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SPECIFICITY OF D-GLUCOSE TRANSPORT BY THE APICAL MEMBRANE OF *NEREIS DIVERSICOLOR* EPIDERMIS *

STEN ALBRECHTSEN and JØRGEN GOMME **

August Krogh Institute, Zoophysiological Laboratory A, University of Copenhagen, 13 Universitetsparken, DK-2100 Copenhagen Ø (Denmark)

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(1) The specificity of D-[6-^{3}H]glucose influx by a Na^+-dependent and phlorizin-sensitive transport system in the apical epidermal membrane of the polychaete worm, *Nereis diversicolor*, was investigated in vivo. (2) The inhibitory effect of eleven D-glucose analogues on D-[6-^{3}H]glucose influx from a 5 μM external concentration was recorded. The inhibitors (each tested at 5, 50, 500 and 5000 μM) were selected to illuminate the configurational requirements for interaction with the D-glucose transport system. (3) The following compounds were found to be significant inhibitors: methyl α-D-glucoside, methyl β-D-glucoside, D-galactose, 3-*O*-methyl-D-glucose, 2-deoxy-D-glucose, D-xylose, *myo*-inositol, β-D-fructose; the effect was graded according to inhibitor concentration. L-Glucose also inhibited D-glucose influx but to the same extent at all four concentrations tested, suggesting transport site heterogeneity. D-Mannose and L-arabinose did not inhibit influx. (4) The most potent inhibitor, methyl-α-D-glucoside, was itself a substrate, and its transport was inhibited by phlorizin and D-glucose, as well as by substitution of Na^+ in the incubation medium with Li^+ or choline$^+$. (5) We conclude that the specificity of the Na^+-dependent D-glucose transporter in the apical epidermal membrane of *Nereis* is similar to that in the apical membrane of vertebrate small intestinal and proximal tubular epithelium, and in the tapeworm integument.

Introduction

The epidermis of the marine polychaete worm, *Nereis diversicolor*, accumulates D-glucose across the apical membrane, even from such low concentrations as found in the natural habitat [1]. The small organic compounds absorbed via the external surface of 'soft-bodied' marine invertebrates generally are believed to supplement the nutrient uptake through the alimentary tract [2–8]. It is difficult, however, to assess the energetic significance of integumentary nutrient uptake, mainly because convincing evidence for a trans-epidermal net uptake of the compounds is lacking [9–11]. Alternatively, accumulating transport systems in the apical epidermal membrane may, provided a diffusion-restraining cuticle is present, serve to decrease the effective integumentary permeability to diffusional loss of substrates from the animal to the environment [12].

D-Glucose influx across the apical membrane of *Nereis* epidermis is Na^+-dependent; it is inhibited by harmaline and phlorizin, whereas phloretin is less potent; and cytochalasin B has no effect [1]. These characteristics are similar to D-glucose

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** To whom correspondence should be addressed.

Abbreviation: 65% ASW, artificial sea water diluted to 65% of the salinity of ocean water [1].

transport in the apical membrane of certain vertebrate epithelia, such as small intestine and proximal kidney tubule * [13–25]. To further evaluate the extent of similarity between D-glucose transport in these seemingly diverse epithelia, we studied the inhibitory effect of a range of D-glucose analogues on epidermal D-glucose influx in *Nereis*.

Methodological limitations preclude a conventional kinetic analysis of D-glucose transport in *Nereis* epidermis. In order to avoid a troublesome batch-to-batch and within-batch variability of D-glucose influx [1], the inhibition experiments were designed allowing each animal to serve as its own control. D-Glucose influx was estimated indirectly, and the D-glucose analogues were ranked according to their inhibitory potency.

In the course of this investigation it became apparent that methyl α-D-glucoside was the most efficient inhibitor. Previous studies, based on the initial-rate method, showed that this glucoside also is transported by *Nereis* epidermis [1]. Hence the investigations were extended to test the reciprocal situation, i.e. the possible inhibitory effect of D-glucose and phlorizin on methyl α-D-glucoside influx.

