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The potential use of tomato lectin for oral drug delivery. 1. Lectin binding to rat small intestine in vitro

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Abstract

Tomato lectin, an apparently non-toxic dietary glycoprotein with a molecular mass of 71 kDa, was isolated from ripe tomato fruits. Its potential as an intestinal bioadhesive was examined using an in vitro organ culture system and Western blotting. The lectin was shown to bind avidly to rings of small intestine and this binding could be inhibited with specific competing sugars. After electrophoretic separation and transfer onto nitrocellulose, several of the proteins located on the small intestinal brush border membranes were found to bind tomato lectin, particularly those in the 160–116 kDa molecular mass range. These correspond to intestinal brush border digestive enzymes, which are known to be complex glycoproteins. The specific binding of tomato lectin to the enterocyte cell surface may give it potential for increasing the gastrointestinal residence time as a component of an oral drug formulation.

Key words: Bioadhesion; Oral drug delivery; Tomato lectin; Small intestine; Intestinal ring; Brush border membrane

1. Introduction

Oral administration is the most common and preferred route for drug delivery. Oral medications are relatively cheap to produce, and tablets and capsules offer the most convenient form of drug administration, thus increasing patient compliance. However, the interaction of the gastrointestinal (GI) tract with pharmaceuticals is highly complex and dynamic, and the efficiency with which a drug is absorbed in the GI tract is a function of many variables, particularly physiological variables affecting drug residence time, and the physical nature of the dosage form. Controlled release systems for oral drug delivery have been designed so that a drug will be released from a preparation at a specific site or through the whole length of the GI tract. Accordingly, gastric residence time and intestinal transit time are the predominant physiological factors affecting the bioavailability of these preparations (Mori et al., 1989).

With a small intestinal transit time of approximately three hours (Davis et al., 1987; Mundy et al., 1989), the residence time of a drug formulation within the small intestine is often too short to allow complete absorption of a drug. Thus, a

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delivery system may pass rapidly through the small intestine, the area of maximum drug absorption, and release most of its drug in the colon, a non-optimal site. If the residence time of a drug in the small intestine could be increased, this may lead to improved drug bioavailability and allow less frequent dosing.

One approach for attempting to delay intestinal transit is 'bioadhesion' in the GI tract. Bioadhesion has many definitions, but for drug delivery purposes, it implies attachment of a drug carrier to a specific biological location (Jiménez-Castellanos et al., 1993). Most bioadhesives tested to date have been synthetic macromolecules in the form of polymers, which are believed to interact with either the mucosal cell surface or, more frequently, with the mucus present in the GI tract, and are refered to as 'mucoadhesive'. The interactions between these synthetic polymers and cell surfaces or mucus are very complex and influenced by the chemical composition of the polymer and the milieu of the intestine. Park and Robinson (1984) found that polyanions were the best bioadhesives, and in recent years there have been a number of studies using anionic polyacrylic acid derivatives, known as polycarbophils. These have been shown to increase gastric residence considerably (Smart and Kellaway, 1989), and also to delay transit of microspheres in the rat small intestine (Lehr et al., 1990).

We have been developing an alternative approach to bioadhesion for oral drug delivery, exploiting natural 'biological' principles rather than the physicochemical interactions described above. This approach utilizes the properties of a lectin, a naturally occurring plant glycoprotein. Lectins occur widely in nature and have the unique ability to recognise and bind to exposed carbohydrate residues on glycoproteins (Sharon and Lis, 1972), such as those that are exposed on the surface of intestinal epithelial cells. The present study was designed to investigate the potential of a lectin from the tomato plant (*Lycopersicon esculentum*) as an intestinal bioadhesive for use in oral drug delivery. This lectin is a dietary glycoprotein of approx. 71 kDa, and has already been shown to bind to both rat and human intestine without disruption of villus integrity (Kilpatrick et al., 1985). Circumstantial evidence suggests that it is non-toxic to the majority of the human population, and consumption of the purified lectin by human volunteers produced no ill-effects (Kilpatrick et al., 1985). The purpose of the study was to evaluate the binding of tomato lectin to the adult rat small intestine and to identify the binding sites for the lectin on the mucosal cell surface. Preliminary reports of this work have been presented at meetings of the Controlled Release Society (Woodley and Naisbett, 1988, 1989).

