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AN ANALYSIS OF THE D-GLUCOSE INFLUX KINETICS OF IN VITRO HAMSTER JEJUNUM, BASED ON CONSIDERATIONS OF THE MASS-TRANSFER COEFFICIENT

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SUMMARY

1. A study designed specifically to investigate the effects of unstirred layers on the apparent glucose-influx kinetics of hamster jejunum was conducted.

2. The apparent V was 12.81, 10.71, 9.75, 10.17 and 9.33 μmol/cm^2 · h while the apparent K_m was 7.42, 3.95, 1.87, 0.93 and 0.5 mM, respectively, when the rate of shaking the incubation flasks was 40, 80, 120, 160 and 200 cycles/min.

3. Extrapolation of the slope and reciprocal intercept of Lineweaver-Burke plots of the data to infinite shaking rate is mathematically justified to yield the slope and intercept of a Lineweaver-Burk plot which is uncomplicated by unstirred layers. These extrapolations were found to have a regression coefficient = 1 when plotted as (intercept)$^{-1}$ or slope = $b_0+b_1b^{-(\text{shake})^2}$ where $b = 2.764$ for the slope plot and 6.626 for the (intercept)$^{-1}$ plot. From the values of b_0 one obtains a K_m of 0.41 and a V of 9.35 which should represent the true kinetic parameters for glucose influx into this tissue under the experimental conditions employed.

4. Values of the theoretical flux expected on a basis of unstirred-layer thickness which was calculated from the relation C_b (for $J = V/2$) $= K_m + 0.5\ V/K_d$ agreed with the experimental values of J in some instances but the 95 % confidence interval of the theoretical and experimental values did not overlap in many instances at low shaking rates and low concentrations of glucose.

5. A factor θ representing the error between the theoretical and experimental values was found to fit the relationship ln(theoretical J) $= -3.8 + 5.77\ (1/\theta)$ with a regression coefficient of 0.98 and was proposed to be due to one or more of the following parameters: (1) a villus tip to base gradient of transport (influx) activity; (2) a dependence of brush-border influx area on substrate concentration in the bulk incubation media; and (3) an end-product inhibition of the overall transport rate.

6. It is apparent from the data that the flux of glucose across the unstirred layer is ordinarily the rate-limiting step in the trans-brush-border transport of this sugar by hamster jejunum when less than saturating concentrations of glucose are used. At high shaking rates the contribution of the unstirred layer is reduced.

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INTRODUCTION

The presence of unstirred water layers at solid-liquid interfaces was demonstrated to be a real physical characteristic of such interfaces by direct microscopic examination in 1930 [1] and more recently [2]. Since it is virtually impossible to stir a solution so that complete mixing occurs right up to the interface [3], the presence of unstirred layers must be considered in studies of membrane permeation for all but the slowest of permeating substances [4]. In fact, considerable attention has been given to the effect of unstirred layers on water permeability across synthetic [5–8], plant [3], and animal [9, 10] membranes, and the results of these studies have been an important factor in the debate on the presence or absence of water filled pores in membranes and in our understanding of membrane-water-transport related processes.

In addition, attention has been given to the role that unstirred layers play in determining the availability of O_2 to tissues incubated in vitro (ref. 11 and Dugas, M. and Crane, R. K., unpublished) and in the process of membrane permeation by sugars across synthetic membranes [12] and cellular membranes of suspended cell preparations [13, 14], fatty acids across in vitro intestinal preparations [15, 16], and bile acids across in vitro intestine [17].

A recent in vivo study of the effect of perfusion rate on the kinetics of active sugar and amino acid absorption briefly deals with the question of unstirred layers but no evaluation of this parameter was performed [18]. While the present paper was being prepared, an additional report appeared in the literature [19] which demonstrated an in vitro effect of unstirred layers on the influx kinetics of several substrates into rat jejunum and ileum. This investigation approached the problem of unstirred layers from the standpoint of surface areas and utilized techniques of analysis which were dependent upon diffusion of substrates up to the mucosal surface acting as diffusion up to a flat surface. Later investigations [20] revealed that the assumption of diffusion up to a flat surface cannot be applied indiscriminately to the mucosal surface with its many villi and the validity of using the method employed by these investigators in obtaining measures of unstirred-layer thickness with respect to active-transport mechanisms is open to question. In addition, no attempt has been made at determining how closely the theoretically predicted influx values agreed with the experimental influx values nor at estimating a limiting value for the transport-kinetic constants when unstirred layers are absent. The present study reports such an attempt, and analysis of the results indicates that a substantial discrepancy exists between the theoretically predicted influx and the experimentally determined influx, particularly at low concentrations of substrate and large unstirred-layer dimensions. The discrepancy can be explained in terms of one of several of the following mechanisms: (1) a villus tip to base gradient of transport (influx) activity; (2) a dependence of mucosal brush-border influx area on substrate concentration in the bulk incubation media; and (3) an end-product inhibition of the overall transport rate. Although the former two possibilities are discussed, it is not possible at the present time to distinguish between any of these factors.

