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Pathological changes in growing dogs fed on a balanced cassava (*Manihot esculenta* Crantz) diet

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Studies were carried out to determine the effects of the toxic principle linamarin, a cyanogenic glucoside, in a diet containing cassava (*Manihot esculenta* Crantz) in the form of gari fed to growing dogs for 14 weeks. There were three groups of dogs, each comprising six animals. One group was fed on a control diet with rice as the carbohydrate source, the second group was fed on cassava (gari) as the carbohydrate source and which was expected to release 10·8 mg HCN/kg cooked food, the third group was fed on the control diet to which enough NaCN was added at feeding time to release 10·8 mg HCN/kg cooked food in order to monitor the effects of the HCN released from gari. All diets contained 130 g crude protein (N × 6·25)/kg and were supplemented with vitamins and minerals. Each animal was given approximately 100 g diet/kg body weight for the duration of the experiment. The biochemical variables investigated were plasma electrolytes, serum proteins, plasma-free amino acids, plasma enzymes and urine protein, and the histology of some metabolically active tissues, namely liver, kidney, myocardium, testis and adrenal gland, was studied. The gari diet caused an elevated plasma thiocyanate concentration ($P < 0.01$), elevated 24 h urinary thiocyanate excretion and elevated urinary protein excretion ($P < 0.01$), lowered serum albumin ($P < 0.05$), a plasma-free amino acid profile which resembled that found in kwashiorkor, lowered plasma K and Ca ($P < 0.05$). The rice + cyanide diet caused an elevated plasma thiocyanate ($P < 0.01$) and a 24 h urinary thiocyanate excretion that was significantly higher ($P < 0.01$) than that of the dogs fed on gari, but caused a urinary protein excretion that was significantly lower than that of the dogs fed on gari ($P < 0.01$), lowered serum albumin ($P < 0.05$), a plasma-free amino acid profile that indicated that the amino acids were not being utilized to the same extent as in the control (rice) group but were accumulating. Neither diet had an effect on plasma γ-glutamyltransferase (*EC* 2.3.2.2), alanine aminotransferase (*EC* 2.6.1.2) or isocitrate dehydrogenase (*EC* 1.1.1.42) activities, plasma Na, Mg, and P concentrations. The gari diet caused generalized congestion and haemorrhage, periportal vacuolation of the liver, swelling, vacuolation and rupture of the epithelial cells of the proximal convoluted tubules of the kidney, myocardial degeneration and adrenal gland degeneration. In the testes there were occasional abnormal germ cells in the seminiferous tubules, and occasional seminiferous tubules denuded to basement membrane of germ cells but with remnants of Sertoli cells. Spermatogenesis, however, appeared to be normal since the percentage of round tubules in stage 8 of the spermatogenic cycle was not significantly different from that of the control dogs. The rice + cyanide diet caused nephrosis and a significantly reduced relative frequency of testicular tubules in stage 8 of the spermatogenic cycle ($P < 0.01$). There was also marked testicular germ cell sloughing and degeneration. There was adrenal gland hyperplasia and hypertrophy. It was concluded that the observed changes which occurred when the gari diet was consumed were not entirely due to cyanide.

Linamarin: Pathological changes: Cassava (*Manihot esculenta* Crantz): Dog

Linamarin is the cyanogenic glucoside of linseed, (*Linum usitatissimum* L.), cassava (*Manihot esculenta* Crantz) and red clover (*Trifolium protense* L.). The root of the cassava plant is an important staple food for millions of people in tropical countries. Linamarin is hydrolysed in the intestinal tract of both man and animals by the microbial flora (Winkler, 1958), and the HCN released is rapidly absorbed from the gastrointestinal tract (Conn, 1973). The absorbed HCN at sublethal levels is converted to thiocyanate.

Barrett *et al.* (1977) reported that a significant amount of linamarin administered orally to rats is absorbed intact and excreted in urine.

Toxic reactions in the human receiving orally-administered sodium or potassium thiocyanate as a hypertensive drug include hepatic necrosis, nephrosis, palpitations and muscle cramp (Nickerson, 1970).

The purpose of the present study was to determine the effects of a balanced diet containing cassava on plasma electrolytes, serum proteins, plasma-free amino acids, selected plasma enzymes and urine protein, and on the histology of the liver, kidney, myocardium, testes and adrenal gland after an experimental period of 14 weeks, when compared with a control diet based on rice.

MATERIALS AND METHODS

Management of the animals and diets fed were as described previously (Kamalu, 1991).

There were three groups of dogs, each consisting of six animals. One group was fed on a control diet with rice as the carbohydrate source; the second group was fed on cassava (gari) as the carbohydrate source, which was expected to release 10·8 mg HCN/kg cooked food; the third group was fed on the control diet to which enough NaCN was added at feeding time to release 10·8 mg HCN/kg cooked food in order to monitor the effects of HCN released from gari. All diets contained 130 g crude protein (N × 6·25)/kg and were supplemented with vitamins and minerals. The details of the composition and proximate analysis of the diets are shown in Tables 1 and 2. Rice was used as the carbohydrate source in the pre-experimental and control diets because rice has no cyanide. The cooked diets were analysed for their HCN content (Association of Official Analytical Chemists, 1970).

For the duration of the experiment the dogs were fed 100 g diet/kg body weight once daily. Clean water was available *ad lib.*

Biochemistry

Blood samples were obtained from each dog before the experimental diets were given and then at weeks 1, 3 and 14 of the experiment. Urine collections (24 h) were obtained at weeks 1, 3, 5, 7, and 14 of the experiment. Plasma and urinary thiocyanate concentrations were determined (Bowler, 1944; Chilaka, 1983). Plasma and urine of a control animal were each used as the standard in order to eliminate the endogenous thiocyanate. Urinary protein concentrations were determined by the dip-and-read technique using Rapignost® Total Screen test strips.

The Biuret method was used to estimate the total serum protein, albumin and globulin for each animal (Gornall *et al.* 1949). The activities of L-γ-glutamyltransferase (*EC* 2.3.2.2), alanine aminotransferase (*EC* 2.6.1.2; ALT) and isocitrate dehydrogenase ($NADP^+$) (*EC* 1.1.1.42; ICDH) for each animal were determined on heparinized plasma using kits supplied by Boehringer Mannheim, Germany.

At week 14, using heparinized plasma samples from each animal, concentrations of Ca^{2+} and Mg^{2+} were determined using a Pye Unicam SP 90 A Series atomic absorption spectrophotometer, while concentrations of inorganic P were determined colorimetrically using a Fisher Electrophotometer II, and concentrations of Na^+ and K^+ were determined using a Gallenkamp Flame Analyser. Concentrations of plasma-free amino acids were determined from pooled samples taken at week 14 using a Beckman 121 M amino acid analyser.

Table 1. *Composition of diets (g/kg dry matter)*

Ingredients	Diets		
	Control	Cassava (gari)	Rice + cyanide
Rice	779	—	779
Cassava (gari) (*Manihot esculenta* Crantz)	—	700	—
Lean pork	130	210	130
Bone meal	75	75	75
Salt	15	15	15
Vitamin and mineral supplement* (ml/kg food)	1	1	1
Cyanide (mg HCN/kg food)	—	10·8	—
Cyanide (ml/kg food)† (NaCN 8·8 mg/ml)	—	—	2

* Supplied mg/ml vitamin and mineral syrup (ViDalyn-M®; Abbott Laboratories, S.A., Spain); retinol 0·18, thiamin hydrochloride 0·3, riboflavin-5-sodium phosphate 0·32, ascorbic acid 10, nicotinamide 2, pyridoxine hydrochloride 0·2, pantothenol 1·0, ferrous gluconate 5·2, potassium iodide 19·6, calcium lactate 30, calcium hypophosphite 16·5, manganese gluconate 0·9, zinc glucoheptonate 0·85, magnesium gluconate 11·1, choline bitartrate 2·08, myoinositol 1·0, cyanocobalamin 0·6 μg, cholecalciferol 2·0 μg.

