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Long-Term and Carry-Over Effect of Dietary Inorganic Cyanide (KCN) in the Life Cycle Performance and Metabolism of Rats

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Long-Term and Carry-Over Effect of Dietary Inorganic Cyanide (KCN) in the Life Cycle Performance and Metabolism of Rats. TEWE, O. O., AND MANER, J. H. (1981). *Toxicol. Appl. Pharmacol.* 58, 1–7. A long-term study was carried out to evaluate the cumulative effects of adding 500 ppm KCN to a cassava root flour-based diet. High dietary level of KCN did not have any marked effect in gestation and lactation performance of female rats. No carry-over effect of level of cyanide fed during gestation was observed on lactation performance. The high cyanide-containing diet, however, significantly reduced feed consumption and daily growth rate of the offspring when fed during postweaning growth. Protein efficiency ratio was not only reduced by the high cyanide diet during the postweaning growth phase but also there was a carry-over effect from gestation. Serum thiocyanate was significantly increased in lactating rats and their offspring during lactation and in the postweaning growth phase of the pups. No apparent carry-over effect was noticed on this parameter. Rhodanese activity in liver and kidney was unaffected by feeding the high cyanide diet during gestation, lactation, and/or during postweaning growth.

The cyanogenic glucosides are among the naturally occurring toxic agents in feeds consumed by man and animals. Ekpechi (1963) has demonstrated the goitrogenic activity of the cyanogenic plant, cassava, in rats. Delange (1974) also reported goiter and endemic cretinism in the Idjwi Island of the Zaire Republic. Flux *et al.* (1963) showed that lambs born of ewes fed cyanogenic clover manifested goitrogenic effects in that their thyroid glands were significantly heavier than those of lambs of ewes who were grazed on ryegrass pastures.

It, however, appears to us that some anomalies in which the cyanogenic glucosides have been implicated need some clarification. Indeed an experiment carried out in the swine programme of the Centro Internacional de Agricultura Tropical (CIAT) in Colombia by Maner and Gomez (1973) showed that no thyroid lesions were observed in weanling rats fed nutritionally balanced fresh cassava diets containing 196 ppm HCN in a trial lasting 4 months.

The present study attempts to clarify whether inorganic cyanide (KCN) when fed independently of other dietary deficiencies can effect a transmission of its toxic influences to the offspring. Observations were carried out on Wistar rats during gestation, lactation, and postweaning growth.

EXPERIMENTAL PROCEDURES

Forty adult female rats with an average body weight of 273 g were randomly alloted from blocks matched for litter and body weight to two treatment groups. The control group received a basal diet prepared from the meal of a low-HCN cassava variety while the other received the basal diet with 500 ppm cyanide added as KCN. The basal diet contained

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TABLE 1

GROSS COMPOSITION OF EXPERIMENTAL DIETS BASED ON LOW-HCN VARIETY[a] CASSAVA MEAL

Ingredient	Percentage
Cassava meal	60.3
Soybean meal	28.7
Corn oil	5.0
Minerals[b]	44.0
Vitamins[c]	2.0
Total	100.0

[a] A low-HCN cassava diet cultivar-Venezuela variety was used for preparing the diets. The dried cassava meal contained 21 mg/kg HCN; 500 ppm cyanide (1.25 g KCN/kg diet) was first mixed with the minerals in treatment 2 of the experiment.

[b] Grams supplied per kilogram of diets: NaCl, 5.6; KH_2PO_4, 15.7; $MgSO_4$, 2.3, $CaCO_3$, 15.4; $FeSO_4 \cdot 7H_2O$, 1.1; KI, 0.03; $MnSO_4 \cdot 2H_2O$, 0.18; $ZnCl_2$, 0.012; $CuSO_4 \cdot 5H_2O$, 0.02; $COCl_2 \cdot 6H_2O$, 0.008.

[c] Milligrams supplied per kilogram of diet: retinyl acetate, 90, cholecalciferol, 5; tocopherol, 100; ascorbic acid, 900; inositol, 100; choline chloride, 1.5; menadione, 45; *p*-aminobenzoic acid, 1.0 g, niacin, 90; riboflavin, 20; pyrodxine hydrochloride, 20; thiamine hydrochloride, 20; calcium pantothenate, 60; biotin 0.4; folic acid, 1.8; vitamin B_{12}, 0.03.

