Biophysical Chemistry 12 (1980) 107–119

TWO ENZYME ACTIVE TRANSPORT IN VITRO WITH pH INDUCED ASYMMETRICAL FUNCTIONAL STRUCTURES. II. PROPERTIES OF THE PUMP AND EXPERIMENTAL ILLUSTRATIONS.

Eric SELEGNY and Jean-Claude VINCENT

Laboratory of Charged and Reactive Polymers and of Biomimetics (E.R.A. 471). Université de Rouen, 76130 Mont-Saint-Aignan, France

Received 24 July 1979

The theory and practice of the pump are illustrated. With graphs drawn using the analytical equations developed in Part I, the optimizing effect of reactive and diffusive parameters of the membrane or of pH gradients are discussed as a function of boundary conditions. Profile symmetries, extractive and accumulating powers, time constants and stereoselectivities are examined. An experimental glucose pump demonstrates the feasibility of the theoretical predictions. A mixture of hexokinase (optimal pH = 8.2) and phosphatase (optimal pH = 6.7) was immobilized in an agarose membrane. When asymmetry was induced by a pH gradient (between 6 and 9), ATP was consumed, D-glucose accumulated on one side and was separated from the L-isomer. Further generalizations and extensions are commented on.

1. Introduction

Recently (part I) [1] we have discussed asymmetrical chemical cell type systems in which active transport is produced by the association of a sink and a source reaction. With "leaky" systems a one-wave space profile of the transported species is obtained. It was predicted that a pH-gradient through the mixture of two enzymes (E1 and E2) of different pH dependance can induce the asymmetrical distribution of the reactions. In the simple model proposed, the "membrane" (of total thickness $E = (n+2)e$) is subdivided by a pH gradient into two active layers (of thickness e) separated by an inactive layer (of thickness ne). The potential enzyme activities of the two enzymes are equal in the "square model". The kinetic behaviour of this model has been analytically described. The obtained equations have been regrouped in table 2 of part I, for high substrate concentrations S and zero order kinetics and in table 3 for low S with regard to the Michaelis constant K_m and first order reactions.

In the present article we are concerned with interpretations and theoretical and experimental illustrations of the pump.

The theoretical examination utilizes the previously established equations mostly in the reduced or dimensionless form and also a few of their combinations. The equations are not unnecessarily repeated here [‡]. The understanding of the results is made possible with the help of graphs and the constants and parameters of the list of symbols. For details part I should be consulted.

The optimizing or inhibiting effects of the reactive and diffusing membrane characteristics on the pumping are compared for various boundary conditions. Some typical properties of the pump are illustrated.

The questions examined proceed from different inspirations. These include the symmetry elements of concentration and flux profiles, the potential of the pump to create concentration (chemical potential) gradients and the evolution of yields. Behind the regulatory effects of the pH gradient is found the interesting question, whether an inert layer is convenient for the replacement of a selective separator to make the pump less "leaky"?

Concentration regulations of the pump, its

[‡] Reference will be made to tables 2 and 3 of part I; in order to avoid confusion, the tables of this paper have been numbered starting from 4.

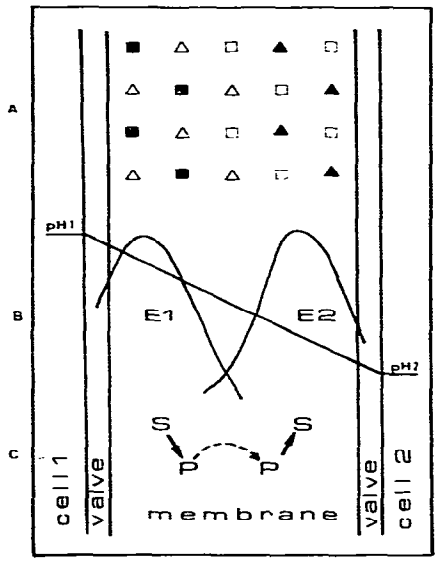


Fig. 1. Schematic diagram of active transport membrane with asymmetric functional structure: A) Homogeneously distributed enzymes E1 (□) and E2 (△) and activity distribution with pH gradient: active (■▲) and inactive (□△). B) Activity profile of enzymes E1 and E2 in function of pH. C) Scheme of reactions.

Michaelian behaviour or stereospecificity have a biophysical interest. This model, however, has yet an intentionally simplified form and is not an imitation of any particular biological pump.

Misunderstanding of the conclusions can be avoided by assuming that this research belongs to the field of "Biomimetics" which concentrates on artificial systems that show equivalent features to biological systems, and by locating the fundamental conceptual question in the properties that interactions of gradients and enzymes can communicate to systems.

Experimental life has been communicated to the theory with a pH gradient induced glucose pump. It uses the same "inversible reaction" set [1] as the originally published "permanent structure" version where separate enzyme layers were soldered together [2]:

glucose + ATP

$$\xrightarrow[pH_{opt} = 8.2]{\text{hexokinase } (Mg^{++})} \text{glucose-6-phosphate + ADP}$$

glucose-6-phosphate + H_2O

$$\xrightarrow[pH_{opt} = 6.7]{\text{Phosphatase}} \text{glucose + phosphate}$$

The carrier is "phosphate" and the chemical energy is furnished by the hydrolysis of ATP added to the system. The two enzymes possess a difference in activity with respect to pH that makes the spacial differentiation of their activity possible.

In distinction with the theoretical model the first reaction generates an H^+ ion that could modify the local pH, but this modification is easily avoided by buffering. (The feed back regulations and ion transports in absence of buffer have been studied separately and will be reported elsewhere).

The enzymes have been immobilized in an agarose membrane of high water content, slightly crosslinked by the glutaraldehyde technique [3–5]. This membrane was covered with polyacrylic acid-agarose valve films, quite inpermeable to anions (namely to glucose-phosphate) but permeable to glucose. The details are given in the experimental part.

2. General aspects of the profiles (graphs)

2.1 Infinite donor and acceptor cell volumes ($\Delta S = 0$)

The calculated results clearly show a space-wave substrate concentration profile in the membrane (fig. 2),

With zero order kinetics (fig. 2a) the mid plane is a symmetry plane both for the fluxes and the concentrations. If $\Delta S = 0$ a symmetry point is observed as well for infinite cell volumes (fig. 3) as for limited ones (fig. 4), for all activities (figs. 3a and 4a) and all different n values (figs. 3b and 4b).

When first order kinetics are assumed the symmetry disappears (fig. 2b). With increased enzyme activity (σ') the deviation from symmetrical profiles increases for S (fig. 5a), for P (fig. 5b) and for the substrate flux (fig. 5c); the shapes of these curves are very different from the zero order ones.

Fluxes are increased by higher activities (σ or Σ) and if membrane thickness E is maintained constant by lower n values.

