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# A MODEL FOR SUGAR TRANSPORT ACROSS RED CELL MEMBRANES WITHOUT CARRIERS

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SUMMARY

1. An alternative to the carrier hypothesis is considered in which fluxes across red cell membranes are likened to diffusion of solutes within a lattice. The membrane lattice is an array of fixed binding sites situated within the water-filled channels which span the membrane. One dimensional diffusion takes place between the sites, and exchange between different solutes is permitted between neighbouring binding sites or between the solution on either side and the boundary sites.

2. A computer simulation of solute fluxes across the membrane is implemented with a Monte Carlo method. The flux kinetics obtained closely resemble the kinetics of sugar flow across red cell membranes in the following ways: (a) saturation kinetics of net flow; (b) saturation kinetics of accelerated exchange flow; (c) uphill counterflow; (d) relatively greater accelerated exchange flux is obtained between a slowly permeating solute and a rapidly permeating solute, than between a slowly permeating solute and another slowly permeating solute; (e) an explanation is obtained for the fact that the flux parameters $K_m$ (accelerated flow) and $K_s$, the dissociation constant, exceed the $K_m$ (net flow).

3. The kinetics of competitive exchange sorption fit the observed kinetics of sugar flows more completely than the kinetic predictions of the carrier hypothesis.

## INTRODUCTION

There is kinetic evidence that the passage of sugars across red cell membranes is mediated by mobile carriers. First, sugar flows conform to saturation kinetics[1]. Second, the flux rates of various stereoisomers vary widely suggesting some form of membrane specificity[1]. Third, there is accelerated exchange diffusion, which is an increased rate of unidirectional flux caused by the presence of sugar on the other side of the membrane[2–4]. A related phenomenon is when a gradient of one sugar across the membrane produces a counterflow of another sugar leading to the formation of a transient concentration gradient[5]. Although a mobile carrier accounts for these observations there are some results which are hard to explain on this basis.

MILLER[6,7] showed that the efflux of [$^{14}$C]glucose from pre-loaded human red cells was accelerated more by extracellular mannose and galactose than by glucose, which suggests that the complex of the carrier with either galactose or mannose can move faster than the carrier–glucose complex. External mannose should therefore

accelerate the efflux of internal [$^{14}$C]mannose or [$^{14}$C]galactose more than [$^{14}$C]-glucose efflux. However, the acceleration of [$^{14}$C]glucose efflux by external mannose and galactose is significantly greater than the acceleration of [$^{14}$C]mannose or [$^{14}$C]-galactose efflux. There are several other inconsistencies. The apparent $K_m$ for net glucose flow across the red cell membrane is an order of magnitude less than the apparent dissociation constant $K_s$, for the carrier, as measured by inhibition of sorbose efflux from red cells[8,6], yet theory predicts that the $K_m$ should exceed $K_s$. Moreover, STEIN[9] has pointed out that the disparity between the $K_s$ and $K_m$ increases as the temperature is reduced; the $K_s$ rises with reduction in temperature, whereas the $K_m$ falls with reduction in temperature[8,10]. A related inconsistency is that the $K_m$ of the carrier for sugars as measured by net flow is more than order of magnitude less than the $K_m$ as measured by isotope exchange[6,4]. If the same system is responsible for net flux and the binding of sugars then the $K_m$ values should be identical, and $K_m$ should exceed $K_s$.

In view of these inconsistencies an alternative model for sugar movements has been examined. This model is a lattice membrane containing fixed binding sites situated within water-filled channels which span the membrane (see Fig. 1). Sugars bind to the sites and are transported by one dimensional diffusion between occupied and vacant sites. Exchange is permitted between bound sugar molecules of different molecular species bound either to neighbouring sites or present in the adjacent solutions.

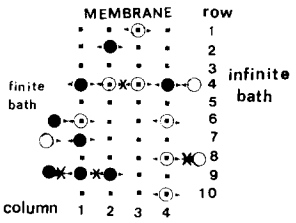


Fig. 1. Diagram showing lattice membrane. →← represents trial transitions which may result in a change of configuration. →×←, represents trial transitions where no change of configuration may result.

Computer solutions have been obtained using this model for the flow of solutes through the lattice membrane, and the kinetics obtained are closely similar to the kinetics of sugar flows across red cell membranes.

## EXPERIMENTAL

### *Computer solutions of interdiffusion across a sorbing lattice membrane*

The simulation of random motion of individual particles depends on the generation of pseudo-random numbers[11]. A two dimensional array 4 × 10 represents the lattice membrane (see Fig. 1). The column on the left (Column 1) is the boundary layer adjacent to a finite bath (inner pool) and the boundary layer on the right (Column 4), adjacent to an infinite pool. The initial conditions set the concentrations of the two solutes in the pools and the transition probabilities of molecular motion within the lattice. The concentration terms determine the probability of a particular molecular species hitting the boundary layer within a finite time interval. When a

molecule enters or leaves the finite pool the concentration within this pool is increased or decreased according to the pool size. The concentration of the components within the infinite pool remains fixed. Randomness of the arrival times of solute at the boundary layer from the pools is attained by generation by the computer of random numbers $\geqslant 0$ and $\leqslant X_i$, where $X_i$ is a real variable determining concentration. If the number generated equals 0, then no molecule arrives at the boundary layer. When $X_i$ is $>0$, a trial hit is made. Once a trial hit is signalled another random number between 1 and 10 is called, this number indicates the number of the row at which the trial is to be made. The appropriate boundary site is examined and, if it is unoccupied, the molecule is placed on the site; however, if the lattice site is already occupied by another molecule of the same species then, since no change in configuration can occur even if exchange were to take place, the molecule is replaced in the pool and no change is registered. Alternatively, if the lattice site is occupied by a molecule of another species, then another random number is called. This new number determines the probability of exchange.

