

## Unstirred Water Layers in Rabbit Intestine: Effects of Pectin (41860)

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**Abstract.** Pectins have been shown to affect the absorption of several different nutrients in clinical studies; however, the mechanisms for decreased absorption have not been defined. A possibility not studied with regards to pectin, but previously demonstrated to be important in absorption, is the effect of change in the unstirred water layer. As the unstirred water layer increases in thickness, the rate of absorption decreases for certain nutrients. The effect of pectin on the unstirred water layer in the lumen of rabbit jejunum was examined by previously described techniques. It was observed that: (1) increases in pectin concentration resulted in an increased thickness of the unstirred water layer; (2) for any stir rate, the addition of pectin increased the thickness of the unstirred water layer; and (3) stir rate is inversely related to the thickness of the unstirred water layer. It was concluded from these results that pectin increases the thickness of the unstirred water layer in rabbit jejunum. This mechanism may explain, in part, the reduction of the rate of absorption of certain nutrients seen following pectin ingestion.

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There exists a layer of relatively unstirred water next to all biological membranes through which solute molecules must move by simple diffusion (1). With regards to the small intestine this unstirred water layer consists of a series of water lamellae extending out from the brush border membrane of the absorptive enterocytes, each progressively more stirred, until they blend in with the bulk water phase of the intestinal contents (2). The boundary between the bulk water phase and the unstirred water layer is not stringently defined, but a finite, functional thickness of this layer can be assessed for the evaluation of various absorptive or secretory transport phenomena in the intestine (2, 3). Therefore, during absorptive phenomena in the small intestine, solutes must penetrate both an unstirred water layer and the brush border membrane of the enterocyte. Both of these barriers may exert significant resistance to absorption such that the unstirred water layer may be rate limiting in the overall absorptive process (4).

Pectin is a type of fiber (a large, complex polysaccharide) found in many fruits and vegetables and has been shown in clinical studies to affect the absorption of several different nutrients (5, 6). Previous studies in our laboratory involving possible interaction between bile salts and pectin showed no binding of bile salts to pectin, *in vitro*, but bile salt absorption was reduced in the small intestine (7). The question concerning the role of pectin relative

to decreased bile salt absorption prompted us to perform studies using pectin in order to elucidate its effect on the functional thickness of the unstirred water layer.

**Materials and Methods.** Adult New Zealand rabbits weighing from 2.5 to 4.0 kg were used in this investigation. After the rabbits were sacrificed by decapitation, a segment of jejunum was rapidly removed and placed in a ice-cold (0°C) Krebs-bicarbonate buffer bubbled with 95% O<sub>2</sub>:5% CO<sub>2</sub>. The jejunum was then opened along the mesenteric border and the mucosal surface was thoroughly flushed with a jet of cold Krebs-bicarbonate buffer from a syringe to remove visible detritus and mucus. Then the intestine was further sectioned into 30 experimental samples and stored in iced (0°C) Krebs-bicarbonate Ringer before preincubation in the various experimental solutions. Preincubation of the intestinal tissue was done in Krebs-bicarbonate Ringer at 37°C containing either 0, 0.25, or 1.0% orange pectin (Sun-Kist Growers Inc.). The jejunal sections were then mounted as a flat sheet in a modified Ussing chamber as first described by Lukie *et al.* (8) and were bathed by the same Krebs-bicarbonate solution (37°C) as in the preincubation step. The incubation solutions were mixed at stirring rates (0, 500, or 1000 rpm) with circular magnetic bars, and stirring rates were precisely adjusted by means of a strobe light (Power Instruments).

