

Electrogenic glucose absorption in untreated and treated coeliac disease

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SUMMARY Using a method for measuring changes in transmural potential difference across the human jejunum *in vivo*, the operational kinetic parameters of 'Apparent Km' and PD max for the active electrogenic component of glucose absorption were estimated in a group of healthy volunteers and in patients with coeliac disease. Both the 'Apparent Km' (17 ± 2 mM; mean \pm SEM) and the PD max (8.6 ± 0.7 mV) in nine patients with untreated coeliac disease were significantly lower ($P < 0.005$) than in the control group ('Apparent Km' = 74 ± 5 mM; PD max 12.8 ± 0.9 mV, $n = 20$). Treatment of five coeliac patients by gluten withdrawal for less than three months increased significantly the values of both the 'Apparent Km' (35 ± 6 mM) and the PD max (11.4 ± 1.2 mV). Treatment of five patients for more than six months caused a further increase in the values of both kinetic parameters ('Apparent Km' = 108 ± 13 mM; PD max = 15.6 ± 2.7 mV) to levels which exceeded those in healthy subjects. The possible interpretations of the differences in the kinetic characteristics of electrogenic glucose transport between coeliac patients and healthy subjects are discussed.

In a previous study we described a technique for characterising the active electrogenic component of glucose absorption in man (Read *et al.*, 1974). We have now applied this method to investigate changes in electrogenic glucose absorption in patients with active and treated coeliac disease.

Methods

SUBJECTS

Tests of electrogenic glucose absorption were carried out on a group of 10 male and 10 female young healthy volunteers and on patients with coeliac disease, who were diagnosed on the criteria of clinical evidence of malabsorption, the typical biopsy appearance of total or subtotal villous atrophy, and response to treatment by gluten withdrawal. In the coeliac group tests were carried out on nine patients who had received no treatment, five patients treated for less than three months, and five patients treated for more than six months. These subgroups included the patients in Table 3 who were tested on more than one occasion. All the treated patients had evidence of either clinical or histological

response to withdrawal of gluten at the time of testing.

The method for characterising electrogenic glucose absorption by measuring jejunal transmural potential differences (PDs) was identical with that previously described (Read *et al.*, 1974). In brief, an intestinal tube was swallowed and positioned with its tip approximately 5 cm beyond the duodeno-jejunal flexure. An intramuscular injection of 30 mg propantheline bromide (Pro-banthine) was then administered. This reduced the normal fluctuations in the record, which varied in amplitude up to 10 mV, to levels (less than 1 mV) sufficiently stable for the measurement of small changes in PD. The solutions shown in Table 1 were infused at a constant rate of 5 ml per minute and the resultant changes in PD between the flowing intraluminal electrode and subcutaneous reference electrode were recorded. A typical record obtained from a patient with untreated coeliac disease is shown in Fig. 1. The infusion of solution 1, containing mannitol but no glucose, provided a baseline PD with the lumen a few millivolts positive to the subcutaneous reference site. Subsequent infusion of a series of solutions containing increasing concentrations of glucose (Table 1) caused the lumen to become progressively

Solution	Sodium chloride (mM)	Glucose (mM)	Mannitol (mM)
1	104	0	100
2	104	10	90
3	104	20	80
4	104	50	50
5	104	100	0

Table 1 Solutions infused into jejunal lumen

more negative. After a change in solution the PD usually achieved a new plateau within five minutes. The value of the PD for any infusion was obtained by drawing a straight line through the middle of the record after a plateau had been maintained for 10 minutes. The glucose transfer PD was taken as the difference between the PD level obtained during the infusion of any glucose solution and the baseline obtained during the infusion of solution 1. The total infusion time required for this method never exceeded 90 minutes. The incorporation of a biopsy capsule (Crosby and Kugler, 1957) enabled a specimen of jejunal mucosa to be obtained immediately after completion of the electrical recordings.

Because of the possible inaccuracies of the double

reciprocal plot (Lineweaver and Burk, 1934) estimates of 'Apparent Km' (the half saturation constant of the electrogenic mechanism) and PD max (the theoretical maximum transfer PD) were obtained from the electrical data by the additional methods of the direct linear plot (Eisenthal and Cornish-Bowden, 1974) and an unweighted iterative method for a Wang 700 desk computer (Wang programme No. 3504). On the assumption that the parameters are normally distributed, differences between 'Apparent Km' and PD max were evaluated by the appropriate paired or unpaired *t* test.