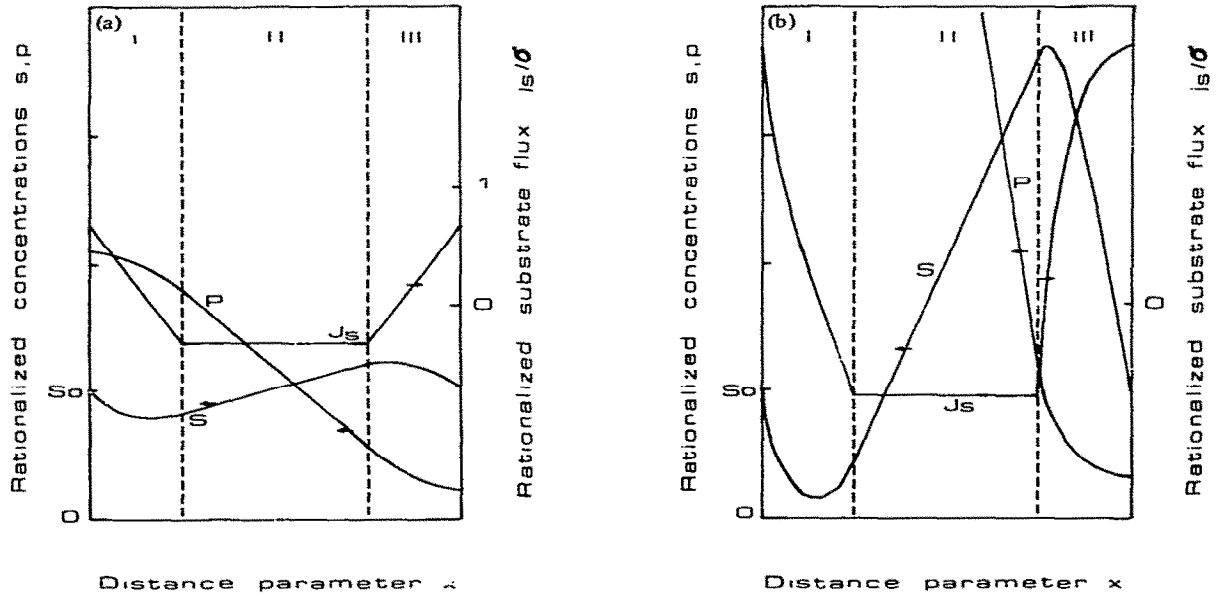


Fig. 2. Concentration profiles and fluxes in stationary state conditions with $\Delta S = 0$ for a) *zero order* reactions and b) *first order* reactions: Substrate concentration profile (S); Product concentration profile (P); Substrate flux profile (J_S).

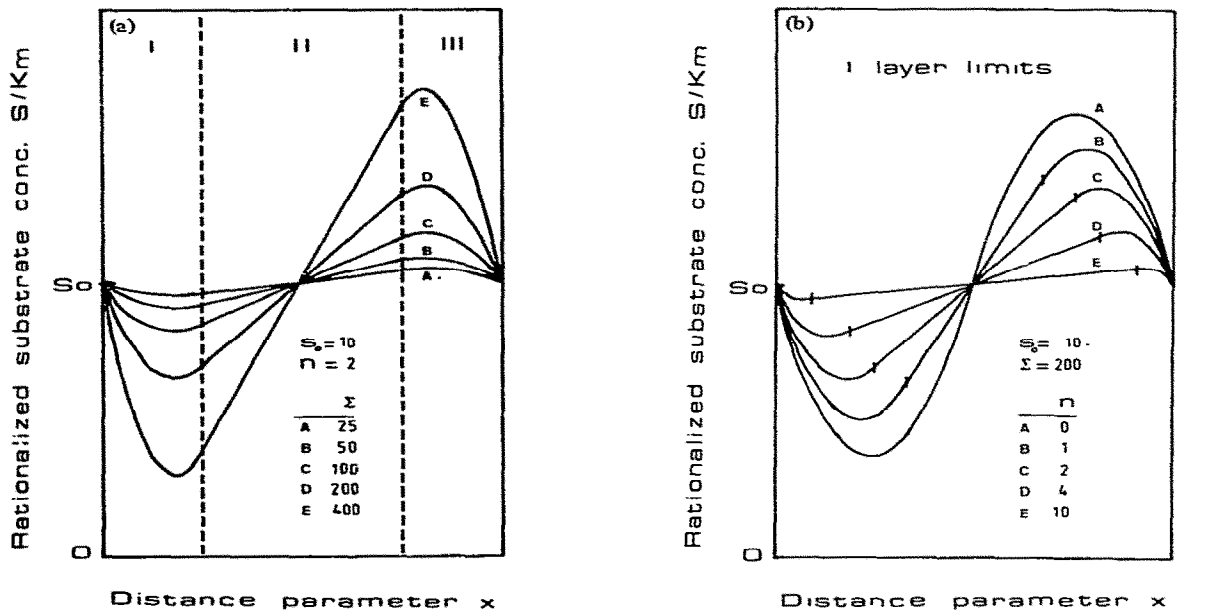


Fig. 3. Effects of a) activity and of b) relative inactive layer thickness on substrate concentration profile for *zero order* in the stationary state with *infinite* cell volumes ($\Delta S = 0$).

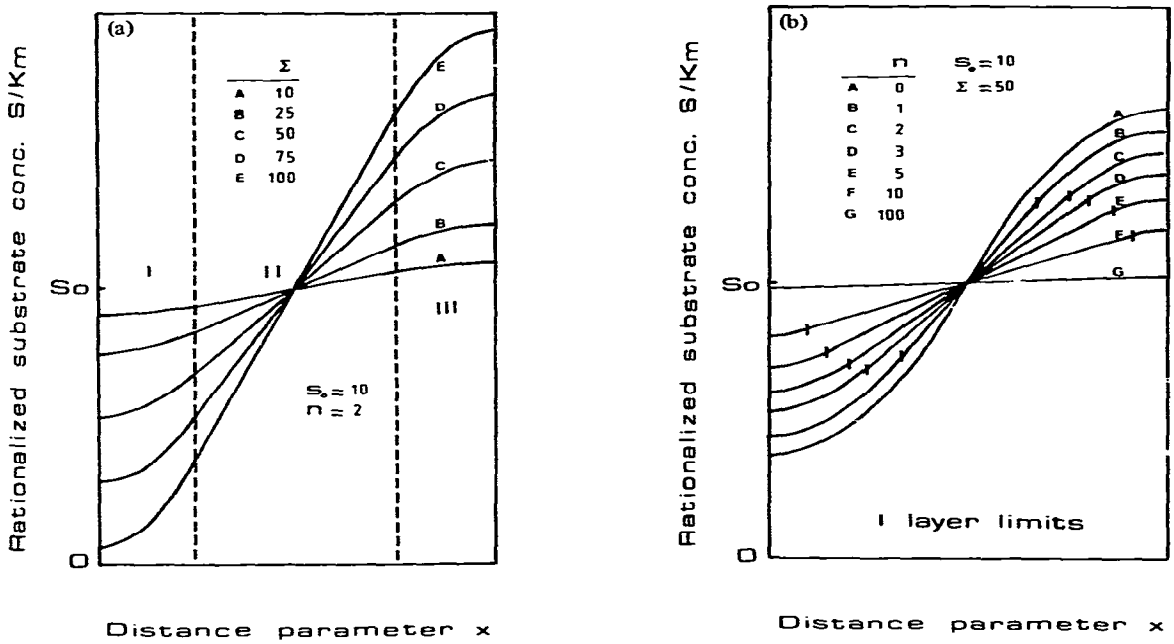


Fig. 4. Effects of a) activity and of b) relative inactive layer thickness on substrate concentration profile for *zero order* in the stationary state at infinite time and *limited* cell volumes.

