# Cellulose and pectin alter intestinal β-glucuronidase (*EC* 3.2.1.31) in the rat

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1. Groups of rats were given a fibre-free diet containing none or one of the three fibre components: pectin, cellulose or galactomannan.

2. After feeding for 16 weeks, total protein level and β-glucuronidase (*EC* 3.2.1.31) activity in the contents and mucosa of jejunum and ileum, and in the contents only of the caecum, were determined.

3. The pectin supplement reduced protein concentration in jejunal contents while cellulose reduced protein concentration in the ileal and caecal contents.

4. β-Glucuronidase activity of caecal contents was significantly reduced in both the pectin- and cellulose-fed groups.

5. Cellulose affected the β-glucuronidase activity of both the ileal contents while pectin reduced the β-glucuronidase of the ileal but not the jejunal contents.

6. Dietary fibre components did not significantly affect jejunal or ileal mucosal β-glucuronidase activity.

There is evidence suggested from epidemiological studies that a diet high in animal fat and low in fibre increases the risk of the development of large bowel cancer (Armstrong & Doll, 1975). In the rodent, fat is a promoter of 1,2-dimethylhydrazine colon cancer (Reddy *et al.* 1976). The addition of fibre to the rodent diet will reduce the incidence of chemically-induced bowel cancer (Freeman *et al.* 1978; Clapp *et al.* 1980). β-Glucuronidase activity in the faeces is raised in human colon cancer (Reddy *et al.* 1977). β-Glucuronidase (*EC* 3.2.1.31) is an enzyme which hydrolyses glucuronides, possibly in the lower small bowel but certainly within the colon. The activity of β-glucuronidase is dependent on bile flow (Roberton *et al.* 1982). While a high-fat diet would be expected to increase bile flow, and therefore indirectly β-glucuronidase activity, it is uncertain what effect, if any, dietary fibre would have on β-glucuronidase activity in both the mucosa and bowel contents. We have investigated the effects of pectin, cellulose and galactomannan on β-glucuronidase activity of the intestinal contents and of the mucosal activity in the small bowel.

## EXPERIMENTAL

### *Design*

Twenty-four weanling SPF Wistar rats from litters bred in our laboratory, were divided into randomly selected groups of six rats each and maintained in a temperature-controlled, air-conditioned room under a 12 h light–12 h dark cycle. Coprophagia and the consumption of fibre-containing materials was prevented by housing the animals in cages with wire-mesh floors and by providing the mothers and litters with a fibre-free diet after parturition.

At weaning (23 d), groups of rats were fed *ad lib.* on one of four diets. These diets were a basic fibre-free diet (NF) containing 80 g fat/kg, an NF diet containing 50 g pectin/kg (NFP), an NF diet containing 150 g cellulose/kg (NFC) and an NF diet containing 4 g galactomannan/kg (NFG) (Sigma Co., St Louis, MO). The chemical composition of the basic diet is summarized in Table 1.

* For reprints.

Table 1. *Composition of the fibre-free diet (mg/kg, unless otherwise indicated)*

| Ingredients | | Source |
|---|---|---|
| Skim-milk powder | 69·5 g | Anchor |
| Dried egg-white powder | 69·5 g | Zeagold Products |
| Maize starch | 723 g | NZ Starch Products Ltd |
| Water | 400 ml | |
| Safflower oil | 80 g | International Foods (1976) Ltd |
| Vitamins | | |
| Ascorbic acid | 400 | Cow & Gate* |
| Thiamine hydrochloride BP | 10 | |
| Riboflavin BP | 10 | |
| Pyridoxine hydrochloride BP | 3·32 | |
| Nicotinamide BP | 33·2 | |
| D-Biotin | 0·32 | |
| Calcium-D-pantothenate USP | 20 | |
| Folic acid BP | 2·52 | |
| Cyanocobalamin BP | 0·04 | |
| Acetomenaphthone BP | 5 | |
| D-α-Tocopheryl acetate | 49·6 | |
| Ferrous sulphate BP | 163·4 | |
| Zinc sulphate BP | 109·2 | |
| Manganese sulphate BP | 0·8 | |
| Copper sulphate BP | 5·32 | |
| Ammonium molybdate | 0·44 | |
| Potassium iodate | 0·84 | |
| Synthetic vitamin A | 3·44 | RA vitamin tablets† |
| Calciferol | 0·75 | Sterogyl 15‡ |
| Mineral mix | | |
| Cobalt chloride | 0·0011 g | Koch-Light Labs |
| Calcium carbonate | 18·31 g | Technical grade |
| Sodium chloride | 6·686 g | Technical grade |
| Potassium dihydrogenorthophosphate | 18·67 g | Analar BDH |
| Choline bitartrate | 0·2 g | Sigma |

* Vitamins equivalent to forty supplementary vitamin tablets for infants.
† Equivalent to 0·2 tablets.
‡ Equivalent to 0·05 ampoules.

