The influence of dietary carbohydrate and fat on kidney calcification and the urinary excretion of *N*-acetyl-β-glucosaminidase (*EC* 3.2.1.30)

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The influence of dietary carbohydrate and fat on kidney calcification and the urinary excretion of *N*-acetyl-β-glucosaminidase (*EC* 3.2.1.30)

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1. Male Sprague–Dawley rats were fed on diets containing either sucrose or starch as the carbohydrate component. In one experiment, the diets also contained 200 g either butter or polyunsaturated margarine/kg; in a second experiment, the diets contained less fat in the form of 20 g maize oil/kg.

2. Over a period of 11 months assays were made in the urine of several ions and of the activity of the enzyme *N*-acetyl-β-glucosaminidase (β-2-acetamido-2-deoxy-β-D glucoside acetamidodeoxygluco-hydrolase; *EC* 3.2.1.30); at 13 months, examination was made of some of the abdominal viscera, especially of the kidneys.

3. In rats fed on the higher amount of fat, dietary sucrose produced a higher activity of the enzyme than did dietary starch, and a greater excretion of inorganic phosphate.

4. With both the higher and lower amounts of dietary fat, sucrose led to an increase in the weight of the liver and of the kidneys, and an increase in the concentration of calcium and of phosphate in kidney tissue. With the higher amount of fat, sucrose also produced an increase in the concentration of magnesium in the kidney. There was no difference in the concentration of any of the ions assayed in the plasma or, apart from inorganic phosphate, in the urine.

5. The kidneys of the sucrose-fed rats showed nephrocalcinosis, mostly in the cortico-medullary region, and basophilic deposits in the tubules. Attention is drawn to this unusual occurrence of nephrocalcinosis in male rats.

It is becoming increasingly recognized that starch and sucrose, the items that make up most of the carbohydrate in most human diets, produce different effects when metabolized. For example, compared with starch, sucrose leads to an increase in liver weight involving an increase in liver water, glycogen, protein and fat, and an increase in cell number as well as cell size (hyperplasia as well as hypertrophy) (Bender *et al.* 1972; Bruckdorfer *et al.* 1974). The effect of sucrose on the kidney was first described by Cohen & Rosenmann (1971) and Rosenmann *et al.* (1971), who reported the development of diffuse intercapillary glomerulosclerosis. As with the liver, the kidney is also increased in weight by sucrose (Kang, 1973). In this report, we describe the effect of sucrose on the activity of *N*-acetyl-β-glucosaminidase (β-2-acetamido 2-deoxy-β-D glucoside acetamidodeoxygluco-hydrolase; *EC* 3.2.1.30) excreted in the urine, an increase of which is an indication of damage to the kidneys and especially to the tubules (Dance *et al.* 1970; Price *et al.* 1971). In addition, we have looked for nephrocalcinosis both by histological examination of the kidney and by the chemical assay of some of the mineral elements. A brief report of some of our findings has already been made (Kang *et al.* 1977*a*, *b*).

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

	Expt A				Expt B	
Diet no....						
Ingredients	1	2	3	4	5	6
Sucrose	550	0	550	0	700	0
Starch	0	550	0	550	0	700
Butter	200	200	0	0	0	0
Margarine	0	0	200	200	0	0
Casein	160	160	160	160	190	190
Maize oil	0	0	0	0	20	20
Mineral mix*	45·7	45·7	45·7	45·7	45·7	45·7
Vitamin mix†	20	20	20	20	20	20
Cellulose (Solka floc)	30	30	30	30	30	30

* $CaHPO_4.2H_2O$ 16·4 g; $CaCO_3$ 8·2 g; KCl 8·2 g; Na_2HPO_4 7·4 g; $MgSO_4.7H_2O$ 4·99 g; $MnSO_4.4H_2O$ 237 mg; ferric citrate 173 mg; $CuSO_4.5H_2O$ 23·5 mg; $ZnCO_3$ 30 mg; KIO_3 1 mg.

† Cellulose 17·6 g; choline bitartrate 1·8 g; vitamin concentrate 0·602 g (comprising ascorbic acid 75 mg; nicotinic acid 60 mg; cyanocobalamin 0·05 mg in 50 mg mannitol; Ca-D-pantothenate 0·04 mg; thiamine hydrochloride 10 mg; riboflavin 10 mg; pyridoxine 10 mg; folic acid 5 mg; D-biotin 1 mg; menaphthone 1 mg; Rovimix A 500 12 mg; Rovimix AD_3 500/100 12 mg; Rovimix E25 3 mg; cellulose 12 mg; Roche Products Ltd, London, W.1, UK).

