The hypocholesterolaemic effects of pectins in rats

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1. The hypocholesterolaemic effects of pectins were studied in a series of five experiments in weanling Sprague–Dawley rats.

2. Expt A examined the effects of differing levels of dietary fat on plasma and liver lipids. Rats were given diets containing 100 g pectin, National Formulary (NF)/kg, and either 50,100, 150 or 200 g oil/kg. All diets contained 10 g cholesterol/kg. Plasma cholesterol levels were lower in all pectin-fed groups compared with rats given the control diet containing 100 g Solkafloc and 100 g oil/kg. Liver lipid and cholesterol levels increased with increasing fat content of the diet, reaching a plateau at 150 g fat/kg diet, but were still significantly lower in all pectin-fed groups compared with the control group.

3. Expt B. The effects of molecular weight and degree of methoxylation of pectins were studied in five groups of rats given either a control diet containing 100 g Solkafloc/kg or high molecular weight, high methoxyl pectin (HMW HMP); high molecular weight, low methoxyl pectin (HMW LMP); low molecular weight, high methoxyl pectin (LMW HMP); low molecular weight, low methoxyl pectin (LMW LMP). All diets in this and subsequent experiments contained 100 g fat and 10 g cholesterol/kg. Plasma cholesterol levels were significantly lower than control values only in the HMW HMP group. Compared with controls, animals given HMW pectins had lower levels of liver lipid and liver cholesterol; on the LMW HMP diet the liver cholesterol, but not the liver lipid, was lower.

4. Expt C. An attempt was made to clarify the possible effect of degree of methoxylation by feeding diets containing either 100 g Solkafloc/kg, 100 g pectin NF/kg or 100 g very high methoxyl pectin/kg. Plasma cholesterol levels were significantly reduced by both pectins but there was no difference in effect between the two. Both had similar viscosities suggesting that this is a more important factor than methoxyl content.

5. Expts D and E. Effects of dose on hypocholesterolaemic effects of HMP and LMP were studied. Diets containing 50 or 100 g Solkafloc, HMP or LMP/kg were given in Expt D, and 25 g Solkafloc or HMP/kg, 50 g Solkafloc, HMP or LMP/kg and 100 g Solkafloc or LMP/kg in Expt E. Plasma cholesterol levels were significantly reduced in groups given 50 or 100 g HMP/kg and in groups given 100 g LMP/kg.

6. HMP were found to be more effective at lowering plasma cholesterol levels than LMP. LMW pectins were not effective. This suggests that the hypocholesterolaemic effects are at least partly due to viscosity.

7. Gut length and weight was increased in pectin-fed animals compared with controls despite their lower body-weight. The weight of small intestinal contents at death was also greater in pectin-fed rats (Expts A and B), particularly in the distal small intestine.

Pectins, complex colloidal polysaccharides which occur in all higher plants, are considered as part of the dietary-fibre complex. They have been shown to be effective hypocholesterolaemic agents in both man (Kay *et al.* 1978) and animals (Lin *et al.* 1957; Wells & Ershoff, 1961, 1962; Leveille & Sauberlich, 1966; Riccardi & Fahrenbach, 1967; Mokady, 1973) but the mechanisms of this effect are still unclear.

Pectins are partially esterified rhamnogalacturonans having an α-1-4-linked D-galacturonan chain with L-rhamnopyranosol-rich areas and side chains which include D-glucuronic and galacturonic acids. Molecular weights vary from 30000 to 300000 (Pilnik & Voragen, 1970). The carboxyl groups of the galacturonic acids are esterified to varying degrees, usually with methoxyl groups, and pectins can be described as high or low methoxyl, the latter having less than 50% of the possible carboxyl groups as methyl esters. The theoretical maximum percentage of methyl ester in pure galacturonic acid is 16% and, allowing for impurities, the 50% ester content is taken as 7% (Kertesz, 1963).

The properties of pectins depend largely on molecular weight (MW) and degree of

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esterification. They form viscous solutions with viscosity increasing with increase in MW but also affected by degree of esterification and electrolyte concentration (Rees, 1967; McKay *et al.* 1969; Pilnik & Voragen, 1970).

The gel-forming properties of pectins are also related to the degree of methoxylation. High methoxyl pectins (HMP) form gels with sugar and acid; low methoxyl pectins (LMP) form gels in the presence of calcium ions. In addition, the degree of esterification influences the rate and temperature at which a gel will form (Pilnik & Voragen, 1970).

The type of pectin used for most studies of its effects on plasma lipids has been pectin National Formulary (NF) with a degree of esterification of approximately 70 (American National Formulary XIII, 1970). It has been suggested that only pectins with such high degrees of methoxylation ($> 10{\cdot}1\% = 63°$) are effective hypocholesterolaemic agents (Ershoff & Wells, 1962; Anderson & Bowman, 1969; Mokady, 1973). Other workers have suggested, however, that viscosity is an important variable (Kay & Truswell, 1977), not only for pectin but for other gel-forming polysaccharides such as guar gum.

Early work has also suggested that pectin is only effective in animals made hypercholesterolaemic by the addition of cholic acid (to suppress bile salt formation) as well as cholesterol to the diets (Lin *et al.* 1957; Wells & Ershoff, 1961; Fausch & Anderson, 1965; Fisher *et al.* 1967), but we have found a hypocholesterolaemic effect in rats when 100 g pectin NF/kg was added to the diet without the addition of cholesterol or cholic acid (Judd *et al.* 1977).

The present study was therefore designed to examine the hypocholesterolaemic effects of a range of pectins under varying conditions in order to help clarify the mechanism of action of gel-forming polysaccharides. Observations on gut morphology were also included as it had been noticed in preliminary experiments that pectin-fed animals had apparently larger guts than control animals.