The data were presented in a preliminary form at the 14th FEBS Meeting in Edinburgh, April, 1981.

Methods

Specimens of *Nereis diversicolor* Müller (150–700 mg) were obtained and kept in the laboratory as previously described [1,11]. Animals were acclimated for 5–15 days to 65% ASW, and to a temperature of 15°C. The same salinity and temperature were used for all incubation media.

Animals of known wet weight were preincubated for 30–60 min in unlabelled 65% ASW and then transferred to individual beakers with 5 ml medium containing 5 μM D-[6-^{3}H]glucose (spec. act. approx. 5.6 MBq · μmol^{-1}) and approximately 1 μM D-[1-^{14}C]mannitol (spec. act. 2.3 MBq · μmol^{-1}) (first experimental period). The latter compound served as a volume marker (see below). The incubation medium was stirred by air-bubbling. After 120 min incubation, each animal was quickly transferred to 5 ml fresh medium, containing the same initial concentrations of D-[6-^{3}H]glucose and D-[1-^{14}C]mannitol, but now supplemented with 0–5000 μM of an unlabeled presumptive inhibitor (second experimental period). Each treatment was repeated with two animals. In all double-label experiments, 10 μl medium aliquots were drawn at equal intervals throughout each incubation period for the determination of ^{3}H- and ^{14}C-activity.

All samples were pipetted onto small pieces of lens paper, which were left at room temperature in empty liquid scintillation counting vials until complete dryness. Then, 50 μl distilled water, and subsequently 10 ml scintillation fluid (300 ml 2-ethoxyethanol, 150 g naphthalene and 7 g 2,5-diphenyloxazole in 1000 ml toluene) were added, and the ^{3}H- and ^{14}C-activity in the resulting homogeneous system [26] were determined in triplicate by liquid scintillation counting.

Exploiting the fact that D-mannitol is not taken up by *Nereis* [1], the ^{14}C-activity in the samples should deviate only in relation to the volume drawn. The ^{3}H-values were therefore recalculated to a basis of constant ^{14}C-activity, ensuring that the only source of analytical error remaining was caused by the Poisson counting statistics ([28]; cf. Bamford, D.R. and Gomme, J., unpublished data).

Since non-volatile degradation products of D-[6-^{3}H]glucose are not released to the medium [1], the corrected ^{3}H-activity of the dry aliquots was used to calculate D-[6-^{3}H]glucose concentration (c); the specific activity at $t = 0$ served as the basis.

The rate of decrease of D-[6-^{3}H]glucose in the incubation medium (the slope of a c-t curve) was used to estimate D-glucose influx. In some cases, influx values were scaled to the size of a standard (300 mg) animal, using Eqn. 2 in Ref. 1.

The fractional inhibition of D-glucose influx was expressed as:

$$I = 1 - \frac{J_i}{J - \Delta} \qquad (1)$$

in which J is the (uninhibited) D-glucose influx in the first period, J_i D-glucose influx into the same

* There is ambiguity about the action of cytochalasin B on D-glucose transport by the luminal membrane in mammalian intestine, cf. Discussion in Ref. 24.

animal during the second period, and Δ the depression of D-glucose influx in the second period not caused by substrate inhibition (see Results). J and J_i, as well as the standard error of estimate, $s(J)$ and $s(J_i)$, were determined in each experiment by conventional linear regression analysis of c vs. t. Δ and $s(\Delta)$ are global values obtained in separate experiments ($N = 23$), conducted without inhibitor during the second incubation period. Assuming normal distributions and independence of J, J_i, and Δ, the propagated standard deviation [27] of I was estimated as

$$s(I) = \frac{J_i}{J - \Delta}\left[\frac{s^2(J_i)}{J_i^2} + \frac{s^2(J) + s^2(\Delta)}{(J - \Delta)^2}\right]^{1/2} \quad (2)$$

The table of normal values was used to find the cases of I being significantly different from zero.

