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# The Fate in the Rat of Ingested Dhurrin Present in Sprouted Sorghum Grain

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*ABSTRACT*

*Female albino rats, which had been starved for 24 h, were each fed 5 g of sprouted sorghum containing 1·83 mg bound cyanide and 0·91 mg free cyanide.*

*All rats consumed the sprouted sorghum within 5 h but bound cyanide could not be detected in all parts of the gastrointestinal tract wall, plasma or faeces. By contrast, bound cyanide was present in gastrointestinal contents and urine.*

*The hydrolysis of a substantial amount of bound cyanide in the gastrointestinal contents was shown by the increase in proportion of free cyanide relative to bound cyanide.*

*Thiocyanate was increased in the plasma and urine of rats fed sprouted sorghum, and there was increased secretion of this radical into the stomach contents of the rat with increase in time following feeding of sprouted sorghum.*

## INTRODUCTION

Sprouting of sorghum has been recommended as a means of improving the nutritional quality of the grain (Wu & Wall, 1980). This is because the major limiting amino acid, lysine, is increased as a result of sprouting. Furthermore, the nutritional benefit to be obtained from a lysine increase is

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expected to be enhanced by the substantial decrease in sorghum tannins (Okoh & Kubiczek, 1985) during sprouting.

Recent studies, however, have shown that the toxic component, cyanide, is substantially increased in malted sorghum grains (Panasiuk & Bills, 1984; Ikediobi *et al.*, 1987; Dada & Dendy, 1987). The cyanide formed in sprouted sorghum grain is present largely in the form of bound cyanide, dhurrin, which accounts for about 75% of the total cyanide present (Olugboji, 1987).

Acute toxicity from cyanide is due to the inhibitory effect of this substance on the respiratory enzyme, cytochrome oxidase. The occurrence of acute toxicity following ingestion of a meal containing dhurrin will therefore depend, not only on the amount present, but also on the rate of release of free cyanide from the dhurrin *in vivo*. If sprouted sorghum is to be used as a human and animal food, then it will be of interest to determine the fate in animals of dhurrin formed during sprouting of the grain.

## MATERIALS AND METHODS

### Sprouting of sorghum

The sorghum variety used in this study was obtained from the Institute for Agricultural Research, Ahmadu Bello University, Zaria. Seeds were germinated in the room with daylight for 4 days according to the Wu & Wall (1980) method after retarding mould growth with 0·2% formaldehyde at an average temperature of 30°C. Sprouted grains were dried at 50°C for 24 h and then ground in a Wiley mill, before using for animal experiment or analysis.

### Animal experiment

Twelve female albino rats weighing between 70 and 80 g were each assigned to individual metabolism cages which allowed for the collection of faeces and urine separately. Three of the rats were used as controls and were fed commercial diet only. Each of the remaining nine experimental rats were starved for 24 h and were then fed 5 g of sorghum that had been germinated for 4 days. Five grams was about the amount each rat could consume within 3 to 5 h after starvation. The rats were killed 5, 7 and 9 h after the start of feeding; three rats belonging to each group of time interval. Killing of rats was achieved by anaesthetizing with chloroform in a desiccator and removing blood by heart puncture using a heparinized syringe. The blood was centrifuged rapidly at 2700*g* and the plasma removed by means of a pasteur pipette.

The gastrointestinal tract (GIT) was carefully dissected out, and separated into stomach, small intestine and large intestine portions. The contents of each portion were removed; the weights of wall and contents were recorded and they were stored separately. All samples were stored frozen in screw-capped bottles until required for analysis.

**Assay of free and bound cyanide in sprouted sorghum, tissues, plasma and urine**

Approximately 1 g of dried ground sprouted grains was extracted thrice with 5 ml aliquots of 0·1 M sodium phosphate buffer, pH 6·8. The extract was then stored frozen in a tightly stoppered vessel until assayed for free and total cyanide. In the case of parts of the gastrointestinal wall and contents, the total separated portion was extracted similarly with 0·1 M phosphate buffer after mincing and homogenizing in the same buffer. The cyanide in various extracts was determined by a procedure based on the colorimetric method of Epstein (1947) as described below.

To an aliquot of each extract was added 1 ml of 0·1 M NaOH in a 15 ml test tube. The mixture was incubated for 30 min at room temperature to achieve total hydrolysis of the extracted cyanogenic glucoside, dhurrin (Ikediobi *et al.*, 1987). At the completion of hydrolysis, the solution was made up to 8 ml with 50 mM phosphate buffer, pH 6·0.

