The effect of dietary bagasse on the activities of some key enzymes of carbohydrate and lipid metabolism in mouse liver

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1. The effects of a 100 g/kg diet substitution of bagasse on the body-weight gain, food consumption and faecal dry weight of mice given a high-sucrose diet and on the activities of hepatic glucose-6-phosphate dehydrogenase (*EC* 1.1.1.49), 6-phosphogluconate dehydrogenase (*EC* 1.1.1.44), malate dehydrogenase (oxaloacetate-decarboxylating) ($NADP^+$) (*EC* 1.1.1.40), ATP-citrate (*pro-3S*) lyase (*EC* 4.1.3.8), 6-phosphofructokinase *EC* 2.7.1.11), pyruvate kinase (*EC* 2.7.1.40) and fructose-1,6-bisphosphatase (*EC* 3.1.3.11) were studied.

2. Bagasse had no effect on body-weight gain, food consumption or faecal dry weight.

3. Bagasse decreased the activities of glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and phosphofructokinase expressed on a wet weight basis and on a protein basis.

4. Bagasse decreased the activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase expressed on a body-weight basis.

5. These results suggest that bagasse decreases the flux through some pathways of hepatic lipogenesis when mice are given high-sucrose diets.

Dietary fibre has been defined as 'the plant polysaccharides and lignin which are resistant to hydrolysis by the digestive enzymes of man' (Trowell *et al.* 1976). This definition includes two major types of fibre, namely the gel-forming polysaccharides such as guar gum, pectin and gum tragacanth and the natural fibre preparations such as wheat bran and bagasse. It is well established that gel-forming polysaccharides have effects on carbohydrate and lipid metabolism in healthy human subjects and diabetics (for a review, see Jenkins, 1979). These effects of guar gum on human metabolism prompted us to investigate its effects on the metabolism of mouse liver, in order to discover whether the effects observed in the blood, urine and faeces of man were accompanied by changes in the metabolism of specific tissues. We found that guar gum increased the activities of a number of key enzymes of carbohydrate and lipid metabolism in the livers of mice given high-carbohydrate diets (Stanley & Newsholme, 1985).

Some natural fibre preparations also have effects on metabolism. Bagasse, which is that part of sugar cane remaining after the commercial extraction of all soluble carbohydrates (Morgan *et al.* 1974), has effects on carbohydrate and lipid metabolism. It has been shown that bagasse binds bile acids in vitro (Morgan *et al.* 1974); this property probably accounts for its effects on lipid metabolism in vivo. Thus bagasse increases the faecal excretion of bile acids and neutral steroids in the rat (Morgan *et al.* 1974) and of acid steroids and fat in man (Walters *et al.* 1975). Loss of bile acids in the faeces is compensated for by an increase in the rate of their synthesis in the liver to maintain the bile acid pool (Morgan *et al.* 1974). Hence, the activity of the enzyme cholesterol 7α-monooxygenase (*EC* 1.14.13.17) which determines the rate of bile acid synthesis from cholesterol (Myant & Mitropoulos, 1977) increases in the livers of rats given bagasse (Morgan *et al.* 1974). Bagasse also has effects on carbohydrate metabolism since it raises the blood glucose concentration in human subjects during an oral glucose-tolerance test (Jefferys, 1974).

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Table 1 *The effect of bagasse on the food consumption and faecal dry weight of mice*

(Mean values for six mice per diet. Body-weight, food consumption and faecal dry weight of six mice per group were measured daily during the first 7 d of the 50 d feeding period)

Diet ...	Control	Bagasse	Difference	SED (10 df)
Body-wt (g)	25·1	25·4	0·3	1·17
Food consumption:				
g/d	6·5	6·0	−0·5	0·41
g/d per kg body-wt	260	237	−23	17·5
Faecal dry wt:				
g/d	1·3	1·3	0	0·12
g/d per kg food consumption	199	214	15	10·6

SED, standard error of difference.

Values for the bagasse diet were not significantly different from the corresponding values for the control diet (Student's *t* test).

We have applied our experimental approach (Stanley & Newsholme, 1985) to a study of the effects of the natural fibre preparation, bagasse, on the metabolism of mouse liver. The results of this study are reported here.

EXPERIMENTAL

Except for the following minor differences the mice used in the present study were as described previously (Stanley & Newsholme, 1985). At the beginning of the 50 d feeding period the mice were divided into two groups so that each group had a mean initial body-weight of 24·4 g. All mice were 162 d old at this time.

The high-sucrose control diet used in the present study has been described previously (Stanley & Newsholme, 1985). Bagasse was prepared by Tate and Lyle Ltd, London. The composition of the bagasse preparation used in the present work is given by Morgan *et al.* (1974) as (g/kg): moisture 72, ash 19, total nitrogen 2, pectic substances 5, hemicelluloses 313 and cellulose plus lignin 492. Lignin was extracted from the bagasse as described by Eastwood & Hamilton (1968) for wood lignin, to give a yield of 234 g/kg bagasse. Bagasse was shredded in an Osterizer liquidizer and material which passed through a sieve of mesh size 1·5 mm was used to prepare the diet. The experimental diet consisted of the control diet thoroughly mixed with 100 g bagasse/kg.