METHODS

Animals

Male weanling rats of the Sprague–Dawley strain were used. Weighing between 40 and 50 g on arrival, they were allowed to acclimatize to animal-house conditions for 2–3 d and were given stock diet (Oxoid breeding diet) before commencing the experimental diets. The rats were assigned to experimental groups such that the mean weight of each group was similar. They were housed in pairs in stainless-steel and plastic cages with wire bottoms to facilitate collection of spilt food and faeces and prevent coprophagy. Animals were weighed on alternate days for the first week and subsequently each week.

At the end of each experiment the animals were anaesthetized with diethyl ether and bled from the heart, using a heparinized needle. Blood was centrifuged and the plasma stored at $-20°$ for subsequent analysis. In Expts A and B livers were excised, blotted dry, weighed and stored at $-20°$ for analysis.

In Expts A, B, C and E observations were made on gut weight, length and content. A solution (5 ml) containing atropine sulphate (5 μg/l) was injected into the abdomen of the anaesthetized animals to minimize intestinal movement. After death the small intestine was clamped at the pyloric sphincter (PS) and the ileo-caecal valve (ICV) and at intervals between. After cutting between the caecum and ICV and the stomach and PS the following measurements were made: (1) length; the small intestine was gently separated from the mesentery and measured, without stretching, against a metre rule; (2) the small intestine was weighed after being cut into short sections and the contents either squeezed out (Expts A and B) or washed out with saline (9 g sodium chloride /l) and the tissue blotted dry; (3) in Expts A and B gut contents were examined; stomach, caecum and colon were cut

open and the contents scraped out, the contents of the small intestine were collected by dividing the gut into small sections and 'milking' them into polycarbonate tubes, weights of contents were recorded and the samples centrifuged in an MSE High Speed 18 centrifuge for 45 min at 28000 ***g*** and 25°. The supernatant fraction and precipitate were weighed and examined separately.

Diets

Semi-synthetic diets were used to enable manipulation of the amount and type of dietary fibre. The basic diet contained (g/kg): casumen (Unigate Ltd, UK) 150, groundnut oil 100, sucrose 100, vitamin mix 20, mineral mix 40, cholesterol 10, maize starch 580. Solkafloc (850 g cellulose/kg and 150 g hemicellulose/kg; Brown & Co., Ltd, New Hampshire, USA) or pectins were substituted for maize starch in the experimental diets. Cholesterol was dissolved in the warmed oil (80°) before being added to the dry ingredients. Diets were mixed for 30 min in a 5 kg Hobart blender. Samples of each diet were oven dried at 105° for 48 h for subsequent analysis.

Food intake and faecal collections

Diets were given *ad lib.* and food intake measured on a dry-weight basis after collection, drying and measurement of spilt food. Faeces were collected for 48–72 h during the last 7 d of an experiment. The collections were made daily at the same time and frozen immediately to minimize changes due to drying out or bacterial degradation. Faeces were freeze-dried in an Edwards Model EFO3 freeze dryer, ground and stored as a dry powder for analysis.

Analytical methods

Nitrogen in diet and faecal samples was measured by the Kjeldahl method (Bradstreet, 1965) and gross energy was measured by bomb calorimetry (Miller & Payne, 1959). Apparent N and energy digestibilities could then be calculated as $(100 \times \text{intake} - \text{excretion})/\text{intake}$.

Total plasma cholesterol was measured by the method of Abell *et al.* (1959). Duplicate samples were saponified in alcoholic potassium hydroxide, extracted with redistilled light petroleum (b.p. 60–80°), dried in a stream of air and assayed by the Liebermann–Burchard colour reaction. Cholesterol standard was obtained from BDH (Poole, Dorset).

Total lipid in liver (central lobe) and faeces was measured gravimetrically after extraction by the method of Folch *et al.* (1957). Cholesterol was measured by redissolving the lipid extract in chloroform, and taking portions of this for analysis by the Abell method (Abell *et al.* 1959).

Statistical methods

Student's *t* test for independent samples was used to detect differences when two groups of animals were involved. When more than two groups of animals were used, one-way analysis of variance was used to test homogeneity of the data. When a difference was detected between groups, Duncan's multiple-range test was used to find the position and significance of the differences (Duncan, 1955).

Expt A. The effect of different levels of fat on the hypocholesterolaemic activity of pectin

Forty animals were divided into five groups. Maize starch (100 g) was replaced by 100 g Solkafloc in the control diet and by 100 g HMP in the four other groups (for details of all pectins used, see Table 1). In addition, the fat content was varied such that the four pectin groups had 50, 100, 150 or 200 g fat/kg, all changes being made at the expense of maize starch. Animals were fed for 21 d.

Table 1. *Details of pectins used*

Expt	Type	Degree of methoxylation*	Percentage uronic acid†	Inherent viscosity‡
A	NF	70	87	500
B	HMW HMP	67	87	560
	HMW LMP	34	89	350
	LMW HMP	65	87	200
	LMW LMP	38	87	200
C	NF	69	90	560
	VHMP	81	90	550
D	HMP	71	88	500
	LMP	37	92	350
E	HMP	72	87	540
	LMP	33	90	340

NF, National Formulary; HMW, high molecular weight; LMW, low molecular weight; VHMP, very high methoxyl pectin; HMP, high methoxyl pectin; LMP, low methoxyl pectin.

All LMW pectins were prepared from the HMW product by maintaining the latter in solution at pH 1–2 for several days at a temperature of 40°. All pectins were supplied and analysed by H. P. Bulmer Ltd, Hereford.

* Percentage of possible uronic acid residues esterified with methoxyl groups.

† Percentage of uronic acid groups in molecule.

‡ Viscosity in ml/g of a 1 g/l solution (McKay *et al.* 1969).