At 16 weeks of age laparotomy was performed on non-fasting animals under Nembutal anaesthesia (60 mg pentobarbitone sodium/g body-weight) and the intestines removed by stripping from the mesentery. The length of the intestines from the ligament of Treitz to the ileo-caecal valve was measured. The first 10% of jejunum was taken immediately for histological studies to be reported elsewhere. The next 20% of jejunum was immediately placed on ice. Similarly, the last 10% of the ileum was taken for histological studies and the 20% proximal to that was immediately placed on ice. The 20% jejunal and ileal segments and the caecum were split open and their lumen contents carefully removed. The jejunal and ileal segments were then washed clean with ice-cold saline (9 g sodium chloride/l), dried by gently blotting and the mucosa carefully scraped off, weighed and stored at −70° for the later homogenization and enzyme analysis.

*Analytical procedures*

Lumen contents and mucosal scrapings were homogenized in ice-cold saline (ten times weight) using a Virtis homogenizer for 1 min at low speed (approximately 150 rev./min).

Table 2. *Mean weights (g) of rats at weaning and 16 weeks of age*
(Mean values and standard deviations)

| Dietary group† | At weaning | At 16 weeks Mean | At 16 weeks SD | Average wt gain |
|---|---|---|---|---|
| No fibre (NF) | 55·3 | 377·5 | 41 | 322 |
| NF + pectin | 55·8 | 364·8 | 14 | 309 |
| NF + cellulose | 51·8 | 322·7* | 25 | 271 |
| NF + galactomannan | 53·3 | 376·3 | 21 | 323 |

* Significantly different from NF value: $P < 0·05$.
† For details, see p. 21.

The homogenate was spun at 2000 ***g*** for 10 min and the supernatant fraction removed and stored at −70° until analysis.

Total protein of the homogenates was determined in duplicate using the modified Lowry method (Markwell *et al.* 1978).

β-Glucuronidase activity in the supernatant fraction was maintained at pH 5·5 with citrate phosphate buffer and assayed using 0·01 M-phenolphthalein–glucuronic acid as the substrate and measuring the amount of phenolphthalein liberated. The method was carried out using a Technicon AutoAnalyzer (model AA1). Peaks of activity were read using a microcomputer. Tests were done in duplicate and controls run with each batch of tests.

### *Statistical analysis*

Values were analysed using the two-tailed unpaired Student's *t* test.

## RESULTS

Throughout the experimental feeding period the rats maintained good health. At 16 weeks there was a difference between the weight of the rats given the NF and the NFC diets (Table 2).

### *Protein measurement*

Ileal and caecal contents of the cellulose-fed rats had a protein concentration which was significantly lower than that of all the other dietary groups (Table 3). Similarly, the total protein of jejunal contents of the NFP group was lower than those fed on the NFG diet. Although there were some apparent differences in the jejunal mucosal protein compared with the other groups, these differences were not statistically significant.

### *β-Glucuronidase measurements*

β-Glucuronidase activity of lumen contents increased serially from small intestine through to caecum and followed the know numerical gradient of bacterial flora (Gorbach *et al.* 1967) (Table 4). Animals fed on NFP and NFC had reduced β-glucuronidase activity in both ileal and caecal contents ($P < 0·001$) compared with other dietary groups. In NFC-fed animals the β-glucuronidase activity in the jejunal contents was also lower. The jejunal mucosal β-glucuronidase activity (Table 4) of animals on the cellulose-supplemented diet was significantly lower than that for those on an NF diet ($P < 0·001$).

Table 3. *Effect of dietary fibre component on protein concentration (mg/g wet weight) in intestinal contents and mucosae of rats*

(Mean values and standard deviations)

| | Intestinal contents | | | | | | Mucosae | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Jejunum | | Ileum | | Caecum | | Jejunum | | Ileum | |
| Dietary group† | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| NF | 41·4 | 2·24 | 29·2 | 4·9 | 15·5 | 3·4 | 42·9 | 6·6 | 32·0 | 2·6 |
| NF + pectin | 32·2** | 5·8 | 31·2 | 7·8 | 16·7 | 4·3 | 31·0 | 10·5 | 38·6 | 6·3 |
| NF + cellulose | 32·8** | 3·1 | 17·9* | 4·9 | 5·0*** | 1·5 | 38·6 | 7·0 | 33·7 | 6·0 |
| NF + galactomannan | 46·4 | 1·7 | 22·7 | 7·0 | 12·7 | 2·8 | 44·7 | 7·4 | 32·0 | 9·7 |

NF, fibre-free diet.
Values significantly different from NF value: * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.
† For details, see p. 21.

