The influence of carbohydrate gelling agents on rat intestinal transport of monosaccharides and neutral amino acids in vitro

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Summary
1. In the present investigation with rings of everted rat small intestine, carbohydrate gelling agents (plant polysaccharides) such as guaran, pectin, tragacanth, carubin and carrageenan were employed to study their direct effect on intestinal absorption of α-methyl-D-glucoside, D-galactose, L-leucine and L-phenylalanine.
2. Inhibition was found to correlate with the viscosity of the incubation medium, a function only of the polysaccharide concentration, and was independent of other properties of the carbohydrate gelling agents.
3. Reversal of this inhibition was achieved either by washing the tissue free of polysaccharide or by raising tissue agitation.
4. Uptake kinetics in polysaccharide-containing solutions revealed a marked increase of the apparent Michaelis constant although the maximal transport capacity remained essentially unaltered.
5. Since there was no binding of the substrate by the polysaccharides under experimental conditions as judged by a membrane filtration technique, it is concluded that carbohydrate gelling agents may impair intestinal absorption by means of an increased unstirred layer resistance.

Key words: amino acid absorption, binding, carbohydrate gelling agents, dietary fibre, intestine, monosaccharide absorption, transport kinetics, unstirred layer.

Introduction
Carbohydrate gelling agents, plant polysaccharides such as guaran, pectin, tragacanth, carubia and carrageenan, the last-named an algal product, are partially related and contribute to the soluble part of dietary fibre. These substances exhibit physico- and bio-chemical properties which may affect or impair intestinal absorption of nutrients or their final cleavage products. When taken orally some of these carbohydrate gelling agents were found to influence post-prandial blood glucose levels (Jenkins, Goff, Leeds, Alberti, Wolever, Gassull & Hockaday, 1976; Jenkins, Leeds, Gassull, Cochet & Alberti, 1977a; Jenkins, Gassull, Leeds, Metz, Dilawari, Slavin & Blendis, 1977b; Jenkins, Wolever, Leeds, Gassull, Haisman, Dilawari, Goff, Metz & Alberti, 1978). At present little is known of the mechanisms of the gastrointestinal interactions of these polysaccharides although there are several possibilities. Carbohydrate gelling agents may delay gastric emptying (Holt, Heading, Carter, Prescott & Tothill, 1979; Leeds, 1979) or impair absorption within the small intestine itself. A reduced absorption may be caused either by an altered diffusion rate or by binding as was found for bile acids with some fibre constituents (Eastwood & Hamilton, 1968; Birkner & Kern, 1974; Kritchevsky & Story, 1974). The aim of this study is to characterize and elucidate the effects of carbohydrate gelling agents on the intestinal uptake of monosaccharides and neutral amino acids in vitro.

Methods
Materials
L-[U-14C]Leucine, α-methyl-D-[U-14C]glucoside and D-[N-1-3H]mannitol were obtained from NEN Chemicals GmbH (Dreieich, Germany), L-[U-14C]phenylalanine and D-[1-14C]galactose from Amersham Buchler GmbH.
Incubation technique

The present experiments were performed by incubation of rings of everted rat small intestine \textit{in vitro} according to previously described and well-established methods (Crane & Mandelstam, 1960; Caspary, Stevenson & Crane, 1969; Alvarado & Mahmood, 1974). Mid small intestine of fed Wistar rats (weighing 150-200 g) was everted and cut into 0.5-1 cm pieces which were then randomly distributed into 25 ml Erlenmeyer flasks containing 5 ml of Krebs–Henseleit phosphate buffer (KH buffer) with \(^{14}\)C-labelled substrates and the appropriate carbohydrate gelling agents at pH 7.2-7.4 and 37°C. Incubations were made in a shaking incubator (model 3047, Kottermann, Hanigsen, Hannover, Germany) having a total horizontal excursion of about 1 cm at rates between 0 and 350 cycles/min. Usually incubation was for 3 min, since within this period tissue uptake was linear both in the controls and polysaccharide-containing solutions. The short time period also minimizes the influence of intracellular accumulation on the results, thus reflecting mainly initial uptake rates. The following compounds were employed as substrates: \(\alpha\)-methyl-D-glucoside (\(\alpha\)-Me-Glc), D-galactose (Gal), L-leucine (Leu) and phenylalanine (Phe).