Expts B and C. To ascertain the effect of degree of methoxylation and molecular weight on the hypocholesterolaemic effect of pectins

Expt B. Fifty animals were assigned to five groups and fed for 21 d. The control diet was the basal diet with 100 g maize starch/kg replaced by Solkafloc and the experimental diets contained 100 g of each of the pectins described in Table 1 per kg diet. Combinations of high (HMW) and low molecular weight (LMW) and high (HMP) and low methoxyl pectins (LMP) were used. At each level of methoxylation the LMW product was prepared from a portion of the HMW type by maintaining it at 40° in a solution at pH 1–2 for several days.

Expt C. The effect of pectin with a very-high methoxyl (VHMP) content was measured using three groups of twelve rats, fed for 21 d on diets containing 50 g of either Solkafloc, pectin NF or VHMP/kg.

Expts D and E. Effects of dose on the hypocholesterolaemic effect of HMP and LMP

Expt D. Six groups of ten rats were used and fed for 21 d. Two control diets were given, containing either 50 or 100 g Solkafloc/kg, and the corresponding experimental diets contained 50 or 100 g of either HMP or LMP/kg.

Expt E. Seven groups of ten rats were used and fed for 21 d. The control groups contained 25, 50 or 100 g Solkafloc/kg and the experimental diets 25 or 50 g HMP/kg or 50 or 100 g LMP/kg.

RESULTS

Expt A. Effect of level of dietary fat on the hypocholesterolaemic effect of pectins

Weight gain and food intake. These were reduced in all pectin-fed animals compared with the control animals (Table 2). The effect was more pronounced as dietary fat content increased. Feed efficiency was also decreased in the 150 g and 200 g fat/kg groups, although no statistical analysis was attempted due to the small number of samples.

Table 2. *Expt A. The effect of varying dietary fat level on growth, food conversion efficiency and plasma and liver cholesterol in rats given pectin National Formulary (NF)*

(Mean values with their standard errors for eight rats/group. All diets contained 100 g fibre/kg)

Group...	Control†		Pectin NF							
Fat content of diet (g/kg)...	100		50		100		150		200	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Original wt of rats (g)	66	0·9	66	0·6	75	0·9	78	0·9	78	1·8
Change in wt (g)	105	4	69**	4	60**	3	50**	3	42**	3
Food intake (g)	261	7	189	25	185	7	185	21	186	22
Food conversion efficiency‡	0·41	0·01	0·36	0·03	0·33	0·03	0·29	0·05	0·23	0·04
Plasma cholesterol (mmol/l)	3·16	0·32	1·67**	0·06	1·73**	0·08	2·01**	0·04	2·22**	0·17
Liver										
Weight (g)	8·68	0·22	5·98	0·22	6·37	0·20	5·14	0·24	4·60	0·17
Lipid (mg/g)	92	7	41**	3	54**	4	79	45	72	7
Lipid (mg/rat)	776	60	245**	24	327**	27	397**	9	340**	30
Cholesterol (mg/g)	22	3	3**	0·6	5·2**	1·1	13·2**	1·7	13·3*	1·7
Cholesterol (mg/rat)	189	23	18**	2	33**	7	66**	7	64**	8

Significantly different from control: * $P < 0·05$, ** $P < 0·01$.

† Control group fibre was Solkafloc.

‡ Weight gain (g)/food intake (g).

Plasma and liver lipids. Plasma cholesterol levels were significantly lower in all groups given pectin compared with the control group (Table 2). There was no correlation between plasma cholesterol levels and weight gain or food intake.

Livers were larger and paler in the control animals compared with the pectin-fed animals at all levels of fat in the diets and liver weight was found to correlate with animal weight in groups consuming 50, 150 or 200 g fat/kg diet ($P < 0{\cdot}01$ in control, 50 and 150 g fat/kg diet groups and $P < 0{\cdot}05$ in 200 g fat/kg diet group). Liver total lipid and cholesterol were significantly lower in all pectin-fed groups but increased with increasing fat intake reaching a plateau at 150 g fat/kg diet.

Cholic acid was not used in the diet and the results of this preliminary experiment suggested that a significant reduction in plasma cholesterol could be achieved without the necessity of inducing extremely high values by including it. This was, therefore, also omitted from the diets in subsequent experiments. A level of 100 g fat/kg diet was chosen for subsequent experiments as 100 g pectin NF/kg appeared to be an effective hypocholesterolaemic agent at this level and rats showed no ill-effects.

Expt B. Effect of MW and degree of methoxylation of pectins on the hypocholesterolaemic effect

Food intake and weight gain. Weight gain was reduced in all four pectin-fed groups but was significant only in HMW groups (Table 3). There were significant differences also between LMW and HMW pectins with similar methoxyl content. Food intake was also reduced in the HMW groups, but not in LMW groups, suggesting that MW and hence viscosity are factors affecting food intake and hence weight gain. Food conversion efficiency was significantly reduced only in the HMW high-methoxyl groups ($P < 0{\cdot}01$).

Plasma lipid levels. Plasma cholesterol levels were significantly lower than control values only in the HMW HMP group. The value for the HMW LMP group appeared anomalous considering the low food intake and weight gain but similar results were given by re-analysis. There was no correlation between final weight or weight gain of animals and plasma cholesterol in any group. Plasma cholesterol levels in the control group were found to correlate with food intake ($P < 0{\cdot}01$), but there was no such effect in the pectin groups, suggesting that reduced food intake and weight gain *per se* are not the main mechanisms of the hypocholesterolaemic action of pectins.

Liver lipid levels. Good correlation was again seen between liver and body-weights of animals ($P < 0{\cdot}01$ for all groups except HMW HMP where $P < 0{\cdot}05$). Compared with controls, animals given HMW pectins had lower levels of liver lipid and liver cholesterol; on the LMW HMP diet the liver cholesterol, but not the liver lipid, was lower. Within the HMW groups those animals fed on HMP had lower (non-significant) levels of liver lipid and cholesterol than those fed on LMP, which suggest that degree of methoxylation has some effect, although this was not observed in the LMW groups.

