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"CARRIER" THEORY AND THERMODYNAMICS OF IRREVERSIBLE PROCESSES

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SUMMARY

A theory of transport via a "carrier" based on Wyman's theory on multiple equilibria is presented. By taking into account the detailed balance principle, it is possible to simplify the flux expressions and their coupling coefficients. In this way, Onsager's rules are derived. An experimental approach to the model is proposed.

INTRODUCTION

Transport across membranes and enzymatic phenomena present strong similarities, especially in specificity with respect to substrates and in the analytical form of the kinetic laws. Such observations soon led research workers to the concept of transport by a "carrier", i.e. a macromolecule confined within a membrane, capable of binding molecules on either side and carrying them through the membrane. This concept has inspired a large number of models which demonstrate coupled transport when the same "carrier" is capable of binding several kinds of ligands together (see Schultz and Curran [1]). There have been many experimental studies [2] referring to such models, but theoretical studies have been relatively limited. Perhaps the difficulties encountered by the experimentalists have discouraged theoreticians. Most models deal with particular situations of binding or with the proximity of equilibrium where linear laws are applicable. However, a thorough study was carried out by Heinz et al. [3] on the transport of a molecule by a carrier capable of binding an agonist and an antagonist.

The present paper attempts to combine the carrier theory and some of the advancements made in the multiple equilibria theory, in particular those made under the impetus of Wyman [4, 5]. The result is a greater simplicity in treating general cases in conjunction with the linear theory of irreversible processes. A detailed study is made of coupling between transports. The coupling between chemical reaction sand transport will be the object of a future work.

For lack of conventional notation, that of Heinz [3] is used in part. An appen-

dix indicates how our notation relates to the concepts and notations used in other papers.

CARRIER NOTION, GENERAL RELATIONS

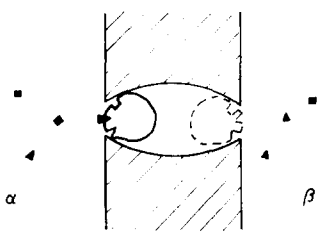
The general hypotheses which support the carrier theory have a rather arbitrary character. The assumptions made in this paper are frequently encountered in the literature but they are not the only ones possible. They are:

(1) The membrane separates two phases, α and β. It is assumed that the carrier movement across the membrane is slow enough to be considered as constituting the limiting stage of the transport and that the carrier is in equilibrium when it is in contact with each phase, α or β.

(2) The time scale considered makes it possible to establish a steady state for transport.

(3) The total number of carrier molecules X_t is constant. The various carrier states are denoted X_i. They differ either in their conformation or in the number of bound ligands, or both. Each carrier molecule is in relation with one of the phases; $X_{i\alpha}$ and $X_{i\beta}$ designate the corresponding quantities of state X_i. Any intermediate state is assumed to be negligible in quantity. Carrier movement is thus described by:

$$X_{i\alpha} \underset{k'_i}{\overset{k_i}{\rightleftharpoons}} X_{i\beta} \tag{1}$$



where k_i is the rate constant of the transit of X_i from α to β and k'_i is the rate constant for the reverse process.

The electrical potential difference is assumed to be maintained at zero, under which conditions k_i and k'_i are true constants. The influences of a potential difference on transport will be examined in a further paper. X_α is the total carrier quantity on side α, X_β on side β:

$$X_\alpha = \sum_i X_{i\alpha} \qquad X_\beta = \sum_i X_{i\beta} \qquad X_t = X_\alpha + X_\beta \tag{2}$$

The special case in which the rate constants are not dependent on the carrier state is called the "Affinity model" by Heinz et al. [3]:

