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CHANGES IN pH AND LEVELS OF β-GLUCOSIDASE, β-GLUCURONIDASE AND REDUCING ACTIVITY AS FOOD RESIDUE PASSES ALONG THE MOUSE COLON

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The contents were collected from successive regions of the colon of Swiss mice. Analyses show that the pH rises by about 0.55 pH units between the cecum and the distal colon. The level of β-glucuronidase falls sharply, typically 2 to 3 fold, as food residue leaves the cecum. This is followed by a large rise in the distal colon, around 1.6 to 4 fold, about half of which is due to the concentrating effect caused by loss of water. Measurements were also made of nonspecific reducing activity, the level of which rises by about 74% along the colon mainly because of water loss. For each of the above parameters similar results were observed using old and young mice and with diets high and low in fat. Studies indicated that the reducing activity is nonenzymic.

INTRODUCTION

To gain a better understanding of gastrointestinal function, particularly in regard to colon carcinogenesis, many analytical studies have been made of the contents of the cecum and colon (eg 1–4). However, relatively little attention has been paid to changes in composition of food residue as it passes along these organs. Such changes could be of considerable significance. For instance, faeces expelled from a human or experimental animal might have important differences from that present in regions of the colon where tumors develop.

In this study samples were collected from the cecum and from the remaining colon of Swiss mice. Each sample was analyzed for pH, β-glucuronidase, β-glucosidase and nonspecific reducing activity. The study utilized mice fed both high and low fat diets and also both old and young mice. Reducing activity was partially characterized.

MATERIALS AND METHODS

Mice

Swiss mice were used from a colony maintained in our animal house.

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Table. *Composition of diets.*

Ingredients	*Diet, % composition by weight*		
	Control	*F6*[a]	*F23*[a]
Chow[b]	100	67	81
Corn oil[c]		1.9	18.5
Oleic acid[d]			0.28
Wheat starch[e]		31	
Fat content[f]	5.5	5.5	23
Nutrient/calories[g]	100	61	61

[a] The diets were prepared approximately once per week by mixing the ingredients with water until a soft consistency was obtained. They were stored at 4°C.
[b] Rodent Laboratory Chow (Ralston Purina Co, St Louis, Mo).
[c] 'Mazola' (Best Foods, San Juan, Puerto Rico).
[d] From Fisher Scientific Co, Fair Lawn, NJ.
[e] From United States Biochemical Corp, Cleveland, Ohio.
[f] Final content of fat calculated on a dry weight basis.
[g] Relative content of vitamins, minerals, protein and fiber per 100 calories.

Diet and treatment

Mice were housed in a temperature-controlled room with a 12-h light-dark cycle. At age 33 days they were fed one of 3 diets (see Table) for a period of approximately 6.5 mo. Another group of mice were fed the control diet until aged approximately 17 mo.

Preparation of sample

Mice were sacrificed by cervical dislocation. The cecum was opened and the entire contents collected (sample A). The rest of the colon was divided into 3 sections of equal length and all contents were collected from each section. These are samples B (proximal), C(mid-colon) and D(distal). Each sample was mixed and then divided in two. One part was used for pH determination. The remaining sample was homogenized in 100 mM sodium phosphate, pH 7.0. A supernatant sample was prepared by extraction with 0.1% Triton X-100 using the procedure described previously (4) except that the first centrifugation step was at 1500 × g for 1 min. Where indicated, a supernatant sample was also prepared from the contents of the last 10 cm of the ileum.

pH determination

The sample was vigorously mixed with water (0.9 ml per 100 mg) and the pH measured using a glass calomel combination electrode (Sigma Chemical Co, St Louis, Mo).

Reducing activity

Nonspecific reducing activity was determined in fecal supernatant samples by measuring the reduction of 2-(p-iodophenyl)-3-(p-nitrophenyl)-5-phenyltetrazolium chloride hydrate (INT). The method was described previously ('INT reductase', reference 4) except that the pH was 10.0 and the incubation time was 13 min.

β-Glucosidase

The method was based on that of Goldin & Gorbach (5). The incubation was in a volume of 0.5 ml with a final concentration of 3 mM p-nitrophenyl-β-D-glucoside

(Sigma) 100 mM sodium phosphate, pH 7.0, and 0.2 ml of fecal supernatant sample. Control incubations lacked substrate. After 1.5 h at 37°C the reaction was stopped by adding 2.25 ml of 10 mM NaOH and the extinctions taken at 420 nm.

β-Glucuronidase

Fecal supernatant samples were assayed according to Reddy *et al.* (6) and Goldin & Gorbach (5). The incubation was in a volume of 0.5 ml with a final concentration of 4 mM phenolphthalein glucuronide (Sigma), 90 mM sodium phosphate, pH 7.0, and 0.25 ml of sample. Control incubations lacked substrate. After incubation for 2 h at 37°C the reaction was terminated by addition of 2.25 ml of stopping mixture (56 mM glycine-NaOH in 1.1% trichloroacetic acid (TCA)) giving a final pH of approximately 10.3. Extinctions were then measured at 550 nm.