When an exchange is called, the molecule on the boundary site is replaced by the incoming molecule, and the displaced molecule enters the adjacent pool. Since the energy levels of the two components may differ, the probability of exchange of A for B may differ from the probability of exchange of B for A. The variables determining the exchanges can be set independently.

Extension of this kind of procedure is used to simulate diffusion within the lattice. One dimensional random movement is achieved by a random variable which signals either go left, or go right instructions.

Following this instruction the nearest-neighbour configuration of the appropriate side is noted to determine if a move, or no move, or an exchange is to be made. After all four columns and the two pools have been examined in turn, the whole process is repeated. Following a given number of iterations, *e.g.* 500, the state of the matrix is printed along with the number of particles of each species in the finite pool, and the total number of particles of each species which have been present in each column during the iteration period. These last figures give a measure of the percentage occupancy of sites in each column by each species, during each iteration period. By this means the distribution of each molecular species across the thickness of the membrane is computed. It is possible to alter independently the transition probabilities in the first two and the second two columns. The programme was written in Algol 60 and solutions were obtained on the University of Leicester's Elliott 4100 computer.

## RESULTS

The intrinsic properties of the lattice membrane give rise to one dimensional diffusion, competitive adsorption to the binding sites and saturation kinetics of solute flow across the membrane. If these properties of the lattice model are the only determinants of sugar flow across red cell membranes then the kinetics of flow across the lattice membranes should closely resemble the observed kinetics of sugar flow across red cell membranes.

Initially the lattice is entirely free of solute, and consequently there is a time delay before steady-state fluxes across the membrane are established.

The first point to be examined was the steady-state flow rate. With column transition probabilities ranging from $p = 0.5$–$0.16$ per iteration, for each species it was found that a steady-state flow rate was established within 1000 iterations and the percentage occupancy of the sites reached a steady state within 500 iterations. This means that internal equilibrium is achieved before steady-state flow is established, as HILL[12] predicts. As there are only ten sites in each lattice column, greater accuracy in estimation of the percentage occupancy is achieved by summing the total number of sites occupied by each species over 2500 iterations.

A feature of the working program is that substitution of a different series of pseudo-random numbers makes an almost undetectable difference to the overall flow rates even over as short a period as 500 iterations.

*Net flow: Saturation kinetics*

The net rate of loss of a component A from the finite pool was measured. As is to be expected, raising the concentration of A in the outer pool decreases the initial flux rate of A. From Fig. 2 it can be seen that the rate of loss of A is a saturable function of the external concentration of A. The small basal loss is caused by solute flow into the initially empty lattice.

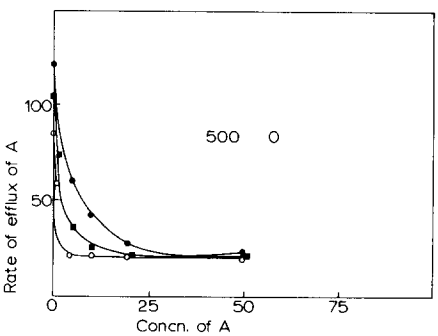


Fig. 2. The rate of loss of species A from inner pool with varying concentrations of A in outer pool. The rate is measured by total loss of A during the first 2500 iterations. Initial conditions: Concentration of A in finite bath = 500. Concentration of A in outer infinite bath varies from 0 to 50. The plots show effect of varying the free transition probability of species within the membrane. ●—●, (free transition probability of A)/iteration = ½; ■—■, (free transition probability of A)/iteration = $^1/_3$; ○—○, (free transition probability of A)/iteration = $^1/_6$.

*Coupled flow phenomena*

*Accelerated exchange flux.* A test was made to see whether the efflux of A from the inner pool is accelerated on increasing the concentration of B (an isotope of A) in the outside pool. Fig. 3a shows that in the absence of A in the outer bath, addition of B to the outer bath slightly retards the initial loss of A from the inner pool. From Fig. 3b it can be seen that the concentration gradient of A across the membrane is not altered, although the amount of A within the membrane is slightly reduced by high concentrations of B in the outer pool. Superficially it would appear that accelerated exchange efflux is not a property of lattice flow. However, the conditions described above do not accurately reflect the biological situation. In practice, it is impossible to obtain a suspension of red cells, pre-loaded with glucose, in which the external solution is entirely free of glucose, because of the rapid efflux of glucose

