A METHOD FOR THE ADMINISTRATION OF POTASSIUM CYANIDE IN NORMAL RAT DIET

By

P.N. OKOH

Department of Animal Science,
Ahmadu Bello University, Zaria, Nigeria.

SUMMARY

Potessium cyanide added to normal rat diet moistened with water was exensively lost partly by diffusion as HCN and partly by transformation to formate and other product. However, when potassium cyanide was added to the diet mixed with 5% arachis oil instead of moistening with water, the loss of cyanide was not significant (P 0.3). Using this diet, it was possible to show that KCN given to groups of rats at a dose level of 77 umoles/rat/day in the diet did not diminish weight gain or feed intake but resulted in elevated thiocyanate levels in plasma and urine. This method therefore represents a most effective way for the administration of known amounts of cyanide to rats or other experimental animals in the diet.

INTRODUCTION

Cyanide is ingested by man and animals either in the form of free cyanide (non-glycosidic) or as bound cyanide (cyanogenic glycoside). Both types of cyanide will have differing toxicities especially as it has been shown that a substantial amount of bound cyanide (in the form of cyanogenic glycoside, linamarin) given orally to rats was excreted unchanged in the urine (Barrett *et al.* 1977). For this reason, estimation of cyanide toxicity by the administration of diets containing cyanogenic glycosides to experimental animals (Osuntokun, 1970) may not provide a good index, as the actual amount of cyanide absorbed will depend on the mode of metabolism of the glycoside — whether they are converted to free cyanide or not (Oke, 1969).

The use of potassium and sodium cyanide (KCN or NaCN) for cyanide toxicity study has been limited by their proven instability in aqueous solution due to hydrolysis.

$$KCN + H_2O \rightleftharpoons KOH + HCN.$$

When potassium (or sodium) cyanide has been used for studies on chronic cyanide intoxication, it has been administered in dilute solutions by repeated injections (Smith and Foulkes, 1966; Mehta and McGinity, 1977) — a method which is physiologically unrelated to the normal mode of ingestion.

Experiments on the addition of potassium cyanide to the diet of rats have long been reported (Clark, 1939). To date, however, there is no information on the stability of alkali cyanide added to the diets of experimental animals. Accordingly, I have studied the availability of potassium cyanide added to the rat diet. A method for achieving improved stability of cyanide in normal rat diet is suggested.

MATERIAL AND METHODS

Diets:

Four types of diets were used in this study. Two are semi-synthetic — one based on extracted soyabean meal (SB-diet) and the second based on skimmed milk powder (SM-diet). The third was obtained by grinding up cubes of a commercial diet (CM-diet). The fourth diet designated. As 'oil diet' (OD-diet) refers to the soyabean diet to which 5% by weight of arachis oil has been added.

Addition of cyanide to diet:

To study the stability of cyanide in diets, two methods were used: In one method, cyanide solution of known concentration was added to known weight of

the diet being studied in a 100ml beaker. This was mixed thoroughly and stored for 18 hours. In a second method, known weight of solid potassium cyanide was added to 10g diet, mixed thoroughly in 100ml beaker and also stored for 18 hours.

Animal Experiment:

Twelve male albino rats (from the stock colony of the Department of Biochemistry, University of Liverpool, England) weighing about 100g each were randomly assigned to individual cages and divided into two groups of six rats each. Both groups of rats were maintained on the OD-diet. Potassium cyanide was added to the diet of each rat in one group to provide 5mgKCN per rat per day for three weeks. The cyanide was added to 10g diet which was given to the rat in the evening. Ten gram was about the amount of diet each rat consumed overnight. By the following morning, each rat was given the OD-diet without cyanide *ad libitum* for the rest of the day. The rats in the second group served as control and were fed similarly except that no potassium cyanide was added to their diet. The daily weight of each animal and the amount of diet consumed by each were recorded. At the end of the experiment, each rat was anaesthetised with chlorofarm, blood taken by heart puncture for thiocyanate estimation.

Estimation of cyanide and thiocyanate:

Estimation of cyanide in diets containing 200mg KCN per 10g diet was carried out by distilling off the cyanide into a standard sodium hydroxide solution using the Markham apparatus, followed by titration with standard silver nitrate solution (A.O.A.C. 1970). In diets containing 5mg KCN per 10g diet, cyanide was estimated by the method of Epstein (1947) after separating this radical into 0.05M NaOH by the nitrogen aeration of Boxer and Rickards (1951). Thiocyanate in plasma and urine (after removal of cyanide by nitrogen aeration) was oxidized to cyanide by mild oxidation with permanganate, separated into 0.05M NaOH by nitrogen aeration (Boxer and Rickards 1951) and estimated by the method of Epstein (1947).

