, since the cyanide/cobalt ratio was less than 6, that these transitory symptoms of cyanide poisoning were to be attributed to slight dissociation of the cobaltocyanide, but in any case the experiment shows that there are grounds for the belief that the cobalt ion can, under the most favourable conditions, antidote the theoretical amount of cyanide.

These expectations were only in part borne out by experiment on mice, as shown in Table 11. These results show that when the dose of hydrocyanic acid is low ($3 \times$ LD50 or less) and the cyanide/cobalt ratio is 6 or less, the expectations from theory hold approximately, but with four or more times the LD50 and cyanide/cobalt ratios above 4 the results are less good.

With mixtures of hydrocyanic acid with hydroxocobalamin or cobinamide at ratios of 1 to 1.5, no symptoms at all were seen ; this may be taken to indicate that with these two antidotes the union with the cyanide ion, once it was formed, was firm enough to keep it out of the ambit of the cytochrome oxidase. With every other compound, initial convulsions occurred, even in those animals that ultimately recovered, and with cobalt acetate at high doses these were succeeded by tremors, indicating a residual effect of cobalt, in the animals that recovered.

Dicobalt edetate was ineffectual against $4 \times$ LD50 of hydrocyanic acid at all ratios of cyanide/cobalt above 2, and only fully effectual at a ratio of unity ; in this it differed from the cobalt acetate, in which a ratio of 4 to 6 was effective. This was surprising in view of the expectation that the second atom of cobalt in the

TABLE 11

EFFECTS ON MICE OF INJECTION OF PREMIXED HYDROCYANIC ACID AND COBALT COMPOUNDS

Injections were intraperitoneal. Volume of mixture injected 0·1 ml./10 g. Numbers in brackets after the numbers of mice died give the numbers of mice tested

Hydrocyanic acid			Cobalt	Cyanide/			
Dose (μmoles/kg)	Multiple of LD50	Cobalt compound	dose (μmoles/kg)	cobalt ratio	Number died Total	%	Remarks
333	3·0	Acetate	56	6·0	0 (4)	0	Convulsions, then tremors
444	4·0	Acetate	38	12·0	2 (2)	100	—
444	4·0	Acetate	74	6·0	3 (3)	100	—
444	4·0	Acetate	89	5·0	3 (5)	60	Convulsions, then tremors
444	4·0	Acetate	112	4·0	0 (3)	0	Convulsions, tremors
150	1·35	Hydroxocobalamin	167	0·9	0 (2)	0	No symptoms
150	1·35	Hydroxocobalamin	104	1·45	0 (2)	0	No symptoms
150	1·35	Hydroxocobalamin	87	1·73	0 (3)	0	Convulsions
150	1·35	Hydroxocobalamin	36	4·20	0 (2)	0	Convulsions
444	4·0	Hydroxocobalamin	444	1·0	0 (4)	0	No symptoms
444	4·0	Hydroxocobalamin	222	2·0	5 (5)	100	—
444	4·0	Cobinamide	444	1·0	0 (3)	0	No symptoms
444	4·0	Cobinamide	222	2·0	5 (5)	100	
444	4·0	Dicobalt edetate	74	6·0	4 (4)	100	—
444	4·0	Dicobalt edetate	112	4·0	4 (4)	100	—
444	4·0	Dicobalt edetate	148	3·0	4 (4)	100	—
444	4·0	Dicobalt edetate	222	2·0	1 (4)	25	Convulsions
444	4·0	Dicobalt edetate	444	1·0	0 (4)	0	Convulsions
166	1·5	None	—	—	3 (3)	100	—

edetate was fully ionized, would behave as such, and fix 6 moles of hydrocyanic acid. It accords with the estimate by Paulet, Chary & Bocquet (1959), however, that 0.1 mg of cyanide is neutralized by 0.8 mg of cobalt edetate (3.9 μmoles/1.96 μmoles, ratio of 2).