Feeding techniques have been described previously (Stanley & Newsholme, 1985). Throughout the feeding period the body-weight of each mouse was measured every morning immediately before giving the diet. During the first 7 d of the 50 d feeding period the food consumption and faecal dry weight of six mice in each group were measured daily.

Methods for preparing liver extracts, assaying enzyme activities and protein, and statistical methods were as described previously (Stanley & Newsholme, 1985).

RESULTS

The effects of a 100 g/kg diet substitution of bagasse on the food consumption and faecal dry weight of mice given a high-carbohydrate diet are shown in Table 1. The group of mice given the bagasse diet had a lower mean food consumption than the control group but this difference was not significant.

The effect of a 100 g/kg diet substitution of bagasse on the body-weight gain of mice given a high-carbohydrate diet throughout the 50 d feeding period is shown in Table 2. The

Table 2. *The effect of bagasse on the body-weight, final 24 h food consumption, liver weight and liver protein concentration of mice*

(Mean values for seven mice per diet)

Diet ...	Control	Bagasse	Difference	SED (12 df)
Initial body-wt (g)	24·4	24·4	0	0·59
Final body-wt (g)	29·9	30·1	0·2	0·60
Food consumption:				
g/d	5·5	5·6	0·1	0·21
g/d per kg body-wt	187	187	0	7·4
Liver wt:				
g	1·8	1·7	−0·1	0·09
g/kg body-wt	60	57	−3	3·2
Liver protein concentration (mg/g liver)	230	244	14	10·2

SED, standard error of difference.

Values for the bagasse diet were not significantly different from the corresponding values for the control diet (Student's *t* test).

Table 3. *The effect of bagasse on the activities of some mouse liver enzymes expressed on a wet weight basis (μmol/min per g)*

(Mean values for seven mice per diet)

Diet ...	Control	Bagasse	Difference	SED (12 df)
Glucose-6-phosphate dehydrogenase (*EC* 1.1.1.49)	1·0	0·6	−0·4**	0·13
6-Phosphogluconate dehydrogenase (*EC* 1.1.1.44)	2·0	1·7	−0·3*	0·12
Malate dehydrogenase (oxaloacetate decarboxylating) ($NADP^+$) (*EC* 1.1.1.40)	8·5	10·1	1·6	0·81
ATP-citrate (*pro-3S*)-lyase (*EC* 4.1.3.8)	1·9	2·2	0·3	0·21
Pyruvate kinase (*EC* 2.7.1.40)	63·8	67·9	4·1	4·65
6-Phosphofructokinase (*EC* 2.7.1.11)	2·3	2·1	−0·2*	0·09
Fructose-1,6-bisphosphatase (*EC* 3.1.3.11)	13·5	14·8	1·3	1·23

SED, standard error of difference.

Values for the bagasse diet were significantly different from the corresponding values for the control diet (Student's *t* test): * $P < 0.05$, ** $P < 0.01$.

body-weight gain of the two groups was very similar, suggesting that the control and experimental groups of mice were closely matched.

The effects of 100 g/kg diet substitution of bagasse on the final 24 h food consumption, liver weight and liver protein concentration of mice given a high-carbohydrate diet is shown in Table 2. Preliminary experiments established that the activities of some hepatic lipogenic enzymes are directly proportional to the final 24 h food consumption of mice given high-carbohydrate diets (results not shown). The mean final 24 h food consumptions of the two groups of mice in the present work were not significantly different. Consequently, the differences in enzyme activity observed (see p. 418) can be confidently attributed to bagasse.

Table 4. *The effect of bagasse on the activities of some mouse liver enzymes expressed on a protein basis (nmol/min per g)*

(Mean values for seven mice per diet)

Diet ...	Control	Bagasse	Difference	SED (12 df)
Glucose-6-phosphate dehydrogenase (*EC* 1.1.1.49)	4·6	2·3	−2·3**	0·66
6-Phosphogluconate dehydrogenase (*EC* 1.1.1.44)	8·8	6·8	−2·0*	0·74
Malate dehydrogenase (oxaloacetate decarboxylating) ($NADP^+$) (*EC* 1.1.1.40)	36·8	41·5	4·7	3·25
ATP-citrate (*pro-3S*)-lyase (*EC* 4.1.3.8)	8·4	9·0	0·6	1·06
Pyruvate kinase (*EC* 2.7.1.40)	279	279	0	24·3
6-Phosphofructokinase (*EC* 2.7.1.11)	10·1	8·7	−1·4*	0·59
Fructose-1,6-bisphosphatase (*EC* 3.1.3.11)	58·3	60·7	2·4	4·81

SED, standard error of difference.

Values for the bagasse diet were significantly different from the corresponding values for the control diet (Student's *t* test): * $P < 0.05$, ** $P < 0.01$.

