Collagen alteration in an animal model of colonic diverticulosis

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Abstract

Background—Changes in the structure and integrity of the colon dependent on collagen content and crosslinkage occur with age.

Aims—This study using an animal model examines colonic collagen content and crosslinkage over the lifetime of rats on fibre deficient and higher fibre diets.

Methods—Two groups of 20 rats were fed either a fibre deficient diet (1.7 g NSP (non-starch polysaccharide)/100 g) or a higher fibre diet (13.3 g NSP/100 g) for 18 months. Diverticula were identified by postmortem examination. Caecal and colonic contents were weighed and assayed for short chain fatty acids. Collagen solubility in weak acid was measured to give an indication of the nature and amount of crosslinks in the collagen of the bowel wall.

Results—The incidence of colonic diverticula was greater (42.1% fibre deficient rats; 0% higher fibre rats). Colonic collagen solubility index in fibre deficient rats was significantly lower than higher fibre diet fed rats ($p < 0.001$ in all four sections of the large bowel). Rats with diverticula had the lowest solubility index ($p < 0.001$ in all four sections of the large bowel). Higher fibre diet rats had increased caecal and colonic contents, caecal and colonic tissue wet weights, and greater caecal short chain fatty acids. Fibre deficient diet fed rats had more pathological abnormalities.

Conclusions—This animal model permits a study of the relation between collagen crosslinkage and the development of colonic diverticulosis. A higher fibre diet protects against collagen crosslinking and this is related to a decreased incidence of diverticula.

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Keywords: collagen, colon, crosslinking, diverticulosis, fibre, SCFA.

Colonic diverticulosis is a human benign condition particularly of the sigmoid colon in an elderly population, a frequent concomitant of the aging process. The condition is rare before the age of 30 years in Britain, but a third of the population have colonic diverticulosis by the sixth decade and a half by the ninth decade.¹ Aging is also associated with a progressive deterioration in the strength of the colonic wall and may have a synergistic influence on the development of colonic diverticula.² There is a

thickening of the circular and longitudinal muscle coats together with a progressive increase in the thickness of collagen and elastin tissue of the colon wall.³

Painter has suggested that the development of colonic diverticulosis was due to a deficiency of dietary fibre, which led to higher pressures in the colon because of the strong contractions necessary to propel and expel the small hard stools associated with a very low fibre diet.¹ The increased intracolonic pressure causes mucosal transmural herniation at the vulnerable regions where blood vessels enter the colonic wall.

Collagen is the most abundant protein in mammals and serves to maintain the structural integrity of connective tissues. The mechanical properties of the colonic wall depend on the individual components of its various coats, and their juxtaposition and interaction. The viscoelastic properties of collagen and elastin give the colon its expansibility, strength, and maintenance of shape.^{2,3} The submucosa of the large bowel is composed almost exclusively of collagen fibrils⁴ and as such this is the layer that plays the most important part in maintaining the integrity and properties of the colonic wall.

Pathological changes in the colonic wall also develop as a feature of the aging process and could be secondary to a decline in the structural and mechanical integrity of the various layers of the large bowel wall.²

Collagen is known to have inter and intramolecular crosslinks that stabilise, and give strength to the tissue in which it is located. Two pathways of crosslinking have been identified in collagen, one based on lysine aldehydes and the other on hydroxylysine aldehydes. Lysine and hydroxylysine are deaminated to produce allysine and hydroxyallysine respectively. The aldehyde is then free to react spontaneously in a variety of ways. The reaction of either type of aldehyde with the ϵ -amino group of lysine or hydroxylysine results in the production of reducible intermolecular crosslinks. Initially all possible reactions produce a Schiff base type crosslink also known as an aldimine type linkage. These intermediate forms are susceptible to cleavage by dilute acid. Hydroxyallysine derived intermediate crosslinks can also undergo a further spontaneous reaction to form a ketoimine type structure in vivo. This is known as an Amadori rearrangement. The ability for the intermediate crosslink to form the acid stable ketoimine, depends on whether it was derived from allysine and hydroxylysine or hydroxyallysine and lysine. The first of these is unable to form the ketoimine, the second is able to do this. The

