

Intra-Protein Interactions Across a Fluid Membrane as a Model for Biological Transport

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ABSTRACT A model is proposed for the mechanism of action of the glucose transport system of the human erythrocyte. The model is based on the possibility of there being interaction through the membrane between superficially disposed protein subunits, these units being embedded within the bimolecular lipid layer, anchored to the aqueous phase, perhaps mobile in the plane of each face of the membrane. The subunits have the ability to bind sugar and, when associated with the symmetrical protein at the opposite face of the membrane, transfer sugar across the membrane. Evidence for the model is presented. The possibility that this model may also be a model for the cell membrane as such is briefly touched upon.

(*Note Added in Proof.* The paper presented at the Symposium described a detailed model for glucose transport within the general framework of the ideas developed below. Insofar as work subsequent to the meeting has shown that the original version of the model did not give a correct description of the kinetics, the kinetic treatment of that model has been omitted. The current version of the model will be submitted for publication elsewhere.)

In this paper I present a model for the cell membrane as a bimolecular layer of lipid molecules interspersed with protein subunits, the latter extending only halfway through the membrane (Fig. 1). The proteins are considered to possess a hydrophobic surface where they interact with lipid molecules or other proteins, but a hydrophilic surface where they interact with the aqueous phase. The proteins, like the lipids, are therefore amphiphilic and are anchored in the surface of the membrane. It is a special feature of the model that the cell membrane is essentially fluid, although highly viscous, so that the molecular components of the membrane might be free to move in the plane of the membrane parallel to the surface. I propose that intra-protein interactions can occur *across the membrane*, normal to the surface, between protein subunits anchored in opposite faces of the membrane. These interactions

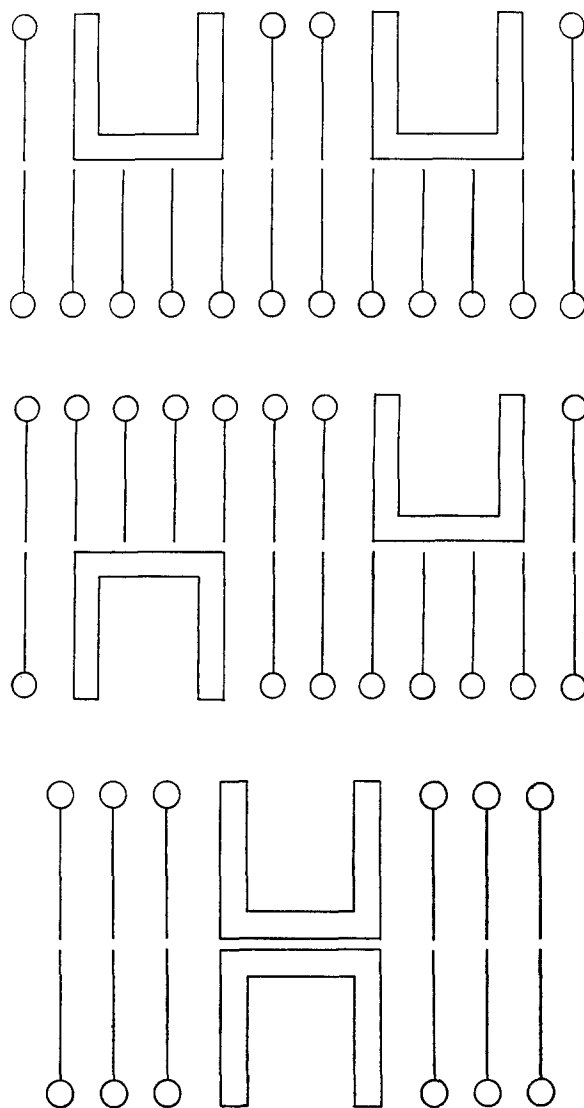


FIGURE 1. A model of a fluid, amphiphilic membrane. Lipids are represented as lollipops, with circles as charged groups. Proteins are represented as U-shaped objects. Bonding between protein and lipid is hydrophobic. Top, two protein subunits are on the same side of the membrane; middle, the two subunits are arranged symmetrically; and bottom, the two subunits are associated.

are again essentially similar to such intra-protein interactions as are well known to occur in aqueous solution. A particular case of such intra-protein interaction across the membrane involves the transfer of a substrate from one subunit to another. I suggest that this is the mechanism of facilitated diffusion. The model is presented in terms of the glucose transport system of the human erythrocyte, for which system the model was first considered. I propose the model, however, as a general solution to problems of cell membrane structure and function.