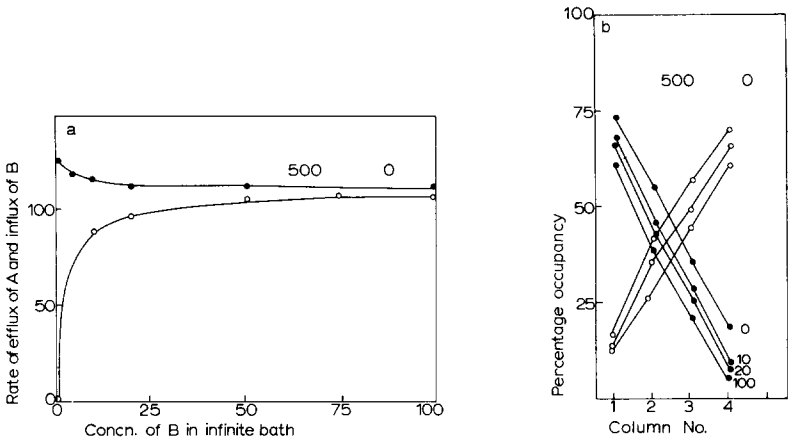


Fig. 3. a. Effect of varying the concentration of B in the outer pool on the rate of efflux of A from the inner pool. Rate measured as loss during the first 2500 iterations. Initial conditions: Concentration of A in inner pool = 500. Concentration of A in outer pool = 0. Concentration of B in outer pool varied from 0 to 100. Concentration of B in inner pool = 0. Free transition probability A = ½/iteration at all lattice sites. Free transition probability of B = ½/iteration at all lattice sites. Exchange probability following A–B or B–A collision = ½. ●—●, rate of efflux of A; ○—○, rate of influx of B into finite pool. b. Effect of varying the concentration of species B in outer infinite bath on the percentage occupancy of species A and B in the lattice columns. Initial conditions: Identical to those shown in a. As concentration of B is increased A falls. Figures at the side of lines ●—● indicate concentrations of B in infinite bath. ○—○, concentration of B in lattice.

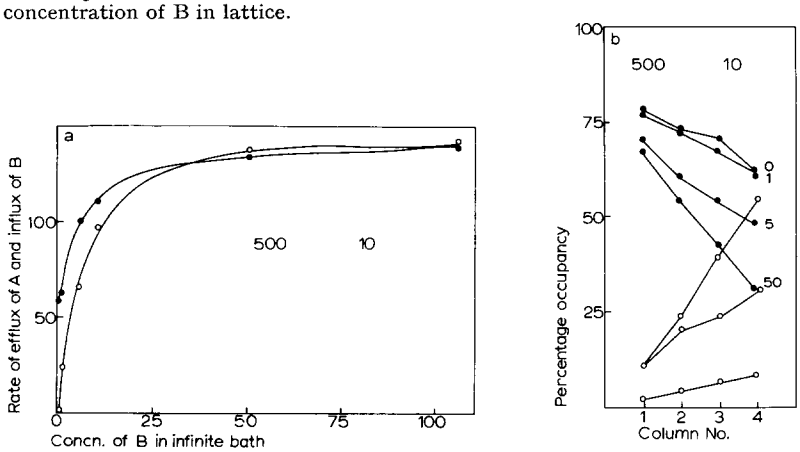


Fig. 4. a. Effect of varying the concentration of B in the outer pool on the rate of influx of A from the inner pool. Rate measured as loss during first 2500 iterations. Initial conditions: Concentration of A in inner pool = 500. Concentration of B in inner pool = 0. Concentration of A in outer pool = 10. Concentration of B in outer pool varies from 0 to 100. Free transition probability of A = ½/iteration at all lattice sites; free transition probability of B = ½/iteration at all sites. Exchange probability following A–B and B–A collisions = ½. ●—●, rate of efflux of A. ○—○, rate of influx of B into finite pool. b. Effect of varying concentrations of B in outer pool on the percentage occupancy of species A and B in the lattice columns. Initial conditions: Identical to those in a. As B increases A falls. Figures at side of ●—● indicate concentration of B in outer pool. ○—○, concentration of B in lattice.

across the membrane which gives rise to an 'unstirred layer' effect, resulting in a higher local concentration of emerging solute in the immediate vicinity of the external membrane surface, than in the remainder of the extracellular fluid[13].

If the initial conditions for computer analysis are altered so that the outer pool contains low concentrations of A as well as varying amounts of B, then the initial rate of efflux of A from the inner pool is now accelerated by the presence of B in the outer solution (Figs. 4a, 5a). Relatively greater acceleration in efflux was found with higher concentrations of A in the outer pool. These figures also show that stimulation of efflux is a saturable function of the external concentration of B. Comparison of Figs. 4b and 5b with 3b shows that with A on both sides of the membrane the presence of B on the outside surface causes a marked alteration in the concentration profile of A within the membrane.

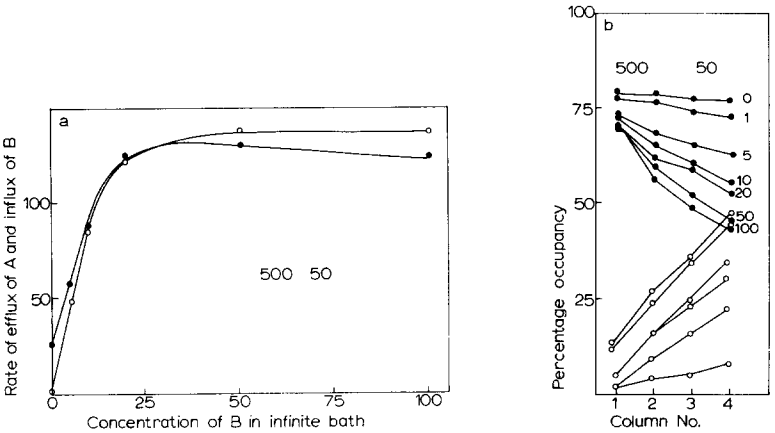


Fig. 5. a. Effect of varying the concentration of B in the outer pool on the rate of efflux of A from the inner pool. Rate measured as the total loss of A during the first 2500 iterations. Initial conditions: The same as in 4a, except that concentration of A in the outer pool = 50. b. Effect of varying the concentration of B in the outer pool on the concentration profile of A within the lattice membranes. Percentage occupancy estimated by total occupancy over the first 2500 iterations. Initial conditions as in a.