12 mg HCN/kg mixed diet. There were five replicates of four female rats per treatment during the gestation period. Twenty male rats were alternated daily for mating the ut groups. All rats were kept in well-ventilated wire cages.

Using a factorial arrangement, the rats were allotted to the treatment groups during pregnancy, then each group was subdivided during lactation and the postweaning growth periods as shown in the results in Tables 3 and 4.

Management of Rats in Gestation

The respective diets were offered to the female rats at the start of the experiment, that is as soon as breeding with male rats began. They therefore had the rations throughout gestation. The prepregnancy duration of feeding the control diet was 16.3 ± 1.1 and 19.7 ± 1.8 days for the test diet.

Feed intake was controlled during gestation by limiting the intake of the control diet to that of the diet containing KCN. To ensure that the male rats did not consume the experimental diets, feeds were removed from the female cages during mating. The mating period was 9 PM to 8 AM daily. Males were offered commercial pellets during their resting periods (i.e., 8 AM to 9 PM daily). The rat pellets were obtained from Ralston Purina Company, St. Louis, Missouri. Water was supplied *ad libitum*. Pregnant rats were separated in the last week of gestation. They were kept in plastic cages and offered 20 g of their respective diets until parturition. Weekly feed intake and body weights were recorded in the two experimental groups.

Management of Rats in Lactation

After parturition, the females were offered their respective diets and water *ad libitum*. Dams and their pups were weighed weekly and total feed intake recorded at each weighing. Mortality was also recorded throughout the 21-day lactation period. At weaning, two female pups with body weights nearest to the mean weight of their respective litter were selected for growth studies. The lactating rats and the remaining weanling rats were anesthetized with chloroform and blood obtained by heart puncture for thiocyanate determination by the method of Bowler (1944). Livers and kidneys were removed from each rat and frozen for rhodanese activity determinations by a modification of the Sorbo (1951) method as described by Tewe (1975). Protein determinations were also carried out using the Biuret method.

Management of Rats in the Postweaning Growth Phase

The two female weanling rats selected per litter were randomly allotted to the two experimental diets. Feed and water were offered *ad libitum*. Feed intake and body weights are recorded twice weekly. At the end of the 28-day growth trial, the rats were anesthetized with chloroform and blood obtained by heart puncture for serum thiocyanate determination. Liver and kidneys were also removed, weighed, and frozen for rhodanese activity determinations.

Preparation of diets. As a high-HCN-containing cassava variety could not be obtained, potassium cyanide (KCN) was used to augment the cyanide content of the test diet as shown in Table 1. Proximate analysis of the feeds were carried out and this gave 14.7 and 15.8% crude protein for the control and test diets, respectively. The level of 500 ppm was chosen for the test diet following observations made by Maner and Gomez (1974) in the experimental station (CIAT). They reported that deaths of rats occurred when they were fed diets containing 960 ppm KCN and above. Differences in growth and feed consumption were, however, observed with a level of 480 ppm KCN in the diet as compared to a control diet.

TABLE 2

'ERFORMANCE OF RATS (DAMS) FED A CONTROL AND TEST DIET CONTAINING 500 ppm ADDED CYANIDE, DURING GESTATION AND LACTATION[a]

	Diet fed during gestation:	Control		Test	
ams	Diet fed during lactation:	Control	Test	Control	Test
eed intake during lactation, g/day		37.5 ± 1.7	36.4 ± 2.0	33.1 ± 1.4	34.5 ± 2.3
et weight change during lactation, g		15.9	−21.0	21.4	−18.0
eight of fresh tissues—at 21 days of lactation, percentage of body weight					
Liver		4.1 ± 0.2	3.4 ± 0.3	4.2 ± 0.3	3.9 ± 0.1
Kidney		0.6 ± 0.02	0.6 ± 0.03	0.7 ± 0.02	0.6 ± 0.03
verage number of offspring per litter at birth		12	12	10	11
ercentage mortality at birth		0	3.9	2.3	1.0
ody weight at birth, g		6.1	6.0	6.3	6.1
otal weight gain during suckling, g		21.5 ± 1.4	22.8 ± 2.2	24.8 ± 2.4	21.8 ± 1.6

[a] Each value represents the mean of 10 litters ± SE during the lactation period.