This was followed by the addition of 0·4 ml of 0·5% chloramine-T solution and incubation of the resulting solution in a water bath at 0°C for 5 min. The absorbance of the resulting blue colour was read at 620 nm against a reagent blank. To determine the amount of free cyanide in the extract, the above experiment was repeated except that addition of 0·1 M NaOH was omitted in the incubation solution. The value for bound cyanide was routinely obtained, if required, by subtracting free cyanide from total cyanide values.

For plasma and urine, cyanide present in an aliquot was quantitatively transferred by nitrogen aeration into 4·0 ml of 0·1 M NaOH (Okoh & Pitt, 1982; Okoh, 1983; Lundquist *et al.*, 1985) and the separated cyanide assayed by the colorimetric method of Lundquist *et al.* (1985).

**Assay of thiocyanate**

Thiocyanate in serum, urine and tissue extracts was assayed by the method of Lundquist *et al.* (1979). This involved separating thiocyanate from interfering compounds by first adsorbing it on a 2·5 × 0·7 cm Amberlyst A21 (Rohm and Hass Co., Philadelphia, PA) anion-exchange column. The resin was ground in a Tecator model 1090 mill using the finest adjustment before

use. Elution of the adsorbed thiocyanate was achieved with sodium perchlorate solution after washing the column twice with 5 ml of 0·1 M HCl to remove interference due to cyanide (Lundquist *et al.*, 1979). The eluted thiocyanate was quantified by the modified Konig reaction (Lundquist *et al.*, 1979).

## RESULTS

### Free and bound cyanide in the gastrointestinal tract (GIT) faeces, urine and plasma

The mean weights of different parts of the gastrointestinal tract wall and their contents at different times following administration of sprouted sorghum are shown in Table 1. The content of the distal parts of the gastrointestinal tract, especially the large intestine, was found to be low, even 9 h after feeding. This can be attributed to the slow rate of gastric emptying of the rats during the daytime when the experiment was carried out.

The amounts of free and bound cyanide found in the GIT, faeces, urine and plasma at different times following administration of sprouted sorghum to rats are given in Tables 2 and 3. All parts of the GIT of rats fed sprouted sorghum were found to contain free cyanide but only some had bound cyanide. Bound cyanide was indeed not detected in any part of the GIT wall. In contrast, the contents of all parts of the GIT contained bound cyanide, the highest being in the stomach contents, followed by the small intestinal contents.

The total amount of bound cyanide in all parts of the GIT at different times was calculated to be 48·7 $\mu$g in the 5 h-group; 50·5 $\mu$g in the 7 h-group and 47·3 $\mu$g in the 9 h-group, while free cyanide levels at the different times

**TABLE 1**
Mean Weights of Tissues from Control Rats and Rats fed Malted Sorghum (g)

| *Tissue or sample* | *Control rats* | *Rats killed at different times after a meal of sprouted sorghum* | | |
|---|---|---|---|---|
| | | *5 h* | *7 h* | *9 h* |
| Stomach wall | 0·95 | 0·91 | 0·90 | 0·90 |
| Stomach contents | 1·43 | 6·16 | 4·90 | 3·60 |
| Small intestine wall | 2·09 | 2·04 | 1·87 | 2·00 |
| Small intestine contents | 1·82 | 2·45 | 2·60 | 2·60 |
| Large intestine wall | 1·36 | 1·33 | 1·14 | 1·30 |
| Large intestine contents | 1·94 | 1·25 | 1·72 | 1·40 |

**TABLE 2**
Free Cyanide in Parts of Gastrointestinal Tract, Faeces, Urine and Plasma after a Meal of Sprouted Sorghum ($\mu$g per total tissue weight)

| *Tissue or sample* | *Control rats* | *Rats killed at different times after a meal of sprouted sorthum* | | |
|---|---|---|---|---|
| | | *5 h* | *7 h* | *9 h* |
| Stomach wall | ND | 42·7 ± 3·30[a] | 45·2 ± 3·77[a] | 39·9 ± 2·89* |
| Stomach contents | ND | 72·5 ± 3·12 | 79·9 ± 5·91 | 65·4 ± 6·37 |
| Small intestine wall | ND | 51·0 ± 6·72 | 57·0 ± 2·92 | 60·9 ± 7·60 |
| Small intestine contents | ND | 48·5 ± 3·20 | 51·0 ± 0·72 | 40·5 ± 3·50 |
| Large intestine wall | ND | 35·6 ± 2·55 | 34·8 ± 1·94 | 33·9 ± 1·12 |
| Large intestine contents | ND | 51·6 ± 2·10 | 47·6 ± 0·75 | 36·6 ± 6·40 |
| Faeces | ND | Trace | Trace | Trace |
| Urine[b] | 0·38 ± 0·05 | 1·32 ± 0·63 | 0·390[c] | 1·06 ± 0·38 |
| Plasma[b] | 0·25 ± 0·06 | 0·35 ± 0·13 | 0·36 ± 0·05 | 0·38 ± 0·09 |