MATERIALS AND METHODS

Sixty male Sprague–Dawley rats (Charles River (UK) Ltd, Margate, Kent) weighing between 90 and 110 g were fed on a standard laboratory diet (41 b; Dixon Ltd, Ware, Herts.) for 1 week before being put on to the experimental diets. Water and diets were given *ad lib.*

The rats were divided at random into six groups each of ten animals. For Expt A, each of four of the groups was given a diet with either starch or sucrose as the carbohydrate, and with either butter or polyunsaturated margarine as the fat (Table 1). For Expt B, each of the remaining two groups was given a low-fat diet with starch or sucrose as the carbohydrate; in these diets, the fat was a small quantity of maize oil.

At intervals, the animals were transferred individually into metabolic cages (Flexicage; Jencons Scientific Ltd, Hemel Hempstead, Herts.) for periods of 24 h for the collection of urine and the measurement of food intake. Urine samples were collected at 2, 6 and 11 months for analysis of calcium, magnesium, sodium, potassium and chlorine, and at 3·5, 5·5 and 11 months for the assay of the activity of *N*-acetyl-β-glucosaminidase. The assay was carried out fluorimetrically according to the procedure of Robinson *et al.* (1967).

After 13 months, the rats were anaesthetized with diethyl ether, killed by exsanguination from the aorta, the major organs removed and weighed, and plasma prepared from the heparinized blood. One kidney was fixed in formaldehyde (370 ml/l sodium chloride (9 g/l methanol (100 ml/l)), dehydrated and stained with haematoxylin and eosin, periodic acid–Schiff (PAS) reagent, and Masson's trichrome. The other kidney, and samples of liver and diets, were weighed, ashed at 600° (since completing this study it has been drawn to our attention that there is some loss of mineral when ashing is carried out at 600° and that 500° is a more desirable temperature), dissolved in concentrated nitric acid, and filtered. Protein-free plasma was prepared by the addition to 1 ml of 0·5 ml trichloroacetic acid (200 ml/l) and centrifugation for 15 min at 3000 *g*.

Ca and Mg were assayed in these preparations by atomic absorption spectrophotometer (Pye-Unicam Ltd) according to the procedure based on the methods of Willis (1961). Inorganic phosphate was measured by the method of Fiske & SubbaRow (1925) and Na, K and Cl by flame photometry or with the chloride meter (Corning EFL, Scientific Instruments Ltd, Halstead).

RESULTS

Urinary enzyme excretion

In Expt A there were no significant differences in urine volume between the four dietary groups. The excretion of *N*-acetyl-β-D-glucosaminidase increased with age (Table 2). The activity of the enzyme was significantly higher in the rats fed on sucrose at each of the time intervals when it was assayed. On the other hand, no statistically significant effects of fat on enzyme excretion were observed, although after 6 months there was a statistically significant interaction of dietary fat and carbohydrate on the excretion of the enzyme. In margarine-fed rats the differences in enzyme activities between rats receiving starch and those receiving sucrose diminished in the later stages of the experiment.

In Expt B the differences in *N*-acetyl-β-D-glucosaminidase excretion between starch-fed and sucrose-fed animals did not reach statistical significance, thus suggesting some role of dietary fat on the effects of sucrose.

Food intake, body-weight and organ weight

The increase of body-weight in the different groups was not affected by differences in diet (Table 3). The greater weight of food eaten in experiment B is accounted for by the fact that, having a lower fat content, the diets had a lower energy density. In both experiments, sucrose produced a greater liver weight than did starch; there was no effect of dietary fat. The kidney weight was also greater in the sucrose-fed animals receiving butter or margarine, and the differences on the low-fat diet were also significant. There was no significant difference in the weight of testes, spleen, or adrenal gland produced by the different diets.

Histology of kidney

Sections of the kidney were stained with haematoxylin and eosin. No abnormalities were seen in starch-fed rats. Sections from all the sucrose-fed rats examined showed the presence of basophilic material in the lumina of the medullary tubules in the cortico-medullary region. The concretions made the cutting of the sections difficult, and often led to damage of the adjacent cellular structure. A representative section is shown in Plate 1.

Content of mineral elements in tissues and urine

With the high-fat diets, sucrose produced a considerable increase in the concentration of Ca in the kidney, a lesser though still substantial increase in the concentration of phosphate, and a smaller increase in the concentration of Mg (Table 4). The nature of the dietary fat did not affect these differences. With the low-fat diets in Expt B, smaller but still significant increases in the concentrations of Ca and phosphate occurred with the sucrose diets but no increase in the concentration of Mg. In the livers and the plasma, sucrose had no effect on the concentration of these cations.