The test diet was prepared every other day and kept tightly closed transparent plastic bags. Cyanide alysis was carried out on each batch of prepared et by the method of Wood (1966). Preliminary terminations of cyanide showed no remarkable ss of added cyanide from diets when tested over 48-hr period

atistical Analysis

Variance analysis was run on the gestation param-ers by the method of Snedecor and Cochran (1967). actation and postweaning growth parameters were atistically analyzed using the factorial method of teel and Torrie (1960).

RESULTS

The performance of rats fed diets con-aining different levels of cyanide during estation and lactation is presented in Table and Fig. 1. In pregnant rats, although not tatistically significant, the total body veight gain during gestation was greater vhen the control diet was fed (49.7 ± 8.0 g) han when the test diet was fed (41.6 ± 7.5). Litter size and birth weights of the pups were also not significantly different between groups.

Feed consumption during lactation was also similar in all groups. Factorial analysis carried out on the body weight of pregnant rats at parturition and after 21 days in lactation shows that the differences in mean body weights were not statistically sig-nificant. The cyanide treatment also had no

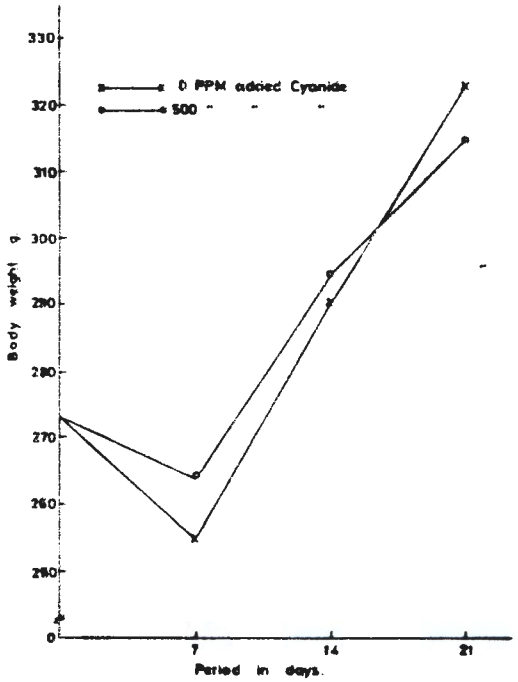

FIG. 1. Body weight of female rats on cassava diets containing two levels of cyanide during gestation.

measurable effect on weights of maternal livers and kidneys when these were expressed as percentages of total body weight. No carry-over effect of cyanide level fed during gestation were observed on the parameters measured during lactation. There was no consistent treatment effect of cyanide level during gestation or lactation on the weaning weights or mortality rate of the offspring (Table 2).

Performance of offspring during the postweaning growth phase is shown in Table 3. The feeding of cyanide during the postweaning growth phase caused significant reductions in the daily feed consumption and growth rates of rats. These parameters did not, however, reveal any carry-over effect from gestation or lactation treatments. Although there was no significant effect over gestation, lactation, and/or postweaning growth periods, the offspring of rats fed the control diet during gestation showed a tendency to have a higher feed/gain ratio when the control diet was fed during the postweaning growth phase. No such trend was observed in offspring of rats fed the test diet during gestation.

A high dietary level of cyanide in the postweaning growth phase significantly ($p < 0.01$) reduced protein efficiency ratio as determined from the calculated true protein. Cyanide fed during gestation caused a significant ($p < 0.05$) reduction in the liver weight in the growing rats showing a carry-over effect.

Table 4 shows metabolic changes in lactating rats and their offspring at weaning and after 28 days of postweaning growth. Serum thiocyanate concentration was significantly increased feeding added cyanide during the postweaning growth phase. No apparent carry-over effect of added cyanide fed during gestation and lactation was noticed in this phase of growth. Rhodanese activity of liver and kidney were similar in all treatment groups.

