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The transport of amino acids, amino acid derivatives and ions across ion-exchange membranes

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The passage of inorganic salts, glucose, amino acids and peptides across polystyrene-backed double membranes (negative-positive fixed-charge junctions) was studied in a two-compartment cell and compared to a known cellular system, the Ehrlich-Lettre ascites carcinoma. It was concluded that passage proceeds by a 1:1 exchange of diffusing ions in the membrane. The more rapidly transported systems reflected an increased probability of exchange in all cases, as evidenced both by a saturation effect and by the degree to which the space charge was perturbed at the membrane. The amino group (as NH_3^+) of the amino acid involved in the exchange process was vital for transport. The presence of a second amino group, either ionized or as $-NH_2$, accelerated the exchange. The presence of electron-attracting groups on the side chain or of a methyl group on the alpha carbon also facilitated passage. Cation dependence was seen. Passage was hindered by a second carboxyl group, an alcoholic group, or a lengthened side chain. Use of double membranes permits experimental electrodic transport modelling and may facilitate design of a drug delivery system.

Introduction

Biological membranes are organized as a fluid mosaic consisting of a phospholipid bilayer in which are dispersed a variety of protein complexes existing in dynamic equilibrium [1]. Lipids and protein complexes can move laterally in the plane of the membrane [2]. Charges transfer across the membrane of topologically closed vesicles [3]. Membrane surfaces are highly charged, orienting molecules in narrow intermembrane space [4]. A membrane potential is required for structural maintenance [5].

Active transport across certain biological membranes has been well demonstrated. Solutes recognized as 'actively' transported are in fact driven by coupling to the downhill movement of specific ions: sodium ions in animal cells and protons in prokaryotic cells and walled eukaryotes [6]. The sodium ions or protons are in turn driven uphill through the same membranes by coupling to ATP hydrolysis [7]. The molecular devices that accomplish coupling have become known as cotransport systems (the 'driver' ion and coupled solute move in the same direction) or countertransport systems. Initial observations that many transport systems exhibit Michaelis-Menten kinetics led to attempts to describe carrier-mediated transport in terms analogous to enzyme kinetics. Experiments with a wide range of cellular membranes have revealed great kinetic heterogeneity in cotransport and countertransport mechanisms, with the proliferation of a variety of ad hoc explanations [8].

Recently Sanders et al. [9] described a minimal model of cotransport. The model consists of a single transport loop linking six discrete states: one trans-membrane charge-transport step and one step each for binding of substrate and driver ion

at each side of the membrane. Specific behavior of the model depends upon the size of individual reaction constants among the whole set in the single transport loop proposed. The model does not require most postulates of special mechanisms in cotransport. The observed experimental diversity in cotransport kinetics reflects control-related selection of reaction rate constants.

These considerations lead to the conclusion that transitions or junctions of fixed charge densities of opposite sign will present highly localized space charge regions and confer upon the system the conservative property of capacitance. The property of asymmetrical conductance is related to the concentration of the ions in solution, determined by the field created by the junctions of fixed charges.

The Ehrlich-Lettre ascites carcinoma has been well studied. Maintained cell lines are employed for comparative drug testing. Free cells are able to accomplish the inward transfer of amino acids from the suspending medium against considerable concentration gradients [10]. Most of the accumulated amino acid remains unbound [11]. The uptake was noted to be exponential with time and also exponentially dependent upon the ratio of intracellular to extracellular amino acid concentration [12]. Amino acid distribution was found to approach a time-independent value within 15 min after addition of glycine to the suspending medium [13]. Inward transport is dependent upon the presence of a cation gradient in the opposite direction [14]. A proton group is released in the transport reaction [15].

The purpose of the present study is to examine the phenomenon of exchange transport in a model system that is controlled and metabolically independent and to compare that transport to a known cellular system. Knowledge gained may facilitate design of a drug delivery system. To achieve this purpose, the movement of inorganic salts, glucose, amino acids and amino acid derivatives across polystyrene-backed ion-exchange double membranes was examined.