Restoration of oxygen utilization

In seventeen experiments the oxygen utilization of rats anaesthetized with a mixture of allobarbitone and urethane was measured by the use of a small-scale Krogh respirometer, and the effect of an intraperitoneal dose of hydrocyanic acid, as large as possible without causing arrest of respiration, was observed; cobalt derivatives of various kinds were then injected intravenously, and the subsequent oxygen usage measured over a sufficient period. In several experiments, the cobalt injection was given too late, but when the animal survived the cobalt injection restoration of oxygen usage was prompt and complete (Figs. 3 and 4) and was often followed by increased usage, presumably the expression of oxygen lack.

Interaction between cobalt salt and hydrocyanic acid given orally

When hydrocyanic acid or a cyanide is taken by mouth, one obvious line of treatment would be to give a cobalt salt by mouth immediately, or as soon as possible after any appropriate intravenous therapy had been applied.

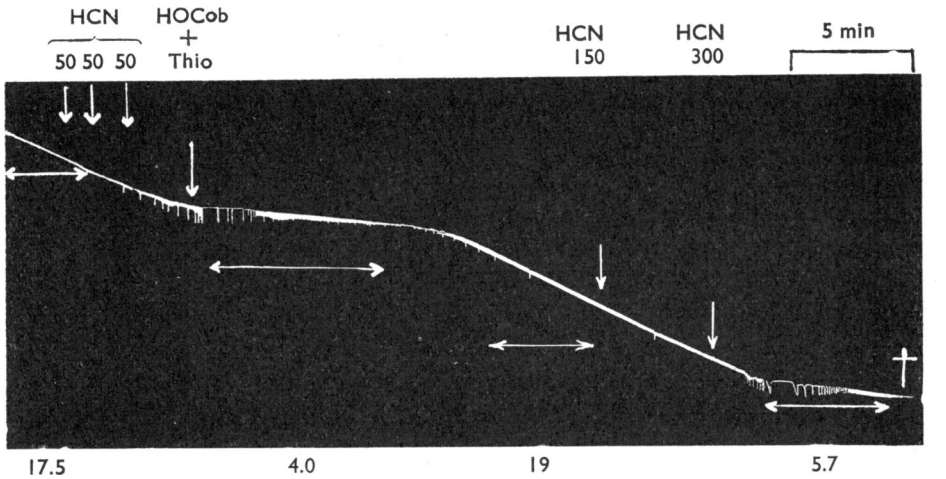


Fig. 3. Record of oxygen uptake of a rat anaesthetized with Dial-urethane, by a Krogh spirometer, showing the reduced uptake after 150 μmoles/kg of hydrocyanic acid (HCN) injected intraperitoneally and the restoration after 25 μmoles/kg of hydroxocobalamin (HOCob) and 0·4 g/kg of sodium thiosulphate (Thio). A subsequent dose of 150 μmoles/kg of hydrocyanic acid had no effect, but a further 300 μmoles/kg caused death. Figures below the record give oxygen uptakes in ml./kg/min for the periods indicated by the horizontal arrows. Time scale, 5 min.