METHODS AND MATERIALS

Theoretical basis

Although an analysis of the time course of diffusion and streaming potentials

has been used successfully in the past to obtain measurements of unstirred-layer thickness [9, 10], the equations which are pertinent to this technique were derived on the basis that a flat surface was being considered [21]. Since the presence of villi on the intestine make the relationship between unstirred-layer thickness and the half-time for build-up of a diffusion potential dubious, another technique of obtaining a measure of intestinal functional unstirred layers was employed.

The basic equations for this approach have been described previously [22, 23], but require slight modifications before they can be applied.

The steady-state diffusional flux of glucose (j_G) across the unstirred layer is given by Fick's law:

$$j_G = K(C_b - C_i) \tag{1}$$

where C_b = concentration of glucose in the bulk incubation media, C_i = interfacial concentration of glucose at the membrane, and K = mass-transfer coefficient = D/δ where D = free diffusion coefficient of glucose at 37 °C and δ = length of the functional unstirred layer across which the assumed linear concentration gradient of glucose exists [3]. The unidirectional influx of glucose across the brush-border membrane is given by:

$$j_G = \frac{VC_i}{K_m + C_i} \tag{2}$$

where V is the maximum velocity of transport per unit influx area and K_m is the Michaelis constant for glucose influx. However, since influx data are expressed in terms of the serosal area, Eqn 2 becomes:

$$j_{G_s} = \frac{V_s \theta C_i}{K_m + C_i} = J \tag{3}$$

where j_{G_s} denotes flux per unit measured serosal area and is defined as J, V_s the maximal velocity of glucose influx in terms of unit serosal area under our conditions, and θ = a parameter representing the dependence of V_s on: (1) variations in available brush-border influx area; (2) a gradient of influx activity from villus tip to base; and (3) end-product inhibition of influx activity resulting from cellular accumulation of substrate. θ may vary with variations in the aforementioned parameters, or may be invarient and equal to unity. Since the diffusional flux is expressed in terms of the diffusional area, multiplication of Eqn 1 by $(A_d)/(A_s)$ where A_d = the diffusional area and A_s = the available serosal flux area yields:

$$C_i = C_b - \frac{J}{K_d} \tag{4}$$

where $K_d = K(A_d/A_s)$. Substituting for C_i in Eqn 3 yields:

$$J = \frac{V_s \theta \left(C_b - \frac{J}{K_d}\right)}{K_m + \left(C_b - \frac{J}{K_d}\right)} \tag{5}$$

which can be rearranged to yield:

$$\frac{1}{J} = \frac{K_m C_b}{V_s \theta \left(C_b - \frac{J}{K_d}\right)} \frac{1}{C_b} + \frac{1}{V_s \theta} \tag{6}$$

As has been pointed out previously [20], a plot of $1/J$ versus $1/C_b$ will yield a somewhat straight line which gives a false value for $V\theta$ and K_m when obtained by the conventional Lineweaver-Burke technique. However, it should be clear that as the δ decreases, $C_b - J/K_d$ will approach C_b and when $\delta = 0$ these terms will cancel in Eqn 6. In a like fashion, the extrapolated value of $V_s\theta$ will approach the limiting value of V_s since at infinite C_b and $\delta = 0$, θ will equal unity for the influx conditions employed. Thus, a plot of the slope and the reciprocal of the intercept obtained at different perturbation rates versus some function of the perturbation rate should yield a straight line giving an estimate of the limiting values of the slope and intercept at infinite perturbation rate. From these values, the estimated limiting value of V_s and K_m can be obtained* and thus the value of K_d can be obtained from the previously described [22, 23] relation

$$C_b \left(\text{for } J = \frac{V_s}{2}\right) = K_m - \frac{0.5 V_s}{K_d} \tag{7}$$

From these values of C_b, V_s, K_m and K_d the flux of glucose (J') that one should have obtained with $\theta = 1$ and K_d as described can be calculated [22] from:

$$J' = K_d \left[0.5 \left(K_m + C_b + \frac{V_s}{K_d}\right) - \sqrt{0.25 \left(K_m - C_b + \frac{V_s}{K_d}\right)^2 + C_b K_m}\right] \tag{8}$$

