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A Model for Erythrocyte Sugar Transport Based on Substrate-Conditioned "Introversion" of Binding Sites

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Summary. A number of kinetic incompatibilities with classical carrier theory, previously noted in the behavior of the monosaccharide transport system in human erythrocytes, are accommodated by a revised picture of the arrangement of the sugar-recognizing entities in the cell membranes. The new schema, an extension from that recently proposed by Naftalin (*Biochim. Biophys. Acta* **211**:65, 1970), replaces the mobile carriers with binding sites fixed in a two-column matrix (bilayer). These sites are in a state of equilibrium between a more interfacially disposed conformation and a more "introverted" state not in direct contact with the adjacent aqueous pool (cell interior or outside medium); adsorbed sugars may migrate between the two layers only when the apposed sites are both in the introverted orientation. Occupation of the sites by any given sugar characteristically shifts the position of the introversion-extroversion equilibrium. Computer simulation of this model, under certain restricting conditions, shows reasonable correspondence with observations on the biological system which have been difficult to bring into accord with mobile-carrier theory, particularly the dissonance in the apparent binding characteristics given by several accepted experimental approaches. Moreover, the single "introversiveness" characteristic distinguishing the several substrates may alone serve (in place of differing binding affinities and inter-site transition probabilities) as the basis for the sugars' comparative "saturation" characteristics, and for their patterns of mutual competitive or accelerative interplay.

The mobile-carrier mechanism for monosaccharide transport, proposed in 1952 by Widdas [33] originally as a working model for his observations on the kinetics of glucose movements through the placenta in sheep, was quickly recognized [34] as offering also a quantitatively consistent framework for interpretation of the various aspects of sugar transfer in human red blood cells which had been noted as deviating from simple diffusion behavior [7, 12, 13, 35]. During the subsequent decade, the special technical accessibility of the red cell system led to its further experimental kinetic analysis in unprecedented detail [29], firmly establishing the success of the mobile-carrier model in fitting systematically a wide diversity of observations on sugar inflow and outflow in these cells, and in reducing to quite simple terms a number of superficially more complex phenomena which

otherwise seemed intuitively anomalous. In consequence, the application of the Widdas model to this particular transport system has become the standard illustration used in didactic descriptions of the presumed operation of a "facilitated-diffusion" mechanism.

Widdas had envisioned a rapid reversible adsorption-desorption equilibrium between substrates (sugars) and membrane carriers, symmetrically at the two interfaces, allowing trans-membrane migration by way of a rate-limiting diffusion of the sugar-carrier complexes and the free carriers. Thus, in this original form of the schema, a sugar's translocation behavior (in a given medium and temperature) is totally defined by specification of two constants: (a) the rate constant for trans-membrane migration, set by the population density of the carriers and their mobility within the barrier, and (b) an "affinity" constant which characterizes the particular substrate's association with the carriers. One minor complication in this simple schema did appear to be necessitated by two independent demonstrations in 1965 [16, 21] of the phenomenon of "*trans*-acceleration", suggesting that the mobility of carriers loaded with glucose substantially exceeds that of the unattached carriers (and adding a third constant to specify the magnitude of this effect for each substrate). With this single refinement, however, the Widdas schema accords well with the diversity of types of observation listed in Section A of Table 1.

More recently, however, a number of serious discrepancies between this schema and experimental observation have been brought out. Table 1 B lists these, and designates their most explicit expression in the 1968 reports by D. M. Miller [22, 23]. Applying a systematic battery of concurrent tests to identical cell preparations under closely parallel conditions, Miller minimized the possible role of subtle procedural distinctions in generating the quantitative discord that had been reported, and showed that it was genuinely impossible to reconcile within this framework the several classes of data on glucose tracer behavior (each of which was *separately* readily interpretable by the mobile-carrier analysis). Especially notable was the glaring contrast in the magnitude of the glucose affinity constant given by the standard procedure of Sen and Widdas [28] and by three equally valid alternative procedures which should have been simply mutually complementary. Thus (Table 1, item B.1), in a standardized medium at 20 °C, the concentration of D-glucose required in the medium to halve the rate of net glucose exit from heavily loaded cells (Miller's Type I experiment, equivalent to the Sen-Widdas procedure) was found to be less than one-tenth the concentration required to give 50% inhibition of the uptake of the low-affinity substrate, L-sorbose (Type II experiment). Moreover, these two experi-

Table 1. Compatibility of mobile-carrier schema with kinetic observations

A. Consistencies	References
1. Systematic hierarchy of apparent affinities among substrates as indicated by	
a) interplay in competitive inhibition	[12]
b) comparative saturation behavior in net uptake	[8, 9, 35]
c) relation between isotopic equilibration rates and net uptake rates at high concentrations	[10, 14]
2. Relative constancy of apparent V_{max}'s of all substrates in face of widely differing apparent K's	[9]
3. Counterflow-coupling phenomena; notably,	
a) induction of transient "uphill" countertransport	[27]
b) enhancement of one-way flux upon adding *trans*-substrate	[16, 21]