Faecal analysis. Faecal lipid excretion was apparently higher in all pectin-fed groups (highest in HMP groups) compared with the control group, but these effects did not reach statistical significance. The HMW LMP group had the lowest lipid excretion of the pectin-fed groups; possibly explaining its high plasma cholesterol level. When expressed as a 'balance', i.e. lipid retained in the body, this was reduced in all pectin-fed groups, particularly those fed on the HMW form.

Cholesterol balance was negative or low in all pectin-fed groups, i.e. there may have been excretion of endogenous as well as dietary cholesterol.

Table 3. *Expts B and C. Effect of pectins of differing molecular weight (MW) and methoxyl content (M) on growth, food conversion efficiency and plasma and liver lipids in rats*

(Mean values with their standard errors for ten (B) or twelve (C) rats/group. All diets contained 100 g oil/kg. Fibre content of diets in Expt B was 100 g/kg and in Expt C 50 g/kg)

	Expt B										Expt C					
Group...	Control†		HMW HMP		HMW LMP		LMW HMP		LMW LMP		Control		Pectin NF		VHMP	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Original wt of rats (g)	51	0·8	53	0·5	54	1·0	56	0·7	56	0·7	72	2	72	2	72	2
Change in wt of rats (g)	126	6	69**^{b}	3	79**^{a}	6	111^{b}	5	102^{a}	5	119	4	89**	4	82**	4
Food intake (g)	306	9	224**	3	208**	11	298	8	319	18	297	13	241*	10	237*	10
Food conversion efficiency‡	0·39	0·02	0·31**	0·02	0·37	0·02	0·38	0·01	0·34	0·03	0·40	0·01	0·36*	0·01	0·35*	0·01
Fat balance§ (g)	29	1·1	15·9**^{b}	1·8	18·3**^{a}	1·8	22·6*	0·7	27·5*^{ab}	1·9	24·6	1·2	15·3**	1·2	16·9**	0·3
Cholesterol balance§ (g)	1·5	0·2	−0·4**	0·3	0·04*	0·4	−0·6*	0·2	−0·4	0·5	1·5	0·1	0·9*	0·1	1·0*	0·1
Plasma cholesterol (mmol/l)	3·23	0·32	1·98*	0·14	3·74	0·48	3·36	0·21	2·83	0·22	3·59	0·15	2·53*	0·11	2·52*	0·11
Liver																
Lipid (mg/g)	113	7	60**^{b}	6	67**	3	98^{b}	5	87*	6	—	—	—	—	—	—
Lipid (mg/rat)	990	92	295**^{b}	30	398**^{a}	34	862^{b}	64	745^{a}	56	—	—	—	—	—	—
Cholesterol (mg/g)	37	2	8**^{c}	2	10**^{b}	1	25**^{c}	2	30^{b}	2	—	—	—	—	—	—
Cholesterol (mg/rat)	327	23	41**^{c}	5	60**^{b}	8	223*^{c}	26	255*^{b}	24	—	—	—	—	—	—

HMW, high molecular weight; LMW, low molecular weight; HMP, high methoxyl pectin; LMP, low methoxyl pectin; VHMP, very high methoxyl pectin.
Significantly different from control: * $P < 0·05$, ** $P < 0·01$.
Within a horizontal row, values with the same superscript letters were significantly different: ^{a} $P < 0·05$, ^{b,c} $P < 0·01$.
† Control diet contained Solkafloc.
‡ Weight gain (g)/food intake (g).
§ Fat and cholesterol balance calculated as intake − excretion.

Expt C. Effect of VHMP

Weight gain and food intake. Weight gain, food intake and feed efficiency were reduced in both pectin-fed groups (50 g/kg NF and VHMP) by similar amounts compared with the controls (Table 3).

Fat and cholesterol balance were also reduced in both groups. The lower fat balance appeared to be due to increased faecal fat and the reduced cholesterol balance to continued cholesterol excretion despite the lower food intake.

Plasma cholesterol. Feeding both pectins resulted in a significant reduction in plasma cholesterol levels. VHMP was no more effective than pectin NF, despite the high degree of methoxylation (81% *v.* 69–70%), possibly because the viscosities were similar (see Table 1).

Expt D. Effects of dose of HMP and LMP on hypocholesterolaemic effect

Weight gain and food intake. Weight gain was significantly reduced in the 100 g LMP/kg group and in both the 50 and 100 g HMP/kg groups compared with their respective controls (50 and 100 g Solkafloc/kg) (Table 4). This was related to food intake, especially at the 100 g/kg level, but feed efficiency also appeared lower, significantly so only for 100 g HMP/kg ($P < 0{\cdot}01$).

Plasma cholesterol levels. Plasma cholesterol levels of all pectin-fed groups were lower than their respective controls. The hypocholesterolaemic effect was greater as dose increased and within each dose level the HMP caused the greatest fall. Again there was no correlation between final weight or change in weight except in the group given 50 g HMP/kg where change in weight correlated with cholesterol levels (R 0·77, $P < 0{\cdot}01$).

Expt E. To confirm the effect of LMP and find the lowest effective dietary concentration of HMP

Food intake and weight gain. Food intake and weight gain were similar in all three groups (25, 50 and 100 g Solkafloc/kg) but reduced in all pectin-fed groups except for the group given 50 g LMP/kg (Table 5). There was considerable variation in food conversion efficiency in this experiment.

Apparent N digestibility was reduced by 50 g HMP/kg and 100 g LMP/kg. Energy digestibility was reduced by 50 g HMP/kg and metabolizable energy (ME) intake was lower, both in this and the 100 g/kg group.

Fat and cholesterol balances were reduced in both 25 and 50 g HMP/kg and 100 g LMP/kg groups. Again, the decrease in fat balance appeared to be due to increased faecal fat levels in these animals, whereas cholesterol excretion was similar in all groups, i.e. the reduction in balance appeared to be caused by the reduced food intake. The increase in faecal fat would have contributed to the lower available metabolizable energy in these groups.