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ketoimine type crosslink is stable to weak acids. The amount of acid labile crosslinks decreases with the maturation of a tissue.⁵ The initial aldehyde derived crosslinks undergo maturation to produce more stable forms, which are stable in weak acids. The mature products of crosslink formation are a complex area of study. By stabilising the molecular arrangement within collagen fibrils, intermolecular crosslinks confer stability to the collagen containing tissue.^{5,6}

Collagen solubility in weak acids is indirectly related to the amount of acid labile crosslink forms in the collagen of the tissue under study. In this study solubility index is defined as a ratio of acid soluble collagen to acid insoluble collagen. A lower solubility index indicates a higher degree of acid stable crosslink forms. We have previously carried out a study on the solubility of colonic collagen from the human large bowel in relation to aging and colonic diverticulosis.⁷ The solubility of collagen in weak acids is known to be decreased with advancing age in certain tissues – that is, skin, vascular adventitia, and chordae tendinae of heart valves,⁶ and this is one of the bases for this study. Acid solubility gives an estimate of the strength of the collagen and hence the tissue in which it is located.

The hypothesis that colonic diverticulosis is caused by a low fibre diet was supported by recent work in which rats were fed diets containing low or high amounts of dietary fibre for 18 months.⁸ In these experiments the low fibre fed rats had a higher incidence of colonic diverticular than the high fibre fed rats.

In this study we used this animal model⁸ to measure the amount and structure of the colonic collagen and have related these measurements to the development of colonic diverticula.

Methods

ANIMALS

Forty male Wistar rats bred at the Western General Hospital Animal Unit were maintained in cages, on refined cat litter to avoid intake of the bedding. They were housed in groups of five and maintained on one of two

diets for 18 months. The rats were fed ad libitum for the duration of the study. There were 20 rats in each dietary group fed fibre deficient or higher fibre diets (Table I) similar to those used by Fisher⁸ (Special Diet Services, Lavendar Mill, Poole, Dorset, UK, prepared from one batch). Non-starch polysaccharides in the diet were measured by the Englyst method.⁹ The two diets used in the experiment differed in the fibre, protein, and utilisable carbohydrate content. The fibre deficient diet contained per 100 g dry weight, 1.7 g/100 g non-starch polysaccharide, 17.8 g protein, and 73.0 g utilisable carbohydrate. The higher fibre diet contained 13.3 g non-starch polysaccharide, 20.5 g protein, and 57.5 g utilisable carbohydrate. The lipid content of both diets was small and equal. The fibre deficient diet was fed in a paste form with added water and the higher fibre diet was eaten dry. The rats all had adequate supplies of protein at 17.8 g and 20.5 g/100 g in the two diets. The fibre deficient diet was based on wheat gluten, casein, and dried egg supplemented with lysine and threonine. The protein in the higher fibre diet was derived from wider sources. The utilisable carbohydrate in the fibre deficient diet was readily digested starch and present in greater amounts than the utilisable carbohydrate in the higher fibre diet which was of wider origins. The recommendations from the Zeneca 1994 working party¹⁰ were that a rodent diet should contain by weight at least 12.0±1.7% protein, 2.0±1% fat, 60±15% utilisable carbohydrate, and 3.6±2% fibre. The diets used in this experiment more than met these requirements.

The rats were bred specifically for this study and the parents of experimental animals were fed the appropriate diet for a period of one month prior to mating. Each group of rats were weaned onto the chosen diet after 17 days suckling and remained on that diet thereafter.

All rats were weighed and monitored at weekly intervals and the terminal body weight was measured. The health of the animals at the end of the experiment was assessed at post-mortem examination and weekly examinations of their fur, teeth, gait, mobility, and general activity were carried out for the duration of the study.