Results

The glucose transfer PDs in the untreated coeliac and control groups are compared in Fig. 2. The transfer PD generated by 10 mM glucose was very much higher in patients with untreated coeliac disease ($P < 0.0001$). At 20 mM ($P < 0.005$) and 50 mM ($P < 0.05$) the differences in PD become less significant until at 100 mM the transfer PDs were virtually identical. Treatment of the coeliac patients for less than three months caused a reduction in the transfer

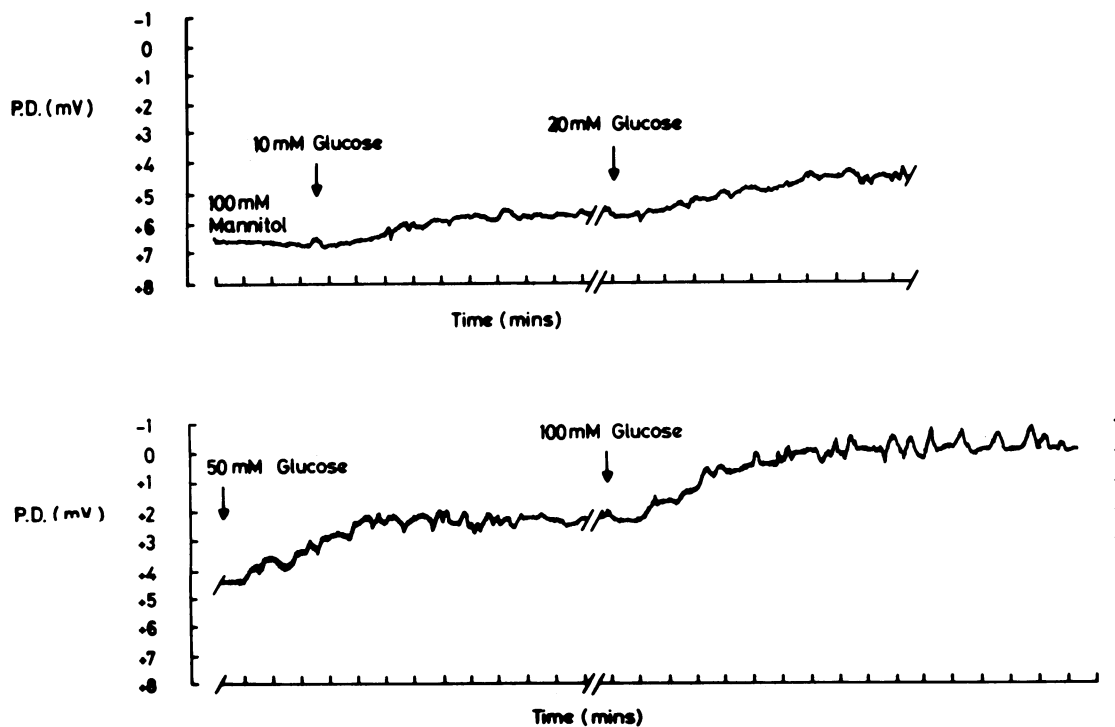


Fig. 1 Tracing of a typical record, taken from a patient with untreated coeliac disease. The full length of the PD plateau for each infusion is not shown in this figure.