Work with Radioactive Cyanide:

In experiments when Na^{14} CN was added to OD diets, the amount of cyanide that could be recovered was also separated by nitrogen aeration into sodium hydroxide solution (Boxer and Rickards, 1951). The radioactivity in the alkaline solution was counted in toluene scintillation fluid mixed with NCS solubilizer, using a liquid scintillation counter (Mode SL40 Intertechnique Ltd. Bringhton, England). Appropriate corrections were made for quenching by the external standard method. The mixture in the aeration vessel after removal of the recoverable cyanide was cnetrifuged to precipitate large food particles. Thin-layer chromatography (T.L.C.) was performed on the diet supernatant fraction. The absorbent was silica gel G, type 60 (Merck Ltd.), while the solvent system was ethamol: ammonia: water (80:4:61). This system is known to separate formic acid from other fatty acide (Prey *et al.* 1962).

RESULTS

Because of the practical difficulties of detecting small amounts of cyanide in diet, the effect of diet on the loss of cyanide from aqueous solution of KCN was studied. The results are shown in table 1. About 50% of cyanide was lost when 0.5g of either SB-diet or CM-diet was added to 10ml of KCN solution containing 10mg/ml. When this was compared with the loss of cyanide from a control KCN solution (table 1), it was seen that the addition of SB or CM-diets resulted in increased loss of cyanide from the KCN solution by about 12%. In contrast, the loss of cyanide from KCN solu-

tion when 0.5g of SM-diet was added is much the same as that lost from the control KCN solution with no diet added. When the amount of each diet added to the KCN solution was increased to 1g, greater loss of KCN (about 70% in the case of SB- or CM-diet and about 80% in the case of SM-diet, table 1) occured.

TABLE 1

The effect of diet on the recovery of cyanide from KCN solution added to 0.5g or 1.0g of the diet.

Type of diet	*Storage time (h)*	*% of added KCN recovered (mean ± S.E.M.)*	
		0.5g diet	*1.0g diet*
Diet based on Soyabean	0	95.5 ± 0.9	
	18	48.5 ± 1.9	29.8 ± 1.0
Diet based on Skimmed milk	0	94.3 ± 1.4	
	18	63.0 ± 1.4	19.4 ± 1.4
Commercial diet	0	94.0 ± 1.6	
	18	52.5 ± 0.9	31.3 ± 2.2
Control (no diet added)	0	99.6 ± 1.1	
	18	64.6 ± 6.3	

TABLE 2

Recovery of cyanide from soyabean meal diet containing 5% arachis oil (OD-diet) to which KCN was added

Storage time (h)	*Amount of KCN added per 10g diet*	*% of added KCN recovered mean ± S.E.M., n = 4*
0	200mg	95.0 ± 0.9
18	200mg	93.0 ± 1.8
0	5mg	94.4 ± 2.7
18	5mg	90.5 ± 1.1

Since KCN is readily hydrolysed in aqueous solution, it was thought that the loss of KCN from diet could be due to the presence of water. The mixing of diets with arachis oil was therefore considered as a means of reducing hydrolysis of KCN. Preliminary studies showed that rats preferred SB-diet plus arachis oil than any of the other two diets (SM- or CM-diet) to which arachis oil was added. This explains why further studies on the effect of arachis oil in stabilizing KCN in diets was carried out on the SB-diet alone.

The stability of KCN in the SB-diet mixed with arachis oil (OD-diet) was studied using two levels of KCN (200mg/10g diet and 5mg/10g diet). The recovery of cyanide from the OD-diet after storage in an open beaker was close to the recovery from the diet before storage (P 0.3), table 2). The KCN was therefore stable when mixed in the diet containing 5% arachis oil.

Results on the test of the availability of KCN in the OD-diet conducted with rats are shown in table 3. Rats on the OD-diet plus cyanide had significantly elevated thiocyanate content in both plasma and urine compared with rats on OD-diet alone. However, giving KCN in the OD-diet at the level of 77 umoles per rat per day, did not reduce the growth of the rats relative to that of control group (P 0.90). Furthermore, the OD-diet with cyanide was found to be acceptable to rats, Since the rats are adequate amount of the diet compared with controls without cyanide (table 3).

TABLE 3

Feed intake, body weight gain, and thiocyanate content of the plasma and urine of rats fed KCN (77 umoles/rat/day) in the diet values are means ± S.E.M.