† Approximately equivalent to the level found in the cooked cassava (gari) diet and added at the time of feeding.

Table 2. *Proximate analysis of wet diet fed to dogs (g/kg)*

Diet*...	Control	Cassava (gari) (*Manihot esculenta* Crantz)	Rice + cyanide
Water	709·6	727·3	709·6
Crude protein (N × 6·25)	129	131	129
Diethyl ether extract	46	53	46
N-free extract	—	—	—
Crude fibre	1·5	5·5	1·5
Ash	—	—	—
Energy (kJ/g)	25·27	26·65	25·27

* For details of composition, see Table 1.

Histopathology

At the end of the experimental period the animals were killed under deep anaesthesia. Pieces of liver, kidney, myocardium, testis and adrenal gland were fixed in buffered formalin (100 ml/l), routinely processed and embedded in paraffin wax. Sections were cut at 5 μm thickness, stained with haematoxylin and eosin and examined by light microscopy.

Statistics

The means with their standard errors were calculated where necessary. Analysis of variance (ANOVA) was used to test for differences between treatments. Duncan's (1955) multiple range test was used to separate the means which differed significantly.

RESULTS

Analysis of the diets for their HCN content showed that the linamarin in the gari diet released 10·8 mg HCN/kg compared with none in the rice diet.

Table 3 shows the effects of foods containing cyanide on plasma thiocyanate and urinary thiocyanate concentrations. The plasma thiocyanate and urinary thiocyanate in the control group remained undetectable throughout the experimental period because the plasma and the urine of a control animal were used as thiocyanate-free standards in order to cancel out levels of endogenous thiocyanate.

Results of the analysis of urine protein concentrations are shown in Table 3. The effects of diet and time on urine protein levels were highly significant ($P < 0{\cdot}01$). Both the gari diet and the rice+cyanide diet caused protein to appear in urine, while the dogs fed on the control diet showed no urinary protein throughout the experimental period.

The effects of the dietary treatments on total serum proteins, albumin and globulins are summarized in Table 4. There were no significant differences in total serum protein, albumin and globulins between the groups at the beginning of the experiment. The concentrations of the total serum protein rose significantly with time in the three groups up to the end of the experiment and there were no significant differences between the groups. By week 3, serum albumin concentration of the control group was not significantly different from the basal level, while albumin had increased over the basal levels in the gari group ($P < 0{\cdot}05$) and in the rice+cyanide group ($P < 0{\cdot}01$). By week 14, albumin had significantly increased in the controls over the concentration at week 3 ($P < 0{\cdot}05$), but had decreased in the gari- and rice+cyanide-fed dogs and had become significantly lower than that of the control dogs ($P < 0{\cdot}05$). By week 3 the concentration of globulins in the control dogs was not significantly different from the basal level, while the concentrations of globulins were at this time significantly lower for both the gari- and rice+cyanide-fed dogs than those of the control dogs ($P < 0{\cdot}01$). By week 14 the concentration of globulins in the control dogs had decreased from the level in week 3 ($P < 0{\cdot}01$) while the concentrations of globulins in the gari- and rice+cyanide-fed dogs were not different from those at week 3 or the control values.

The effects of the diets on the concentrations of plasma-free amino acids and on plasma-free amino acid ratios in the dogs after 14 weeks of feeding are shown in Table 5. The concentrations of plasma-free amino acids were higher in the rice+cyanide group of dogs than in the control group and the gari group indicating that amino acids were accumulating and not being utilized. In the gari group of dogs, to which 60% more animal protein was fed compared with the control diet, the concentrations of many of the plasma-free amino acids were lower than the concentrations of the same amino acids in the control group of dogs. The value of the plasma free glycine, serine, glutamic acid: leucine, isoleucine and valine ratio in the gari group of dogs was increased to a level about four times (400%) greater than that of the control group of dogs, while the value of the corresponding ratio in the rice+cyanide group of dogs was the same as in the control group of dogs. The plasma-free essential amino acids: non-essential amino acids in the gari group of dogs decreased to a value that was approximately half that of the control group of dogs, while the corresponding value in the rice+cyanide group was greater than that in the control group of dogs.

The effects of the diets on selected plasma enzymes are summarized in Table 6. γ-Glutamyltransferase activity was not influenced by either the dietary treatments or time and there was also no significant time × diet interaction. Dietary treatments had no effect on plasma ALT concentrations ($P > 0{\cdot}05$). However, the time effect was highly significant ($P < 0{\cdot}01$). By week 14 the ALT concentrations for all groups were almost double those at

Table 3. *Effect of dietary treatment on thiocyanate concentrations (μmol/ml) in the plasma and 24 h urine excretions and on urine protein (μmol/ml) concentrations of growing male dogs*

(Values are means with their standard errors for six dogs)

Period of treatment (weeks)	Dietary group‡	Plasma SCN (μmol/ml)		Urine SCN (μmol/ml)		Urine protein (μmol/ml)	
		Mean	SE	Mean	SE	Mean	SE
1	Control (rice)	0	0	0	0	0	0
	Cassava (gari)§	0·0908	0·009	24·04	3·98	150·0**	7·1
	Rice + cyanide	0·080**	0·007	132·0**††	0·45	0	0
3	Control (rice)	0	0	0	0		
	Cassava (gari)	0·0682**	0	33·49**	5·11		
	Rice + cyanide	0·0850**	0·005	84·40**	19·12		
5	Control (rice)			0	0	0	0
	Cassava (gari)			18·20**	4·60	766·7**	14·76
	Rice + cyanide			93·47**	13·87	200·0**††	6·32
7	Control (rice)			0	0		
	Cassava (gari)			27·5**	9·29		
	Rice + cyanide			50·05**††	13·25		
14	Control (rice)	0	0	0	0	0	0
	Cassava (gari)	0·0486**	0·016	3·07	1·18	766·7**	14·76
	Rice + cyanide	0·119**††	0·019	59·10**††	15·04	483·3**††	17·01

Mean values were significantly different from those of the control (rice) group for the same week: ** $P < 0.01$.
Mean values were significantly different from those of the cassava (gari) group for the same week: †† $P < 0.01$.
‡ For details of diets and dietary regimen, see Tables 1 and 2 and p. 922.
§ *Manihot esculenta* Crantz.