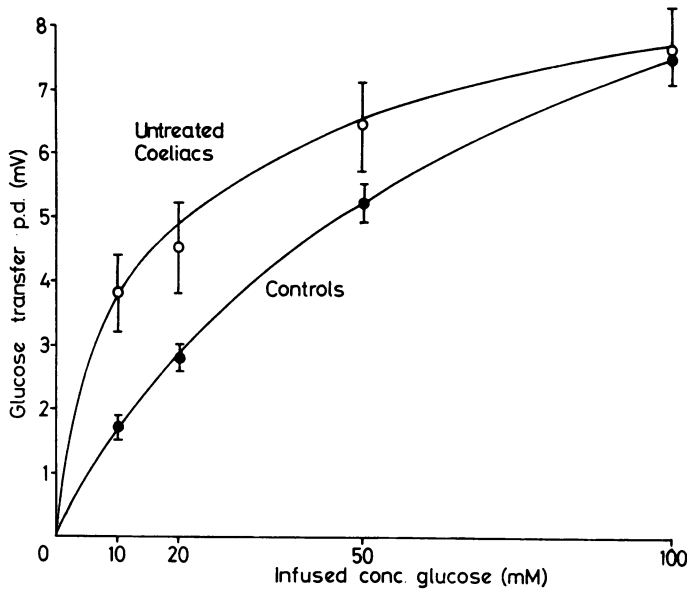


Fig. 2 Plot of glucose transfer PD against infused glucose concentration in nine patients with untreated coeliac disease and 20 normal control subjects. The results are expressed as means \pm SEM.

PDs at 10 mM and 20 mM glucose but an increase at 100 mM (Fig. 3). After six months' treatment the transfer PD at each glucose concentration was not significantly different from the corresponding value in the control subjects.

The operational kinetic parameters estimated from these data by the three different methods are

listed in Table 2. Despite the fact that the values of 'Apparent K_m ' and PD max obtained from the direct linear plot are medians while those from the other methods are means, there was no significant difference between the results from the three methods. Whatever the method of estimation, the 'Apparent K_m ' from the untreated coeliac group

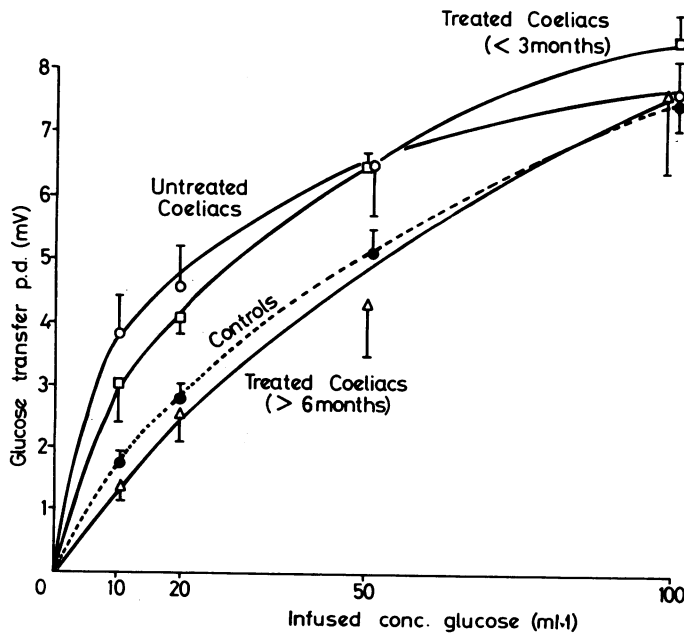


Fig. 3 Plot of glucose transfer PD against infused glucose concentration in nine patients with untreated coeliac disease, five treated by gluten withdrawal for less than three months and five treated for more than six months. For comparison the results from 20 normal control subjects are included. To avoid confusion some of the points are slightly offset along the abscissa. The results are expressed as means \pm SEM.

were strikingly lower than those from the controls ($P < 0.005$). Similarly, the values for the PD max were also very much lower in untreated coeliac disease ($P < 0.01$). Treatment of the coeliac patients for less than three months induced a significant rise in both the 'Apparent Km' ($P < 0.002$) and the PD max ($P < 0.02$) compared with the untreated group. After six months' treatment there was an additional significant increase in 'Apparent Km' ($P < 0.005$) and an insignificant increase in PD max ($P > 0.05$) to levels above those obtained in the control group. The changes in the kinetic parameters upon treatment of the coeliac patients, who were tested on more than one occasion, were accompanied by corresponding improvements in clinical state and jejunal morphology (Table 3).

