Soluble polysaccharide and biomass of red microalga *Porphyridium* sp. alter intestinal morphology and reduce serum cholesterol in rats

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The present study investigated the effects of the red microalga *Porphyridium* sp. on gastrointestinal physiology and lipid metabolism in male Sprague-Dawley rats. Diets containing dietary fibre from pelleted red microalgal cells (biomass) or their sulfated polysaccharide, pectin or cellulose (control) were fed to rats for a period of 30 d. All three fibre-supplemented diets increased the length of both the small intestine and colon, with a significantly greater effect in rats fed the algal polysaccharide. The polysaccharide also increased mucosa and muscularis cross-sectional area of the jejunum, and caused hypertrophy in the muscularis layer. The algal biomass significantly lowered gastrointestinal transit time by 44 % in comparison with the control rats. Serum and mucosal cholecystokinin levels were lower in rats on the pectin and polysaccharide diets, while cholecystokinin levels in rats fed algal biomass were not different from those in the control animals. In comparison with the control diet, all the experimental diets significantly lowered serum cholesterol levels (22–29 %). Feeding of non-fermentable algal polysaccharide or biomass significantly increased faecal weight and bile acid excretion compared with pectin-fed or control rats. The algal polysaccharide and biomass were thus shown to be potent hypocholesterolaemic agents active at low concentrations in the diet. Both metabolic and morphological changes were observed following consumption of algae, suggesting several possible mechanisms by which the alga affects lipid metabolism. The results presented in the present study encourage the use of red microalga as a functional food.

Dietary fibre: Red microalga: Cholesterol: Image analysis

Since ancient times, seaweeds have served as a food source, primarily in Asia. It is now well known that algal biomass serves as a rich source of proteins, lipids, carbohydrates, dietary fibre, minerals and vitamins (Fabregas & Herrero, 1990; Lopez-Alonso *et al.* 1992). Consequently, there has been a revival of interest in marine algae as an alternative source of nutrients in Western countries (Mabeau & Fleurance, 1993). Seaweeds that are rich in undigestible polysaccharides (such as agar, carrageenan and alginate) are widely used as food additives. These algae also constitute potential sources of dietary fibre that differ chemically and physico-chemically from those of land plants, and thus may have different physiological effects on the human body (Lahaye, 1991; Lahaye & Jegou, 1993). The addition of fibre-rich algal products to the diet could increase overall dietary fibre intake, which has been hypothesized to decrease the prevalence of diseases associated with low dietary fibre intake such as obesity, diabetes, heart disease and cancer (Southgate, 1990).

Red microalgae constitute a new source of dietary fibre.

The cells are encapsulated in a cell wall composed of sulfated polysaccharides (about 50–70 % of the biomass), of which the external part dissolves in the medium. The cell-wall polysaccharides are heteropolymers in which the main sugars are xylose, glucose and galactose (Malis Arad, 1988; Geresh & Malis Arad, 1991). The presence of sulfate groups and glucuronic acid confer a negative charge on these molecules. In addition, red microalgae contain eicosapentaenoic acid (20:5*n*-3; Bajpai & Bajpai, 1993; Nuutila *et al.* 1997).

Consumption of dietary fibre induces both local and systemic responses. Morphological changes in the gastro-intestinal tract occur following direct exposure to dietary fibre, while systemic changes occur in carbohydrate and lipid metabolism (Davidson & McDonald, 1998). The unique chemical and physical characteristics of each fibre source dictate their effects in the body. In addition, the mechanisms by which fibre induces physiological changes are related to its viscosity, fermentability, water-holding capacity, bile acid-binding ability, cation-exchange capacity and faecal-bulking ability (Eastwood & Morris, 1992). Consumption of soluble fibre is associated with changes in lipid metabolism, including a hypocholesterolaemic effect (Anderson *et al.* 1994).

The present investigation was undertaken to elucidate the metabolic and morphological effects of polysaccharides and biomass from the red microalga *Porphyridium* sp. in rats. Results described in the present study indicate that consumption of algae may have great potential as a therapeutic agent.

Materials and methods

Source of algae and culture conditions

The unicellular red alga *Porphyridium* sp. (UTEX 637) was obtained from the culture collection of the University of Texas, Austin, TX, USA. The algae were cultivated outdoors for 21 d in artificial sea water (Jones *et al.* 1963), in polyethylene sleeves (Cohen & Malis Arad, 1989).