$k_i = k; \qquad k'_i = k' \qquad$ for any i value

Local equilibrium

The hypothesis of the local equilibrium of each phase implies that the distribution of X_α in the various X_i may be described by a partition function ϕ_α. The quantity of each state $X_{i\alpha}$ is proportional to a term of the partition function ϕ_α. When the

solutes are ideal, ϕ_α is a polynomial of their concentrations $c_{j\alpha}$. Thus by letting ν_{ij} be the number of ligands of type j fixed on state i and $a_{i\alpha}$ the polynomial coefficient corresponding to state i, the partition function can be written:

$$\phi_\alpha(\ldots, c_{j\alpha}, \ldots) = \sum_i a_{i\alpha} c_{1\alpha}^{\nu_{i1}} \ldots c_{r\alpha}^{\nu_{ir}} \tag{3}$$

where r is the ligand type number.
$X_{i\gamma}$ is given by:

$$X_{i\alpha} = \frac{a_{i\alpha} c_{1\alpha}^{\nu_{i1}} \ldots c_{r\alpha}^{\nu_{ir}}}{\phi_\alpha} X_\alpha \tag{4}^\star$$

If a j ligand is not in an ideal solution, its concentration must be replaced by its activity.

Values in phase β are defined in the same way: ϕ_β, $a_{i\beta}$, $c_{j\beta}$, etc.

Detailed balances

The constants a_i and k_i must be compatible with an equilibrium when the concentrations (or activities) $c_{j\alpha}$ are respectively equal to the concentrations $c_{j\beta}$, which we will denote by $(\alpha) = (\beta)$.

The $X_{i\alpha}$ values must thus satisfy the detailed balance relation which is an expression of "microscopic reversibility":

$$(\alpha) = (\beta) \Rightarrow k_i X_{i\alpha} = k'_i X_{i\beta} \tag{5}$$

Before explaining this detailed balance it should be noted that ϕ_α and ϕ_β are both determined except for an arbitrary factor. These factors can be selected so that at equilibrium the overall partition function ϕ is simply the sum of $\phi_\alpha + \phi_\beta$. Thus, at equilibrium, when $(\alpha) = (\beta)$:

$$\begin{aligned} X_{i\alpha} &= \frac{a_{i\alpha} c_1^{\nu_{i1}} \ldots c_r^{\nu_{ir}} X_t}{\phi} \\ X_{i\beta} &= \frac{a_{i\beta} c_1^{\nu_{i1}} \ldots c_r^{\nu_{ir}} X_t}{\phi} \end{aligned} \tag{6}$$

Hence the detailed balance (5) is reduced to the fundamental relation:

$$k_i a_{i\alpha} = k'_i a_{i\beta} \tag{7}$$

Let us call ψ the polynomial derived from ϕ_α by replacing each coefficient $a_{i\alpha}$ by $k_i a_{i\alpha}$ (or derived from ϕ_β by replacing $a_{i\beta}$ by $k'_i a_{i\beta}$). Its numerical values $\psi(\alpha)$ and $\psi(\beta)$ will play an important role later on.

For the affinity models, this relation (7) can be reduced to:

$$k a_{i\alpha} = k' a_{i\beta} \tag{8}$$

In this case, the polynomials ϕ_α, ψ_β, ϕ and ψ are proportional.

$\star$ The numerical value $\phi_\alpha(\ldots, c_{j\alpha}, \ldots)$ will be noted $\phi_\alpha(\alpha)$ and, when there can be no confusion, will be abbreviated ϕ_α which normally designates the polynomial defined by its coefficients.

