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Transient Proteinuria and Aminoaciduria in Rodents Following Uranium Intoxication

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Urinary uranium analysis for occupationally exposed workers remains unsatisfactory for quantitative burden calculations. Notwithstanding its deficiencies, urine bioassay remains the method of choice in most uranium facilities (AECB Canada 1981; NRC/RG 8.22. 1978). Additional biological information examining the relationships between exposure levels, tissue deposition and urinary excretion burdens is of particular importance in comparative evaluation of both urine and alternative potential bioassay techniques. The available literature has been extensively reviewed (NRC 1978, Durbin and Wrenn 1975).

Intravenously administered uranyl salts in rabbits (Hursh and Spoor 1973), dogs and rodents (Morrow et al. 1982) give induction of proteinuria, catalasuria, and disturbance of nitrogen excretion at concentrations as low as 10 $\mu g\ Kg^{-1}$ body weight (Yuile 1973). Morrow et al. have concluded that intravenous doses above a threshold of 10 $\mu g\ Kg^{-1}$ are effective in producing renal injury, and furthermore 0.3 $\mu g\ g^{-1}$ fresh kidney tissue uranium is the threshold concentration for renal injury in dogs. Direct extrapolation of this data to Reference Man (ICRP 23 1975) would indicate transient injury at the 700 μg intake dose level. This level is below the present permissible limits (ICRP 30 1978).

Human data from intravenously administered uranium doses of between 70–100 $\mu g\ Kg^{-1}$ produced transient renal injury in the Rochester and Boston studies (Hursh and Spoor 1973), whereas data from below this level (6–70 $\mu g\ Kg^{-1}$) did not. Boback's data (Boback 1975) from accidentally exposed uranium workers with soluble intakes estimated to be 100–200 $\mu g\ Kg^{-1}$ (corresponding to an initial urine excretion of 7–14 mg day^{-1}) appeared not to exhibit renal injury as measured by protein, sugar, pH, SG or microscopic examination.

Alternative human bioassay procedures for uranium are being investigated. These include examination of hair deposition (Bentley and Wyatt 1980; Bentley et al. 1983) and β-microglobulin excretion (Thun et al. 1981). Aminoaciduria in uranium hexafluoride and uranium mill employees has been examined (Clarkson and Kench 1956). Although not conclusive, the results suggest that

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chronic human urinary uranium concentrations of 30-50 μg L^{-1} produce marked changes in the amino acid excretion profile although no consistent patterns emerged.

As part of a program to examine alternative bioassay techniques for occupationally exposed uranium workers and to assist in interpretation of amino acid data obtained from human "incident" exposures (peak uranium concentration in urine >80 μg L^{-1}), we have examined the occurrence of transient aminoaciduria following uranium intoxication in female rats.

MATERIALS AND METHODS

Four experiments were performed to examine transient aminoaciduria; two experiments using natural uranium nitrate and the others uranium nitrate prepared from uranium foil of isotopic composition ^{234}U: 0.72%, ^{235}U: 92.83%, ^{238}U: 6.42%. The ^{235}U enriched material was used in the 13-50 μg Kg^{-1} dose range so that rapid direct uranium analysis by the delayed neutron method could be carried out with adequate detection limits. Each experiment was performed on five groups of female albino Wistar rats (six animals per group) aged 11-13 weeks. Small groups of animals were acclimatised in metabolic units several days before experimentation. The rats were cube fed ("Barastoc", Melbourne, Australia) with *ad lib* access to food and water.

The animals received a single parenteral dose of 50-500 μg Kg^{-1} (uranium natural) or 13-50 μ Kg^{-1} body weight (^{235}U-enriched). The injection solutions were prepared in 0.2 M nitric acid and citrate buffered to pH 4.2 immediately before use to minimise coloid formation. Urine and faeces were collected daily, the equipment being thoroughly cleaned after each collection. The animals were regularly weighed and fluid intake determined. Only on days one to five at the 250 and 500 μg Kg^{-1} doses was significant loss of weight 1.2-1.5% day^{-1}) and elevation of fluid intake noted.

Urine and fecal uranium excretion was measured directly by delayed neutron analysis, after triple containment in sealed polyethylene vials (Amiel 1962). Urinary creatinine was determined using phosphate buffered picrate (Bartels 1972). Total protein was measured in 100 μl samples following the methods of (Lowry et al. 1951) as adapted by Clark to the Eppendorf microlitre system (Clark and Jakoby 1970).