B. Inconsistencies	Miller's "Expt. Type" [a]	References
1. a) Marked discrepancies in apparent K for glucose given by different procedures:		
K for *trans*-suppression of net exit	I	[22, 23, 28]
$\ll K$ for competitive inhibition of sorbose,	II	[15, 22, 23]
or for zero-*trans* exit	VI [b]	[6]
$< K$ for isotopic exchange	IV	[17, 22, 23]
b) Opposite temperature-dependencies in above	I, II	[15]
	I, IV	[17]
2. Time-course of glucose uphill countertransport (too fast, peak too low)	V	[22, 23]
3. Paradoxical inter-specific relations in flux enhancement by *trans*-substrate	III	[22, 23]

[a] These Roman-numeral designations are used throughout the text for shorthand identification of the several classes of experiment.
[b] Extension of Miller's code to cover "zero-*trans*" experiments.

mental parameters varied in opposite directions as a function of temperature [17].[1] Even higher than the apparent Type-II K are the apparent 50%-saturation levels for isotopic-exchange fluxes (Type IV) [17, 22] and for efflux into an infinite sink (the "zero-*trans* experiments [6], which will here be designated, in extension of Miller's nomenclature, as "Type VI").

Miller also called attention to a specific complication in the *trans*-stimulation phenomenon: the enhancement of isotopic D-glucose exit was found to be significantly greater when the *trans*-sugar was D-mannose or D-galactose than when it was D-glucose itself (Type III experiments). Since comparably high exit fluxes were not seen in the simple equilibrium self-

1 This is evidently strictly true only at reasonably low temperatures, since Levine, Levine and Jones [15] recently report that the glucose K in Type II experiments passes through a minimum at 25 to 30 °C.

exchange situation with either of these two inducing sugars, this finding appears paradoxical regardless of what relative mobilities are assigned to the several species of carrier-sugar complexes. (Levine, Oxender & Stein [16], however, felt that their qualitatively similar observations were accountable in terms of competition for exit by the sugar that had entered the cells during the period of measurement.)

Finally, although the Widdas model fairly adequately predicts the (Type V) observed course of the transient "countertransport" of labeled mannose or galactose into cells heavily pre-loaded with the unlabeled form of the sugar [24], the comparable experiments with glucose (in the same laboratory) could not be similarly fitted (Table 1, item B.2). The tracer accumulation observed peaked too soon and at too low a level, and the entire transient passed away far too rapidly to be reconcilable with the transport parameters given by the other procedures [23].

In view of these inconsistencies, Naftalin [25] has proposed an alternative model for the transport apparatus, retaining the concept of stereospecific membrane-bound sugar-adsorbing sites, but replacing their "carrier" (mobility) characteristic with the capacity to accept transfer of adsorbed sugars from adjacent sites in a *fixed matrix*. Specifically, Naftalin proposes a lattice membrane in which fixed sites lie along (or comprise in themselves) aqueous channels spanning the membrane; bound sugars migrate by one-dimensional diffusion between occupied and vacant sites, or by exchange of occupants between adjacent sites or between the terminal (interfacial) sites and the adjacent aqueous compartment.

To arrive at theoretical flux patterns for the several experimental situations, Naftalin employed a Monte Carlo method in a computer simulation with a 4×10 array of such sites, assigning various combinations of values to the relative likelihood of occurrence of the several steps in the hypothetical process. Thus, pseudo-random numbers were generated to make such decisions as when and where a molecular impact occurred, whether a given occupancy shifted to the right or left, whether adjacent groups exchanged, etc., in accordance with assigned numerical thresholds (expressing the probability) for each type of event. Within a few thousand iterations, this simulation generated in each case a steady-state flux; and the parallel between the patterns of these fluxes in some of the critical model experiments and those observed in the biological system was sufficiently close to encourage serious consideration of this sort of schema in place of the classical model. However, to mimic the coupled-flow phenomenon of acceleration of exchange flux [16, 21], it was necessary to assume a significant "unstirred-layer effect" at the outer membrane surface; and the anomalous

aspects of this phenomenon when heterologous substrates are involved (Type III studies) must in part be ascribed to the change in the magnitude of this complicating consideration with change in the external sugar's affinity for the transport sites.