After 18 months the rats were killed by overdose of ether. Immediately after death a post-mortem examination was carried out by a consultant pathologist (AB). The condition of the intestines, in particular the colon, and the presence or absence of diverticulosis were recorded. The postmortem examination also examined all body layers, body contents, and the genitalia. The animals were identified by colour coding on their tails and were killed in batches – that is, the animals in each of the cages were killed in close sequence but in no specific overall order. The pathologist was not informed of the significance of the colour code. The postmortem macroscopical examinations were carried out systematically, in that the abdominal and pelvic cavities were first examined and then the other body cavities. The small bowel was released along its mesentery and examined in its totality. After removal

TABLE I Dietary composition of the two diets used in this experiment. The fibre, utilisable carbohydrate, protein, fat, and vitamin and mineral content are shown as g per 100 g of the total weight of the diet. Low fibre diet is mJ/kg wet weight as fed including the water content

Fibre deficient diet		Higher fibre diet	
Component	g/100 g dry wt	Component	g/100 g dry wt
Utilisable carbohydrate	73.0	Utilisable carbohydrate	57.5
Non-starch polysaccharide	1.7*	Non-starch polysaccharide	13.3*
(Soluble)	1.2	(Soluble)	3.1
(Insoluble)	0.5	(Insoluble)	10.2
Protein	17.8	Protein	20.5
(Wheat gluten)	8.9	(L-leucine)	1.4
(Casein)	8.0	(L-lysine)	1.0
(L-lysine hydrochloride)	0.3		
Corn oil	2.0	Crude oil	2.4
Vitamin mix	1.0	Vitamin mix	1.0
Mineral mix	4.5	Mineral mix	4.5
		(Calcium)	0.8
Metabolisable energy wet weight as fed	12.5 mJ/kg	Metabolisable energy wet weight as fed	11.9 mJ/kg

*Non-starch polysaccharide was measured by Englyst,⁹ other data from manufacturer's data base.

of the mesocolon the whole colon was displayed and examined along its length.

Histological assessment

In accordance with the work by Fisher *et al.*,⁸ three types of protrusion of the mucosa were identified: (1) Predisiverticular – that is bulging of the mucosa towards a gap in the muscle wall, but no actual protrusion. (2) Diverticula – that is a true protrusion of the mucosa through the intestinal muscle wall. (3) Bulges – that is protuberances through the gut wall through which all layers of the gut were present.

Only those bulges that fall into categories 1 and 2 above were recognised as diverticular in this study. The presence of a single diverticulum did not constitute diverticulosis. The diagnosis was based on the presence of multiple diverticula.

Other tissues removed for further analysis: (a) Caecum. This was washed in buffered mammalian Ringer's solution, measured, weighed, and retained for collagen measurements. (b) Colon. The colon was removed, washed, measured, and weighed and then divided into the defined quarters before being retained for collagen measurements. The defined quarters were termed ascending, transverse, descending, and sigmoid and corresponded to four equal sections from the caecum to the anal sphincter. In addition a section of each of these specimens was fixed and removed for further histological examination. (c) Tumours and organs and tissues with any other pathological abnormalities. These were removed and placed in formalin fixative for further detailed histological and pathological examination.

Caecal and colonic tissue and contents

Caecal and colonic contents were collected, weighed wet, and then freeze dried and reweighed. Short chain fatty acids were analysed by gas liquid chromatography.¹¹ The caecum and colon were carefully dissected and divested of any fat. The tissues were then rinsed in isotonic saline, blotted, and weighed.

Collagen content and solubility

The resected sections were washed in mammalian buffered Ringer's solution and 1 gram sections were homogenised and extracted for 48 hours in 0.5 M acetic acid (1 ml/100 mg wet weight tissue) at 4 °C to extract the acid soluble collagen.¹² The homogenate was centrifuged at 36 000 rpm for 60 minutes and the pellet and supernatant removed and stored separately. The supernatant was analysed for collagen content by measuring the hydroxyproline content.¹³ This fraction was termed acid soluble collagen.

