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Unstirred layer and kinetics of electrogenic glucose absorption in the human jejunum *in situ*

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SUMMARY Using an electrical technique we estimated the thickness of the unstirred layer in the human jejunum during kinetic studies of electrogenic glucose absorption. The unstirred layer in seven healthy volunteers (632 ± 24 μm: mean ± SEM) was significantly thicker than in 10 patients with active coeliac disease (442 ± 23 μm) but not significantly different in seven patients who had responded to treatment by gluten withdrawal (585 ± 49 μm). There were similar differences in the values of 'Apparent Km' for electrogenic glucose absorption between healthy control subjects (36 ± 6 mM) active coeliac patients (11 ± 1 mM) and treated coeliac patients (31 ± 5 mM). The changes in PDmax however, showed a different pattern. The PDmax in the active coeliac group (6·8 ± 0·7 mV) was lower than in controls (7·6 ± 0·6 mV) but not significantly so, while the PDmax in the treated coeliac group (10·6 ± 0·9 mV) was significantly higher than in both the active coeliac and control groups. It should be noted that both operational kinetic parameters obtained in the present study are much lower than those obtained previously (Read *et al.*, 1976b) because of the use of siphonage. Analysis of the results using a computer simulation indicates that the reduction in Apparent Km in active coeliac disease can be caused by the interaction of the decreased maximal absorption rate for glucose (Jmax) with the attenuated unstirred layer. In these circumstances it is not necessary to postulate any change in the affinity of the transport mechanism for glucose ('Real Km'). It is remarkable that the disease process produces an Apparent Km which is much closer to the Real Km than that found in health.

Part 1: Measurement of functional unstirred layer thickness

We have previously described a technique for measuring changes in transmural potential difference (PD) across the human jejunum *in vivo* in response to infusion of solutions containing glucose (Read *et al.*, 1974). Using this technique we observed that the operational kinetic parameters ('Apparent Km', PDmax) for the active electrogenic component of glucose absorption were significantly lower in untreated coeliac disease than in healthy controls (Read *et al.*, 1976b). It is possible that these results could be caused by differences in the thickness of the layer of unstirred fluid which lies adjacent to the mucosal epithelium.

In this present paper we used an electrical technique for estimating the thickness of this unstirred layer in the human jejunum *in situ* during kinetic studies of electrogenic glucose absorption. An abstract of this work has already been published (Read *et al.*, 1976a).

Methods

SUBJECTS

Studies were carried out on seven healthy volunteers, 17 patients with coeliac disease, and 28 patients who, despite a clinical presentation suggesting malabsorption, had normal jejunal histology. The patients with coeliac disease were diagnosed on the basis of a flat jejunal biopsy and subsequent histological and clinical improvement after withdrawal of dietary gluten. They were subdivided into active and treated groups. The active group all had a flat or convoluted jejunal biopsy at the time of the electrical studies and included five untreated patients and five

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patients who had either shown a poor response to treatment or had initially responded well and subsequently relapsed. The treated group were seven patients who had shown a good clinical and histological response to gluten withdrawal and had a biopsy appearance consisting of finger and leaf-shaped villi.

Techniques

The apparatus and method for measuring the jejunal transmural PD was similar to those described in our previous papers, but incorporated the following modifications: (1) the intestinal tube was altered so that solutions could be continuously siphoned from the infusion site (Fig. 1). The rate of siphonage always matched the infusion rate of 5 ml per minute. Thus, pooling of successive solutions in the jejunum was minimised, allowing rapid change from one solution to the next; (2) intraluminal pressure was measured at the infusion site by means of a transducer (Bell and Howell, model 4-327 L221) connected to the side arm of the infusion column. This allowed us to check that intestinal motility, suppressed by propantheline bromide (Read *et al.*, 1974, 1976b; Brown *et al.*, 1976), remained quiescent during the recording period; (3) both the pressure and the PD signals passed through electronic filters with five-second time constants to suppress fluctuations in the record caused by the infusion pump, respiration, electrocardiogram, intestinal myoelectric activity, and 50 Hz mains interference.

The infusion protocol is summarised in Table 1. The infusion of solution 1 was continued until a stable baseline had been maintained for at least 10 minutes. Subsequent infusion of solution 2, which contained a lower concentration of sodium chloride than solution 1, caused the lumen to become more positive because it created a diffusion gradient for sodium chloride *via* cation-selective pathways in the epithelium (Wright, 1966). The measurement of the half time for the attainment of this diffusion potential (Fig. 2) was used to estimate the thickness of the unstirred layer by the method of Diamond (1966)—namely,

$$d = \frac{t_{\frac{1}{2}} D}{0{\cdot}38}$$

where d is the unstirred layer thickness, $t_{\frac{1}{2}}$ is the half time for the development of the diffusion PD and D is the diffusion coefficient for 125 mM sodium chloride in free solution ($12{\cdot}4 \times 10^{-6}$ cm^{-2} s^{-1}; Diamond, 1966). The infusion of solutions 3 to 6, which contained increasing concentrations of glucose, generated a series of transfer potentials causing the lumen to become progressively more negative