Isolation of biomass and polysaccharide

Algal cultures were harvested by continuous centrifugation (Cepa Z-41; Carl Parberg, Lahr, Schwarzwald, Germany). The pellet containing the algal cells (biomass) was washed with distilled water and centrifuged. The supernatant fraction, containing the soluble polysaccharides excreted by the algae during growth, was dialyzed (to remove salts) and the polysaccharide concentrated by ultrafiltration. Both the polysaccharide and biomass were dried by lyophilization and powdered for use in the present experiment.

Dietary fibre sources and analysis

Four sources of dietary fibre were used: (1) cellulose (Solka-folc; James River Corp., Hackensack, NJ, USA); (2) pure citrus pectin, methoxy concentration 10 g/100 g (Sigma, St Louis, MO, USA); (3) *Porphyridium* sp. cells (biomass); and (4) sulfated polysaccharide of the red

Table 1. Composition of experimental diets* (g/kg)

Diet Ingredient	Algal polysaccharide	Algal biomass	Pectin	Control
Casein	178	166	196	200
Sucrose	232	232	232	232
Maize starch	338	304	394	430
Maize oil	67	59	70	70
Fibre source	140†	190‡	60§	20‖
Vitamin mix*	10	10	10	10
Mineral mix*	35	35	35	35
DL-methionine	3	3	3	3

* Vitamin mix and mineral mix was AIN 76A (American Institute of Nutrition, 1977, 1980).
† Polysaccharide contained (g/kg): protein 150, carbohydrate 265, lipids 25, ash 110, dietary fibre 450; total dietary fibre in diet 67 g/kg.
‡ Biomass contained (g/kg): protein 180, carbohydrate 270, lipids 60, ash 135, dietary fibre 355; total dietary fibre in diet 63 g/kg.
§ Pectin contained (g/kg): protein 70, ash 41·4, dietary fibre 889; total dietary fibre in diet 53 g/kg.
‖ Cellulose contained (g/kg): protein 23, ash 1, dietary fibre 976; total dietary fibre in diet 20 g/kg.

microalga *Porphyridium* sp. (prepared as described earlier). Dietary fibre analysis was carried out according to the Association of Official Analytical Chemists standard method (Prosky *et al.* 1988).

Animals

Male Sprague-Dawley rats (Harlan, Jerusalem, Israel), each weighing 130–140 g, were housed in individual suspended stainless-steel cages in a controlled environment (22–24°C and 12 h light–dark) with free access to food and water. Animals were kept according to the guidelines laid down by the Animal Care Committee of the Institutes for Applied Research, Ben-Gurion University of the Negev, Israel.

Experimental procedures

Twenty-eight rats were divided into four groups such that the mean weights of each group were similar. Rats were fed the diets shown in Table 1 (American Institute of Nutrition, 1977, 1980) for a 30 d period. Every 2–3 d the animals and faeces were weighed, and food intake was assessed. During the last 4 d of the experiment, faeces were collected. Gastrointestinal transit time (GTT) was determined during the third week of the experiment. Plasma cholecystokinin (CCK) levels were measured during the fourth week of the experiment and in samples collected at the end of the experiment. After 30 d of the experiment, food was withheld from the rats overnight (12–14 h), and animals were anaesthetized with chloral hydrate (100 g/l). Blood samples were taken from the heart and placed in tubes with or without heparin. Samples were then centrifuged for 10 min at 1000 *g*, and plasma or serum was frozen at −20°C until analysed. The entire intestine from the pyloric sphincter to the rectum was removed, extended on a flat surface with minimal stretching, and the length measured. Jejunum and colon samples were taken for histopathological examination. Caecal contents were removed and stored frozen (−20°C).

Methodologies

Gastrointestinal transit time. Rats were intubated with 1·5 ml carmine solution (10 g/l) as a marker. The length of time between administration and the first appearance of the marker in the faeces was taken as the GTT.

Determination of plasma lipids. Total plasma cholesterol content was measured by the enzymic colorimetric methods as described by Allain *et al.* (1974) and Lopes-Virella *et al.* (1977). Plasma triacylglycerol levels were determined by the method of Fossati & Prencipe (1982).