2.2. Finite donor and acceptor cell volumes

With limited cell volumes the boundary conditions become time dependent as the cell concentrations change from initial equality to the stationary state values.

Assume zero order kinetics and all the successive quasi-stationary profiles as a function of the dimensionless time χt show a symmetry point (fig. 6a). The entering substrate flux decreases with time (fig. 6b) while the concentration gradient approaches its maximum (fig. 7a) and the net stoichiometric yield tends to zero (fig. 7b).

For constant membrane thickness E and activity Σ a large increase in n results in decreased flux, concentration gradient and yield and leads to the inactivation of the pump.

3. Influence of the various parameters on the pump

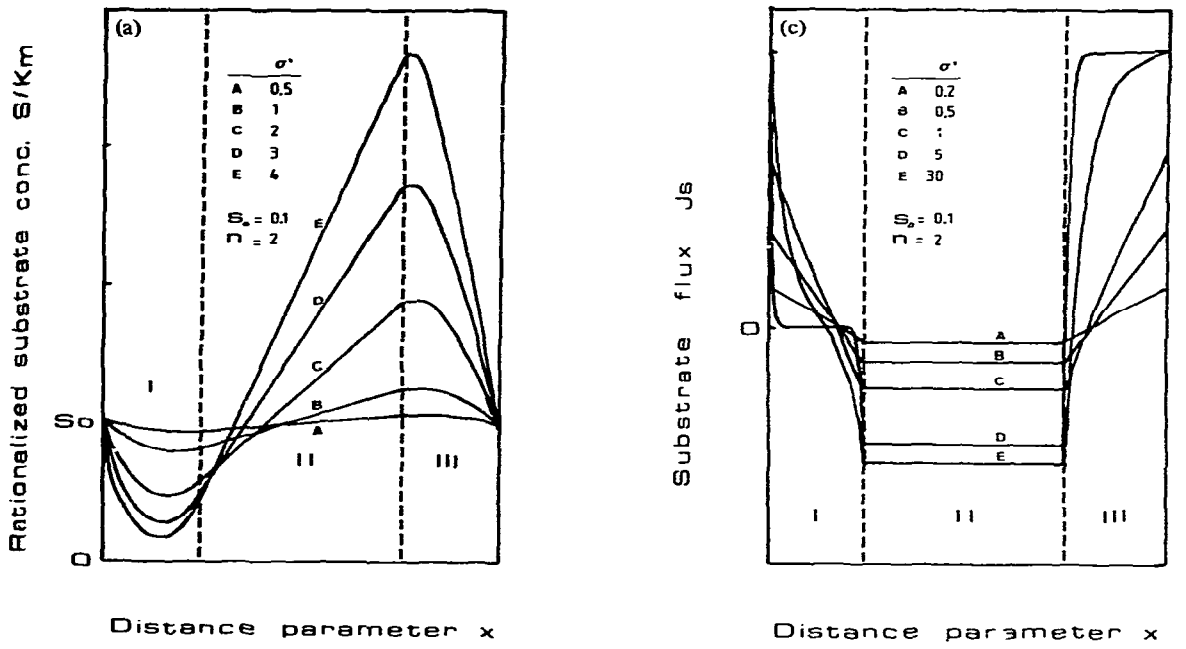
The description of the pump and of its work needs three types of variables: a) the Thiele type enzyme diffusion-reaction parameter that is composed of diffusion and reaction terms; b) n, a function of the enzymes and of the pH gradient; c) the external boundary concentrations and cell volumes.

3.1. The effect of substrate concentration (S or S/K_m).

The level of concentration S determines the order of reaction. Low values of external S_0 lead to first order kinetics. The corresponding equations indicate that with high enzyme activity (αe or σ') the initial flux is proportional to S_0. In the stationary state the transfer of substrate to the acceptor compartment tends to be total.

On the other hand, high values of S_0 result in zero order kinetics with high transport flux for large donor and acceptor compartments or high concentration differences with small acceptor compartments.

If we define the extracting power of a system with finite donor and acceptor compartments as $(S_2 - S_1)_{max}/S_0$, we can conclude that first order kinetics result in high extracting power and zero order kinetics in high accumulating power.



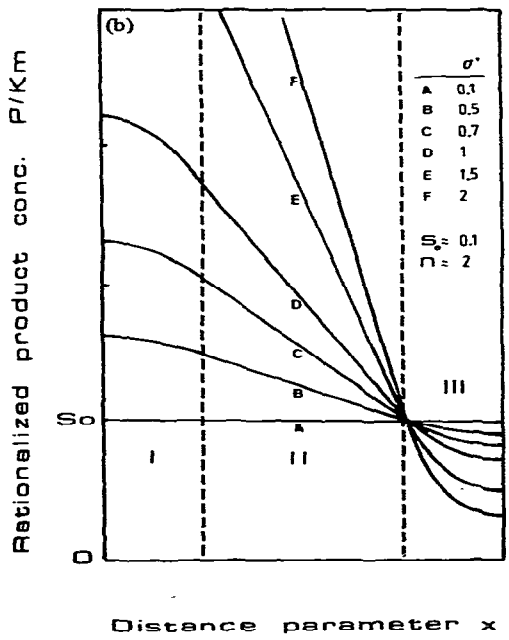


Fig. 5. Effects of *first order* activity on concentration and flux profiles inside the membrane in the stationary state with *infinite* cell volumes: a) Substrate concentration profile; b) Product concentration profile; c) Substrate flux profile.

The time necessary for the establishment of the stationary state decreases when the concentration rises. The first order time constant θ is always greater than the zero order time constant χ.

3.2. *Effect of diffusive properties* (E^2/D)

The global diffusion parameter E^2/D has the dimension of time and depends on membrane thickness and its resistance to diffusion. The load of the pump increases when the value of this diffusion parameter increases. This is shown by the increase of Σ with E^2/D. Σ is indicative of the global active transport/back-diffusion ratio and controls the maximum load (figs. 4, 9 and 10).