GLUCOSE TRANSPORT IN HUMAN RED CELLS

There is abundant evidence that glucose and other sugars are transported across the cell membrane of the human erythrocyte by a specific system (1). This is one of the "facilitated diffusion" systems, which enable their substrates to reach rapidly an equilibrium distribution across the membrane. A number of such systems exist in the erythrocyte membrane. In addition, there are present active transport systems, which bring about the concentration of their substrates within the cell. Although the model of a "mobile carrier" has been very successful in accounting for many of the properties of these systems, there are a number of pieces of evidence (which I list below) which are difficult to reconcile, or indeed cannot be reconciled, with this model. In addition the model does not fit naturally into the available models of the cell membrane.

I shall first list briefly certain experimental observations which are at variance with the carrier model and then postulate the new model in a formal manner, considering how it accounts for the available experimental data. Finally, I shall discuss some of the consequences of the model.

OBSERVATIONS WHICH ARE DIFFICULT TO RECONCILE ON THE "MOBILE CARRIER" MODEL

1. The value of the Michaelis constant K_m , which describes how the rate of glucose transport varies with the concentration of sugar, depends on the experimental situation used to determine K_m , and is often substantially smaller than K_i , the inhibition constant for the sugar. Such is the case where K_m is measured by the procedure of Sen and Widdas (2), namely, by observing the net outflow of sugar from preloaded cells exposed to external solutions varying in their sugar concentration. K_m here decreases as the temperature is lowered reaching a value of 1.1 mM at 13°C and, by extrapolation, some 0.5 mM at 5°C. In contrast, the inhibition constant, K_i , for glucose inhibiting the movement of sorbose, increases slowly as the temperature is lowered and reaches a value of 19 mM at 13°C (3). If K_m is measured in equilibrium tracer experiments, where sugar is present at the same concentration on both sides of the membrane, its value parallels the K_i values, being 20 mM

at 13°C and as high as 44 mm at 5°C (3). I have tried repeatedly to resolve this paradox but have not been able to devise a model based on a mobile carrier which can account for a discrepancy of this proportion. In particular, if one assumes, as has been suggested for enzyme kinetics (4), that the carrier substrate complex is not in equilibrium with free carrier or substrate, i.e. that the rate of transport of the carrier is considerable, one arrives at the result that $K_m = K_s + k_2/k_{-1}$, where k_2 is the rate of transport of the carrier substrate complex, k_{-1} the rate of its breakdown, and K_s the dissociation constant. Hence K_m is *greater* than K_s , not considerably less than K_s , as we find. We will see that the resolution of this paradox lies in the realization that the binding of glucose at one face of the membrane to a carrier, enhances the ability of a second glucose molecule at either face of the membrane to be transported by the carrier.

2. Glucose transport is inhibited by reaction of the cells with 2,4:fluorodinitrobenzene (FDNB) and the inhibition follows some extraordinary patterns. Bowyer and Widdas (5) found, and we have repeatedly confirmed, that the rate of disappearance of reactivity to transport sugar is proportional to the square of the concentration of FDNB. In addition, however, this rate is reported to be proportional to the square of the transporting activity still remaining to be inactivated. The former finding suggests merely that the carrier contains a pair of reacting groups per molecule and that these groups cooperate somehow in reaction. But the latter finding, a consideration of which provided a clue to the understanding of this system, suggests that pairs of carrier molecules interact at some stage during the process we are considering. In particular, the evidence demands not only that pairs of "carriers" interact, but that any carrier can react with any other carrier—since the reaction rate is higher than first order over the whole course of the inactivation. This can only mean that the carriers are indeed mobile—but in a direction parallel to the membrane surface. (In addition, the inactivation reaction has an unusually high Q_{10} ($Q_{10} = 7$) (5).)