Faecal bile acid analysis. Faecal samples were collected over the last 4 d of the experiment, weighed, and frozen at −20°C. Samples were then dried by lyophilization, and stored in a desiccator for later analysis. Dried faecal matter (100 mg) was extracted overnight with 10 ml chloroform–methanol (2:1, v/v; Folch *et al.* 1957). KCl (3·7 g/l; 2 ml) was then added, and the sample was centrifuged at 1500 g for 10 min. The upper layer was removed, evaporated to dryness, and dissolved in 1 ml methanol (500 ml/l). Total bile acids were determined by enzymic analysis using a modified method of Sheltawy & Losowsky (1975). Each tube content was as follows: phosphate buffer pH 10·5, 2 mmol NAD/l and 100 μl sample. The reaction was initiated by adding 3α-hydroxysteroid dehydrogenase (0·1 IU per reaction) in a final volume of 1·5 ml. After 30 min at room temperature, the absorbence was measured at 340 nm to detect NADH formed.

Faecal neutral sterol analysis. A modified method of Searcy & Bergquist (1960) was used to determine faecal neutral sterol concentrations. Dried faecal samples (50 mg) were saponified with 3 ml ethanolic KOH (100 g/l) for 1 h at 65°C. Samples were cooled to room temperature and neutral lipids were extracted with 3 ml water and 5 ml light petroleum (b.p. 40°C–60°C). Mixtures were vortexed for 30 s and the solvent removed. Second and third extractions with 5 ml and 3 ml light petroleum were then carried out and extracts pooled. The samples were dried under N_2 and a colorimetric reaction was carried out in 3 ml acetic acid saturated with $FeSO_4$ and 1 ml H_2SO_4. Absorbence was measured at 490 nm to detect sterol concentrations.

Cholecystokinin. In week 3 of the experiment food was withheld overnight and rats were fed 5 g of their respective diets over a 2 h period. At 1 h after removing the food, blood samples were collected from rat tail tips in pre-heparinized Eppendorf tubes. Samples were then centrifuged for 10 min at 1000 g and plasma was frozen at −20°C. Mucosal samples were prepared as follows: duodenal sections were placed on ice and the mucosa scraped off. The mucosal samples were freeze–thawed twice, acidified with 10 g trifluroacetic acid/l in distilled water, homogenized for 1 min, and centrifuged at 40 000 g for 20 min. Extraction of peptide from the mucosa was carried out with a C_{18} SEP-COLUMN as described in the general protocol for radioimmunoassay kit (Peninsula Laboratories Inc., Belmont, CA, USA). CCK levels in the blood and in the mucosa samples were also measured using this kit. Protein levels were determined in each mucosa sample, using method of Bradford (1976).

Caecal content viscosity. Frozen caecal contents were defrosted to room temperature and 1 g (wet weight) was diluted with 10 ml distilled water. Samples were centrifuged at 30 000 g for 30 min. The viscosity of the supernatant fraction was measured at 22°C with a Brookfield Digital Viscometer (Brookfield Engineering, Stoughton, MA, USA). Samples with low viscosity (<0·1 Pa.s) were measured with spindle 18 and shear rate of 39·6/s. Samples with high viscosity were measured with spindle 31 and shear rate of 9·3/s.

Histopathology. At the termination of the experiment, samples of 10 mm segments from the jejunum and mid-colon were cut out and preserved in buffered formaldehyde (40 ml/l). Particular attention was paid to quick fixation of the tissues to prevent autolysis; the maximum time from killing to fixing being 5 min. Transversal slices of the intestinal segments, perpendicular to the mucosal surface, were prepared, dehydrated and embedded in paraffin wax. Sections were cut to a thickness of 4−5 μm and stained with haematoxylin and eosin. The tissue was well orientated, and the plane of the section passed vertically through the epithelium. In each section, image analysis (GALAI SuperCue3/ScanArray; GALAI, Migdal-Haemek, Israel) was used to determine the area of the mucosa including the villi and crypts, and the underlying submucosa. This layer was easily distinguished from the *tunica muscularis* layer.