Influx of methyl α-D-[U-^{14}C]glucoside was estimated by the initial-rate method previously described [1].

Labeled compounds were purchased from the Amersham International, U.K. All other chemicals used were reagent grade. The purity of labelled as well as unlabelled sugars were checked by thin-layer chromatography, using the solvent system isopropanol/*n*-butanol/redistilled H_2O (5 : 3 : 2, v/v).

Results

The concentration of non-volatile tritiated material (D-glucose) in the incubation medium was a linear function of time over a period of 120 min (Fig. 1). Presumably, the uptake rate (dc/dt) decreases with decreasing concentration (since $c < K_t$). One might hence expect a non-linear relationship between c and t. In practice, however, such a phenomenon was not observed because the concentration changes (Δc) in the course of the experiments were small. Linearity was confirmed by visual inspection, and the average rate of removal of non-volatile labeled material was estimated from the slope. This parameter, in turn, was used to calculate D-glucose influx.

Variability of D-glucose influx is a major concern in studies with *Nereis*. Even after having used the ^{14}C-counting rate of medium samples to correct for methodological errors, the scatter around the c-t curve could not be accounted for by Poisson counting statistics alone. Thus some of the short-term fluctuations in D-glucose influx must have been caused by biological factors. However, $s(J)$ was sufficiently low to allow detection of non-zero influxes down to 10% of the control level.

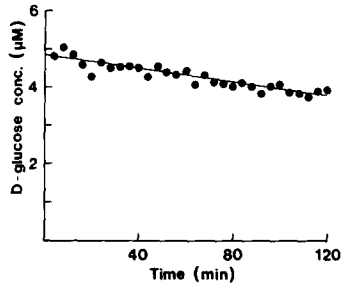

Fig. 1. D-Glucose concentration in the medium as a function of incubation time. The initial concentration of D-[6-^{3}H]glucose was 5.0 μM. Animal weight: 456 mg. Medium volume: 5 ml. D-Glucose concentration at $t > 0$ was determined from the concentration of non-volatile ^{3}H-radioactivity in the medium as compared to the initial radioactivity. The slope of the line is -0.0090 ± 0.0007 $\mu M \cdot min^{-1}$, which corresponds to an influx of 99 ± 8 $fmol \cdot mg^{-1} \cdot min^{-1}$.

Without added inhibitor (first incubation period), D-glucose influx was 226 ± 86 $fmol \cdot mg^{-1} \cdot min^{-1}$ (mean $\pm$ S.D., $n = 112$, values scaled to the size of a 300 mg animal). This result is about twice the similarly scaled apical D-glucose influx determined by the initial-rate method under otherwise identical conditions [1]. For the present results, the coefficient of variation of 38% illustrates the considerable animal-to-animal variability. Even then, the spread of values is less than anticipated from initial-rate experiments [1], probably because the uptake rate is averaged over a longer period (120 min as compared to 3–4 min).

In 23 of the experiments mentioned in the preceeding paragraph, inhibitor concentration in the second period was 0 μM. Theoretically, influx during the first and second period then should be identical. However, a paired t-test demonstrated a significant ($P < 0.001$) depression of the influx during the second period, the percentage reduction amounting to $9.1 \pm 0.6\%$. Thus, apart from fluctuations of D-glucose influx within the scale of a few minutes, a slower, gradual depression occurs which must be corrected for; hence the introduc-

TABLE I

THE INHIBITORY EFFECT OF ELEVEN MONOSACCHARIDES ON D-GLUCOSE INFLUX

D-Glucose influx as per cent of the uninhibited control, 100 $(1 - I)$ %. The initial concentration of radiolabelled D-glucose was 5.0 μM in all cases. Inhibitor concentrations were 5, 50, 500 and 5000 μM. Inhibition was ascertained by comparing D-glucose influx in the second period in the presence of inhibitor (J_i) with D-glucose influx in the first period, taking into account the inevitable influx depression not resulting from inhibition per se ($J - \Delta$). For each inhibitor concentration, the results of two experiments are shown. [a] denotes cases of both duplicates causing a significant inhibition at the 5% level.