The schema now to be proposed emerged directly from consideration of certain simplifications in Naftalin's computer simulation method which were adopted to facilitate further analysis of the model's predictions by use of the "DAC-512" data-reduction attachment supplied with a Packer Nuclear liquid scintillation counter (Liquimat 330) in the author's laboratory. To this end, the finite matrix (4×10) was at first supplanted by an infinitely extended 4-column array, whereby the Monte-Carlo one-molecule-at-a-time, random-number approach could be replaced, for each type of event, by an expression of its frequency (or probability at each iteration). One aspect of this conversion was the explicit setting of odds that a given occupant of the lattice would move to the left or right (inwards or outwards). Although a simple 50:50 likelihood would presumably be anticipated at an interior site with symmetrically disposed neighbors, there is obvious basis for possibly marked asymmetry at the surface columns. Pursuit of this consideration soon brought out the unexpectedly critical prominence of this particular probability parameter in defining the overall patterns of transfer behavior of the model, to the extent that perhaps no other variable property among the substrates need be postulated at all to provide a hypothetical basis for the experimental findings. Furthermore, this emphasis on an interfacial-site characteristic makes the interposition of the inner columns of the lattice an unnecessary complication introducing no qualitative change in behavior. Accordingly, the schema as diagrammed in Fig. 1 as a *bilayer* of fixed sites, somewhat comparable to Stein's *hemiports* [30], is now proposed as a working hypothesis for the operation of this transport mechanism, on the grounds of the extreme simplicity and paucity of the assumptions which allow substantial accord with the observations.

This model pictures the adsorbing sites in each layer as in a state of conformational or orientative equilibrium, between a more superficial "extroverted" state (allowing interplay with the adjacent aqueous pool) and a more "introverted" state which might allow trans-membrane contacts but no immediate further interaction with the aqueous pool. The critical postulate distinguishing this schema is that it is *only the state of occupancy* of the sites that governs their equilibrium distribution between introversion and extroversion. Thus a single parameter, the *introversiveness*, expresses the equilibrium characteristic for each occupancy state; no affinity differences nor other distinctions among the substrates need be postulated.

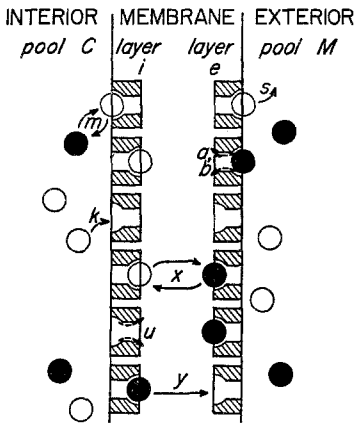

Fig. 1. Substrate-conditioned introversion model for equilibrative transport. The *hemiport*-like sites indiscriminately adsorb all suitable substrates (two species here indicated as black and white balls), by processes k, s and m, as further characterized in text. Tendencies to introvert (processes a, b, u) are set by state of occupancy and identity of occupant. Representation of introversion here as reversal of orientation of grommet-like sites is for diagrammatic convenience only. Model does not distinguish between individual coupling of apposed sites as a single trans-membrane component [18], and random transient coupling during two-dimensional diffusion within each liquid monolayer [30]. Trans-membrane interaction between apposed introverted sites can lead to one-way substrate migration (y) or to bidirectional exchange (x)

A preliminary report on this model has been published in abstract form [11].

Formal Model

Any two substrate (sugar) species are designated A and B; the subscripts i and e refer respectively to the interior and exterior layers of membrane sites, while the two aqueous pools are identified by subscripts C (intraCellular) and M (extracellular Medium). Thus, the pool sugar *concentrations* (as millimolarities) are symbolized as A_C, B_C, A_M, and B_M, while A_i, B_i, A_e and B_e indicate the corresponding sugar *occupancies* of each layer of sites, expressed as a *fraction* (from 0 to 1) of the total sites in the layer.

The *introversiveness* characteristic (from 0 to 1) expresses the *probability* that a site of a given occupancy class will be found in the introverted state (i.e., the *fraction of time* that such sites spend in the more internalized orientation); this is given as a for sites occupied by species A, b for sites occupied by B, and u for unoccupied sites. Thus, the fraction of inner-layer sites which are occupied by species B and are at any instant extroverted (hence available for interaction with the intracellular pool) would be $(1-b)\,B_i$; the fraction of introverted, unoccupied sites in the exterior layer is $u(1-A_e-B_e)$; and so on.

As pictured in Fig. 1, there are five classes of events (molecular interplays) identified in the operation of the system, each expressible in terms of a single probability per cycle:

k: *fixation* of substrate from a pool onto a surface site;

s: spontaneous *detachment* of substrate from a surface site into the adjacent pool;

m: *displacement* of substrate from a surface site by another species (detachment *plus* fixation);

x: trans-layer *exchange* of substrates (migration of an apposed pair in opposite directions);

y: simple trans-layer *migration* of an occupant substrate (into an apposed vacancy).

Here k is expressed as the probability in a cycle that fixation will occur at a given surface vacancy, per unit concentration (mM) of substrate in the adjacent pool; this is taken as a fixed constant on the presumption that the frequency of effective impacts at the surface is essentially linear with concentration up to the maximum of about 250 mM involved in any of the present applications; (the "cycle" time-unit should accordingly be of an order of magnitude comparable to the duration of the fixation process.) Similarly, x and y express the likelihood that exchange (or migration) between a suitably occupied pair of apposed introverted sites will occur during a cycle; and m and s are the corresponding probabilities of detachment at the surface when the occupied site is, respectively, encountering an effective impact or remaining unimpacted. With this formulation, the one-cycle unidirectional fluxes (expressed as J with the appropriate subscripts) are as follows, for substrate A:

$$ {}_AJ_{C\to i}=k\,A_C[(1-u)\,(1-A_i-B_i)+m(1-b)\,B_i] \tag{1a}$$

$$ {}_AJ_{i\to C}=(1-a)\,A_i[s(1-k\,A_C-k\,B_C)+m\,k\,B_C] \tag{1b}$$

$$ {}_AJ_{i\to e}=a\,A_i[u\,y(1-A_e-B_e)+b\,x\,B_e] \tag{1c}$$

$$ {}_AJ_{e\to i}=a\,A_e[u\,y(1-A_i-B_i)+b\,x\,B_i] \tag{1d}$$

$$ {}_AJ_{e\to M}=(1-a)\,A_e[s(1-k\,A_M-k\,B_M)+m\,k\,B_M] \tag{1e}$$

$$ {}_AJ_{M\to e}=k\,A_M[(1-u)\,(1-A_e-B_e)+m(1-b)\,B_e]. \tag{1f}$$

These fluxes thus give the following net transfers (T's) for A at the three interfaces, $C{:}i$, $i{:}e$, and $e{:}M$:

$$\begin{aligned} {}_{A}T_{C\to i} = {} & k A_C[(1-u)(1-A_i-B_i)+s(1-a)A_i+m(1-b)B_i] \\ & +k(s-m)(1-a)A_iB_C-s(1-a)A_i \end{aligned} \tag{2a}$$

$${}_{A}T_{i\to e} = a[(bx-uy)(A_iB_e-A_eB_i)+uy(A_i-A_e)] \tag{2b}$$

$$\begin{aligned} {}_{A}T_{e\to M} = {} & s(1-a)A_e-k(s-m)(1-a)A_eB_M \\ & -kA_M[(1-u)(1-A_e-B_e)+s(1-a)A_e+m(1-b)B_e]. \end{aligned} \tag{2c}$$

In the above form, the model would permit such manipulations of the parameters as Naftalin [25] has employed to illustrate the fit of his polar-creep schema with the experimental findings; thus, it is feasible to propose that the trans-bilayer migration is encouraged by occupancy of the apposed site (i.e., that $x > y$), or analogously that surface displacement may specifically enhance the detachment process (that $m > s$). However, for the present applications no such special postulations are required to allow reasonable accord with the critical observations, and it has accordingly been assumed here that the probabilities for the basic events are *not modified* by the concurrent counter-events (i.e., that $x \equiv y$, and $m \equiv s$). These restrictions allow elimination of two of the constants, and simplification of the net-transfer equations for substrate A to the following forms:

$$\begin{aligned} {}_{A}T_{C\to i} = {} & k A_C[(1-u)(1-A_i-B_i)+s\{(1-a)A_i+(1-b)B_i\}] \\ & -s(1-a)A_i \end{aligned} \tag{3a}$$

$${}_{A}T_{i\to e} = ax[(b-u)(A_iB_e-A_eB_i)+u(A_i-A_e)] \tag{3b}$$

$$\begin{aligned} {}_{A}T_{e\to M} = {} & s(1-a)A_e-kA_M[(1-u)(1-A_e-B_e) \\ & +s\{(1-a)A_e+(1-b)B_e\}]. \end{aligned} \tag{3c}$$

The transfers for substrate B are of course described by the symmetrical equations wherein all A, a and B, b symbols in Eq. (3a, b, c) are interchanged.

No method for definitive assignment of values to each fixed constant by analysis of experimental data is evident, but appropriate combinations of relative values may readily be examined to evaluate whether the schema might be applicable, and to extrapolate predictions accordingly to new experimental test situations. From preliminary scanning of the patterns of transfer behavior given by the schema with a variety of assigned values for the constants, it appeared that the most satisfactory mimicking of the experimental system was achieved by the assumption of an intermediate degree of introversiveness for the unoccupied sites (u), and a quite low

likelihood of trans-membrane exchange (so that x becomes the rate-limiting process, in good analogy with the comparable step of diffusion of the carrier-complex in the application of mobile-carrier theory to this same system). Specifically, for all illustrations given in the several figures in this report, the following fixed probabilities were adopted: $k = 0.002$ (per mM); $s = 0.5$; $x = 0.001$; and $u = 0.3$. The admittedly simplistic compartmental treatment applied by Naftalin [25] has also been retained for this first approximation: a finite intracellular fixed volume (ignoring the osmotic changes that would accompany sugar transfer), and an infinite extracellular pool (so that A_M and B_M are invariant with time). For the computer simulation, a unit delivery of substrate into the C pool was equated to a concentration increment of 100 mM, to achieve significant progression of the transfer within a reasonable number of cycle iterations (a few thousand). This unrealistically low relative capacity for the intracellular compartment imposed some degree of artificial latency in the transfer curves generated, as may be appreciated from the examples in Fig. 3, where a brief initial concavity upward is apparent.[2]