The rate of access of solute A to the outer layers of the membrane from the inner pool is limited by the presence of solute adsorbed to the sites in the inner layer. Thus, the addition of A to the outer pool increases the amount of A adsorbed to sites in the outer layers. This gain in solute by the outer layers flattens the solute concentration profile within the membrane and retards the efflux of A from the inner pool. The presence of B in the outer pool reduces the total amount of A in the membrane, but particularly in the outer layers of the membrane.

Coupled flow arises from inequality in the direction of solute movements by exchange interaction. Since one dimensional movement within the lattice is random, the cause of the inequality in the direction of exchange movement within the lattice results from the concentration gradients within the membrane. Thus steepening of the concentration gradient of A across the membrane by competitive exchange displacement by B gives rise to coupled flow, and it is this which accounts for the accelerated efflux.

*Counterflow.* Uphill counterflow will occur across a sorbing membrane if the solute concentration gradient within the membrane caused by a concentration difference between the inner and outer pool is reversed by competitive displacement

on addition of a larger amount of B into the pool containing the higher concentration of A (see Figs. 6a and 6b).

Addition of B to the outer pool will not always lead to acceleration of efflux. If only small amounts of A are present in the outer layers addition of B reduces the number of free lattice sites. If the increase in A–B, B–A collisions and decrease in A–A collisions does not offset the decrease in flux due to decrease in the number of vacant sites, no acceleration in flow will be observed, in fact flow may well be retarded, as is seen in Fig. 3a.

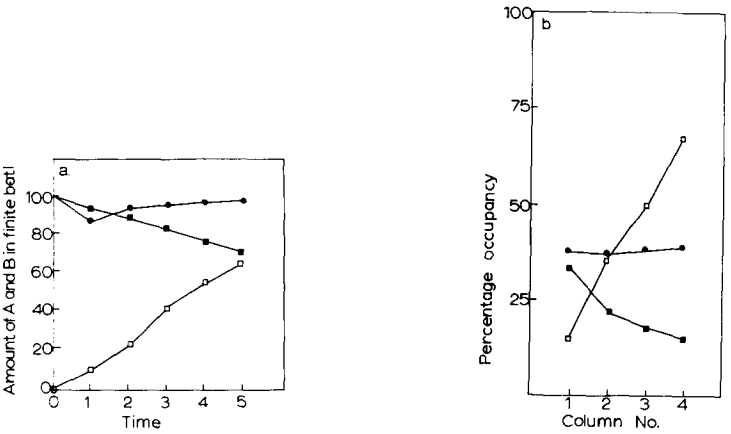


Fig. 6. a. Effect of addition of B on the efflux of A from the inner pool. ●—●, changing amounts of A in inner pool. Each time point represents an interval of 500 iterations. Initial conditions: Concentration of A in inner and outer pools = 2. Concentration of B in inner and outer pools = 0. Free transition probability of A = ½. ■—■ shows changing amount of A in inner pool during the course of 2500 iterations on addition of B to outer pool at time = 0. Initial conditions for ■—■ : Concentration of A in inner and outer pools = 2. Concentration of B in outer pool = 50. Concentration of B in inner pool = 0. Free transition probability of A/iteration at all lattice sites = ½. Free transition probability of B/iteration at all lattice sites = ½. Exchange probability following A–B and B–A collision = ½. □—□, increasing of amounts of B in inner pool on addition of B to outer pool. b. Concentration profiles of A and B in membranes during the first 1500 iterations of the computer. Initial conditions as shown in a. ●—●, percentage occupancy of A in the lattice columns in the absence of B; ■—■, percentage occupancy of A in the lattice columns with B present in outer pool; □—□, percentage occupancy of B in lattice columns.

*Mannose–glucose exchange anomaly.* So far the lattice has been shown to reproduce the two major kinetic features of the carrier model, *i.e.* saturation kinetics with both net flow and accelerated exchange flow. The third property of biological membranes, substrate specificity, may using similar assumptions to those determining carrier binding specificity, be considered to be a function of the binding characteristics of the substrate for the lattice sites. A more rigorous test of the sorbing lattice model is to determine whether or not it reproduces the phenomena which the carrier hypothesis cannot explain.

Mannose and galactose have higher $K_m$'s for transport across red cell membranes than glucose[14,15]. Mannose and galactose have also been observed to equilibrate across red cells more rapidly than glucose[16,17]. In terms of the carrier model the latter finding has been attributed to the lower affinity of mannose and galactose for the carrier and not to an increased rate of translocation across the membrane[14]. However, in terms of the sorbing lattice membrane a species with a lower binding affinity has

a higher inter-site transition probability and consequently moves faster within the lattice, although not necessarily faster across the lattice since this depends on the concentration gradient within the lattice (see Fig. 2). It can be seen from Fig. 7a that the acceleration in efflux of a slow substance A by saturating concentrations of a fast substance B is relatively greater than the acceleration of slow A produced by saturating concentrations of slow B, the reason being that the number of A–B, B–A exchanges within the membranes increases as a function both of the number and mobility of the particles within the lattice. Thus a greater acceleration of efflux is produced by a fast moving solute than by equal concentrations of slow moving solute.

