The effect of a nutritionally-balanced cassava (*Manihot esculenta Crantz*) diet on endocrine function using the dog as a model 1. Pancreas

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Growing dogs were divided into three groups and were fed on a control (rice) diet, a diet in which cassava (*Manihot esculenta Crantz*; gari) was used as the carbohydrate source, and the rice diet to which cyanide (equivalent to that present in gari) was added. Each group consumed its diet for 14 weeks, during which plasma thiocyanate concentration and plasma lipase (*EC* 3.1.1.3) activity were monitored. Plasma free amino acids were determined from pooled samples taken at the end of the experimental period, and the insulin status of the dogs was evaluated using the gluconeogenic index. The dogs were killed and the pancreas examined for histopathology. Dogs fed on both gari diet and the rice + cyanide diet generated significant amounts of thiocyanate when compared with the controls, with the rice + cyanide group having higher plasma thiocyanate than the gari group ($P < 0.01$). Plasma lipase activity rose significantly at the end of the experimental period in the dogs fed on gari ($P < 0.05$). Gluconeogenesis from protein was greatest in the dogs fed on gari, five times greater than that in the control dogs, while gluconeogenesis from protein in the dogs fed on rice + cyanide was approximately twice as high as that of the control dogs. Histopathological examination of the pancreas showed haemorrhage, necrosis, fibrosis and atrophy of the acinar tissue and fibrosis of the islets of Langerhans in the dogs fed on gari. The pancreas of the dogs fed on rice + cyanide showed similar lesions but haemorrhage was not prominent and fibrosis was more marked. The present study indicated that a hypoinsulinaemia developed which was more severe in the animals fed on gari than in the dogs fed on the rice + cyanide diet and that the condition was not related to the level of plasma thiocyanate or the histopathology observed.

Pancreatopathy: Cassava diet: Dog

The cassava plant (*Manihot esculenta Crantz*) is widely cultivated in tropical countries where its root tubers constitute a principal source of dietary carbohydrate. It is a staple food in southern Nigeria.

All varieties of cassava contain the cyanogenic glucoside, linamarin, 2-(β-D-glucopyranosyloxy)isobutyronitrile (Nartey, 1968). Hydrolysis of linamarin by the endogenous β-glucosidase enzyme linamarase (*EC* 3.2.1.21), produces D-glucose, acetone and hydrocyanic acid (Conn, 1969). Linamarin is hydrolysed in the intestinal tract of both man and animals by the microbial flora (Winkler, 1958). The cyanide released is rapidly absorbed from the gastrointestinal tract. In addition, the liver contains enzymes capable of hydrolysing absorbed linamarin to a metabolite which can dissociate to release cyanide ion (Maduagwu & Umoh, 1986).

Cassava-based diets have been implicated in the development of tropical pancreatitis and diabetes mellitus in man. The disease has been observed in tropical areas of Asia, Africa and South America. It is believed that low protein intake combined with cassava-based diets may be one of the causes of the disease (Sarles, 1973; Geevarghese, 1982; Abu-Bakare *et al.* 1986).

The purpose of the present study was to investigate the effect of the cyanide in a nutritionally-balanced gari diet on the pancreas using the growing dog as the model.

MATERIALS AND METHODS

Animals

Eighteen male dogs of approximately 6 weeks of age were acquired from village markets. They were washed in warm water with medicated soap containing monosulfiram (250 ml/l) (Tetmosol®; Chemical and Allied Products Ltd, Nigeria) then dipped in a solution of 1 g *O*,*O*-diethyl-*O*-(3-chloro-4-methyl-7-coumarinyl)-phosphorithioate (Asuntol® 50; Bayer, Germany)/l, as treatment for ectoparasites. They were given single oral doses of praziquantel (Droncit®; Bayer, Germany) for tapeworms, and pyrantel pamoate (Combantrin®; Pfizer Products Ltd, Nigeria) for hookworms and roundworms. They were given oxytetracycline hydrochloride (50 g/l; Adamycin®; Assia Chemical Laboratories, Israel) intramuscularly for 3 d as antibiotic treatment for occult disease. They were also given iron dextran (Ronidex®; Teva Ltd, Israel) and vitamin B_{12} (Gedeon Richter Ltd, Hungary) intramuscularly, as haematinics. The dogs were housed individually in metabolism cages. Treatments for ectoparasites and endoparasites were repeated towards the end of the pre-experimental period. All animals were fed on a nutritionally-balanced diet for growing dogs with rice as the carbohydrate source, in quantities based on body-weight, for 4 months before the start of the experiment (Gaines Dog Research Center, 1968). Clean water was available *ad lib*.