2. Materials and methods

2.1. Purification of tomato lectin

Tomato lectin was purified to homogeneity from the locular fluid of ripe tomatoes, in which it is the major protein, using ammonium sulphate precipitation and chromatofocusing, as described by Kilpatrick et al. (1983). Lectin activity was measured by the agglutination of untreated human erythrocytes (Kilpatrick and Yeoman, 1978). An 8-fold purification was obtained over the starting locular fluid. 5 mg of purified lectin was radiolabelled with 0.5 mCi of $Na^{125}I$ (preparation IMS 30, Amersham International Plc, U.K.), using Iodobeads (Pierce Chemical Co., IL, U.S.A.) as described by Kilpatrick et al. (1985). Following the labelling reaction, free [^{125}I]iodide was removed by extensive dialysis against 1% (w/v) sodium chloride. The amount of free [^{125}I]iodide in the reaction mixture and resultant preparations was estimated using paper electrophoresis (Whatman No. 1 filter paper, run in 0.05 M sodium barbitone buffer, pH 8.6, for 25 min at 400 mV, 10–15 mA). The specific activity of the ^{125}I-labelled lectin was approx. 30 μCi/mg.

2.2. Binding of lectin to intestinal rings

Adult male Wistar rats were starved for 24 h, humanely killed, and the small intestine quickly excised and placed in warm (37°C), oxygenated tissue culture medium 199 (TC 199; ICN Flowlabs, Herts, U.K.). The intestine was washed through with TC 199 and half the length was everted on a

notched glass rod. Everted and non-everted intestine was cut into segments (rings), approx. 0.5 cm in width, and the rings were incubated at 37°C for 15 min in an oscillating (70 strokes/min) water bath, in 10 ml of TC 199 plus or minus heat-inactivated foetal calf serum (FCS) (Gibco, Uxbridge, U.K.), in the presence of 10 μg/ml ^{125}I-labelled tomato lectin or control macromolecules, [^{125}I]BSA (BSA, bovine serum albumin) and [^{125}I]PVP (PVP, polyvinylpyrrolidone).

After incubation, gut rings were washed four times in ice-cold saline (0.85% w/v), blotted dry and completely digested in 1 M NaOH. Duplicate samples of the digest were taken for the measurement of protein, using the Peterson (1983) modification of the method of Lowry et al. (1951). Multiple samples were also taken for radioactive counting. Binding of radiolabelled substrate to the intestinal rings was expressed as ng of substrate bound per mg of ring protein.

2.3. Competition studies

^{125}I-labelled tomato lectin (10 μg/ml; 0.14 μM) was preincubated in TC 199 (15 min, 37°C) with a range of competing sugars, all present in 10-fold concentration excess of the lectin, (1.4 μM). Preincubated lectin and competing sugar were then incubated with non-everted intestinal rings as before, and binding of the lectin to the rings was calculated.

2.4. Antibodies

Polyclonal antibodies against tomato lectin were raised in rabbits according to the method of Reid (1988). Briefly, the animals were injected subcutaneously on day 1 (with complete Freund's adjuvant), days 14 and 28 (with incomplete Freund's adjuvant), and intramuscularly on day 42. Each dose contained 1 mg of purified lectin. The rabbits were bled on day 49 and IgG was purified from serum using protein A-Sepharose, according to the manufacturer's instructions (Pharmacia). Other antibodies and peroxidase complexes mentioned in these experiments were obtained from Amersham International Plc, U.K.