[a] Mean of duplicate analysis ± SD from three rats.
[b] Cyanide in $\mu g\,ml^{-1}$.
[c] Value for one sample.
ND = Not detectable.

**TABLE 3**
Dhurrin Cyanide in Parts of Gastrointestinal Tract, Faeces, Urine and Plasma after a Meal of Sprouted Sorghum ($\mu$g per total Tissues weight)

| *Tissue or sample* | *Control rats* | *Rats killed at different times after a meal of sprouted sorghum* | | |
|---|---|---|---|---|
| | | *5 h* | *7 h* | *9 h* |
| Stomach contents | ND | 43·1 ± 7·13[a] | 37·7 ± 1·50[a] | 33·0 ± 2·09[a] |
| Stomach wall | ND | ND | ND | ND |
| Small intestine wall | ND | ND | ND | ND |
| Small intestine contents | ND | 3·66 ± 0·70 | 6·60 ± 2·50 | 8·10 ± 1·99 |
| Large intestine wall | ND | ND | ND | ND |
| Large intestine contents | ND | 1·95 ± 0·87 | 6·27 ± 1·70 | 6·18 ± 2·30 |
| Faeces | ND | ND | ND | ND |
| Urine[b] | ND | 40·4 ± 16·90 | 111[c] | 28·2 ± 42·80 |
| Plasma[b] | ND | ND | ND | ND |

[a] Mean of duplicate analysis ± SD from three rats.
[b] Values in $\mu g\,ml^{-1}$.
[c] Value for one sample.
ND = Not detectable.

were: 302; 316 and 277 μg, respectively. Free cyanide showed a decrease in both the stomach and intestinal contents from 7 h to 9 h, but this was only statistically significant ($P < 0.05$) for the intestinal contents. The free cyanide in the walls of the stomach and large intestine showed a decrease from 7 to 9 h while there was an increase in free cyanide of the small intestinal wall from 5 to 9 h. However, these changes were not statistically significant ($P > 0.05$).

A substantial amount of bound cyanide was found in the urine of rats fed sprouted sorghum while it was not detectable in the plasma and faeces. In the control rats, only the urine and the plasma contained low levels of free cyanide (Table 2).

## Thiocyanate in gastrointestinal tract (GIT) faeces, urine and plasma

The amount of thiocyanate found in various parts of the GIT, faeces, urine and plasma is shown in Table 4. All parts of the GIT contained thiocyanate in both control rats and rats fed sprouted sorghum. The highest amount of thiocyanate was found in the stomach contents of all the groups of rats and this increased with time following feeding of sprouted sorghum. Indeed, the increase in the amount of thiocyanate present in gastric contents at 9 h was highly significant ($P < 0.01$) when compared with control rats. The

**TABLE 4**

Thiocyanate in Parts of Gastrointestinal Tract, Faeces, Urine and Plasma after a Meal of Sprouted Sorghum (μg per total tissue weight)

| *Tissue or sample* | *Control rats* | *Rats killed at different times after a meal of sprouted sorghum* | | |
|---|---|---|---|---|
| | | *5 h* | *7 h* | *9 h* |
| Stomach wall | 0·79 ± 0·17[a] | 1·69 ± 0·44[a] | 1·34 ± 0·57[a] | 1·21 ± 0·60[a] |
| Stomach contents | 13·3 ± 2·37 | 15·0 ± 0·89 | 16·8 ± 1·29 | 19·7 ± 2·85 |
| Small intestine wall | 0·66 ± 0·24 | 0·56 ± 0·27 | 1·18 ± 0·59 | 1·37 ± 0·56 |
| Small intestine contents | 0·47 ± 0·01 | 1·16 ± 0·10 | 1·36 ± 0·47 | 1·25 ± 0·30 |
| Large intestine wall | 0·44 ± 0·11 | 0·50 ± 0·01 | 0·46 ± 0·01 | 0·42 ± 0·01 |
| Large intestine contents | Trace | 0·99 ± 0·16 | 0·51 ± 0·39 | 0·37 ± 0·25 |
| Faeces | ND | Trace | Trace | Trace |
| Urine[b] | 2·60 ± 0·62 | 4·32 ± 0·59 | 6·20[c] | 5·90 ± 1·69 |
| Plasma[b] | 1·08 ± 0·66 | 1·53 ± 0·72 | 2·09 ± 0·77 | 2·17 ± 0·72 |