Analytical methods and calculation of results

Processing of the incubated tissue was accomplished by the methods previously described (Crane & Mandelstam, 1960). \(^{3}\H\)Mannitol was used to correct for the extracellular space. Radioactivity was assayed with a Searle liquid-scintillation system (mark III) by using an automatic quench correction and a counting program for double-labelled samples. Tissue accumulation or uptake of \(^{14}\)C-labelled substrate was expressed as a distribution ratio:

\[
\text{Tissue uptake} = \frac{\text{substrate concentration in intracellular fluid volume (} S_I \text{)}}{\text{substrate concentration in incubation medium (} S_M \text{)}}
\]

assuming an intracellular fluid volume of 80% of the tissue net weight (Crane & Mandelstam, 1960; Alvarado & Mahmood, 1974). Tissue uptake rates as used in the evaluation of transport kinetics are expressed as substrate concentration in the intracellular fluid achieved per min. The unpaired Student’s \(t\)-test was used to determine whether significant differences occurred in the indices measured.

Polysaccharide solutions

Polysaccharide-containing incubation media were prepared by dispersing the commercially available powder compounds by vigorous stirring in hot KH buffer. After boiling for 5 min solutions were filtered through cheesecloth, allowed to cool, and, if necessary, adjusted to pH 7.2-7.4. Tissue incubations as well as binding studies were performed with these polysaccharide solutions.

The relative viscosity of the polysaccharide solutions was determined at 37°C with a capillary viscosimeter (viscosity in KH buffer was taken arbitrarily as 1). For this purpose polysaccharide solutions had to be free of the solid particles in trace amounts commonly present in the commercial products (particularly in guaran, carubin and tragacanth). To remove insoluble residues the carbohydrate gelling agents were dissolved in distilled water by boiling for 5 min, and, after centrifugation at 15 000 \(g\) for 20 min, solutions were freeze-dried to obtain partially purified polysaccharides. Some fractionation of the polysaccharides may occur as a result of this procedure, but major changes are unlikely. Only for tragacanth may the soluble portion, subsequently referred to as tragacanthin, exhibit slightly altered properties.

Binding studies

In order to examine binding of low-molecular-weight substrates to soluble polymers, techniques are available which enable the separation of bound and unbound substrate on the basis of their different molecular weights. In the present study, ultrafiltration on membranes having a general retentivity for molecular weights greater than 1 000 (BM-10, Berghof GmbH, Tübingen, Germany) was employed. A portion of polysaccharide solution (8 ml) containing radioactive substrate (1 mmol/l; approx. 5 0Ci/l) was placed in a 10 ml ultrafiltration cell (Amicon Corporation, Lexington, MA, U.S.A.) and the filtrate was collected in three consecutive 0.5 ml fractions in which substrate concentration was determined by measuring radioactivity. Because some buffer was left in the filter system, causing an initial dilution, the first fraction was rejected.
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FIG. 1. Effect of increasing concentrations of carbohydrate gelling agents in the incubation medium upon the tissue uptake of monosaccharides and neutral amino acids. Substrates (mmol/l): (a) α-methyl-d-glucoside (3), (b) D-galactose (2), (c) L-leucine (1), and (d) L-phenylalanine (1). Polysaccharide concentrations were varied between 0 and 5 g/l for guaran (+0), carubin (++), and galactomannan (LU), between 0 and 10 g/l for carrageenan (O-O-O), between 0 and 12.5 g/l for tragacanthin (V-V) and between 0 and 15 g/l for pectin (∆-∆-∆). The abscissa represents the corresponding viscosities on a log scale. Everted segments of rat small intestine were incubated in KH buffer with or without polysaccharides at a shaking rate of 120 cycles/min at 37°C for 3 min. The viscosity of the KH buffer (control) was arbitrarily taken as 1. Values are means of four determinations; the coefficient of variation averaged 10%.

Results

Increasing concentrations of carbohydrate gelling agents in aqueous solutions led to an exponential increase of the viscosity. For substrates actively transported in rat small intestine this caused a significant reduction in the initial tissue uptake in vitro (Fig. 1). The abscissa represents the viscosity on a logarithmic scale which would coincide with a linear plot of the polysaccharide concentration. As Fig. 1 shows, there is no significant difference between the effects of the individual polysaccharides on the tissue uptake if their concentration is expressed as the resulting viscosity. In all these experiments a relative viscosity between 45 and 70 centipoises reduced the tissue uptake to about 55% of the control value. According to the nature of the carbohydrate gelling agents different amounts were needed to produce solutions with similar viscosity (see the legend to Fig. 1).
Reversibility of this inhibition was tested simply by washing the tissues. In these experiments the everted segments of rat small intestine were exposed to the polysaccharide for an even longer period (15 min). After a 12 min intermediate incubation either in polysaccharide containing KH buffer or in KH buffer alone (to wash off the adherent polysaccharide) tissue accumulation was determined in a final 3 min incubation with or without polysaccharide. Washing of the tissue restored the tissue uptake completely as exemplified by Fig. 2 for guaran as polysaccharide and α-methyl-D-glucoside as substrate. Complete reversal of the inhibition was also obtained with carrageenan and α-methyl-D-glucoside as well as with guaran and L-leucine. These results were thought to provide sufficient evidence for a more general mechanism, so other combinations of gel and substrate were not tested further.