Methods and Materials

All measurements were performed in a lucite cell consisting of two chambers ($9 \times 7 \times 7$ cm) of approx. 400 ml capacity, divided by a lucite partition in the center of which was an aperture (2×2 cm) which could be covered by the membrane used. Furthermore, there was a removable stainless steel shutter which covered the aperture and separated the solutions on either side of the partition until time zero. The two chambers were filled with stirred solutions of the amino acid, glucose, or the electrolyte to be studied. One pair of electrodes was arranged to measure the voltage across the junction, one pair to measure the current.

Electrical measurements were obtained at $24 \pm 1°C$ with a Radiometer TTT-1a pH meter connected to an SBR2C recorder and a Simpson 260 microammeter. The amino acid solutions were made up with 0.1 M NaCl and maintained at a pH of 6.8 with potassium phosphate buffer throughout the electrical measurements.

The extent of amino acid transport was determined by titrating aliquots of the amino acid solutions in each compartment at various time intervals with biuret reagent. The extent of other electrolyte movement was determined in analogous fashion with the use of a Beckman flame photometer with an internal lithium standard. Glucose concentrations were determined on a two-channel Technicon autoanalyzer using the ferric cyanide method. The results were plotted and the rates of diffusion were determined graphically from the slopes of the lines drawn by the method of least squares.

The transport of amino acids and their derivatives, inorganic salts, and glucose across single anionic or cationic membranes, and across a junction of negative-positive fixed charge in which cation and anion exchange membranes were employed as closely apposed pairs, was studied.

The membranes used were polystyrene-backed (Nepton, Ionics, Inc., Cambridge, MA, U.S.A.), one containing negative fixed charge, SO_3^-, the other a positive fixed charge, NH_3^+. The negative-positive fixed charge junctions of the model system were formed by cementing together membranes (70 μ-thick) of each charge type with dimethylformamide, allowing the combination to dry for 24 h under pressure before use.

The amino acids and derivatives used were commercial preparations of the highest purity. Inorganic compounds and glucose used were all of

analytical reagent grade. Deionized, distilled water was used to prepare all solutions.

Experimental results

The capacitance of the constructed negative-positive fixed charge junction measured 1 $\mu F/cm^2$ with a shunt resistance of $5 \cdot 10^4/cm^2$. Hydrogen ion is many times more mobile than is any other cation in this water-solvated system [16]. Pore size as an influence is negligible [17].

The variation of ion flux with concentration gradient through a negative fixed charge membrane (using sodium chloride) was found to be exponential. The results indicate that the rapidity of the exchange is related to the availability of exchangeable opposing ions. The membrane was then soaked in electrolyte for 24 h and the variation of ion flux with the concentration of charge at the membrane was determined. Rapidity of exchange was determined to relate to the availability of exchangeable sites at the membrane. The number of sites, not the ion present, is important. Transport proceeds by a 1 : 1 exchange of diffusing ions at the membrane, the more rapidly transported systems reflecting the increased probability of exchange. The addition of charge, as ions to be exchanged, results in an ohmic drop across the membrane as a whole, a reflection of the accelerated transport rates. The ability to exchange is the limiting process.

TABLE I

RATES OF ION MOVEMENT THROUGH SINGLE MEMBRANES

K^+, Na^+ and Li^+ were present as their chloride salt; Cl^- and Br^- were present as their sodium salt. *n* represents the number of ions in the hydration sphere of the cation [18].

Ion	Relative rate	Measured rate (mol/min)	*n*
Cation exchange membranes (negative fixed charge junction)			
K^+	1.00	$46 \cdot 10^{-4}$	1.9
Na^+	0.70	$30 \cdot 10^{-4}$	3.5
Li^+	0.54	$25 \cdot 10^{-4}$	7.1
Anion exchange membranes (positive fixed charge junction)			
Cl^-	1.00	$30 \cdot 10^{-4}$	
Br^-	0.86	$26 \cdot 10^{-4}$	