At the end of the pre-experimental period, the dogs were randomly divided into three groups comprising six individuals. The dogs were left in the metabolism cages into which they were initially placed and fed on the experimental diets for a period of 14 weeks.

Each dog was weighed on day 1 of the experimental period and every seventh day thereafter.

Diets

The diets used in the experiment were wet-type cooked food (Tables 1 and 2). Analysis of the diets for their HCN content showed that the gari contained 10·8 mg HCN/kg compared with none in the rice diet. In order to monitor the true effects of cyanide, an equivalent amount of free HCN was added to rice as a test diet.

The control diet, in which rice provided the carbohydrate source was fed to dogs in the control (rice) group; the diet in which cassava was used as the carbohydrate source was fed to dogs in the gari group, while the rice diet to which cyanide was added was fed to the dogs in the rice + cyanide group. For the duration of the study the dogs were fed on 500 g diet once daily. The quantity of food was based on body-weight. Clean water was available *ad lib*.

Biochemistry

Blood samples were taken using heparinized syringes before the experimental diets were fed, and then at intervals after the beginning of the experiment. The plasma was separated from the cells in a refrigerated centrifuge and then stored in a freezer.

Plasma thiocyanate concentrations of the dogs were determined using the method of Bowler (1944) as modified by Chilaka (1984). The activity of plasma lipase (*EC* 3.1.1.3) was determined using kits supplied by Boehringer Mannheim.

Plasma free amino acid concentrations for each group of dogs were determined from pooled samples taken at the end of the experimental period using a Beckman 121M amino acid analyser. The gluconeogenesis index from protein was calculated according to the method of Barej *et al.* (1981).

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

Ingredients	Diets		
	Control	Cassava (gari)	Rice + cyanide
Rice	779	—	779
Gari	—	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, ml/mg food† (NaCN 8·8 mg/ml)	—	—	2

* Supplied mg/ml of 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, 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, myc-inositol 1·0, cholecalciferol 2·0 μg, cyanocobalamin 0·6 μg.

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

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

Diet* ...	Control	Cassava (gari)	Rice + cyanide
Water	709·6	727·3	709·6
Crude protein (nitrogen × 6·25)	129	131	129
Diethyl ether extract	46	53	46
Nitrogen-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

The dogs were killed under deep anaesthesia. The pancreas of each dog was removed and pieces 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 and Duncan's multiple range test was used to separate the means which were highly significant.

RESULTS

Animals

The dogs appeared clinically healthy for the duration of the experiment. Dogs fed on the rice + cyanide diet grew less well than the other groups.

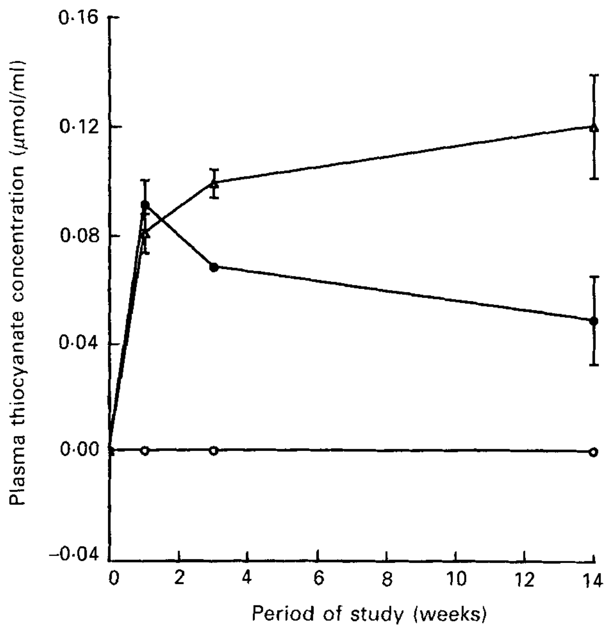

Fig. 1. Variations in plasma thiocyanate with time during the experimental period. (○) Dogs fed on control (rice) diet; (●) dogs fed on cassava (gari) diet; (△) dogs fed on rice+cyanide diet. For details of diets and dietary regimen, see Tables 1 and 2 and p. 366. Values are means with their standard errors represented by vertical bars.