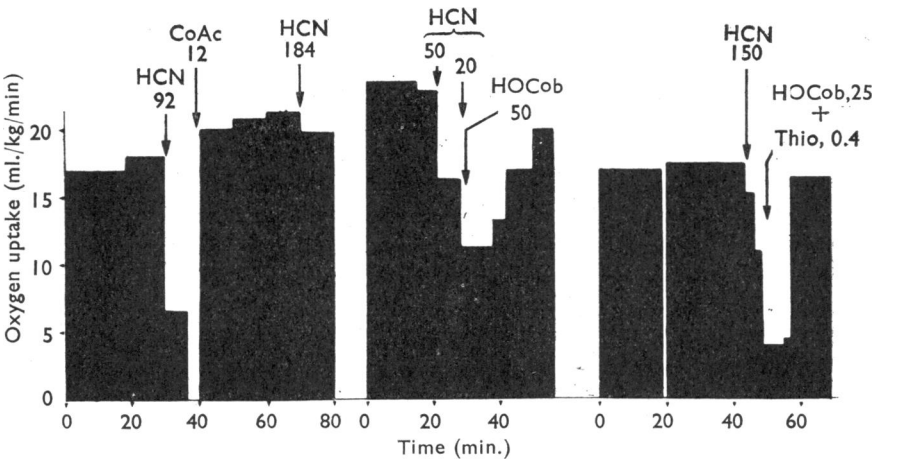


Fig. 4. Histogram of three experiments on rats anaesthetized with Dial-urethane, showing restorative effect of cobalt acetate (Co Ac), of hydroxocobalamin (HOCob), and of hydroxocobalamin plus thiosulphate (Thio), on oxygen uptake depressed by hydrocyanic acid (HCN). The last experiment was taken from the tracing shown in Fig. 3. The oxygen uptake figures are not corrected for temperature, barometric pressure or water vapour pressure. All doses in μmoles/kg, except for that of thiosulphate which is in g/kg.

An experiment was carried out by giving hydrocyanic acid by stomach tube to mice, and then following this up after an interval of time by administering by the same route a dilute cobalt acetate solution. The results were rather irregular, as might be expected, but among them were as follows:

Oral HCN (μmoles/kg)	Multiple of LD50	Time between HCN and cobalt (min)	Oral cobalt (μmoles/kg)	Ratio died/used	Mortality (%)
233	1.5	—	—	2/2	100
465	3.0	3.0	75	1/2	50
310	2.0	4.0	75	1/6	17

It would seem from this that cobalt acetate solution, given by mouth within a short time of ingestion of cyanide, might be helpful; it would normally be followed by a wash-out of the stomach, or by chelating any excess of cobalt by giving by mouth a solution of the sodium edetate, 0.5 mg per mg of cobalt acetate administered, and there would then be little danger from toxic effects due to absorption of cobalt into the blood stream (Ebyl, Sýkora & Köcher 1959; Saccà, Aragona & Ceruso, 1959; Smith, 1962).

DISCUSSION

The consequences of administration of cyanide depend mainly on the rate at which it enters the blood stream, a maximal effect resulting when the dose is high and the rate of entry rapid, as for example, when a high concentration of hydrocyanic acid vapour is breathed or when a large dose is injected into a systemic vein, in either of which cases serious symptoms ensue in a matter of seconds and death within minutes. Even if attempts at resuscitation appear to be successful, permanent damage to the central nervous system may remain if the period of tissue anoxia has been more than a few minutes.

As the rate of entry of cyanide into the circulation is slowed, as by slower administration (Lendle, 1964), another factor enters into consideration, namely, the rate of destruction of cyanide in the body by conversion into thiocyanate, a change which takes place mainly in the liver by the action of the enzyme rhodanese. The use of thiosulphate depends on the provision by it of available sulphur for this reaction; the change seems to be somewhat slow, so it is better as a prophylactic than as an antidote. Since cyanide when given orally or intraperitoneally enters initially into the portal circulation, it will go direct to the liver, so that destruction is facilitated and larger doses can therefore be tolerated. If the rate of eventual entry into the systemic circulation is slow enough, as on subcutaneous, oral or respiratory administration of small doses, the effects are less serious, or may even be absent altogether. The mode of administration therefore has a definite effect on the LD50, and as shown, the LD50 in increasing order is intravenous—intramuscular—intraperitoneal —subcutaneous—oral, with inhalation, which is a common industrial risk, comparable with administration by the intravenous route.

Another factor which influences the outcome is whether the antidote precedes or follows the administration of the cyanide and, if the latter, after what interval of time. It would be expected that the maximal antidotal effect would be seen when poison and antidote were mixed before administration, and next to this when the two were injected simultaneously into the circulation, or when antidote preceded the cyanide by a very short time; the minimal effect would be seen when the antidote was given slowly, for example subcutaneously, and at a time when the animal

was near to death, when it would be all but useless. In nearly every instance in the present experiments, the antidote was administered intravenously, but intraperitoneal injection has also been found effective. For practical reasons the cobalt antidote was usually given to mice intravenously a few seconds before the dose of hydrocyanic acid, intraperitoneally or intramuscularly.

