Mucus Gel Thickness and Turnover in the Gastrointestinal Tract of the Rat: Response to Cholinergic Stimulus and Implication for Mucoadhesion

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The thickness of the mucus gel and its turnover rate were measured in the stomach, proximal jejunum, cecum and proximal colon of the rat, using microscopy and staining techniques. The specific mucus-secretory responses to carbachol-induced cholinergic stimulus in these locations were also studied. The mucus gel was found to be the thinnest (18±1 microns) in the cecum, and the thickest in the stomach (39±14 microns). The effect of carbachol on mucus secretion was profound and dose dependent in the stomach, and less profound, although still dose dependent, in the proximal jejunum. The least responsive organs were the cecum and the proximal colon, where no effect was observed after three doses of carbachol. Mucus secretion rate was significantly higher in the jejunum (1.1±0.5 μg glucose equivalent min^{-1} cm^{-2}) than in the colon (0.5±0.2 μg glucose equivalent min^{-1} cm^{-2}). Also, the proximal jejunum was more responsive to the carbachol stimulus (mucus secretion rate of 5.4±2.2 μg glucose equivalent min^{-1} cm^{-2} after carbachol treatment) than the colon (mucus secretion rate of 1.0±0.4 μg glucose equivalent min^{-1} cm^{-2} after carbachol treatment). *In vitro* mucoadhesion studies with Polycarbophil disks were performed in the mucosal tissues of the stomach, jejunum, cecum and proximal colon of the rat with and without cholinergic (carbachol) stimulus. The adhesion force in the cecum and the colon was significantly stronger than in the stomach and proximal jejunum when the studies were performed at pH 2. Carbachol treatment did not significantly change the mucoadhesion of Polycarbophil disks. It is concluded that in the gastrointestinal tract of the rat the colon and the cecum are more suitable locations for the mucoadhesion than the stomach and the jejunum because: (1) their mucus turnover is lower, (2) their sensitivity to mucus secretory stimulus is lower, and (3) their Polycarbophil adherence properties are stronger.

KEY WORDS: mucoadhesion; gastrointestinal tract; mucus turnover; cholinergic stimulation.

INTRODUCTION

The successful attachment of polymers to the epithelial mucosa in the intestine depends, among other parameters, upon the strength of hydrogen-bonding between the polymer and the mucus lining and the ionic charge of the polymer (1). The mucus gel which covers the entire surface of the gastrointestinal (GI) tract is made of highly hydrated mucin-containing sialic or sulfonic acid residues that confer its dense negative charge (2). Typically, the attachment of mucoadhesives to the mucus layer is stronger than the attachment among the mucin layers (3,4). Therefore, drug-delivery residence time at the mucosa of the GI tract, is rate limited by mucus-turnover rather than by tightness of polymer adherence to the mucus lining (5). The mucus turnover time in the GI tract is highly versatile. Lehr *et al.* (6) estimated the turnover time of the intestinal mucus gel layer in the rat to be between 47 and 270 minutes. In the dog, especially in the fasted state, the turnover time may be a matter of several minutes to a few hours (7). Reproducible therapy using mucoadhesion techniques depends on minimal variations in mucus turnover rate and would be useful in those organs where the turnover rate is minimal.

A relatively large number of polymers have been evaluated for their ability to adhere to mucin. Materials such as polyacrylic acid, polymethacrylic acid, and their derivatives, *e.g.* Polycarbophil (a cross-linked derivative of polyacrylic acid), as well as natural gums possess mucoadhesive properties (3,8–10). Recently, the use of fucosylamine containing HPMA copolymers as colonic bioadhesives was suggested by Kopecek *et al.* (11) and Rihova *et al.* (12). The rationale was to mimic the lectin-like mucosal adhesins that cause fucose-sensitive attachment of some enteropathogenes in the presence of calcium. The measured adherence of those copolymers was better in the mucosa of the colon than that in the small intestine of the rat. However, when isolated enterocytes were analyzed, no difference in the polymer attachment was observed (12). This observation raises a general question regarding the role of regional mucus turn-over characteristics in the GI tract and the possible implication of these characteristics for mucoadhesion. If, indeed there is a slower mucus turnover rate in the intestine than in the stomach, the former organ may be more suitable for mucoadhesion.