Biochemistry

The effect of dietary treatment on plasma thiocyanate concentration is shown (Fig. 1 and Table 3). The plasma thiocyanate concentration in the control group remained undetectable throughout the period of the experiment. The feeding of gari and rice+cyanide elevated plasma thiocyanate concentrations.

The effects of the diets on plasma lipase are summarized (Table 3). Plasma lipase activity was not significantly affected by dietary treatment and time ($P > 0{\cdot}05$). However, there was a significant diet × time interaction ($P < 0{\cdot}05$). Significant interaction occurred because of the differences in the direction of response with time, thus in the gari group there was elevation of lipase activity while lipase activity was depressed in the control group and the rice+cyanide group at week 14. Consequently, mean comparisons at week 14 showed statistical significance between gari and control groups, and between the gari and rice+cyanide groups ($P < 0{\cdot}05$).

The plasma free concentrations of some amino acids are shown in Table 4. Alanine concentration was decreased in both the dogs fed on the gari diet and the dogs fed on the rice+cyanide diet to 203·9 and 745·9 μmol/ml respectively when compared with 1214·59 μmol/ml in the control dogs. Alanine was being utilized in the gari-fed dogs to a much greater extent than in the other groups. The concentrations of leucine, isoleucine and valine in the dogs fed on rice+cyanide had increased above those of the control dogs, while in the dogs fed on gari, the concentrations of leucine, isoleucine and valine had decreased. However, the concentrations of 3-methyl histidine in both the gari and rice+cyanide-fed dogs had increased and again the concentration in the rice+cyanide dogs was greater.

The index for evaluating gluconeogenesis from protein (Table 5) indicated that, when

Table 3. *Effect of dietary treatment on plasma thiocyanate concentration and plasma lipase (EC 3.1.1.3) activity*

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

		Plasma SCN concentration μmol/ml		Lipase activity U/l‡	
Week	Diet§	Mean	SE	Mean	SE
0	Control (rice)	—	—	84·00	17·00
	Cassava (gari)	—	—	84·00	17·00
	Rice+cyanide	—	—	81·33	5·91
1	Control (rice)	0·000	0·000	—	—
	Cassava (gari)	0·091*	0·009	—	—
	Rice+cyanide	0·080*	0·007	—	—
3	Control (rice)	0·000	0·000	—	—
	Cassava (gari)	0·068*	0·000	—	—
	Rice+cyanide	0·085†	0·005	—	—
14	Control (rice)	0·000	0·000	66·00	4·99
	Cassava (gari)	0·049*	0·016	175·30	54·02
	Rice+cyanide	0·119*†	0·019	72·55	22·20

* Mean values were significantly different from those of the control group for the same week: $P < 0{\cdot}005$.
† Mean values were significantly different from those of the cassava (gari) group for the same week: $P < 0{\cdot}001$.
‡ International Units per litre.
§ For details of diets and dietary regimen, see Tables 1 and 2 and p. 366.

Table 4. *Concentration of plasma free alanine, the branched-chain amino acids and 3-methyl histidine in growing dogs after 14 weeks on experimental diets, using pooled samples*

		Concentration (μmol/ml)		
Amino acid	Dietary group ...	Control	Cassava (gari)	Rice+cyanide
Alanine		1215	204	746
Leucine		102	97	136
Isoleucine		56	55	67
Valine		172	128	184
3-Methyl histidine		1631	1951	2336

* For details of diets and dietary regimen, see Tables 1 and 2 and p. 366.