In the urine, dietary sucrose with the high-fat diets had no effect on the excretion of Ca and Mg, but, except in the urine collected at 11 months, the concentration of inorganic phosphorus was significantly increased (Table 5). The concentration of Na, K and Cl in the urine was the same with all diets. With the low-fat diets, there was no difference in any of the mineral element concentrations.

DISCUSSION

Dietary sucrose produces increases in the size of the liver and the kidney of rats (Bruckdorfer *et al.* 1972; Kang, 1973). Unpublished work from these laboratories has not detected any clear-cut effect of dietary sucrose on renal function, as demonstrated for example by

Table 2. *The activity (μmol/h per 24 h urine sample) of* N-*acetyl-β-*D-*glucosaminidase in urine of rats fed on high-fat or low-fat diets with starch or with sucrose*‡

(Mean values with their standard errors for ten rats/treatment)

Diet	Expt A								Expt B			
	Sucrose–butter		Starch–butter		Sucrose–margarine		Starch–margarine		Sucrose		Starch	
Duration of feeding (months)	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
3·5	7·0	0·6	3·0	0·3	5·8	0·8	3·3	0·4*	5·2	0·4	4·7	0·5
5·5	6·7	0·4	3·8	0·5	5·4	0·4	5·1	0·5*†	3·5	0·4	4·2	0·6
11	10·3	0·8	7·1	0·9	10·4	1·0	9·3	0·6*	5·8	0·5	7·0	0·5

* Values for sucrose diets were greater than those for starch diets: $P < 0{\cdot}01$ (analysis of variance).
† Carbohydrate–fat interaction was found to be significant by analysis of variance.
‡ For details, see Table 1.

Table 3. *Food intake, body-weight and organ weights of rats fed on high-fat or low-fat diets with starch or with sucrose*‡

(Mean values with their standard errors for ten rats/treatment. The livers and kidneys were weighed at 13 months)

Diet	Expt A								Expt B			
	Sucrose–butter		Starch–butter		Sucrose–margarine		Starch–margarine		Sucrose		Starch	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Food intake after 6 months (g/24 h)	12·5	1·1	10·5	1·4	11·7	0·7	10·3	1·0	14·2	1·3	14·9	1·6
Body-wt. (g):												
After 6 months	570	17	579	24	674	16	576	24	426	16	454	15
After 13 months	713	24	694	20	751	24	705	20	622	20	539	25
Liver wt. (g)	16·6	0·81	14·2	0·6	16·5	0·6	14·5	0·8*	19·8	0·7	14·7	0·6††
Left kidney wt. (g)	2·16	0·12	1·67	0·07	2·12	0·05	1·85	0·09**	1·90	0·07	1·63	0·05†

Values for sucrose diets were greater than those for starch diets; * $P < 0{\cdot}01$, ** $P < 0{\cdot}001$ (analysis of variance), † $P < 0{\cdot}05$, †† $P < 0{\cdot}001$ (Student's t test).
‡ For details, see Table 1.

Table 4. *Concentration of calcium, magnesium and inorganic phosphate (as phosphorus) in liver, kidney and plasma of rats fed on high-fat or low-fat diets with starch or with sucrose*‡

(Mean values with their standard errors for ten rats/treatment. Analyses were made after 13 months on diets)

	Expt A								Expt B			
Diet	Sucrose–butter		Starch–butter		Sucrose–margarine		Starch–margarine		Sucrose		Starch	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Liver (mg/g)												
Ca	0·09	0·01	0·11	0·01	0·09	0·02	0·1	0·01	—		—	
Mg	0·14	0·02	0·2	0·01	0·15	0·02	0·18	0·02	—		—	
P	3·6	0·2	4·7	0·2	4·3	0·1	4·2	0·4	—		—	
Kidney (mg/g)												
Ca	1·41	0·3	0·28	0·1	1·16	0·4	0·33	0·1*	0·27	0·03	0·16	0·01†
Mg	0·19	0·01	0·15	0·01	0·19	0·02	0·13	0·02*	0·25	0·03	0·21	0·02
P	9·7	1·3	4·8	0·5	9·5	2·6	4·5	0·3*	5·9	0·2	3·5	0·04†
Plasma (mg/l)												
Ca	130	4	137	4	135	3	140	5	130	9	123	9
Mg	23	1·8	25·6	0·9	27·5	0·6	29·5	1·2	26·5	3·2	25·7	3·3
P	80	8	75	11	69	8	67	4	81	8	71	12

Values for sucrose diets were greater than those for starch diets; * $P < 0.01$ (analysis of variance), † $P < 0.05$ (Student's t test).
‡ For details, see Table 1.