Total non-protein nitrogen analysis was by the microkjeldahl method following protein precipitation with 10% sulphosalicylic acid. Urine and plasma amino acid concentrations were determined using physiological amino acid analysis (Beckman 120CL) with a lithium buffering system (Dewolfe et al. 1967).

Experiments examining organ deposition from 4 hours to 21 days post-injection were carried out. Groups of animals (3 per dose/time) identically treated to the metabolic experiment groups were sacrified by pentobarbital narcosis and tissue was immediately

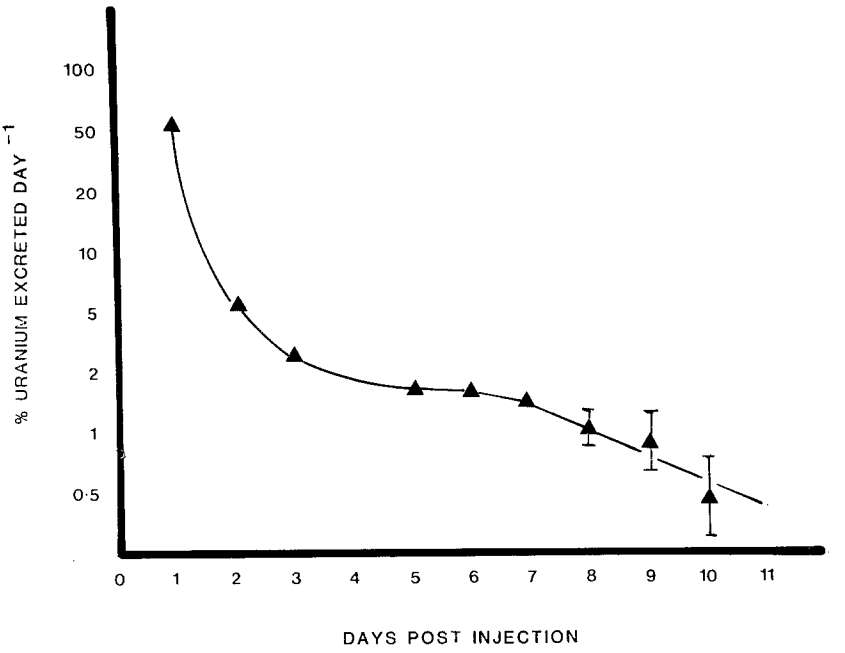


Figure 1. Daily uranium excretion following parenteral administration of uranium salts (13-100 µg Kg^{-1}).

removed for analysis. Initially uranium determinations were carried out on bone and most soft tissues for all of the low dose ^{235}-uranium enriched animals, and the 250 and 500 µg Kg^{-1} U^{6+} (natural) administered rats. This analysis was later reduced to bone (femur), kidney, and liver as negligible accumulation remained 48 hours post exposure in other soft tissues.

Specimens of kidney tissue were prepared for histopathology, using hematoxolin-eosin or periodic acid - Schiff 5% phosphate buffered formalin fixed staining of 7 µm paraffin embedded serial sections (Lillie and Fulmer 1977). Uranium distribution was determined by neutron induced autoradiography (Wilson and Bentley 1981).

RESULTS AND DISCUSSION

Twelve hours after parenteral injection of U^{6+} less than 3% remained at the injection site indicating a rapid and complete disperson of the citrate buffered uranium solution. The injection doses were from 13-500 µg Kg^{-1} body weight. No significant differences could be detected in the tissue deposition or excretion of the uranium natural or 235 enriched material at the overlap injection dose of 50 µg Kg^{-1} body weight. The change-over between uranium natural and enriched ^{235}U at the 50 µg Kg^{-1} dose was to permit direct tissue uranium determination by delayed neutron analysis.