Steady state

As in Enzymology a steady state may be established every time the concentrations of ligands can be held constant, the steady state must satisfy the general balance:

$$\sum k_i X_{i\alpha} = \sum k'_i X_{i\beta} \tag{9}$$

If expressions (4) for $X_{i\alpha}$ and $X_{i\beta}$ and the definition of ψ are substituted into Eqn 9, it becomes:

$$\frac{\psi(\alpha)X_\alpha}{\phi_\alpha} = \frac{\psi(\beta)X_\beta}{\phi_\beta} \tag{10}$$

Then, by way of conservation Eqn 2 we can write:

$$\begin{aligned} X_\alpha &= \frac{\phi_\alpha \psi(\beta) X_t}{\phi_\alpha \psi(\beta) + \phi_\beta \psi(\alpha)} \\ X_\beta &= \frac{\phi_\beta \psi(\alpha) X_t}{\phi_\alpha \psi(\beta) + \phi_\beta \psi(\alpha)} \end{aligned} \tag{11}$$

For the affinity models, Eqn 11 can be reduced to:

$$\begin{aligned} X_\alpha &= \frac{k' X_t}{k + k'} \\ X_\beta &= \frac{k X_t}{k + k'} \end{aligned} \tag{12}$$

Transport flux at the steady state

Let us consider first the case of one ligand only. This permits us to do away with the index j. The corresponding flux can be expressed in the following manner.

$$J = \sum \nu_i k_i X_{i\alpha} - \sum \nu_i k'_i X_{i\beta} \tag{13}$$

If $X_{i\alpha}$ and $X_{i\beta}$ are then expressed in terms of Eqn 4 for only one ligand:

$$J = \frac{\sum \nu_i k_i a_{i\alpha} c_\alpha^{\nu_i} X_\alpha}{\phi_\alpha} - \frac{\sum \nu_i k'_i a_{i\beta} c_\beta^{\nu_i} X_\beta}{\phi_\beta} \tag{14}$$

We can recognize $c_\alpha \, \partial\psi(\alpha)/\partial c_\alpha$ and $c_\beta \, \partial\psi(\beta)/\partial c_\beta$ in the numerators.
If X_α and X_β are replaced by the values expressed for them in Eqn 11, then:

$$J = \frac{X_t}{\phi_\alpha \psi(\beta) + \phi_\beta \psi(\alpha)} \left[\psi(\beta) \frac{\partial \psi(\alpha)}{\partial \log c_\alpha} - \psi(\alpha) \frac{\partial \psi(\beta)}{\partial \log c_\beta} \right] \tag{15}$$

which can also be written:

$$J = \frac{\psi(\alpha)\psi(\beta)X_t}{\phi_\alpha \psi(\beta) + \phi_\beta \psi(\alpha)} \left[\frac{\partial \log \psi(\alpha)}{\partial \log c_\alpha} - \frac{\partial \log \psi(\beta)}{\partial \log c_\beta} \right] \tag{16}$$

For simplicity's sake, let:

$$\Phi = \frac{\psi(\alpha)\psi(\beta)X_t}{\phi_\alpha\psi(\beta)+\phi_\beta\psi(\alpha)} \tag{17}$$

$$\zeta(\alpha) = \frac{\partial \log \psi(\alpha)}{\partial \log c_\alpha}$$

$$\zeta(\beta) = \frac{\partial \log \psi(\beta)}{\partial \log c_\beta} \tag{18}$$

Φ is the quantity of carrier crossing the membrane in a given direction during one unit of time, and Φ/X_t is the frequency of turn-over.

The flux J can now be written:

$$J = \Phi[\zeta(\alpha) - \zeta(\beta)] \tag{19}$$

The unidirectional fluxes are respectively:

$$\vec{J} = \Phi\zeta(\alpha) \; ; \; \overleftarrow{J} = \Phi\zeta(\beta) \tag{20}$$

from α to β from β to α

These expressions can be simplified in affinity models to:

$$J = \frac{kk'X_t}{k+k'}\left[\frac{\partial \log \phi_\alpha}{\partial \log c_\alpha} - \frac{\partial \log \phi_\beta}{\partial \log c_\beta}\right] \tag{21}$$

The value appearing in the brackets corresponds to the difference of the average number $\bar{r}$ of ligands which the carrier binds on each side: $\bar{r}(\alpha) - \bar{r}(\beta)$.