2.5. Electrophoresis and Western blotting

Brush border membranes (BBMs) were prepared by the standard method of Kessler et al. (1978) from the adult rat small intestine. BBMs were electrophoresed on 7–17% gradient, sodium dodecyl sulphate polyacrylamide gels following the method of Laemlli (1970). After electrophoresis, the gels were stained for protein with Coomassie blue R (0.2% w/v in 50% ethanol (v/v) and 10% glacial acetic acid (v/v) for 30 min) and destained in 5% ethanol (v/v) containing 10% acetic acid (v/v). Proteins from unstained gels were transferred onto a nitrocellulose immobilizing matrix using an LKB Novablot semi-dry flat bed blotter (Pharmacia Ltd, U.K.) (90 min, 0.8 mA/cm^2 of gel in a continuous Tris/glycine buffer system). Blots were then incubated with tomato lectin (1 mg/ml) in phosphate-buffered saline (PBS), pH 7.2, supplemented with 10% (v/v) FCS and 0.2% (v/v) Triton X-100, for 2 h at 37°C. Blots were washed three times in PBS/FCS/Triton (washing buffer) and then were incubated with anti-tomato lectin polyclonal antibodies (2 h, 37°C). After washing in buffer as before, blots were incubated, firstly with biotinylated anti-rabbit IgG (1 : 1000 dilution) and, after further washing in buffer, streptavidin-biotin-peroxidase complex (1 : 1000 dilution). Finally, after washing three times in washing buffer and three times in PBS, the bands to which the lectin had bound were visualized by incubation with 10 ml PBS containing diaminobenzidine (0.5 mg/ml) and 2 μl 60% (v/v) hydrogen peroxide. Competition studies were performed by preincubating the tomato lectin with a 10-fold molar excess of *N*-acetylglucosamine tetramer, $(GlcNAc)_4$, prior to incubation with the blots. The $(GlcNAc)_4$ was a kind gift from Dr Helen Marcan, University of Hull, U.K.

3. Results and discussion

3.1. Lectin binding to intestinal rings

In order to evaluate the potential of tomato lectin as an intestinal bioadhesive, the binding of

radiolabelled lectin to rings of small intestine was compared with the binding of two control macromolecules of similar molecular mass, [^{125}I]BSA, a degradable protein, and [^{125}I]PVP, an inert polymer. The effect of the presence or absence of heat-inactivated FCS in the incubations, and eversion/non-eversion of the rings was also investigated. The results of these experiments are shown in Fig. 1. It can be seen that tomato lectin binding was up to 6-times greater than that of BSA and up to 14-times higher than that of PVP. There was no statistically significant difference in binding of the lectin when the rings were everted or non-everted, or when the medium was supplemented with serum. This was also true for BSA and PVP.

FCS had previously been shown to be necessary for the long-term incubation of gut sacs, as it contains essential fatty acids and growth factors not present in the tissue culture medium, (Bridges, 1980). However, for this short-term incubation of intestinal rings, addition of FCS did not affect (neither increase nor decrease) the binding of the macromolecules. These experiments also showed that the macromolecules bound equally well to everted and non-everted

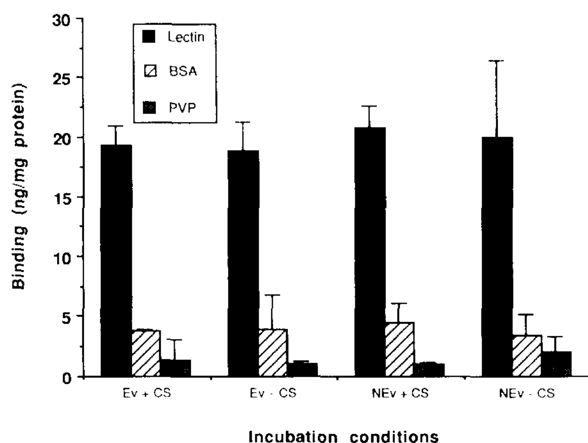

Fig. 1. Binding of macromolecules to intestinal rings. (Ev + CS) everted intestine with foetal calf serum in the medium; (Ev-CS) everted intestine minus foetal calf serum; (NEv + CS) non-everted intestine with foetal calf serum in the medium; (NEv-CS) non-everted intestine minus calf serum. Bars represent standard errors of the mean ($n = 16$).

intestine. However, this did not necessarily imply that the macromolecules could bind to the serosal surface of the small intestine. When 0.5 cm rings were cut from non-everted intestine, the ends of the gut ring turned 'inside out' to such an extent that, in effect, the majority of the mucosal surface was exposed to the bathing medium, thus giving an everted appearance. Conversely, when rings were cut from everted intestine, the rings remained flat and did not turn inside out, thus leaving the entire mucosal surface exposed. All subsequent experiments reported in this study were carried out using non-everted rings, as this involved less handling of the tissue, and without FCS.