The thickness of the unstirred water layer was measured according to the method of Diamond (9). Briefly, the technique consists of measuring the change in the transmural potential difference (PD) caused when the mucosal surface of the intestine suddenly was exposed to a hyperosmotic solution (Krebs-bicarbonate + 150 mM sucrose). The time required to achieve half the new steady-state potential difference,  $t_{1/2}$ , is a function of the thickness of the unstirred water layer,  $d$ , and the diffusivity of the molecule used to induce the change in potential difference,  $D$  (free-diffusion coefficient of sucrose in 150 mM NaCl =  $6.2 \times 10^{-6}$  cm<sup>2</sup> sec<sup>-1</sup>). Thus, the thickness of the unstirred water layer can be calculated from the formula:

$$d = (Dt_{1/2}/0.38)^{1/2}$$

To measure the  $t_{1/2}$  values, the PD was monitored continuously by means of KCl bridges placed in the serosal and mucosal compartments of the chamber and connected via calomel half-cells to a voltmeter (Radiometer Inst.) and rapid response chart recorder (Yewtec Corp). To induce a change in the PD, the chamber was rapidly transferred from the incubation solution to another beaker where sucrose had been added to the Krebs-bicarbonate buffer to increase its osmolality. After the new steady-state potential difference was achieved, the chamber was transferred back to the original incubation solution: thus, the  $t_{1/2}$  could be measured repeatedly for the build-up and decay of the change in potential difference in the same tissue preparation. These values were used to calculate the effective “ $d$ ”

under different experimental conditions and these thicknesses are reported in micrometers ( $\mu\text{m}$ ).

All data are reported as means  $\pm$  SEM. Differences between means were analyzed statistically using the nonparametric rank sums test (10). The 0.01 level of probability was used as the criterion of significance.

**Results.** Figure 1 demonstrates typical examples of different stir rates and pectin concentrations (B, C, D) on the rate of decay of PD (half-times =  $t_{1/2}$ ) from control values (A). As can be seen, the greater the stir rate (B, C, or D) or the less the pectin concentrations in the perfusate (B, C), the less the  $t_{1/2}$ , which is a direct reflection of the thickness of the unstirred water layer.

Table I summarizes the calculated data of the unstirred water layer thickness relative to concentration of pectin in the perfusate and stirring rate. As can be seen, at 0% pectin concentration the effect of increasing the stirring rate from 0 to 500 rpm significantly decreased the calculated unstirred water layer thickness by 45% ( $P < 0.01$ ), whereas increasing the stirring rate to 1000 rpm further decreased the unstirred layer thickness by 55% compared to control (0% pectin and 0 rpm). At 0.25% pectin concentration, the effect of increasing the stirring rate from 0 to 500 rpm significantly decreased the calculated unstirred water layer thickness by 46% ( $P < 0.01$ ), whereas increasing the stirring rate to 1000 rpm further decreased the unstirred water layer thickness by 58% compared to the concentration-based control (0.25% pectin and 0 rpm). At 1.00% pectin concentration, the effect of increasing

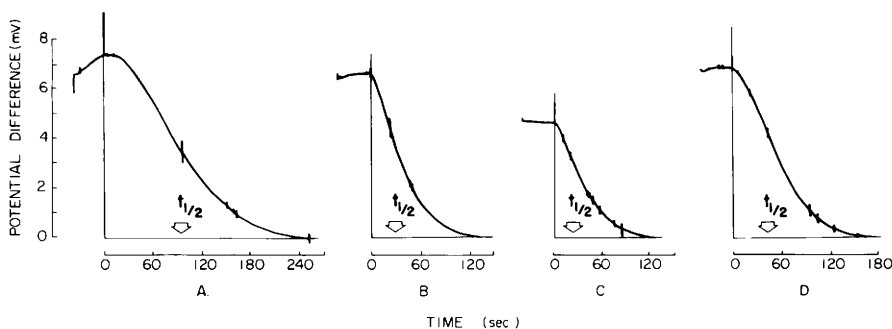


FIG. 1. Representative recordings of potential difference as a function of time in seconds. A: 0 rpm, 1.00% pectin; B: 500 rpm, 0.25% pectin; C: 1000 rpm, 0.25% pectin; D: 1000 rpm, 1.00% pectin.

TABLE I.