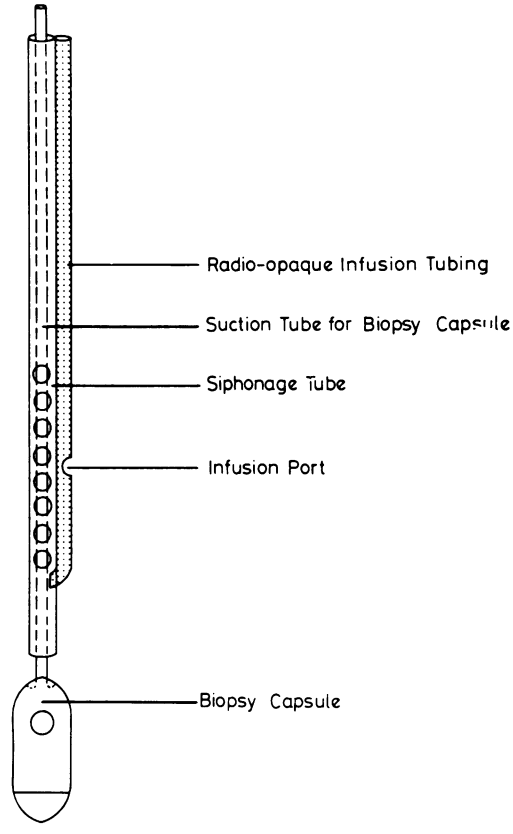

Fig. 1 *A diagram of the distal end of the intestinal tube. Solutions were infused at 5 ml per minute via the infusion port and allowed to siphon immediately from the same site via the siphonage ports arranged around the infusion orifice. The incorporation of a biopsy capsule (Crosby and Kugler, 1957) enabled a specimen of jejunal mucosa to be obtained immediately after the electrical recordings were completed.*

(Fig. 3). Finally, after return to solution 1 the osmotic potential produced by a hypertonic solution of 100 mM mannitol in normal saline (solution 7) was recorded in some subjects. The half time for the attainment of this potential provided a further estimate of the thickness of the unstirred layer by using Diamond's formula but substituting the diffusion coefficient for mannitol ($9{\cdot}48 \times 10^{-6}$ cm^{-2} s^{-1}). Before the tube was finally withdrawn a specimen of jejunal mucosa was obtained by means of the biopsy capsule (Crosby and Kugler, 1957). The duration of the experiment never exceeded 90 minutes from the time the tube was positioned in the proximal jejunum.

To confirm the influence of siphonage on the estimations of unstirred layer thickness and opera-

Table 1 *Solutions infused into jejunal lumen*

Solution	*NaCl (mM)*	*Mannitol (mM)*	*Glucose (mM)*	*PD measured*
1	154	0	0	Endogenous PD
2	104	100	0	Diffusion PD
3	104	90	10	Glucose Transfer PDs
4	104	80	20	
5	104	50	50	
6	104	0	100	
1	154	0	0	Endogenous PD
7	154	100	0	Osmotic PD

tional kinetic parameters for glucose absorption, measurements were also carried out on seven subjects in whom solutions were not siphoned from the lumen.

Values of the operational kinetic parameters of the PDmax (the maximum glucose transfer PD attainable) and the Apparent Km (the concentration of glucose which generates half the PDmax) were estimated by two graphical methods (Lineweaver and Burk, 1934; Eisenthal and Cornish-Bowden, 1974) and an iterative method for a computer (Wang programme number 3504). As there was no significant difference between the results from each method, the values quoted are derived from the direct linear plot (Eisenthal and Cornish-Bowden, 1974).

The possible effect of the electronic filter with a five-second time constant on measurements of the diffusion PD half times was assessed *in vitro* by means of a linear sweep generator (Aldous, 1972). When connected to our recording system this device generated a signal which changed from zero to full scale with a duration which could be varied to simulate the diffusion transients. Changing the time constant on the electronic filter from 0 to five seconds had no significant effect on the time taken to attain half the maximum signal when this was varied over a range from 20 to100 seconds.

Results

The results from the control and the active and treated coeliac groups are shown in Table 2. The unstirred layer in the healthy control group was significantly higher ($P < 0.001$) than in patients with active coeliac disease, but not significantly different from that found in the treated coeliac group. The Apparent Km was also significantly higher ($P < 0.001$) in the control group than the active coeliac group, but there was no significant difference between the control and treated coeliac groups. The PDmax in active coeliac disease, although lower, was not significantly different from the control value, while in the treated group it was very significantly

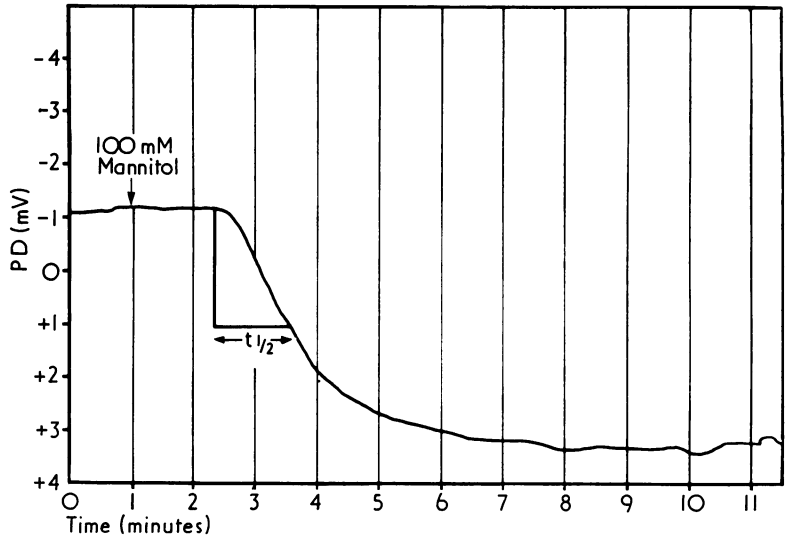

Fig. 2 *The diffusion potential used to measure the thickness of the unstirred layer. At the point indicated by the arrow, the solution containing 154 mM NaCl was replaced by solution 2, which contained 104 mM NaCl. The half time for the generation of the resulting diffusion potential was determined from the moment that the PD first began to change. This value was then used to calculate the unstirred layer thickness (see text).*