In spite of its high local gradients P, being restricted to the membrane, has only limited influence on the rate of the process. The results illustrate our previous remarks (Part I) i.e. "the back diffusion of S through the membrane is the major rate-and-yield limiting factor in this leaking imperfect chemical cell model".

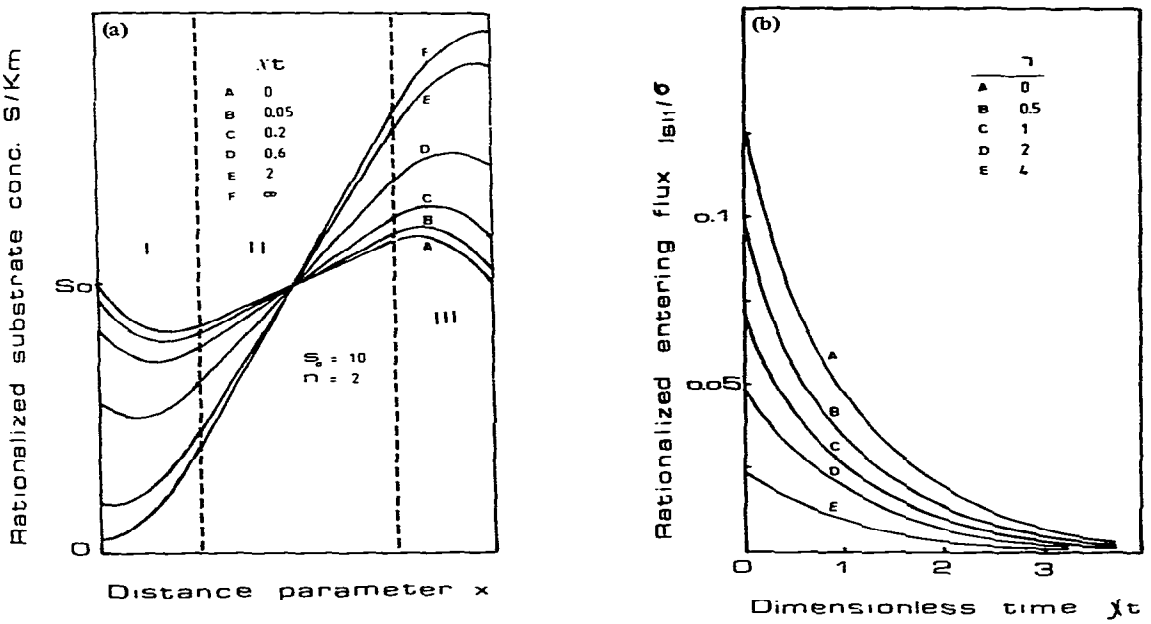


Fig. 6. Evolution of substrate concentration profile and of substrate entering flux of an active transport membrane for *zero order*. a) Rationalized substrate concentration profile inside the membrane in function of time parameter χt; b) Substrate entering flux in function of time parameter χt for different values of relative inactive layer thickness n.

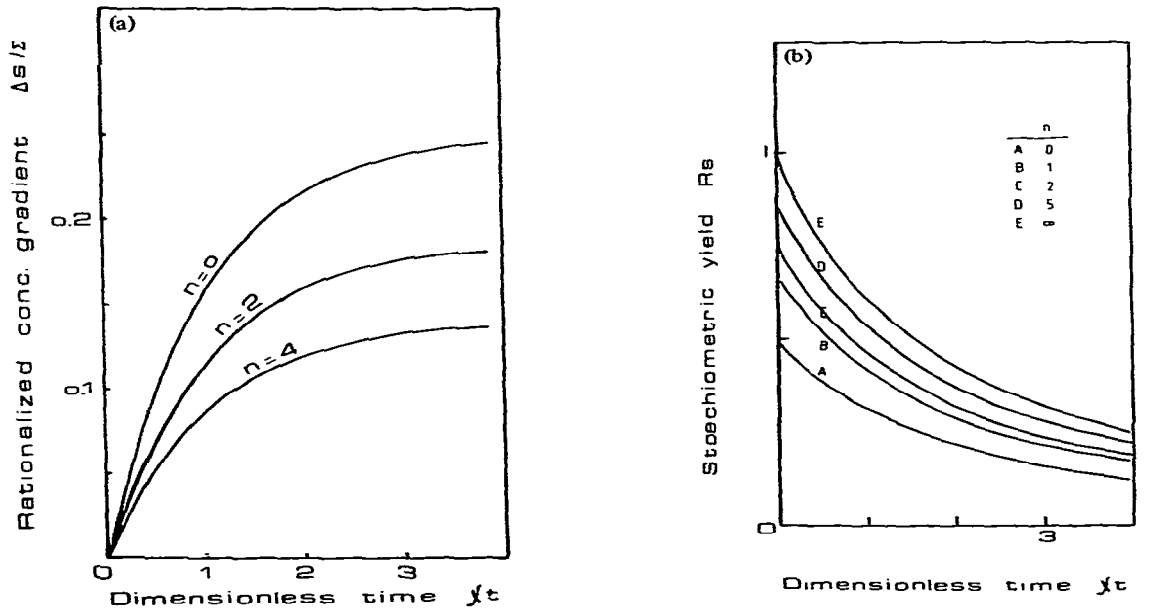


Fig. 7. Evolution of *zero order* substrate concentration gradient and of stoichiometric yield of the pump: a) Rationalized concentration gradient in function of time parameter χt for different values of relative inactive layer thickness n. b) Stoichiometric yield in function of time parameter χt for different values of n.

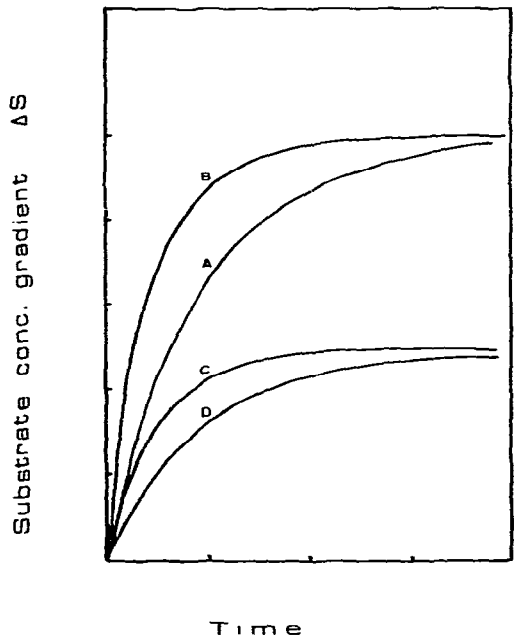


Fig. 8. Influence of the different membrane parameters on active gradient and transport kinetics: A) Reference conditions. At n = Cst *division by a factor of two* of: B) Cell volume parameter v_c/v_m. C) Diffusion time parameter E^2/D. D) Reaction rate parameter V_m/K_m.