Table 4. *Effect of dietary treatment on concentration of serum total protein, albumin and globulins (g/l) of growing male dogs*

(Values are means with their standard errors for six dogs)

Period of treatment (weeks)	Dietary group§	Total protein (g/l)		Albumin (g/l)		Globulins (g/l)	
		Mean	SE	Mean	SE	Mean	SE
0	Control (rice)	46·66	0·90	24·66	1·99	22·16	1·81
	Cassava (gari)‖	49·16	2·27	24·33	1·92	24·83	1·53
	Rice + cyanide	48·16	3·07	24·16	3·42	24·33	3·10
3	Control (rice)	67·00**	2·02	34·16	2·33	32·83	1·97
	Cassava (gari)	61·83***	2·96	38·33*	4·35	23·50††	5·05
	Rice + cyanide	65·83**	0·59	42·33**†††	1·12	23·83††	1·06
14	Control (rice)	62·83**	0·89	43·66†	0·80	20·83††	2·20
	Cassava (gari)	62·33**	2·40	36·66‡	2·16	25·83††	3·08
	Rice + cyanide	64·50**	2·35	37·83‡	2·20	26·66††	4·32

Mean values were significantly different from those at week 0: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.02$.
Mean values were significantly different from those of control (rice) group at week 3: † $P < 0.05$, †† $P < 0.01$, ††† $P < 0.02$.
Mean values were significantly different from those for control (rice) group at week 14: ‡ $P < 0.05$.
§ For details for diets and dietary regimen, see Tables 1 and 2 and p. 922.
‖ *Manihot esculenta* Crantz.

Table 5. *The effects of experimental diets on the concentrations of plasma-free amino acids ($\mu mol/ml$) and on plasma-free amino acid ratios in growing male dogs after 14 weeks on experimental diets*

(Measurements were made on pooled plasma samples from six dogs in each group)

		Dietary group*			
		Cassava (gari) (*Manihot esculenta* Crantz)		Rice + cyanide	
Plasma-free amino acid	Control (rice)		Percentage change from control		Percentage change from control
Aspartic acid	20·44	32·23	57·68↑	43·80	114·28↑
Threonine	167·84	34·34	79·5↓	172·04	2·5↑
Serine	734·6	2364·60	221·88↑	729·69	0·6↓
Glutamic acid	173·62	293·81	69·22↑	319·99	84·30↑
Proline	232·84	196·35	15·67↓	213·73	8·20↓
Glycine	336·35	1552·33	361·52↑	378·16	12·43↑
Alanine	1214·59	203·93	83·20↓	745·90	38·58↓
Valine	171·81	128·18	25·39↓	183·77	6·96↑
Methionine	17·16	22·72	32·40↑	37·40	117·94↑
Isoleucine	55·56	55·11	0·80↓	67·30	21·13↑
Leucine	101·98	97·40	4·49↓	135·58	32·94↑
Tyrosine	45·20	30·68	21·12↓	66·94	48·09↑
Phenylalanine	33·66	46·55	38·29↑	70·76	110·21↑
Lysine	333·65	214·16	35·81↓	305·20	8·52↓
Histidine	95·30	120·03	25·94↑	106·06	11·29↑
3-Methylhistidine	1631·18	1950·58	19·58↑	2336·47	43·23↑
Arginine	114·35	91·79	19·72↓	137·08	19·87↑
Glycine + serine + glutamic acid : leucine + isoleucine + valine	3·78	15·00	396·82↑	3·69	1·43↓
Essential amino acids : non-essential amino acids	0·288	0·123	57·46↓	0·355	23·12↑

↑, Percentage increase, ↓, Percentage decrease.
* For details of diets and dietary regimen, see Tables 1 and 2 and p. 922.

week 0. ICDH concentrations were not affected by the dietary treatments ($P > 0{\cdot}05$). However, the time effect was highly significant ($P < 0{\cdot}01$). In all the groups ICDH concentrations were significantly lower at week 14 than at week 0.

Table 7 shows the effects of the diets on plasma electrolytes after a 14-week feeding period. The dietary treatments had a highly significant effect on plasma Ca levels. The gari group had a significantly lower Ca level ($P < 0{\cdot}05$) and also the highest P concentration; however, the group differences were not statistically significant ($P > 0{\cdot}05$). Differences in Mg and Na concentrations were not statistically significant ($P > 0{\cdot}05$). Plasma K concentration was highest in the control group and lowest in the gari group. The differences between these two groups were statistically significant ($P < 0{\cdot}05$).

The histopathological changes seen in the kidney consisted of congestion, vacuolation, swelling and rupture of the epithelial cells of the proximal tubules in the dogs fed on the gari diet (Plate 1). There were also desquamation of cells of the tubular epithelium in some areas, and casts. In the dogs fed on the rice + cyanide diet the epithelial cells of the proximal tubules showed no swelling or vacuolation. Casts were present in the lumina of the tubules and there was occasional desquamation.

Table 6. *Effect of dietary treatment on the activities of some plasma enzymes (U/l) of growing male dogs after 14 weeks on experimental diets*

(Values are means with their standard errors for six dogs)

		Activities (U/l)			
		Week 0		Week 14	
Plasma enzyme	Dietary group*	Mean	SE	Mean	SE
γ-Glutamyltransferase (*EC* 2.3.2.2)	Control (rice)	7·67	1·80	5·75	0·944
	Cassava (gari)†	5·33	1·82	7·33	0·989
	Rice + cyanide	7·83	1·38	8·50	1·088
Alanine aminotransferase (*EC* 2.6.1.2)	Control (rice)	9·19	1·38	16·98	2·92
	Cassava (gari)	9·17	1·38	15·41	2·36
	Rice + cyanide	7·96	0·81	16·99	3·34
Isocitric dehydrogenase ($NADP^+$) (*EC* 1.1.1.42)	Control (rice)	8·8	0·90	6·4	0·92
	Cassava (gari)	8·96	1·06	4·8	0·83
	Rice + cyanide	8·26	0·96	6·7	1·26

* For details of diets and dietary regimen, see Tables 1 and 2 and p. 922.
† *Manihot esculenta* Crantz.

Table 7. *Effect of dietary treatment on plasma concentrations of some electrolytes (mmol/l) of growing male dogs after 14 weeks on experimental diets*

(Values are means with their standard errors for six dogs)

		Concentration (mmol/l)	
Electrolyte	Dietary group†	Mean	SE
Ca	Control (rice)	3·58	0·053
	Cassava (gari)‡	3·08*	0·083
	Rice + cyanide	3·58	0·053
P	Control (rice)	1·00	0·090
	Cassava (gari)	1·39	0·122
	Rice + cyanide	1·11	0·351
Mg	Control (rice)	1·19	0·025
	Cassava (gari)	1·16	0·032
	Rice + cyanide	1·19	0·498
K	Control (rice)	7·19	0·508
	Cassava (gari)	5·94*	0·302
	Rice + cyanide	6·19	0·232
Na	Control (rice)	59·07	0·595
	Cassava (gari)	57·59	0·932
	Rice + cyanide	57·13	0·753

Mean value was significantly different from that for control (rice) group: * $P < 0·05$.
† For details of diets and dietary regimen, see Tables 1 and 2 and p. 922.
‡ *Manihot esculenta* Crantz.

The only histopathological changes in the liver were found in the dogs fed on the gari diet. There was congestion and periportal vacuolation (Plate 2).

Histopathological changes were seen in the myocardium of the dogs fed on the gari diet. Haemorrhage was a significant feature and was seen between the muscle fibres (Plate 3). The muscle fibres showed swelling, some nuclei showed pyknosis with vacuoles in the surrounding cytoplasm. Striations were still present in the muscle fibres.

Table 8. *The effect of dietary treatments on the relative frequency of stage 8 of spermatogenesis (%) in dogs after 14 weeks on experimental diets*

(Values are means with their standard errors for six dogs)

	Frequency of stage 8 (%)	
Dietary group†	Mean	SE
Control (rice)	14·4	0·94
Cassava (gari) (*Manihot esculenta* Crantz)	13·1	0·66
Rice + cyanide	1·6*	1·07

Mean value was significantly different from that for control (rice) group: * $P < 0{\cdot}01$.
† For details of diets and dietary regimen, see Tables 1 and 2 and p. 922.