Plasma cholesterol. Only the rats consuming the 50 g HMP/kg and 100 g LMP/kg diets showed significantly lower plasma cholesterol levels. However, the mean cholesterol level for the 50 g/kg control group was low compared with previous experiments. This can be seen in Fig. 1 which shows the relation between the level of Solkafloc or HMW pectin in the diets and plasma cholesterol in all the experiments described previously.

Observations on gut size and gut contents

Gut length and weight. Although it was intended that rats in which these measurements were made should be of similar weight, this was impossible to achieve due to the reduced weight gain in pectin-fed animals. The largest animals in each pectin group were therefore chosen, and the results expressed per kg body-weight.

Table 4. *Expt D. The effects of high and low methoxyl pectins at doses of* 50 *and* 100 *g/kg diet on growth, food conversion efficiency and plasma cholesterol levels in rats*

(Mean values with their standard errors for ten rats/group)

Group...	Control		HMP		LMP		Control		HMP		LMP	
Fibre content of diet (g/kg)...	50		50		50		100		100		100	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Original wt of rats (g)	62	1	72	3	73	2	59	4	85	3	86	1
Change in wt of rats (g)	126	3	98*	3	121	5	130	7	89**	5	107*	4
Food intake (g)	297	9	247*	13	293	10	297	16	270	7	266	8
Food conversion efficiency†	0·42	0·01	0·39	0·02	0·41	0·01	0·44	0·02	0·31**[a]	0·01	0·41[a]	0·03
Plasma cholesterol (mmol/l)	3·80	0·32	1·96*	0·26	2·77	0·16	3·14	0·30	1·45**	0·07	2·17*	0·15

HMP, high methoxyl pectin; LMP, low methoxyl pectin. Control diets contained Solkafloc.
Significantly different from the control: * $P < 0·05$, ** $P < 0·01$.
Within a horizontal row, values with the same superscript letters were significantly different: [a] $P < 0·01$.
† Weight gain (g)/food intake (g)

Table 5. *Expt E. The effects of high and low methoxyl pectins at dose levels of 25, 50 and 100 g/kg diet on growth, food conversion efficiency, fat, energy and nitrogen digestibilities and plasma cholsterol in rats*

(Mean values with their standard errors for ten rats/group)

Group...	Control†		HMP		Control		HMP		LMP		Control		LMP	
Fibre content of diet (g/kg)...	25		25		50		50		50		100		100	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Original wt of rats (g)	61	2	60	1	59	2	61	1	60	2	60	1	61	2
Change in wt of rats (g)	141	6	110***	3	141	4	116*^{b}	4	143^{b}	5	145	5	122**	3
Food intake (g)	369	33	266*	16	309	23	218*^{b}	18	341^{b}	16	334	9	280*	16
Food conversion efficiency‡	0·39	0·02	0·40	0·01	0·48	0·01	0·55	0·04	0·43	0·03	0·42	0·01	0·40	0·04
Apparent N digestibility	0·95	0·006	0·93	0·006	0·91	0·008	0·82**^{b}	0·022	0·90^{b}	0·006	0·91	0·007	0·85**	0·007
N absorbed (g/rat)	7·8	0·6	6·5	0·05	5·6	0·3	4·1*^{b}	0·5	7·1^{b}	0·5	7·1	0·2	5·4**	0·4
Apparent energy digestibility	—	—	—	—	0·92	0·005	0·82*	0·031	0·90	0·004	0·87	0·007	0·86	0·005
Metabolizable energy intake (MJ/rat)	—	—	—	—	5·4	0·2	3·3**^{b}	0·4	5·3^{b}	0·3	5·5	0·2	4·4**	0·3
Fat balance§ (g)	40	3	27**	2	31	1	18**^{b}	2	33^{b}	2	35	1	23**	1
Cholesterol balance§ (g)	2·7	0·3	1·7*	0·2	1·9	0·1	1·1*^{b}	0·2	2·4^{b}	0·2	2·3	0·2	1·7*	0·2
Plasma cholesterol (mmol/l)	3·95	0·41	3·13	0·32	2·94	0·16	2·12*	0·16	2·65	0·12	3·16	0·24	2·52**	0·17

HMP, high methoxyl pectin; LMP, low methoxyl pectin.
Significantly different from control: * $P < 0·05$, ** $P < 0·01$, *** $P < 0·002$.
Within a horizontal row, values with the same superscript were significantly different:^{b} $P < 0·01$.
† Control diet contained Solkafloc.
‡ Weight gain (g)/food intake (g).
§ Fat and cholesterol balances calculated as intake − excretion.

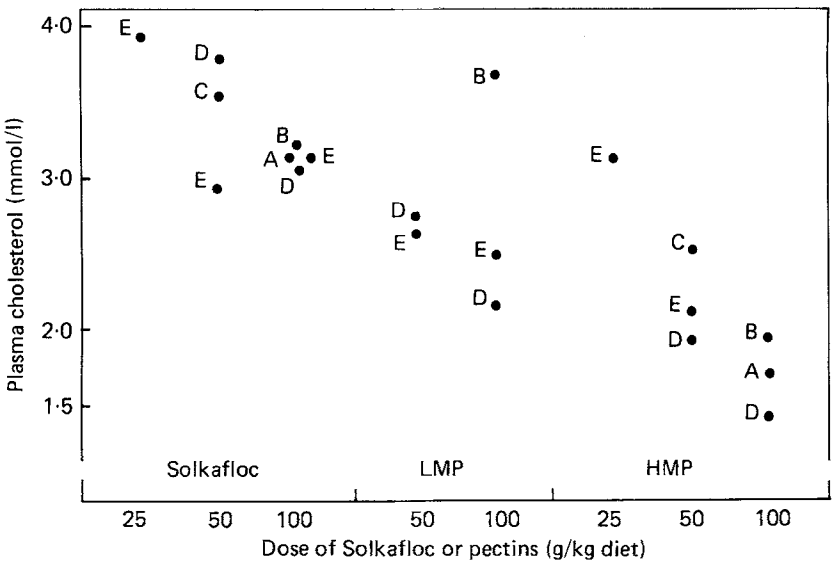

Fig. 1. Effect of dose level of high molcular weight pectins (g/kg diet) and Solkafloc on plasma cholesterol level. Each point represents the mean of ten to twelve rats. A–E, Expts A–E.