The pellet from the acetic acid extraction was also analysed for hydroxyproline content by the above method after hydrolysis for 24 hours at 110°C with 6N HCl. This fraction was termed acid insoluble collagen. The total

collagen content of the tissues (mg/100 mg wet weight tissue) was calculated by the addition of acid soluble and acid insoluble fractions. The ratio of soluble collagen to insoluble collagen (the solubility index) was calculated using the methodology above and gives an indirect measurement of the degree of crosslinkage.

Statistics

The effect of colonic diverticulosis, rat weight, and the presence of tumours and any other abnormalities on the total collagen content and the collagen solubility was determined by multiple regression analysis and analysis of covariance.

Results

General health of animals

The health of all of the rats was assessed throughout the experiment, by examining the teeth, skin, movement, and body weight on a weekly basis. The animals fed on the fibre deficient diet weighed significantly more than those fed on the higher fibre diet at 18 months (Figure). Three rats from the fibre deficient diet group developed middle ear infection and were removed from the study.

Postmortem examination

All of the rats in the fibre deficient group had a bezoar in the stomach whereas none of the rats in the higher fibre group had a bezoar. Of the final 17 fibre deficient diet fed rats, seven (41.2%) had colonic diverticula compared with none in the higher fibre group. The diverticula were multiple in all instances. The diverticula were all found in the regions corresponding to the transverse and the descending

The weight gain of rats fed either a higher fibre (13.3 g NSP/100 g) or fibre deficient (1.7 g NSP/100 g) diet for 18 months. Age in weeks is plotted against weight in grams.

TABLE II Effect of lifelong (18 months) fibre deficient diet or higher fibre diet on the caecal and colonic contents of the rat. The wet tissue weights after blotting dry are shown together with the wet and dry weight of contents after freeze drying. Results are expressed as mean (SEM), and the *p* values relate to Student's *t* test

	Fibre deficient diet (n=17)	Higher fibre diet (n=20)	<i>p</i> Value
Caecal wall tissue weight (g)	1.17 (0.02)	1.45 (0.17)	<i>p</i> <0.001
Colonic wall tissue weight (g)	1.40 (0.02)	2.05 (0.06)	<i>p</i> <0.001
Liver weight (g)	19.6 (0.7)	18.7 (0.4)	NS
Caecal contents			
Wet weight (g)	3.49 (0.22)	5.93 (0.19)	<i>p</i> <0.001
Dry weight (g)	1.35 (0.13)	1.90 (0.08)	<i>p</i> <0.01
% Water	62.5 (1.65)	67.9 (0.8)	<i>p</i> <0.01
Colonic contents			
Wet weight (g)	2.05 (0.2)	3.56 (0.31)	<i>p</i> <0.001
Dry weight (g)	1.15 (0.14)	1.56 (0.15)	<i>p</i> <0.05
% Water	44.9 (2.8)	56.1 (1.46)	<i>p</i> <0.01

TABLE III Short chain fatty acid (SCFA) content of caecal and colonic contents of rats fed lifelong (18 months) fibre deficient or higher fibre diets. Results are expressed as mean (SEM) and *p* values relate to Student's *t* test, fibre deficient compared with higher fibre rats