The value of J' obtained from Eqn 8 divided into the experimental values of J will yield the value that θ would have had to be to yield the experimental flux since:

$$J' = \frac{V_s C_i}{K_m + C_i} \tag{9}$$

$$\therefore J = J'\theta \tag{10}$$

Incubation technique

8–12-week-old male Syrian Golden Hamsters which were allowed free access to food and water were used in these studies. After sacrificing the animal by cervical crushing, jejunal tissue located 6–12 inches from the pyloric sphincter was removed from the animal, cleaned with the buffer being used, and everted. Since measurements of the unidirectional influx of D-[^{3}H]glucose across the brush border membrane of the epithelial cells were desired, complications [24] which could arise from the movement of sugar from the serosal surface into the tissue made it necessary to use small sealed flat segments of everted jejunum which had only the mucosal surface exposed to the test molecule. These sacs were prepared by tying off small lengths of everted jejunum at both ends. The lumen of the segments was empty since this was found to

* The "limiting" values of V_s and K_m so obtained would equal "true" values were the conditions employed ideal for the influx process. Because the ideal conditions cannot be explicitly stated, the term "limiting" is used to describe the values of V_s and K_m which would prevail when no unstirred layers are present.

facilitate measuring the length of each sac. Four such sacs were prepared from each animal; and, although the sacs were prepared from a restricted region of the jejunum, randomization of whatever animal or tissue variations might occur was performed by placing each of the four sacs from any given animal into separate beakers containing 10 ml of sugar-free buffer being gassed with 95 % O_2/5 % CO_2 at 25 °C. This usually took about 4 min from time of sacrifice. Krebs-Ringer bicarbonate buffer [25] was used throughout. Sacs prepared from additional animals were distributed into similar beakers until each beaker held three such sacs from different regions of the jejunum of different animals. The sacs were then transferred to 125-ml Ehrlenmeyer flasks containing 50 ml of pregassed buffer at 37 °C plus glucose at a specified concentration plus D-[^{14}C]mannitol which was used as a marker for adhering volume [26]. The flasks were then shaken for 40 s (this time of incubation will be justified later) at a variable but specified rate in a shaking incubator which had an excursion of 1½ inches. The jejunal segments were recovered by decanting the flasks contents through highly porous cloth. The segments were then blotted on Whatman No. 1 filter paper to remove excess adsorbed liquid and measured separately for length. The tissue between the two ligatures was obtained, weighed separately, and placed in separate grinding tubes containing 1 ml of 0.19 M $ZnSO_4$. After homogenization, 1 ml of 0.15 M $Ba(OH)_2$ was added to each tube and the deproteinized supernatant obtained as previously described [26]. 1 ml of the deproteinized solution was then added to 10 ml of the scintillation fluid described by Eichholz et al. [27], and assayed for the ^{14}C and ^{3}H cpm according to the discriminator ratio method of Okita et al. [28]. After correction for D-[^{14}C]mannitol adsorption [26], the influx of D-glucose was calculated from the equation:

$$\frac{\mu\text{mol D-glucose/segment}}{2 \cdot L \cdot W \cdot 0.0111} = \text{influx of D-glucose in } \mu\text{mol/cm}^2 \text{ serosal surface per h.}$$

where L and W are the length and width of the segments and 0.0111 is a conversion factor from 40 s to 1 h.

Since the width of the flat jejunal tissue varied only slightly from animal to animal in any given experiment, this value was recorded for each animal and the average of the values used as the width of the sacs for any given experiment.

Non-labeled D-glucose and all salts were obtained from Matheson, Coleman and Bell, East Rutherford, N.J., while D-[1-^{3}H]glucose (3 μCi/mmol) and D-[1-^{14}C]-mannitol (45–55 Ci/mol) were obtained from Calbiochem, LaJolla, Calif.