In contrast to Naftalin's [25] computer simulation which began each test situation with the matrix completely free of any substrate regardless of the initial sugar distribution in the two pools, the present simulation more realistically presumes complete initial equilibration with the intracellular levels of A and B, and triggers the experimental transfer by an instantaneous change in the fixed concentrations in the medium (A_M, or B_M, or both). Essentially, all the critical types of experiment with which the schema must deal were aimed at estimations of *initial* rates, but in actual practice these of course entailed recording of some appreciable displacement from the initial conditions to provide the rate estimation; accordingly, the point taken in the present analysis for reading of the transfer rate was in every case the "20%-time" (at which A_C had just attained a value one-fifth of the way between its initial setting and its final equilibrium value equal to A_M). Thus, the DAC-512 data-reduction unit was programmed to reiterate the single-cycle transfers given by Eq. (3a, b, c) and the corresponding three ${}_BT$ equations, until A_C reached this one-fifth point, and then to print out the current transfer rates. This procedure was used to generate the theoretical data for experiments of Types I, II, III, IV and VI. For Type V (uphill

2 It has also become apparent subsequently that this seemingly rather inconsequential speeding up of the intracellular compartment's concentration changes may be quite essential to the quantitative mimicking by the model of some of the critical experimental findings. The potentially significant implications of this development in the further interpretation of the kinetic observations are currently under examination.

countertransport) experiments, a continuing record of A_C, B_C, A_i, B_i, A_e and B_e was provided at suitable multiples of the iteration. The choice of specific initial and final substrate levels set up for each class of experiment was dictated by the standardized tests adopted by Miller [22, 24] in his critical experimental evaluation of the anomalies; and within that framework, by consideration of which ranges provided optimal resolution of the slopes and intercepts in the several types of nominally rectilinear plots by which such data were conventionally analyzed in terms of mobile-carrier models. Details regarding the selected test concentrations are provided in the appropriate figure legends.

Application to Published Experimental Data

Of the several failings in the application of mobile-carrier theory to the monosaccharide transport behavior of human red cells, perhaps the most striking is the very large divergence in the affinity constant for glucose-carrier interaction given by the several accepted experimental approaches (as summarized under B.1 in Table 1). The lower panel of Fig. 2 illustrates the correspondingly divergent values for this imaginary K parameter that would be given by forcing the behavior of the present model into the classical analyses based on the mobile-carrier hypothesis, for the experimental situations identified as Types I, II, IV and VI. The apparent dissociation constant is presented here (on a logarithmic scale) as a function of the introversiveness of the test sugar; only the upper range of introversiveness (approaching unity, corresponding to high apparent affinity under the classical schema) is covered in the figure, because (a) the experimental anomalies deal essentially with glucose, a high-affinity substrate, (b) some of the analytic procedures (I, II) are designed for application only to substrates of high apparent affinity, and (c) in any case, the curves tend to plateau to the left as is evident in Fig. 2. The K given by the net-exit procedure of Sen and Widdas [28] (Type I) is seen to approach closely the actual 50%-saturation concentration for any sugars which induce nearly complete introversion, but falls somewhat short of this half-saturation level as the introversiveness diminishes. In contrast, a marked *over*estimation is seen in the apparent K calculated in the conventional manner by any of the other procedures, and each differs characteristically from the others.

The arrows to the right of the figure mark the respective experimental values reported for the D-glucose K from data taken at 20 °C, pH 7.4. There is some discrepancy in the reported figures in Type IV experiments, and even for Miller's data alone, no precise fit of all the observations can be