	Fibre deficient diet (n=10)	Higher fibre diet (n=10)	<i>p</i> Value
Caecum			
SCFA n=10			
μmol/g dry wt	60.2 (10.3)	296.6 (18.4)	<i>p</i> <0.001
μmol/g wet wt	22.7 (3.0)	96.4 (6.8)	<i>p</i> <0.001
μmol/caecum	88.4 (12.9)	597 (49.6)	<i>p</i> <0.001
%acetic	70.0 (1.2)	61.9 (1.4)	NS
%propionic	16.1 (0.97)	12.95 (1.2)	<i>p</i> <0.01
%butyric	5.9 (0.4)	20.3 (0.53)	<i>p</i> <0.001
%isobutyric	3.0 (0.2)	2.8 (0.53)	NS
%valeric	3.2 (0.2)	1.6 (0.3)	<i>p</i> <0.001
%isovaleric	1.9 (0.1)	0.6 (0.07)	<i>p</i> <0.001
Colon			
SCFA n=10			
μmol/g dry wt	24.7 (3.5)	135.9 (12.1)	<i>p</i> <0.001
μmol/g wet wt	6.9 (2.2)	52.3 (3.1)	<i>p</i> <0.001
μmol/colon	19.9 (5.9)	34.6 (4.2)	<i>p</i> <0.001
%acetic	64.0 (3.6)	71.5 (2.1)	NS
%propionic	12.1 (0.64)	10.4 (1.3)	NS
%butyric	6.3 (0.65)	14.6 (1.48)	<i>p</i> <0.001
%isobutyric	5.9 (0.97)	1.0 (0.1)	<i>p</i> <0.001
%valeric	4.2 (0.55)	1.5 (0.17)	<i>p</i> <0.001
%isovaleric	6.2 (1.13)	0.84 (0.07)	<i>p</i> <0.001

regions of the colon. Histological examination showed no muscular thickening: there were no tinctorial changes in the collagen of the lamina propria or submucosa. No inflammatory changes of the acute type were seen but some of the diverticula were surrounded by chronic inflammatory cells. In general inflammation was not a prominent feature.

The fibre deficient diet fed rats also had significantly more pathological abnormalities and tumours of body tissues than the higher fibre fed rats (48 fibre deficient *v* nine higher fibre). In the higher fibre group there were

eight testicular tumours and one mesenteric fibrous nodule. In the fibre deficient group all rats had a small caecum, six rats had nodular and reticulated lungs (due to histologically confirmed desquamative pneumonitis), 14 rats had a pale or nodular liver (due to fatty infiltration and portal fibrosis), eight rats had a pale or nodular pancreas (due to an interlobular fibrosis), and 12 rats had testicular tumours. Eight rats were so obese that they had developed anterior extensive abdominal wall abrasions with local infection.

The wet weight, dry weight, and percentage water content of caecal and colonic contents were significantly less in the fibre deficient group compared with the higher fibre fed rats (Table II). The total caecal and colonic SCFA concentrations and amount per caecum or colon were less in the fibre deficient group (Table III, *p*<0.001). The molar proportion of butyric acid was higher and the proportion of propionic acid lower, in the caecal and colonic samples of the higher fibre group compared with the fibre deficient group (Table III), although this did not reach statistical significance in the colonic contents.

The caecal and colonic tissue weights were significantly higher in the rats fed higher fibre diet than those fed the fibre deficient diet (Table II, *p*<0.001).

Colonic collagen

The total collagen content as measured by hydroxyproline content was similar in both groups and was not affected by the presence of colonic diverticulosis (Table IV).

The acid solubility indices were significantly lower in the fibre deficient group than the higher fibre group for all four sections of the large bowel (Table IV, *p*<0.001). Those rats with colonic diverticulosis in the fibre deficient group had significantly lower solubility indices than those without diverticulosis (Table IV, *p*<0.001 for all four sections of the large bowel). The acid solubility index in the fibre deficient group decreased as the more distal portions of the gut were examined, but not in the higher fibre group (Table IV). The solubility index was significantly different between the ascending colon from fibre deficient diet fed rats with no diverticulosis compared with sigmoid colons in these rats and also ascending

TABLE IV Results of the analysis of total collagen and crosslinkage of collagen in the intestine of rats fed either a fibre deficient diet or higher fibre diet. Results are expressed as mg/100 mg wet weight tissue. Results are expressed as mean (SEM)