Inhibitor	D-Glucose influx $(100\,(1 - I) \pm s(I)$ %)			
Inhibitor concentration:	5 μM	50 μM	500 μM	5000 μM
Methyl α-D-glucoside	100.4 ± 6.8	53.7 ± 7.1 [a]	0.6 ± 7.7 [a]	0.1 ± 10.6 [a]
	86.0 ± 6.0	57.0 ± 12.7 [a]	7.0 ± 8.4 [a]	0.1 ± 8.0 [a]
Methyl β-D-glucoside	107.6 ± 14.6	58.5 ± 15.2	20.1 ± 7.0 [a]	5.6 ± 6.3 [a]
	88.6 ± 6.4	64.9 ± 24.8	28.1 ± 17.6 [a]	−2.1 ± 5.9 [a]
D-Galactose	86.5 ± 7.6	97.7 ± 9.7	56.7 ± 4.2 [a]	14.1 ± 13.3 [a]
	89.9 ± 9.0	94.2 ± 9.9	81.7 ± 5.7 [a]	26.3 ± 11.2 [a]
3-*O*-Methyl-D-glucose	92.7 ± 15.1	93.9 ± 9.4	60.3 ± 16.9 [a]	47.3 ± 2.8 [a]
	91.6 ± 19.3	88.2 ± 12.1	79.4 ± 9.3 [a]	38.7 ± 5.1 [a]
2-Deoxy-D-glucose	100.8 ± 7.4	102.3 ± 11.9	81.1 ± 23.3	27.4 ± 3.9 [a]
	95.4 ± 7.5	100.6 ± 6.7	105.8 ± 12.3	18.4 ± 2.8 [a]
D-Xylose	105.5 ± 7.7	88.4 ± 6.2	65.5 ± 8.1	42.2 ± 6.0 [a]
	91.9 ± 7.8	103.9 ± 6.9	93.0 ± 5.9	48.3 ± 3.9 [a]
myo-Inositol	89.7 ± 14.7	82.7 ± 5.8	102.2 ± 14.4	43.1 ± 6.7 [a]
	75.5 ± 16.8	91.4 ± 7.3	142.0 ± 81.4	41.9 ± 10.6 [a]
β-D-Fructose	79.8 ± 7.9	60.5 ± 17.8	94.4 ± 9.7	74.4 ± 7.5 [a]
	80.9 ± 11.4	88.1 ± 14.0	96.8 ± 10.3	81.4 ± 7.3 [a]
L-Arabinose	86.0 ± 6.7	66.6 ± 8.5 [a]	91.8 ± 6.0	107.7 ± 10.7
	92.5 ± 8.7	79.0 ± 7.6 [a]	82.6 ± 8.1	92.4 ± 7.1
D-Mannose	88.6 ± 6.9	90.1 ± 14.2	101.7 ± 5.2	69.4 ± 11.9
	75.7 ± 8.3	89.8 ± 6.7	95.1 ± 15.3	98.7 ± 21.6
L-Glucose	25.0 ± 6.7 [a]	29.9 ± 6.8 [a]	52.0 ± 4.7 [a]	18.0 ± 4.3 [a]
	17.5 ± 7.6 [a]	8.0 ± 6.6 [a]	19.4 ± 5.1 [a]	18.0 ± 5.4 [a]

tion of the quantity Δ in Eqns. 1 and 2.