Water content

In order to compare samples, attention must be paid to the decrease in water content as food residue passes from the ileum to the anus. The results (except for pH) were therefore calculated both with and without correcting for differences in water content and in each case are presented relative to sample D. The mean value of the water content at each of the intestinal regions was calculated by collecting samples as above except that different mice were used. Each sample was weighed and dried at about 75°C until it reached a constant weight.

Statistical analyses

Data were initially analyzed by two-way analyses of variance (one-tailed). Significant differences were further analyzed by the Student-Newman-Keuls test. Where indicated, the two-tailed Student's paired *t*-test was used. In all cases P values less than 0.05 were considered significant.

RESULTS

Samples A–D were prepared from the intestinal contents of mice and were analyzed for β-glucosidase, β-glucuronidase, reducing activity and pH. In young mice the observed water content was 79.3, 79.2, 82.7, 70.7 and 56.2% in ileum and in samples A–D respectively.

Figure 1 depicts the results using mice fed the control diet for 6.5 mo (group 1). Analyses were also done on: male and female mice treated as above except that the diet was F6 or F23 (15 mice in total, group 2); and, 8 female mice fed the control diet for approximately 17 mo (group 3). In each case, the results were similar to those shown in Fig. 1 except where indicated.

As food residue passes from the cecum (sample A) and along the remaining colon (samples B, C and D), there is a rise in pH. In old mice (group 3) the pH of sample B was lower than that of sample A by 0.27 pH units. For young mice (groups 1 and 2), the average rise in pH between sample A and samples C and D was 0.55 pH units ($P<0.001$ using the paired *t*-test).

The data for β-glucosidase and β-glucuronidase resemble each other and indicate a sudden drop in wet wt activity from the cecum to the proximal colon (sample B) followed by a rise in the distal colon (sample D). However, about one-half of the latter rise reflects a simple concentrating effect due to the decrease in water content. In old mice (group 3) the values of β-glucosidase in samples A and B were lower (59

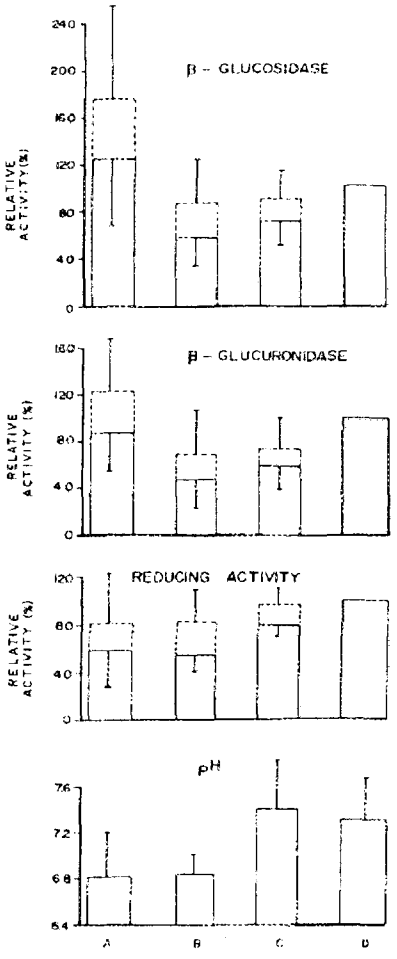

Fig. 1. *Relative activity of β-glucosidase, β-glucuronidase and reducing activity in samples A–D from 5 male and 5 female mice fed the control diet for 6.5 mo (D = 100%).* The results are shown as activity per mg wet wt (continuous line) and after correcting for differences in the water content between each sample and that of sample D (discontinuous line). The actual activity in sample D was 3.92 ± 1.45 nmoles/h/mg (mean ± SD; β-glucuronidase, 14.5 ± 5.3 nmoles/h/mg (β-glucosidase), and 10.4 ± 3.4 nmoles/13 min/mg (reducing activity). Activities are calculated in terms of original faeces used for sample preparation. The pHs of samples A–D are also shown. All trends (by two-way analyses of variance) show $P<0.005$ except that after correcting for differences in water content β-glucuronidase shows $P<0.0025$ and reducing activity is no longer significant. β-Glucosidase and β-glucuronidase (wet wt activity) and β-glucosidase (after water correction) all show B *vs* A and B *vs* D $P<0.001$. Also, β-glucosidase (wet wt activity) A *vs* D shows $P<0.01$. β-Glucuronidase (after water correction) shows A *vs* D $P<0.025$, B *vs* D $P<0.01$ and B *vs* A $P<0.001$. For wet wt reducing activity A *vs* D and B *vs* D both $P<0.001$. For pH A *vs* C and B *vs* C both $P<0.001$; A *vs* D and B *vs* D both $P<0.005$.

and 26% of D respectively for wet wt activity; both show $P<0.001$ compared with D). In comparison with the mice depicted in Fig. 1 the mice of groups 2 & 3 showed a more pronounced fall in β-glucuronidase activity between samples A and B (an approximately three-fold drop) and the value of B was lower (21–35% for D for wet wt activity).