	Ileum	Caecum	Ascending	Transverse	Descending	Sigmoid
Total collagen (mg/100 mg wet weight tissue)						
High fibre diet						
No diverticulosis (n=20)	13.43 (0.25)	13.29 (0.29)	13.46 (0.25)	13.40 (0.29)	13.49 (0.31)	13.51 (0.24)
Low fibre diet						
No diverticulosis (n=10)	13.27 (0.23)	13.13 (0.24)	13.36 (0.25)	13.35 (0.24)	13.47 (0.32)	13.33 (0.32)
Diverticulosis (n=7)	13.37 (0.30)	13.23 (0.28)	13.19 (0.26)	13.33 (0.24)	13.36 (0.24)	13.51 (0.16)
Acid solubility index (soluble collagen: insoluble collagen)						
High fibre diet						
No diverticulosis (n=20)	0.075 (0.0016)	0.075 (0.0013)	0.075 (0.0020)	0.073 (0.0015)	0.073 (0.0016)	0.073 (0.0015)
Low fibre diet						
No diverticulosis (n=10)	0.065 (0.0018)** ³	0.058 (0.0015)* ²	0.047 (0.0007) ¹	0.043 (0.001)* ¹	0.037 (0.0006)*** ¹	0.032 (0.0007)*** ¹
Diverticulosis (n=7)	0.054 (0.0008)* ²	0.051 (0.0006)* ¹	0.039 (0.0012) ¹	0.033 (0.0003)* ¹	0.033 (0.0003)* ¹	0.028 (0.0002)*** ¹

***=*p*<0.001, **=*p*<0.01, *=*p*<0.05 compared with ascending region. ^a=*p*<0.001, ^b=*p*<0.01, ^c=*p*<0.05 diverticulosis compared with healthy colon. ¹=*p*<0.001, ²=*p*<0.01, ³=*p*<0.05 fibre deficient diet fed group compared with higher fibre diet fed group. The results were compared by Student's *t* test after analysis of variance.

colon in fibre deficient diet fed rats with colonic diverticulosis ($p < 0.001$). There was also a significant difference between the solubility index from animals fed a fibre deficient diet without diverticulosis and those with diverticulosis ($p < 0.001$).

Statistical analysis

Statistical analysis shows that neither rat body weight or tumours affect the total collagen content or the acid solubility index in fibre deficient diet fed rats or higher fibre fed rats. The only significant effect is from colonic diverticulosis in the fibre deficient fed rats.

Discussion

Studies of diverticulosis have tended to concentrate on colonic pressure and motility.^{1 14} There have been few studies of the structure and strength of the colonic wall in this disease. The strength and elasticity of the colonic wall is known to deteriorate with age.² Thomson *et al* have shown an increase in the collagen fibril numbers and a decrease in the collagen fibril diameter in the left colon of these patients.⁴ In a necropsy study we have shown that the extent of crosslinkage of collagen of the colon increases from the right side of the colon to the left where the colonic diverticula mainly occur.¹⁵

This study uses an animal model of dietary regimens that has been shown to protect against colonic diverticulosis in the rat.⁸ We therefore developed and used a rat model to study the effect of low and high fibre diets on colonic wall structure, collagen solubility in dilute acid, and bacterial fermentation in the adult offspring. This enables a study of total collagen content and solubility in these two animal groups with or without colonic diverticulosis. The study shows a similar profile of collagen solubility along the colon of rats fed a fibre deficient diet. In the fibre deficient diet fed rats the solubility index was decreased in the rats with colonic diverticula compared with those without colonic diverticula. The collagen solubility was significantly higher in the rats fed a higher fibre diet and there was no difference in solubility along the colon from proximal to distal sites. This suggests that rats fed a higher fibre diet retained a more flexible and elastic colon wall.

Colonic collagen is in a dynamic state, being produced and degraded continuously. Aging is associated with increased crosslinking between collagen molecules,⁵ which results in decreased solubility in weak acids, increasing stiffness, and resistance to enzymic digestion *in vitro*. The measurement of collagen solubility in weak acid, although not a direct measure of the amount of crosslinks present, was considered reasonable as an indicator for the analysis of the effect of colonic diverticulosis and diet on colon collagen. The measurement of collagen solubility gives an indirect, inverse measure of the amount of crosslinks that are insoluble in weak acid. The insoluble residue can be regarded as due to ketoimine crosslinks,

mature crosslinks of both the allysine and hydroxyallysine pathways, and advanced glycation products. The acid solubility of a crosslink in collagen depends on the presence of the crosslink in a Schiff base form. The mechanism by which a higher fibre diet can change this process is unclear.