Pectin-fed animals had larger guts than control animals (Plate 1), the differences being in both length and weight (Table 6). Significant differences in length/kg body-weight were shown by HMW pectins in Expts B and C and by HMP and 100 g LMP/kg in Expt E.

Significant differences in weight/kg body-weight were also shown by pectin NF and VHMP in Expt C and by HMP and 100 g LMP/kg in Expt E.

Observations on gut contents (*Table 7*). Despite lower food intakes in most pectin-fed groups in the 24 h before death the total weight of gut contents was always greater (the major part of this being in the distal small intestine). Weight of faeces was higher in the control groups compared with HMW, probably due to undigested cellulose. Pectin has been reported to be largely digested by gut bacteria (Werch & Ivy, 1941; Cummings *et al.* 1979) and the bulking effect would therefore be expected to be abolished at this stage. This was not apparent with LMW, however, where faecal weights were similar to those of the control group.

When the gut contents were centrifuged there were obvious physical differences between those of control and pectin-fed groups. A supernatant liquid always separated in the contents from control groups but only occasionally in those from pectin-fed groups. When separation did occur in the contents from the pectin-fed groups, the amount of supernatant was always less than that from the control group and had an oily appearance. The appearance and texture of the pellets were also different. In control animals the pellet was white, grainy and firm and cut cleanly; in pectin-fed animals it was yellow, smooth, shiny and pasty looking; when cut it was soft and sticky.

Other observations were that pectin-fed animals always had full, swollen stomachs whereas control stomachs were empty, suggesting slowing down of gastric emptying by the pectin 'gel', and pectin-fed animals always had large gas-filled caecums possibly due to bacterial breakdown there.

Table 6. *Observations on weight and length of small intestine (SI) related to body-weight (BW) of rats*

(Mean values with their standard errors. No. of rats/group in parentheses)

Dietary regimen	Fat (g/kg diet)	Final BW (g) Mean	SE	Wt of SI (g) Mean	SE	Length of SI (mm) Mean	SE	Wt of SI (g)/BW (kg) Mean	SE	Length of SI (mm)/BW (kg) Mean	SE
					Experiment A						
Control†	100	162	3	—	—	101	3	—	—	6·2	0·1
Pectin NF	50	144	3	—	—	109	2	—	—	7·6	0·2
Pectin NF	100	141	1	—	—	104	2	—	—	7·5	0·2
Pectin NF	150	132	5	—	—	106	6	—	—	8·1	0·5
Pectin NF (4)	200	123	3	—	—	110	3	—	—	8·9	0·3
					Experiment B						
Control	—	165	9	—	—	90	5	—	—	5·5	0·1
HMW HMP	—	127	3	—	—	101	4	—	—	7·9**^{c}	0·3
HMW LMP	—	148	4	—	—	99	1	—	—	6·7**^{ba}	0·1
LMW HMP	—	171	7	—	—	100	3	—	—	5·8^{bc}	0·2
LMW LMP (5)	—	163	8	—	—	95	4	—	—	6·0^{a}	0·2
					Experiment C						
Control	—	191	6	7·24	0·5	108	2	0·38	0·03	5·7	0·1
Pectin NF	—	161	7	8·94*	0·6	120*	3	0·57**	0·03	7·5**	0·2
VHMP (12)	—	155	7	7·88	0·6	119*	3	0·50**	0·02	7·7**	0·2
	Fibre (g/kg diet)				Experiment E						
Control	25	202	8	7·9	0·6	113	3	0·39	0·02	5·6	0·2
HMP	25	170	4	8·5*	0·4	118	2	0·50*	0·03	7·0**	0·2
Control	50	200	5	8·6	0·3	110	2	0·43	0·01	5·5	0·1
LMP	50	203	5	8·2	0·3	121	2	0·40	0·01	5·5	0·2
HMP	50	177	3	9·7	0·6	121	3	0·54*	0·03	6·8**	0·1
Control	100	205	5	7·7	0·3	115	2	0·37	0·01	5·6	0·1
LMP (10)	100	183	4	8·7**	0·3	122**	3	0·47*	0·02	6·7**	0·2

HMW, high molecular weight; LMW, low molecular weight; NF, National Formulary; HMP, high methoxyl pectin; LMP, low methoxyl pectin; VHMP, very high methoxyl pectin.

Significantly different from control: * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$.

Values with the same superscript letters were significantly different: ^{a} $P < 0{\cdot}05$, ^{b,c} $P < 0{\cdot}01$.

† Control diets contained Solkafloc.

DISCUSSION

Hypocholesterolaemic effects of pectins

The hypocholesterolaemic activity of pectins has been demonstrated in these studies under various conditions using the rat as an experimental model. HMW HMP lowered cholesterol levels whenever used, significant effects being found at 50 g or more/kg diet; LMP was shown to be effective at the 100 g/kg level. LMW HMP, however, was not an effective hypocholesterolaemic agent. Fig. 1 shows a (non-significant) trend towards lower cholesterol levels with increasing dose of HMP, LMP and Solkafloc.