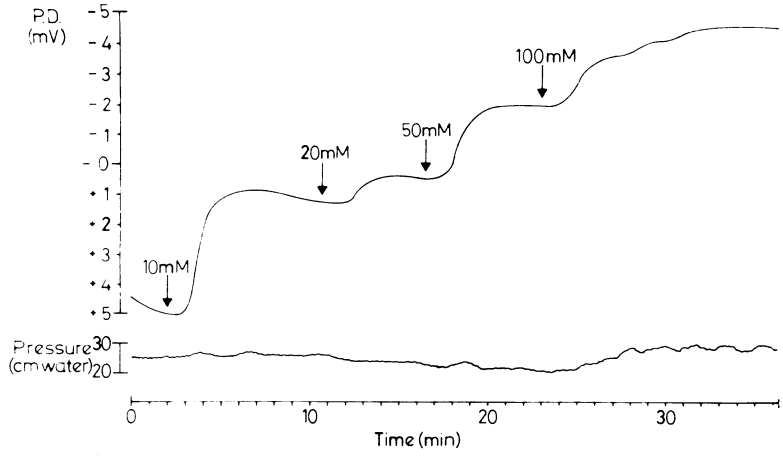

Fig. 3 *The changes in potential difference produced by infusion of solutions containing increasing concentrations of glucose in one patient with active coeliac disease. Initial infusion of an isotonic solution containing 100 mM Mannitol (solution 2), but no glucose, produced a baseline against which the other PDs were compared. Subsequent infusion of solutions 3 to 6 containing increasing concentrations of glucose (see Table 1 for composition of solutions) caused the lumen to become progressively more negative. The difference between the steady state PDs produced by each of these solutions and the baseline obtained with solution 2 was the glucose transfer potential which was used to calculate the Apparent Km and PDmax.*

Table 2 *Thickness of jejunal unstirred layer estimated from measurements of half time for generation of diffusion PD, together with Apparent Km and PDmax for electrogenic component of glucose absorption and magnitude of diffusion PD in healthy controls and patients with active and treated coeliac disease*

Group	*Unstirred layer thickness (d) (μm)*	*Apparent Km (mM)*	*Apparent PDmax (mV)*	*Diffusion PD (mV)*	*n*
Control	632 ± 24	36 ± 6	7·6 ± 0·6	4·3 ± 0·4	7
Active coeliac	442 ± 23	11 ± 1	6·8 ± 0·7	5·8 ± 0·7	10
Treated coeliac	585 ± 49	31 ± 5	10·6 ± 0·9	5·8 ± 0·4	7

Results are expressed as mean ± SEM. 'n' refers to the number of subjects tested in each group.

higher ($P < 0.001$). The mean diffusion PDs were identical in both active and treated coeliac groups and these were significantly higher ($P < 0.05$) than the diffusion PD in the control group. There was no correlation between the magnitude of the diffusion PD and the half time for its generation.

The changes in kinetic parameters in coeliac disease reported here were similar to those published in our previous study (Read *et al.*, 1976b) where solutions were not siphoned from the lumen. However, it can be seen from Table 3 that the absolute values of Apparent Km and PDmax were significantly lower in the present study where siphonage was employed.

Values for Apparent Km (21 ± 3 mmol/l), PDmax (7·5 ± 0·5 mV), and unstirred layer thickness (490 ± 17 μm) were obtained in 28 patients with conditions other than coeliac disease. There was no obvious correlation between the results of any clinical test (Hb, serum folate, serum B_{12}, Schilling B_{12} excretion test, serum iron, faecal fat, xylose (five hour) tolerance test), the unstirred layer thickness, and the kinetic parameters. Those patients presenting with diarrhoea, however, were a particularly interesting group. As shown in Table 4, those with a known pathological cause for diarrhoea (ulcerative colitis, Crohn's disease and hypolactasia), designated the 'pathological diarrhoea' group, have significantly lower Apparent Kms and thinner unstirred layers than the control group. However, the Apparent Km and unstirred layer thickness in those patients where no significant cause for diarrhoea could be demonstrated diarrhoea) are not significantly different from the

Table 3 *Comparison of Apparent Km and PDmax for electrogenic glucose absorption in present series of experiments where solutions were siphoned continuously from lumen with results from non-siphoned previous series (Read* et al., *1976b)*

Group	*Apparent Km (mM)*	*Apparent PDmax (mV)*	*n*
Control			
Siphoned	36 ± 6	7·6 ± 0·6	7
Non-siphoned	69 ± 5	12·9 ± 1·0	20
	P < 0·001	P < 0·005	
Active coeliac			
Siphoned	11 ± 1	6·8 ± 0·7	10
Non-siphoned	20 ± 3	8·7 ± 0·7	9
	P < 0·05	P < 0·05	
Treated coeliac			
Siphoned	31 ± 5	10·6 ± 0·9	7
Non-siphoned	106 ± 15	15·4 ± 2·4	5
	P < 0·001	P < 0·05	

Results are expressed as mean ± SEM. 'n' refers to the number of subjects in each group. The statistical significance of the differences between the siphoned and the non-siphoned groups was calculated using Student's *t* test for unpaired data.