For reducing activity there is a rise of about 74% in wet wt activity from the cecum and proximal colon to the distal colon. After correction for water content the true rise is no more than 20%.

The question arises as to whether the reducing activity present in the cecum is derived from that organ or whether it is already present in the ileal contents. Samples were therefore prepared from the contents of the distal ileum. The activity of these samples was roughly 36% of that in cecal samples of the same mice. We estimate that of the total activity present in faeces about 30% comes with ileal contents, about 54% from the cecum and about 16% is produced in the remaining colon.

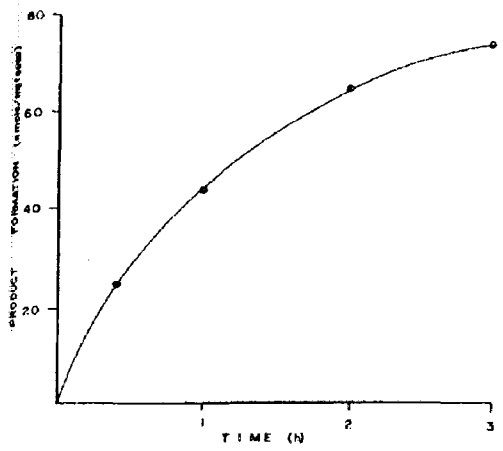

Fig. 2. *Relationship between product formation (INT oxidation) and incubation time after a faecal sample was assayed for reducing activity.*

Studies on reducing activity

When faecal homogenate was centrifuged (24 000 × g for 11 min) without Triton X-100, about 79% of the reducing activity was detected in the supernatant. Most of the activity is therefore not localized inside bacteria.

The activity has a pH peak of approximately 10.0. Three observations demonstrate that it is nonenzymic. First, it is fairly stable to heat, only about 20% being lost after preincubation at 100°C for 30 min. Secondly, the reaction rate increases with temperature. It is about 4–5 times faster at 62°C than at 25°C and is about 1.5–3 times faster at 98°C than at 62°C. Thirdly, about 64% of the activity is dialyzable (molecular weight cut-off: 6000–8000). Neither the dialyzable nor the nondialyzable components are enzymes as indicated by a similar response to temperature (25, 68, 98°C).

The reaction rate increases linearly with the quantity of faecal supernatant sample added. However, the rate steadily slows as the incubation time rises. Fig. 2 show a typical result. Even after 3 h there was still appreciable activity and it was therefore not practicable to measure the total quantity of activity by allowing it to be fully consumed. Measuring the reaction rate over the first 13 min of incubation, as used in the assays reported here, is considered a valid method to quantitate the activity.

DISCUSSION

The results presented here show that as food residue moves from the cecum and through the colon the wet wt concentration of nonspecific reducing activity rises by about 74%. This is largely accounted for by loss of water. Of the activity present in faeces about 30% originates from the ileum, 54% from the cecum and only about 16% from the rest of the colon.

Other observations reported here indicate that 79% of the activity is recovered from the supernatant when faecal homogenate is centrifuged in the absence of detergent. The response of the activity to temperature and to dialysis show it to be nonenzymic. Its most likely origin seems to be an intestinal wall secretional though we cannot discount the possibility that a bacterial secretion is at least a partial source.

β-Glucosidase and β-glucuronidase are bacterial enzymes and gave similar results to each other. The large fall in activity as food residue moves out of the cecum was unexpected. It suggests that the main source of each activity is the cecum but that as the cecal contents move further along the colon they become diluted by food residue with a low bacterial count passing directly from the ileum. As this material moves towards the distal colon, the activity of both enzymes increases about 1.6 to 4.8 fold (ie between samples B and D). After correcting for the concentrating effect due to water absorption the rise is about 1.1 to 2.9 fold.

For young mice there was an average rise in pH of 0.55 as food residue moves from the cecum to the distal colon. Jacobs & Lupton (3), in their study of rats, reported a pH rise of about 0.2 pH units as food residue passes from the proximal to the distal colon. However, they also observed an apparent pH fall between the cecum and the proximal colon; this was seen by us only in old mice, not in young.

In general, diet and the age of mice had no pronounced effect on the gradients along the colon of the biochemical parameters under study. We will report elsewhere our observations on the effect of the three diets studied here on the actual values of pH and of the three activities analyzed.

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