Table 5. *Plasma amino acid index of gluconeogenesis after 14 weeks on experimental diets, using pooled samples*

Dietary group ...	Control	Cassava (gari)	Rice+cyanide
Leu+Isoleu+Val : Ala	0·271	1·376	0·518

* For details of diets and dietary regimen, see Tables 1 and 2 and p. 366.

compared with the dogs on the control diet, in the ratio 0·271:1·376:0·518, more protein was utilized for gluconeogenesis by the dogs fed on the gari diet than those fed on the rice+cyanide diet. This means that gluconeogenesis from protein was 5·077 times greater in the gari-fed dogs and 1·912 times greater in the rice+cyanide-fed dogs than in the control dogs. This suggested that the insulin status was lowest in the dogs fed on the gari diet.

Histopathology

The pancreas in the dogs fed on gari showed haemorrhage (Plate 1), necrosis (Plate 2), fibrosis and atrophy of the acinar tissue. There was fibrosis of the islets of Langerhans (Plate 3). The pancreas in the dogs fed on rice+cyanide showed lesions that were similar to those found in the pancreas of the dogs fed on gari but haemorrhage was not prominent and fibrosis was more marked (Plates 4 and 5).

DISCUSSION

An elevation in serum thiocyanate concentration after ingestion of organic compounds is good evidence that some degradation to HCN must have taken place. Sublethal levels of cyanide combine with thiosulphate to form thiocyanate and sulphite in a reaction catalysed by the enzyme rhodanese (thiosulphate cyanide sulphurtranferase; *EC* 2.8.1.1) (Cosby & Summer, 1945). Cyanide and thiocyanate are selective poisons and in low concentration cause necrosis 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) preventing the use of oxygen in an irreversible inhibition, and thiocyanate inhibits the enzyme fumarate hydratase (*EC* 4.2.1.2) in the tricarboxylic acid cycle (Massey & Alberty, 1954). Philbrick *et al.* (1977) reported reduced cardiac cytochrome oxidase activity and an increased lactate:pyruvate ratio in the blood due to an increase in the level of lactic acid in rats given daily doses of linamarin for 5 weeks. In the present study, the cyanide released and thiocyanate formed from the gari and rice+cyanide diets may have been responsible for the pathology observed.

There was a difference in the patterns of elevation of plasma thiocyanate levels in the two groups. For the gari diet, plasma thiocyanate levels rose during the first few weeks and then declined, whereas, in the rice+cyanide group, plasma thiocyanate levels continued to rise throughout the experiment. It is difficult to explain these differences; however, it is possible that feeding of cassava over several weeks might lead to changes in the gut microflora which could alter microbial hydrolysis of linamarin resulting in reduced release of free cyanide for absorption from the gastrointestinal tract. It might also cause adaptation of the activity of any liver enzyme capable of hydrolysing absorbed intact linamarin.

Adams *et al.* (1958) found that in rodents, the organs most affected by cassava diets were those with the most rapid turnover in protein, such as the pancreas and liver. Tissues with a rapid turnover of protein also have a rapid turnover of cell contents generally (Munro & Allison, 1964), and consequently a rapid metabolic rate. The effects of inhibition of enzymes associated with oxidation and oxidative phosphorylation are, therefore, likely to be seen in these tissues first. The loss of oxidative phosphorylation and ATP generation have widespread effects on many systems within the cell, culminating in cell death or necrosis with leakage of intracellular enzymes across the abnormally-permeable plasma membrane into the blood.

Lipase, triacylglycerol acylhydrolase, is secreted by the pancreas and is a specific index for measurement of pancreatic integrity. However, many workers have shown the presence of normal lipase activity despite severe pancreatic pathological changes (Strombeck *et al.* 1984). This phenomenon was observed in the dogs fed on rice+cyanide. The statistically significant high lipase activity in the gari group of animals indicated that gari induced pancreatic acinar injury even in a balanced diet, causing large quantities of lipase to leak out of the cells. This was confirmed by the histopathological picture of haemorrhage, necrosis and fibrosis. Since the histopathological picture of the pancreas in the dogs fed on rice + cyanide was similar to, but with more fibrosis than, that of the gari-fed dogs, and the plasma thiocyanate level was greater in the rice + cyanide-fed dogs than in the gari-fed dogs, it would appear that pancreatic acinar injury was caused by cyanide and its metabolite thiocyanate. Also, since the islets of Langerhans of both the gari-fed dogs and the rice + cyanide-fed dogs were replaced by fibrous connective tissue, it would appear that cyanide and thiocyanate also caused the destruction of the cells of the islet of Langerhans. It would seem, therefore, that the pancreatic injury observed in the present study is related to the concentration of cyanide released from the gari and the level of plasma thiocyanate formed, and not to protein deficiency.