The relative rates of movement for various cations through a negative fixed charge membrane as determined in this system correlated with the number of oppositely charged ions or dipoles in the hydration sphere, a reflection of the relative extent to which the diffusing ion perturbs the space charge at the membrane. These are shown in Table I, where the relative diffusing rates of various ions through a positive fixed charge membrane are also detailed. These data taken together indicate that the nature of the electrolyte is unimportant when an exchange of mobile species can be made at the membrane. When a second electrolyte with a lesser ability to accommodate oppositely charged ions in its ionic sphere and consequent inability to perturb the space charge at the membrane surface is added to the system, a retardation of transport results. Analogously, an acceleration of transport may be induced when an ion electrolyte which can further perturb the membrane charge is added. A shunt resistance of $13/cm^2$ was measured for the anion exchange membrane (chloride form).

The rates of movement of various amino acids and derivatives through a negative fixed charge membrane and through a positive fixed charge

TABLE II

RATES OF DIFFUSION THROUGH SINGLE MEMBRANES

Amino acid	Relative rate	Exchange membrane, measured rate (10^4 mol/min)	
		cation	anion
Glycine	1.00	1.25	0.55
Alanine	0.63	0.79	0.33
Asparagine	0.58	0.72	0.30
Serine	0.56	0.70	0.33
Aspartic acid	0.54	0.68	0.35
Cysteine	0.2	0.65	0.29
Arginine	0.52	0.65	0.28
Lysine	0.50	0.63	0.27
Diglycine	0.47	0.59	0.26
Glycine ethyl ester	0.43	0.54	0.28
Methionine	0.45	0.56	0.25
Histidine	0.48	0.53	0.25
Phenylalanine	0.30	0.38	0.20
Leucine	0.16	0.20	0.09
Triglycine	0.13	0.16	0.07
N-Acetylglycine	0.01	0.01	0.01

membrane as determined in this model system are presented in Table II. These too are exponential for both membrane types. Variations in the companion electrolytes used to swamp the system did not appreciably affect the transport times of the various amino acids. Removal of the electrolyte reduced transport times considerably, a consequence of the necessity for exchange at the membrane surface. The decrease in the rate of movement of glycine peptides parallels the increase in the dielectric permittivity of the diffusing solution. The data in Table II indicate that the rates of transport of those amino acids with short chains were greater than those with long side chains, which was apparently not a consequence of 'pore size', as glycylglycine moves readily in this system. The response of the membrane to the various amino acids resembles that to the various ion types.

At no time could transport be demonstrated with glucose solutions alone.

In the presence of abrupt transition regions of fixed charge, a negative-positive fixed charge junction formed by cementing anionic and cationic membranes together, striking phenomena were observed in the transport of sodium ion and potassium ion. The concentration of potassium ion increased, that is K^+ flowed into compartment two, regardless of whether it was present in greater or lesser concentration in the other compartment. Sodium ion was concentrated also, even in the presence of high potassium ion concentration in that compartment, though not as rapidly as potassium ion. The difference was apparently related to the relative ability to be exchanged with hydrogen ions at the membrane surface. The membrane response is given in Fig. 1, which shows the ion flux, and Fig. 2, which reflects the capacitance and rectification properties of the system in which small perturbations of charge at the membrane surface lead to nonohmic alterations in resistance. It appears that the cell is in nonequilibrium with respect to concentrations in the outer solution at all times.

Analogous phenomena were also observed in the transport of amino acids. The transport rates of several amino acids and derivatives are given in

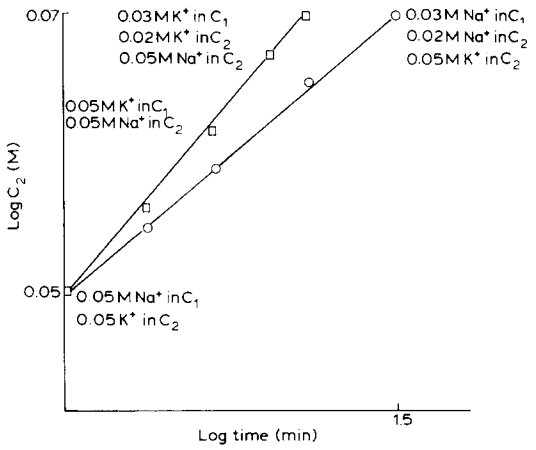

Fig. 1. Ion flux with double membrane (positive-negative fixed charge junction). Initially, C_1 contained 0.5 M KCl or 0.5 M NaCl, while C_2 contained 0.5 M NaCl or KCl. Finally, C_1 contained 0.3 M KCl or NaCl, while C_2 contained the original 0.5 M KCl or NaCl plus 0.2 M of the KCl or NaCl which was transported.