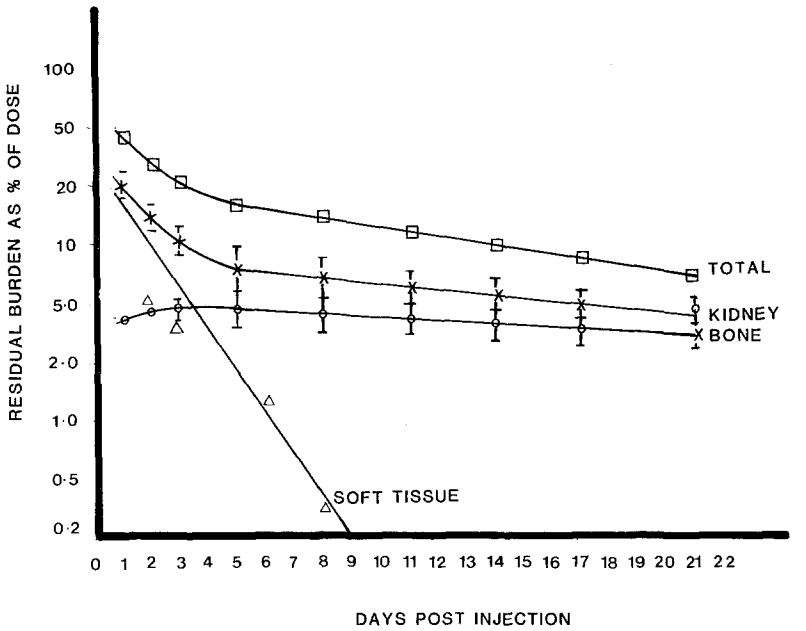


Figure 2. Residual burdens as a percentage of initial dose in carcase, kidney, bone and soft tissues.

The rats were held in metabolic units for 11 days post exposure at which time the transient urinary biochemical changes had reverted to normal values. Additional groups of animals identically treated were sacrified between 4 hours and 21 days post exposure for uranium tissue burden measurements.

The percentage of initial dose recovered from urinary excretion of uranium is summarised in Figure 1. The results are expressed in terms of the mean of groups of six animals. For the dose range examined all groups have comparable excretion rates. The 250 and 500 $\mu g\ Kg^{-1}$ data using uranium natural injections have much larger errors with the analytical technique used. Excretion of parenterally administered uranyl salts in faeces is a minor pathway. Only 1-2% of the injection dose was detected in the highest dose groups. The fecal uranium was probably derived from biliary excretion. These results are similar to those reported for some rodent and human data (Yuile 1973) but are not in agreement with the canine results at higher dose levels (Morrow et al. 1982).

The skeletal uranium values are derived by extrapolation from femur data assuming skeleton 11.5% of total body weight. Kidney and liver uranium measurements wre carried out throughout the experiment. Analysis of uranium from other soft tissues (spleen, muscle, heart, lung, brain) was discontinued after 72 hours as no

sample contained more than 0.2 percent of the absorbed dose after 24 hours. For the 250 and 500 μg Kg^{-1} doses the percentage residual burden in kidney and bone remained higher throughout the experiment.

The kidney data show increasing uranium accumulation for the first 12 hours ; (4 hours 25.8 ± 1.9%; 8 hours 27.3 ± 1.6%; 12 hours 29.4 ± 2.3%; 24 hours 21.3 ± 2.7% initial dose) followed by a steady decline with the 24-504 hour data (Fig. 2). Table 1 summarises the residual tissue burden half-lives for the 13-100 μg Kg^{-1} injection dose groups.

Table 1. Residual Tissue Burden Half-Times Following Parenteral Injection of 50 μg Kg^{-1} Uranyl Nitrate

TISSUE	TIME PERIOD	T 1/2 DAYS
Total	>4 days	14
Liver	1-3 days	1.5
Kidney	1-21 days	2.3 (Component 1) 13 (Component 2)
Bone	>4 days	21
Soft Tissue (Blood)	1-8 days	1.1
Urine	<5 days >5 days	0.5 2.4

Renal injury has frequently been reported following uranium intoxication (Magee and Foreman 1958; Griswold and McIntosh 1973). The dose-response characteristics are well established for a number of species (e.g. Durbin and Wrenn 1975). Our results show marked renal morphological alterations for Wistar strain rats at the 50 μg Kg^{-1} initial dose (10 μg g^{-1} kidney; 24 hour post exposure) becoming increasingly prominent with higher doses. At the 500 μg Kg^{-1} initial dose, necrosis of the convoluted tubules involved most *tubuli renales recti*. The necrotised tubules contained much hyaline material, and cell debris. The tubular epithelial cells were without nuclei and denuded. Glomerular lesions with randomly oriented fibre deposits were present at the higher dose levels. Homogenous eosinophilic, periodic acid-Schiff positive deposits were present in the mesangial cells.