Table 4 *Apparent Km and PDmax for electrogenic glucose absorption and thickness of the jejunal unstirred layer in nine patients with diarrhoea of known aetiology (ulcerative colitis, Crohn's disease, and constitutional hypolactasia) and five patients with functional diarrhoea*

Group	*Unstirred layer thickness (μm)*	*Apparent Km (mM)*	*Apparent PDmax (mV)*	*n*
Functional diarrhoea	557 ± 34 (NS)	36 ± 9 (NS)	9·2 ± 1·5 (NS)	5
Pathological diarrhoea	416 ± 16 (P < 0·001)	12 ± 2 (P < 0·001)	6·6 ± 0·6 (NS)	9
Control	632 ± 24	36 ± 6	7·6 ± 0·6	7

Results are expressed as mean ± SEM. 'n' refers to the number of subjects tested in each group. The statistical significance of the differences between each of these groups and the healthy control group was calculated using Student's *t* test for unpaired data. Where applicable the P values are given in parentheses.

Table 5 *Comparison of unstirred layer thickness determined from measurements of half times for osmotic PD compared with values determined from diffusion PD half times in healthy controls and patients with active and treated coeliac disease*

Group	*Unstirred layer thickness (μm)*	
	From diffusion PD	*From osmotic PD*
Control	632 ± 24 (7)	393 ± 14 (6)
Active coeliac	442 ± 23 (9)	344 ± 28 (5)
Treated coeliac	585 ± 26 (7)	398 (2)

Results are mean ± SEM. The figures in parentheses refer to the number of subjects tested in each group. Although the results obtained from the diffusion PD are significantly lower in active coeliac disease compared with controls (P < 0·001) the differences between the results from the osmotic PD did not achieve statistical significance (P < 0·1).

control values. The patients with hypolactasia were all relatively fit, with only mild atrophic changes in the jejunal biopsy, yet their unstirred layers were thinner than any other group studied (395 ± 33 μm).

It should be noted that the thickness of the unstirred layers quoted in Tables 2, 4, and 5 were determined from the half times for the diffusion PD. As these were always measured at the beginning of the experiment when conditions were most stable, they were considered a more reliable index of unstirred layer thickness than the half times for the osmotic PDs measured some 50 to 90 minutes later, towards the end of the experiment. However, it can be seen from Table 5 that the unstirred layer thickness estimated from measurements of the osmotic PD showed changes in coeliac disease similar to those obtained from diffusion PDs, although the absolute values were significantly lower. This discrepancy could be caused by progressive thinning of the unstirred layer during the course of the experiment, as suggested by duplicate measurements of the half time for the diffusion PD in six subjects. This was investigated further in one patient with normal jejunal histology by making repeated estimations of unstirred layer thickness using both methods over the period of a normal experiment. The results in Fig. 4 show that the unstirred layer in this subject did become thinner as the experiment progressed, but this fact alone does not account for the difference in the thickness of the unstirred layer estimated by the two methods.

In the studies where solutions were not siphoned from the intestinal lumen, the thicknesses of the unstirred layers were very high and these were associated with correspondingly high values of Apparent Km and PDmax (Table 6).

Discussion

The layer of unstirred fluid lying adjacent to the mucosal surface of the intestine provides a barrier through which solutes must move by diffusion. Several studies have indicated that the presence of this unstirred layer introduces significant errors in the values of kinetic parameters for active transport processes (Dietschy *et al.*, 1971; Winne, 1973; Dietschy and Westergaard, 1975). Wilson and Dietschy (1974) showed that reduction of the thickness of the unstirred layer of the rat jejunum *in vitro* by stirring the bulk incubation solution was associated with a decrease in the values of the Km for several actively transported solutes. Similar results have since been reported in the hamster jejunum (Dugas *et al.*, 1975).

We used an electrical method to estimate the thickness of the unstirred layer in the human

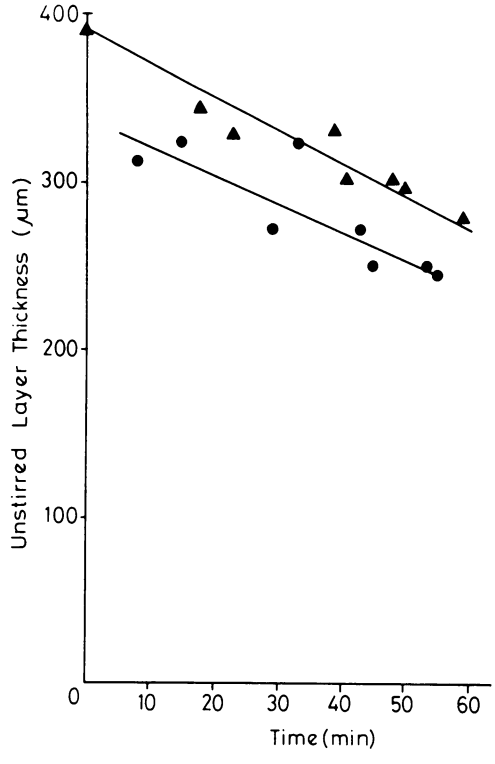

Fig. 4 *Repeated estimations of the unstirred layer thickness determined by measurements of the half times for the diffusion PD (▲) and the half time for the osmotic PD (●) plotted against the duration of the experiment in one patient with anaemia but normal jejunal histology. Using either method the unstirred layer appears to become progressively thinner during the course of the experiment.*

jejunum *in situ* and have shown that the reduction in the Apparent Km for electrogenic glucose absorption in active coeliac disease is associated with a significant attenuation of this layer.