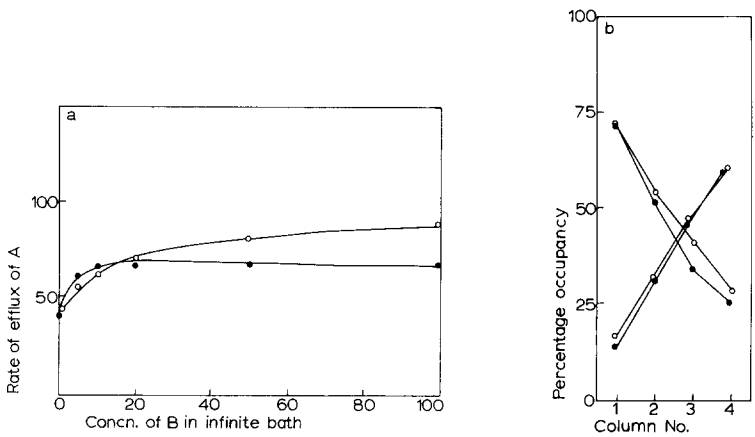


Fig. 7. a. Effect of variation in the concentration of B in the outer pool and of variation of the transition probability of B within the lattice on the rate of efflux of A from the inner pool. ●—●: slow A–slow B. Initial conditions: Concentration of A in inner pool = 500. Concentration of A in outer pool = 5. Concentration of B in inner pool = 0. Concentration of B in outer pool varied from 0 to 100. Free transition probability of A/iteration at all lattice sites = $^1/_3$. Free transition probability of B/iteration at all lattice sites = $^1/_3$. Exchange probability following A–B and B–A collisions = ½. ○—○, slow A–fast B. Initial conditions: same as above except that free transition probability/iteration at all sites of fast B = ½. b. Concentration profiles of species A and B within the lattice membrane with saturated concentration of B in outer pool. Calculation made from total occupancy of each species during the first 2500 iterations. Initial conditions as shown in a. ●---●, slow A–fast B; concentration of fast B in outer bath = 100. Lines showing concentration profiles of A have high concentration in inner columns. Lines showing concentration profiles of B have higher concentration in outer columns.

More efficient solute displacement leaves more sites available for free movement or for further additions of solute. It follows that a relatively greater acceleration in efflux is caused by displacement of slow A by fast B than by either slow A by slow B or fast A by fast B as shown in Figs. 7a, 8 and 7b.

Additionally, since the fast moving particles bind less strongly to the lattice, low concentrations of fast A in the outside medium will retard the efflux of fast A from the inner pool relatively less than the same concentration of slow A in the outer pool will retard the efflux of slow A from the inner pool, *i.e.* the unstirred layer effect will be greater with strongly binding substances than weakly binding ones. In this situation saturation concentrations of fast B in the outer pool will displace less fast A from the lattice than an equal concentration of slow B will displace slow A. This results in fast B accelerating the efflux of fast A relatively less than slow B accelerates

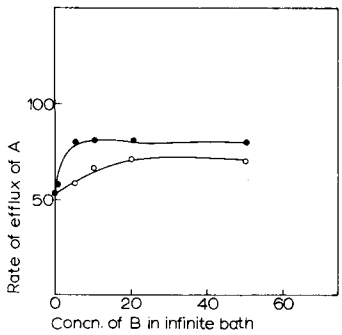


Fig. 8. Effect of variation in the concentration of B in the outer pool and the transition probability of B within the lattice on the rate of efflux of A from the inner pool. Rate measured as loss of A from inner pool during the first 2500 iterations. Initial conditions: Concentration of A in inner pool = 500. Concentration of A in outer bath = 5. Concentration of B in inner bath = 0. Concentration of B in outer bath varies from 0 to 100. Figure shows only effect of varying B. from 0 to 50. ●—●, fast A–fast B. Free transition probability/iteration at all lattice sites of A = ½. Free transition probability/iteration at all lattice sites of B = ½. ○—○, fast A–slow B. Free transition probability of A = ½. Free transition probability of B = $^1/_3$.

TABLE I

(a) EFFECT OF ADDITION OF A SATURATING CONCENTRATION OF B TO OUTER BATH ON EFFLUX OF A FROM INNER BATH (see Figs. 7a and 8)

| | | *Acceleration* (%) |
|---|---|---|
| Slow A efflux accelerated by slow B | | 170 |
| Slow A efflux accelerated by fast B | | 220 |
| Fast A efflux accelerated by fast B | | 140 |
| $K_m$ (net) for slow A and slow B | 1.5 | |
| $K_m$ (net) for fast A and fast B | 2.5 | |
| Experimental results ref. 6 | | |
| Acceleration of [$^{14}$C]glucose efflux by 130 mM external glucose | | 190 |
| Acceleration of [$^{14}$C]glucose by 130 mM external mannose | | 260 |
| Acceleration of [$^{14}$C]glucose by 130 mM external galactose | | 250 |
| Acceleration of [$^{14}$C]galactose by 130 mM external galactose | | 150 |
| Acceleration of [$^{14}$C]mannose by 130 mM external mannose | | 180 |