Substance added to diet	*Average diet consumed per rat per day (g)*	*Mean body weight gain (g)*	*Plasma SCN- umoles per per 100ml*	*Urinary SCN- umoles per day*
Hone	13.6 ± 0.9	100 ± 6	2.9 ± 0.5	1.5 ± 0.1
KCN	14.0 ± 0.6	101 ± 4	15.1 ± 2.8	39.3 ± 3.8

TABLE 4

Recovery of 14_{CN} added to 2g basal Soya bean meal diet.

ug CN⁻	*Total activity dpm*	*% of added CN⁻ recovered mean ± S.E.N. n = 4*
12.5	52.500	7.8 ± 0.1
20,000	52,500	8.5 ± 0.3
40,000	52,500	43.5 ± 1.1
20,000	3.0×10^6	40.6 ± 1.0

Studies with 14C-cyanide (in sealed tubes that prevented loss of cyanide as HCN) confirmed that only a proportion of cyanide activity in SB-diet moistered with water is recovered as HCN (table 4). Furthermore, lower percentage recovery of HCN was obtained at low dietary cyanide concentration compared with diets containing high amounts of cyanide (table 4). Result of the thin-layer chromatography of the activity remaining in the SB-diet after removal of the HCN is shown in fig. 1. The radioactivity was distributed over the plate but large amounts co-chromtographed with maker and carrier formic acid. Location of formic acid on the chromatogram was achieved by spraying with alcoholic methyl red indicator (Lynes, 1964). In this system formic acid had an Rf value of 0.57.

DISCUSSION

The work described here has established that potassium cyanide cannot be recovered when added to the diet of experimental rats which has been moistened with water to minimize spilling. Investigation revealed that the cyanide was lost by transformation to other products and perhaps also to volatile HCN. This preliminary study has shown that a number of products may be formed, including formate. Clearly, further studies on the nature of cyanide transformation in the diet to which water has been added may prove interesting, knowledge acquired from such studies might help to improve the method of cassava processing with a view to reducing its cyanide content.

Extensive loss of cyanide either as volatile HCN or by transformation to other products was prevented by the addition of arachis oil to SB-diet (OD-diet). It is noteworthy that rats given OD-diet plus KCN had not only elevated plasma thiocyanate but also excreted large amounts of this radical in the urine. Since elevated thiocyanate in plasma or urine is accepted as an evidence of exposure to chronic cyanide intake (Pettigrew and Fell, 1972; Tewe *et al.* 1977), then the cyanide added to the OD-diet must be stable.

Although cyanide in the form of KCN has been given to rats in drinking water in a reasonably stable form (Okoh, 1978), this substance will be best administered to experimental rats in the diet. This is because rats on KCN diet ate adequate amounts of the diet like controls while the addition of KCN to the drinking water caused a reduction of water intake in the rats compared with controls (Okoh, 1978).

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REFERENCES

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, 1970. Official methods of analysis. Association of Official Agricultural Chemists, Washington D.C.

BARRETTE, M.D., HILL, D.C., ALEXANDER, J.C. and ZITNAK, A., 1977. Fate of orally dosed linamarin in the rat. Can. J. Physiol. Pharmacol. **55**; 134—136.

BOXER, G.E. and RICHARDS, J.C., 1951. Chemical determination of vitamin B_{12}. The quantitative isolation and colorimetric determination of millimicrogram quantities of cyanide. Archs. Biochem. Biophys. **30**; 372—381.

CLARK, A.J., 1939. A preliminary note on the inhibitory effects of chronic poisoning by foods containing cyanic substances. J. Trop. Med. Hyg. **42**; 65—72.

LYNES, A., 1964. The analysis of straight-chain (n-c_1-cq) carboxylic acids by a thin-layer chromatographic method. J. Chromatoq. **15**; 108—110.

METHA, C.S. and McGNITY, J.W., 1977. Chronic administration of cyanide: Urinary excretion of thiocyanate in male and female rats. Acta Pharmacol. Toxical. 41: 49 — 52.

OKE, O.L., 1969. Role of hydrocyanic acid in nutrition, Wld. Rev. Nutr. Dietetics. 11: 170—198.

OKOH, P.N., 1978. Aspects of the metabolism of cyanide in the rat. Ph.D. Thesis, University of Liverpool.

OSUNTOKUN, B.O., 1970. Cassava diet and cyanide metabolism in Winstar rats. Br. J. Nutr. 24: 797—799.

PREY, V. BERBALK, H. and KAUSZ, M., 1962. Beitrage Zur Papierchromatographie organischer substanzen. Mikrochimica Acta. 449—454.

PETTIGREW, A.R. and FELL, G.S. 1972. Simplified colorimetric determination of thiocyanate in biological fluids and its application to investigation of the toxic amblyopia. Clin. Chem. 18, 966—970.

SMITH, A.D.M. and FOULKES, M., 1966. Cyanide excretion in the rat. Nature, Lond. 209: 919—920.