For the 13-100 μg Kg^{-1} groups initiation of tubular regeneration was apparent 5-6 days post exposure. After 12 days regeneration appeared complete with the original tubular epithelial cells replaced by flattened basophilic epithelium. The renal uranium concentrations were approximately 2-5 μg g^{-1} fresh kidney five days post exposure. Neutron-induced autoradiography of thin (7 μ) sections of renal cortex were used to calculate approximate molar

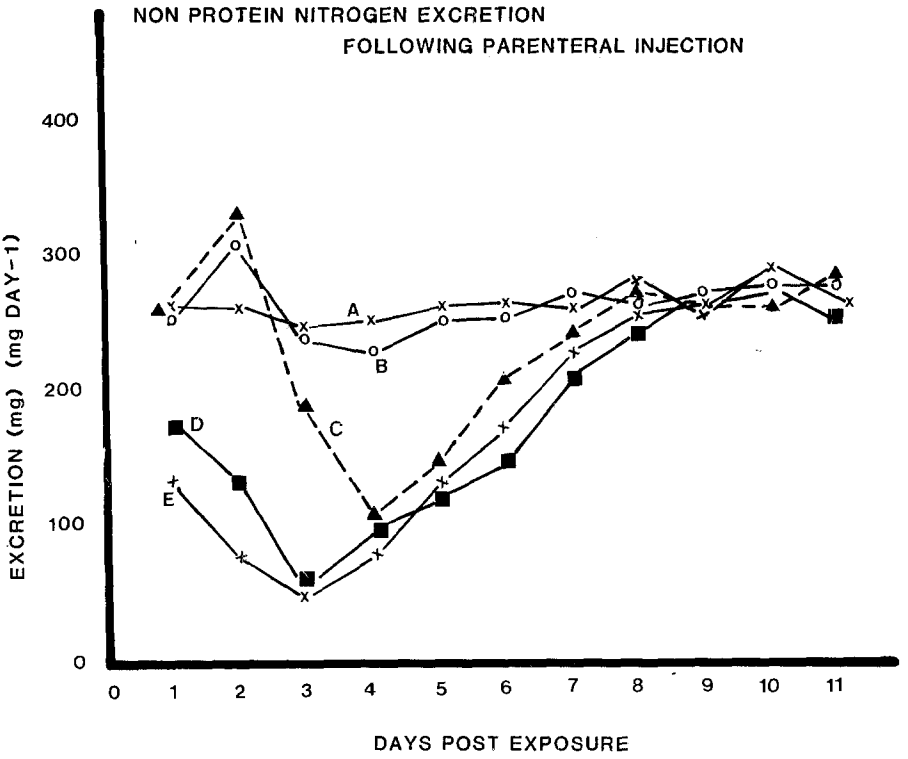


Figure 3. Non protein nitrogen excretion following parenteral administration of uranium salts. A: control; B: 13-50 $\mu g\ Kg^{-1}$; C: 100 $\mu g\ Kg^{-1}$; D: 250 $\mu g\ Kg^{-1}$ and E: 500 $\mu g\ Kg^{-1}$ initial dose.

concentrations of uranium adjacent to the epithelial cells of the proximal convoluted tubules. Peak concentrations of about 6-8 M uranium occurred in the 12-hour post exposure samples in the 500 $\mu g\ Kg^{-1}$ dosed animals.

Transient disturbance of urinary protein, nitrogen base and amino acid excretion occurs shortly after parenteral injection as a result of impairment of tubular resorption and excretion. Both the rate and duration of response is dose-dependent with a maximum perturbation 4-5 days post exposure. For the dose range examined (13-500 $\mu g\ Kg^{-1}$) the urine composition appears to revert to normal limits 8-9 days post exposure.

Non-protein nitrogen retention is increased following uranium exposures greater than 50 $\mu g\ Kg^{-1}$ dose levels (Fig. 3). The reduction in ammonia excretion is probably the cause of urinary acidosis. Urinary creatinine excretion appears only marginally depressed at the uranium dose levels examined, while serum creatinine values remain unaffected.