Discussion

This study has shown clearly that the operational

kinetic parameters of electrogenic glucose absorption, no matter how calculated, are greatly affected by coeliac disease. The 'Apparent Km' is dramatically decreased while the PD max is also significantly reduced. On treatment with a gluten-free diet the 'Apparent Km' and PD max return to normal levels concomitant with restoration of normal structure (Table 3). In the group treated with a gluten-free diet for more than six months the 'Apparent Km' was significantly greater than that observed in healthy controls but the PD max was not significantly higher. Whether this enhanced 'Apparent Km' has a functional significance is as yet unknown. The actual dose response curve for electrogenic glucose absorption (Fig. 3) for these treated patients is not significantly different from that for normal subjects and, in general, the higher the values for 'Apparent Km' the less meaningful becomes the functional significance of differences of this parameter.

The method of characterising intestinal absorption

Operational parameter	Method	Groups			
		Control	Untreated	Treated 3/12	Treated 6/12
'Apparent Km' (mM)	1	74 ± 5	17 ± 2	35 ± 6	108 ± 13
	2	69 ± 5	20 ± 3	29 ± 3	106 ± 15
	3	80 ± 8	19 ± 3	32 ± 8	124 ± 22
PD max (mV)	1	12.8 ± 0.9	8.6 ± 0.7	11.4 ± 1.2	15.6 ± 2.7
	2	12.9 ± 1.0	8.7 ± 0.7	11.0 ± 0.8	15.4 ± 2.4
	3	13.6 ± 1.0	8.8 ± 0.7	10.8 ± 1.1	16.6 ± 2.6
n		20	9	5	5

Table 2 Operational kinetic parameters of 'Apparent Km' and PD max for active electrogenic glucose absorption in normal subjects and patients with coeliac disease

These parameters are calculated from the same data by three different methods. (1) the double reciprocal plot of Lineweaver and Burk (1934); (2) the direct linear plot of Eisenthal and Cornish-Bowden (1974); (3) an unweighted iterative method for a Wang desk computer. The results are expressed as mean ± SEM.

Patient, sex, age (yr)	Period of gluten withdrawal	Weight (kg)	Haemoglobin (g/dl)	Biopsy appearance	'Apparent Km' (mM)	PD max (mV)
L.B.	Untreated	51	13.4	Convolutated	16	8
F 23	4 weeks	52	14.5	Convolutated	24	10
H.F.	Untreated	64	9.8	Flat	18	8
M 44	6 weeks	67	13.0	—	48	11
	1 year	67	14.0	Leaves	125	10
I.T.	Untreated	56	11.8	Flat	26	10
F 47	11 months	60	14.0	Leaves	86	13
G.M.	Untreated	58	8.1	Flat	26	9
M 47	8 weeks	62	14.4	Flat	28	10
	6 months	75	15.0	Convolutated	90	11
C.W.	6 weeks	64	11.3	Flat	52	16
M 59	1 year	70	14.7	Leaves	91	22

Table 3 Clinical data and operational kinetic parameters of electrogenic glucose absorption determined by method of Lineweaver and Burk, from five patients with coeliac disease before and at varying intervals after initiation of treatment by dietary gluten withdrawal

The biopsy appearance relates to the gross morphology as seen under the low power ($\times 25$) of a dissecting microscope. Changes in morphology were always associated with corresponding changes in histological appearance as seen with the light microscope ($\times 60$).

by the determination of saturation kinetic parameters was initially borrowed from studies on enzyme substrate interactions (Fisher and Parsons, 1953; Smyth, 1971). Its further application to electrical measurements of transfer activity came from the *in vitro* studies of Asano (1964), Schultz and Zalusky (1964), and Lyon and Crane (1966). Debnam and Levin (1975a, b) extended its application to *in vivo* studies and showed that the 'Apparent Km' for the active electrogenic component of glucose absorption is the same whether calculated from measurements of hexose transfer PDs or from the chemically measured loss of glucose from the lumen. This study suggests that *in vivo* electrical and chemical methods can be used to characterise the same process.