Table 5. *Urinary excretion (mg/24 h) of calcium, magnesium and inorganic phosphorus by rats fed on high-fat or low-fat diets with starch or sucrose*‡

(Mean values with their standard errors for ten rats/treatment)

	Expt A								Expt B			
Diet	Sucrose–butter		Starch–butter		Sucrose–margarine		Starch–margarine		Sucrose		Starch	
Period of experiment (months)	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
2 Ca	0·09	0·02	0·09	0·02	0·11	0·02	0·10	0·01	0·14	0·04	0·07	0·01
Mg	1·23	0·16	1·12	0·18	1·57	0·24	1·15	0·24	1·34	0·21	0·86	0·12
P	39·5	2·6	27·0	1·9	38·5	3·4	25·1	1·4*	32·9	2·7	32·9	3·3
6 Ca	0·15	0·03	0·11	0·02	0·17	0·03	0·15	0·02	0·33	0·07	0·18	0·05
Mg	0·94	0·18	0·6	0·12	1·24	0·23	1·29	0·19	0·75	0·13	0·98	0·20
P	18·7	2·0	14·2	1·9	19·2	2·2	11·5	2·3*	6·3	0·9	7·66	1·12
11 Ca	0·14	0·03	0·12	0·02	0·11	0·02	0·12	0·02	0·24	0·04	0·17	0·04
Mg	1·39	0·25	1·29	0·24	1·21	0·14	1·58	0·25	1·42	0·25	1·07	0·13
P	28·3	3·9	25·3	4·2	28·7	3·4	17·7	3·1	15·7	2·8	21·4	3·1

* Values for sucrose diets were greater than those for starch diets; $P < 0.01$ (analysis of variance).
‡ For details, see Table 1.

the clearance of creatinine, insulin or *p*-aminohippuric acid (Kang, Whaler & Yudkin, unpublished results). This finding nevertheless cannot be taken as demonstrating that sucrose in the diet leaves renal function intact, since the kidney has considerable reserve functional capacity. It was for this reason that we have examined the urinary excretion of *N*-acetyl-*β*-D-glucosaminidase, since an increase in the activity of this enzyme is a fairly sensitive index of renal damage (Dance *et al.* 1970; Price *et al.* 1971).

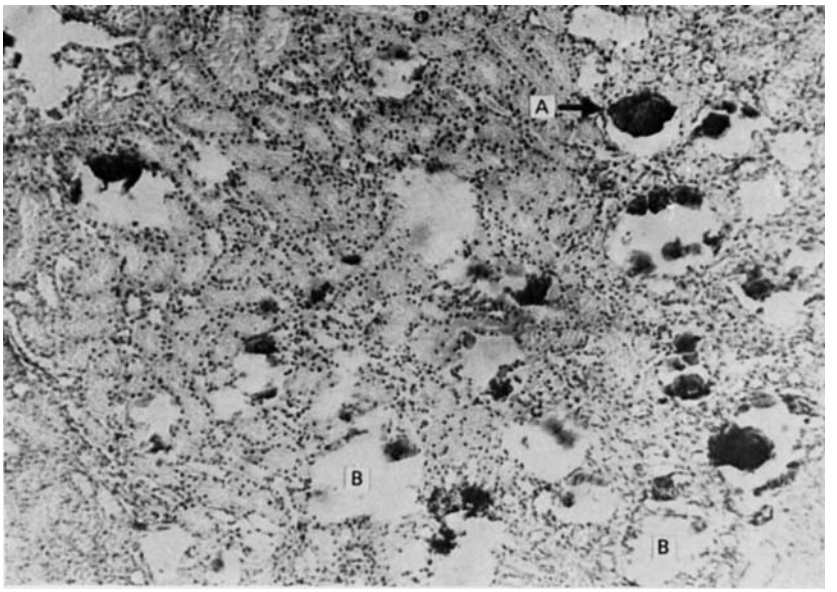
Nephrocalcinosis has repeatedly been demonstrated in rats fed on semi-purified diets. It occurs more readily in female rats than in male rats (Cousins & Geary, 1966). It has been produced by the inclusion of casein in the diet (Kaunitz & Johnson, 1976), as well as by diets deficient in Mg (Meyer & Forbes, 1968; Goulding & Malthus, 1968); it has also been produced by the injection of sodium phosphate (Haase, 1975). Du Brun (1972) concludes that 'kidney calcification was induced by feeding phosphorus at dietary levels exceeding certain critical levels, the latter depending on dietary magnesium content, the higher the magnesium level, the higher the phosphorus level had to be to induce calcification'. Whereas the condition can be prevented by parathyroidectomy, the administration of parathyroid extract did not aggravate the condition (Goulding & Malthus, 1971). In the experiments described in this paper, the content of Mg, which was 640 mg/kg, was greater than that considered to be nutritionally adequate for rats; it is now recognized however that higher levels may be necessary to prevent nephrocalcinosis in female rats (AIN Standards, 1977).