One possible explanation for the observed decrease in acid solubility is that the turnover rate of colon collagen has been affected by some mechanism to produce a higher proportion of intermediate cross link forms that are acid labile. The mechanism may be that on a higher fibre diet there is increased mechanical stimulation of the colon wall, which may in turn increase the turnover rate. This does not, however, explain the differences in acid solubility seen in the fibre deficient group, which were all fed the same diet.

There may be many mechanisms working in unison or separately for the development of colonic diverticulosis. These include: (a) Dietary components (fibre, protein, utilisable carbohydrate). It has been shown that dietary fibre deficiency is a causative agent in the development of colonic diverticulosis. Protein in the diet may influence the collagen content and structure. The nature of the diet may also play a part in the function and perhaps structure of the colon wall. (b) Body weight of rats. Obesity may have a part to play in the development of colonic diverticulosis. There are two main reasons for obesity, overeating or eating the wrong type of food. There may be differences in the rate of absorption of nutrients in the small intestine from the two different types of diets. On an enriched fibre diet, the absorption of glucose may be extended over a longer time compared with fibre deficient diets. (c) Tumours and any other organ and tissue abnormalities. The presence of tumours may be related to structural changes in the bowel or may be secondary to structural changes general in the body.

Although our diets were isocaloric and differed in dietary fibre content, they also differed in protein and utilisable carbohydrate. The percentage difference between the two diets was 15% for the protein and 27% for the utilisable carbohydrate. The higher fibre diet was a commercial pelleted laboratory diet for rats. It proved impossible to compose a diet low in dietary fibre without changing the other major dietary constituents. The fibre deficient diet was supplied as a dry powder and was then reconstituted in water before feeding. It is possible but unlikely that these other constituents may have had a part to play in the development of the diverticula. The difference in the composition and consistency of the two diets may influence the function of the colon. The rheological properties of the contents of the colon are likely to be different in the two diets. The percentage water value for colonic contents is different on a fibre deficient diet, 44.9% against 56.1% (Table II, $p < 0.01$). The caecal content was 62.5% water and on the higher fibre diet 67.9% water (Table II, $p < 0.01$). The wet weight and dry weight of the colonic contents was significantly greater in the

higher fibre group. It is also possible that the short chain fatty acids in the colonic contents may also influence the consistency of colonic contents. The consequences of lifelong exposure to a fibre deficient diet are also shown in the differences in the caecal and colonic weights and contents. The differences in the diets may have some bearing on the colonic function and hence movement of contents along the intestine and may affect the turnover rate of collagen and hence lead to the production of more acid soluble crosslink forms.

At 18 months the high fibre diet had increased the colonic contents by 70% and the caecal short chain fatty acid concentrations were increased over fivefold. The change in the physical properties of the colonic contents and the additional force and effort needed to propel them may well have some effect on the development of crosslinks. There is no obvious link at present between colonic collagen crosslinkage and the colonic luminal nutrition provided by SCFA. Indirectly, the difference in colonic collagen solubility could be related to the metabolism of nutrients coincidental with the fibre content of the diet.

It seems that there may be many factors that could influence the decrease in collagen solubility in weak acids. The two groups of rats were maintained in exactly the same way and were the same age at the end of the experiment. So it is obvious that this is not merely an aging effect as none of the higher fibre fed rats had colonic diverticulosis. The effect of weight was tested statistically and was found to be non-significant as was the number of tumours present in the animals. It is apparent that the major difference between the two groups was the diets that they were fed for their lifetime. If the protein was the causative agent then one would expect the total collagen content to be

changed, and this is not the case. Dietary fibre may have an important part to play in the alteration of collagen and in the development of colonic diverticulosis.

In conclusion, this study has shown an association between a longterm fibre deficient diet, the development of colonic diverticulosis, and an associated decrease in the solubility of colonic wall collagen in dilute acid.

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