3. I have argued elsewhere (6) that a "mobile carrier" which diffuses through the lipid from face to face of the membrane cannot account for the high turnover number of glucose transport.

These considerations, together with the inherent symmetry of the facilitated diffusion systems, led to the formulation of the following model.

A MODEL FOR FACILITATED DIFFUSION

I propose that facilitated diffusion is the interchange of substrate between two subunits of a protein, the subunits being symmetrically disposed about the membrane. The proteins are probably free to move in the plane formed by this essentially symmetrical membrane, although confined to this plane. In detail:

1. The cell membrane is a bilayer, each layer being composed in part of

lipid molecules, arranged as in the now classic Davson-Danielli model, and in part of protein (Fig. 1). These proteins are confined to one side of the bilayer, being, like the phospholipid molecules of the bilayer, amphiphilic—having a hydrophobic surface which inserts within the lipid molecules and a hydrophilic surface anchoring the protein to the aqueous phase. The membrane is

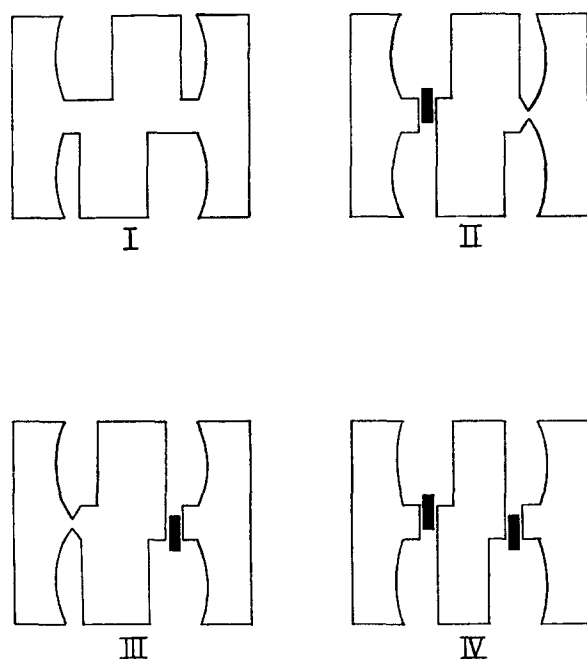


FIGURE 2. Transfer of substrate through a protein plug formed by the association of two subunits, symmetrically disposed about the membrane. In I, the two subunits associate; in II, they have bound a lozenge-shaped molecule of substrate from the outer face; in III, they have so bound, from the inner face; in IV, two substrate molecules have been bound.

fluid and both proteins and lipids in a particular half of the membrane are able to move more or less freely within this plane.

2. Certain of these proteins (Fig. 1, bottom) can form complexes with their symmetric fellows on the opposite face of the membrane. Within the membrane, I suggest, one will find a range of associating protein systems, just as in the aqueous phase one finds the oligomeric associating proteins. Each species of protein can, of course, recognize and associate only with its respective partner. There will be a range of values for the association constants.

3. Of these associating symmetric subunits, certain will have the property of binding substrates entering the protein from the aqueous phase. But unless such a subunit-substrate complex is bound to the subunit from the *trans*-face, the substrate cannot cross the membrane (Fig. 1, middle) being opposed by a layer of lipid or indifferent protein. The pores are blind. When the cor-

rect associating partner is encountered, a symmetric dimer (or tetramer) is formed (Fig. 2), the substrate can pass from one subunit to the other and thus traverse the membrane.