Liver lipids were also shown to be reduced in rats given the pectin diets, which is consistent with the observations of other workers (Wells & Ershoff, 1961; Leveille & Sauberlich, 1966;

Table 7. *Expts A and B. Observations on food intake, gut contents and faecal output, for four (Expt A) or five (Expt B) rats/group*

Dietary regimen	Fat (g/kg diet)	Final wt of rat (g)		Mean wet food intake (g)		Wt of gut contents (g)						Pellet: supernatant fraction			Wet wt of faeces (g)
						Upper SI		Lower SI		Colon					
		Mean	SE	Last 24 h/d		Mean	SE	Mean	SE	Mean	SE	Upper SI	Lower SI	Colon	
								Experiment A							
Control†	100	162	3	13	35	1·1	0·1	1·9	0·2	0·4	0·1	0·67	1·06	2·79	3·0
Pectin NF	50	144	3	10	22	2·0	0·9	3·6	0·8	0·2	0·1	3·53	(XS)	(5·02)[2]	1·8
Pectin NF	100	141	1	10	22	1·2	0·2	3·4	0·4	0·2	0·1	2·40	(3·06)[2]	(3·71)[1]	1·5
Pectin NF	150	131	5	10	20	1·9	0·1	2·8	0·3	0·3	0·1	(0·51)[3]	(3·02)[3]	(6·13)[1]	1·5
Pectin NF	200	123	3	10	25	1·9	0·2	3·0	0·1	0·4	0·1	2·63	(15·04)[3]	(2·41)[2]	1·4
								Experiment B							
Control†	100	165	8	13	16	1·2	0·3	1·7	0·1	0·8	0·2	1·59	2·18	2·47	3·1
HMW HMP	100	127	3	10	10	1·7	0·2	3·0	0·2	0·5	0·1	0·71	(6·55)[1]	(6·03)[1]	1·8
HMW LMP	100	148	3	9	12	2·2	0·2	4·9	0·7	0·7	0·1	1·88	14·32	2·50	1·7
LMW HMP	100	171	7	13	16	1·8	0·2	2·4	0·2	0·7	0·1	0·88	2·65	(2·26)[4]	2·9
LMW LMP	100	163	8	14	14	1·6	0·2	3·5	0·4	0·7	0·1	1·09	4·26	7·11	2·8

Fibre content of diets was 100 g/kg in Expts A and B.
NF, National Formulary; HMW, high molecular weight; LMW, low molecular weight; HMP, high methoxyl pectin; LMP, low methoxyl pectin; SI, small intestine.
Values in parentheses indicate that no supernatant separated in all (XS) or some samples on centrifugation. Superscripts indicate the number of samples not separating.
† Control diet contained Solkafloc.

Riccardi & Fahrenbach, 1967). Total lipid and cholesterol excretions were measured in Expts B, C and E. Total lipid excretion was increased in animals given the pectin diets compared with those given the control diets in all the experiments and, in Expt B, cholesterol excretion was also increased giving negative cholesterol balance which may indicate losses of endogenous cholesterol as well as reduced absorption. Mokady (1973) has similarly shown a fivefold increase in fat excretion and a twofold increase in steroid excretion in animals given pectin diets compared with controls.

The experiments described have indicated that HMP are more potent hypocholesterolaemic agents than LMP, as have other workers (Ershoff & Wells, 1962; Anderson & Bowman, 1969; Mokady, 1973), and that LMP is effective in high doses. Viscosity seems to be an important factor as well. In most cases where inherent viscosity was greater than 34 ml/g there was a lowering of cholesterol levels, irrespective of degree of methoxylation, the exception to this being HMW LMP in Expt B. Mokady (1973) had also suggested that this might be important. The methoxyl groups in the molecule contribute to the viscosity (Kertesz, 1951; Rees, 1967; McKay *et al.* 1969) so that HMP generally have higher viscosities (see Table 1) but this is overcome by degrading the molecule. It is not, however, possible to produce a pectin in which all free acid groups are methoxylated (Kertesz, 1951) and attempts to do this result in degradation. This may account for the lack of effect of VHMP (81% methoxylated) whose viscosity was similar to that of the pectin NF (69% methoxylated). The low viscosity of HMP in Expt B may also have contributed to its lack of effectiveness.

Pectin has been shown to increase faecal bile acid excretion in man (Kay & Truswell, 1977). It has been suggested that bile-acid binding may depend on the number of uronic acid groups rendered permanently un-ionized by the presence of methyl groups (Eastwood & Mowbray, 1976); HMP would therefore be expected to be more effective.

However, other gel-forming polysaccharides such as guar gum (Ershoff & Wells, 1967; Riccardi & Fahrenbach, 1967) and konjac mannan (Kiriyama *et al.* 1969), which do not have methoxyl groups, also have hypocholesterolaemic activity.

Further investigation of this may be useful as in vitro studies have shown that maximum bile-acid binding occurs at low pH (Eastwood & Hamilton, 1968). The pH of bile is 7·0–7·4 but solutions of pectins themselves have an acid pH. The pectins comprised a significant proportion of the diet in this study and they may have provided a suitable environment, with stomach acid perhaps trapped in a gel within which bile-acid binding could occur.

Bile salts may have been partitioned into the gel phase of the gut contents in this experiment, lowering their concentration in the absorptive phase. Suggestive evidence for this is seen in the results of centrifuging gut contents in Expts A and B. Centrifugation has been used to separate the micellar component of gut contents (McIntyre *et al.* 1971) and the fact that no liquid phase could be separated in pectin-containing digesta may indicate that micelle formation was disrupted or that the micellar component was bound within a gel.

The decreased retention of fat and cholesterol may also point to some disruption of absorptive processes. Other workers have shown decreased absorption of dietary cholesterol (Lin *et al.* 1957; Hyun *et al.* 1963; Kiriyama *et al.* 1969; Kelly & Tsai, 1978) and the fact that pectin is effective as a hypocholesterolaemic agent when it is given on alternate days, cholesterol being given on the remaining days, suggests (as do the results of Expt B here) that endogenous cholesterol is affected too (Wells & Ershoff, 1961; Leveille & Sauberlich, 1966). Mokady (1974) has demonstrated increased synthesis of cholesterol in the liver in pectin-fed rats on a cholesterol-free diet, which would further indicate reduction of endogenous cholesterol absorption. Kelly & Tsai (1978), however, showed that increased turnover only occurred when cholesterol was present in the diet. These differences may be partly explained by the composition of the diet.