3.2. *Competition studies*

To determine whether the binding of tomato lectin to the small intestine was true lectin binding or simply non-specific binding to the mucosa, lectin was preincubated with a range of competing sugars prior to incubation with gut rings. *N*-Acetylglucosamine tetramer ($(GlcNAc)_4$) and *N*-acetylglucosamine monomer (GlcNAc) were chosen, as tomato lectin had previously been shown to recognise and bind to these sugars (Kilpatrick, 1980). *N*-Acetyllactosamine (LacNAc) was also included as it had also been reported to bind tomato lectin (Merkle and Cummings, 1985). Other sugars were selected to include the small number of monosaccharides to which other lectins are known to bind, including mannose, galactose, *N*-acetylgalactosamine and fucose (Sharon and Lis, 1989). The structures of these sugars are shown in Fig. 2. Table 1 shows results of the binding of tomato lectin to intestinal rings, after preincubation with competing sugars. $(GlcNAc)_4$ and GlcNAc reduced lectin binding by 83 and 80%, respectively. Preincubation with LacNAc also reduced lectin interaction with the rings by 75%. The other sugars tested gave values which were not significantly different from the control value.

Tomato lectin is known to recognise *N*-acetylglucosamine, but it was previously thought that only oligomers (di-, tri- or tetrasaccharides) of that sugar would inhibit its binding activity

(Kilpatrick, 1980), and that inhibition of binding increased with the molecular size of the oligomer. The results in Table 1 show that the monomer of GlcNAc also inhibited tomato lectin binding as effectively as the tetramer. *N*-Acetyllactosamine (LacNAc) also reduced lectin binding significantly (75%). Although this disaccharide is not generally reported to be a substrate for tomato lectin, it has been observed that when linked to Sepharose, the lectin binds glycoproteins containing poly(*N*-acetyllactosamine) residues with high affinity (Merkle and Cummings, 1985; Callaghan et al., 1990). *N*-Acetyllactosamine is a disaccharide of galactose and *N*-acetylglucosamine (Fig. 2), so it is possible that the lectin was able to recognise the GlcNAc residue and bind to it, thus reducing the lectin interaction with GlcNAc residues on the intestinal mucosa. The fact that LacNAc is a disaccharide containing galactose does not necessarily preclude tomato lectin binding to it, as lectins can vary considerably in their ability to interact with derivatives of the monosaccharides for which they are specific. Many lectins, as well as being able to recognise the parent monosaccharide, continue to recognise it in the linked form even when the C4 linkage is in a different configuration from the C4 hydroxyl group in the free monosaccharide, normally an important prerequisite for lectin-sugar specificity (Sharon and Lis, 1989).

Table 1
Binding of tomato lectin to gut rings after preincubation with competing sugars

Competing sugar	Binding (± S.E.) (ng/mg protein) [a]	% inhibition of binding
None	18.7 ± 7.6	–
N-Acetylglucosamine	3.6 ± 1.3	80.8 [b]
N-Acetylglucosamine tetramer	3.1 ± 1.4	83.4 [b]
Glucose	17.4 ± 6.6	7.0
N-Acetyllactosamine	4.6 ± 0.3	75.4 [b]
Mannose	17.8 ± 1.0	4.8
N-Acetylgalactosamine	17.3 ± 6.2	7.5
Galactose	18.4 ± 5.9	1.6
Fucose	19.1 ± 8.3	–

[a] Data are presented as the mean ± standard error, where $n = 16$.
[b] Differences were assigned to be significant for values of $p < 0.001$.

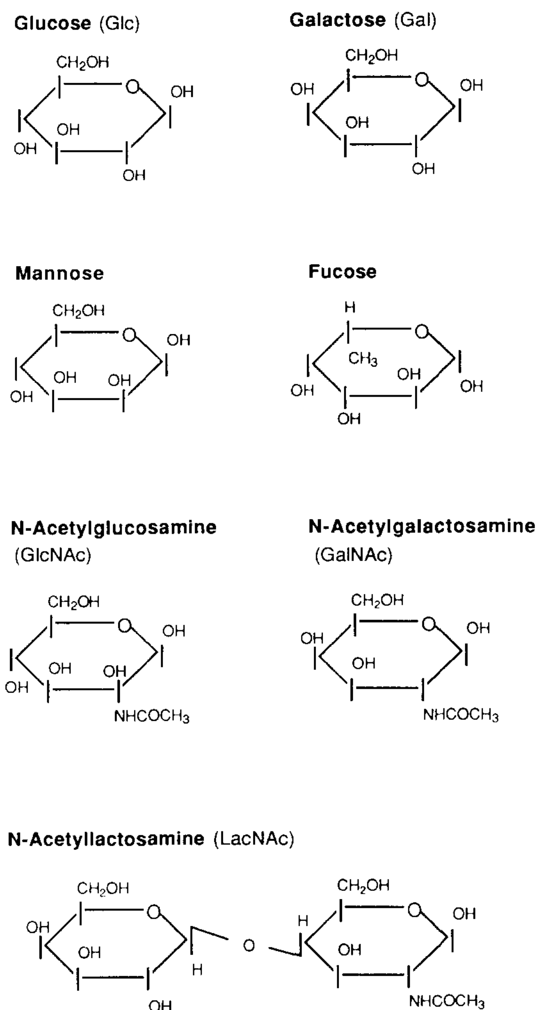

Fig. 2. Structures of sugars used in the lectin competition experiments.