THE KINETICS OF THE MODEL

(*Note Added in Proof.* In the oral presentation of this paper there followed a kinetic analysis of a transport model in which it was assumed that the subunits on either side of the membrane were mostly to be found dissociated and that transport followed upon the coming together of subunits already bound to substrate. I am indebted to Prof. P. G. Le Fevre and to Dr. W. R. Lieb for pointing out to me that that model indeed failed to give the correct kinetics. That model did not account for the discrepancy between the K_m values referred to above and did not allow for a sufficient rate of transport—the number of interactions which can occur between isolated subunits is too low. To account for the observed rates of transport most of the subunits must, at any one time, be in the oligomeric form. Dr. Lieb and I are completing the theoretical analysis of a more satisfactory model, within the framework of the ideas discussed above, and the results of our analysis will be submitted for publication elsewhere.)

It may be as well to summarize the key observations that support the present model. These are (*a*) that the rate of inactivation by FDNB depends to a higher than first order on the transporters still available for reaction. It follows therefore that the binding sites interact in pairs and hence are freely mobile parallel to the membrane surface—but not that glucose transport depends on this particular directional component of carrier velocity; (*b*) that K_m as measured by Sen and Widdas is, at low temperatures, substantially less than K_m measured by tracer exchange. It follows that the binding of glucose at the *trans*-face affects the transport of glucose from the *cis*-face. Hence it follows that the glucose-binding sites are in communication across the membrane—but not that the glucose-binding sites are free to dissociate and reassociate. It might well be possible to construct a more classical model which accounted for both of these findings.

IMPLICATIONS OF THE MODEL

While no other system seems to have been studied with the same intensity as has the glucose transport system, there are a number of indications that the model here presented might be more widely applicable. I might mention the second order dependence of uptake on substrate concentration found for proline transport in pancreas slices (10), arabinose transport in rat heart (11), glucose transport in adipose tissue cells in the absence of insulin (12), and in leukocytes and tumor cells (13); and the second order dependence on the concentration of inhibitor or activator in the cases of proton and copper

inhibition of glycerol transport in human red cells (14), competitive inhibition of proline uptake in pancreas slices (10), activation of tryptophan uptake by other amino acids in ascites tumor cells (15), and finally the sodium ion activation of glycine uptake by pigeon erythrocytes (16). The present model would predict this type of finding but other models may do so as well. It is of much interest, however, that for sugar uptake by mouse fibroblasts in tissue culture (17), a consistent finding that K_m was an order of magnitude less than the inhibition constant K_i was reported.

The wider implications of the model are, however, its possibly more general relevance to the problem of membrane structure. The model as suggested accommodates the recently accumulated data on the presence of hydrophilic interaction between protein and lipid in membranes (18, 19) and also the report that there is insufficient lipid in the membrane to cover completely a bimolecular layer (21). There seems for the red cell to be only sufficient lipid to cover 70 % of each surface, leaving 30 % of each face to consist of proteins arranged as in Fig. 1. The model accounts also for the data on the ratio of the hydrophilic to apolar amino acid content of the membrane structural protein, a point I hope to be able to develop further elsewhere.

Two other points should perhaps be mentioned. The model includes the concept of protein subunits being perhaps mobile within the membrane and of the possibility of reactions taking place in the membrane in a hydrophobic environment, shielded from the aqueous phase. The importance of this consideration for electron transport and photosynthesis is clear. In such a hydrophobic phase, hydrophobic bonds will be weak, hydrogen bonds strong, reversing the familiar order found from considerations of aqueous solutions. It appears that it may be necessary to develop methods for studying reactions and interactions in such hydrophobic environments. The high viscosity of the membrane will slow the diffusion-controlled steps of such reactions, but since the amphiphilic reactants are highly concentrated within the small volume that is the membrane, and are restricted to move in two dimensions rather than three, as in the bulk solution, reaction rates comparable with those in free solution might yet be found.