Table 5. *The effect of bagasse on the activities of some mouse liver enzymes expressed on a body-weight basis (μmol/min per kg)*

(Mean values for seven mice per diet)

Diet ...	Control	Bagasse	Difference	SED (12 df)
Glucose-6-phosphate dehydrogenase (*EC* 1.1.1.49)	65	32	−33**	10·3
6-Phosphogluconate dehydrogenase (*EC* 1.1.1.44)	122	94	−28*	10·5
Malate dehydrogenase (oxaloacetate decarboxylating) ($NADP^+$) (*EC* 1.1.1.40)	507	568	61	41·8
ATP-citrate (*pro-3S*)-lyase (*EC* 4.1.3.8)	116	123	7	13·2
Pyruvate kinase (*EC* 2.7.1.40)	3888	3822	−66	381·6
6-Phosphofructokinase (*EC* 2.7.1.11)	141	120	−21	11·4
Fructose-1,6-bisphosphatase (*EC* 3.1.3.11)	800	836	36	64·1

SED, standard error of difference.

Values for the bagasse diet were significantly different from the corresponding values for the control diet (Student's *t* test): * $P < 0.05$, ** $P < 0.01$.

There were no significant differences between the mean liver weight and mean liver protein concentration of the two groups of mice.

A variety of units have been used to express enzyme activities in nutritional studies (Freedland, 1967). In the present work, enzyme activities have been expressed on a liver fresh weight basis (Table 3), on a liver protein basis (Table 4) and on a body-weight basis

(Table 5). The values presented in Tables 3–5 show that a 100 g/kg diet substitution of bagasse significantly decreased the activities of glucose-6-phosphate dehydrogenase (*EC* 1.1.1.49) and 6-phosphogluconate dehydrogenase (*EC* 1.1.1.44) regardless of the units used and decreased the activity of 6-phosphofructokinase (*EC* 2.7.1.11) when expressed on a fresh weight or on a protein basis.

DISCUSSION

Glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase are key enzymes of the pentose phosphate pathway, one of whose functions is the synthesis of NADPH (Krebs & Eggleston, 1974). The most striking effect of bagasse was to decrease the activities of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. This observation indicates that the flux through the pentose phosphate pathway decreases when mice are given bagasse, presumably because the demand for NADPH in the liver has fallen.

Reducing equivalents in the form of NADPH are required for fatty acid, cholesterol and bile acid synthesis. Morgan *et al.* (1974) have shown that hepatic synthesis of cholesterol from acetate and the activity of cholesterol 7α-monooxygenase, which catalyses the rate-limiting step in bile acid synthesis (Myant & Mitropoulos, 1977), increase when rats are given bagasse. The increased rate of bile acid synthesis from cholesterol allows the bile acid pool to be maintained in the face of an increased rate of excretion of bile acids in the faeces. Hence, it might be expected that the demand for NADPH by the pathways of cholesterol and bile acid synthesis would increase in the livers of animals given bagasse. However, we find a decrease in the activities of the pentose phosphate pathway dehydrogenases in mice given bagasse. The results of our study could be reconciled with those of Morgan *et al.* (1974) if the increased demands for NADPH by the pathways of cholesterol and bile acid synthesis are more than compensated for by a decreased demand for NADPH by the pathway for fatty acid synthesis. However, studies of the effect of bagasse on the rate of hepatic fatty acid synthesis have not been reported so there is no experimental support for this suggestion. Alternatively the increased demand for NADPH for bile acid synthesis in animals given bagasse may be provided by increased flux through the pyruvate–malate cycle. In the present work the activities of two key enzymes of the pyruvate–malate cycle, malate dehydrogenase (oxaloacetate-decarboxylating) ($NADP^+$) (*EC* 1.1.1.40) (Rongstad & Katz, 1979) and ATP-citrate (*pro-3S*)-lyase (*EC* 4.1.3.8) (Löwenstein, 1971), increased in the livers of mice given bagasse but these changes were not statistically significant. Nevertheless, it is possible that a modest increase in flux through the pyruvate–malate cycle would be sufficient to provide the extra NADPH required in bagasse-fed animals.

Liver glycogen metabolized via the glycolytic pathway is an important precursor for fatty acid synthesis (Salmon *et al.* 1974). The activity of the key glycolytic enzyme, 6-phosphofructokinase, also decreased in the livers of mice given bagasse. This implies that glycolytic flux decreases in these animals, presumably because the demand for carbon for fatty acid synthesis has fallen.

The main finding of this work is that bagasse, like guar gum (Stanley & Newsholme, 1985), can change the activities of key lipogenic enzymes in the liver. However, whereas bagasse decreases the activities of some key hepatic lipogenic enzymes, guar gum increases them. Neither bagasse nor guar gum affected the activity of the key gluconeogenic enzyme fructose-1,6-bisphosphatase (*EC* 3.1.3.11). The definition of dietary fibre proposed by Trowell *et al.* (1976) includes both the natural fibre preparations, such as bagasse, and the gel-forming polysaccharides, such as guar gum. The very different effects of guar gum and

bagasse on pathways of hepatic lipogenesis reported here and previously (Stanley & Newsholme, 1985) emphasize the dangers of generalizing about metabolic effects of dietary fibre.

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