Table 9. *Effect of dietary treatments on the adrenal gland of growing male dogs after 14 weeks on experimental diets*

(Values are the mean values with their standard error for six dogs)

	Thickness of cortex (mm)		Thickness of zona glomerulosa (mm)	
Dietary group†	Mean	SE	Mean	SE
Control (rice)	0·3386	0·042	0·0603	0·003
Cassava (gari) (*Manihot esculenta* Crantz)	0·3346	0·047	0·0622	0·02
Rice + cyanide	0·5388	0·078	0·1023*	0·015

Mean value was significantly different from that for control (rice) group: * $P < 0{\cdot}05$.
† For details of diets and dietary regimen, see Tables 1 and 2 and p. 922.

Stage 8 of the spermatogenic cycle in the dog testes is characterized by the lining of the seminiferous tubular lumen by elongated spermatids (Foote *et al.* 1972). The relative frequency of stage 8 tubules in the three groups of dogs determined by classifying 1200 cross-sections of round seminiferous tubules for each group (six dogs × one testis × two locations/testis × 100 tubular cross sections/location) are shown in Table 8. There was no significant difference between the frequency of stage 8 tubules in the control dogs and the gari-fed dogs ($P > 0{\cdot}05$), while the frequency of stage 8 tubules in the rice + cyanide-fed dogs was significantly lower than that of the control and gari-fed dogs ($P < 0{\cdot}01$). There was the occasional occurrence of two types of abnormal cells, one with a single large pyknotic nucleus and eosinophilic cytoplasm (Plate 4), and the other multinucleated and with golden yellow cytoplasm (Plate 5) in the seminiferous tubules of the gari-fed dogs; there was also the occasional occurrence of seminiferous tubules devoid of normal germ cells but with numerous Sertoli cells and a few abnormal cells. The abnormal cells were of more frequent occurrence in the seminiferous tubules of the rice + cyanide group of dogs, where extensive sloughing of germ cells was observed.

The widths of the adrenal cortex and the widths of the zona glomerulosa of the three groups of dogs are shown in Table 9. There were no significant differences between the widths of the adrenal cortex in the three groups of dogs ($P > 0{\cdot}05$) but the increase in the width of the rice + cyanide-fed dogs compared with the other two groups did approach significance. The widths of the zona glomerulosa of the dogs fed on the control diet and on the gari diet were not significantly different ($P > 0.05$), but the width of the zona glomerulosa in the dogs fed on the rice+cyanide diet was significantly greater than those in the other two groups ($P < 0.05$), indicating that hyperplasia and hypertrophy had occurred in the aldosterone-secreting zona glomerulosa in the rice+cyanide-fed dogs.

In the zona glomerulosa pathological changes occurred in the dogs fed on the gari diet. Significant features were haemorrhage, areas of cloudy swelling and areas of fibrosis (Plate 6). In the dogs fed on the gari diet there was significant haemorrhage in the zona fasciculata and zona reticularis.

The adrenal medulla of the control and the rice+cyanide-fed dogs appeared normal. The adrenal medulla of the gari-fed dogs showed significant pathological changes. There was haemorrhage in the corticomedullary region, congestion throughout the medulla, small groups of chromaffin cells with pyknotic nuclei and a significant increase in the amount of fibrous connective tissue when compared with the adrenal medulla of the control dogs.

DISCUSSION

The present study shows that the ingestion of either inorganic cyanide or cyanogenic glucosides elevated plasma and urinary thiocyanate concentrations. The decrease in the amount of urinary thiocyanate in 24 h excretions by both the dogs fed on the gari diet and the dogs fed on the rice+cyanide diet with increase in size was probably due to the fact that thiocyanate was being more efficiently reabsorbed by the kidney resulting in the accumulation of very high levels of thiocyanate in the plasma. Pullman & McClure (1954) have shown that renal excretion of thiocyanate in the normal human male is slow due to efficient reabsorption of the thiocyanate and that thiocyanate accumulates when repeated doses are given. The urinary thiocyanate concentrations in 24 h excretions of dogs fed on the gari diet was generally lower than that of dogs fed on the rice+cyanide diet. This was probably due to the fact that all the linamarin ingested is not usually hydrolysed to HCN. Barrett *et al.* (1977) observed intact linamarin in the urine of rats. This finding correlates with the finding that the plasma thiocyanate concentration of the dogs fed on the gari diet became progressively lower while the plasma thiocyanate of the dogs fed on the rice+cyanide diet rose progressively after week 1 of the experiment.

Cyanide and thiocyanate are selective poisons. In sublethal concentrations they cause cell death by interfering with oxidative production of energy from glucose, fatty acids and amino acids. Cyanide inhibits the enzyme cytochrome oxidase (*EC* 1.9.3.1), thereby preventing the use of O_2, while thiocyanate inhibits the enzyme fumarase hydratase (*EC* 4.2.1.2) in the Krebs citric acid cycle (Massey & Alberty, 1954). Intact linamarin, in sublethal concentrations, inhibits the activity of Na^+–K^+ ATPase (*EC* 3.6.1.37) (Hill, 1977) and decreases the intracellular K^+ concentration (Philbrick *et al.* 1977) in cardiac tissue.

In the present study the dogs fed on the gari diet showed hypokalaemia which resulted in swelling, vacuolation and rupture of cells in different tissues, some being more severely affected than others. These findings are related to those of both Philbrick *et al.* (1977) and Hill (1977).

A gari diet containing 120 g protein/kg, on a dry matter basis, was fed to rats for 4 weeks and vacuolation in the liver was observed (Ononogbu & Emole, 1978). The distribution of the vacuolation was not stated. The finding in the present study was vacuolation in the periportal region of the liver. Evidence that the periportal vacuolation in the liver seen in the present study was not caused by cyanide derived from cassava or the metabolite thiocyanate was demonstrated by the fact that, although the level of plasma thiocyanate was higher in the group of dogs fed on the rice+cyanide diet than in the group of dogs fed on gari, there was no vacuolation in the liver of the dogs fed on rice+cyanide. It can be concluded, therefore, that some factor in cassava other than cyanide, probably intact linamarin, caused the observed periportal vacuolation in the liver.

The periportal vacuolation of the liver that occurred in the study did not cause abnormal hepatic function. This was demonstrated by the fact that the activities of the liver enzymes in the dogs fed on the gari diet did not differ significantly from that of the control dogs.

The very early and significantly greater concentration of urinary protein excreted by the dogs fed on gari compared with those fed on the rice+cyanide diet suggested that some factor in cassava in addition to cyanide was responsible for a very early and a more severe expression of nephrosis. The factor was probably intact linamarin.

The protein loss in urine progressively increased in concentration until the serum albumin levels fell in both groups of dogs, with serum albumin concentration always higher in the rice+cyanide-fed dogs, although not significantly so. However, by week 14 the concentrations of the serum albumin in the dogs fed on gari and the dogs fed on rice+cyanide became significantly lower than concentrations in the control dogs ($P < 0{\cdot}05$).

The hypocalcaemia in the dogs fed on gari was related to protein loss in the urine. Approximately 41% of the plasma Ca is bound to protein and the loss of large amounts of serum albumin results in a lowering of serum Ca level.