Finally, the concept of subunits interacting across the membrane allows for the passage of information across the membrane without the passage of substances. This would be consistent with theories of the mechanism of action of the protein hormones which depend on the possibility of their interaction with superficial receptors, which then communicate with specific receptors at the inner face of the membrane. The signal that a substance X is present outside the cell is transmitted into a conformational change in the receptor of X , at the outer face and in turn this affects the behavior of Y , an X receptor, that is, an associating subunit of X , present at the inner face.

In summary, I propose a model for the glucose transport system of the hu-

man erythrocyte based on the possibility of interaction through the membrane of superficially disposed protein subunits, these units being embedded within the bimolecular lipid layer, anchored to the aqueous phase and confined to the plane of each face of the membrane. The subunits have the ability to bind sugar and, when associated with the symmetrical protein at the opposite face of the membrane, transfer sugar across the membrane. The possibility that this model may also be a model for the cell membrane as such is briefly touched upon.

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REFERENCES

1. STEIN, W. D. 1967. *The Movement of Molecules Across Cell Membranes*. Academic Press, New York.
2. SEN, A. K., and W. F. WIDDAS. 1962. *J. Physiol. (London)*, **160**:392.
3. LEVINE, M., and W. D. STEIN. 1966. *Biochim. Biophys. Acta*. **127**:179.
4. DIXON, M., and E. C. WEBB. 1964. *Enzymes*. Longmans, Green, London.
5. BOWYER, F., and W. F. WIDDAS. 1956. *Disc. Faraday. Soc.* **21**:251.
6. STEIN, W. D. 1968. *Nature*. **218**:570.
7. USSING, H. H. 1949. *Acta Physiol. Scand.* **19**:43.
8. LEVINE, M., D. L. OXENDER, and W. D. STEIN. 1965. *Biochim. Biophys. Acta*. **109**:151.
9. BOWYER, F., and W. F. WIDDAS. 1958. *J. Physiol. (London)*. **141**:219.
10. BEGIN, N., and P. G. SCHOLEFIELD. 1965. *Biochim. Biophys. Acta*. **104**:566.
11. MORGAN, H. E., E. CADENAS, D. M. REGEN, and C. R. PARK. 1961. *J. Biol. Chem.* **236**:262.
12. CAYGILL, C. R. 1968. Doctorate thesis. University of Manchester, Manchester, England.
13. LUZZATTO, L., and G. LEONCINI. 1961. *Ital. J. Biochem.* **10**:249.
14. STEIN, W. D. 1962. *Biochim. Biophys. Acta*. **59**:47.
15. JACQUEZ, J. A. 1963. *Biochim. Biophys. Acta*. **71**:15.
16. VIDAVER, G. A. 1964. *Biochemistry*. **3**:662.
17. CRANE, R. K., R. A. FIELD, and C. F. CORI. 1957. *J. Biol. Chem.* **224**:649.
18. WALLACH, D. F. H., and P. H. ZAHLER. 1968. *Biochim. Biophys. Acta*. **150**:186.
19. CHAPMAN, D., V. B. KAMAT, and R. J. LEVENE. 1968. *Science*. **160**:314.
20. KORN, E. D. 1966. *Science* **153**:491.
21. MILLER, D. M. 1965. *Biophys. J.* **5**:417.
22. BRITTON, H. G. 1964. *J. Physiol. (London)*. **170**:1.
23. HARRIS, E. J. 1964. *J. Physiol. (London)*. **173**:344.
24. WIDDAS, W. F. 1954. *J. Physiol. (London)*. **125**:163.
25. LEFEVRE, P. G., and G. F. MCGINNISS. 1960. *J. Gen. Physiol.* **44**:87.

Discussion from the Floor

Dr. Pardee: I want to thank Dr. Stein for a most elegant presentation of a very lovely model. It suggests some experiments, which I think is most important. Rather than using the format of this morning, I will open this talk to questions now. We will limit the time for questions; if there is time at the end of the entire session, we can have further questions.