The overall goal of this study was to set criteria for locating the best site for mucoadhesion in the GI tract. More specifically the study aims were: (*a*) to measure the amounts of mucus gel in the stomach, proximal jejunum, cecum and proximal colon of the rat, (*b*) to compare the mucus turnover rate in the jejunum and proximal colon of the rat, (*c*) to study the mucoadhesion properties of Polycarbophil in various locations of the GI tract of the rat, (*d*) to measure how secretory stimulus, which results in increased mucus secretion, affects the mucus thickness, turnover and mucoadhesion in the measured organs.

MATERIALS AND METHODS

All materials and reagents were purchased from Sigma, St. Louis, MO, unless otherwise mentioned in the text. Polycarbophil (Goodrich) was donated by Teva Pharmaceutical Industries, Jerusalem.

Stationary Conditions Studies

Segmental Sampling of Epithelial Tissue. Fed male Sabra rats (13), weighing between 200–250 g, were sacrificed with an overdose of ether. Slices of epithelial tissue were cut from (*a*) the stomach, (*b*) the proximal jejunum (6 cm distal to the stomach), (*c*) the cecum, and (*d*) the proximal colon (1 cm distal to the cecum). The cutting was performed with a specially designed double blade cutter that yielded equal squares of 0.5×0.5 cm. Three slices were taken from each segment.

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Mucus Staining. Alcian blue (AB) is a cationic histological dye that binds to the acidic mucin fraction in the mucus by creating an insoluble complex with its soluble polysaccharides (14). Because the dye is hydrophilic, it does not penetrate into the epithelial cells. Therefore, it can be used to specifically stain the mucus attached to the epithelial layer over the entire gastrointestinal tract (15,16). The precut epithelial square slices were then soaked in a petri dish containing 0.1% (w/v) AB in an acetate buffer solution, pH 5.8 (0.05 M). To achieve isotonicity, 0.25 M of sucrose was added (16).

Determination of Soluble Mucin Fraction. After exactly 1 hour the soaked tissue was taken out from the AB bath and homogenized (tissue homogenizer, 5 min) in 1.5 ml of 1 M $MgCl_2$ in a 0.05 M acetate buffer, pH 5.8. The homogenized mixture was shaken for 2 hours at 37°C to dissolve the resulted insoluble complex. After centrifugation the supernatant was separated and its absorbance was measured at 603 nm (Uvikon 930, Kontron Instruments, Switzerland). The results were then normalized to the mucus thickness of the stomach for each corresponding rat. Three measurements were taken from each of the four organs (stomach, proximal jejunum, cecum and proximal colon). The study was repeated in five rats.

Microscopy of the Stained Tissues. Slices of epithelial tissue, 0.5 mm thick each, were cut from the stomach and colon using a double blade cutter. The epithelial tissues were bathed in 0.1% (w/v) AB solution, this time for 15 minutes only (17). Microscopic preparations were carried out without fixation as follows: each slice was rinsed with saline and analyzed in an inverted phase microscope (Olympus LH 50A) (×100 magnification). The mucus lining of each epithelial segment could be visualized clearly as a translucent layer (demonstrated in Figure 1).

The thickness of the mucus layer of each epithelial slice was assessed visually as follows: The area of the colored mucus was graphically bordered and then measured using a linear (metric) graph paper. The results were normalized to each slice length. In this way four measurements were taken from each segment of three individual rats (altogether 12 measurements per segment).

Mucus Turnover Under Cholinergic Stimulus. Carbachol is an acetyl choline agonist which increases the mucus secretion in the GI tract (18–20). It was used in the present study to check the regional secretory response of various segments along the alimentary canal of the rat to cholinergic stimulation and its implication for mucoadhesion. In separate experiments, rats were dosed (i.p.) with 100, 300 or 500 μg/Kg body weight of carbachol (each dose was administered to five rats). Thirty minutes after the injection the rats were sacrificed with an overdose of ether, and a laparotomy was immediately performed. Epithelial slices from the respective gastrointestinal segments (i.e. stomach, proximal jejunum, cecum and proximal colon) were taken and bathed in AB solution for 60 minutes after which the staining intensity of each tissue was measured as described above both in the test and the control animals. The value obtained for each test experiment was normalized to its control (saline). This normalization to control animals was required to account for the elevation in the mucus layer thickness caused by the increasing doses of the carbachol, in the various regions of the rat GI tract. The relative mucus thickness (RMT) was calculated according to the following equation:

$$\text{RMT} = \frac{\text{AB abosorbance of an epithelial tissue after dosing with carbachol}}{\text{AB absorbance of similar tissue of the control (saline dosed) animal}}$$

Each experiment was performed in two rats, one served as the control while the other was treated with carbachol. This procedure was repeated five times.