DISCUSSION

The cyanide in the KCN-containing diet caused a striking rise in serum thiocyanate of both the lactating dams and their suckling offspring as shown in Table 4. This significantly higher serum thiocyanate indicates that some organic sulfur has been used in detoxifying the ingested cyanide. However, the growth rate was similar in all groups. These results suggest that rats can maintain some thiocyanate in the circulatory system without apparent effects on their performance.

TABLE 3

PERFORMANCE DURING THE POSTWEANING GROWTH PHASE OF GROWING RATS FED A CONTROL AND TEST DIET CONTAINING 500 ppm ADDED CYANIDE[a]

Diet fed during gestation:	Control				Test			
Diet fed during lactation:	Control		Test		Control		Test	
Diet fed during postweaning phase:	Control	Test	Control	Test	Control	Test	Control	Test
Daily feed intake, g	12.3 ± 0.3	11.5 ± 0.2	12.2 ± 0.5	12.1 ± 0.6	12.7 ± 0.4	11.3 ± 0.5	12.7 ± 0.8	11.5 ± 0.5
Daily weight gain, g	3.9 ± 0.1	3.5 ± 0.2	3.7 ± 0.1	3.5 ± 0.1	3.6 ± 0.1	3.4 ± 0.1	3.6 ± 0.2	3.5 ± 0.1
Feed efficiency	3.2 ± 0.1	3.3 ± 0.2	3.3 ± 0.1	3.5 ± 0.2	3.5 ± 0.2	3.3 ± 0.2	3.6 ± 0.2	3.3 ± 0.2
Protein efficiency ratio (PER)	2.2 ± 0.07	1.8 ± 0.07	2.0 ± 0.08	1.7 ± 0.11	2.0 ± 0.12	1.9 ± 0.13	1.9 ± 0.12	1.9 ± 0.08
Fresh weight of tissues, percentage of body weight								
Liver	4.4 ± 0.12	4.6 ± 0.19	4.4 ± 0.11	4.5 ± 0.13	4.6 ± 0.20	4.9 ± 0.16	4.4 ± 0.11	4.5 ± 0.18
Kidney	0.8 ± 0.03	0.9 ± 0.02	0.9 ± 0.02	0.9 ± 0.03	0.8 ± 0.02	0.9 ± 0.03	0.9 ± 0.02	0.7 ± 0.2

[a] Each value represents the means from 10 rats ± SE during the postweaning growth phase.

Factorial analysis showed that the only statistically significant effects were those of postweaning growth HCN level of food intake, body weight gain, and PER and that of the interaction of gestational HCN level × postweaning HCN level on PER at ($p < 0.05$). Details are shown in Table 5.

TABLE 4

METABOLIC CHANGES IN RATS FED A CONTROL DIET AND TEST DIET CONTAINING 500 ppm ADDED CYANIDE DURING GESTATION, LACTATION, AND POSTWEANING GROWTH[a]

Diets fed during gestation:	Control		Test	
Diet fed during lactation:	Control	Test	Control	Test
Lactating dams—21 days of lactation				
Serum thiocyanate, mg/100 ml	2.42 ± 0.13	3.56 ± 0.39	2.40 ± 0.10	3.22 ± 0.30
Rhodanese activity, mg thiocyanate/min/g protein				
Liver	28.4 ± 0.8	30.3 ± 1.0	30.0 ± 1.0	27.2 ± 1.1
Kidney	22.5 ± 1.1	23.5 ± 1.1	23.5 ± 1.7	22.3 ± 1.0
Weanling rats				
Serum thiocyanate, mg/100 ml	1.84 ± 0.19	2.67 ± 0.28	1.85 ± 0.13	2.08 ± 0.27
Rhodanese activity, mg thiocyanate/min/g protein				
Liver	30.4 ± 1.5	31.0 ± 1.1	33.6 ± 1.5	30.9 ± 1.4
Kidney	24.3 ± 0.7	24.2 ± 0.6	24.0 ± 0.7	24.4 ± 0.6