COUPLED TRANSPORT

We are now ready to look at the central problem of coupled transport. In doing so, we will limit ourselves to relations involving two ligands, labeled 1 and 2. It is easy to generalize for a larger number of ligands.

If J_1 and J_2 are used to denote the respective fluxes of constituents 1 and 2 and $c_{1\alpha}$, $c_{1\beta}$, $c_{2\alpha}$ and $c_{2\beta}$ their concentrations on both sides, then by substitution into Eqn 19:

$$J_1 = \Phi\,[\zeta_1(\alpha) - \zeta_1(\beta)] \tag{22}$$
$$J_2 = \Phi\,[\zeta_2(\alpha) - \zeta_2(\beta)]$$

Before studying coupling itself, let us first define an independence criterion.

Independence criterion

One condition necessary for independence is:

$$\frac{\partial J_1}{\partial c_{2\alpha}} = 0 \quad \text{and} \quad \frac{\partial J_1}{\partial c_{2\beta}} = 0$$

that is,

$$\begin{cases} \dfrac{\partial \Phi}{\partial c_{2\alpha}} [\zeta_1(\alpha) - \zeta_1(\beta)] + \Phi \dfrac{\partial \zeta_1(\alpha)}{\partial c_{2\alpha}} = 0 \\ \dfrac{\partial \Phi}{\partial c_{2\beta}} [\zeta_1(\alpha) - \zeta_1(\beta)] - \Phi \dfrac{\partial \zeta_1(\beta)}{\partial c_{2\beta}} = 0 \end{cases}$$

These relations must be true when $(\alpha) = (\beta)$ (equilibrium) and therefore:

$$\frac{\partial \zeta_1}{\partial c_2} = 0$$

This implies the decomposition of ψ into factors:

$$\psi(c_1, c_2) = \psi_1(c_1)\psi_2(c_2) \tag{23}$$

and finally that

$$\frac{\partial \Phi}{\partial c_{2\alpha}} = 0$$

and the nullity of the other derivatives of Φ which is thus constant. This is an affinity type system in which:

$$\frac{\partial \bar{v}_1}{\partial c_2} = 0 \qquad \text{etc.}$$

in other words, the two ligands are independently bound by the carrier. These conditions are, of course, sufficient.

Coupling coefficients

The generally accepted definition of coupling coefficients makes use of unidirectional fluxes [1].

coupling coefficient

$$CC2/1 = \left(\frac{\partial \vec{J}_2}{\partial \vec{J}_1}\right)_{c_{2\alpha}} \tag{24}$$

This can also be expressed as:

$$CC2/1 = \frac{\dfrac{\partial \log \Phi}{\partial \log c_{1\alpha}} \zeta_2(\alpha) + \dfrac{\partial \zeta_2(\alpha)}{\partial \log c_{1\alpha}}}{\dfrac{\partial \log \Phi}{\partial \log c_{1\alpha}} \zeta_1(\alpha) + \dfrac{\partial \zeta_1(\alpha)}{\partial \log c_{1\alpha}}} \tag{25}$$

A symmetrical expression exists for *CC* 1/2.
For the affinity models, the expression can be simplified to:

$$CC2/1 = \frac{\partial \bar{v}_2(\alpha)}{\partial c_{1\alpha}} \Big/ \frac{\partial \bar{v}_1(\alpha)}{\partial c_{1\alpha}} \tag{26}$$

and the notion of coupling in transports coincides with that of cooperativity in binding:

$$\frac{\partial J_1}{\partial c_{1\alpha}} = \frac{kk'X_1}{k+k'} \frac{\partial \bar{v}_1(\alpha)}{\partial c_{1\alpha}} \quad \text{and} \quad \frac{\partial J_1}{\partial c_{2\alpha}} = \frac{kk'X_1}{k+k'} \frac{\partial \bar{v}_1(\alpha)}{\partial c_{2\alpha}} \tag{27}$$

$\partial J_1/\partial c_{1\alpha}$ has the same sign as $\partial \bar{v}_1(\alpha)/\partial c_{1\alpha}$ and is thus positive.
However, $\partial \bar{v}_1(\alpha)/\partial c_{2\alpha}$ can have either sign:

A + sign denotes that the bindings are cooperative and the transports are agonist.