The plasma-free amino acid concentrations for the gari-fed dogs and the rice+cyanide-fed dogs in the present study were compared with the findings of Grimble & Whitehead (1970*b*) in their study of kwashiorkor in children, those of Grimble & Whitehead (1970*a*) in their study of experimentally protein-deficient pigs up to week 14 of the experiment, and those of Grimble & Whitehead (1969) in their study of amino acid ratios in experimentally protein-deficient pigs. Because pooled samples were used in the present study the statistical significance of the changes seen cannot be discussed but only the direction of changes and how the patterns of change resemble those seen in kwashiorkor.

In the present study, 130 g crude protein/kg, on a dry matter basis, was fed. Because gari is very low in protein, 60% more lean pork was added to the gari diet than was added to the control (rice) and rice+cyanide diets. Amino acids in the diets were, therefore, not a limiting factor as in kwashiorkor or the low-protein diets fed to the pigs. In addition, lean pork is a good source of essential amino acids.

Plasma concentrations of threonine, alanine, valine, tyrosine, lysine and arginine decreased in the gari-fed dogs when compared with those of the control and rice+cyanide-fed groups of dogs. This is similar to Grimble & Whitehead's (1970*a,b*) findings in kwashiorkor and in protein-deficient pigs at week 14, except that alanine was elevated in the pigs.

In the gari-fed dogs there appeared to be a slight tendency for plasma concentrations of isoleucine and leucine to decrease when compared with those of the control and rice+cyanide-fed dogs. This trend is in agreement with the findings in kwashiorkor and in the experimentally protein-deficient pigs.

The concentrations of methionine, phenylalanine and histidine were elevated in the gari-fed and in the rice+cyanide-fed dogs. The findings of Grimble & Whitehead (1970*b*) showed that in the early stages of kwashiorkor the concentrations of phenylalanine and histidine increased and then decreased in severe kwashiorkor, while methionine decreased steadily with time. The concentration of methionine in the experimentally protein-deficient pigs also decreased with time (Grimble & Whitehead, 1970*a*).

Thus, with the exception of methionine, the pattern showed by the essential amino acids in the present study is generally in agreement with the findings of Grimble & Whitehead

(1970*a*,*b*) for essential amino acids in kwashiorkor in children and in experimentally protein-deficient pigs.

Delange *et al.* (1982) found severe protein–energy malnutrition, with an amino acid pattern of normal serum levels of methionine but low levels of valine, leucine and isoleucine, together with low serum levels of albumin, in the population in Kivu, Zaire, where due to a food shortage there was a marked increase in the consumption of cassava. The amino acid pattern in Kivu is similar to the pattern in the present study.

The plasma concentration of glycine was most markedly elevated in the gari-fed dogs at week 14 when compared with that of the control dogs. This is in agreement with the findings of Grimble & Whitehead (1970*a*,*b*) in kwashiorkor in children and experimentally protein-deficient pigs. The plasma concentration of serine was also markedly elevated in the gari-fed dogs when compared with the control dogs. This is in agreement with the findings in experimentally protein-deficient pigs at week 14. The findings for kwashiorkor (Grimble & Whitehead 1970*b*) showed an elevation of serine just before the onset of severe kwashiorkor. The plasma concentration of aspartic acid was elevated in the gari-fed dogs and in severe kwashiorkor. In the rice+cyanide-fed dogs the plasma concentrations of glycine and serine were little affected when compared with the changes in the gari-fed dogs. Aspartic acid, however, was markedly elevated.

Grimble & Whitehead (1970*b*) found that at the beginning of protein inadequacy in children the plasma concentration of valine starts to fall first among the branched-chain amino acids and glycine starts to rise. This resembles the findings in the gari-fed dogs in the present study.

The glycine+serine+glutamic acid:leucine+isoleucine+valine value was about 400% higher in the gari-fed dogs than that in the control dogs, while the ratio was relatively unaffected in the rice+cyanide-fed dogs. This elevation is similar to findings in Uganda where the corresponding ratios in severe kwashiorkor are as much as 400–800% higher than those found in healthy children (Grimble & Whitehead, 1970*b*), and also to the findings in protein-deficient pigs (Grimble & Whitehead, 1970*a*). The value for essential:non-essential amino acids in the gari-fed dogs showed a decrease of 57% when compared with the controls, whereas the corresponding ratio in the rice+cyanide dogs showed an increase of 23%.

The distorted amino acid pattern and amino acid ratios in the gari-fed dogs after 14 weeks on diet had some features of both early protein deficiency and severe kwashiorkor. The distortion in the amino acid ratios was due to the great increase in the plasma concentration of glycine and serine rather than a major decrease in the concentration of the branched-chain amino acids. This is a feature of early protein deficiency. Usually the concentration of alanine increases at the onset of protein inadequacy and the concentration of lysine remains normal. The concentrations of both fall as kwashiorkor becomes severe. In the present study alanine was being used up in gluconeogensis (Kamalu, 1991). The fall in the concentration of lysine cannot be explained. The amino acid pattern and amino acid ratios in the gari-fed dogs became distorted in the presence of normal liver function. This agrees with the findings of Grimble *et al.* (1969) in malnourished rats.

Since plasma albumin levels, plasma-free amino acid patterns and amino acid ratios in the present study, in which adequate protein was fed with gari as the carbohydrate source, resemble those found in protein–energy malnutrition, there appears to be a need to re-evaluate the carbohydrate source when such observations are being made in areas where cassava is the dietary staple, because it is apparent that there is protein loss from the body as a result of some factor in cassava which would exacerbate dietary protein deficiency. Based on the results of the present study and in Kivu, Zaire, cassava would constitute the primary factor in the degree of protein–energy malnutrition.

The high concentrations of plasma-free amino acids in the presence of low serum albumin in the dogs fed on rice + cyanide was probably partially due to the relatively low insulin levels (Kamalu, 1991), partially due to the relatively low levels of total serum 3,5,3′-triiodothyronine (T_3) (Kamalu & Agharanya, 1991), and partially to the possible increase in glucocorticoids related to the enlargement of the adrenal cortex observed in this group of dogs. Protein synthesis is usually depressed when insulin levels or T_3 levels are abnormally low, or when glucocorticoid levels are abnormally high (Millward *et al.* 1976).

It has been reported that linamarin causes a decrease of intracellular K in cardiac tissue, reduces cardiac N^+–K^+ ATPase activity (Hill, 1977), and causes a significant decrease in cardiac cytochrome oxidase activity (Philbrick *et al.* 1977). These previous findings explain the findings of swelling, vacuolation, and degeneration of myocardial fibres in the hypokalaemic gari-fed dogs.

Most tissues of the gari-fed dogs showed congestion and haemorrhage. Several factors appear to be related to this phenomenon. Degeneration and swelling of the myocardial fibres appeared to have affected the efficiency of the pumping action of the heart so that generalized chronic passive congestion was present, which agrees with the findings of Philbrick *et al.* (1977) who showed that systolic blood pressure was reduced in a group of rats receiving a sublethal dose of linamarin daily for 5 weeks. In addition, the apparent inhibition of the Na^+–K^+ ATPase could have caused swelling and rupture of the endothelial cells, making haemorrhage prominent.

The presence of replacement fibrosis in the adrenal cortex of the gari-fed dogs indicated that the factors causing cloudy swelling were severe.