The insulin status of the dogs was evaluated at the end of the experimental period using the gluconeogenic index proposed by Barej *et al.* (1981). Low insulin secretion in diabetes mellitus depresses amino acid uptake and protein synthesis, and increases the protein catabolism and the output of amino acids from muscle. It leads to the increase of free amino acid concentration, mainly branched-chain amino acids, in blood plasma (Felig *et al.* 1977; Prior & Smith, 1982). The low insulin secretion in diabetic patients also leads to increased utilization of alanine for glucose synthesis (Felig *et al.* 1977). Barej *et al.* (1981) suggested the use of the sum of plasma isoleucine, leucine and valine:alanine ratio as a measure of gluconeogenic index increases. Inspection of the gluconeogenic indices for pooled plasma free amino acids from each group of dogs showed that gluconeogenesis from protein was highest in the group of dogs fed on the gari diet; this was five times higher than gluconeogenesis from protein in the dogs fed on the control diet, while gluconeogenesis from protein in dogs fed on the rice + cyanide diet was almost twice as high as that of the control animals. These results indicated that the existence of a hypoinsulinaemia produced as a consequence of necrosis and fibrosis of the islets of Langerhans in the two groups of dogs was caused by the effects of cyanide and its metabolite thiocyanate. The severity of hypoinsulinaemia in the two groups of dogs at the end of the experiment was not related to the concentration of plasma thiocyanate or to the degree of histopathological change in the groups, because the same pattern did not exist. Findings of the present study suggested that some factor in gari, in addition to cyanide, was responsible for the marked hypoinsulinaemia. Philbrick *et al.* (1977) have suggested that the Na^+, K^+-ATPase (*EC* 3.6.1.37) pump is inhibited by the intact linamarin. This would result in depletion of intracellular potassium, swelling and bursting of cells and, therefore, interference with the synthesis of insulin. Barrett *et al.* (1977) showed that a portion of orally administered linamarin was absorbed intact and excreted in urine.

The present study showed that pancreatic degeneration and hypoinsulinaemia will occur when a gari diet containing 10·8 mg HCN/kg and 130 g protein/kg dry matter is fed consistently to dogs over a sufficiently long period in a nutritionally balanced diet, and that the hypoinsulinaemia produced by the gari diet was only partly due to the cyanide released from the cyanogenic glucoside linamarin, and the thiocyanate formed.

Cassava is the staple food in many areas where tropical diabetes occurs. The histopathological picture of the pancreas in man suffering from tropical diabetes is characterized by diffuse fibrosis with widespread dissociation and disorganization of acinar tissue, and destruction of the islets of Langerhans ranging from virtual absence to almost normal islet structure with areas of atrophy (Abu-Bakare *et al.* 1986). The histopathological picture in the dog of haemorrhage, necrosis, fibrosis and atrophy of the acinar tissue and fibrosis of some of the islets of Langerhans, after 14 weeks on a balanced cassava diet, can be considered an early expression of the end-stage picture seen in humans. Strombeck *et al.* (1984) showed that the dog is able to survive for a relatively long period after severe acute pancreatitis has been induced and that it might be used as a model to study chronic pancreatitis.

Turner & Bagnara (1971) reported that the metabolic defects occurring in the depancreatized dog are the same as those appearing in diabetic man. The blood amino acid profile of the dogs fed on cassava showed that a more severe hypoinsulinaemia was present and that more alanine was used up for gluconeogenesis than in any other group in the study. These biochemical changes resemble those found in the human diabetic.

The present study suggests that the histological lesions found in the pancreas of dogs fed on cassava-based diets and the concurrent biochemical changes may not be entirely due to cyanide and increased plasma thiocyanate levels or protein malnutrition. The present study shows that the dog might be considered to be a good model for studying the effect of a cassava diet on the pancreas of man.