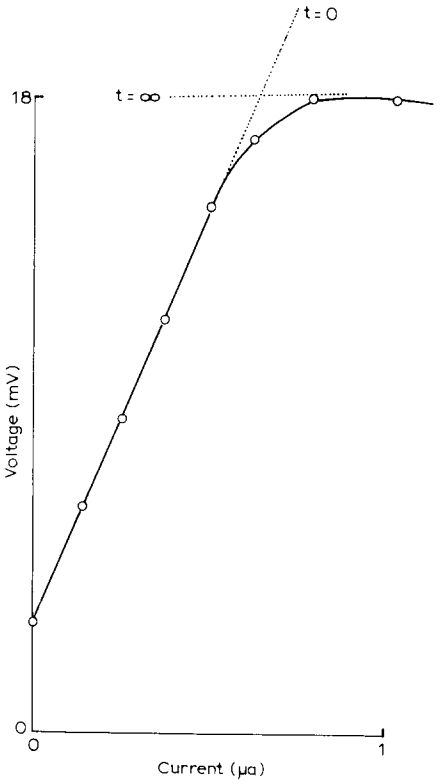

Fig. 2. Conductance properties of double membrane (positive-negative fixed charge junction).

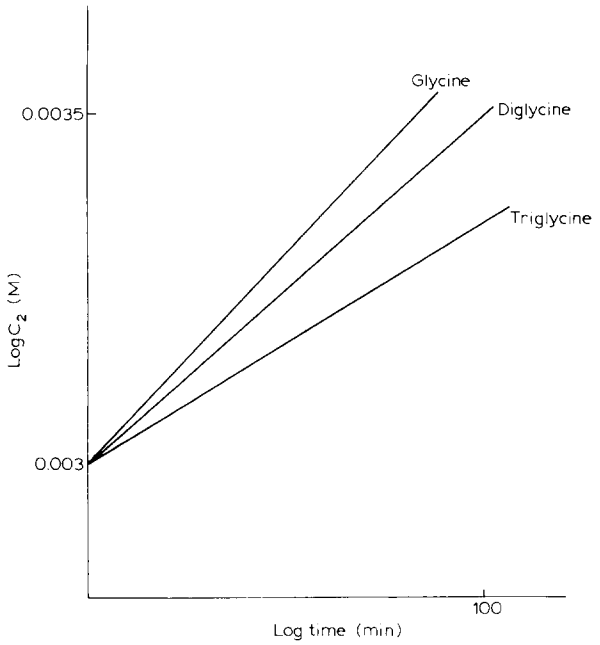

Fig. 3. Flux of glycine and derivatives across a negative-positive fixed charge junction.