Dr. Harden McConnell (Stanford University, Calif.): There is very solid direct physi-

cal evidence for highly fluid regions in membranes, obtained by dissolving paramagnetic molecules in these membranes; molecules as large as steroids undergo very rapid and nearly isotropic motion in the membrane, so that part of your model is not an assumption.

Dr. Stein: Thank you very much, Sir. We have been able from the temperature dependence of glucose transport, on the basis of the hemiport model, to get some idea of the actual rates at which these diffusions are occurring. Apparently, there is an activation energy of 40 kcal/mole, which we can compare with your data. These are essentially very high numbers compared with the 5's and 10's found for aqueous diffusion.

Dr. I. Bihler (University of Manitoba, Canada): Dr. Stein, I am sure you must have calculated the activation energy of binding or transport and its relation to the number of hydrogen bonds severed. How consistent are the results with your hemiport model?

Dr. Stein: I did the following: Calculated from the temperature dependence of the binding constant K_s for the binding of one hemiport with glucose, that there is of the order of 5 kcal/mole of binding between a substrate and the hemiport itself. The association, i.e. the overall association of two hemiports with one another, is governed by a reaction which has a Q_{10} —an activation energy—of 40 kcal/mole. But as for the binding constant for this reaction, if the reaction occurs, we haven't been able of, course, to begin to understand how we might measure that.

I might say just one thing, while I am talking about Q_{10} 's, and that is that the rate of inactivation of transport by fluorodinitrobenzene has another peculiarity—an astonishingly high Q_{10} of 7. And if there is time later, I will come back to that.

Dr. F. H. Wallach (Harvard Medical School, Boston, Mass.): The hemoglobin analogy is a good one from several points of view, and one of them is, that the hemoglobin tetramer has a pore running through it. It is 5–10 Å units in diameter, is filled with water, and has some other interesting properties.

Also Dr. Weinstein at the Massachusetts Hospital has done some rather higher resolution freeze-cleaving electron microscopy of erythrocyte ghosts and has shown that a significant number of membrane-associated particles actually penetrate right through the membrane.

Dr. Stein: Yes, the hemoglobin analogy was useful and might indicate that there are permanent pores in the membrane. But, indeed, as far as the second point is concerned, it is only for the glucose that I had to assume that the two hemiports are completely, or most of the time dissociated. And in the published paper, I make it clear that this assumption for that system is certainly incorrect.

Dr. V. S. Vaidyanathan (State University of New York, Buffalo, N.Y.): I would like to add that I welcome Dr. Stein's suggestion regarding the fluid nature of membranes with not so well-defined structure, and that this has been the basis for theoretical treatment of transport across membranes in a number of papers published in the *Journal of Theoretical Biology*. In these papers, the membrane system is viewed as a multicomponent fluid system with the restriction that a subset of these components have zero mobility.

Dr. Stein: Well, how big are these subsets?

Dr. Vaidyanathan: Multicomponent system essentially means any number of components that one may identify. Subset only means that the number of such components

whose mobility is conceived as vanishing should be less than the total number of components. If the components in a subset equal the total components and these are fixed, then one approaches solid state. If the subset equals just one component then this may refer to the membrane being considered as a single component. This type of approach facilitates application of equations of hydrodynamics and the current statistical theory of transport in multicomponent fluid mixtures and electrolytes to transport of ions and neutral molecules across the membrane.

Dr. Alvin Essig (Tufts Medical School, Boston, Mass.): It seems to me that the isotope exchange flow and the net flow which you have described are, in principle, of completely different character, as shown by the fact that the "permeability coefficients" for two such processes are often far from equal. I don't understand therefore why you expect that the two Michaelis constants should be similar.

Dr. Stein: I don't really understand the argument. If one sets up the conventional models, based on a mobile carrier or a reorientating carrier, then one cannot accommodate the phenomena that the experiments reveal. We have looked into this very thoroughly. In one case we have merely an isotopic change, in the other case we are seeing a sum total of flows in two directions across the membrane.

Dr. Essig: But the permeability coefficients are often very different.

Dr. Stein: Oh, no, I never say that they are the same.