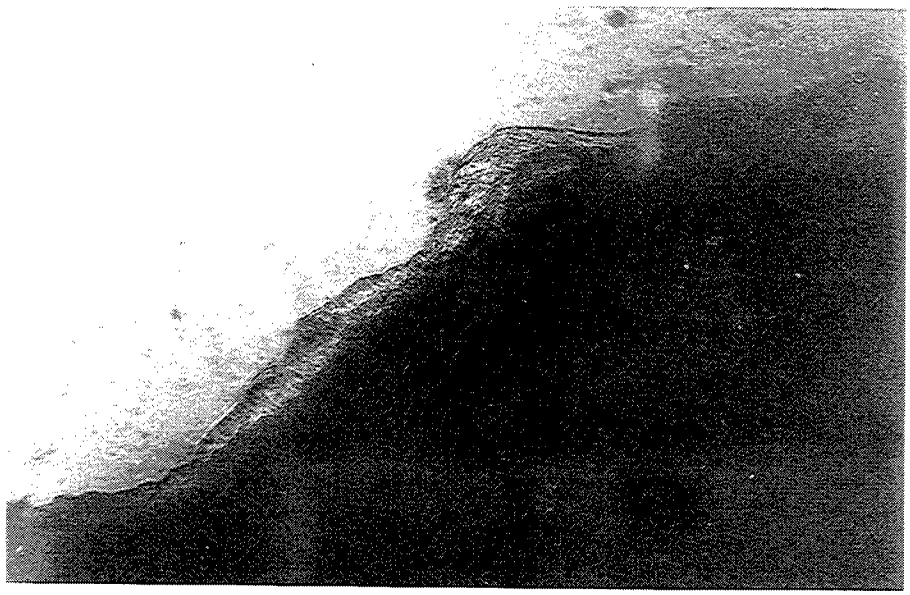

Figure 1: A typical inverted phase micrograph (magnification: ×100) taken from the gastric mucosa (AB stained) of the rat, demonstrating the epithelial tissue (dark tissue at the bottom) covered by translucent mucus gel (top).

Perfusion Studies

The proximal jejunum or proximal colon of five anesthetized rats (i.p. Equitensin solution equivalent to 6 mg/100 g body weight sodium pentobarbitone) was perfused with 15 ml of saline in a closed loop system (5–7 cm) as described elsewhere (21) over 2.5 hours. Samples of 1 ml were withdrawn in 30 minute intervals and analyzed for neutral hexoses content as described below. At the end of each experiment the rats were sacrificed with an overdose of ether. The perfusion rate was 1.0 ml/min. This study was performed in non-treated rats and, separately, in rats that were dosed twice with 10 μg/Kg of carbachol 60 and 30 minutes prior to each perfusion study. Because a large single dose of carbachol would have killed the rats before the end of the experiment, the same dose of carbachol was painted on the serosa of the perfused intestinal segment every 15 minutes during the perfusion.

Neutral Hexoses Content Determination. Each 1 ml perfusate sample was mixed with 1.5 ml of distilled water, 2.5 ml of concentrated H_2SO_4, and 0.1 ml of orcinol (60 mg/ml). After incubation at 80°C for 15 min, the absorbance was determined at 420 nm, using 5 μg of glucose as a standard (22). The slopes of the total hexoses amount *v.s.* time curve in the proximal jejunum or proximal colon were calculated separately for each rat. The obtained values were normalized to the measured surface areas.

Bioadhesion Studies

Disks (12 mm, diameter) of Polycarbophil were prepared by compressing 100 mg of the polymer at 5 ton (manual Perkin Elmer IR press). The upper surface of each Polycarbophil disk was glued to a disk of polypropylene with an equal diameter and balanced to a total weight of 5 g. The glued Polycarbophil disks were allowed to swell in PBS buffer (pH 7) or citrate buffer (pH 2) for 10 minutes.