The width of the zona glomerulosa was significantly increased in the rice + cyanide dogs when compared with the control dogs and the gari-fed dogs ($P < 0.05$). The hypertrophy and hyperplasia of cells in the zona glomerulosa of the adrenal glands of the dogs fed on the rice + cyanide diet indicate that this zone was induced by some stimulus to increase the amount of aldosterone secreted. The reason is not understood but it is possible that it is as a result of the hypothyroidism observed in the rice + cyanide-fed dogs which was caused by the cyanide ingested and the thiocyanate formed (Kamalu & Agharanya, 1991), since in hypothyroid animals blood volume is considerably reduced (Turner & Bagnara, 1971). It is possible that the renin–angiotensin–aldosterone system had become activated to increase the extracellular fluid volume and to cause a long-term increase in arterial blood pressure.

It has been reported that the adrenal glands respond to stressful stimuli by increased production of adrenocortical steroids, and produce the required increase by enlargement and hyperactivity of the cortex (Appleby & Sohrabi, 1978). In the present study, in the dogs fed on the rice + cyanide diet the increase in the width of the region of the cortex below the zona glomerulosa may indicate that the free cyanide at the level fed in the diet for the duration of the experiment and its metabolite thiocyanate constituted a stress on the dogs when compared with the effect of the cyanide and thiocyanate originating from linamarin in the gari diet.

The relative frequencies of stage 8 tubules in the control dogs and the dogs fed on gari in the present study are not significantly different from that of normal Beagle dogs (Foote *et al.* 1972). The occasional presence of abnormal cells in the seminiferous tubules and tubules denuded to the basement membrane in the dogs fed on gari and those fed on rice + cyanide, together with a significantly lower relative frequency of stage 8 tubules in the dogs fed on rice + cyanide, could indicate that cyanide and thiocyanate had an adverse effect on spermatogenesis. The precise manner in which spermatogenesis has been interfered with is not clear and further work will be required to study the details in order to identify at which stage of the cycle this interference is effected.

The present study showed that cassava may exert a cytotoxic effect on metabolically active tissues by two routes; (1) by causing electrolyte imbalance resulting in swelling, vacuolation and rupture of cells, and (2) by causing cell death through the selectively poisonous actions of its metabolites cyanide and thiocyanate. The present study also showed that cassava diets caused a condition allied to kwashiorkor largely because of protein loss as a result of nephrosis, and that the toxic effects on the kidney were not entirely due to the release of HCN.

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REFERENCES

Appleby, E. C. & Sohrabi, I. (1978). Pathology of the adrenal glands and paraganglia. *Veterinary Record* **102**, 76–78.

Association of Official Analytical Chemists (1970). *Official Methods of Analysis*, 11th ed., p. 438. Washington, DC: Association of Official Analytical Chemists.

Barrett, M. D. F., Hill, D. G., Alexander, J. C. & Zitnak, A. (1977). Fate of orally dosed linamarin in the rat. *Canadian Journal of Physiology and Pharmacology* **55**, 134–136.

Bowler, R. O. (1944). The determination of thiocyanate in blood serum. *Biochemical Journal* **38**, 385–386.

Chilaka, F. C. (1983). An investigation of the effects of high and prolonged cassava diets on some drug metabolizing enzymes of rat liver. MSc Thesis, University of Nigeria, Nsukka.

Conn, E. E. (1973). Cyanogenic glycosides. *Agricultural and Food Chemistry* **17**, 519–526.

Delange, F., Bourdoux, P., Colinet, E., Courtois, P., Hennart, P., Lagasse, R., Mafuta, P., Seghers, P., Thilly, C., Vanderpas, J., Yunga, Y. & Ermans, A. M. (1982). Nutritional factors involved in the goitrogenic action of cassava. In *Cassava Toxicity and Thyroid: Research and Public Health Issues. Proceedings of a Workshop*, Ottawa *International Development Research Centre Monograph* 207e, pp. 17–26 [F. Delange and R. Ahluwalia, editors]. Ottawa: International Development Research Centre.

Duncan, D. B. (1955). Multiple range and multiple F tests. *Biometrics* **11**, 1–42.

Foote, R. H., Swierstra, E. E. & Hunt, W. L. (1972). Spermatogenesis in the dog. *Anatomical Record* **173**, 341–352.

Gornall, A. G., Bardawill, C. J. & David, M. M. (1949). Determination of serum proteins by means of the biuret reaction. *Journal of Biological Chemistry* **177**, 751–766.

Grimble, R. F., Sawyer, M. B. & Whitehead, R. G. (1969). Time relationships between the elevation of serum amino acid ratio and changes in liver composition in malnourished rats. *British Journal of Nutrition* **23**, 879–888.

Grimble, R. F. & Whitehead, R. G. (1969). The relationship between an elevated serum amino acid ratio and the development of other biological abnormalities in the experimentally malnourished pig. *British Journal of Nutrition* **23**, 791–804.

Grimble, R. F. & Whitehead, R. G. (1970*a*). Changes in the concentration of specific amino acids in the serum of experimentally malnourished pigs. *British Journal of Nutrition* **24**, 557–564.

Grimble, R. F. & Whitehead, R. G. (1970*b*). Fasting serum-amino acid patterns in kwashiorkor and after administration of different levels of protein. *Lancet* **i**, 918–921.

Hill, D. C. (1977). Physiological and biochemical responses of rats given potassium cyanide or linamarin. *Cassava as an Animal Feed. Proceedings of a Workshop held at University of Guelph*, 1977. *International Development Research Centre Monograph* 095e, pp. 33–42 [B. Nestel and M. Graham, editors]. Ottawa, Canada: International Development Research Centre.

Kamalu, B. P. (1991). The effect of a nutritionally balanced cassava diet on endocrine function using the dog as a model. 1. Pancreas. *British Journal of Nutrition* **65**, 365–372.

Kamalu, B. P. & Agharanya, J. G. (1991). The effect of a nutritionally balanced cassava (*Manihot esculenta* Crantz) diet on endocrine function using the dog as a model. 2. Thyroid. *British Journal of Nutrition* **65**, 373–379.

Massey, V. & Alberty, R. A. (1954). Ionisation constants of fumarase. *Biochimica et Biophysica Acta* **13**, 354–359.

Millward, D. J., Garlick, F. J., Nnanyelugo, D. O. & Waterlow, J. C. (1976). The relative importance of muscle protein synthesis and breakdown in the regulation of muscle mass. *Biochemical Journal* **156**, 185–188.

Nickerson, M. (1970). Antihypertensive agents and the drug therapy of hypertension. In *The Pharmacological Basis of Therapeutics*, 4th ed., pp. 735–736 [L. S. Goodman and A. Gilman, editors]. New York: Macmillan.

Ononogbu, I. C. & Emole, I. (1978). The effect of gari on rat plasma cholesterol. *Atherosclerosis* **31**, 101–104.

Philbrick, D. J., Hill, D. C. & Alexander, J. C. (1977). Physiological and biochemical changes associated with linamarin administration to rats. *Toxicology and Pharmacology* **42**, 539–551.

Pullman, T. N. & McClure, W. W. (1954). The renal tubular reabsorption of thiocyanate in normal man. *Journal of Laboratory and Clinical Medicine* **43**, 815–823.

Turner, C. D. & Bagnara, J. T. (1971). *General Endocrinology*, 5th ed., p. 210. Philadelphia: W. B. Saunders Co.
Winkler, W. O. (1958). Report on methods for glucosidal HCN in lima beans. *Journal of the Association of Official Agricultural Chemists* **41**, 282–287.

Plate 1. Kidney of growing male dog fed on cassava (gari) (*Manihot esculenta* Crantz) diet showing cytoplasmic strands from ruptured epithelial cells stretching across the lumen of proximal tubules (haematoxylin and eosin). For details of diet, see Tables 1 and 2 and p. 922. In plates 1–5, scale bar = 1 μm.