A − sign denotes that the bindings are anticooperative and the transports are antagonist.

Note finally that the Wyman theorem on $\bar{v}_1$, $\bar{v}_2$ can also be applied to the fluxes:

$$\frac{\partial J_1}{\partial \log c_{2\alpha}} = \frac{\partial J_2}{\partial \log c_{1\alpha}} \tag{28}$$

Strict coupling

This is a special case frequently encountered when the ratio between J_1 and J_2 is constant. Such a situation obviously implies a constant ratio between ζ_1 and ζ_2 as well:

$$c_1 \frac{\partial \psi}{\partial c_1} = hc_2 \frac{\partial \psi}{\partial c_2} \tag{29}$$

where h = constant. The equality 29 must be written term by term. A term like $c_1^m c_2^n$ with a non-zero coefficient gives:

$$m = h\,n$$

Hence any carrier state has ligands 1 and 2 fixed in a constant proportion. This condition is obviously sufficient. The coupling coefficients are equal respectively to h and $1/h$.

Onsager relations

We will adopt for variables in this paragraph the chemical potentials ($\mu_{1\alpha}$ etc.) instead of the concentrations ($c_{1\alpha}$ ...). As we know, the expression of the entropy production for a binary transport between two phases is:

$$\sigma = \frac{J_1}{T}(\mu_{1\alpha} - \mu_{1\beta}) + \frac{J_2}{T}(\mu_{2\alpha} - \mu_{2\beta}) \tag{30}$$

and σ must be positive. When a term of σ is negative, the corresponding transport is designated "active". The expressions 22 of the fluxes are developed using conditions α close to conditions β as the origin. After derivation with respect to $\mu_{1\alpha}$, $\mu_{2\alpha}$ we get:

$$\begin{cases} J_1 = \Phi \left[\dfrac{\partial \zeta_1}{\partial \mu_1}(\mu_{1\alpha} - \mu_{1\beta}) + \dfrac{\partial \zeta_1}{\partial \mu_2}(\mu_{2\alpha} - \mu_{2\beta}) \right] \\ J_2 = \Phi \left[\dfrac{\partial \zeta_2}{\partial \mu_1}(\mu_{1\alpha} - \mu_{1\beta}) + \dfrac{\partial \zeta_2}{\partial \mu_2}(\mu_{2\alpha} - \mu_{2\beta}) \right] \end{cases} \tag{31}$$

Thermodynamic fluxes and forces can be defined in many ways; one of them consists of keeping J_1 and J_2 for the fluxes and $(\mu_{1\alpha} - \mu_{1\beta})/T$ and $(\mu_{2\alpha} - \mu_{2\beta})/T$ for the forces.

The phenomenological coefficients which appear in the linear relations 31 are thus:

$$L_{11} = \Phi T \frac{\partial \zeta_1}{\partial \mu_1} \qquad L_{12} = \Phi T \frac{\partial \zeta_1}{\partial \mu_2}$$
$$L_{21} = \Phi T \frac{\partial \zeta_2}{\partial \mu_1} \qquad L_{22} = \Phi T \frac{\partial \zeta_2}{\partial \mu_2} \tag{32}$$

The Onsager relation is derived from:

$$\frac{\partial \zeta_1}{\partial \mu_2} = \frac{1}{RT} \frac{\partial^2 \log \psi}{\partial \log c_1 \partial \log c_2} = \frac{\partial \zeta_2}{\partial \mu_1}$$

and therefore

$$L_{12} = L_{21} = \frac{\psi X_t}{R\phi} \frac{\partial^2 \log \psi}{\partial \log c_1 \partial \log c_2} \tag{33}$$

Hence, the carrier theory is linked to the thermodynamics of irreversible processes. Compatibility results from the existence of the function Ψ which in turn comes from the detailed balances. In relations 32 the phenomenological coefficients are expressed as a function of the coefficients of polynomials ϕ_α, ϕ_β and ψ, that is, of the intrinsic properties of the carrier as well as of the concentrations of ligands.