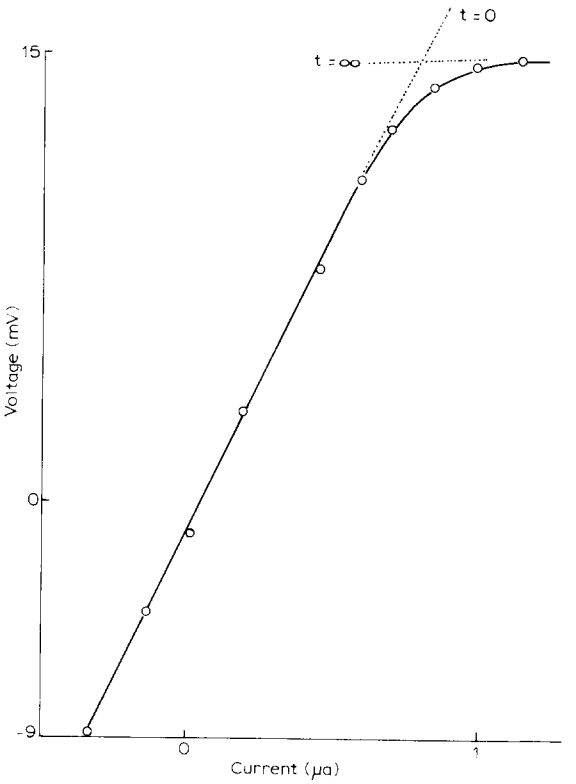

Fig. 4. Conductance properties of double membrane (negative-positive fixed charge junction) with glycine.

Fig. 3. The corresponding current and voltage changes are shown in Fig. 4. Here too, with small perturbations at the membrane surface the resulting alterations in resistance are nonohmic, i.e., the relation between current and voltage is nonlinear. The membrane unit exhibits both the property of capacitance and the property of rectification. It is immediately apparent that with the proper orientation of the membrane, an amino acid or peptide may be transported against a concentration gradient. The phenomenon is modified with the substitution of constituent electrolyte, potassium stimulating transport, and bromide depressing transport slightly. This is shown in Table III. It was noted that substitution of anions did not modify the phenomenon to the degree by which it was modified by cation substitution. It was found that the cations would tend to concentrate against the amino acid gradient no matter which cation was present with the amino acid. The transport phenomenon itself was abolished upon removal of the added electrolyte.

Several general points are apparent from the data on the transport of amino acids in this system. The amino group (as NH_3^+) is the portion of the amino acid involved in the exchange process at the membrane. If the amino group is bound, as in *N*-acetylglycine, transport of the amino acid either with or against the concentration gradient is severely retarded (Table IV). The presence of a second amino group accelerates the exchange of amino acid. The second group may be ionized or present as $-NH_2$. It should be noted that the second amino group present in an amide link in asparagine also accelerates the exchange of the

TABLE III

RATES OF GLYCINE DIFFUSION THROUGH DOUBLE MEMBRANES

Measured rate (10^4 mol/min)		Electrolyte
negative-positive fixed charge junction	positive-negative fixed charge junction	
0.50	1.60	KCl
0.38	1.10	NaCl
0.29	0.87	LiCl
0.35	0.99	NaBr

TABLE IV

RATES OF DIFFUSION THROUGH DOUBLE MEMBRANES

Amino acid	Relative rate	Fixed charge junction, measured rate (10^4 mol/min)	
		negative-positive	positive-negative
Glycine	1.00	0.50	1.6
Alanine	0.62	0.31	0.98
Asparagine	0.58	0.29	0.94
Serine	0.56	0.28	0.92
Aspartic acid	0.54	0.27	0.87
Cysteine	0.52	0.26	0.85
Arginine	0.52	0.26	0.84
Lysine	0.50	0.25	0.82
Diglycine	0.50	0.25	0.81
Glycine ethyl ester	0.42	0.21	0.67
Methionine	0.40	0.20	0.66
Histidine	0.40	0.20	0.66
Phenylalanine	0.30	0.15	0.51
Leucine	0.16	0.08	0.25
Triglycine	0.16	0.08	0.24
N-Acetylglycine	0.01	0.005	0.01

amino acid to some degree. The presence of electron-attracting groups such as are found in methionine also accelerates the exchange of the amino acid. Transport is retarded if a second carboxyl group or an alcoholic group is present on the amino acid; however, if the only carboxyl group is bound, as in glycine ethyl ester, no effect on transport is noted. Finally, the increasing length of the side chain of an amino acid is directly related to an increase in time required for transport across the double membrane unit. This may reflect hydophobic interaction between the resin and the amino acid side chains.

TABLE V

COMPARISON OF RELATIVE RATES DETERMINED IN VARIOUS SYSTEMS

Relative rates were determined with: A, single membrane (negative fixed charge junction); B, double membranes (negative-positive fixed charge junction); and C, Ehrlich ascites tumor cells [19].