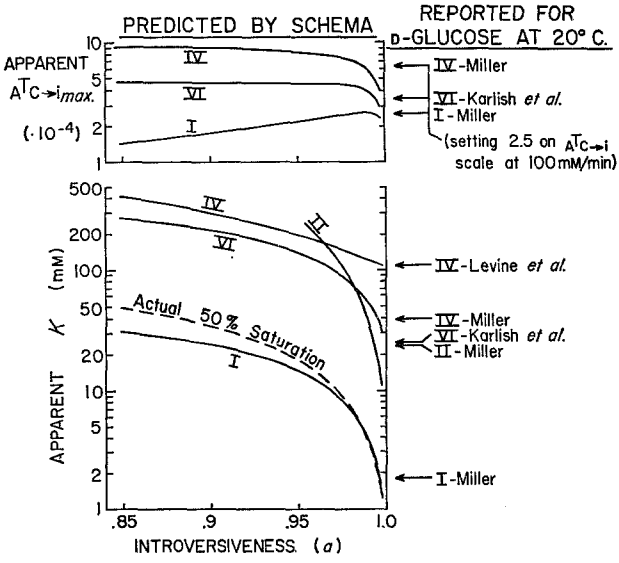

Fig. 2. Predictions of introversion model, in respect to calculation of classical transport parameters by several accepted procedures. V_{max}'s, calculated conventionally (as detailed below) in terms of the computed ${}_AT_{C\to i}$'s (upper panel) and dissociation constants, *K's* (lower panel), both on logarithmic scales, are shown as a function of the introversiveness of the test sugar, for the indicated procedures identified in Table 1. Fixed parameters of model are set at values given in Formal Model section. Test situations and analyses are those applied in Miller's experiments [22, 23]: I. Initial A_C=130 mM; A_M varied (0 to 30 mM); plot of ${}_AT_{C\to i}{}^{-1}$ *vs.* A_M. II. Initial $A_C=A_M$ (0 to 100 mM); initial $B_C=0$; B_M=230 mM; b=0.4; plot of ${}_BT_{i\to C}$ *vs.* A_M, and K estimated by interpolation for A_M halving the rate in absence of A. IV. Initial $A_C=B_M$=varied (40 to 250 mM): initial $B_C=A_M=0$; plot of ${}_AT_{C\to i}{}^{-1}$ *vs.* $A_C{}^{-1}$. VI. Initial A_C varied (22 to 200 mM); $A_M=0$; plot of ${}_AT_{c\to i}{}^{-1}$ *vs.* $A_c{}^{-1}$. For I, IV, and VI, 5 to 8 levels were chosen for the variable, falling at approximately regular intervals along the abscissa in the plot applied, and confined to the range giving fluxes at least 1/3 of the maximum for that procedure (since very little deviation from the rectilinearity predicted by mobile-carrier theory was evident in this range); apparent K's and V_{max}'s were estimated from slopes and intercepts of regression lines in the standard plots. Alignment, on the ${}_AT_{C\to i}$ scale, of the logarithmic spacing of the three experimental V_{max} figures (arrows) at right of upper panel, such that ${}_AT$ of 0.00025 equates to 100 mM/min, was by simple visual inspection for a reasonably satisfactory fit

claimed at any one position along the abscissa; however, it is clear that the predictions of the schema are reasonably consonant with the observations if D-glucose is taken as inducing *nearly complete introversion* (*a* of perhaps 0.99, as the system is set up here; it should be noted that the precise optimal figure would be shifted somewhat by assumption of different values for the fixed constants). This 0.99 figure is also in good accord with the reported relative differences in apparent V_{max}, as shown in the upper panel of Fig. 2.

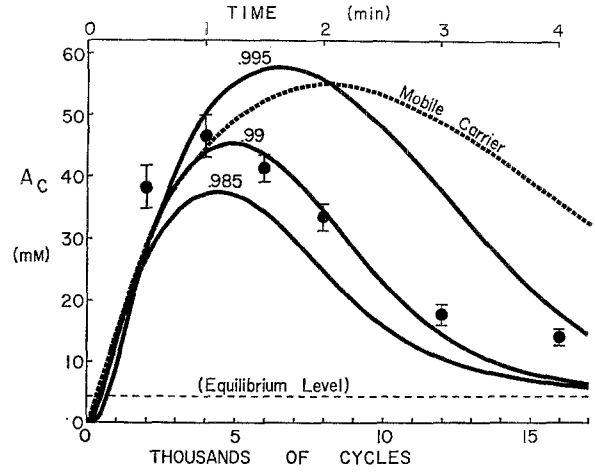

Fig. 3. Predictions of introversion model for Miller's Type V (countertransport) experiments with D-glucose. A=labeled glucose; B=ordinary glucose; initial $A_C=B_M=0$; initial $B_C=130$ mM; $A_M=4.3$ mM. Time scale refers to experimental points [22] and to simple mobile-carrier theoretical curve; cycle scale for introversion-model curves is equated on basis of conversion factor from upper panel of Fig. 2. Numbers above each curve signify introversiveness (a, b) assigned to glucose

The quantitative compatibility of the conclusions drawn from Fig. 2 with Miller's data [22] on the time-course of glucose countertransport is illustrated in Fig. 3. These Type V experiments concern the "uphill" accumulation of tracer glucose from a relatively low external concentration into cells preloaded with ordinary glucose (as detailed in the figure legend). The decided failure of the mobile-carrier model parameters (taken from the Type I measurements) to fit the Type V observations is shown by the position of the dashed curve in relation to the experimental points; the predicted peak falls at a distinctly higher level and later time, and the decay back toward equilibrium is much too delayed. The other (unbroken) curves show the introversion model's predictions, with three values in the indicated range for glucose's introversiveness. Though some systematic deviation is still apparent, it is obvious that again (for the adopted set of fixed constants) an introversiveness of about 99% yields a great improvement in fit over that seen with the classical schema.

Fig. 4 shows the predictions of the new model in respect to the one remaining experimental anomaly (Type III) stressed by Miller: the seemingly paradoxical relations observed in the extent of exchange-flux enhancement ("*trans*-acceleration") when the inducing (*trans*) sugar is of a different chemical species, rather than an isotopically labeled form of the test sugar.