Rodents have a normal spontaneous age and sex linked progressive elevation in protein excretion. Protein excretion is higher in

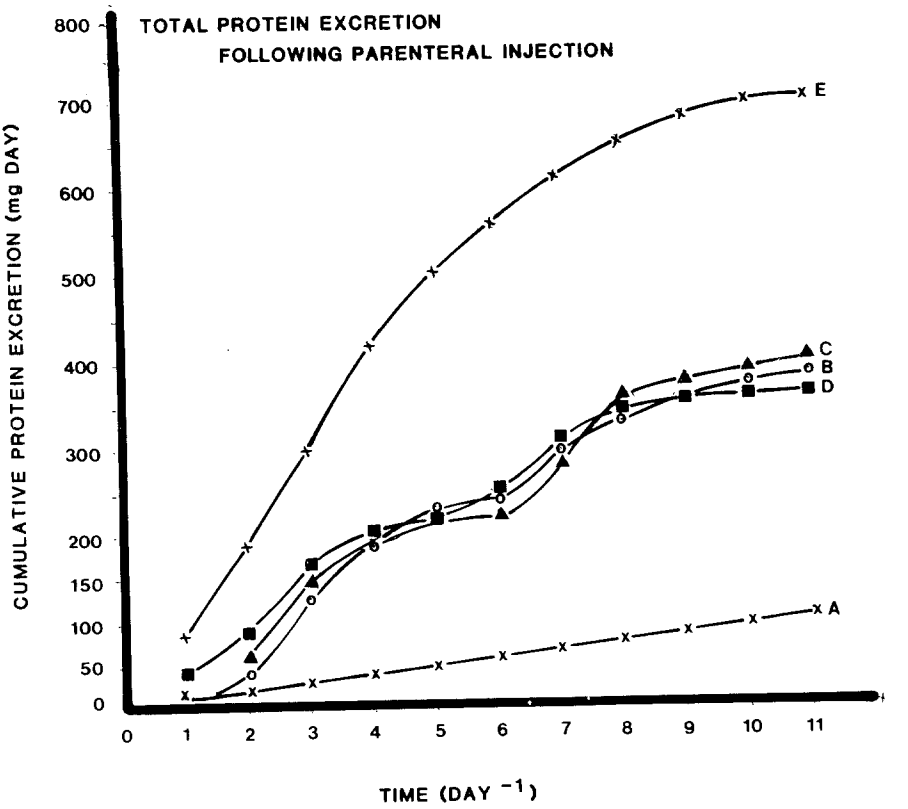
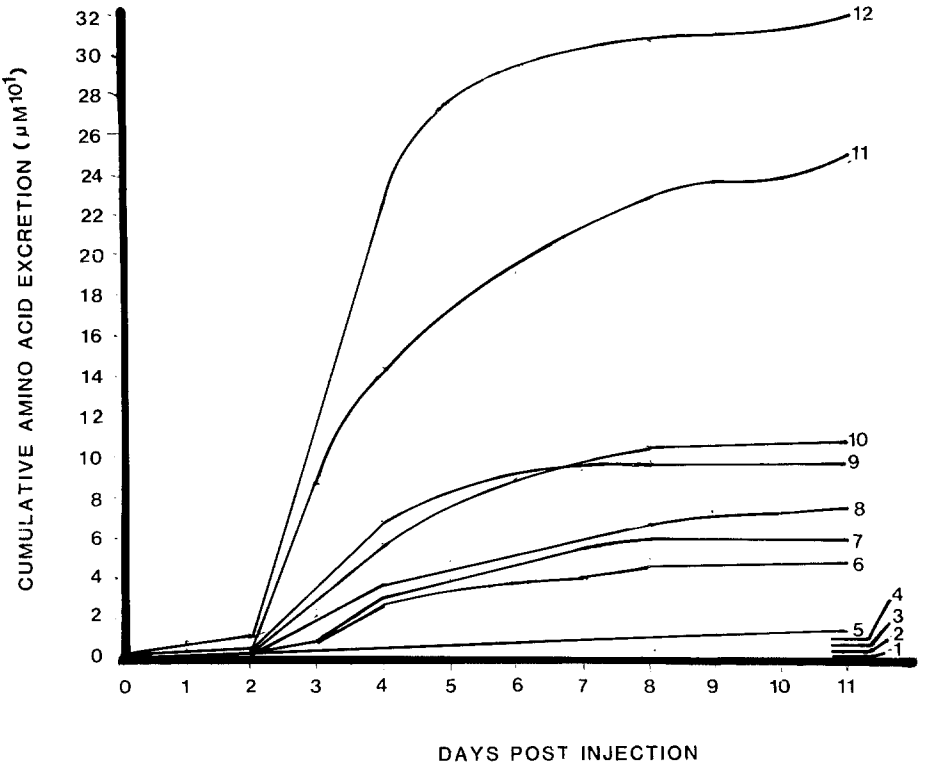