Other sugars preincubated with the lectin caused no reduction in binding to the gut rings. These sugars were all lacking in either an acetylamine group at the C2 position or had the wrong configuration of the C4 hydroxyl group.

3.3. Western blotting

Since tomato lectin had been shown to bind to the small intestine in vitro and that this was true lectin binding, experiments were carried out to

determine which components of the brush border membrane were involved in the binding process. After gel electrophoresis, blotting to nitrocellulose, and incubation with tomato lectin, the components of the brush border to which the lectin had bound were visualised immunochemically and tentatively identified. The experiments were repeated in the presence of competing sugar, $(GlcNAc)_4$, to show that the binding was lectin-specific.

Fig. 3 shows the pattern obtained on SDS-polyacrylamide gels after electrophoresis of small intestinal brush borders and staining for protein with Coomassie Blue. Many bands were visible with molecular masses ranging from 200 kDa down to less than 15 kDa, with the majority of the bands being in the higher molecular mass region of the gel. Fig. 3 also shows the tomato lectin binding pattern obtained for small intestinal BBMs. Strong lectin binding was detected to the brush border glycoproteins, with bands ranging in size from almost 200 to 37 kDa. The heaviest staining was found in a region containing proteins of molecular mass 160–116 kDa.

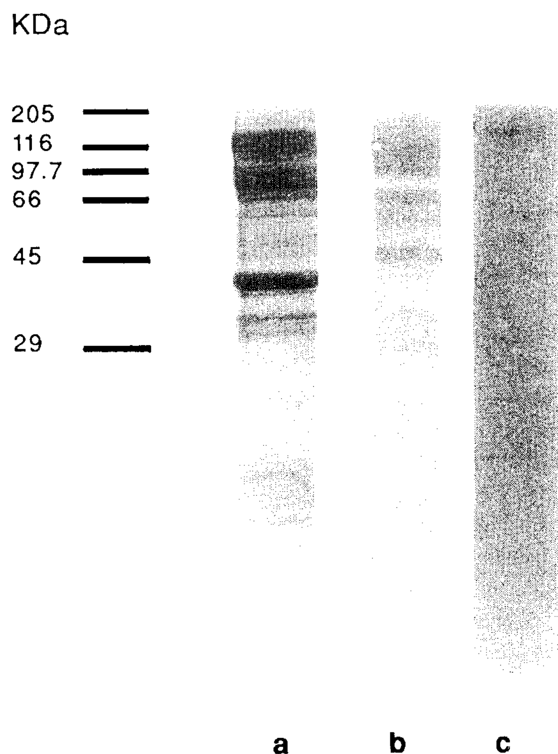

Fig. 3. Binding of tomato lectin (TL) to small intestinal brush border membrane (BBM) glycoproteins. (a) SDS-polyacrylamide gel of BBMs, stained with Coomassie Blue; (b) Western blot of BBMs incubated with TL, followed by lectin detection using anti-lectin antibodies; (c) control blot of BBMs incubated with TL and competing sugar. Numbers refer to molecular mass markers.