Plate 2. Liver of dog growing male dog fed on cassava (gari) (*Manihot esculenta* Crantz) diet showing periportal vacuolation (haematoxylin and eosin). For details of diet, see Tables 1 and 2 and p. 922.

Plate 3. Myocardium of growing male dog fed on cassava (gari) (*Manihot esculenta* Crantz) diet showing haemorrhage (haematoxylin and eosin). For details of diet, see Tables 1 and 2 and p. 922.

Plate 4. Seminiferous tubule of growing male dog fed on cassava (gari) (*Manihot esculenta* Crantz) diet showing large abnormal cells each with one pyknotic nucleus (haematoxylin and eosin). For details of diet, see Tables 1 and 2 and p. 922.

Plate 5. Seminiferous tubule of growing male dog fed on cassava (gari) (*Manihot esculenta* Crantz) diet showing tubule with several layers of germ cells lost and large abnormal multinucleated cell in the lumen (haematoxylin and eosin). For details of diet, see Tables 1 and 2 and p. 922.

Plate 6. Adrenal gland of growing male dog fed on cassava (gari) (*Manihot esculenta* Crantz) diet showing degeneration and fibrosis of the adrenal cortex (haematoxylin and eosin). For details of diet, see Tables 1 and 2 and p. 922. Scale bar = 4 μm.

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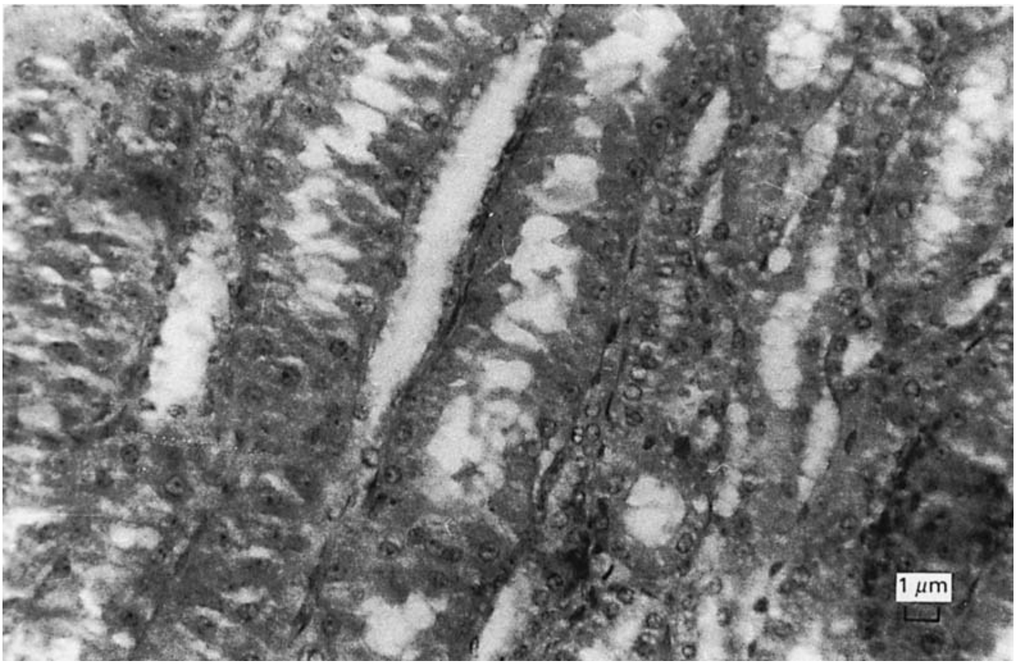
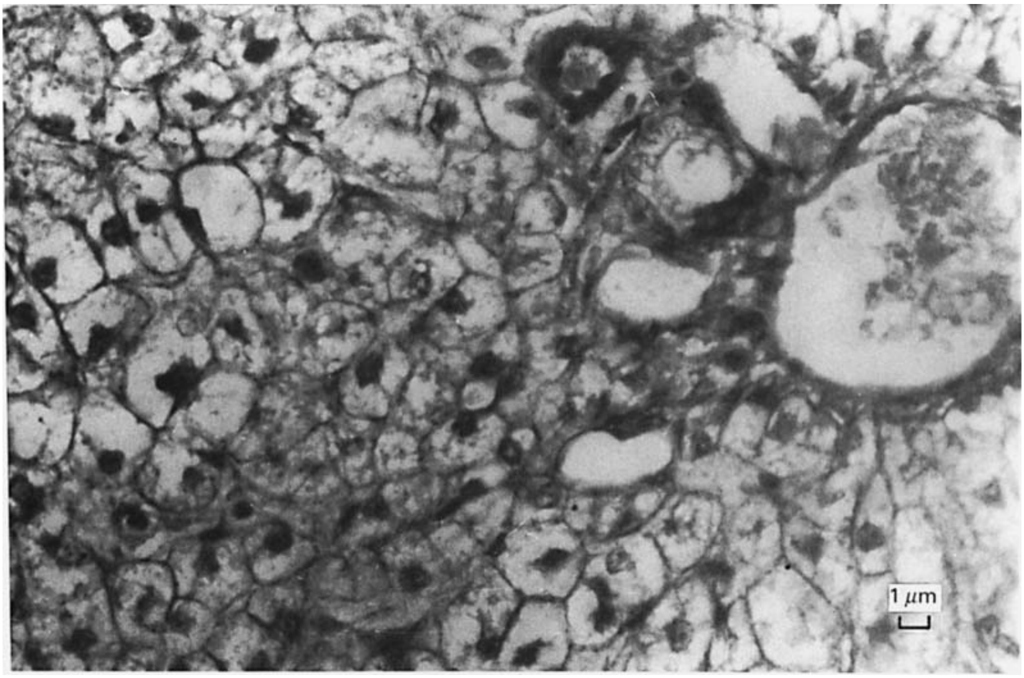