(b) EFFECT OF SIMULATED REDUCTION OF TEMPERATURE ON FLUX PARAMETERS OF SPECIES B (see Fig. 9)

| | $K_m$ (*net*) | $K_s$ |
|---|---|---|
| Higher temperature | 2.5 | 6 |
| Lower temperature | 1.5 | 12 |

(c) EFFECT OF INCREASING THE UNSTIRRED LAYER EFFECT ON $K_m$ (ACCELERATED FLUX) (see Figs. 4a and 5a)

| $K_m$ (*net flow*) *Both species* | $K_m$ (*accelerated flux*) | |
|---|---|---|
| | *Small unstirred layer effect* | *Large unstirred layer effect* |
| 4.5 | 6 | 10 |

the efflux of slow A (Figs. 8 and 7a). These results explain why fast mannose accelerates [$^{14}$C]mannose efflux less than slow glucose accelerates [$^{14}$C]glucose efflux (see Table I).

*The flux parameter anomalies*

*Disparity between $K_m$ (net flux) and $K_m$ (accelerated flux).* Computer solutions obtained from the lattice model can be used to measure Michaelis constants for net flow and accelerated flow, and also the dissociation constant, $K_s$. Measurement of the efflux rate of a species from the inner pool present at concentration levels more than sufficient to saturate the inner surface of the membrane with varying concentrations of the same species in the outer pool, will give a value for the operational $K_m$ for net flow of the species across the membrane: the $K_m$ (net) being the concentration of the species in the outer pool which reduces the efflux to the half maximal level. This simulates the experimental conditions used by SEN AND WIDDAS[10] to measure the $K_m$ of sugar fluxes across red cell membranes.

Since net flow occurs by movement of a single species from occupied to vacant lattice sites, the $K_m$ (net flow) of a substance is a measure of its efficiency in occupying free lattice sites. The meaning of $K_m$ (net flow) in the lattice model system closely resembles the carrier $K_m$.

The movement across a lattice of a single species in the presence of another is influenced by the coupled flow caused by exchange movement of the species between lattice sites as well as by movement between occupied and free sites. Consequently, in the case of accelerated flux, the $K_m$ now has a different significance from that ascribed by the carrier theory.

Acceleration of one species by addition of another as previously mentioned only takes place if coupled flow can offset the reduction in flow caused by the increased occupancy of the free sites. Acceleration of efflux is dependent on the concentrations of both species in the outer pool, and on their binding efficiencies to the lattice site and on their exchange probabilities; the $K_m$ (accelerated flux) will be a function of all these factors.

The measured $K_m$ of B for acceleration of A efflux increases as the concentration of A in the outer pool increases (see Figs. 4a and 5a and Table I).

The reason for this is that coupled flow is dependent on the inequality in the direction of exchanges. Increase in concentration of A in the outer compartment increases the amount of A in the outer layers of the lattice and so decreases coupled flow. Since there will be a finite amount of both species in all the columns, coupled flow will never be 100 % efficient, thus the $K_m$ (net) will always be less than the $K_m$ (accelerated).

The discrepancy between $K_m$ (net) and $K_m$ (accelerated) will be an index of the "coupling efficiency".

*Disparity between the dissociation constant $K_s$ and $K_m$ (net flux).* Measurement of $K_s$, the dissociation constant, by inhibition of net flow of A, requires that equal concentrations of B should be present in both pools.

Since the gradient of A across the membrane will induce a gradient of B, within the membrane, A will pass across the membrane by coupled exchange flow as well as by passage *via* free lattice spaces. Consequently, the concentration of B required to inhibit flow of A by 50 % "$K_s$" will be substantially greater than the $K_m$ (net) (see Fig. 9).

*The effect of temperature on the flux parameters.* Reduction in the temperature will lower the energy of the molecules bound to the lattice sites, so reducing the transition probability and consequently increasing the affinity for the lattice sites. Reduction in temperature normally increases the adsorption efficiency of any binding solute for the above reason[18]. Thus, lowering the temperature leads to an overall slowing in permeation rates ($v_{max}$) through the membranes, and reduction in $K_m$ (net). This effect has been observed with sugar flow across red cell membranes[10].

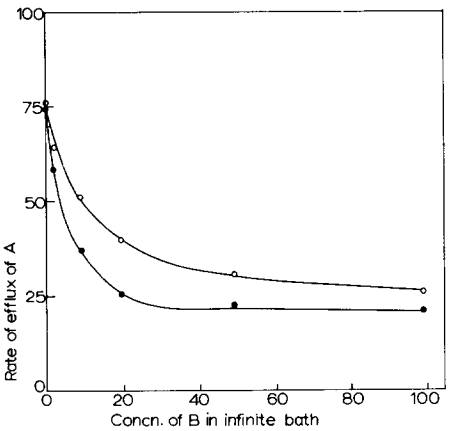


Fig. 9. Effect of simulated temperature reduction on efflux of a weakly bound species A from the inner pool with varying concentrations of B in both inner and outer pools. Rate of efflux measured as loss of A from the inner pool during the first 2500 iterations. ●—●, lattice at the higher temperature. Initial conditions for ●—●: Concentration of A in inner bath = 5. Concentration of A in outer bath = 0. Free transition probability of A within lattice = ½. Concentrations of B in both baths as indicated in figure varies from 0 to 100. Free transition probability of B = $^1/_3$. Exchange probability following A–B and B–A collision = ½. Simulation of reduction of temperature: ○—○, increased binding of A to lattice is simulated by increasing the concentrations of A in both baths. Decrease in rate of permeation and increase binding efficiency of B is modelled by reduction in transition probability of B. Initial conditions: Concentration of A in inner bath = 20. Concentration of A in outer bath = 2. Free transition probability of A remains at ½. Concentration of B in both inner and outer bath as indicated in the figure. Free transition probability of B = $^1/_6$. Exchange probability following A–B and B–A collision = ½.