Figure 4. Cumulative protein excretion following parenteral injection of uranium salts. A: control; B, C, D: 13-50, 100 and 250 µg Kg^{-1}, respectively and E: 500 µg Kg^{-1} initial dose.

males and animals older than 5-6 months (Alt et al. 1980). Following uranium intoxication a major part of the new protein excreted in the urine appears to result from failure of convoluted tubular epithelium to resorb serum proteins from the glomerular filtrate. The excretion of serum proteins may arise by diffusion through capillaries of denuded tubules rather than increased glomerular permeability. Other proteins may have arisen from damaged tubular cells either by leakage from or sloughing of the cells into the tubular lamina.

The pattern of protein excretion appears complex. Preliminary investigation by immunoelectrophoresis to define the origin and identity of the components shows distinct differences both in the concentrations and species excreted between days 1-4 and 5-9 for all doses examined. The second rise in total protein (days 5-9) is accompanied by reduced albumin excretion but a proliferation of minor low molecular weight proteins. Similarly, for gross protein excretion, Figure 4 indicates that two sequential dose-independent mechanisms are present for doses between 13 and 100 µg Kg^{-1}. At doses of 250 and 500 µg Kg^{-1} total breakdown of protein filtration is apparent. These results are consistent with the histologically visible time sequence of cellular damage and repair.



No.	Amino acid	No.	Amino acid
1	Isoleucine	7	Threonine
2	Leucine	8	Alanine
3	Tyrosine	9	Lysine
4	Phenylalanine	10	Citrulline
5	Histidine	11	Glycine
6	Serine	12	Glutamic acid

Figure 5. Cumulative amino acid excretion in rodents ($\mu M \times 10^1$) at the 50 $\mu g\ Kg^{-1}$ dose

Uranium intoxication at the 38-500 μg dose absorbed levels is accompanied by transient aminoaciduria. The elevation of individual amino acid releases suggests a complex mechanism not solely related to changes to be anticipated by passive alteration in renal tubule permeability. Although not readily apparent from the cumulative amino acid plots (Fig. 5), results from measurements of daily excretion of urinary amino acids at each dose level have a distinct valley between days 4-6. The ratio between the excretion concentrations of individual amino acids also differs between days 1-4 and 4-8 (Table 2).

Our results for amino acid excretion suggest a similar response to that of the individual and total protein excretion data and may be similarly interpreted as a consequence of the sequential tissue damage and repair mechanism.

Table 2. Percentage of Total Amino Acids

AMINO ACIDS	NORMAL		URINE 50 μg Kg^{-1} U	
	Plasma	Urine	Days 2-4	Days 4-8
Serine	0.6	5.6	3.8	5.2
Threonine	33.0	5.6	4.5	9.4
Glutamic acid	5.8	17.1	37.0	13.5
Glycine	8.0	30.8	19.3	24.1
Alanine	15.1	12.6	5.2	7.8
Citrulline	0.6	2.1	8.4	14.8
Valine	4.2	1.9	2.8	1.9
Isoleucine	3.0	1.1	1.1	1.0
Leucine	5.3	0.9	1.7	1.2
Tyrosine	1.3	1.3	1.3	1.3
Phenylalanine	2.0	3.1	1.2	1.8
3-Methylhistidine	0.6	2.0	0.4	0.9
Histidine	10.5	2.3	2.1	4.6
Lysine	8.3	9.2	9.4	9.8
Arginine	0.8	2.9	0.9	1.9
α-Amino-butyric acid	0.6	1.3	0.7	0.6

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