The cell size in the inner circular smooth muscle layer of the *tunica muscularis* was determined by counting the number of nuclei in a defined area. Three fields were randomly selected, providing a total area of 4·1 mm². The fields for measurement were selected at random. The principles of the technique used for this computer-aided microscopy are outlined in Slavin *et al.* (1980). Before applying morphometric quantifications, cross-sections were closely examined for the technical quality (i.e. grade of staining intensity of the nuclei in order to enable accurate identification and discrimination from the cytoplasm background). Before each measurement session the number of nuclei in a given area was manually counted, and compared with the data obtained by using automated image analysis in three histological samples. Since haematoxylin and eosin staining was adequate to discriminate between nuclei and cytoplasm of the muscle cells, automated image analysis counting of the nuclei was possible. Mucosal height was measured at five different and spaced sites. In the jejunum the height represents a perpendicular axis passing from the tip of the villus to the base of the crypt. Colonic measurements signify a perpendicular axis passing from the tip of the gland to the base of the crypt. All sections were coded so that the origin of the section was unknown to the observer. Before morphometric quantifications cross-sections were examined closely, and no signs of atrophy were observed in any of the experimental groups. Damaged tissue was not included in the image analysis.

Statistical analysis

Data are expressed as means with their standard errors. Group means were compared by ANOVA. Means were considered significantly different at $P \leq 0.05$, as determined by Fisher's protected least significant difference

Table 2. Weight gain, faecal dry weight, gastrointestinal transit time (GTT), faecal bile acids and faecal neutral sterols in rats fed control, algal polysaccharide, algal biomass, or pectin-rich diets for 30 d*

(Values are means with their standard errors for seven rats per group)

Diet. . .	Algal polysaccharide		Algal biomass		Pectin		Control	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Wt gain (g/30 d)	103[b]	6	131[a]	2	119[a]	5	123[a]	6
Faecal dry wt (g/d)	2·78[a]	0·07	2·17[b]	0·06	0·75[d]	0·02	0·94[c]	0·03
GTT (h)	13·31[a]	0·74	6·56[b]	0·27	16·21[a]	1·55	15·06[a]	1·82
Faecal bile acids (μmol/d)	7·6[b]	0·3	12·2[a]	0·7	3·7[c]	0·3	2·4[c]	0·3
Faecal neutral sterols (μmol/d)	39·0[a]	3·0	ND		16·5[b]	0·4	12·9[b]	0·5

ND, not determined.
[a,b,c,d] Mean values in a row with unlike superscript letters were significantly different ($P < 0.05$).
* For details of diets and procedures, see Table 1 and p. 470.

method. For caecal viscosity measurements, data were log transformed to equalize variance.

Results

Analysis of dietary fibre and fermentability

The dietary fibre content of the algal biomass consisted of 27 % insoluble and 8·5 % soluble dietary fibre on a dry weight basis, whereas that of the polysaccharide was 37 % soluble and 8 % insoluble dietary fibre (Table 1). *In vitro* fermentation experiments (24 h) demonstrated that the algal polysaccharide was resistant to bacterial breakdown (data not shown). In addition, the dietary fibre recovery in faecal samples was 80–105 % for the cellulose-, biomass- and polysaccharide-fed rats, while only 30 % in rats fed the fermentable fibre pectin.

Body weight gain and faecal dry weight

Significantly lower weight gain ($P < 0.005$) was observed in polysaccharide-fed rats as compared with the control, biomass and pectin-fed rats (Table 2). Furthermore, faecal dry weight was significantly higher in the polysaccharide-fed rats than all other experimental groups ($P < 0.05$). Biomass also significantly increased faecal dry weight in comparison with the pectin and control diets ($P < 0.05$).

Gastrointestinal transit time

The GTT was reduced ($P < 0.05$) in biomass-fed rats (51–60 %) compared with rats fed polysaccharide, pectin or control diets (Table 2).

Plasma lipid levels

Plasma cholesterol levels were significantly higher ($P < 0.001$) in control rats (2·93 (SE 0·23) mmol/l) than in rats fed the biomass (2·29 (SE 0·08) mmol/l), polysaccharide (2·08 (SE 0·09) mmol/l) or pectin (2·28 (SE 0·09) mmol/l) diets. Overall, polysaccharide feeding had the greatest hypocholesterolaemic effect. A trend towards lower plasma triacylglycerols was observed in rats fed polysaccharide, biomass and pectin in comparison with the control rats: 0·17 (SE 0·02) mmol/l, 0·17 (SE 0·01) mmol/l, 0·17 (SE 0·01) mmol/l and 0·24 (SE 0·04) mmol/l respectively.