However, reduction in temperature will have a much smaller effect on exchange. The kinetic energy of the entering molecule will raise the energy of the molecule on the lattice site making the probability of exchange following collision less temperature sensitive than the simple transition probability of a molecule moving to a vacant site.

On reduction in temperature both species will be bound more strongly to the membranes, consequently in the absence of the strongly bound species B, the total amount of the weakly bound species A within the membrane will be increased; providing that A is present at a subsaturation concentration at the higher temperature. Also reduction in temperature will increase the amount of A bound to the outside of the membrane because of the unstirred layer effect. Since there is now more A present within the membrane and the exchange probability is relatively unaffected by temperature, A will displace more B from the lattice than at the higher temperature, consequently the concentration of B required to reduce the flux of A to 50 % of maximal rate will be increased. The lattice model shows that $K_s$ increases as the

temperature is decreased, which explains the similar experimental findings of LEVINE AND STEIN[8].

## DISCUSSION

The essential features of the mobile carrier model for sugar flows across the red cell membrane are that the total amount of carrier is constant and that the distribution of carrier molecules within the membrane can be altered by gradients of solute across the membrane. With these assumptions the model satisfactorily explains the phenomena of saturation kinetics of net flow, accelerated exchange flow, and uphill counterflow. A drawback with the mobile carrier model is that it does not explain why the $K_m$ for net flow is less than the $K_m$ for accelerated flow, and also less than the $K_s$ value (the concentration of sugar A which reduces the flow of sugar B by 50%). Another difficulty relates to the enhancement of movements of [$^{14}$C]glucose and [$^{14}$C]mannose by mannose. Theoretically, mannose should accelerate the efflux of [$^{14}$C]mannose to a greater extent than it accelerates [$^{14}$C]-glucose, whereas the experimental observations show the contrary. In view of these difficulties an attempt has been made to formulate an alternative model for sugar transport. The lattice membrane model is an extension of a model suggested by ZIERLER[19], who proposed that sugar moved across red cell membranes through pores; the movement of sugars was hindered by the presence of sugar molecules within the membrane preventing further access to the pores. This model explains saturation kinetics of net flow but was discounted as a mechanism for sugar flow as it does not explain accelerated exchange flux or counterflow. The lattice model described above envisages flow paths across the lattice which can be considered as pores whose walls are covered with binding sites able to adsorb sugar molecules (see Fig. 10). This model has been analysed by Monte Carlo methods[20].

The application of Monte Carlo methods to the problem of diffusion flow through a lattice was first suggested by KING[21] and extension of this method provides numerical solutions for diffusion of two or more interacting solutes through a finite lattice membrane. With this method fluxes may be examined in conditions displaced far from equilibrium, when analytical solutions for flow are mathematically ex-

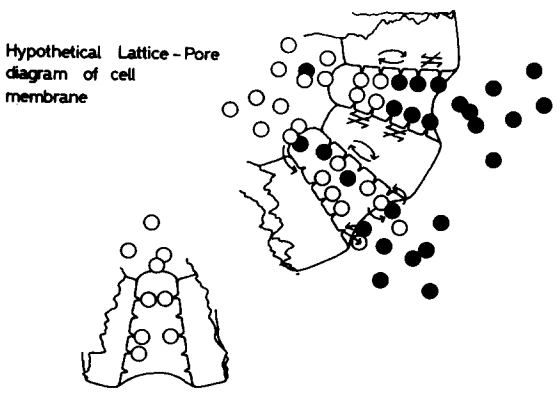


Fig. 10. Diagram showing proposed structure of water-filled channels across the red cell membrane.

tremely complex[22–25]. This procedure retains the simplicity of the statistical mechanical interpretation of diffusion and coupled flows in terms of binary molecular collisions and has the additional advantage of being applicable to complex situations[26].

Like the mobile carrier model the computer solutions of the lattice model can account for saturation kinetics of net flow, accelerated exchange flow and also uphill counterflow. However, unlike the carrier model, the lattice model accounts for the experimental observation that mannose accelerates the efflux of [$^{14}$C]glucose to a greater extent than it accelerates [$^{14}$C]mannose efflux[6,7]. Furthermore, the lattice model shows that the operationally defined parameters $K_m$ (accelerated flow) and $K_s$ exceed the $K_m$ (net flow) and the difference between $K_m$ (net flow) and $K_s$ is increased by simulation of reduction in temperature[6–9]. Consequently the kinetics of competitive sorption and diffusion within a lattice more closely resemble the real situation than carrier kinetics.

The lattice model assumes only that solute may be bound to immobile sites within the membrane, no assumptions are necessary concerning the distribution or specificity of solute binding sites other than that they should be spread across the membrane. It is also no longer necessary to postulate conformational changes in the binding material once binding has taken place.