As in the experiments with the intestinal rings, tomato lectin was then preincubated with an excess of competing sugar, $(GlcNAc)_4$, prior to incubation with small intestinal blots. The results are shown in Fig. 3. Binding of the lectin to the brush border components was substantially reduced, with only faint traces of residual binding at approx. 160 kDa. These results provide further evidence for the specific nature of the binding of tomato lectin to intestinal glycoconjugates. Many workers have measured the glycoprotein content of the small intestinal brush border membranes, and it has been shown that these membranes have a high incorporation of *N*-acetylglucosamine into their glycoproteins (Weiser, 1986). Morita et al. (1986) have shown that the proximal and distal rat small intestine contains 62.2 and 76.4 nmol of GlcNAc per mg of gut protein, respectively. Many of these GlcNAc residues can be found in the brush border enzymes. The majority of brush border enzymes have been shown to be glycoproteins and contain between 13 and 35% carbohydrate (Kenny and Maroux, 1982), with *N*-acetylglucosamine and glucose as the most abundant carbohydrates in the glycoprotein structure. This is also true for intestinal glycolipids (Kenny and Maroux, 1982). Four brush border membrane hydrolases, aminopeptidase N, dipeptidyl peptidase IV, sucrase-isomaltase and alkaline phosphatase, have previously been shown to bind the lectin wheat germ agglutinin, which binds to GlcNAc and sialic acid residues (Weiser, 1973; Naim et al., 1986). These four enzymes, which are known to contain GlcNAc, are within the molecular mass range of those bands found to bind tomato lectin in the Western blotting studies detailed here. Table 2 lists those enzymes found in the brush border which contain carbohydrate and may be possible binding sites for tomato lectin. Data in this table are taken from Holmes and Lobley

Table 2
Human brush border enzymes containing carbohydrate [a]

	Enzyme	Molecular mass (kDa)
Glycosidases:	sucrase-isomaltase	145 + 151
	lactase-phlorizin hydrolase	160
	trehalase	80
Peptidases:	aminopeptidase A	170
	aminopeptidase N	162
	aminopeptidase W	130
	carboxypeptidase P	130
	endopeptidase 24.18	100
	endopeptidase 24.11	96
	γ-glutamyl transferase	62
Phosphatases:	alkaline phosphatase	86

[a] Taken from Holmes and Lobley (1989).

(1989) and are with reference to human intestinal enzymes. There will be some slight variation in the enzyme molecular masses for the rat.

4. Conclusions

For a macromolecule such as tomato lectin to have potential as an intestinal bioadhesive for use in oral drug delivery, it is necessary for it to adhere to the mucosal surface. This binding to the intestinal wall may then be used to delay the intestinal transit time of drugs by inclusion of the lectin in an oral formulation.

The results in this report are promising. Tomato lectin bound avidly to the small intestinal epithelial surface and this binding was shown to be specific and mediated through GlcNAc-containing glycoconjugates, tentatively identified as high molecular mass brush border membrane hydrolases. These data indicate that tomato lectin should be a good candidate as a bioadhesive in the GI tract.

The contribution of secretory glycoconjugates such as mucin to lectin binding has not been considered in this initial study. Whilst secretory glycoconjugates are undoubtedly an important factor in vivo, as many GlcNAc residues are present in mucus secretions, their contribution to tomato lectin binding in vitro is likely to have been negligible. The tissue preparation procedures for both the intestinal ring incubations and Western blotting experiments removed the majority of the mucus from the tissue. The importance of tomato lectin interactions with mucus in vivo will be reported and discussed in a later paper.

References

Bridges, J.F., Uptake of macromolecules by rat small intestine in vitro. Ph.D. Thesis, University of Keele, UK, 1980.

Callaghan, J.M., Toh, B.H., Pettitt, J.M., Humphris, D.C. and Gleeson, P.A., Poly-*N*-acetyllactosamine-specific tomato lectin interacts with gastric parietal cells. Identification of a tomato-lectin binding 60–90 kDa membrane glycoprotein of tubulovesicles. *J. Cell Sci.*, 95 (1990) 563–576.

Davis, S.S., Khosla, R., Wilson, C.G. and Washington, N., Gastrointestinal transit of a controlled-release pellet formulation of tiaprofenic acid and the effect of food. *Int. J. Pharm.*, 35 (1987) 253–258.

Holmes, R. and Lobley, R.W., The intestinal brush border revisited. *Gut*, 30 (1989) 1667–1678.

Jiménez-Castellanos, M.R., Zia, H. and Rhodes, C.T., Mucoadhesive drug delivery systems. *Drug. Dev. Ind. Pharm.*, 19 (1993) 143–194.

Kenny, A.J. and Maroux, S., Topology of microvillar membrane hydrolases of kidney and intestine. *Physiol. Rev.*, 62 (1982) 91–128.

Kessler, M., Acuto, O., Storelli, C., Murer, H., Muller, M. and Semenza, G., A modified procedure for the rapid preparation of efficiently transporting vesicles from small intestinal brush border membranes. Their use in investigating some properties of D-glucose and choline transport systems. *Biochim. Biophys. Acta*, 506 (1978) 136–154.