A controversial point concerning values of 'Apparent Km' and PD max is whether they can ever be used as indices of carrier affinity and transfer capacity respectively. If they can, it would allow inferences to be made about the effects of coeliac disease on absorption mechanisms. One possible way of investigating this appealing prospect is to see whether the changes in electrical parameters determined in our study are matched by corresponding changes in chemical parameters in man. At present we have found that it is technically impossible to design a satisfactory protocol to measure accurately glucose absorption both chemically and electrically during the same experiment. This is because the probanthine required to obtain satisfactory stability for electrical recording makes quantitative recovery of infusions containing glucose from a site lower down the intestine extremely variable. Two previous perfusion studies have, however, measured the disappearance of glucose from the jejunal lumen of normal and coeliac subjects at several infused concentrations (Schedl and Clifton, 1961; Holdsworth and Dawson, 1965). Both studies showed a decrease in maximal glucose absorption (Jmax) in the untreated coeliac groups compared to normal controls. The data of Holdsworth and Dawson (1965), moreover, shows that the absorption curve in the untreated coeliac group clearly saturates at a much lower concentration of glucose than the controls and thus has a lower 'Apparent Km'. Therefore, there is a clear association in untreated coeliac disease between decreases in Jmax and PD max and also reductions in chemical and electrical 'Apparent Kms'. While such associations can never be regarded as proof of a causal relationship, it is more than tempting to suggest that the changes in the electrical data are concomitant with the changes in glucose absorption, measured chemically.

Kinetic parameters, however, whether obtained by chemical or electrical techniques are affected by a

number of experimental complications. Among these is the thickness of the unstirred water layer through which glucose has to diffuse before being actively transferred by the enterocyte. This has been shown to exert a profound influence on experimental values for Km and Jmax *in vitro* (Winne, 1973; Wilson and Dietschy, 1974; Dugas *et al.*, 1975). By extrapolation of these data, it is possible that the reduction in the operational electrogenic parameters in coeliac disease (Km, PD max) could arise from attenuation of the unstirred layer.

The decreased PD max observed in untreated coeliac disease may be due not only to changes in the ion-linked active transfer mechanisms, but also to alteration in intestinal mucosal resistance. No measurements of this resistance have been made in coeliac disease even *in vitro* and it is unlikely that any simple technique could be applied to assess it *in vivo*. However, studies have shown that *in vivo* the jejunal mucosa in this condition exhibits a greater resistance to the diffusional movements of several solutes (Fordtran *et al.*, 1967). While there is no proven direct correlation between diffusional and electrical resistances, the data do not support the possibility that the observed low PD max in coeliac disease may be due to low mucosal resistance.

Other factors affecting the values of 'Apparent Km' and PD max are technical, such as the possible reduction of the effective glucose concentration by pooling of the serially infused solutions. Because the electrogenic absorption curve appears to exhibit saturation kinetics, the PDs obtained at low glucose concentrations are liable to be lowered more by pooling than those obtained at the higher concentrations, near saturation level. Thus conditions in which there is increased pooling alter the shape of the absorption curve and lead to higher experimental values for Km and PD max. All the available evidence indicates that there is greater possibility of pooling in coeliac disease compared with normal subjects, in that there is less absorption of solute and water (Schedl and Clifton, 1961; Holdsworth and Dawson, 1965), and more secretion (Fordtran *et al.*, 1967; Schmid *et al.*, 1969; Russell *et al.*, 1972) while the jejunal tone and motility are reduced (Ingelfinger and Moss, 1943; Ritchie and Salem, 1965). The fact that the 'Apparent Km' and PD max are actually lower in untreated coeliac disease strongly suggests that pooling does not account for the observed differences between this group and the controls.

In conclusion, despite the uncertainties in interpretation of kinetic parameters for electrogenic glucose transfer, the changes found in coeliac disease are qualitatively similar to those found by perfusion techniques in which glucose absorption was measured chemically. The total perfusion time for such

chemical kinetic studies is much longer, being a minimum of five hours as compared with 90 minutes for the electrical test. The real meaning of the kinetic parameters obtained by either method is still subject to debate, but data obtained previously in the rat suggests that the electrical method might even give more accurate information about active glucose transport than the chemical method which does not discriminate between active and passive glucose absorption. The narrow single lumen tube used for the electrical method involves no more discomfort than that used for jejunal biopsy, which can indeed be carried out at the end of the test. It thus compares favourably with the four lumen tube used currently for the chemical method. Clearly, a considerable amount of work is required before the electrical test could be advocated for routine clinical usage but at present it provides a useful research tool for the further exploration of absorptive pathophysiology.

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