Reversible solute binding and solute exchange at binding sites have been observed on carbon and on glass surfaces[27,28]. It has also been shown that accelerated exchange flux is not a unique property of biological membranes since accelerated exchange flows have been observed in two non-biological membrane systems[29,30]. Moreover, there is good evidence that glucose and other monosaccharides bind reversibly to red cell membranes and to phospholipid extracts of red cell membranes[31,32]. However, binding by itself provides supporting evidence for both the mobile carrier model and for the lattice model, so that the validity of either model can only be checked by determining which model has kinetics which most closely resemble the observed behaviour of sugar flows across red cell membranes.

An additional feature of the lattice model which differentiates it from the carrier model is that it suggests a possible explanation for the observation that monosaccharides reduce the hydraulic permeability[33] and stabilize red cells against osmotic haemolysis[34]. Sorption of glucose within the membrane will reduce the activity of water in the hydrophilic regions of the membrane, both by volume exclusion and by hydrogen bonding of water to the bound sugar molecules. It has already been pointed out that reduction of the hydraulic permeability of the membrane will stabilize a viscoelastic cell membrane[35].

The present results with the lattice model appear to account rather better than the carrier model for the observed kinetics of sugar flow which suggests that the mechanism of sugar transport across red cell membranes is simpler than previously supposed.

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REFERENCES


1 W. F. WIDDAS, *J. Physiol. London*, 125 (1954) 163.
2 L. LACKO AND M. C. BURGER, *Nature*, 191 (1961) 882.
3 R. C. MAWE AND H. G. HEMPLING, *J. Cellular Comp. Physiol.*, 66 (1965) 95.
4 M. LEVINE, D. L. OXENDER AND W. D. STEIN, *Biochim. Biophys. Acta*, 109 (1965) 151.
5 T. ROSENBERG AND W. WILBRANDT, *J. Gen. Physiol.*, 41 (1957) 189.
6 D. M. MILLER, *Biophys. J.*, 8 (1968) 1329.
7 D. M. MILLER, *Biophys. J.*, 8 (1968) 1339.
8 M. LEVINE AND W. D. STEIN, *Biochim. Biophys. Acta*, 127 (1966) 179.
9 W. D. STEIN, *J. Gen. Physiol.*, 54 (1969) 815.
10 A. K. SEN AND W. F. WIDDAS, *J. Physiol. London*, 160 (1962) 404.
11 A. R. EDWARDS, *Computer J.*, 2 (1959) 181.
12 T. L. HILL, *Thermodynamics for Chemists and Biologists*, Addison Wesley, Reading, Mass., 1968, p. 137.
13 R. I. SHAAFI, G. T. RICH, V. W. SIDEL, W. H. BOSSERT AND A. K. SOLOMON, *J. Gen. Physiol.*, 50 (1967) 1377.
14 P. G. LEFEVRE, *Am. J. Physiol.*, 203 (1962) 286.
15 D. M. MILLER, *Biophys. J.*, 5 (1965) 417.
16 S. KOZAWA, *Biochem. Z.*, 60 (1914) 231.
17 W. WILBRANDT, *Arch. Ges. Physiol.*, 241 (1938) 289.
18 J. J. KIPLING, *Adsorption of Non-Electrolytes from Solution*, Academic Press, New York–London, 1965, p. 101.
19 K. ZIERLER, *Bull. Johns Hopkins Hosp.*, 109 (1961) 35.
20 J. M. HAMMERSLEY AND D. C. HANDSCOME, *Monte Carlo Methods*, Methuen and Co., London, 1964.
21 G. W. KING, *Ind. Eng. Chem.*, 43 (1951) 2475.
22 T. L. HILL, *J. Theoret. Biol.*, 10 (1966) 442.
23 J. LANE AND J. S. KIRKCALDY, *Can. J. Phys.*, 42 (1964) 1643.
24 J. LANE AND J. S. KIRKCALDY, *Can. J. Chem.*, 43 (1965) 1812.
25 S. PRAGER, *J. Chem. Phys.*, 21 (1953) 1344.
26 T. L. HILL AND O. KEDEM, *J. Theoret. Biol.*, 10 (1966) 399.
27 R. N. SMITH, C. F. GEIGER AND C. PIERCE, *J. Phys. Chem.*, 57 (1953) 382.
28 J. W. SHEPARD AND J. P. RYAN, *J. Phys. Chem.*, 63 (1959) 1729.
29 R. J. NAFTALIN, *J. Physiol. London*, 204 (1964) 55P.
30 D. VAN BREEMAN AND C. VAN BREEMAN, *Nature*, 223 (1969) 898.
31 A. D. PERRIS AND D. K. MYERS, *Nature*, 207 (1967) 986.
32 W. GOOD AND S. M. ROSE, *Biochim. Biophys. Acta*, 163 (1968) 483.
33 P. G. LEFEVRE, H. I. HABICH, H. HESS AND M. R. HUDSON, *Science*, 143 (1964) 955.
34 H. BOBINSKI AND W. D. STEIN, *Nature*, 221 (1966) 1366.
35 A. KATCHALSKY, O. KEDEM, C. KLIBANSKY AND A. DE VRIES, in A. L. COPLEY AND G. STAINSBY, *Flow Properties of Blood and other Biological Systems*, Pergamon Press, London, 1960, p. 155.