

DIETARY FIBRE AND THE MOUSE COLON: ITS INFLUENCE ON LUMINAL pH, REDUCING ACTIVITY AND BILE ACID BINDING

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SUMMARY

Female, Swiss mice were fed semi-synthetic diets for 33 days. The diets were fibre-free (FF) or supplemented with corn bran (CB) 12%, wheat bran (WB) 12%, alfalfa (AL) 12%, pectin (P) 6%, cellulose (CL) 6%, or lignin (LG) 6%. Fibre caused little hyperplasia of the colon mucosa. The number of cells per crypt was increased 9–13% and the crypt column length by 14–19% in the CL, AL and LG groups. CB caused rather less hyperplasia, WB less again and P caused none. The colon mucosal DNA content was approximately 5–10% lower in mice given supplemental fibre. The pH of the contents of the distal colon was apparently unaffected by fibre. Measurement of a non-specific, non-enzymic reducing activity indicated that activity was doubled by AL and LG, lowered 41% by CB but little changed by WB, CL and P. The deoxycholate binding capacity of the colon contents was increased 3–4-fold by LG, whereas the other fibre sources were without appreciable effect. This high binding capacity by LG was also seen in the material used for diet preparation. Analyses of the contents of the caecum and of the remaining colon indicated that as food residue passes from the caecum to the remaining colon little change in binding capacity occurs.

Key words: Dietary fibre; Mouse colon; Bile acid binding; Colon cell kinetics.

INTRODUCTION

Burkitt's hypothesis that colon cancer is caused by an inadequate intake of dietary fibre has been intensively researched [5]. Studies of chemically-induced colon tumours in experimental animals have indicated that some sources of fibre are indeed protective, such as wheat bran [28,30], cellulose [9,10,39] and perhaps lignin [29]. By contrast other sources of fibre have little effect on experimental colon cancer such as pectin [2,3,10,40] and alfalfa [27,40] while corn bran

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actually seems to enhance tumour formation [1,6,29]. These observations cannot be explained in terms of the various known actions of fibre on colon function. We therefore decided to explore this problem.

In this study mice were fed a fibre-free diet or diets supplemented with various types of fibre. The colon and its contents were then analysed for parameters thought to be relevant to colon cancer:

(a) *pH of colon contents*: Fibre lowers this [8,13,16,18,21,38], an action conjectured to be protective [16,36].

(b) *Non-specific reducing activity*: The colon contents are highly active metabolically and this includes the reduction of numerous compounds [32]. A non-enzymic reducing activity present in the mouse colon has been characterized [35]. Possibly it affects carcinogen formation.

(c) *Binding of deoxycholate*: Bile acids are promoters of colon cancer [7,26,31] and can be bound by dietary fibre but to a highly variable extent [22,33,37]. However, the effect of passage through the intestine on the bile acid binding capacity of fibre is unknown. Some types of fibre, pectin being a possible example, might bind bile acids in the small intestine, so preventing their absorption, and carry them to the colon. There the fibre is partially digested and the bile acids liberated so promoting cancer.

(d) *Colon mucosal cell proliferation (DNA content and crypt size)*: Jacobs and colleagues [15,19,20] reported that wheat bran induces cell proliferation in the rat colon and hypothesised that this has a promoting action [14].

MATERIALS AND METHODS

Animals and diets

Female Swiss (ICR) mice (aged 39 days; mean wt. 22.8 g, S.D. 1.9 g) were used from a colony maintained in the University animal facilities. They were randomised into 7 groups (8 mice/group). The reference diet was fibre-free, semi-synthetic (FF) and contained casein 21.5%, dextrose 16%, starch 50%, corn oil 6%, mineral mix 5% (Bernhart-Tomarelli, from Teklad Diets, Madison, WI; number 170750), vitamin mix 1% (AOAC, from TEKLAD Diets; number 40055), choline 0.27%, inositol 0.025% and L-methionine 0.25%. To this was added corn bran (CB) containing 90% dietary fibre (G-regular, from Staley, Decatur, II), 12%; wheat bran (WB) containing 43% neutral detergent fibre (AACC certified, from Hard Red Spring Wheat), 12%; legal alfalfa meal (AL) containing 26.5% crude fibre (dehydrated whole alfalfa, from Legal Alfalfa, Barrhead, Alberta), 12%; citrus pectin, P (from Eastman Organic Chemicals, Rochester, NY, number P2569), 6%; cellulose, CL (from the General Filtration Division of Lea Chemicals Ltd., Toronto, grade BW 40), 6% or lignin, LG (from Aspen Wood Chips, Stake Technology Ltd., Oakville, Ontario, number 714 WW), 6%. Mice were fed ad libitum on the above 7 diets for 33 days and then killed.