Intestinal tissue (stomach, jejunum, cecum and proximal colon) from previously sacrificed rat was spread and mounted over a petri dish containing either of the buffers.

The polypropylene disks, with the glued, swollen Polycarbophil underneath were placed separately on the mounted tissues for two minutes. The upper surface of the polypropylene was connected to a force gauge (250 mg accuracy) which, in turn, was lifted at a constant rate (23). The adherence force was determined at the time of detachment and the weight of the polypropylene surface was subtracted. The experiments were performed in two different pH values (2, and 7). Each study was performed with and without cholinergic stimulus (carbachol i.p. 500 μg/Kg, 0.5 hour prior each experiment).

Each study was repeated six times.

Statistical Analysis

Data were analyzed by Kruskal-Wallis (non-parametric test). A difference was considered to be statistically significant when the p value was less than 0.05. When the difference between the groups was obtained, a Mann-Whitney U test was used to analyze the significance of the differences between the individual group means ($p < 0.05$).

RESULTS

The thickness (calculated by dividing the measured mucus area by the slice length, and expressed in microns ± S.D.) of the mucus layer of the rat stomach and cecum as visualized and measured microscopically is summarized in Table 1 which shows that the thickness was significantly higher in the stomach of the rat than in its cecum.

The amounts of mucus in the epithelial slices taken from the rat's proximal jejunum, cecum and proximal colon were measured spectrophotometrically after reaction with AB and expressed as a percentage of the amount in the slices taken from the stomach ± S.D. (n=5). In all cases the values measured in the proximal jejunum, cecum and proximal colon were lower than those in the stomach. However, although the values measured in the colon (67±15%) were lower than those measured in the proximal jejunum (82±19%), significant differences were only observed in the cecum (34±9%). This observation, found by measuring the amount of anionic mucins in the mucus layer, supports the finding in Table 1 with microscopy.

Figure 2 shows the influence of three doses of carbachol on the mucus gel thickness in the stomach, proximal jejunum, cecum and proximal colon of the rat, as expressed by the RMT values ± S.D. The stomach was found most sensitive to increases in the dose of carbachol (from initial RMT value of 1.2±0.1 at a dose of 100 μg/Kg body weight, to RMT value of 1.9±0.2 at a dose of 500 μg/Kg body weight). The more caudal the organ, the less responsive it was to carbachol. Among the three organs (proximal jejunum, cecum and proximal colon), the proximal jejunum was the most sensitive (from initial RMT value of 1.0±0.1 at a dose of 100 μg/Kg body weight, to RMT value of 1.4±0.1 at a dose of 500 μg/Kg body weight). The cecum and the proximal colon did not respond to carbachol (from initial RMT value of 1.1±0.1 at a dose of 100 μg/Kg body weight, to RMT value of 1.0±0.1 at a dose of 500 μg/Kg body weight for the cecum, and from initial RMT value of 1.1±0.2 at a dose of 100 μg/Kg body weight, to RMT value of 1.1±0.1 at a dose of 500 μg/Kg body weight for the proximal colon).

The mucus secretion rate in the proximal jejunum and proximal colon during 2.5 hours of saline perfusion was measured with and without carbachol treatment. The mucus turnover rates, as calculated from the slopes of the total hexoses that were accumulated in the perfused saline solutions, are shown in Figure 3 and expressed in μg glucose equivalent min^{-1} cm^{-2} ± S.D. In the proximal jejunum the mucus turnover rate was 2.2 times higher than in the colon (1.1±0.5 compared with 0.5±0.2). Carbachol increased this ratio to 5.4 (5.4±2.2 in the proximal jejunum compared with 1.0±0.4 in the proximal colon). These findings support the observations under stationary conditions that mucus secre-

Table 1: *The thickness of the mucus gel in the stomach and cecum of the rat as measured visually by inverted phase microscope.*

Stomach (microns)	Cecum (microns)
39±14[a]	18±1.4[a]*

[a] Shown are the results of 12 studies ± SD.
* Significantly different compared to the stomach.