If unstirred layer phenomena are responsible for the reduction in tissue uptake an increased agitation of the tissue should abolish the inhibitory effect of polysaccharides in solution. The dependency of the tissue uptake of L-leucine (at a concentration of 1 mmol/l) on the shaking rate was studied for guaran and carrageenan employing two different polysaccharide concentrations. With both polysaccharides similar sigmoid curves were obtained (Fig. 3). At very...
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Table 1: Effect of guaran and shaking rates on the kinetic parameters of the small-intestinal uptake of L-leucine in vitro

Results are means ± SD (n = 4); pairs of parameters (K_m, V_max) were determined by four separate experiments (everted intestinal segments from two male Wistar rats (230–280 g) and five different substrate concentrations, 2, 4, 8, 16 and 32 mmol/l, in duplicates for each experiment) and calculated from corresponding Lineweaver–Burk plots. Incubation was at 37°C for 3 min in KH buffer (pH 7.4) with or without guaran (5 g/l).

Table 2: Increase of transport K_m values by various carbohydrate gelling agents

Experimental conditions were similar to those described in Table 1. The increase of K_m for each substrate and polysaccharide was calculated from corresponding Lineweaver–Burk plots obtained from two separate experiments with everted jejunal segments and a shaking rate of 120 cycles/min.

In order to further characterize the inhibitory effect a detailed determination of the influence of carbohydrate gelling agents on transport indices was performed with guaran and L-leucine. The results obtained (Table 1) show a highly significant increase in the K_m values due to addition of guaran to the incubation medium. Although the increase at the lower shaking rate was about 100%, at 250 cycles/min the effect of guaran was less pronounced, showing an increase of about 50%. Despite the fact that K_m values were lower in the ileum, changes caused by the polysaccharide addition or the altered shaking rate did not differ greatly between the two parts of rat small intestine. Comparing the V_max values, however, a significant difference in the maximal transport capacity was not found in any of these experimental conditions.

To generalize the competitive type of inhibition further experiments were undertaken with combinations of polysaccharides and substrates chosen at random. The preceding experiments led to the selection of a shaking rate of 120 cycles/min, for which significant differences in tissue uptake could be expected. With all substrates and polysaccharides tested similar results were obtained (Table 2). In poly-
Saccharide solutions having a relative viscosity of about 50 centipoises the increase of the apparent Michaelis constant averaged 115%, although the maximal transport capacity remained essentially unchanged.

On the basis of the experimental conditions (short-term incubations), it was unlikely that the results were due to intracellular events. Thus the observed effect of carbohydrate gelling agents should not only apply to actively transported substrates but also to other luminal interactions, e.g. blocking or binding, with the corresponding transport carrier. For this reason the inhibitory effect of phlorizin on the uptake of α-methyl-D-glucoside was determined in the absence and presence of guaran. Phlorizin, which binds only to the intestinal carrier, should be influenced by guaran to the same extent as the monosaccharide. Indeed, kinetic analysis (Fig. 4) revealed that the inhibition constant of phlorizin was increased (from 0.58 to 1.39 μmol/l) by the same factor as the apparent transport constant of α-methyl-D-glucoside (from 4.8 to 11.5 mmol/l).

Substrate binding to the polysaccharides could have been part of the inhibitory effect of carbohydrate gelling agents on the tissue uptake. This possibility was investigated by using an ultrafiltration assay on appropriate membrane filters capable of separating the low-molecular-weight substrate from the polysaccharide. Since the lowest substrate concentration used in the tissue uptake experiments was 1 mmol/l, binding was studied at this concentration. As summarized in Table 3 recoveries of 100% clearly demonstrate the lack of any significant interaction between substrates and polysaccharides. Noteworthy is the observation that in spite of the increasing concentration of the polysaccharide during the ultrafiltration, even tracer amounts of substrate (picomolar range) did not reveal any evidence of binding.

**Table 3. Recovery of monosaccharides and neutral amino acids from binding experiments with various carbohydrate gelling agents**

<table>
<thead>
<tr>
<th>Polysaccharide</th>
<th>α-Me-Glc</th>
<th>Gal</th>
<th>Leu</th>
<th>Phe</th>
</tr>
</thead>
<tbody>
<tr>
<td>Guaran (5 g/l)</td>
<td>100</td>
<td>102</td>
<td>104</td>
<td>101</td>
</tr>
<tr>
<td>Pectin (12 g/l)</td>
<td>100</td>
<td>99</td>
<td>101</td>
<td>99</td>
</tr>
<tr>
<td>Carubin (5 g/l)</td>
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<td>97</td>
<td>100</td>
<td>99</td>
</tr>
<tr>
<td>Carrageenan (8 g/l)</td>
<td>98</td>
<td>102</td>
<td>99</td>
<td>100</td>
</tr>
<tr>
<td>Tragacanthin (12 g/l)</td>
<td>97</td>
<td>98</td>
<td>99</td>
<td>100</td>
</tr>
<tr>
<td>Galactomannan (5 g/l)</td>
<td>100</td>
<td>96</td>
<td>100</td>
<td>98</td>
</tr>
</tbody>
</table>