RESULTS

Fig. 1 shows that the uptake of glucose by flat segments of hamster jejunum from solutions containing either 0.25 or 5 mM D-glucose was linear for at least 60 s, thus indicating that a significant backflux of glucose had not occurred at this time [29]. The fact that uptake values at times shorter than 30 s were higher than the line drawn through the origin may indicate that a significant amount of uptake was occurring after the experimental period but before the tissue was blotted. Thus, an incubation time of 40 s was chosen for further studies as a technically manageable time which represented unidirectional influx of substrate across the brush-border membrane. The finding that the rate of uptake of glucose was reasonably constant during the

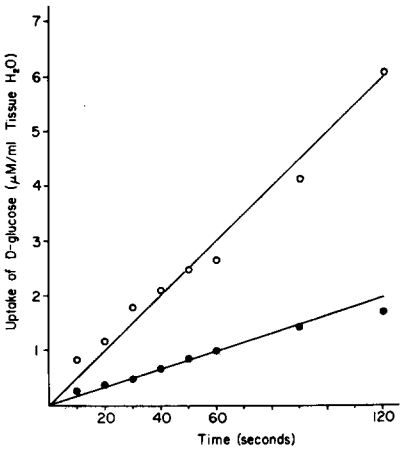

Fig. 1. Uptake of D-glucose by hamster jejunal flat segments from Krebs-Ringer's bicarbonate buffer containing 5 mM (open circles) or 0.25 mM (closed circles) D-glucose. Shaking rate = 200 cycles/min, abscissa = time in s.

period of observation can be taken as an indication that a steady-state flux of substrate across the unstirred layer must be occurring under these conditions. At slower shaking rates than the one used in this study, the length of the unstirred layers would be larger [2] and a consequent increase in the time for attainment of steady-state flux across such layers could be expected. However, similar studies performed at the lowest shaking rate used in the present studies indicate that uptake is linear during at least the first 40 s of incubation (Ramaswamy, K. and Crane, R. K., unpublished observations). There is, therefore, ample reason for believing that a steady-state rate of flux of substrate across the unstirred layers was reached by the fortieth second of incubation at the slowest rate of shaking (40 cycles/min) used in these studies.

Kinetics of D-*glucose influx*

Fig. 2 shows least-squares regression analysis as Lineweaver-Burk plots of the

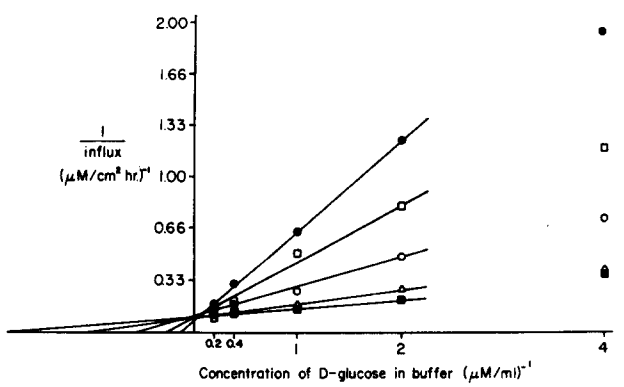

Fig. 2. Lineweaver-Burk plot of D-glucose 40-s influx into segments of hamster jejunum. Lines are least-squares regression lines of data at all but 0.25 mM D-glucose. Shaking rate: (●) 40; (□) 80; (○) 120; (△) 160; (■) 200 cycles/min.

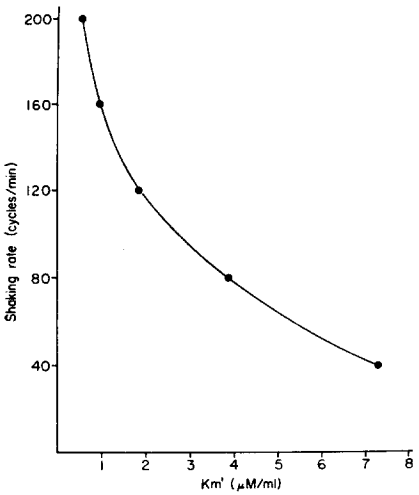

Fig. 3. Plot of apparent Michaelis constant (K_m') obtained from Lineweaver-Burk plots shown in Fig. 2 versus shaking rate.

TABLE I

Apparent kinetic constants (K_m' and V_s') obtained from Lineweaver-Burk plots of the data at various shaking rates, and 95 % confidence intervals for extrapolated limiting K_m, V_s', and mass-transfer coefficient K_d.

Shaking rate (cycles/min)	K_m' (μmol/cm^3)	V_s' (μmol/cm^2 · h)	K_m (μmol/cm^3)	V_s (μmol/cm^2 · h)	K_d (cm/h)	K_d when $J = V/2$
40	7.42	12.81	0.35–0.47	9.13–9.57	1.17–1.26	1.21
80	3.95	10.71			1.69–1.85	1.77
120	1.87	9.75			3.34–3.83	3.57
160	0.93	10.17			10.5–15.1	12.4
200	0.5	9.33			31.3–173	53.2

40-s influx into flat segments of hamster jejunum at different rates of shaking the incubation flasks. The apparent kinetic constants V_s' and K_m' are calculated in the usual manner. As can be seen from Figs 2 and 3 and Table I, as the shaking rate increases, the K_m' and (with the exception of the value at 160 cycles/min) V_s' decrease. As pointed out previously [22, 23], the results are the expected consequence of a decrease in the length of the unstirred layer with an increase in shaking rate.