B. P. Kamalu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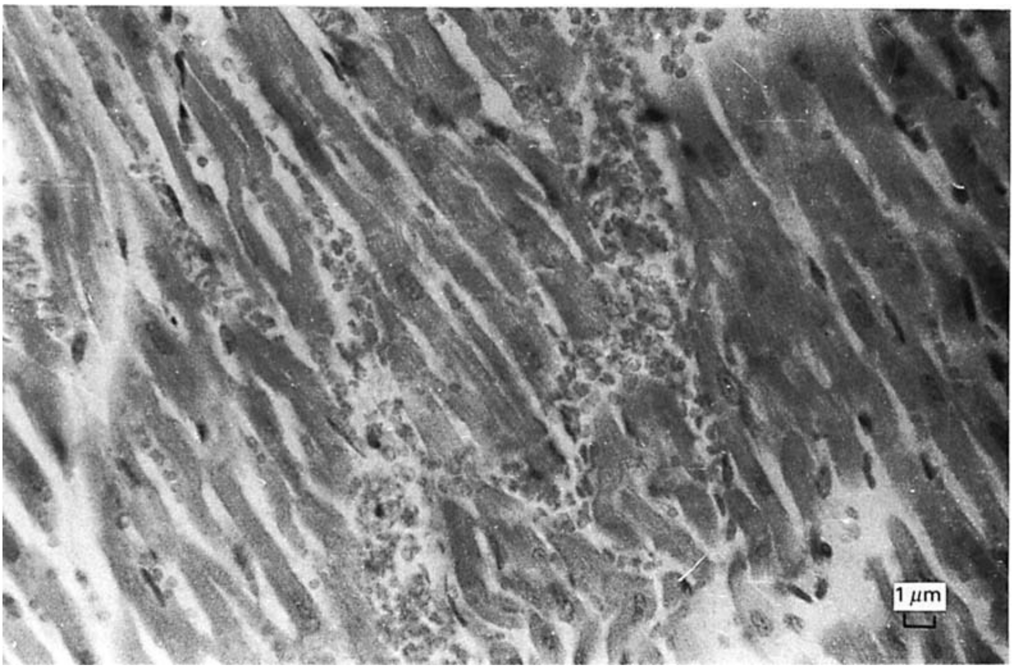
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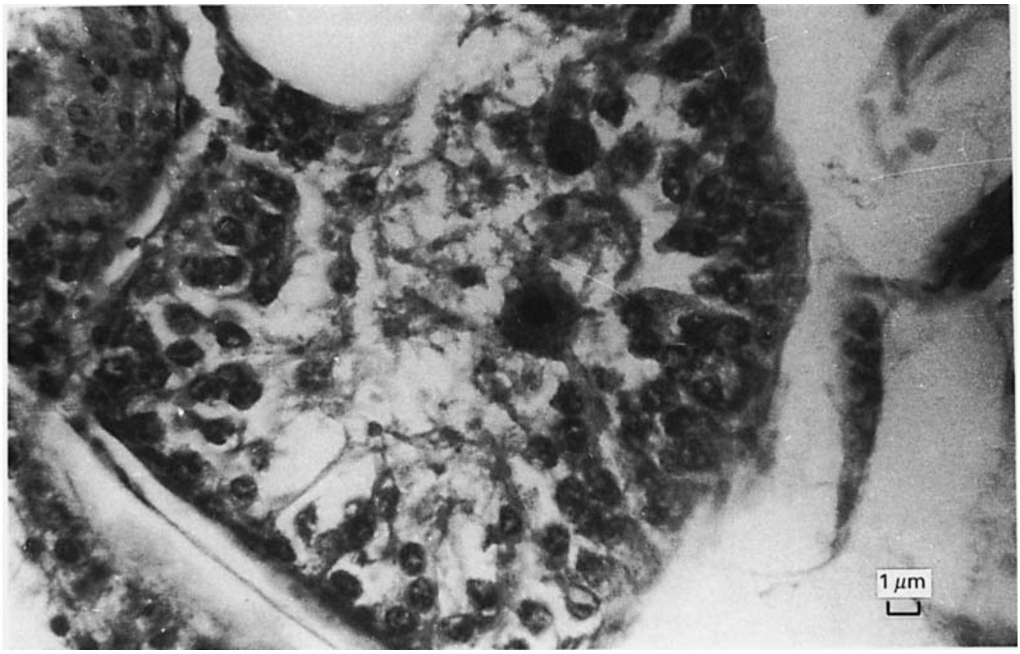
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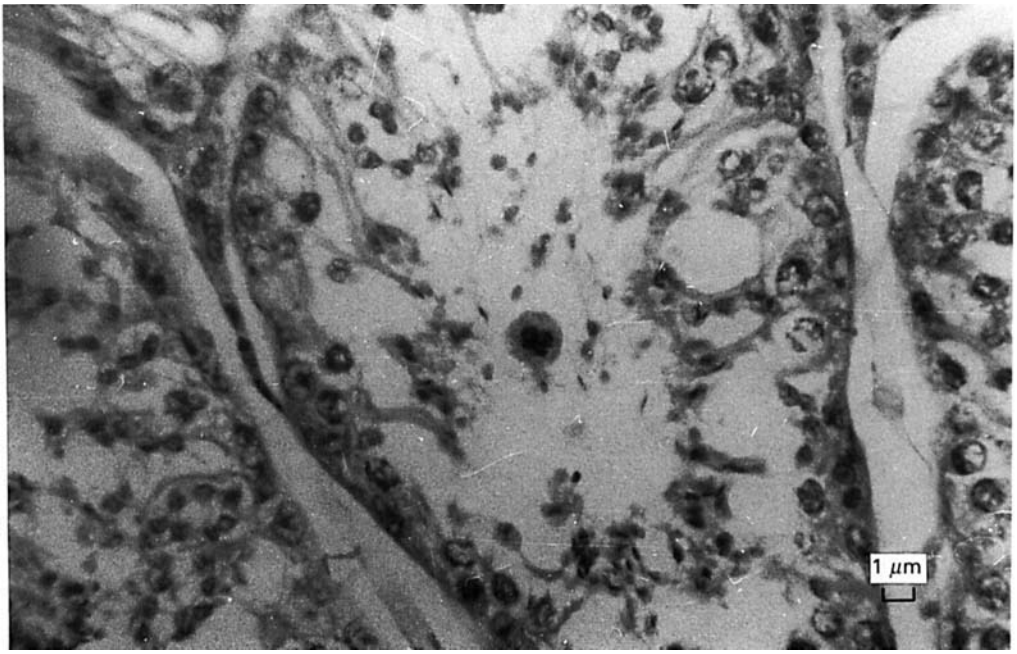
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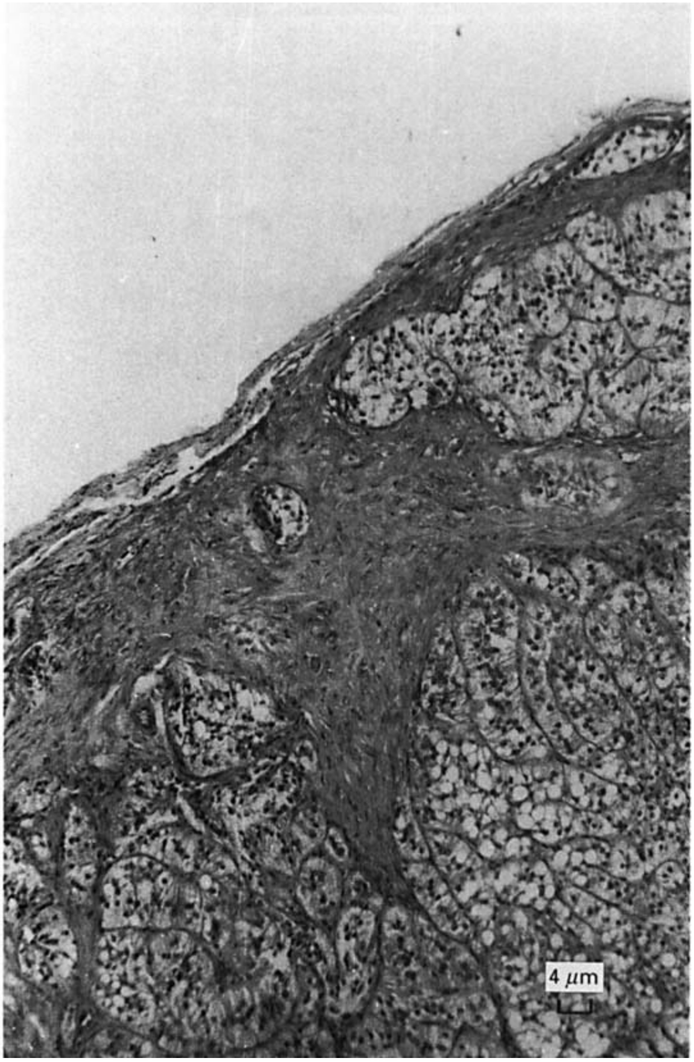
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