It seems likely that dietary factors other than mineral elements are involved in nephrocalcinosis, and especially that the type of protein and of fat may be important (Kaunitz & Johnson, 1976). These workers found that medium-chain triglycerides produced less calcification than did maize oil. Many of the purified diets used in this work contain sucrose, and Luoma *et al.* (1976) showed that a diet rich in sucrose can produce nephrocalcinosis in female rats. Although Woodard (1971*a*) found that the replacement of sucrose by maize starch in the semi-purified diet of female rats did not reduce the incidence of nephrocalcinosis, neither did single changes of types of protein or of fat. Woodard (1971*a*) also showed that substitution of the ash from chow for the mineral mix in the semi-purified diet did not prevent nephrocalcinosis. In our own experiments, there was a clear difference in the severity and frequency of nephrocalcinosis in sucrose-fed and starch-fed rats. The type of fat in the diet had little effect on the severity of nephrocalcinosis, but the amount of fat in the diet considerably enhanced the effect of sucrose. It may be that this is partly due to the fact that, in terms of energy, though not in terms of weight, the concentration of the mineral salts is lower in the high-fat diets of Expt A than in the low-fat diets in Expt B.

The mechanism of calcification by dietary sucrose in male rats is not clear. Like Woodard (1971*b*) we found no relationship between the plasma concentration of the mineral ions and the incidence of nephrocalcinosis. The only observation that may be relevant was that the sucrose-fed rats on the high-fat diet had an increased excretion of phosphate ions during the earliest stages of the experiment, and that this did not occur when low-fat diets were fed.

It was shown by Forbes & Parker (1971) that ethionine exaggerates the calcification of the kidneys of rats with Mg-deficient diets. Acute doses of fructose are also known to reduce the ATP content of rat liver, because of the rapid utilization of ATP consequent on the high activity of fructokinase (*EC* 1.1.99.11) (Mäenpää *et al.* 1968). The kidney also has a high activity of fructokinase, but to our knowledge no information is available on the effect of fructose or sucrose on the ATP of the kidney. With the liver, the concentrations of ATP are restored after prolonged feeding with fructose (Romsos & Leveille, 1974).

The possible involvement of glycoproteins, as proposed by Woodard (1971*b*), was suggested by the presence of PAS-positive material associated with mineral deposition. This would be consistent with parallel observations that disturbances in the glycoprotein metabolism of the kidney led to the thickening of glomerular basement membrane (Price *et al.*


A
B
B

1978). Bunce & Bloomer (1972) proposed an increased endocytosis of Ca by tubular cells, leading to calcification and an involvement of lysozymes. Exocytosis of the contents of secondary lysosomes may lead to extracellular concretions. Such an hypothesis would be consistent with the early appearance of increased *N*-acetyl-β-glucosaminidase activity in the rats with nephrocalcinosis. It has recently been shown that calcification occurs within 5 weeks of the commencement of the sucrose diet (Oxley, Bruckdorfer & Yudkin, unpublished results). Other possible mechanisms involving hormonal effects have not been investigated, but there is evidence that sucrose significantly increases insulin:glucagon in the plasma (Gardner *et al.* 1977), and that fructose increases plasma growth hormone concentrations in man.

It is, however, clear that dietary constituents other than mineral elements are important in the production of calcinosis in the kidney. It is also possible that an early warning of such damage may be given by monitoring the activity of *N*-acetyl-β-glucosaminidase in rat urine.

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EXPLANATION OF PLATE

Histology of rat kidney cortico-medullary region in sucrose fed rats. Note the calcified basophilic concretions (A) which have a laminated appearance. Difficulty was encountered when cutting the calcified tissue, the resultant tears in the tissue can be seen (B). Kidneys were stained with haemotoxylin and eosin. Original magnification $\times 48$.

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