It should be pointed out that the kinetic constants shown in Figs 2 and 3 and Table I were calculated on a basis which did not include the influx values obtained when the concentration of D-glucose in the incubation media was 0.25 mM. These influx values were usually higher than would have been expected from the least-squares regression line calculated from the influx values at higher concentrations of D-glucose, but are an expected consequence of θ being greater than unity at lower concentrations of D-glucose in the incubation media. The mean $\pm$S.E. values of the influx at each C_b and shaking rate are given in Table II.

TABLE II

INFLUX OF D-GLUCOSE (J)

Values are given as mean flux ±S.E. (μmol/cm^2 · h). Number of observations in parentheses.

C_b	Influx of D-glucose				
Shaking rate (cycles/min)	40	80	120	160	200
0.25	0.51±0.04 (6)	0.84±0.08 (3)	1.38±0.23 (8)	2.43±0.35 (6)	2.58±0.32 (7)
0.5	0.81±0.08 (3)	1.23±0.14 (6)	2.01±0.41 (6)	3.54±0.54 (3)	4.65±0.33 (7)
1	1.53±0.72 (3)	1.95±0.22 (6)	3.78±0.43 (8)	5.52±1.47 (3)	6.36±0.80 (7)
2.5	3.12±1.20 (3)	4.92±0.45 (6)	5.13±0.71 (9)	6.42±1.45 (3)	7.50±1.10 (6)
5	5.43±1.35 (6)	5.82±1.10 (6)	6.78±0.80 (12)	9.75±2.09 (3)	8.64±1.92 (6)

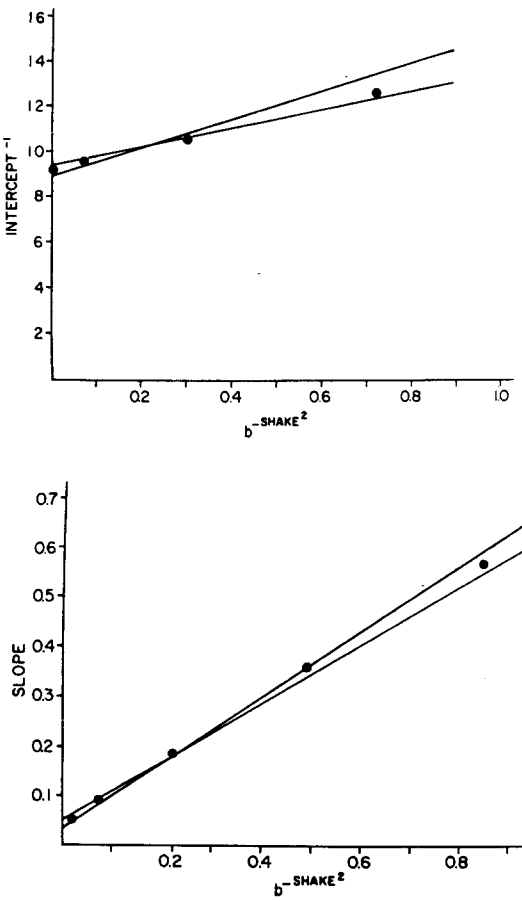

Fig. 4. Plot showing 95 % confidence intervals of (intercept)$^{-1}$ (A) and slope (B) obtained from Lineweaver-Burk plots of Fig. 2 versus $b^{-(\text{shaking rate})2}$ where $b = 6.626$ (A) and 2.764 (B). Regression coefficient = 0.9993 (A) and 1.000 (B).

Fig. 4 shows a plot of the reciprocal of the intercept (V_s') and the slope obtained from Lineweaver-Burk plots of the data against $b^{-(\text{shake})^2}$ where shake = shaking rate in hundreds/min and $b = 2.764$ for the slope plot and 6.626 for the V_s' plot. The values for b were obtained by iteration to yield the best fit of the data to a least-squares regression analysis of the equation

$$\text{slope or } V_s' = b_0 + b_1 b^{-(\text{shake})^2} \tag{11}$$

and yielded correlation coefficients of 1.000 for the slope plot and 0.9993 for the V_s' plot. The V_s' plot did not include the value for V_s' obtained for the study at a shaking rate of 160 cycles/min which lies outside the 95 % confidence intervals [30] shown in Fig. 4a.