In Expt A, the effect of pectin was found to be greater at low levels of dietary fat. Cholesterol absorption is facilitated by high-fat intakes (Kim & Ivy, 1952) and pectin may then be less effective. Chang & Johnson (1976) have noted similar effects and also show that pectin is less effective when the carbohydrate source is sucrose.

Another explanation for lower cholesterol levels in pectin-fed animals could be reduced food intake and smaller weight gain. To test this hypothesis the cholesterol levels of all control animals were compared with food intake, weight change and final weight but no correlations could be found.

Other workers have shown reduced weight gain in pectin-fed animals (Lin *et al.* 1957; Riccardi & Fahrenbach, 1967; Viola *et al.* 1970; Mokady, 1974; Hove & King, 1979) but it is not a consistent effect (Wells & Ershoff, 1961; Leveille & Sauberlich, 1966; Tsai *et al.* 1976) and appears to be minimized by long-term feeding (Ershoff & Wells, 1967). The reduced food intake, which is the major cause of this, may be due to stomach distension and the slowing of gastric emptying which has been demonstrated in rats given guar gum (Leeds *et al.* 1979): non-starved pectin-fed animals always had full stomachs at death, whilst control animals rarely did. Reduced food conversion efficiency was also demonstrated in some pectin-fed groups and the reduction of apparent N and energy digestibilities, demonstrated in Expts C and E when HMP and high doses of LMP were given, would contribute to this.

Effects on gut morphology and gut contents

In Expts A and B the contents of the small intestine were seen to be greater in pectin-fed rats despite lower food intakes. Faecal weight was lower in rats given HMW pectins however. Pectin is known to be digested by gut bacteria (Werch & Ivy, 1941) whereas cellulose is digested to a much lesser extent (Williams & Olmstead, 1938). This, together with the different behaviour of the gut contents on centrifugation, suggests that the effects of pectin are mediated largely in the small intestine, although fermentation of pectin in the large gut may also have peripheral effects due to production and absorption of volatile fatty acids.

It is suggested that the viscous gut contents seen here may retain the bile acid, fat and cholesterol within a gel and prevent absorption and may also present a barrier to efficient lipolytic activity. Diffusion through this mass and the unstirred layer of the small intestine may also be altered (Kay & Truswell, 1977).

The changes in gut morphology may also be significant. Feeding of high-fibre diets has been shown to cause caecal enlargement in rats (Food and Agriculture Organization/World Health Organization, 1975) and increased weights and lengths of colon and rectum were seen in rats given high- compared with low-fibre diets (Younoszai, 1978). Weights and lengths of colon and rectum were not studied here but increased small intestine (SI) length was found in Expts A, B and E and increased weight in Expt E, despite lower body-weights. Similar effects have been shown by other workers (Addis, 1931–2; Brown *et al.* 1978). Scott (1978) compared the effects of pectin and bran on these variables, pair-feeding the animals, and found that only pectin increased the weight and length of the gastrointestinal tract.

The suggestion has also been made that gut muscle layers may be enlarged due to increased physical work required to propel a gel but this has not been borne out by histological studies. Brown & Kelleher showed an increased crypt:villus value (Brown *et al.* 1978) due to shortening of villi and, in their study on paracetamol absorption, suggested that the absorptive area may be increased (Brown *et al.* 1979). Cassidy *et al.* (1981) reported that scanning electron microscopy of the guts of rats given high levels of pectin demonstrated denudation of microvilli in the epithelial cells of the small intestine and a 'cracked clay' appearance in the colon. These effects may be undesirable, as they may affect absorption and therefore merit further study.

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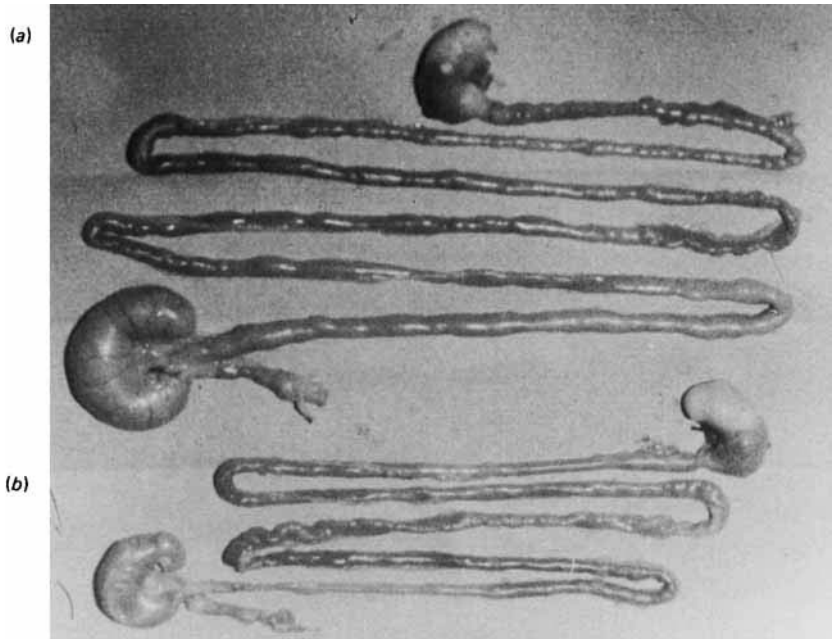

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EXPLANATION OF PLATE

Plate 1. Gastrointestinal tract from (*a*), pectin-fed (body-weight 140 g) and (*b*), control (body-weight 157 g) animals.

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