Table 4. *Effect of dietary fibre components on β-glucuronidase* (EC 3.2.1.31) *activity (μg phenophthalein released/h at 37° per mg wet weight) in intestinal contents and mucosae of rats*

(Mean values and standard deviations)

| | Intestinal contents | | | | | | Mucosae | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Jejunum | | Ileum | | Caecum | | Jejunum | | Ileum | |
| Dietary group† | Mean | SD | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
| NF | 1·21 | 0·17 | 2·04 | 0·26 | 6·13 | 1·90 | 2·28 | 0·26 | 1·70 | 0·20 |
| NF + pectin | 1·27 | 0·38 | 1·20*** | 0·12 | 3·35* | 1·10 | 1·88 | 0·54 | 1·46 | 0·38 |
| NF + cellulose | 0·66*** | 0·13 | 0·90*** | 0·38 | 2·05** | 0·70 | 1·50** | 0·38 | 1·80 | 0·38 |
| NF + galactomannan | 1·24 | 0·35 | 1·44 | 0·94 | 7·68 | 1·74 | 2·04 | 0·50 | 1·55 | 0·30 |

NF, fibre-free diet.
Values significantly different from NF value: * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.
† For details, see p. 21.

## DISCUSSION

In the present study, the fibre components pectin, cellulose and galactomannan were added to a basic fibre-free diet. Although the balance of the nutrients was maintained, differing amounts of the fibre components were used. The amount of fibre component added was varied to be consistent with the expected amount in the 'natural' diet.

During the study the rats remained healthy and their growth was satisfactory with average weight gains which were not markedly different between the dietary groups. This satisfactory weight gain and the maintenance of good health over the prolonged period of the present study suggested that possible differences in the palatability of the diet did not lead to malnutrition. With the prolonged period of the experiment (over 16 weeks) in which growth and weight were carefully monitored it was considered unnecessary, as well as impractical, to pair-feed the animals.

The protein concentration and the β-glucuronidase activity of the lumen contents and

the intestinal mucosa are two markers which can be used to monitor changes resulting from different diets. In the present study, cellulose was associated with a reduced protein concentration in the contents of both the ileum and caecum. Differences in protein concentration of the lumen contents may be explained by the variability of gel-forming and protein-binding properties of different fibres, a variability in transit time through the small intestine (Bueno *et al.* 1981) and a variability in fibre digestion. In a series of studies we have shown that pectin and hemicelluloses such as galactomannan are almost completely degraded in the gastrointestinal tract but cellulose is less completely degraded (Holloway *et al.* 1978, 1980, 1983).

Those rats on a cellulose-supplemented diet had significant reductions in the β-glucuronidase activity of the contents in the jejunum, the ileum and the caecum. With pectin supplementation there was a significant reduction of β-glucuronidase activity in the ileum and the caecum. Vegetarian and grain-fed rats have low faecal β-glucuronidase activity (Goldin & Gorbach 1976, 1979; Reddy *et al.* 1977). Attempts to alter the faecal β-glucuronidase activity by dietary means have been equivocal (Goldin *et al.* 1978; Indira *et al.* 1980; Bauer *et al.* 1979). Bile flow is an important factor in regulating β-glucuronidase activity (Roberton *et al.* 1982). Bauer *et al.* (1979) found the β-glucuronidase activity in the faeces of rats given dietary citrus pectin was very high. The 190 g fat/kg diet used by Bauer *et al.* (1979), in contrast to the 80 g fat/kg diet used in the present study, may have accounted for the high β-glucuronidase activity. Thus the apparent differences between these two studies may be related more to the fat content of the diet than to the pectin content.

The origin of β-glucuronidase activity in the lumen contents of the jejunum is uncertain. The very low jejunal bacterial population makes it likely that β-glucuronidase has originated from the mucosa of the jejunum. Studies on the enzyme activity of the various segments along the gastrointestinal tract show that β-glucuronidase activity is higher in the left colon than in the right colon and higher in the duodeno-jejunum than in the ileum but with activities in the large intestine significantly greater than in the small intestine (Celik *et al.* 1983).

Further studies are necessary to determine the origin of intestinal β-glucuronidase under different dietary conditions. The cellulose- and pectin-associated reduction in β-glucuronidase activity in caecal contents may reflect an effect of these substances on bile flow, with a reduction in intestinal neoplasia promoters.

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