Hyperplasia

Histological assessment: A portion of colon 1–2 cm from the anus was

removed, flushed with saline and placed in neutral buffered formalin. It was sectioned longitudinally and stained (H and E). On each slide, the height of 6 separate crypt columns was measured as was the number of cells lining one side of the crypt column [19].

Biochemical analysis: The remaining colon (excluding the caecum) was opened longitudinally. The distal half was washed under the tap and the mucosal layer scraped off using microscope slides. This separation technique was confirmed histologically. The scrapings were weighed and homogenised in 3.5 ml of 0.06 M potassium phosphate (pH 7.8). The homogenate was analysed for DNA by the method of Burton [4] as modified by Giles and Myers [12] and for protein [24].

Analysis of intestinal contents

The contents were collected from the caecum, the proximal half of the colon and from the distal half (up to the anus).

pH: A portion of the contents of the distal colon were mixed with deionized water (71 mg/ml) and the pH measured using a glass-calomel electrode (Sigma Chemical Co., St. Louis, MO; number E-4878).

Reducing activity: After measuring the pH the same sample was adjusted to 0.1 M sodium phosphate (pH 7.0), 0.1% Triton X-100 and homogenised using a Potter-Elvehjem homogeniser. It was centrifuged at $12,000 \times g$ for 22 min. The supernatant was analysed by incubating at pH 10.0 for 12 min at 25 °C with 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium chloride hydrate (INT; Sigma) [34].

Deoxycholate binding

Samples analysed were the contents of the caecum and of the combined proximal and distal colon. A portion of sample (approx. 20–80 mg) was incubated in 1 ml of 0.1 M sodium phosphate (pH 8.0) containing 10 mM [¹⁴C]deoxycholate (Amersham, Oakville, Ontario) for 90 min at 37 °C [25]. Tubes were centrifuged ($1500 \times g$ for 30 min) and an aliquot counted. Binding data are presented as % bound per mg. Data were statistically analysed using Student's *t*-test.

RESULTS

Weight gain

The FF group gained slightly more weight than the other groups (4.3 vs. 2.8–3.8 g).

Colon mucosal DNA content and crypt size

There was no indication of increased cell proliferation induced by dietary fibre when assayed by biochemical analysis (DNA content; Table 1). Indeed, mice given fibre had roughly a 5–10% lower DNA content than FF mice. However, histological measurements showed a small degree of colon mucosal hyperplasia induced by CL, AL and LG (9–13% increase in cells/crypt and 14–19% increase in crypt column length, mostly $P < 0.05$). CB caused somewhat less hyperplasia, WB less still while P had no effect.

TABLE 1

COLON MUCOSAL DNA CONTENT, CRYPT SIZE AND COLON CONTENTS ANALYSES IN RELATION TO DIETARY FIBRE

Diet	Colon contents*		Colon mucosa*			
	pH	Reducing activity	DNA/protein ($\mu\text{g}/\text{mg}$)	DNA/wet wt ($\mu\text{g}/\text{mg}$)	No. of cells/crypt column	Crypt column length (μM)
FF	8.42 \pm 0.21	7.36 \pm 3.38	74.6 \pm 11.7	5.90 \pm 1.10	23.2 \pm 1.8	187 \pm 24
WB	8.19 \pm 0.22	7.44 \pm 1.71	69.6 \pm 14.5	5.48 \pm 1.36	24.3 \pm 1.4	211 \pm 26
CB	8.55 \pm 0.34	4.32 \pm 0.76	70.7 \pm 12.8	5.44 \pm 1.28	24.6 \pm 1.6	219 \pm 23 ^c
CL	8.43 \pm 0.25	7.01 \pm 3.99	67.9 \pm 11.7	5.57 \pm 1.38	25.3 \pm 2.5 ^c	223 \pm 46 ^c
P	8.14 \pm 0.45	6.49 \pm 3.23	71.3 \pm 11.1	5.11 \pm 0.97	23.2 \pm 2.0	195 \pm 10
AL	8.54 \pm 0.24	15.17 \pm 1.63 ^b	67.4 \pm 8.9	5.27 \pm 1.43	26.1 \pm 1.9 ^c	214 \pm 27 ^c
LG	8.42 \pm 0.23	13.00 \pm 4.32 ^c	69.0 \pm 11.1	5.69 \pm 1.00	25.3 \pm 2.6	216 \pm 22 ^c

*Mean \pm S.D.; each mean represents 7–8 observations.