The measurement of unstirred layer thickness by this method indicates the existence of a diffusion barrier, which behaves like a static layer of water similar in thickness to the height of a normal human finger-shaped villus (529 ± 107 μm, Riecken *et al.*, 1976). This measurement, however, cannot convey any knowledge about the structure of the unstirred layer. The current concept incorporates an inner fairly rigid structure induced by the epithelium but becoming superficially less rigid and finally merging imperceptibly with the fluid in the luminal bulk phase. Alternatively, it could be a thin layer of mucus which by limiting the free diffusion of solutes behaves like a much thicker water layer. Another important consideration is that measurements of

Table 6 *Thickness of unstirred layer, obtained from measurements of half time for diffusion PD and operational kinetic parameters for electrogenic glucose absorption in seven non-siphoned subjects (three healthy controls and four patients with normal jejunal histology) compared with siphoned group*

Group	*Unstirred layer thickness (μm)*	*Apparent Km (mM)*	*Apparent PDmax (mV)*	*n*
Non-siphoned	1009 ± 151	120 ± 25	17 ± 3	7
Siphoned	632 ± 24	36 ± 6	7·6 ± 0·6	7
P	< 0·02	< 0·005	< 0·01	

Results are expressed as mean ± SEM. 'n' refers to the number of subjects in each group. P values are derived from Student's *t* test for unpaired data.

the thickness of the unstirred layer are based on the assumption that the epithelium is a flat sheet. While this may be the case in active coeliac disease, it is clearly not true for the irregular villous surface of the normal jejunum. Westergaard and Dietschy (1974) claim that measurements of the thickness of the unstirred layer of the normal jejunum incorporate the lengths of the diffusion pathways to sites on the sides of the villi as well as those on their tips. This may account for the relatively thick unstirred layers found in the healthy control subjects. However, since glucose absorption also takes place on the sides of the villi as well as the tips, the thickness of the unstirred layer in the normal jejunum estimated from our measurements should provide a more accurate index of the length of the diffusion pathway for glucose.

The electrical method used for estimating the thickness of the unstirred layer was originally devised by Diamond (1966) for measurements on the epithelial surface of the gall bladder *in vitro*. Although the technique has been applied to the small intestine *in vitro*, apparently with satisfactory results (Westergaard and Dietschy, 1974; Wilson and Dietschy, 1974), its application to the human jejunum *in vivo* creates several additional problems. First, it is essential for accurate measurements of PD transients that spontaneous fluctuations in the transintestinal PD are absent from the record. This is achieved by administering an intramuscular injection of propantheline bromide before the start of the infusion (Read *et al.*, 1974; Brown *et al.*, 1976). Second, the change from one solution to another must be very rapid compared with the development of the diffusion potential. We have therefore infused the solutions at a relatively rapid rate (5 ml per minute) and maintained a similar rate of siphonage from the site of infusion. In studies without siphonage the large unstirred layers that are obtained are presumably

related in part to the time taken for any new solution to equilibrate with the pool of solution already present in the lumen. The artefactual increase in unstirred layer thickness is associated with very high values of the Apparent Km for the active electrogenic component of glucose absorption. This effect presumably also accounts for the differences in Km between the present study and our previous unsiphoned studies on the kinetics of glucose absorption in coeliac disease (Read *et al.*, 1976a). Third, unlike the data obtained *in vitro* (Westergaard and Dietschy, 1974), estimations of the thickness of the unstirred layer determined by recording osmotically induced PDs are significantly lower than those obtained from diffusion PDs. The results of our experiments suggest that this discrepancy may be caused in part by progressive thinning of the unstirred layer throughout the duration of the experiment either by the effect of the constant infusion or by obliteration of the intervillous spaces by swelling of the villi. The latter has been observed *in vitro* but may be caused in that situation by lack of lymph and blood flow from the intestine (Westergaard and Dietschy, 1974).

Although a theoretical 'minimum unstirred layer thickness' exists at all membrane/fluid interfaces it is unlikely that this can be measured in man *in vivo* because neither the turbulent stirring or the interrupted flow (Winne, 1976) needed to achieve it can be tolerated by a conscious subject. The assessment of the thickness of unstirred layers in the small intestine of conscious subjects is thus highly dependent on specific technical factors such as the use of probanthine, siphonage and the choice of whether to use the half time for the osmotic rather than the diffusion PD. Because of these dependencies, values estimated from our data can apply only in our conditions of assessment. However, the study allows relative values to be determined under the same stable conditions as those used for kinetic studies of electrogenic glucose absorption. Thus the changes in the functional thickness of the jejunal unstirred layer, which we have estimated, may account for the differences in the Apparent Km between controls and patients with coeliac disease. This need not be the only mechanism, nor even the most important. Other possibilities include the effect of changes in the maximal rate of glucose absorption mediated through the unstirred layer and real alterations in the affinity of the transport mechanism for glucose—that is, changes in the Real Km. Thomson and Dietschy (1977) have derived equations that describe the theoretical effects of unstirred water layers on the kinetic parameters of active transport processes in the intestine. In the second part of our paper we have similarly investigated, using a computer simulation, the relative roles of unstirred layers and changes in the maximal rate of glucose absorption in creating the differences, measured *in vivo*, between the Apparent Kms of untreated coeliac patients and controls.

Part 2: Interpretation of changes in operational kinetic parameters by computer simulation

In the second part of this paper we have used a computer simulation to elucidate the role of the unstirred layer in accounting for the changes in operational kinetic parameters for electrogenic glucose absorption in coeliac disease.