Amino acid	Relative rate		
	A	B	C
Glycine	1.00	1.00	1.00
Alanine	0.63	0.62	0.57
Methionine	0.45	0.40	0.53
Phenylalanine	0.30	0.30	0.28
Leucine	0.16	0.16	0.14

Table V illustrates the relative degree to which amino acids are transported into Ehrlich-Lettre ascites carcinoma cells and compares data with the relative transport rates presented earlier.

Discussion

A general minimal transport model compatible with that loop described by Sanders may be proposed on the basis of the results with the present system. It is suggested that the initial step of the exchange process involves the loss of hydrogen ion from the ionized amino portion of the amino acid at the negative interface of the negative-positive fixed charge junction, with the consequent reversion of the amino nitrogen to a relatively more negative form. The amino acid (stripped of the hydrogen ion) must be in the membrane at this intermediate time. It is ejected because of the rather large repulsive forces between the negatively charged portion of the membrane and the relatively negative form of the amino acid. The direction of travel demanded by the rather steep field gradient is to the positively charged portion of the double membrane.

At the positive interface, in a highly polarized medium, a hydrogen ion is regained by the amino nitrogen. That this is possible is due to the greater attraction of the fixed charge for the relatively concentrated charge density of the free anion in the form of swamping electrolyte. A compensatory shift of the cation is thus imperative. At the positive interface the cation is believed to shed its oppositely charged hydration sphere, and is acelerated to the negative interface. The increased saturation of exchange sites at the negative interface by the cation appears across the membrane as a whole where it results in an ohmic drop, and consequently enhances the measured response of the fixed charge junction as both a capacitor and as a rectifier, approaching time independence at maximum saturation.

A controlled double membrane model transport system may be manipulated for electrodic

transport modelling, permitting separation of contributory transport mechanisms for kinetic analysis, thus permitting dissection of complex forms seen in living cells. An understanding of physicochemical mechanisms of transport (and, by extension, membrane stability) facilitates design of drug delivery systems ,with applications of giant liposomes and receptor-directed pharmaceuticals being favored.

References

1 Agutter, P.S. and Suckling, K.E. (1984) Biochem. Soc. Trans 12, 713–718
2 Axelrod, D. (1983) J. Membrane Biol. 75, 1–10
3 Skulachev, V.P. (1985) Trends Biochem. Sci. 9, 182–185
4 Hung, Y.Q. and Junge, W. (1983) Biochim. Biophys. Acta 722, 197–208
5 Ohta, S. and Schatz, G. (1984) EMBO J. 3, 651–657
6 Skulachev, V.P. (1985) Trends Biochem. Sci. 9, 482–485
7 Mitchell, P. and Moyle, J. (1974) Biochem. Soc. Spec. Publ. 4, 91–111
8 Christensen, H.N. (1985) J. Membrane Biol. 84, 97–103
9 Sanders, D., Hansen, U.-P., Gradmann, D. and Slayman, C.L. (1984) J. Membrane Biol. 77, 123–152
10 Christensen, H.N., Riggs, T., Fischer, H. and Palatine, I. (1952) J. Biol. Chem. 198, 1–17
11 Heinz, E. (1957) J. Biol. Chem. 225, 305–311
12 Heinz, E. (1955) J. Biol. Chem. 211, 781–792
13 Heinz, E. and Mariani, H. (1957) J. Biol. Chem. 228, 97–104
14 Heinz, E. and Walsh, P. (1958) J. Biol. Chem. 233, 1433–1445
15 Oxender, D. and Christensen, H.N. (1963) J. Biol. Chem. 238, 3686–3699
16 Tomblakian, A., Yeh, C. and Graydon, W. (1967) J. Phys. Chem. 71, 435–439
17 Shean, G.M. and Sollner, K. (1966) Ann. N.Y. Acad. Sci. 137, 759–770
18 Stokes, T. (1948) J. Am. Chem. Soc. 70, 1870–1873
19 Oxender, D. and Christensen, H.N. (1959) J. Biol. Chem. 234, 2321–2330