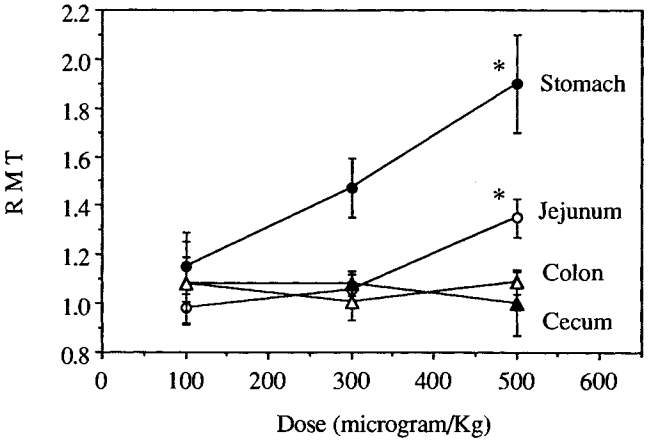

Figure 2: The influence of three doses of carbachol on the relative mucus gel thickness (RMT) in the stomach, proximal jejunum, cecum and proximal colon of the rat. Shown are the results of 5 studies ± S.D.

* Statistically significant as compared to the RMT as measured after dosing with 100 μg/KG body weight of carbachol.

tion and turnover in the colon is much less sensitive to cholinergic stimulation.

The detachment force values in the various GI segments of the rat at two different pH values, with or without carbachol pretreatments are summarized in Figure 4 and expressed in g ± S.D. At pH 7, the detachment force was 1.0±0.2 before and 1.0±0.2 after the carbachol treatment in the stomach, 1.5±0.2 before and 1.25±0.5 after the carbachol treatment in the jejunum, 2.0±0.8 before and 1.5±0.5 after the carbachol treatment in the cecum and 1.75±0.5 before and 1.25±0.25 after the carbachol treatment in the jejunum. At pH 2, the detachment force was 2.0±0.2 before

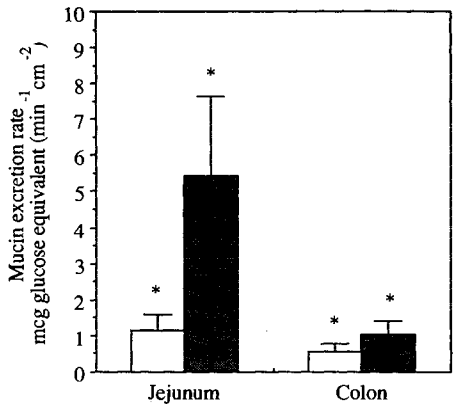

Figure 3: Mucus turnover rates before (white columns), and after (hatched columns) carbachol treatment in the proximal jejunum and proximal colon of the rat. The turnover rates were calculated from the slopes of the total accumulated hexoses (expressed in μg glucose equivalents normalized to surface area) in the perfused saline solutions versus time plots. Shown are mean values of five studies ± S.D.

* —Jejunum compared to jejunum after carbachol treatment: significantly different;
—Jejunum compared to colon: significantly different;
—Colon compared to colon after carbachol treatment: significantly different.

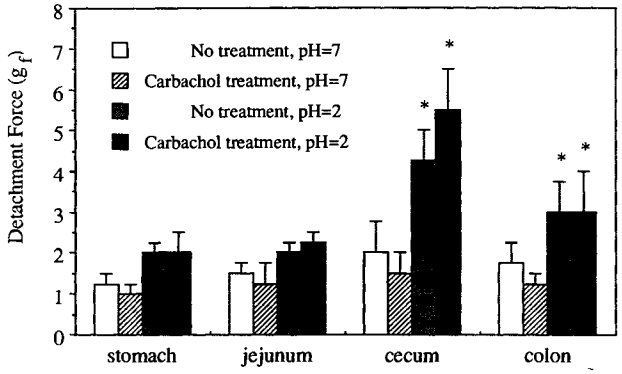

Figure 4: The detachment force of Polycarbophil discs in the various GI segments of the rat at pH 2 and 7, with and without carbachol pretreatment.