Obviously, the value obtained for b_0 describes the estimated limiting V_s and slope of a plot of $1/J$ versus $1/C_i$ and represents the value of these parameters when there is no unstirred layer present. The limiting K_m can be obtained by multiplying the V_s' value of b_0 times the slope value of b_0. Values so obtained were $V_s = 9.35$ and K_m 0.41 and the 95 % confidence intervals for these values are shown in Table I along with the values and confidence intervals of K_d calculated from Eqn 7. Plots of $\delta = (K_d/D)^{-1}$ (obtained by setting $D = 0.030564$ cm/h [31] and solving from K_d) versus shaking rate or the C_b at which $J = V_s/2$ versus δ are shown in Fig. 5. The linearity of the plot of C_b versus δ is as it should be as predicted by Eqn 7. Caution is urged in placing too much emphasis on the values of δ since these were calculated without any knowledge of (A_d/A_s). The values of δ only represent the thickness of the unstirred layer with respect to flux expressed on a serosal area basis.

Using the true values of K_m, V_s, and K_d obtained by assuming θ = unity in Eqn 7, the values of J' were calculated from Eqn 8 and these are shown in Fig. 6 along with the experimental values plotted as reciprocals versus reciprocal C_b.

The obvious discrepancies between the theoretical curves and the experimental

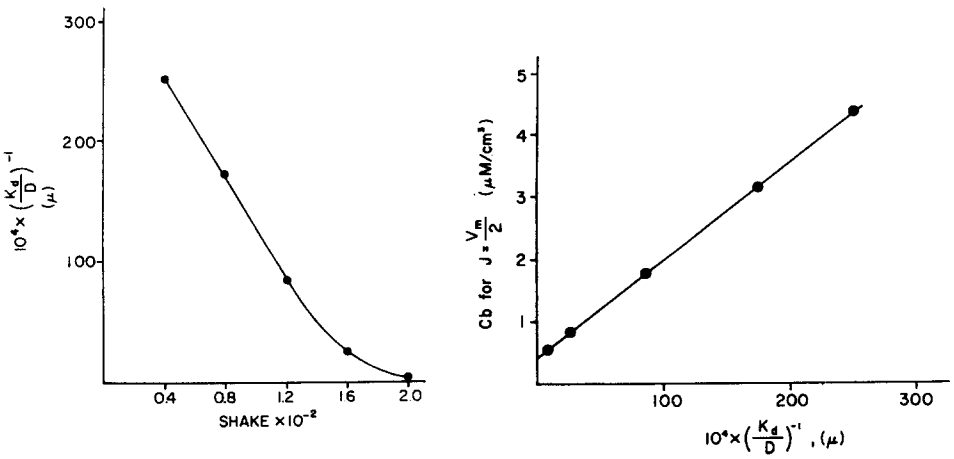

Fig. 5. (A) Plot of functional unstirred-layer thickness = $(K_d/D)^{-1}$ (expressed in terms of serosal flux area) versus shaking rate in hundreds/min. (B) Plot of concentration of D-glucose in bulk incubation media needed to cause influx at various shaking rates to be equal to $V/2$ versus unstirred-layer thickness when expressed on a basis of serosal flux area. Note that intercept = extrapolated value for limiting $K_m = 0.41$.

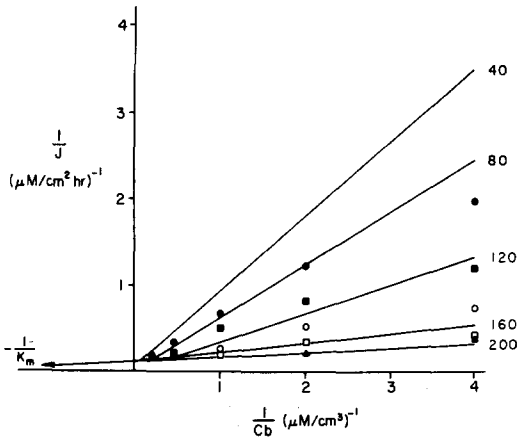

Fig. 6. Plot of (influx)$^{-1} = 1/J$ versus (concentration in bulk incubation media)$^{-1} = 1/C_b$ at various shaking rates. Solid lines are based on values of J as calculated from Eqn 8 in text. Experimental values of J were at shaking rates: (●) 40; (■) 80; (○) 120; (□) 160; (▲) 200 cycles/min.