Faecal bile acids and neutral sterols

Daily faecal bile acid excretion (Table 2) was lowest in the control animals, higher in the pectin-fed rats and significantly higher ($P < 0.05$) in the polysaccharide (two-fold) and biomass fed rats (fourfold) compared with the control rats. Faecal neutral sterol excretion was significantly enhanced ($P < 0.05$) in rats fed the algal polysaccharide in comparison with control and pectin-fed rats.

Cholecystokinin

In week 3 of the experiment, 1 h following food withdrawal from rats, plasma CCK levels did not differ in biomass-fed and control animals. These levels were higher than those measured in both the polysaccharide- and pectin-fed rats (Table 3). A similar pattern was found in the duodenal mucosa at the termination of the experiment; CCK levels were significantly higher ($P < 0.05$) in biomass and control

Table 3. Effects of experimental diets on blood and mucosal cholecystokinin (CCK) levels in rats fed control, algal polysaccharide, algal biomass, or pectin-rich diets for 30 d*

(Values are means with their standard errors for seven rats per group)

Diet. . .	Algal polysaccharide		Algal biomass		Pectin		Control	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
CCK (pg/l)†	145·0	6·44	228·0	6·79	148·0	4·08	193·0	6·31
CCK (pg/l)‡	94·6	13·2	111·9	3·5	80·8	10·3	110·8	5·5
CCK (pg/mg protein)§	0·77[b]	0·82	2·31[a]	1·22	0·68[b]	0·6	2·50[a]	0·76

[a,b] Mean values in a row with unlike superscript letters were significantly different ($P < 0.05$).
* For details of diets and procedures, see Table 1 and p. 470.
† Measured 1 h after ending the experimental diets (the rats were fed the experimental diets for 2 h after food had been withheld overnight).
‡ Measured after food had been withheld overnight.
§ Concentration in the mucosa that had been removed from the duodenum after food had been withheld overnight.

Table 4. Morphometric measurements in rats fed control, algal polysaccharide, algal biomass, or pectin-rich diets for 30 d*

(Values are means with their standard errors for seven rats per group)

Diet...	Algal polysaccharide		Algal biomass		Pectin		Control	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Intestinal length (cm)								
Small intestine	121[a]	2·1	109[b]	3·5	104[b]	3·5	93[c]	2·7
Colon	17·0[a]	0·5	14·0[b]	0·3	14·7[b]	0·4	12·9[c]	0·4
Jejunum (mm^2)								
Total area	6·8[a]	0·73	5·4[ab]	0·59	4·9[b]	0·73	4·3[b]	0·34
Mucosal area	5·5[a]	1·5	4·2[ab]	0·6	3·9[b]	0·3	3·4[b]	0·3
Muscularis area	1·3[a]	0·7	1·2[ab]	0·1	1·0[bc]	0·7	0·9[c]	0·1
Nuclei (/4·1 mm^2)	17[b]	0·9	19[b]	0·1	17[b]	0·9	22[a]	1·0
Colon (mm^2)								
Total area	3·9[b]	0·27	4·9[a]	0·27	4·6[ab]	0·11	4·7[ab]	0·33
Mucosal area	2·8[b]	0·2	3·4[a]	0·2	3·5[a]	0·2	2·9[ab]	0·2
Muscularis area	1·1[b]	0·2	1·5[ab]	0·1	1·1[b]	0·1	1·8[a]	0·2
Nuclei (/4·1 mm^2)	11[b]	1·0	12[ab]	1·0	15[a]	1·0	14[a]	1·0

[a,b,c]Mean values in a row with unlike superscript letters were significantly different ($P < 0.05$).
* For details of diets and procedures, see Table 1 and p. 470.

fed rats compared with polysaccharide- and pectin-fed rats. No significant differences in plasma hormone levels were observed in food-deprived rats.

Viscosity of caecal contents

In control animals, caecal content viscosity was 1·1 (SE 0·05) μPa.s and in pectin-fed animals 1·9 (SE 0·10) μPa.s. Consumption of algal biomass significantly elevated viscosity to 24·0 (SE 3·1) μPa.s. Caecal viscosity in the rats fed the non-fermentable algal polysaccharide was significantly higher ($P < 0.0001$) than that in all other groups, 349 (SE 36·7) μPa.s.