* Significantly different as compared to the stomach and proximal jejunum at pH 2.

and 2.0±0.5 after the carbachol treatment in the stomach, 2.0±0.2 before and 2.25±0.25 after the carbachol treatment in the jejunum, 4.25±0.75 before and 5.5±1.0 after the carbachol treatment in the cecum and 3.0±0.8 before and 3.0±1.0 after the carbachol treatment in the jejunum. No significant difference was observed between the detachment force recorded in the various organs before and after the cholinergic stimulus at pH 7 ($p > 0.1$). However, at pH 2 the polycarbophil adhered to the cecal mucosa stronger than to the colonic mucosa ($p < 0.025$). The attachment in the colon was stronger than the attachment in the stomach and proximal jejunum ($p < 0.05$).

DISCUSSION

In this study the techniques of Whiteman (14), Pipper *et al.* (15), Corne *et al.* (16) and Winzler (22) were used to compare the thickness of the mucus gel and its turnover rate in four different locations in the GI tract of the rat. The bioadhesive force of Polycarbophil in the four regions was also measured (23) at two different pH values. Then, mucus secretion in these locations was increased by cholinergic stimulation, and its effect on the Polycarbophil adherence to the same locations in the GI tract of the rat was measured. The cecal mucus gel was found to be the thinnest, and the gastric mucus gel the thickest (Table 1). The observed values were smaller (39±14 microns) than those found by Allen *et al.* (24) (80±5) probably because of the different methods used to evaluate the thickness of the mucus gel. In our system, the dimensions of the mucus gel were visualized in different regions of the gastrointestinal tract of the rat by a staining procedure that might have reduced the amount of mucus lining in each segment. Our findings were further elaborated under stationary conditions by reacting the mucus with AB and measuring the amount of the stained, negatively charged mucin in the mucus gel of the stomach, proximal jejunum, cecum and proximal colon. Again, the results showed that the values in the cecum were significantly lower than those in the stomach. The finding that the cecal mucus lining is the thinnest (thinner even than the mucus in the colon) can be related to the physiological function of the cecum in the rat: a depot which retains large amounts of

viscous chyme for long periods of time. The dominant parameters in this organ are shear forces and high concentrations of anaerobic bacteria capable of fermenting endogenous mucins, thus leading to a rapid erosion of the cecal mucus lining relative to the colonic mucus lining in the rat.

To compare the effect of cholinergic stimulus on the amount of mucus secreted in the stomach, proximal jejunum, cecum and proximal colon of the rat, the regional amounts of mucus were measured after dosing with carbachol, a drug that mimics a cephalic stimulus. This experimental section was used also to verify the sensitivity of the AB method. The secretory effect expressed by the relative elevation in the thickness of the mucus gel and presented as RMT (relative mucus thickness) was both significant and dose dependent in the stomach (Figure 2). A less profound, although still dose dependent, effect was observed in the proximal jejunum. The least responsive organs were the cecum and the proximal colon where no effect was observed after three different doses of carbachol.

To complete this section of the study the rate of the mucus turnover in the proximal jejunum of the rat was compared to the rate of secretion in the proximal colon with and without cholinergic stimulation. Here, the amount of the uncharged mucin fraction accumulated in the lumen compartment was monitored in a closed intestinal loop. It was found that the mucus secretion rate was significantly higher in the jejunum than in the colon. Moreover, the proximal jejunum was more responsive than the colon to carbachol stimulation (Figure 3).

It can be concluded that the more caudal to the stomach the organ is, the less responsive it is to cholinergic stimulation in terms of mucus secretion. On the other hand, the more responsive the organ is to cholinergic stimulus, the more variations occur in its mucus secretion (Figure 3).

The attachment of Polycarbophil discs was the strongest in the cecum of the rat and weakest in the stomach and jejunum (Figure 4). This observation can be explained by two findings of this study: (*a*) The mucus lining in the cecum is thinner than in the stomach and the jejunum and therefore separation between two adjacent mucus layers is more difficult in the cecum; (*b*) Because of the lower amount of sialic acid in the cecum the mucus in this organ is less negatively charged. This allows for a greater interpenetration of the Polycarbophil chains between the mucin macromolecules (1,25). Our finding that Polycarbophil discs adhere differently onto mucosal tissues of different regions of the GI tract of the rat supports the hypothesis that the attachment of mucoadhesives to the mucus layer is stronger than the cohesive force between adjacent mucin layers. The attachment of Polycarbophil to mucus is typically weaker at pH 7 than at pH 2. Because acidic pH is optimal for mucoadhesion of Polycarbophil, it was postulated in the past that the stomach is an ideal organ for mucoadhesion (3).