Table I shows the inhibitory effect of eleven D-glucose analogues on D-glucose influx from a 5 μM solution. Each compound was tested in duplicate at four concentrations: 5, 50, 500 and 5000 μM. To be considered a significant inhibitor at a given concentration, a substance had to show significant (5% level) reduction of influx in both of the duplicate cases. Neglecting for the moment the case of L-glucose, the following analogues were found to be significant inhibitors when tested at 5000 μM: methyl α-D-glucoside, methyl β-D-glucoside, D-galactose, 3-*O*-methyl-D-glucose, 2-deoxy-D-glucose, D-xylose, *myo*-inositol and β-D-fructose. When the test concentration was reduced to 500 μM, methyl α-D-glucoside, methyl β-D-glucoside, D-galactose and 3-*O*-methyl-D-glucose showed a persistent inhibition. None of the above compounds showed significant inhibition at 50 and 5 μM, and thus the inhibitory effect was graded according to inhibitor concentration. In contrast, L-glucose produced approximately the same inhibition at all concentrations. The inhibition by L-arabinose at 50 μM probably is fortuitous, since this substance had no effect at 500 and 5000 μM.

Methyl α-D-glucoside was earlier shown by the

TABLE II

INHIBITION OF METHYL α-D-GLUCOSIDE INFLUX

(A) Influx determined by initial-rate method, as described earlier [1]. All animals were taken from the same batch to minimize variation. The concentration of methyl α-D-glucoside was 5 μM in all cases. Each entry represents the influx (±S.E. of estimate) based on four time periods and 32 animals. All values are scaled to the size of a 300 mg animal. (B) Influx determination as in A. All Na^+ in the incubation medium was substituted by the ion indicated.

J = initial rate in absence of inhibitor (A) or in presence of Na^+ (B). J_i = initial rate in presence of inhibitor (A) or with substituted ion (B). All units are fmol·mg^{-1}·min^{-1}.

	J	J_i
A. Inhibition		
Control	153.9±9.8	-
D-Glucose (500 μM)	-	9.2±4.3
Phlorizin (25 μM)	-	3.5±4.7
B. Ion substitution		
Control	158.3±12.7	-
Choline$^+$	-	22.0±7.2
Li^+	-	16.6±5.9

initial-rate method to be transported by *Nereis* epidermis [1]. Since this compound was the most efficient inhibitor of D-glucose influx, we decided to test the reciprocal effect of D-glucose on methyl α-D-glucoside influx. However, radiochromatography (unpublished) showed that the glucoside is not metabolized by *Nereis* epidermis, and this may render influx estimates by long-term experiments invalid: Trans-epidermal transport is very low [1,9,11], and the flow across the membrane probably cannot be sustained for a longer period due to the lack of a metabolic sink. Instead, initial-rate measurements were performed. The results (Table IIA) demonstrate that methyl α-D-glucoside influx is completely blocked by 500 μM D-glucose as well as by 25 μM phlorizin. A similar suppression is found, when Na^+ in the incubation medium is replaced by either choline$^+$ or Li^+ (Table IIB).

Discussion

Methodological considerations

In a previous study [1], D-glucose influx across the apical epidermal membrane was evaluated from the initial rate of accumulation of isotopically labelled D-glucose. This method was used here for the experiments of Table II. Unfortunately, the initial-rate method is destructive, while the large animal-to-animal variability makes it desirable to evaluate the effect of inhibitors by using each experimental animal as its own control. Hence, epidermal D-glucose influx was quantified as the decrease of non-volatile radioactivity in the ASW medium over two consecutive periods, serving as 'control' and 'treatment', respectively. At the start of each period, the medium contained 5 μM radioactive D-glucose; during the second one, a known concentration (0–5000 μM) of an unlabelled inhibitor was furthermore added. The validity of this approach derives from the observation [1] that only negligible amounts of non-volatile labelled products (D-glucose or its metabolites) return to the incubation medium from the epidermal cells or deeper tissues, mainly because of effective metabolic trapping [11]. To make sure that this also applies to the long incubations of the present paper, animals were incubated for 240 min in 65% ASW with 5 μM D-[6[^{3}H]glucose, rinsed for 1 min in unlabelled medium, and then subjected to washout for 120 min in 65% ASW with 5 μM unlabelled D-glucose (n = 5). Less than 1% of the absorbed substrate reappeared in the washout medium as non-volatile labelled material. As for the volatile radioactivity, such as tritiated water deriving from a complete metabolism of D-[6-^{3}H]glucose, this was quantitatively removed prior to counting.