3.3. Effect of reaction properties (V_m/K_m)

V_m, the turn over, represents the maximum available activity at a given pH. The reduced turn-over V_m/K_m has the dimension of the inverse of time. An increase in V_m/K_m increases the pumping efficiency, and leads to higher fluxes and maximum load (figs. 4, 9, 10).

The establishment of the stationary state is also accelerated when the reactions are of the first order (lower θ); the zero order time coefficient (χ) remains however unaffected. The modification of V_m/K_m can be the consequence of various mechanisms effecting V_m or K_m as already mentioned in previous publications [2,6,7,9].

3.4. Effect of the pH gradient (n)

Let ΔpH_n be the intermediary pH zone in which the two enzymes have no activity; this zone is depen-

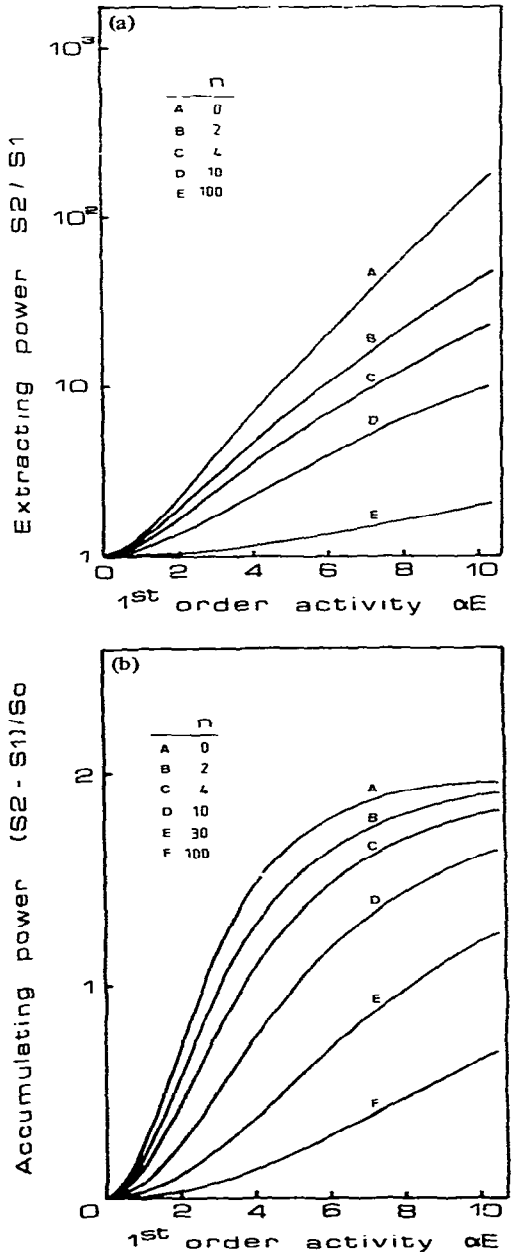


Fig. 9. Effect of *first order* activity (αE) on the a) extracting and b) accumulating powers of the pump at constant total membrane thickness (E) for different values of relative inactive layer thickness n.

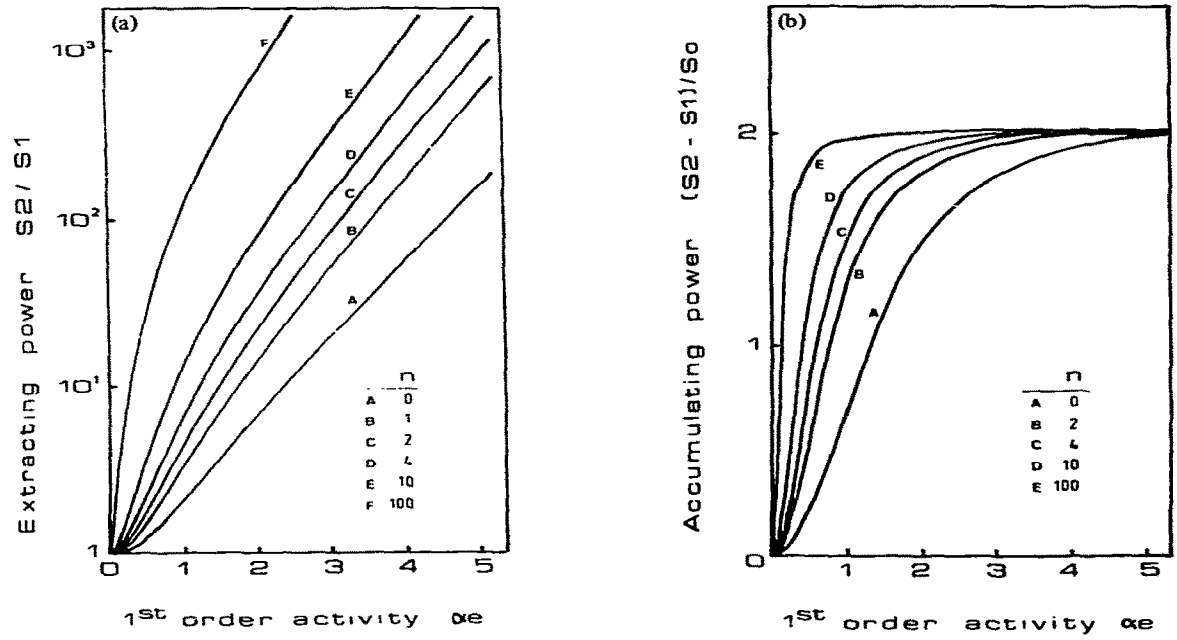


Fig. 10. Effect of *first order* activity (αe) on the a) extracting and b) accumulating powers of the pump at constant active layer thickness (e) for different values of relative inactive layer thickness n.

dent on the nature of the enzymes; ΔpH_n is the gradient through the central inactive layer of thickness ne.

The pH gradient through the whole membrane (ΔpH) creates the functional structure, controls the enzyme activities and their distribution. We have assumed symmetrical and equal activities induced by linear pH profiles, thus we have in first approximation:

$$n = \frac{2\Delta pH_n}{\Delta pH - \Delta pH_n}.$$

When the total membrane thickness (E) and the amount of enzyme are kept constant, a variation in n essentially produces the modification of the fraction of available activity. With increasing n the efficiency of the pump will decrease in terms of flux, maximum load and extracting power, but the time coefficients θ and χ are not changed. For very large values of n the flux progressively becomes zero. (Figs. 3, 4, 6, 7 and 9).

When, on the other hand, different membranes of constant activity eV_m/K_m but varying total thickness E are compared increasing pump efficiencies are found with increasing n values (fig. 10). This effect is due to a diminution of the back diffusion of S with larger inactive layers; as an accompanying effect the pumping becomes slower as shown by the variations of θ and χ constants.