The thickness of the mucus gel that covers the GI epithelium is due to the steady state between the mucus secretion rate and its erosion, namely enzymatic and mechanical degradation. Because of typical gastric motility and proteolytic activity, mucus turnover is most intense in the stomach (26), and therefore, too rate limiting for the rational design of mucoadhesive polymers. Assuming variations in mucus turnover along the gastrointestinal tract, an optimal site for mucoadhesion would be one possessing all of the following properties: (*a*) strong attachment properties, (*b*) a low mucus turnover rate, and (*c*) a low sensitivity to stimuli that might increase mucus secretion. Our studies in the rat indicated that its cecum is the best location for that purpose.

The lack of difference between the observed detachment force before and after carbachol treatment in the rat stomach and jejunum is surprising, and may be explained differently for the cecum and colon than the stomach and jejunum. As was shown in Figure 2, the cecum and the colon are not affected by the induced cholinergic stimulus, and therefore no change in the adherence properties of these two organs is expected. Indeed, this was verified by measuring the mucoadhesion force of Polycarbophil discs following treatment with carbachol as demonstrated in Figure 4. As for the stomach and the jejunum of the rat, it may be assumed that there is a threshold of mucus thickness which restricts mucoadhesion. Above this threshold value the mucus gel thickness does not affect mucoadhesion.

It can be concluded that the colon and the cecum may be more suitable locations for mucoadhesion in the gastrointestinal tract. Furthermore, because in the rat shear forces are likely to be higher in the cecum than in the colon, it is also speculated that the latter is a more appropriate organ for mucoadhesion. Parallel physiological conditions have been observed in the human proximal and transverse colon. The former contains relatively large amounts of liquid, while the latter contains chyme of increasing viscosity (27).

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REFERENCES

1. N. A. Peppas and P. A. Burim. Surface, interfacial and molecular aspects of polymer bioadhesion on soft tissues. *J. Contr. Rel.* **2**: 257–275 (1985).
2. M. R. Neutra and J. F. Forstner. Gastrointestinal mucus—synthesis, secretion and function. In: Johnson L. R. (ed) *Physiology of the Gastrointestinal Tract,* 2nd edn. Raven Press, New York, 1987, pp 975–1009.
3. H. S. Ch'ng, H. Park, P. Kelly, and J. R. Robinson. Bioadhesion polymers as platform for oral controlled drug delivery II: Synthesis and evaluation of some swelling, water insoluble bioadhesive polymers. *J. Pharm. Sci.* **74**: 399–405 (1985).
4. S. A. Mortazavi, and J. D. Smart. An investigation into the role of water movement and mucus gel dehydration in mucoadhesion. *J. Contr. Rel.* **25**: 197–203 (1993).
5. C.-M. Lehr, J. A. Bouwstra, W. Kok, A. G. De Boer, J. J. Tukker, J. C. Verhoef, D. D. Breimer, and H. E. Junginger. Effect of the mucoadhesive polymer polycarbophil on the intestinal absorption of a peptide drug in the rat. *J. Pharm. Pharmacol.* **44**: 402–407 (1992).
6. C.-M. Lehr, F. G. P. Poelma, H. E. Junginger, and J. J. Tukker. An estimate of turnover time of intestinal mucus gel layer in the rat in situ loop. *Int. J. Pharm.* **70**: 235–240 (1991).
7. P. Gruber, A. Rubinstein, V. Hon Kin Li, P. Bass, and J. R. Robinson. Gastric emptying of nondigestible solids in the fasted dog. *J. Pharm. Sci.* **76**: 117–122 (1987).