[a] Mean of duplicate analysis ± SD from three rats.
[b] Values in $\mu g\,ml^{-1}$.
[c] Value for one sample.
ND = Not detectable.

thiocyanate in the GIT progressively decreased from the stomach to the distal portions of the GIT, resulting in the excretion of only trace amounts in the faeces. Increase in urinary and plasma thiocyanate occurred when sprouted sorghum was fed to the rats but was more marked in the urine compared with plasma.

## DISCUSSION

Although the sprouted sorghum fed to each rat contained a greater amount of bound cyanide (67%) than free cyanide (33%) all parts of the GIT showed a higher proportion of free cyanide compared with the amount of bound cyanide present. This indicates that a substantial amount of the dhurrin is hydrolysed to release free cyanide in the GIT. However, the presence of some amount of bound cyanide in all parts of the GIT contents, even 9 h after the administration of the sprouted sorghum, indicates a slow rate of dhurrin hydrolysis in the gut of the rat.

The absence of dhurrin in the faeces may mean that any unhydrolysed dhurrin was completely absorbed in the GIT. Absorption of free cyanide resulting from hydrolysed dhurrin was also efficient resulting in the excretion of only small amounts of free cyanide in the faeces. The absence of dhurrin in the wall of the GIT probably shows that either dhurrin is not absorbed from the lumen of the GIT or the amount absorbed was too small to be within the detection limit of the analytical method employed. The latter is more likely, since dhurrin was found in the urine and could only do so through absorption into the blood stream. Failure to find dhurrin in the plasma (Table 3) also reflects a quite diluted level in that fluid. In the kidney, the low level of dhurrin is efficiently absorbed by the kidney and concentrated in the urine. However, there is also the possibility of temporary binding of dhurrin to plasma proteins in a form not measurable by the analytical methods used. Other researchers (Barret *et al.*, 1977; Umoh *et al.*, 1986) have also observed that another cyanogenic glucoside, linamarin, was absent in the blood but present in the urine when rats were dosed with linamarin. They also found no linamarin in the faeces.

The estimation of the total amount of cyanide excreted as bound cyanide is not possible in this study as very little urine was excreted throughout the experimental period. However, this study has shown that there is slow hydrolysis of dhurrin in the GIT of the rat and that some of the unhydrolysed dhurrin is excreted in the urine.

The minimal lethal dose of cyanide taken orally is 0·25–3·5 mg per kg of body weight (Montgomery, 1969), but the occurrence of acute toxicity depends on the speed with which the cyanide gets to the tissues.

Furthermore, if the cyanide is administered in the bound form (as cyanogenic glucoside), the rate and degree of its hydrolysis to free cyanide is an important factor in the occurrence of acute toxicity. It is important to recall that each rat, weighing about 80 g, consumed, in this study, 2·74 mg cyanide within a 3 to 5 h period. The non-occurrence of acute toxicity in any of the rats must, therefore, be due partly to the slow rate of consumption of the sprouted sorghum and partly to the slow and incomplete hydrolysis of the bound cyanide present.

Thiocyanate was found in the gastric contents of control rats and this must be as a result of normal gastric secretion of this radical (Logothetopoulos & Myant, 1956; Okoh & Pitt, 1982). The increase in the amount of thiocyanate in the rat stomach contents with increase in time following consumption of sprouted sorghum must reflect increased secretion of this radical by the gastric wall of the stomach in response to an increase in the body thiocyanate pool. The increased levels of thiocyanate in the plasma and urine are evidence of an increased thiocyanate pool (Boxer & Rickards, 1952; Okoh & Pitt, 1982; Okoh, 1983).

Okoh & Pitt (1982) have long since reported the existence of a gastrointestinal circulation of thiocyanate following injection of potassium cyanide to rats. We suggest that the increase in gastric secretion of thiocyanate occurs whenever there is an increase in body thiocyanate pool, and that this probably represents a means of removing this radical from tissues where it might be more toxic while allowing time for its elimination via the urine.

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