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EXPLANATION OF PLATES

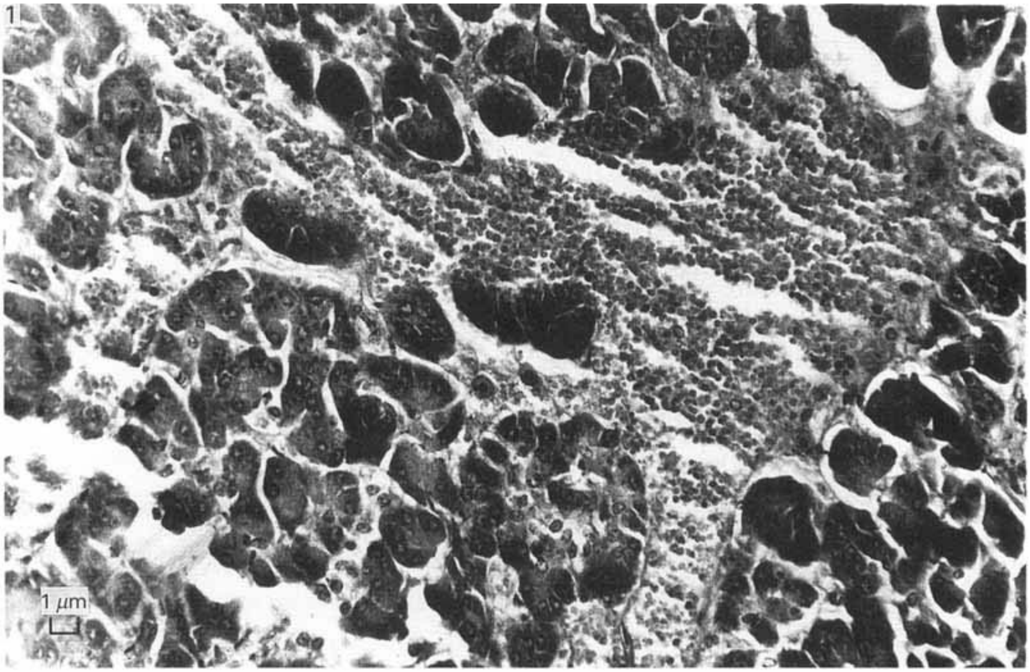
Plate 1. Pancreas of dog fed on balanced cassava (gari) diet showing haemorrhage (haematoxylin and eosin). For details of diet, see p. 366 and Tables 1 and 2.

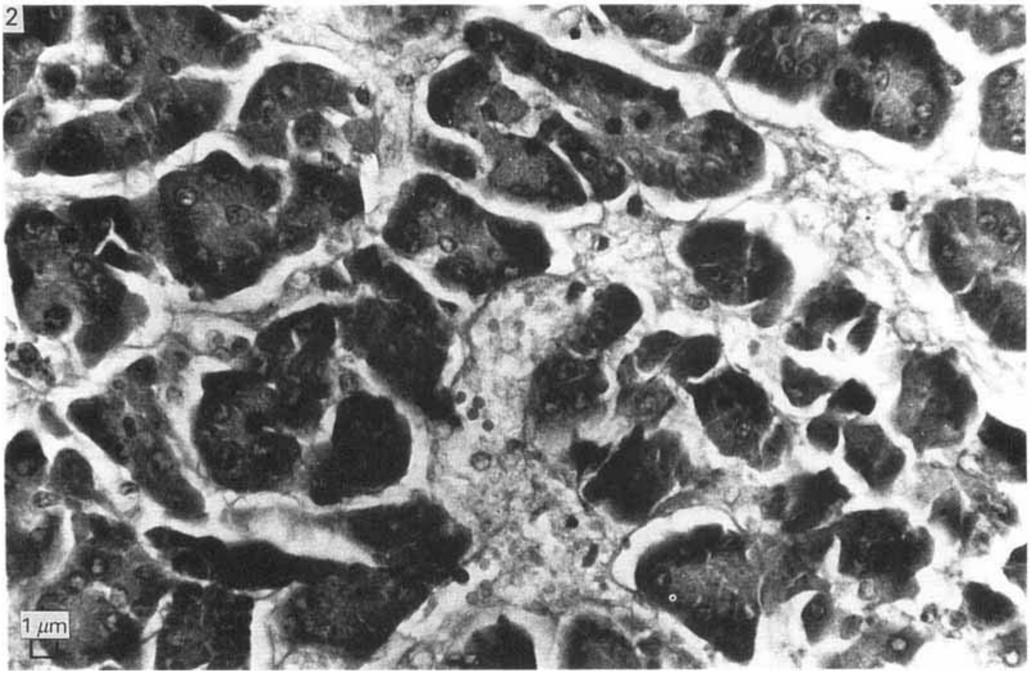
Plate 2. Pancreas of dog fed on balanced cassava (gari) diet showing necrosis of acinar tissue (haematoxylin and eosin). For details of diet, see p. 366 and Tables 1 and 2.