Diets fed during postweaning growth:	Control	Test	Control	Test	Control	Test	Control	Test
Growing rats—28 days postweaning								
Serum thiocyanate, mg/100 ml	1.97 ± 0.07	2.91 ± 0.24	2.11 ± 0.04	3.24 ± 0.30	2.06 ± 0.06	3.28 ± 0.30	2.00 ± 0.13	2.87 ± 0.12
Rhodanese activity, mg thiocyanate/min/g protein								
Liver	36.2 ± 1.2	35.7 ± 1.3	36.1 ± 1.4	36.0 ± 1.0	36.7 ± 1.1	33.8 ± 1.3	34.0 ± 0.9	36.2 ± 1.5
Kidney	27.0 ± 0.9	27.6 ± 0.8	27.7 ± 0.8	27.1 ± 0.9	27.0 ± 1.1	28.5 ± 1.0	27.3 ± 0.5	27.0 ± 0.8

[a] Each value represents the mean of determinations from 10 litters ± SE during the lactation period.

Each value represents the mean of determinations from 10 rats ± SE during the postweaning growth phase.

Factorial analyses of the data for the postweaning period revealed that the only statistically significant effect was that of HCN level during post-weaning growth on serum thiocyanate ($p < 0.01$). (Details are shown in table 5.)

The feeding of cyanide during the postweaning phase caused significant reduction of growth. The poorer growth rate of rats fed added cyanide diet might have been due to the detoxification of cyanide by the sulfur amino acids through the rhodanese pathway. Indeed, Maner and Gomez (1973) demonstrated that there was a progressive decrease in growth rate of rats offered diets containing potassium cyanide from 0 to 8000 ppm.

Although, no correlation in growth rate of rats in the postweaning phase could be traced to a carry-over effect of cyanide consumed by the rats in gestation and lactation. it is remarkable that feed efficiency was significantly reduced by an interaction of high dietary cyanide levels in gestation and postweaning growth phase (Table 5). It therefore appears that when a sublethal dose of cyanide is present in the diet, cumulative effects of cyanide consumed by gestating rats can be observed in their offspring.

The question, however, remains unanswered why the striking rise in serum thiocyanate in the lactating dams and their offspring did not cause a noticeable reduction in growth of pups, while some measureable effect was observed at the postweaning growth phase. The serum thiocyanate concentration was higher in the lactating rats fed the high cyanide diets than in the growing rats (postweaning phase) fed the same diets. That the cyanide diets, however, had more pronounced effect on the pups as compared to the lactating rats suggests that the toxicity of cyanide does not only depend on the quantity ingested and thiocyanate formed, but also on the age of the animal ingesting this poison. A level toxic to young growing rats may therefore have no influence on older rats.

While the metabolism of ingested hydrocyanic acid (HCN) has been shown to be closely related to that of inorganic cyanide in monogastrics (Ermans *et al.*, 1972, 1973), the deleterious effect of ingested inorganic cyanide in the form of KCN may not be the same as that from organic cyanide sources such as cassava. It has been reported by Oke (1969) that when intact glycosides are ingested probably little or no free HCN will be released from the

TABLE 5

SUMMARY OF FACTORIAL ANALYSIS ON PERFORMANCE AND METABOLIC TRAITS OF RATS[a] FED TWO LEVELS OF CYANIDE

	Performance				Metabolic changes		
						Rhodanese activity	
Sources of variation	Feed intake	Body weight gain	Feed efficiency	Protein efficiency ratio	Serum thiocyanate	Liver	Kidney
Gestation (A)							
Growth (B)	*	*		**	**		
Lactation (C)							
AB				*			
AC							
BC							
ABC							

Note. blank space indicates that means are not significant at 5% level.

* Means significant at 5% level.

** Means significant at 1% level.

[a] Rats are offspring of female rats fed two dietary cyanide levels during gestation and lactation.

yanogenic glycoside during its passage ıown the monogastric digestive tract, at east, not before it reaches the bacteria in he lower gut. However, if the cassava meal s cooked, the enzyme normally present n the plant would have been inactivated and incapable of hydrolysing the linamarin.

It will therefore appear necessary to employ pure linamarin or amygdalin in further studies to quantify the effects of glycosides in rats as opposed to those of norganic cyanide.

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