3.5. *The effect of cell volumes (v_m/v_c)*

The cell volume relative to that of membrane v_m/v_c

Table 4
Effects of different structural and functional parameters on the efficiency of the pump.

	increase of parameters				
	S_0	V_m/K_m	E^2/D	n	v_m/v_c
greater maximum load (accumulating power)	+	+	+	−	=
Higher transport flux	+	+	+	−	=
Shorter establishment time	+	=	+	=	−
greater extracting power	−	+	+	−	=

is the factor to be considered. It has no influence on ΔS_{max} as the fluxes through the membrane only depend on its boundary conditions. But both time coefficients θ and χ increase, that is the time required for the establishment of the stationary state decreases, when v_m/v_c is great i.e. the cell volume is small (fig. 8).

3.6. Summary of the effects of the parameters

The positive or negative effects on the pumping properties are summarized in table 4.

The best compromise for a membrane of constant thickness E consists in strong enzyme activity with thin or no intermediate layer and limited diffusibility. Low substrate concentrations are required for complete substrate extraction and high concentrations for large accumulations; small cells give high gradients and big cells high fluxes.

If the enzyme activity eV_m/K_m of the active layers is maintained constant a large intermediate layer gives the higher ΔS_{max} values but needs longer establishment times: the inactive layer acts as an "imperfect separator" (see part I).

4. Some properties of the active transport pump

4.1. Remarks on the global rate of transport (V_M)

The global transport rate V_M is related to the elementary membrane parameters.

These relations are particularly interesting for discussion in bioanalogue terms, as *in vivo* only V_M is attainable. With the limiting assumptions used here Michaelis type global kinetics remain valid as a good approximation. But in order to avoid later misunderstanding let us just mention now that with high enzyme activity allied to more general kinetic conditions significant deviations from Michaelian behaviour can result.

4.2. Regulating effect of the pump

Let us consider an "inside cell" (*small* cell 2) limited by an enzyme membrane ($\Sigma = 100$ and $n = 0$) with two different "external" situations in the large cell 1 and calculate the stationary state S_2 value:

With a 5×10^6 fold change outside, the intracellular concentration changes about 6×10^3 times showing a certain stabilizing effect of the pump on the inside concentration.

external s_1	order of reaction	internal s_2
75 (0.075 M)	zero	125
15×10^{-5} (0.15 μM)	first ($\alpha e = 5$)	2×10^{-2}

4.3. Competitions between active and passive transports

Such competitions can concern different transports of the same compound or of different compounds.

4.3.1. Passive and active transport of the same substrate

When both passive diffusion and active transport of a substrate are possible through the membrane, one or the other can prevail as a function of the external concentration. The ratio of these fluxes can be calculated by proper combination of the equations of tables 2 (zero order) and 3 (first order) (part I)

If we consider a void "internal compartment" and zero order kinetics we get the stationary expression:

$$\frac{J_{SI1}}{J_D} = 1 + \frac{(1+n)\Sigma}{(2+n)^2 s_0}.$$

With external substrate concentration values s_0 high compared to Σ this expression tends to 1 showing that the diffusion flux J_D tends to equal the active flux J_{SI1} entering the membrane.

Similar calculations at first order show that with a small s_0 active transport is predominant and controls the flux.

Remark: there is a qualitative resemblance of these results with some *in vivo* tranports; for example the mechanism of the uptake by active or passive transport of lipoic acid by *E coli* has been found to be dependent on the external substrate concentration by two different techniques and two different groups [6,7].

4.3.2. Stereoselectivity and optical isomer separations

Stereoselective transport is an interesting example of competition.

Enzymes recognize and select one of the two optical isomers, (usually the "natural" one) and neglect its in-

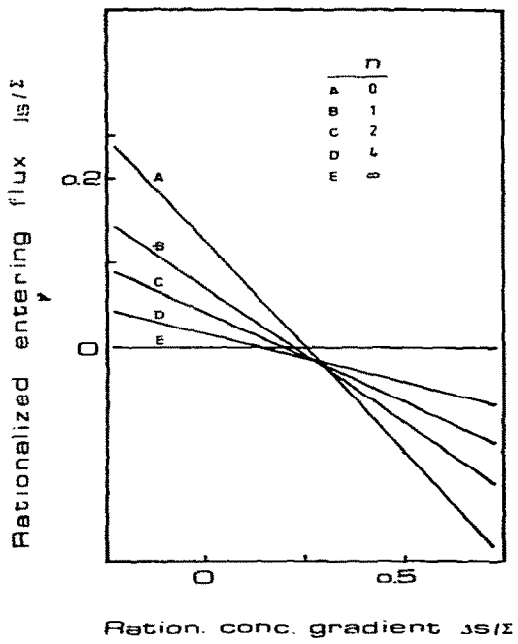


Fig. 11. Effect of initial substrate concentration gradient ($S_2 - S_1$) on entering flux (J_{S11}): behaviour of diffusive (n infinite) and active systems (for different values of n); active pump ($\Delta S_0 = 0$) and active barrier ($\Delta S_0 > 0$).

verse. This is a fundamental property of living systems and also the basis of Pasteur's method of resolution of DL racemic mixtures by fermentation. Moreover theoretical and experimental investigations of isomer transports do not only illustrate the analogies and differences between diffusion and diffusion-reaction processes but are also able to demonstrate that enzyme activity and not the passive pH gradient by itself is the reason for molecular accumulation.

Thus, from the biomimetic point of view it is justified to examine the stereoselectivity of the pump.

To begin with, a solution of a racemic mixture of stereoisomers is placed in the donor and/or acceptor cells.

The actively transported "natural" isomer (say S_D) tends to accumulate in the acceptor cell 2 up to the stationary state plateau ($\Delta S_{D_{max}}$), and the passively diffusing "unnatural" one (say S_L) tends to reach equal concentration in the whole system.

Table 5
Ratio of 'natural" (S_D)/"unnatural" (S_L) isomers. $F = (n+1)\Sigma/2(n+2)^2$; *zero order*, that means $F < S_0$.

	Receiving compartment 2 $(S_2)_D/(S_2)_L$	Giving compartment 1 $(S_1)_D/(S_1)_L$
case 1	$1+\frac{F}{S_0}\left(1-e^{-\chi t}\right)$	$1-\frac{F}{S_0}\left(1-e^{-\chi t}\right)$
case 2	$1+\frac{F}{S_0}$	$1-\frac{F}{S_0}\frac{1-e^{-\chi t}}{1+e^{-\chi t}}$
case 3	$1+\frac{F}{S_0}\frac{1-e^{-\chi t}}{1+e^{-\chi t}}$	$1-\frac{F}{S_0}$
Highest value in all cases	$1+\frac{F}{S_0} \leqslant 2$	for $\Sigma = 800$ and $n = 0$

Starting conditions:
Case 1: Equal distribution of racemic: $(S_{DL})_1 = (S_{DL})_2$; $\Delta S_{t=0} = 0$.
Case 2: Racemic mixture only on giving side 1: $(S_{DL})_1 = 2S_0$; $(S_{DL})_2 = 0$; $\Delta S_{t=0} = 2S_0$.
Case 3: "Active barrier". Racemic mixture only on receiving side 2: $(S_{DL})_1 = 0$; $(S_{DL})_2 = 2S_0$; $\Delta S_{t=0} = -2S_0$.