The efficacy of cobalt ions as an antidote to hydrocyanic acid has long been known, and is here confirmed. The knowledge has not been put into medical practice, despite the fact that desperate states may condone desperate remedies. The toxic action of cobalt ions is the probable reason for this, but this is not great and would largely be offset by the mutual detoxification of the two reactants.

The number of median lethal doses of hydrocyanic acid which can be antidoted by cobalt ions is greater for rabbits than for mice, namely five to six for rabbits and two for mice, with maximal cyanide/cobalt ratios of 5 and 4 respectively. One reason for this is that mice are less sensitive to hydrocyanic acid than are rabbits, while to cobalt ions they are about equal in sensitivity. Doses of cobalt acetate greater than 50 μmoles/kg, though lowering the cyanide/cobalt ratio, were unsatisfactory for mice. Another reason in most of the present experiments is that in rabbits both substances were given intravenously, and so had more opportunity of reacting.

The fact that the cyanide/cobalt ratio can be, with rabbits, effective at ratios as high as 5, indicates that the changes involved in the antidotal reaction are much as expected from theoretical considerations; and the fact that, with high doses of hydrocyanic acid the cyanide/cobalt ratio of effectivity is usually lower, is probably to be explained on the lines that the difference between the affinities of the cyanide ions for cytochrome a_3 and for cobalt are not very large.

The first to try hydroxocobalamin as an antidote were Mushett *et al.* (1952), who used mice given potassium cyanide intraperitoneally and hydroxocobalamin intravenously after respiration had failed, and their report was favourable. The dose of cyanide used was only marginally lethal, however (10 mg/kg or 154 μmoles/kg, about $1.4 \times$ LD50), and they gave 250 mg/kg of hydroxocobalamin (187 μmoles/kg, giving a cyanide/hydroxocobalamin molar ratio of 0.82). Delga *et al.* (1961a, b) also reported favourably on hydroxocobalamin, using the method of slow intravenous infusion of hydrocyanic acid into anaesthetized rabbits and into mice poisoned orally by cyanide; they showed that the antidote given intraperitoneally acted on equimolar amounts of cyanide, and stated that the effect was increased by intraperitoneal injection of thiosulphate up to toleration of three lethal doses. Paulet *et al.* (1963), using the slow infusion method, found hydroxocobalamin to be uncertain in its action and less efficient than the dicobalt edetate.

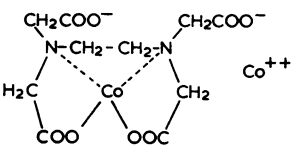
The present results agree with those of the authors mentioned in indicating that 1 mole of hydroxocobalamin can fix 1 mole of cyanide, and the experiments of Table 11 where hydrocyanic acid and antidote were first mixed illustrate that the union is so firm that, where equal numbers of moles were mixed, no symptoms at all were seen. Cobinamide should theoretically be able to fix 2 moles of cyanide, but the results do not bear this out, so that the second mole of cyanide is probably

only loosely held. Hydroxocobalamin differed from cobalt ion in being more efficient in mice than in rabbits, the maximum being 4.5 × LD50 for mice and 2 × LD50 for rabbits, with cyanide/hydroxocobalamin molar ratios of unity in each case. Cobinamide was slightly better for rabbits (maximum 2.5 × LD50, cyanide/cobinamide molar ratio 1).

The results with hydroxocobalamin showed some irregularity, which so far is inexplicable. It may be due to the fact that it is a very reactive substance, as shown by its ready combination with thiosulphate, and it could well be that it is capable of combining with some blood constituent; so far as could be ascertained the irregularity of action is not related to sex, state of digestion, season or environmental temperature. A similar irregularity of action applies to some extent to all the cobalt compounds, but is most conspicuous with the hydroxocobalamin, though the results on any given day are reasonably consistent. The results are further complicated by the mode and timing of the administration of cyanide and antidote, so that application of the results to the treatment of the human subject must be made with caution. An advantage is that the substance, even in such large doses, appears to be harmless, rapidly passes the blood-brain barrier (Worm-Petersen & Poulsen, 1961), and is speedily elmiinated by the kidneys.