Morphometric measurements

Feeding all three fibre-rich diets resulted in a significant elongation ($P < 0.05$) of both the small intestine and colon in comparison to control rats (Table 4). Despite the lower weight gain in the rats fed the algal polysaccharide, intestinal elongation was significantly greater ($P < 0.05$) than all other groups of animals. Small intestine length was 23 % greater than that in the control animals, while colon length was 29 % greater (Table 4). In the jejunum, polysaccharide feeding had the greatest effect on the morphology of both the mucosa and *tunica muscularis* layers. Although pectin- and biomass-fed animals showed a trend towards increased jejunal areas, the polysaccharide diet produced significant differences ($P < 0.05$) in mucosal area, 38 % greater in comparison with the controls. In addition, muscularis area was increased by 30 % (Table 4 and Figs. 1 and 2). Biomass and pectin feeding had a lesser impact on the jejunum mucosal mass, with increased areas of 19 and 13 % respectively. Jejunal muscularis area was greatest in the polysaccharide-fed rats and smallest in the control animals. Furthermore, control rats had significantly more nuclei per mm muscularis area than rats fed the experimental diets (Fig. 2).

In contrast to the jejunum, changes in the mid-colon tissue morphology were less apparent. An 18–20 % increase in mucosal area was observed in the biomass- and pectin-fed animals in comparison with the polysaccharide-fed and control animals. The total cross-sectional areas, therefore, did not differ, while the *tunica muscularis* cross-sectional area was significantly larger in the control group than in the polysaccharide-fed rats ($P < 0.005$). Overall, the effect on morphometric variables was less evident in pectin-fed rats than in the other experimental groups.

Discussion

The present study examined the metabolic and morphological changes in rats following feeding with biomass or polysaccharide from the red microalga *Porphyridium* sp. Both the biomass and the polysaccharide from this alga influenced the intestinal structure and significantly reduced plasma cholesterol levels ($P < 0.05$). The work described here illustrates some of the unique properties of dietary fibre derived from algae, and potential health benefits of incorporating these foodstuffs into the diet.
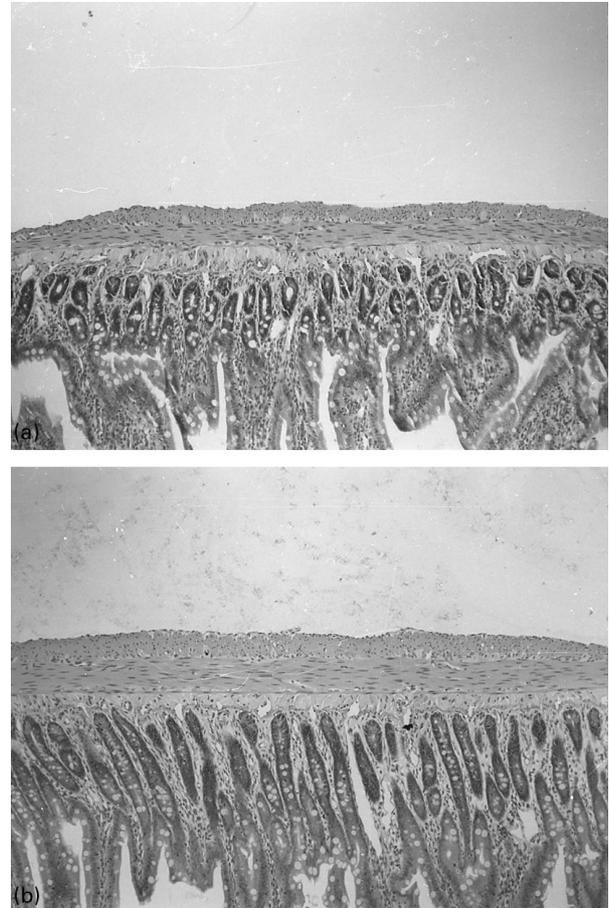
Rats fed the algal polysaccharide gained less weight than rats in other experimental groups, despite similar food intake levels (data not shown). Furthermore, dry faecal weight was more than double in the polysaccharide-fed animals. In general, diets with a fibre content of 50 g/kg do not influence animal growth (Anderson *et al.* 1994). The lower weight gain of the polysaccharide-fed rats may be due to the high viscosity of the intestinal contents following food consumption. The viscosity of the caecal contents of rats fed algal polysaccharide was more than 300-fold higher than that of the control animals and more than 180-fold higher than that of the pectin-fed rats. Highly-viscous chyme is thought to impede nutrient absorption and micelle formation, and may decrease overall lipid absorption, which would result in lower weight gain (Nagengast, 1992; Gallaher *et al.* 1993; Schneeman & Richter, 1993; Lairon, 1996).