Derivation of computer simulation

The active absorption of glucose at the cell membrane conforms to saturation kinetics (Fisher and Parsons, 1953) and is therefore characterised by the equation:

$$J = \frac{Jmax\ C_2}{Km + C_2} \qquad (1)$$

where J is the rate of glucose absorption produced by the concentration of glucose at the cell membrane (C_2), Jmax is the maximal absorption rate, and Km is the concentration of glucose at the cell membrane which yields half the Jmax. The unstirred layer lying adjacent to the cell membrane (Fig. 5) acts like a second membrane through which glucose has to diffuse before reaching the active transport site. Therefore the uptake of glucose from the intestinal lumen must include the expression for diffusion across this unstirred layer

$$J = (C_1 - C_2)\ \frac{D}{d} \qquad (2)$$

where C_1 is the concentration of glucose in the luminal bulk phase, D is the diffusion coefficient for

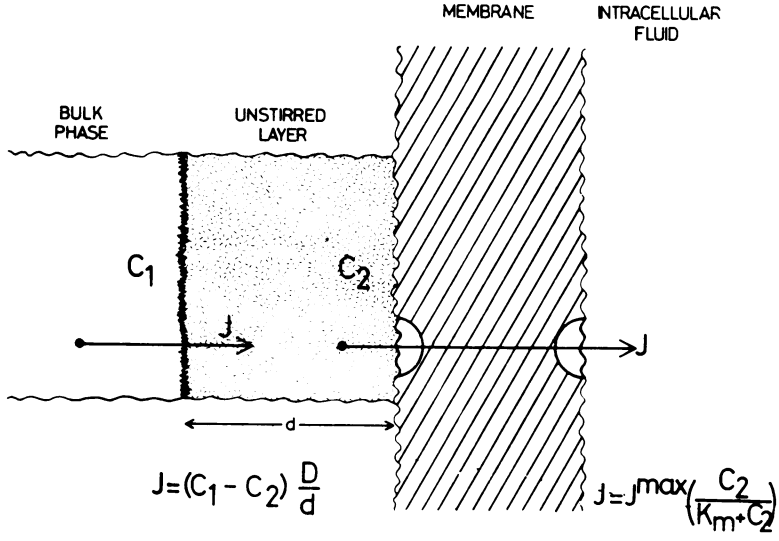

Fig. 5 *A diagram of the mucosal surface of the jejunal epithelium. The fluid in the luminal bulk phase, which is well stirred, is separated from the membrane itself by a layer of relatively unstirred fluid of thickness 'd' through which glucose has to diffuse before reaching the carrier site on the membrane. C_1 and C_2 are the concentrations of glucose in the luminal bulk phase and adjacent to the membrane respectively. J is the rate of glucose absorption. The formulae for the rate of diffusion across the unstirred layer and the rate of active transfer at the membrane are shown at the bottom of this Figure.*

glucose in water, and d is the thickness of the unstirred layer.

At equilibrium the rate of diffusion across the unstirred layer is equal to the rate of active glucose uptake by the cell. Hence, by rearranging (2)

$$C_2 = C_1 - J\frac{d}{D} \qquad (3)$$

and substituting into (1) it is possible to derive an expression for the rate of active glucose absorption from the luminal bulk phase (Winne, 1973; Wilson and Dietschy, 1974) in terms of the bulk phase concentration C_1

$$J = 0{\cdot}5\frac{D}{d}\left[Km + C_1 + \frac{Jmax\ d}{D} - \sqrt{\left(Km + C_1 + \frac{Jmax\ d}{D}\right)^2 - 4\frac{d}{D}(Jmax\ C_1)}\right] \qquad (4)$$

The direct application of this equation to electrical measurements of glucose absorption is difficult because the relationship between the rate of glucose transport (J) and the PD generated is equivocal. This is partly because the glucose transfer PD is influenced by the passive ion conductance of the mucosa as well as the current generated by ion-linked glucose transfer and also because the rate of transfer (J) unlike the PD depends upon the number of functioning enterocytes per unit area. However, if we assume that the shapes of the active absorption curves for glucose are the same whether derived from electrical or chemical data (Debnam and Levin, 1975) then for any glucose concentration . . .

$$\frac{J}{Jmax} = \frac{PD}{PDmax} \qquad (5)$$

and substituting into equation (4)

$$\frac{PD}{PDmax} = \frac{J}{Jmax} = \frac{0{\cdot}5D}{Jmax\ d}\left[Km + C_1 + \frac{Jmax\ d}{D} - \sqrt{\left(Km + C_1 + \frac{Jmax\ d}{D}\right)^2 - 4\frac{d}{D}(Jmax\ C_1)}\right] \qquad (6)$$

In order to study the shape of the absorption curve derived from electrical data, the scale of the PD for practical purposes must be normalised to an arbitrary value of PDmax. In all of the following examples we have used a nominal value of 10 mV for the PDmax. Therefore:

$$PD = \frac{10 \times 0{\cdot}5D}{Jmax\ d}\left[Km + C_1 + \frac{Jmax\ d}{D} - \sqrt{\left(Km + C_1 + \frac{Jmax\ d}{D}\right)^2 - 4\frac{d}{D}\,Jmax\ C_1}\right] \qquad (7)$$

Computer simulation based on equation (7) has enabled us to generate curves of concentration against PD for different values of Jmax, d, and Real Km. Apparent values of Km and PDmax can then be obtained from these curves by the direct linear plot (see Methods section).

PREDICTIONS

The data in Table 7 show that increases in the thickness of the unstirred layer over a range corresponding to our own measurements can produce large increases in the Apparent Km and somewhat smaller increases in the PDmax. A similar effect can be produced by increases in the maximal rate of glucose absorption (Jmax) in the presence of unstirred layer of constant thickness. Table 8 shows the influence of increases in Jmax on the Apparent Km and PDmax when the unstirred layer is fixed at 450 μm, and Table 9 shows that this effect is enhanced if the thickness is increased to 600 μm.