In an experimental demonstration the optical rotation of the two compartments should change in opposite directions and this is what was found [14] with the glucose pump and DL-glucose. The experiments required concentrations high enough to make the optical measurements possible but calculations can consider larger concentration ranges.

Expressions have been developed to compute isomer-compositions as a function of time in both the donor and acceptor compartments for zero order (table 5) and first order (table 6) kinetics. Identical compartment volumes have been assumed on both sides of the mem-

Table 6
Stationary state ratios of optical isomers in the 'giving" compartment in function of Σ. Starting with a racemic mixture; identical cell volumes; S_0 small; *first order*.

Σ	0	2	5	20	100	200
$(S_1)_D/(S_1)_L$	1	0.79	0.59	0.21	0.013	0.002

$(S_1)_D/(S_1)_L = 2/(n\sigma'\sinh\sigma' + 2\cosh\sigma')$, calculated with $n = 0$.

brane. A symmetrical initial situation (case 1) and two different asymmetrical ones (cases 2 and 3) have been considered and specified in table 5.

a) When initially the acceptor compartment does not contain any of the isomers (case 2) the optical purity of the collected material is independent of time. This result is explained by the fact that in these conditions the *"time lags" of pure diffusion and of active transport are the same and the ratio of their time constants is constant.*

b) The ratio of isomers becomes independent of time on the donor side if this compartment is initially free of solutes (case 3). This is understandable as the membrane now acts as an "active barrier" for the substrate but not for its isomer and the transport process is due to unequal but pure back diffusion of the isomers until stationary state and equilibrium concentrations are respectively reached by the isomers.

c) On the receiving side (side 2) a ratio $(S_D/S_L)_2$ not better than 2 can be expected at one stage, whatever the initial conditions. The situation is better in the donor cell where a 99% enrichment of unnatural S_L can be obtained; for this, however, very high values of Σ are required ($\Sigma = 800$ for $s_0 = 100$).

For first order kinetics (table 6) the "extracting power" is much greater. Calculations give a 99% pure "unnatural" isomer in the donor cell for $\Sigma = 100$ and for any sufficiently low s_0.

On side 2 high optical purity $S_D/(S_D + S_L)$ results when the "internal" compartment is small and the external one has infinite volume.

5. Experimental

5.1. Active transport of glucose

The two-enzyme-membrane is of the homogeneous hexokinase-acid phosphatase type:

The two enzymes and ATP were homogeneously distributed in a neutral agarose gel membrane. The enzyme solution was mixed with a 3.5% agarose solution on a water bath at 40°C and casted on a horizontal glass plate and dried at 4°C.

The already self consistent membrane was dipped in a 1% glutaraldehyde solution and cross-linked by the diffusing reactant. The Σ value of the membrane was varied by controlling the thickness and the cross-linking of the membrane, and the amount of enzyme added. Such membranes have good mechanical qualities even with the high water content making them adequate to test diffusion-reaction theories. The details of the preparation techniques and the properties, including pH and cofactor dependences, of individual enzyme membranes have been previously described [5].

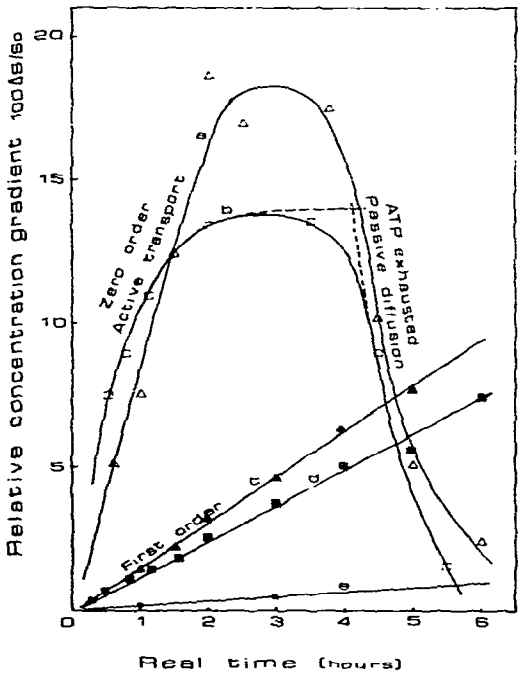


Fig. 12. Experimental glucose pumps working by active transport; energy source ATP: substrate transporter complex Glucose-6-phosphate; Glucose concentration gradient $(S_2 - S_1)/S_0 \times 100$ in function of real time (t); pH: 9 in cell 1 and 6 in cell 2. *Zero order*: Hexokinase and acid phosphatase 1.25 mg; S_0 glucose 3.3 mM; magnesium 50 μM and ATP 475 μM (a) or 400 μM (b). *First order*: Hexokinase and acid phosphatase 0.5 mg; ATP 300 μM; magnesium 70 μM; S_0 glucose 150 μM (c) or 40 μM (d). Passive diffusion of glucose 150 μM (e). Volume of each half cell 40 ml; membrane surface 1 cm^2.

The active membrane was soldered between two thin agarose films containing polyacrylic acid. The passive substrate gradient through these films is easy to take in account in the calculations.

The membrane was inserted between two compartments of a thermostated polymethylmethacrylate

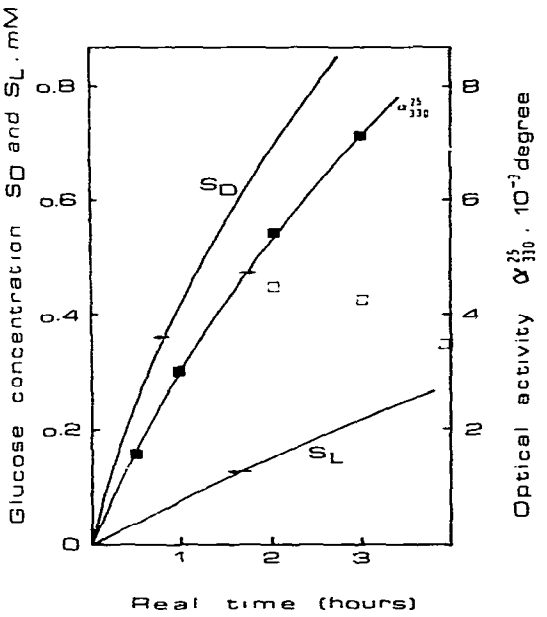


Fig. 13. Experimental separation of optical isomers: Optical activity (right ordinate axis) and concentrations of D and L glucose (left ordinate axis) in function of real time (t) in the receiving compartment; membrane: Hexokinase and acid phosphatase 1 mg, glucose 5 mM, magnesium 50 μM, volume of each half cell 20 ml, membrane surface 1 cm^2; pH 1 : 9.5 and pH 2 : 7; ATP: □ 5 mg, ■ 15 mg.