Morphological changes in the intestine following fibre feeding may also influence absorption and facilitate the metabolic changes induced by fibre feeding (Brown *et al.* 1979; Schneeman & Richter, 1993; Stark *et al.* 1996). Changes in the structure of the villus have been reported
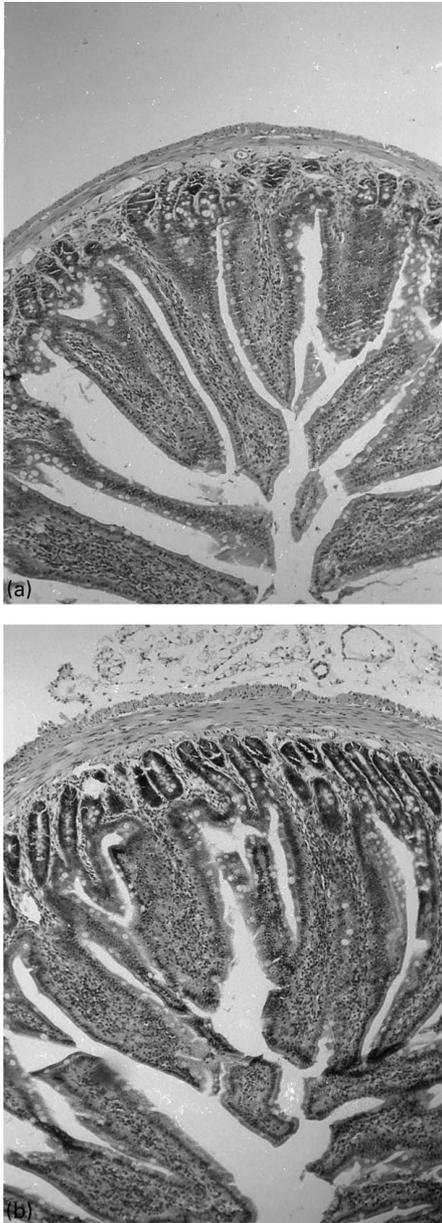
Fig. 1. Photomicrographs of transversal sections of the jejunum from a control rat (A) and from a rat fed the polysaccharide diet (B) for 30 d. Note that the thickness of the *tunica muscularis* layer in (B) is almost double that in the control (A). For details of diets and procedures, see Table 1 and p.470. Haematoxylin and eosin stain; ×200.

Fig. 2. Higher magnification photomicrograph of transversal sections of the jejunum of a control rat (A) and of rat fed the polysaccharide diet (B) for 30 d. Note the number of nuclei per unit area (density) in the inner layer of the *tunica muscularis* layer; it is markedly reduced in the polysaccharide-fed rats (B) than in the control (A). For details of diets and procedures, see Table 1 and p. 470. Haematoxylin and eosin stain; ×350.

following fibre feeding in rats (Cassidy *et al.* 1981; Vahouny & Cassidy, 1986), and it has also been shown that soluble fibre significantly ($P < 0.001$) increases intestinal length (Jacobs, 1983; Judd & Truswell, 1985; Stark *et al.* 1996). Distinct changes in the intestinal morphology were observed following algal polysaccharide, biomass and pectin diets. In all experimental groups lengthening of the small intestine and the colon was observed, with the greatest effect being in polysaccharide-fed rats. Intake of high-fibre diets leads to a downward shift in nutrient absorption (Read & Eastwood, 1992); therefore, intestinal elongation may be an adaptive mechanism to maximize nutrient absorption. Additionally, it appears that the highly viscous nature of the biomass and algal polysaccharide leads to structural modifications in the small intestine necessary to overcome the increased work required to move its contents forward (increased muscularis layer) as well as to greater mucosal volume to maximize nutrient absorption. In the colon total cross-sectional areas of biomass-fed rats were similar to those of the controls, but the relative area of mucosa was greater. This finding may be due to bacterial fermentation in the colon of the biomass-fed rats and increased production of short-chain fatty acids, the preferred source of energy for colonocytes. Pectin, which is fermented in the large intestine, also caused an enlargement in the relative colonic mucosal area, while the non-fermentable polysaccharide did not. By counting the nuclei it was shown that changes in muscularis in both the large and small intestine of rats fed biomass and algal polysaccharide were caused by cell hypertrophy. This finding suggests an adaptive response to dietary intervention rather than a pathological occurrence. It has been demonstrated that hypertrophy signifies an increase in size

or volume of the cell, usually arising from an increased workload (Cheville, 1994). Precisely how morphological changes may modify nutrient absorption and lipid metabolism is still not well understood.