Plate 3. Pancreas of dog fed on balanced cassava (gari) diet showing fibrosis of islet of Langerhans (haematoxylin and eosin). For details of diet, see p. 366 and Tables 1 and 2.

Plate 4. Pancreas of dog fed on balanced rice + cyanide diet showing fibrosis in acinar tissue (haematoxylin and eosin). For details of diet, see p. 366 and Tables 1 and 2.

Plate 5. Pancreas of dog fed on balanced rice + cyanide diet showing fibrosis of islet of Langerhans (haematoxylin and eosin). For details of diet, see p. 366 and Tables 1 and 2.





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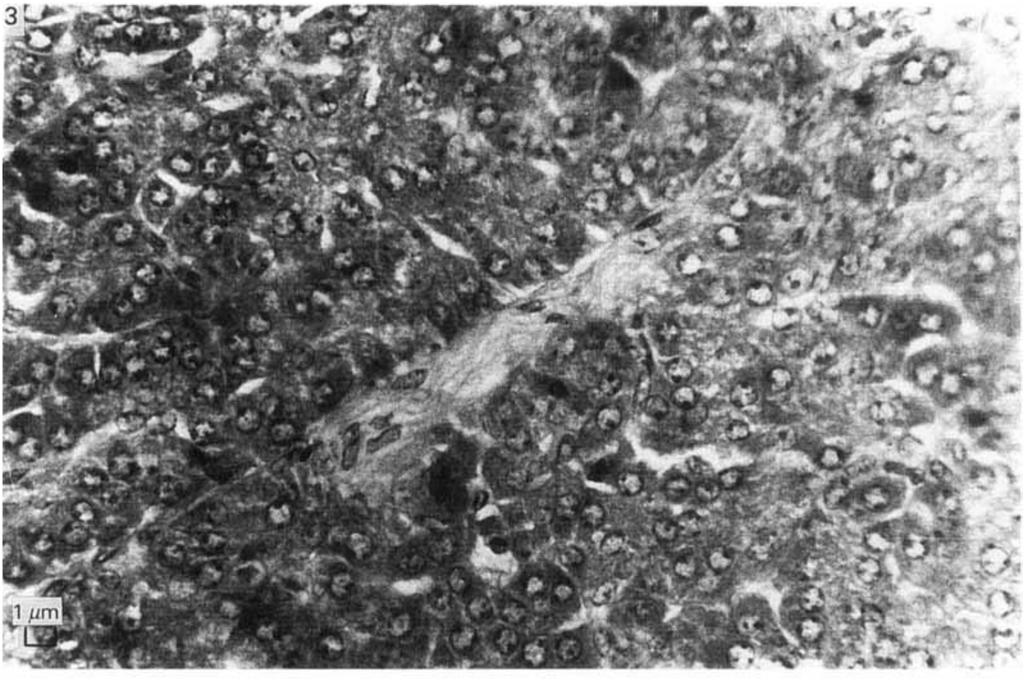
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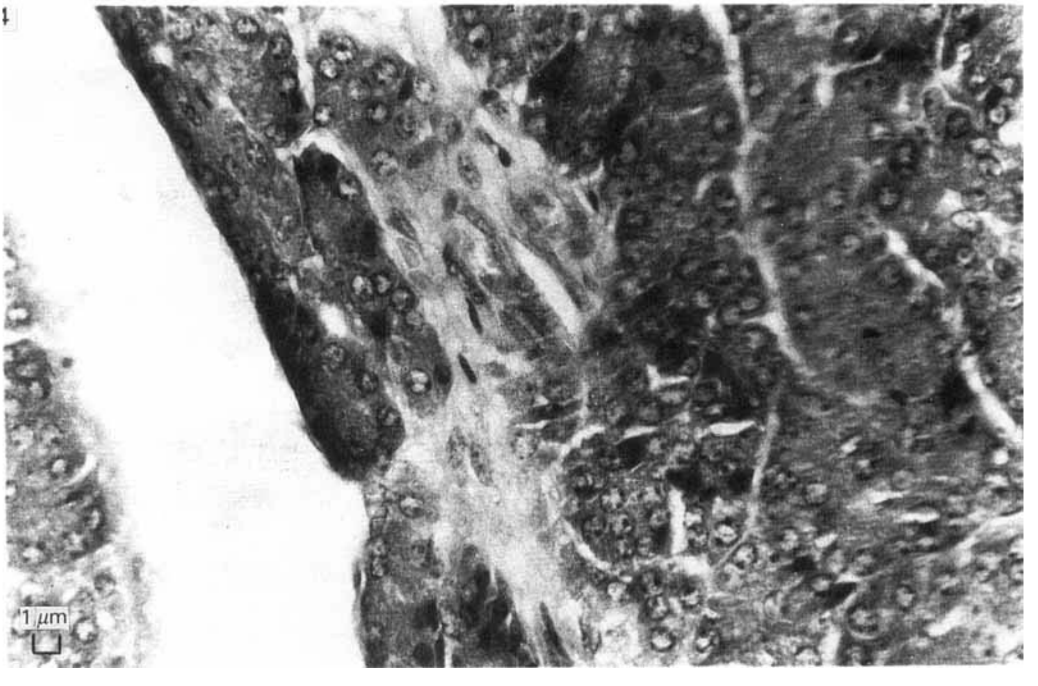
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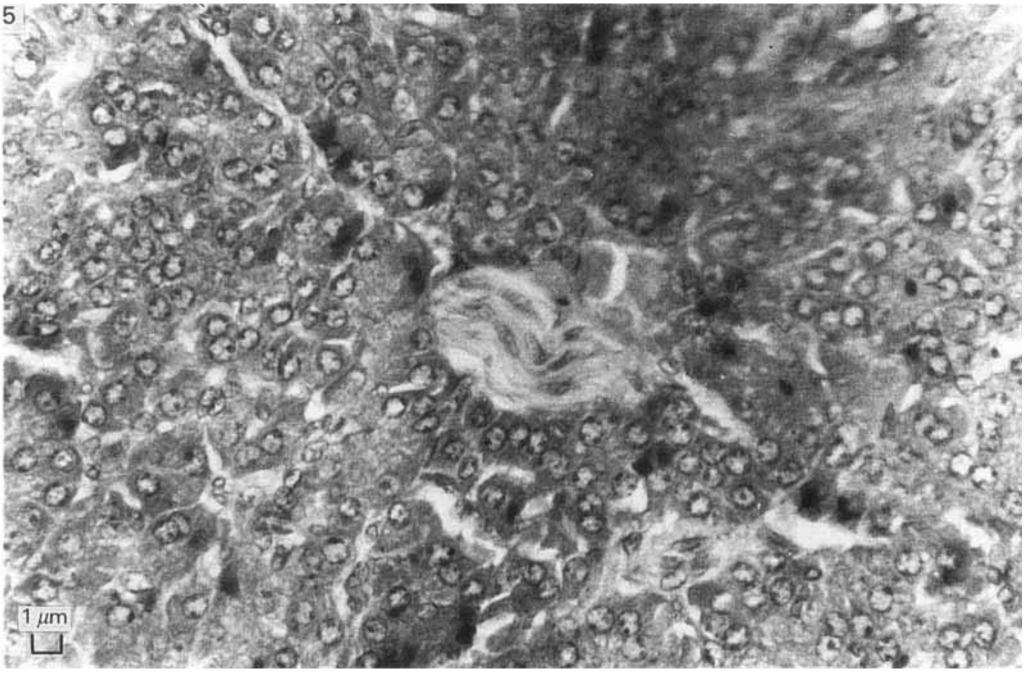
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