**Fig. 4.** Lineweaver-Burk plot of the uptake of α-methyl-D-glucoside and its inhibition by phlorizin in the absence and presence of guaran (5 g/l). Values represent means of two separate experiments (everted jejunal segments from three animals and five different substrate concentrations in duplicates for each experiment). Incubation was at 120 cycles/min for 3 min. Phlorizin was used at a concentration of 2 μmol/l: (1) KH buffer, (2) KH buffer + phlorizin, (3) KH buffer + guaran and (4) KH buffer + guaran + phlorizin. Tissue uptake rate is expressed as mmol/min and substrate concentration is mmol/l.

**Discussion**

The uptake of organic solutes by small-intestinal tissue *in vitro* was found to depend on stirring (Lukie, Westergaard & Dietschy, 1974; Westergaard & Dietschy, 1974) or shaking (Dugas, Ramaswamy & Crane, 1975). Kinetic measurements of active transport revealed a $K_m$ effect due to changes in the unstirred water layer (Wilson & Dietschy, 1974; Dugas et al., 1975), an unstirred
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Diffusion barrier overlying the absorptive surface of the small intestine. By means of this layer, the concentration of any solute in the bulk phase will be lowered at its superficial site of absorption. Lower agitation is accompanied by larger unstirred layers, so that for half-maximal saturation of the carrier system increased concentrations of substrate in the bulk phase are needed.

The unstirred layer resistance represents an additive term by which, under physiological conditions, the 'true' $K_m$ of the transport system will be increased. In a system in which $V_{\text{max}}$ and the geometry of the absorptive surface are kept constant, this term mainly depends on the quotient $\delta/D$, where $\delta$ = effective thickness of the unstirred layer and $D$ = diffusion coefficient of the solute within the unstirred layer (Winne, 1973). If, however, the functional surface area is affected, the equation for correcting the 'true' $K_m$ for unstirred layer resistance must include a term for surface area $s$ and becomes $\delta/Ds$. By increasing $\delta$ and lowering $D$ as well as $s$ an increased viscosity leads to a higher value for the quotient $\delta/Ds$ and consequently to higher $K_m$ values. Therefore it was conceivable that agents exclusively increasing the viscosity of the incubation medium would exert a competitive type of inhibition.

In fact, this investigation shows that carbohydrate gelling agents, which increase the viscosity of aqueous buffer solutions, result in a viscosity-dependent reduction in the tissue uptake of monosaccharides and neutral amino acids. Since inhibition was independent of the nature of the polysaccharide when the tissue uptake was regarded as a function of the viscosity, only a characteristic common to all the polysaccharides studied could have caused the present observations. Since this inhibition is completely reversed by either washing the tissue free of polysaccharides or by increasing the shaking rates during incubation it is concluded that the inhibition of intestinal transport in vitro by carbohydrate gelling agents is due to an enlargement of the unstirred layer resistance. This conclusion is supported by the results of kinetic experiments showing a distinct effect on the apparent Michaelis constant with little or no influence on the maximal transport capacity as is required by theoretical considerations (Winne, 1973) and experimental findings (Wilson & Dietschy, 1974; Dugas et al., 1975).

Further observations confirm that carbohydrate gelling agents reduce intestinal uptake of monosaccharides and neutral amino acids in vitro by means of a single mechanism, i.e. increase of the unstirred layer resistance. Interactions between polysaccharide and intestinal surface, or even intestinal carrier, could be ruled out on the basis of experiments showing reversal of inhibition by increased shaking rates. The same argument applies to the binding between solute and polysaccharide, which was also excluded by means of an ultrafiltration binding assay.

The failure to demonstrate any influence of plant gums on intestinal absorption in a previous study (Förster & Hoos, 1977) is explicable because unstirred layers do not influence the observed $V_{\text{max}}$ values with the application of substrate concentrations far above the $K_m$ (278 mmol/l for glucose) as in that study. These considerations may also be applied to the interpretation of experiments in vivo with glucose tolerance tests where the effect of carbohydrate gelling agents was found to depend mainly on gastric emptying (Holt et al., 1979). Oral application of concentrated glucose solutions produces saturating conditions within the small intestine, particularly early in the test. But as the intraluminal concentration goes down, carbohydrate gelling agents may gain increasing influence and affect later events in absorption, e.g. the occurrence of gentler decay slopes of plasma concentration curves (Taylor, 1979).

Acknowledgments

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References