TABLE III

95 % CONFIDENCE INTERVAL OF J AND J'

C_b Shaking rate (cycles/min)	95 % Confidence interval of J and J'											
	40				80				120			
	J		J'		J		J'		J		J'	
	max	min	max	min	max	min	max	min	max	min	max	min
0.5	0.84	0.78	0.59	0.56	1.59	1.03	0.84	0.79	2.46	1.70	1.56	1.45
1	1.58	1.48	1.18	1.11	2.43	1.62	1.66	1.56	4.60	3.16	3.00	2.81
2.5	3.40	2.88	2.89	2.74	12.48	3.06	3.99	3.79	7.79	4.03	6.21	6.04
5	6.47	4.63	5.50	5.27	32.36	3.18	6.94	6.75	12.65	4.60	8.21	8.03

curves is pointed out in Table III where the 95 % confidence intervals of the influx values are shown for the first three shaking rates employed. Inspection of Fig. 6 and Table III reveals that in general, the confidence intervals do not overlap and that J' is an underestimate of J at the lower concentrations employed. Thus, it would appear that while the concept of unstirred layers accounts for some of the experimental flux values, an additional factor may be involved in determining the experimental values. Calculation of θ from Eqn 10 reveals that $1/\theta$ varies between 0.5 and 1 in 21 out of 25 instances and exceeds 1 by less than 0.2 in the remainder of the cases. By omitting the values of $1/\theta$ which exceeds 1, and the spuriously low value obtained at a shaking rate of 160 cycles/min for $C_b = 5$ mM, one can obtain a fairly linear relationship ($r = 0.98$) by plotting the $\ln \bar{J}'$ versus $(\bar{1}/\theta)$ where $\bar{J}'$ = average J' within the average interval $(\bar{1}/\theta) = 0.1$. This is shown in Fig. 7 and as can be seen, the relationship is not perfect. However, when the relationship which describes the plot of Fig. 7,

$$\ln \bar{J}' = -3.8 + 5.77 \left(\frac{\bar{1}}{\theta}\right),$$

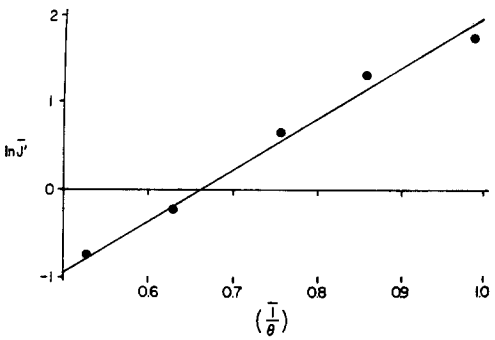

Fig. 7. Plot showing least-squares regression ($r = 0.98$) of ln (average theoretical influx) per 0.1 interval of $1/\theta$ versus average value of $1/\theta$ per 0.1 interval of $1/\theta$. See text for details of calculation.

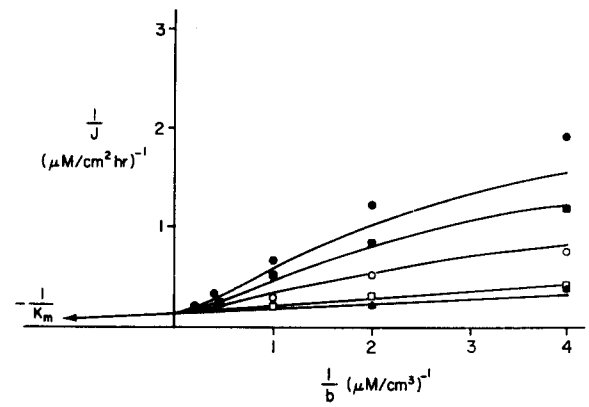

Fig. 8. Plot of reciprocal corrected theoretical influx (solid lines) and experimental influx (symbols) $= 1/J$ of D-glucose versus reciprocal concentration of glucose in bulk incubation media $= 1/C_b$. Symbols are the same as in Fig. 6. See text for details of calculation of corrected theoretical values of influx.