GTT may be an important factor in nutrient absorption. GTT was dramatically reduced in the biomass-fed rats in comparison with the other two experimental groups and control animals. Although fibre intake commonly shortens GTT (Saito *et al.* 1991), it is not clear why the red microalgal biomass had such a marked effect in comparison with other fibre sources (Table 2). Biomass feeding led to higher CCK plasma levels in comparison with pectin and polysaccharide feeding (Table 3). The role of CCK in the regulation of GTT is not yet fully understood and no detailed studies examining the effect of dietary fibre on CCK release have been published. However, it has been observed that this hormone has a potent effect on gut smooth muscle contractility, depending on the region of the gut. CCK also causes contraction of the gall bladder, relaxation of the sphincter of Oddi, relaxation of the gastric fundus and contraction of the intestine (Grider, 1994). Nevertheless, CCK secretion can only partially explain the shortened GTT caused by biomass consumption, since serum and mucosal CCK levels were elevated only in comparison with pectin- and polysaccharide-fed rats. Thus, other factors must be involved in the dramatic decrease in GTT associated with algal biomass consumption.

A significant hypocholesterolaemic effect ($P < 0.05$) was observed following administration of diets containing pectin, algal biomass and algal polysaccharide (all containing approximately 50 g soluble dietary fibre/kg) in comparison with animals fed the control diet. Different dietary fibre varies considerably in its physical properties and chemical composition, therefore characterization of hypocholesterolaemic mechanisms is very complex (Eastwood & Morris, 1992). It has also been suggested that disruption of the entero-hepatic circulation of bile acids leads to increased bile acid excretion and a subsequent decrease in plasma cholesterol levels (Topping *et al.* 1990; Glore *et al.* 1994). Significantly increased bile acid excretion ($P < 0.05$) was measured in rats fed the algal polysaccharide and biomass (Table 2). Daily faecal bile acid excretion was highest in the biomass-fed animals (five times higher than that in the control group) but was also significantly elevated ($P < 0.05$) following polysaccharide consumption. The findings of the present study support the theory that disruption of entero-hepatic circulation of bile acids leads to increased bile acid excretion and a subsequent decrease in plasma cholesterol levels. In addition, neutral sterol excretion was significantly enhanced in algal polysaccharide-fed rats (Table 2), representing another pathway by which cholesterol pools are depleted by fibre feeding.

Other proposed mechanisms of action for the hypocholesterolaemic activity of dietary fibre include direct inhibition of hepatic sterol synthesis. It has been hypothesized that the short-chain fatty acids produced by fermentation of soluble fibre may act in this manner (Chen *et al.* 1984; Kishimoto *et al.* 1995). In an additional study carried out in our laboratory (I Dvir, R Chayoth, U Sod-Moriah, S Shany, A Nyska, AH Stark, Z Madar and S Malis Arad, unpublished results) using algal dietary fibre, levels of hydroxymethylglutaryl-CoA reductase, the key enzyme in cholesterol synthesis, were determined. Algal polysaccharide significantly enhanced endogenous synthesis of cholesterol in comparison with cellulose-fed controls, indicating that direct hepatic inhibition of cholesterol synthesis does not result from algal polysaccharide feeding.

In conclusion, both algal polysaccharide alone and algal biomass, with their unique dietary fibre make-up of sulfated polysaccharide, induce morphological modifications in the intestine and have potent hypocholesterolaemic properties. Several possible mechanisms appear to be involved in the induction of the hypocholesterolaemic effect, including increased faecal bile acid and neutral steroid excretion, increased intestinal viscosity and modified GTT. The red microalga *Porphyridium* sp., a source of unique dietary fibre, may prove to have significant health benefits when consumed in relatively small amounts and provide therapeutic effects similar to, or better than, those of more traditional sources of plant fibre.

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