Stirring rate <sup>b</sup>	Thickness of unstirred water layer <sup>a</sup>		
	0	500	1000
Concentration of pectin			
0.00%	275.1 ± 12.4 (30) <sup>c</sup>	150.4 ± 8.8 (30)	121.9 ± 2.9 (30)
0.25%	319.6 ± 17.5 (30)	173.7 ± 29.2 (30)	132.7 ± 2.8 (30)
1.00%	385.8 ± 5.3 (30)	189.8 ± 5.7 (30)	156.2 ± 4.1 (30)

<sup>a</sup> Units of unstirred water layer in  $\mu\text{m} \pm \text{SEM}$ .

<sup>b</sup> Stirring rate is expressed in rpm.

<sup>c</sup> Number of observations shown in parentheses.

the stirring rate from 0 to 500 rpm decreased the calculated unstirred water layer thickness by 51%, whereas increasing the stirring rate to 1000 rpm decreased the unstirred water layer thickness by 60% compared to the concentration-based control (1.00% pectin and 0 rpm). As is also observed at 0 rpm stirring rate the thickness of the unstirred water layer of the 0% pectin concentration is significantly less than the thickness of the unstirred water layer at 0.25% pectin ( $P < 0.01$ ), which is significantly less thick than the unstirred water layer in the intestine that is perfused with 1.00% pectin ( $P < 0.01$ ). In addition, the same relationship between thickness of the unstirred water layer and stirring rate at 500 or 1000 rpm hold relative to the control perfusate (0% pectin). In summary, the thickness of the unstirred water layer is directly related to the concentration of luminally perfused pectin and inversely related to the magnitude of the stir rate.

**Discussion.** As a molecule is actively or passively absorbed from the bulk aqueous solution of the intestinal contents into the cytoplasm of the enterocyte, it must cross two major diffusion barriers: the unstirred water layer external to the cell and the protein lipid membrane of the microvilli. Experiments in the present study were designed to investigate the thickness of the unstirred water layer and how this thickness could be altered. Measurements of the thickness of the unstirred water layer suggested that the diffusion barrier was several hundred micrometers thick (Table I). The intestinal mucosa contains two structural elements, glycocalyx and mucus, overlying the brush border membrane that could conceivably lead to overestimation errors in

the determination of the thickness of the unstirred water layer. However, it has been demonstrated that unstirred water layers of similar dimensions have been found in other epithelia, such as gallbladder (9, 11), which have less prominent mucus and glycocalyx layers, and in artificial membranes that have no mucus or glycocalyx at all (12). We have therefore concluded that the diffusion barrier is principally a layer of unstirred water.

The finding that increasing the pectin concentration in the luminal perfusate significantly increased the thickness of the unstirred water layer (Table I) suggests that pectin ingestion can reduce both active and passive nutrient absorption by increasing the external diffusion barrier to that nutrient. However, it is obvious that increasing the stirring rate has an inverse effect on the thickness of the unstirred water layer (Table I), as has also been reported in another intestinal epithelium (13), regardless of pectin concentration. This finding may have little physiological benefit if we equate stirring rate in the *in vitro* situation with intestinal motility in the *in vivo* case for, in that situation, there is a decreased time for nutrient absorption. The opposing effects of decreased absorptive time versus decreased thickness of the unstirred water layer may cancel one another. However, it is difficult to imagine gut motility being as vigorous as the experimentally forced stirring rates in the *in vitro* case and, therefore having similar effects on the thickness of the unstirred water layer. Nonetheless, under pectin-ingestion conditions, the unstirred water layer can become totally rate limiting to the uptake of highly permeant nutrient molecules and significantly reduce their uptake.

A possible cause for the pectin-induced unstirred water layer thickness could be the increase in bulk (bathing) fluid viscosity. Viscosity of the bulk fluid increases nonlinearly with increasing concentrations of pectin (14). This phenomenon is usually due to either dissociation of pectin carboxyl groups, hence increased hydrogen bonding, or complex association of pectinic acid molecules (14). Therefore, intestinal unstirred water layer thickness could be directly related to the increased bathing medium viscosity imparted by lumenally perfused pectin. If this premise is true, nutrient absorption by the small intestine is inversely related to luminal fluid viscosity.

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