TABLE IV

TISSUE TO MEDIUM CONCENTRATION RATIOS OF D-GLUCOSE ON THE BASIS OF C_b OR C_i

C_b Shaking rate (cycles/min)	Tissue to medium concentration ratios									
	40		80		120		160		200	
T/M when $M = C_b$	C_b	C_i	C_b	C_i	C_b	C_i	C_b	C_i	C_b	C_i
0.25	0.8	19.4	1.1	14.7	1.7	11.2	3.0	7.4	3.8	4.9
0.5	0.4	7.6	0.8	9.0	1.2	7.5	3.0	6.6	3.0	3.6
1	0.4	7.4	0.6	7.2	1.1	5.6	2.0	3.6	1.9	2.1
2.5	0.3	4.7	0.6	5.1	0.7	2.1	0.9	1.2	1.0	1.0
5	0.3	2.7	0.4	1.6	0.4	0.8	0.7	0.8	0.5	0.5

is used to calculate values of θ at J', and J is calculated from these values and Eqn 10, the curves obtained more adequately describe the experimental values of J. This is shown in Fig. 8.

While there could have been several factors responsible for the discrepancy between J' and J, it cannot be argued that high tissue to medium ratios of glucose was a causitive factor. These ratios are shown in Table IV for medium $= C_b$ or C_i which was calculated from $C_i = C_b - J'/K_d$. Obviously, the higher T/M ratios when $M = C_i$ should have decreased cell accumulation and this would have resulted in experimental values of J which were less rather than greater than the values of J' if this were a factor.

CONCLUSIONS

Although the effects of substrate concentration in the bulk incubation media and shaking rate on the effective influx area were not investigated in this study, evidence is available in the literature which suggests that an increase in influx area with an increase in C_b and/or a decrease in δ is a reasonable likelihood. High-resolution autoradiographic studies of [^{3}H]galactose accumulation by hamster intestine [32] reveals that after 1 min of incubation of in vitro rings of jejunum in 1 mM galactose containing media there is a definite gradient of epithelial cell accumulation of galactose, being greatest at the villus tip and decreasing with distance towards the base of the villi. After 10 min of incubation the gradient of epithelial cell accumulation was less obvious but still detectable. If one considers the fact that a shaking rate of 100 cycles/min was used by these investigators then it would seem reasonable to assume that the increase in C_b of the present studies would have the same effects as an increase in time of exposure to the probe molecule in the autoradiographic studies. In a later investigation [33] of biopsy material from human jejunum these investigators reported that accumulation of [^{3}H]galactose was restricted to the tip of the villi and that phloridzin inhibition was less than had been previously reported [34] for any given concentration of phloridzin. Although the shaking rate employed in the later study was not stated, it is tempting to speculate that a less vigorous shaking rate was employed on the biopsied human jejunum than was employed on the hamster intestine. This speculation is supported by the finding that dilatation of the lateral intercellular spaces occurred only between villus tip epithelial cells in the study of human biopsies [33]. Such a dilatation pattern had been previously reported for rat ileum perfused in vivo [35] under what amounts to mild perturbation conditions. Thus, an increase in absorptive flux area with an increase in perturbation rate is not an unreasonable assumption.

It would appear, therefore, that there is ample justification for believing that the influx area of a villus tissue may increase with increasing C_b and decreasing δ. It should be pointed out, however, that such an increase is likely to be a time-dependent function, and that at an infinitely long time of exposure, the influx area should be the maximum amount available. Even with the increase in influx area as described, a second assumption of a gradient in transport activity along the villus length would have to be made in order to justify the results of the preceding section. This latter assumption is not without precedent in that a villus tip to base decreasing gradient of intestinal disaccharidase activity has been shown to exist in the rat intestine by a technique of sequentially cutting 10 μm thick segments of intestinal cells and making

the appropriate enzymatic assay on the pieces [36]. There were no complicating non-uniform unstirred layers by such a technique. Thus, the apparently greater transport activity associated with a smaller influx area at lower C_b would seem to indicate a villus tip to base decreasing gradient of glucose-influx activity.

Since the present studies were performed in the presence of a saturating concentration of NaCl [37] (145 mM), there should have been no complications arising from Na^+ dependence of glucose influx at the various shaking rates employed. However, when less than saturating concentrations of Na^+ are employed for studies of Na^+-dependent influx kinetics, caution must be exercised in drawing too sweeping a conclusion from the data obtained. A case in particular is that of going from a Na^+-containing to an assumed Na^+-free solution bathing the intestinal mucosa in vivo. Under these conditions, the backflux of Na^+ from the blood through the Na^+-enriched lateral intercellular spaces into the mucosally located unstirred layer might be expected to result in a significant Na^+ concentration in the unstirred region. The presence of the mucosally located unstirred layers also make it possible for the transport of NaCl, or other substrates, across the mucosa followed by the necessary water shifts to very effectively stir the unstirred region thereby leading to a higher C_i and consequently a greater apparent coupled transport of organic substrate.

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