Kilpatrick, D.C., Purification and some properties of a lectin from the fruit juice of the tomato (*Lycopersicon esculentum*). *Biochem. J.*, 185 (1980) 269–272.

Kilpatrick, D.C. and Yeoman, M.M., A lectin from extracts of Datura stramonium. *Plant Sci. Lett.*, 13 (1978) 35–40.

Kilpatrick, D.C., Pusztai, A., Grant, G., Graham, C. and Ewen, S., Tomato lectin resists digestion in the mammalian alimentary canal and binds to intestinal villi without deleterious effects. *FEBS Lett.*, 185 (1985) 5–10.

Kilpatrick, D.C., Weston, J. and Urbaniak, S.J., Purification and separation of tomato isolectins by chromatofocusing. *Anal. Biochem.*, 134 (1983) 205–209.

Lehr, C.-M., Bouwstra, J.A., Tukker, J.J. and Junginger, H.E., Intestinal transit of bioadhesive microspheres in an in situ loop in the rat – a comparative study with copolymers and blends based on poly (acrylic acid). *J. Controlled Release*, 13 (1990) 51–62.

Laemlli, U.K., Cleavage of structural protein during assembly of the head of bacteriophage T4. *Nature*, 227 (1970) 680–685.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., Protein measurement with the folin phenol reagent. *J. Biol. Chem.*, 193 (1951) 262–275.

Merkle, R.K. and Cummings, R.D., Analysis of Poly-*N*-acetyllactosamine sequences in surface glycoproteins in a mouse lymphoma line BW 5147: Binding of oligosaccharides containing these sequences to immobilised tomato lectin. *J. Cell Biol.*, 101 (1985) 62.

Mori, M., Shirai, Y., Uezono, Y., Takahashi, T., Nakamura, Y., Makita, H., Nakanishi, Y. and Imasoto, Y., Influence of specific gravity and food on the movement of granules in the gastrointestinal tract of rats. *Chem. Pharm. Bull.*, 37 (1989) 738–741.

Morita, A., Siddiqui, B., Erickson, R.H. and Kim, Y.S., Comparison of brush border membrane glycoproteins and glycoenzymes in the proximal and distal rat small intestine. *Dig. Dis. Sci.*, 34 (1986) 596–605.

Mundy, M., Wilson, C.G. and Hardy, J.G., The effect of eating on transit through the small intestine. *Nucl. Med. Commun.*, 10 (1989) 45–50.

Naim, H.Y., Sterchi, E.E., Hauri, H.-P. and Lentze, M.J., Purification and biosynthesis of the brush border hydrolases of the human small intestine. *INSERM Symp.*, 26 (1986) 71–74.

Park, K. and Robinson, J.R., Bioadhesive polymers as platforms for oral-controlled drug delivery. *Int. J. Pharm.*, 19 (1984) 47–57.

Peterson, G.L., Determination of total protein. *Methods Enzymol.*, 91 (1983) 95–119.

Reid, D., Studies on the brush border membrane from normal intestine and from coeliac patients. Ph.D. Thesis, University of Keele, UK, 1988.

Sharon, N. and Lis, H., Lectins: cell-agglutinating and sugar-specific proteins. *Science*, 177 (1972) 949–959.

Sharon, N. and Lis, H., *Lectins*, Chapman and Hall, London, 1989, p. 127.

Smart, J. D and Kellaway, I., Pharmaceutical factors influencing the rate of gastrointestinal transit in an animal model. *Int. J. Pharm.*, 53 (1989) 79–83.

Weiser, M.M., Intestinal cell membranes. *Int. Rev. Cytol.*, 101 (1986) 1–58.

Weiser, M.M., Intestinal epithelial cell surface membrane glycoprotein synthesis. *J. Biol. Chem.*, 248 (1973) 2536–2541.

Woodley, J.F. and Naisbett, B., The potential of lectins as vehicles for oral drug delivery: in vivo and in vitro uptake of tomato lectin. *Proc. Int. Symp. Controlled Release Bioactive. Mater.*, 16 (1989) 58–59.

Woodley, J.F. and Naisbett, B., The potential of lectins for delaying the intestinal transit of drugs. *Proc. Int. Symp. Controlled Release Bioact. Mater.*, 15 (1988) 125–126.