Arguing from the fact that in hydroxocobalamin the cobalt atom is chelated, yet can still fix a cyanide ion, Paulet (1958, 1960) studied the properties of other chelates of cobalt, such as the histidine cobalt and the two compounds with edetic acid, one with one atom of cobalt and the other one with two atoms. Of the two chelates with edetic acid, that with one atom of cobalt was ineffective; the dicobalt compound on the other hand was active, and was recommended for use by Paulet (1958) in preference to the very costly hydroxocobalamin.

The dicobalt compound has the structure



and may be regarded as a cobalt salt of the chelate monocobalt compound which would ionize giving one Co^{++} and, according to Bartelheimer (1962b), the dissociation constant of the compound would be expected to be similar to that of cobalt acetate, namely, pK 1.8, whereas the log K_S (also known as β) for the monocobalt compound, disodium cobalt edetate, is 16, and for the cobalt-histidine complex is 13 (Albert, 1960). Accordingly, the toxicity of the monocobalt compound is very low, that of the cobalt-histidine complex comes next, then the dicobalt edetate, and cobalt acetate. This is as shown in Table 2, which incorporates the results of various investigators.

The toxicity of dicobalt edetate in terms of molarity is lower than that of cobalt acetate, but not much lower, and this raises the question whether, in effect, it would be any better than the latter as an antidote. The results of the present experiments indicate that in that respect there is not much to choose between the two. The more favourable results which have been obtained with the compound by

Paulet (1960) present a discrepancy which must be explained; one explanation being that in his experiments cyanide and antidote were given almost simultaneously by intravenous injection, which, as already pointed out, gives very favourable conditions for reaction. One fact is against the view that the compound is to be regarded as an ionizable salt of cobalt, despite the similarity of their respective toxicities when expressed in terms of molarity; namely that the molar cyanide/cobalt edetate ratio at effectivity when no thiosulphate is given is much lower than with cobalt. This is also shown in Table 11 for the experiments with previous mixing, and bears out the findings of Paulet (1960) that the reaction is complete at a molar ratio of about 2, and not at 6. If that is so, then the compound is not an ordinary ionizable cobalt salt, or it reacts with some blood constituent, or else it was not obtainable in as pure a state.

The object of this investigation was to form an opinion as to the best antidote and method of treatment for cyanide poisoning. First, as to the amounts of the various antidotes which would be required for the treatment of a man who had received one LD50 of cyanide, say 50 mg of hydrocyanic acid, or 1,850 μmoles: this would, in accord with the present findings, need 308 μmoles = 77 mg of cobalt acetate (4 H_2O); 930 μmoles = 380 mg of dicobalt edetate (or 25 μl. of Kélocyanor); or 1,850 μmoles = 2.5 g of hydroxocobalamin.

The question is, whether a dose of cobalt acetate of the order of 100 mg would be safe to give intravenously, taking into account the probability that most of its toxicity would be neutralized by the cyanide. The balance of evidence is that it would be a reasonably safe dose under the circumstances. As regards the use of Kélocyanor, the instructions are to give two ampoules of 20 ml. (300 mg) each, and if necessary to follow up with a third ampoule (total of 60 ml. or 900 mg. of dicobalt edetate) and then to give an intravenous injection of hypertonic glucose. Professor Paulet has informed me that a case of oral poisoning has been treated with Kélocyanor by S. Jeretin in Maribor (Jugoslavia); the patient recovered, but the interpretation was not clear-cut, as other remedies were used as well.

The use of oxygen administration has been advised by Paulet (1960), although the only explanation of its value which is offered is that it helps to overcome the large oxygen debt which is seen on recovery from the immediate effects of the poison; at all events, it should be tried. So also should the administration of cobalt acetate by mouth, in cases where cyanide has been ingested. An important aid to treatment is, of course, to keep the subject warm.

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