For the long-term method to be easily applicable, D-glucose influx in the absence of inhibitors during the second period should be identical to that of the first period. Unfortunately, uninhibited D-glucose influx in the second period was found to be systematically lower as compared to the first one. The decrease, however, was constant and could be corrected for with reasonable certainty. The fact that a 120 min incubation of *Nereis* in a close-to-natural D-glucose concentration (5 μM) leads to a significant depression of D-glucose influx, is in itself of interest, and will be further investigated.

The exogenous D-glucose concentration (5 μM) selected for the inhibition experiments appears low as compared to the assumed half-saturation concentration of the transport system (approx. 50

μM). However, a concentration of 5 μM and a medium volume of 5 ml result in the highest amount of D-glucose possible, if a significant decrease of non-volatile radioactivity is to be detected also during partial inhibition. This substrate concentration also is of the same order of magnitude as the D-glucose concentration in the animal's natural habitat.

The rank of D-glucose influx inhibitors

D-Glucose influx across the apical epidermal membrane is not amenable to a detailed kinetic analysis: The influx is quite variable, even under well-defined experimental conditions [1]; with the technique of Fig. 1, the substrate concentration in the medium must be kept considerably below the half-saturation value; and the concentration of substrate and transported inhibitor at the level of the apical membrane are ill-defined because of the large diffusion resistance of the cuticle and the structural complexity of the epidermal/cuticular interface [1,12]. Consequently, we have deferred from describing the inhibition of D-glucose influx using conventional kinetic symbolism [28]. The data, however, allow the compounds to be ranked according to their inhibitory potency (Table I). In the subsequent discussion, we shall assume that a large inhibitory effect indicates a heavy interaction with the rate-limiting step in epidermal D-glucose accumulation.

From the data of Table I, it is possible to establish the following rank of compounds according to their inhibitory effect on apical D-glucose transport: methyl α-D-glucoside > methyl β-D-glucoside > D-galactose > 3-*O*-methyl-D-glucose > 2-deoxy-D-glucose > β-D-xylose = *myo*-inositol > β-D-fructose. L-Glucose is also an inhibitor, but does not fit into the above sequence.

Methyl α-D-glucoside was shown in the initial-rate experiments to be transported across the apical membrane by a Na^+-dependent process inhibitable by D-glucose. Thus, methyl α-D-glucoside, and presumably also the β-form, share a transport pathway with D-glucose. D-Galactose, 3-*O*-methyl-D-glucose and 2-deoxy-D-glucose are moderately effective inhibitors, but firm evidence for their transport by this membrane has yet to be established. The same applies to the weak inhibitors D-xylose, *myo*-inositol, and β-D-fructose. Initial-rate measurements have failed to demonstrate a significant uptake of D-mannose from a 5 μM solution [1], in keeping with the lack of effect of this compound as inhibitor.

Weak effects at high inhibitor concentrations must be viewed with caution. Although the purity of the inhibitors was confirmed chromatographically, a non-discernable impurity of a high-affinity compound (e.g. 1% D-glucose) could distort the 5000 μM data. Hence the affinity of D-xylose, *myo*-inositol and β-D-fructose to the D-glucose transporter may be artifactual.

Under control conditions, epidermal D-glucose uptake from a 5 μM solution in 65% ASW is membrane-limited, but in addition metabolic trapping occurs within the epidermis [1]. In long-term experiments, a reduced uptake rate may result from inhibitory interaction with transport across the apical membrane. Alternatively, an inhibitor, such as methyl α-D-glucoside, might interfere with metabolism, causing an intracellular build-up of D-glucose, in turn reducing the rate of D-glucose accumulation. Considering the reciprocal situation, the glucoside is not metabolized neither by *Nereis* (unpublished) nor by other animals [29,30]; furthermore, its transport across the *Nereis* apical membrane is inhibited by D-glucose (Table IIA) under conditions in which the latter is easily metabolized and therefore does not build up to high intracellular concentrations. Thus, methyl α-D-glucoside and D-glucose seem to interact on the membrane level. For the inhibitors in general, the rank presented in Table I is likewise inconsistent with the known specificity for animal hexokinase (EC 2.7.1.1) [29]. We therefore conclude that inhibition takes place at cell membrane level. Due to the structural analogy between substrate and inhibitors, such inhibition must be caused by competitive interaction at a substrate-binding site, implying that the inhibitors most probably are transported across the apical membrane.