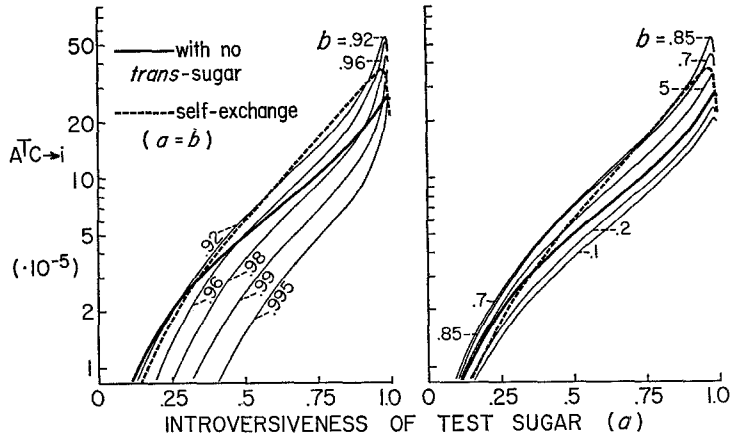

Fig. 4. Predictions of introversion model for Miller's Type III (*hetero-trans*-acceleration experiments. Initial $B_C=A_M=0$; initial $A_C=B_M=130$ mM (except for zero-*trans* reference curve, where $B_M=0$). Exit flux for A, at point when A_C has fallen 20% (to 104 mM) is plotted as function of A's introversiveness (a), with various representative values, as labeled, for introversiveness (b) of the *trans*-sugar (higher range of values in left panel, lower range at right). Continuous heavy line, showing zero-*trans* exit fluxes, delimits *trans*-accelerations (above) from *trans*-inhibitions (below). Heavy broken curve, for self-exchange fluxes, similarly delimits areas of *augmented* acceleration in *hetero*-exchange

As illustrated in the left-hand panel of the figure, a highly introversive sugar like glucose would be expected to attain its highest induced flux with a *trans*-sugar of somewhat lower introversiveness, even though such substrates would not themselves exhibit as great a *self*-acceleration as is evident with glucose. This is precisely what Miller reported [22] in his Type III studies with glucose, mannose and galactose, as summarized in Table 2 for direct comparison with Fig. 4. Moreover, the relative decline in the predicted fluxes for the "zero-*trans*" and "self-exchange" situations, as introversiveness is lowered from 0.99 to about 0.85, parallels reasonably well the glucose →

Table 2. D. M. Miller's Type III findings (rearranged from ref. [22])

Test sugar (A)	Exit rates (mM/min)			
	Trans-sugar (B) =			
	Nil	Glucose	Mannose	Galactose
Glucose	100	190	260	250
Mannose	95	—	170	
Galactose	80	—	—	125

Initial $A_C=B_M=130$ mM; initial $B_C=A_M=0$; 20 °C, pH 7.35.

mannose → galactose progression in Table 2, while the absence of a comparable difference in the accelerated glucose fluxes induced by the latter two sugars is also seen to be consonant with the model.

Discussion

Pursuant to the systematic experimental demonstrations [17, 22, 23, 24] of the reproducible inconsistencies in the application of the classical mobile-carrier schema to the sugar-transport behavior of human erythrocytes, a number of possible alternative models have been brought forth in an effort to resolve or minimize these problems.

(1) Asymmetry in Carrier Parameters

Geck [1] seeks to retain the basic tenets of the mobile-carrier picture by withdrawing the generally accepted restricting assumption of symmetry, and harks back to Regen and Morgan's formulation [26] for the much slower red-cell sugar transfer in rabbits, which had originally allowed the dissociation constants and transition probabilities at the two sides of the membrane to differ substantially. Although Regen and Morgan had concluded that probably no such asymmetry actually obtained in the rabbit cells, Geck stresses new measurements in human erythrocytes (by personal communication from Lacko and associates) pointing to distinct differences in the quantitative characteristics of glucose influx and efflux, and he concludes that the experiments of Types I, III, IV and V (but not II) are reasonably well mutually reconciled by way of the asymmetry analysis. However, this compatibility is illustrated principally only in terms of a crude approximation to the appropriate additivity in the apparent "translocation resistances" in the several test situations, without explicit estimation of the new parameters. Moreover, Lieb and Stein [20] have recently presented (without specific reference to Geck's proposal) a general formal criterion for dismissal of any such asymmetry presumption as a basis for retaining a mobile-carrier model, and find that certain rather complex relations among several of the measured parameters in this case transgress the bounds dictated by the theory.

(2) Complex Oligomeric Transport Units

Vidaver [32] has emphasized that the finding of an augmentation of counterflux (*trans*-acceleration) by a transport substrate need not mean that a carrier-substrate complex is actually translocated, but only that a