Table 7 *Effect of changes in thickness of unstirred layer on Apparent Km and PDmax*

Unstirred layer thickness 'd' (μm)	*Apparent Km* (mM)	*Apparent PDmax* (mV)
300	16	11·2
400	22	12·0
500	31	13·0
600	43	14·4
700	57	16·1

For this computer simulation we have introduced values for parameters based as far as possible on data obtained in man. Thus the Real Km must be lower than our experimental Kms and is given a nominal value of 5 mM, the diffusion coefficient for glucose (D) is $9{\cdot}45 \times 10^{-6}$ cm^{-2} s^{-1}, the range of unstirred layer thicknesses correspond to our own measurements, the real PDmax is ascribed a value of 10 mV and the Jmax is given a value of 4 nM/cm^{-2} s^{-1}, based upon the results of perfusion experiments in man (Modigliani *et al.*, 1973). The values of Jmax from these experiments are expressed in terms of segment length and need to be converted to units of surface area for the computer simulation.

We have assumed that for the active absorption the effective surface area of the diffusion barrier is a little over three times the minimal cylindrical surface area overlying the tips of the villi (Wilson and Dietschy, 1974). A further correction for the valvulae conniventes (× 3; Wilson, 1962) brings our final conversion factor to 10 times the minimal cylindrical surface area ($2\pi rl$; where r is the radius of the jejunum (≏ 2 cm) and l the length of the segment).

Table 8 *Effect of changes in maximal rate of glucose absorption (Jmax) on Apparent Km and PDmax*

Jmax ($nM\ cm^{-2}\ s^{-1}$)	*Apparent Km* (mM)	*Apparent PDmax* (mV)
1	7	10·0
2	10	10·5
3	14	11·0
4	19	11·6
5	26	12·4
6	35	13·5

For this computer simulation the unstirred layer thickness is fixed at 450 μm and the values of Jmax cover a range from 1 to 6 nM cm^{-2} s^{-1}. Real Km, Real PDmax, and D have the same values as in Table. 7.

Table 9 *Effect of changes in Jmax on Apparent Km and PDmax, predicted by computer simulation*

Jmax ($nM\ cm^{-2}\ s^{-1}$)	*Apparent Km* (mM)	*Apparent PDmax* (mV)
1	8	10·0
2	12	10·8
3	19	11·7
4	29	12·8
5	43	14·4
6	60	16·5

This example incorporates the same values for the parameters as Table 8, except for the thickness of the unstirred layer which is fixed at 600 μm.

ANALYSIS OF EXPERIMENTAL DATA

The interaction of Jmax and d in determining the values of Apparent Km is illustrated in Fig. 6. Comparison of the experimental data with these computer predictions, displayed as contour lines, suggests that the Apparent Km between the controls and the patients with active coeliac disease cannot be explained by changes in the unstirred layer thickness alone. If the Real Km for glucose is assumed to

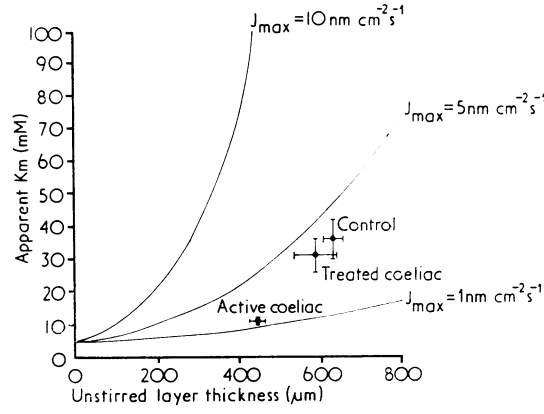

Fig. 6 *Plot of Apparent Km against unstirred layer thickness for three different values of Jmax. This is derived from the computer model and is based on data obtained in man (see caption to Table 7) and an assumed Real Km of 5 mM. These simulations provide a series of contour lines for comparison with experimental data from the control and coeliac groups displayed as the mean ± SEM. It can be seen that the active coeliac group lies just above the 1 nM cm^{-2} s^{-1} contour line, while the control and treated coeliac groups lie just below the 5 nM cm^{-2} s^{-1} contour line. If the assumptions of the simulation are correct, then the difference between the active coeliac and control groups cannot be explained solely by difference in unstirred layer thickness, but also incorporates an approximate four-fold difference in Jmax.*

remain fixed at 5 mM, the separation between the active coeliac and the normal group would indicate an approximately four-fold difference in Jmax corresponding to that estimated experimentally in man (Schedl and Clifton, 1961; Holdsworth and Dawson, 1965).

A more objective approach to the interpretation of differences in Apparent Km involves analysis of the shapes of the experimental curve of PD against glucose concentration. These are governed by three variables, Real Km, Jmax, and d. In practice it is not possible to analyse experimental curves to yield unique values of each of these parameters. However, reduction of the number of variables allows limited analysis to be carried out. Examination of equation (7) shows that it can be simplified using the new expression T, where

$$T = \frac{Jd}{D} \tag{8}$$

and

$$Tmax = \frac{Jmax\ d}{D} \tag{9}$$

Then by substitution into equation (7):

$$PD = \frac{5}{Tmax}\left[(Km + C_1 + Tmax) - \sqrt{(Km + C_1 + Tmax)^2 - 4\ Tmax\ C_1}\right] \tag{10}$$

Substituting values of Tmax and Real Km into this equation allows us to determine the transfer PDs generated by the same glucose concentrations as those used experimentally (10, 20, 50, and 100 mM) and from these data a value of Apparent Km. By this means we define the relationship between Apparent Km and its two component variables, Real Km and Tmax.