^bSignificantly different from FF; $P < 0.001$.

^cSignificantly different from FF; $P < 0.05$.

TABLE 2

DEOXYCHOLATE BINDING BY SOURCES OF DIETARY FIBRE AND BY COLON CONTENTS

Diet or dietary fibre	Binding by fibre (%/mg)	Binding by colon contents ^a	
		Caecum (%/mg)	Remaining colon ^c (%/mg)
FF	—	0.15 ± 0.03	0.20 ± 0.09
WB	0.59	0.14 ± 0.04	0.16 ± 0.03
CB	0.37	0.18 ± 0.05	0.23 ± 0.05
CL	0.14	0.15 ± 0.07	0.14 ± 0.07
P	0.25	0.19 ± 0.08	0.20 ± 0.06
AL	0.45	0.22 ± 0.19	0.21 ± 0.06
LG	1.57	0.61 ± 0.25 ^b	0.61 ± 0.12 ^b

^aMean ± S.D.; each mean represents 7–8 observations.

^bSignificantly different from FF; $P < 0.05$.

^cFrom the end of the caecum to the anus.

Colon contents analysis

Dietary fibre did not significantly affect the pH of the contents of the distal colon. However, non-specific reducing activity was much altered. Compared with mice given the FF diet, activity in those fed CB was lower by 41% ($P = 0.056$) and was approximately doubled by AL ($P < 0.001$) and LG ($P < 0.05$). WB, CL and P were without effect.

Deoxycholate binding by sources of fibre and by colon contents

LG (i.e. the material used for diet preparation) had a several-fold greater deoxycholate binding capacity than the other sources of dietary fibre (Table 2). Of the other types, WB and AL showed the strongest binding and CL the weakest. Compared to mice fed the FF diet the colon contents of mice given LG had a 3–4-fold increase in binding capacity ($P < 0.05$) but the other fibre sources were without appreciable effect. There was little difference between the contents of the caecum and of the remaining colon (i.e. proximal plus distal colon).

DISCUSSION

It was the objective of this study to uncover changes induced in the function of the colon and its contents which might explain why different types of dietary fibre have markedly different effects on experimental colon cancer. However, none of the parameters studied appear to explain this.

Only a small degree of fibre-induced hyperplasia was observed. This was only evident using histological measurement (number of cells per crypt column and crypt column length) but not with biochemical measurement (DNA con-

tent). This hyperplasia was strongest with CL, AL and LG, followed by CB, then WB while P was without effect. Jacobs and colleagues [15,19,20] reported that WB induces hyperplasia of the rat colon while P does not [17]. It is possible that a different picture would have emerged had we measured the rate of cell formation. For instance, Lipkin et al. [23] observed that in various human populations colon cancer risk is associated with an increased replication rate of colon mucosal epithelial cells but not with the total number of cells per crypt.

The failure of supplemental fibre to significantly affect the pH of the colon contents was surprising. In most rat and human studies fibre has been found to lower the pH [8,13,16,18,21,38] but nothing has been previously reported with respect to the mouse. Possibly a higher fibre level would have produced a pH shift.

The non-specific reducing activity of the colon contents was unaffected by WB, CL and P but was doubled by AL and LG and lowered 41% by CB. This activity has been previously characterised [35]. INT, the synthetic electron acceptor used, can accept electrons from $FADH_2$ but not from NADPH. Since numerous compounds are reduced in the colon [32], our observations warrant further investigation to determine their relevance to xenobiotic metabolism in general and to colon carcinogenesis in particular.

Many studies have been made into dietary effects on faecal bile acid output. Since bile acids are promoters of colon cancer [7,26,31], the inference is that an increased faecal concentration will enhance the disease. However, no attention has been paid to the crucial question of the proportion of the bile acids that are bound to the colon contents. This study is apparently the first to consider this important question. The outstanding observation concerning deoxycholate binding is that LG has a strong binding capacity which is still present when food residue reaches the colon. A strong bile acid binding by LG has been previously reported [11,33,37]. Thus LG should sharply reduce bile acid reabsorption in the small intestine. Any increase in faecal bile acid concentration is unlikely to promote colon cancer since much of the extra bile acid is bound. These observations also indicate that lignin may be an effective hypo-cholesterolaemic agent.

With regard to the 5 other sources of fibre none resulted in the colon contents having a significantly raised deoxycholate binding capacity as compared to the fibre-free diet. Also worthy of note is the stability of the binding capacity as food residue moves from the caecum to the more distal colon (i.e. there is no evidence of bacterial metabolism lowering the binding capacity so liberating bound bile acids).

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