barrier-substrate complex of some kind undergoes a change of state such that any ensuing dissociation of the complex frees the substrate into the opposite pool (and that the uncomplexed barrier site similarly shifts between two such states). Along these lines, a promising (but rather intricate) *internal-transfer* model proposed by Lieb and Stein [18, 19] pictures the critical membrane entities as embedded, bi-affine, protein tetramers, which present to each aqueous pool (either simultaneously [18] or alternately [19]) one binding site with high affinity for the substrate, and one with a substantially lower affinity. Occupation of any of the sites by a substrate is presumed to dispose the entire tetramer to undergo a conformational change (not unlike the present schema's "introversion"), so that the occupants are shifted to an "internal cavity" within the membrane where redistribution of substrate between the apposed sites may occur; the reverse conformational change may then effect a trans-membrane migration if such an internal redistribution did take place. Since the several procedures for estimating transport parameters entail differing arrangements of substrates in the two pools, the overall expressions of rate and affinity which will be derived with this schema will sometimes depend on only the high-affinity sites, sometimes on the low-affinity sites, and sometimes on complex combinations of the two. Lieb and Stein find that the pattern of these predictions accords rather well with the contrasting findings in experiments of Types I, II, IV and VI. The model provides no basis for Miller's Type III or Type V observations; but the reality of the Type III anomalies has been questioned recently in connection with a further development of Lieb and Stein's model [20].

(3) Restyling of "Polar Creep" Models

It has been demonstrated [36] that some semblance of saturation and competition phenomena may appear in the passage of particles through a barrier even by way of simple open passages, if these are quite sparsely distributed and are only slightly wider than the permeant particles. Moreover, if passage is allowed only by way of shuttling between adsorption sites on the walls of such pores [31], even more complex flux-coupling phenomena are to be expected [2–5], including uphill countertransport [3]. The lattice-membrane model proposed by Naftalin [25] for the red-cell sugar-transfer system represents a significant modification of these earlier suggestions involving binding sites along the walls of polar pores, in that it specifically requires abandonment of the single-file characteristic [2–5]: Naftalin not only allows the sugar molecules adsorbed within the passageways to pass each other, but suggests that they must do so even more readily than they move into an adjacent vacancy. As noted in the Introduction, this schema

is capable of mimicking much of the kinetic behavior of the biological system; but for the critical comparison of parameters from Type I and Type IV experiments, and for demonstration of the Type III phenomena, it requires the additional assumption of a substantial unstirred-layer effect on the exterior surface of the membrane, of a magnitude which the calculations of Lieb and Stein [20] show to be far beyond realistic limits.

(4) The Present "Introversion" Model

It is clear that, although the schema offered here evolved simply as a remodeling of Naftalin's proposition just discussed, it emerges with the essential form of Vidaver's "allosteric model" [32], with sites exhibiting trans-membrane interaction in the manner of Stein's "hemiports" [30], and with the substrate-conditioned introversion and perhaps "internal cavities" proposed in Lieb and Stein's model [18–20]. If any critical unique feature is to be claimed, it lies in the specific separation of the introversion process from the trans-membrane step proper, so that it provides a partial sequestration from the surface events; i.e., the *cis*-adsorbed sugars are only incompletely available for interaction with the pool even before being committed to the transfer event. Moreover, the apparent "affinity" differences among substrates are here assigned solely to differences in their equilibrium position in this introversion-extroversion process governing their availability for detachment, rather than to their having distinctive basic rates of association *vs.* dissociation.

The extent to which this schema (with the particular values chosen here for the fixed constants) approaches an accord with the critical observations may be judged by examination of Figs. 2–4. One notable failing, however, masked by this summary presentation, is manifest in more detailed examination of the Type II experiments, wherein apparent K's are estimated as K_i's for competitive inhibition of transfer of an apparently "low-affinity" sugar. The present model predicts that at sufficiently reduced concentrations, a highly introversive substrate (A) will *accelerate*, rather than delay, the transfer of a much less introversive substrate (B), because many more exchange sites on the low-B side are made available by the introversion induced by A, thus augmenting transfer of B by the x process. With higher levels of A, this augmentation is of course offset by competition for occupancy of the sites, so that B transfer is reduced. This prediction of initially negative inhibition does not appear to be borne out in the operation of the red-cell sugar-transport mechanism; no enhancement of L-sorbose transfer has been detectable upon adding D-glucose at any range of concentrations.

Fig. 4 calls attention to some as yet untested predictions for the Type III situation which, since not immediately intuitively obvious, are perhaps worthy of mention. The only test sugars which should show their maximal potential flux enhancement in the *self*-exchange situation would be those of intermediately high introversiveness (moderate apparent "affinity"), perhaps in the range of D-galactose, D-xylose or L-arabinose. The flux of less introversive substrates should be maximally enhanced when the *trans*-substrate is of a somewhat *higher* apparent affinity (in diametric contrast with the established behavior of the highly introversive D-glucose). Moreover, *trans-inhibition*, rather than acceleration, may be expected when the inducing substrate is characterized by either a very high or a very low introversiveness. In fact, all substrates of whatever class should show very nearly the same proportional degree of flux inhibition by any *trans*-sugar which induces a *lowering* of the reactive sites' introversiveness (i.e., below that of the unoccupied sites), as might well be the case for some of the substrates showing no evidence of saturation behavior. Levine *et al.* [16] have in fact observed such *trans*-inhibition of glucose egress by both fructose and sorbose, and preliminary tests with a large series of sugars in the author's laboratory appear to fulfill the general pattern predicted in Fig. 4 for the appearance of this phenomenon.

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