Thus, for each experimental value of Apparent Km we can determine the possible combinations of Tmax and Real Km. Moreover, if the Tmaxs are divided by d/D for each subject, we can plot the possible combinations of Jmax and Real Km which would yield the values of Apparent Km. The mean plots with their standard deviations for controls and patients with active coeliac disease are shown in Fig. 7. The lines are clearly well separated confirming that the differences in Apparent Km between the active coeliac and control group clearly cannot be explained by the changes in the thickness of the unstirred layer alone. Moreover, if we accept that the Jmax in coeliac disease is only a fraction of that in health (Schedl and Clifton, 1961; Holdsworth and Dawson, 1965), then it is unnecessary to postulate any change in the real affinity of the glucose carrier

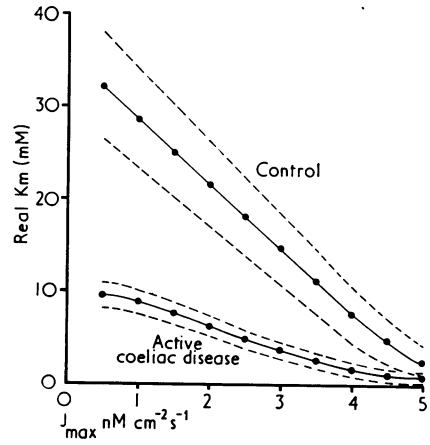

Fig. 7 *A plot of the possible combinations of Real Km and Jmax which interact through the unstirred layer to yield the experimental values of Apparent Km for the control and active coeliac groups. Results are shown as mean ± SD.*

to account for the observed reduction in Apparent Km in coeliac disease.

Although our methods are unable to provide specific values for the Real Km, an estimate can, in theory, be obtained by using the approximation, developed by Parsons (1976)—namely:

$$\text{Apparent Km} = \text{Real Km} + \frac{Jmax\ d}{D} \tag{11}$$

and substituting values for Apparent Km and Jmax, obtained from absorption studies in which glucose absorption was estimated chemically (Modigliani *et al.*, 1973). However, this method is unsatisfactory in practice for the following reasons: (1) as Jmax d/D appears to be very large compared with the Real Km, the errors involved in determining Jmax accurately under *in vivo* conditions and relating it to the functional surface area of the test segment produce very large errors in the estimations of Real Km; (2) at high values of Jmax the approximate relationship of equation 11 becomes inaccurate. Although both Jmax and 'd' are reduced in coeliac disease, reliable extraction of Real Km from chemical absorption data (Schedl and Clifton, 1961; Holdsworth and Dawson, 1965) using Parson's formula is still impossible. However, it is remarkable that as a result of the disease process the Apparent Km in this condition must be much closer to the Real Km than that found in health.

Changes in PDmax are difficult to analyse because the relationship between the rate of glucose transfer (J) and the transfer PD is equivocal. However, it is possible to make some interpretations concerning

the differences in PDmax between controls and patients with coeliac disease. The fact that the reduction in Jmax in untreated coeliac disease is considerably greater than the reduction in PDmax indicates that the former is caused by a loss of enterocytes rather than a decrease in the transport rate per enterocyte. Although the experimental value of PDmax is independent of the number of functioning enterocytes, it should nevertheless respond to changes in Jmax mediated through the unstirred layer as predicted in Tables 8 and 9. The fact that it does not appear to do so suggests that there are also differences in passive ion conductance between coeliacs and controls. We may gain an impression of the relative passive ion conductances from the magnitudes of the diffusion potentials. The increased diffusion PD in coeliac disease indicates that the epithelium is more selectively permeable to sodium, implying that the permeability to other ions and hence the total ion conductance is reduced in that condition. We have attempted to 'correct' our values of PDmax in coeliac disease for differences in total ion conductance by multiplying by the ratio of the diffusion potentials in controls and coeliacs. After applying this correction the changes in PDmax in coeliac disease (Table 10) now correspond to the predictions of the computer simulation.

In conclusion, although the reduced Apparent Km in active coeliac disease could be interpreted as an enhanced affinity of the transport mechanism for glucose, we suggest the following alternative hypothesis. The normal jejunal epithelium has an irregular surface composed of villi covered by large numbers of enterocytes with similar transport characteristics creating a rapid absorption of glucose (high Jmax) per unit area and a relatively thick unstirred layer. These interact to produce a high Apparent Km for active glucose absorption. In coeliac disease there is a considerable reduction in the number of functioning enterocytes which leads to a flattening of the epithelial surface. Thus both the Jmax and the thickness of the unstirred layer are reduced, resulting in a profound decrease in the Apparent Km. Possible reduction in total passive ion conductance in coeliac disease masks a similar effect on the PDmax. Thus the decrease in Apparent Km in coeliac disease could be caused solely by the loss of enterocytes and need not necessarily reflect any change at all in the transport characteristics of the remaining cells.

Table 10 *Values of PDmax for electrogenic glucose absorption corrected for differences in total passive ion conductance in healthy controls and patients with active and treated coeliac disease*

Group	*Unstirred layer thickness* (μm)	*Apparent Km* (mM)	*Apparent PDmax* (mV)	*n*
Control	632 ± 24	36 ± 6	7·6 ± 0·6	7
Active coeliac	442 ± 23	11 ± 1	5·0 ± 0·5	10
Treated coeliac	585 ± 49	31 ± 5	7·9 ± 0·7	7

The thickness of the unstirred layer and the Apparent Km are also shown for comparison. Results are expressed as mean ± SEM. 'n' refers to the number of subjects in each group.

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