(altuglass) cell. The cell was water and gas tight to make possible operations under nitrogen (necessary for some titrations).

Two solutions of identical concentration of glucose, Mg^{++}, ATP and phosphate buffer but of *pH 6 and 9* respectively were added to the compartments (note that at these pH values polyacrylic acid is ionized). The pH is maintained constant throughout the experiment. The resistance to passive diffusion of the membrane (D) was measured in the cell under conditions which inactivate the enzymes. The active transport was followed by glucose titrations as a function of time in both compartments. The determinations were made:

– In the zero order range with "glucose electrodes" based on oxygen electrodes covered by a glucose oxydase film, under nitrogen gas using a Tacussel EPL1 potentiometer and calomel reference electrodes.

– In the more dilute first order range, needing greater precision by radioactive techniques with C^{14}-glucose.

The concentration differences ΔS obtained between the compartments starting from $\Delta S_0 = 0$, were significant and in good agreement with the theoretical predictions. *Membrane specifications and illustrative results* are given in fig. 12.

5.2. Stereoselective separation by active transport in vitro

A demonstrative experiment has already been published [8] (fig. 13). It used the same type of glucose pump as above and racemic DL-glucose was added to compartment 1 only (theoretical case 2). The optical rotation was measured in the receiving compartment 2, at various times, with a Perkin Elmer electronic polarimeter at 330 nm wave length using a micro cell (10 cm optical pathway length, 1 ml volume). The membrane permeability to glucose was determined by diffusion of pure L-glucose.

The starting $2S_0$ concentration was chosen high enough to give sufficient precision to the physical determination of the rotatory power even in the early stages of the active transport.

The agreement with the theoretical previsions was good and in particular the D/L ratio, as predicted, was found independent of time in compartment 2. Higher optical purities are predictable in conditions favouring a greater "extracting power" of the pump (smaller S_0). It has to be mentioned that contrary to fermentation techniques the two isomers are recovered and separated in space by the active transport membrane.

6. Comments

The experimental glucose pump is a particular illustration of the theory.

The theory itself may be perfected, if pH profiles different from linear are considered, or acid or base production and their consequences taken into account. However these extensions will not change the basic concepts exposed.

One of the main interests of the system is to show that pH gradients can induce and regulate molecular transport fluxes *via enzyme activities*. The biological role of such gradients is the subject of intensive modern

research. The nature and behaviour of this coupling needs the greatest attention in order to avoid confusion between "exchange transports" and active ones. The enzyme activities maintaining the pump are directly related to the pH gradient, itself dependent on the pH values on the boundaries of the membrane. The corresponding buffer (acid) flow depends on the permeability to buffer of the membrane (and on eventual H^+ production flows). *Briefly, the functional enzyme activity structure exists even in absence of substrate. It is directly coupled to the pH gradient via enzyme activation and indirectly to externally entertained (acid/base) buffer fluxes.* The primary source of energy is ATP in the glucose pump. The pH gradient can be considered as a sort of an "entropic signal" [9].

The orientation of the pH gradient relative to the active transport depends on the relative values of the optimal pH of the two enzymes. For example let us substitute *alkaline phosphatase* for the acid phosphatase used in the glucose pump. Both enzymes catalyse the same reaction, but respectively at a higher or a lower pH than their hexokinase partner. Thus we can get two similar hexokinase-phosphatase pumps in which however the orientation of the imposed pH gradient should be reversed if the direction of the glucose transport and the directional sequence of the reactions are to be maintained unchanged (fig. 14).

7. Conclusions and developments

The analytical treatment used has the advantage of mathematical exactitude and security and leads to a series of conclusions:

The intrinsic properties of the pump are expressed by the reaction/diffusion parameter Σ and not by membrane thickness.

The global rate of transport V_M can be Michaelian in a number of situations.

The extractive $(S_2/S_1)_{max}$ or accumulative $(S_2 - S_1)_{max}/S_0$ powers of the pump are linked to the external substrate concentration, that is to the order of reaction.

The pump is a regulator of internal concentration as its efficiency, relative to diffusion, decreases when the substrate concentration increases.

The role of compartment volumes leading alternatively to high fluxes or high gradients can be expressed. The stereoselectivity of the pump can be high with a small internal compartment and low (physiological range) substrate concentrations.

The stoichiometric yield was high even experimentally: together with the energy liberated by the reaction (ATP), it determines the energetic yield. The load ΔS decreases the yield due to increased back diffusion, but under specified conditions the inert layer can limit the effects of this imperfection.

Many other situations could be further examined but the analytical treatment of more exact or more complete physical situations becomes rapidly too complicated or impossible. A numerical approach will be the subject of the next paper [10].

Acknowledgements

This work is a part of the Dr. Sc. Thesis of Jean Claude Vincent (June 1980, Rouen). The financial support of the DGRST Grants no 72.7.241 and 76.7.1382 is acknowledged.

References

[1] E. Selegny and J.C. Vincent, Biophys. Chem. 12 (1980) 93.
[2] E. Selegny, G. Broun and D. Thomas, Compt. Rend. A Acad. Sci. Paris, 271 Série D (1970) 1423.
[3] E. Selegny, S. Avrameas, G. Broun and D. Thomas, Compt. Rend. Acad. Sci. Paris, 269, Série C, (1968) 1431; French pat (1968) 2, 028, 722.
[4] E. Selegny, A.M. Meffroy-Biget, M. Labbe, Compt. Rend. Acad. Sci. Paris, 266, Série C (1968) 1329.
[5] J.C. Vincent, Transport actif de glucose in vitro, Dr. Ing. Thesis Rouen (1976).
[6] M. Totskii, Biochemistry (Trad. Biokhimia) (1976) 894.
[7] G.A. Junter, E. Selegny et J.F. Lemeland, Ann. Microbiol. (Inst. Pasteur) 130 A (1979) 295.
[8] E. Selegny, J.C. Vincent and M. Lançon, Tetrahedron Letters 33 (1977) 3171.
[9] E. Selegny, in: Polyelectrolytes, eds. E. Selegny, M. Mandel, U.P. Strauss (Reidel Publ. Co., Dordrecht, Holl., 1974) p. 419.
[10] J.C. Vincent, E. Selegny and M. Metayer, Biophys. Chem., to be published.