8. M. A. Longer, H. S. Ch'ng and J. R. Robinson. Bioadhesive polymers as platform for oral controlled drug delivery III: Oral delivery of chlorothiazide using a bioadhesive polymer. *J. Pharm. Sci.* 74: 406–411 (1985).
9. P. Esposito, E. Sandefer, F. Carli, and G. A. Digenis. New polysaccharide matrices for improved gastric residence. *In vivo* (dogs) scintigraphic studies and correlation with *in vitro* physical properties. *Proc. Int. Symp. Control. Rel. Bioact. Mat.* 17: 319–320 (1990).
10. D. Harris, J. T. Fell, H. L. Sharma and D. C. Taylor, and J. Lynch. GI transit of potential bioadhesive formulations in man: a scintigraphic study. *J. Controlled Rel.* 12: 45–53 (1990).
11. J. Kopecek, P. Kopeckova, H. Brondsted, R. Rathi, B. Rihova, P. Y. Yeh, and K. Ikesue. Polymers for colon-specific drug delivery. *J. Contr. Rel.* 19: 121–130 (1992).
12. B. Rihova, R. Rathi, P. Kopeckova, and J. Kopecek. In vitro bioadhesion of carbohydrate-containing N-2(2-hydroxypropyl) methacrylamide copolymers to the GI tract of guinea pigs. *Int. J. Pharm.* 87: 105–116 (1992).
13. I. Lutsky, F. Aizer, and N. Mor. The Sabra rat: Definition of a laboratory animal. *Is. J. Med. Sci.* 20: 603–612 (1984).
14. P. Whiteman. The quantitative measurement of alcian blue-glycosaminoglycan complexes. *Biochem. J.* 131: 343–350 (1973).
15. D. W. Pipper, D. Whitecross, P. Leonard, and A. Clarke. Alcian blue binding properties of gastric juice. *Gastroenterology* 59: 534–538 (1970).
16. S. J. Corne, S. M. Morrissery, and R. J. Woods. A method for the quantitative estimation of gastric barrier mucus. *Proc. Physiol. Soc.* 116P–117P (1974).
17. S. Kerss, A. Allen, and A. Garner. A simple method for measuring thickness of mucus gel layer adherent to rat, frog and human gastric mucosa: influence of feeding, prostaglandin, N-acetylcysteine and other agents. *Clin. Sci.* 63: 187–195 (1982).
18. S. Tani and N. Muto. Effects of pentagastrin, histamine, carbamylcholine and catecholamines on gastric secretion, and emptying in the rat. *Biochem. Pharmacol.* 31: 3475–3481 (1982).
19. A. Garner, G. Flemstrom, A. Allen, J. R. Heylings, and S. McQueen. Gastric mucosal protective mechanisms: roles of epithelial bicarbonate and mucus secretions. *Scand. J. Gastroenterology* Suppl. 79–86 (1984).
20. P. Alfoldi Jr., F. Obal, E. Toth, and J. Hideg. Capsain pretreatment reduces the gastric acid secretion elicited by histamine but does not affect the responses to carbachol and pentagastrin. *Eur. J. Pharmacol.* 123:321–327 (1986).
21. R. Kohen, A. Kakunda, and A. Rubinstein. The role of cationized catalase and cationized glucose oxidase in mucosal oxidative damage induced in the rat jejunum. *J. Biol. Chem.* 267: 21349–21354 (1992).
22. R. J. Winzler. Methods for determination of serum glycoproteins. *Meth. Biochem. Anal.* 2: 279–307 (1955).
23. M. R. Jimenez-Castellanos, H. Zia, and C. T. Rhodes. Assessment of an in vitro method for measuring the bioadhesiveness of tablets. *Int. J. Pharm.* 89: 223–228 (1993).
24. A. Allen, D. A. Hutton, J. P. Person, and L. A. Sellers. Mucus glycoprotein structure, gel formation and gastrointestinal mucus function. In: *Mucus and Mucosa,* Ciba Foundation Symposium 109, Pitman, London, 1984, pp. 137–156.
25. E. Jabbari, N. Wisniewski, and N. A. Peppas. Evidence of mucoadhesion by chain interpenetration at a poly(acrylic acid)/mucin interface using ATR-FTIR spectroscopy. *J. Contr. Rel.* 26: 99–108 (1993).
26. A. Allen. Structure and function of gastrointestinal mucus. In: Johnson, L. R. (ed) *Physiology of the Gastrointestinal Tract,* Raven Press, New York, 1981, pp 617–639.
27. S. K. Sarna. Physiology and pathophysiology of colonic motor activity, Part 1. *Digest. Dis. Sci.* 36: 827–862 (1991).