The structural requirements for Na^+-dependent glucalogue transport by *Nereis* epidermis may be summarized in a way, which is also in agreement with the main body of evidence relating to Na^+-dependent D-glucose transport in the apical membrane of the vertebrate small-intestinal and proximal tubule epithelia [33–45], as well as the tapeworm integument [46]. The preferred structure is a

pyranoside ring in the C1-conformation [47] with equatorial hydroxyl groups at carbons 2, 3 and 4, and an equatorial CH_2OH at carbon 5. We assume that the substrate is bound to the carrier by hydrogen bonds at the four positions indicated, and that such binding is hindered by tilting the hydroxyl group to an axial position, by methylating it, or by removing it altogether. The detrimental effect of obstructing binding at each of these four positions increases in the order: 4, 3, 2, 5. Modifications at carbons 4 and 5 seem to have additive effects (L-arabinose). The hydroxyl group at position 2 is of special interest: 2-deoxy-D-glucose is a moderately effective inhibitor in the perfused proximal tubule and in *Nereis* epidermis, but according to the classical model of Crane [49] it should not be a substrate for the intestinal Na^+-dependent system. In rabbit renal slices, 2-deoxy-D-glucose was ineffective as an inhibitor of methyl α-D-galactoside uptake [34]. However, D-mannose (the C-2 epimer of D-glucose) is actively transported in frog intestine [50], and the stringent requirement of an equatorial hydroxyl at carbon 2 in Crane's model may have to be relaxed if a common denominator is sought. Alternatively, the effect of 2-deoxy-D-glucose in some preparations may be due to a separate transport system, possibly a Na^+-independent system as known from basolateral membranes [39].

The similarity of substrate-inhibitor interactions in the four different tissues discussed suggest that we may be dealing with a D-glucose transport system of wide distribution in the animal kingdom. The particular D-glucose-accepting site apparently has remained quite conservative during evolution. Only the peculiar role of L-glucose as an inhibitor of the *Neireis* system seems to be at variance with the other tissues. Earlier, L-glucose was found not to be transported by *Nereis* epidermis [1]. A heterogeneity in the set-up of D-glucose binding sites may be the reason for this anomaly; a fraction of the sites would then have a high affinity and the remainder a low affinity to L-glucose. Whether in fact a high-affinity, low-capacity L-glucose transport has gone unnoticed in previous studies, or alternatively the L-glucose-carrier complex cannot translocate, remains to be elucidated. It is worth noting that for another annelid, *Lumbricus rubellus*, evidence has been presented that integumentary D-galactose and 2-deoxy-D-glucose uptake is not affected by the presence of D-glucose [48].

Unlike the other epithelia, the *Nereis* epidermis does not normally undertake a mass trans-cellular transfer of D-glucose [9], and under natural conditions the bulk of the exogenous D-glucose absorbed is metabolized within the epidermis [11]. The biological significance of D-glucose transport by this tissue is not fully understood, but it may serve to supply nutrients to the epidermis, or to reduce diffusional losses incurred by the large D-glucose concentration gradient between the extracellular fluid and the D-glucose-poor habitat of the animal [9]. The latter function, which implies a recycling of D-glucose at the apical face of the integument, presupposes the presence of a diffusion-restraining material peripheral to the apical membrane. The collagenous cuticle of the *Nereis* integument recently has been proposed to fulfill this role [12].

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