Unfortunately, the choice of $CC2/1$ and $CC1/2$ as coupling coefficients does not correspond in a simple manner with the thermodynamic coupling coefficients L_{12} and L_{21}. We can easily see that:

$$CC2/1 = \frac{L_{21}}{L_{11}} \quad \text{and} \quad CC1/2 = \frac{L_{12}}{L_{22}}$$

All these relations are true in the neighbourhood of equilibrium, characterized by:

$$|\mu_{i\alpha} - \mu_{i\beta}| \ll RT$$

These relations do not depend on the particular choice of fluxes or forces made in defining the L_{ij} values.

One final remark: the implication of Eqn 33 that in the neighbourhood of equilibrium,

$$\frac{\partial J_1}{\partial \mu_2} = \frac{\partial J_2}{\partial \mu_1}$$

is no longer true when we are far from equilibrium except for the Affinity models where the relation exists through the entire domain due to Eqn 28.

EXPERIMENTAL APPROACH

Two kinds of experiments can be performed on transport systems:

(1) Those intended to measure the binding of ligands at equilibrium, i.e. titration curves of the carrier by its ligands. For one ligand such an experiment gives the result

$$\bar{\nu} X_t = \left(\frac{\partial \log \phi}{\partial \log c}\right) X_t$$

as a function of c. Hence, one integration gives: ϕ^{X_t}

(2) Those intended to measure unidirectional fluxes. Their ratio is merely:

$$\frac{\vec{J}}{\overleftarrow{J}} = \frac{\zeta(\alpha)}{\zeta(\beta)} \tag{34}$$

When $c_\beta \to \infty$, $\zeta(\beta) \to n$, where n is the number of binding sites on the carrier and the degree of polynomials ϕ and ψ. In this case, one integration with respect to log c_α gives $\psi^{1/n}$.

In order to finish up the determination of ϕ and ψ we need to know X_t or n, since their product nX_t can be experimentally determined as the maximum bounded ligand. If it is not determined by an independent experiment, the minimum value allowing the construction of ϕ and ψ by polynomial approximation from experimental functions will be assigned to n.

The final determination of ϕ_α and ϕ_β is based upon the general expression of unidirectional fluxes:

$$\vec{J} = \Phi\, \zeta(\alpha)$$

$$\phi_\alpha \psi(\beta) + \phi_\beta \psi(\alpha) = \frac{\psi(\alpha)\psi(\beta)\zeta(\alpha) X_t}{\vec{J}} \tag{35}$$

in Eqn 35, the right hand side is known. The coefficients of ϕ_α and ϕ_β, and hence the rate constants k_i and k'_i can be determined.

When dealing with several ligands, the polynomial coefficients obtained with respect to one ligand will appear as polynomial of the other ligands. Repeated calculations will be needed to complete the determination.

Remarks

The proposed experimental approach being based on concentration effects cannot distinguish between two isomers bearing the same numbers of each ligand. Other techniques are required to solve the states of binding in conformation isomers. A possible source of data is the analysis of non-steady-states, in particular, the pre-steady-states. The amplitudes and relaxation times of a carrier system would give a large number of equations for the parameters to be determined.

DISCUSSION

There is no question that the carrier model is capable of explaining a large number of experimental situations. But a transmembrane transport model cannot be expected to have the same reliability and precision as obtained in enzymology, due to special experimental difficulties.

In most cases it is impossible to study an isolated transport system, since the system is usually the entire cell and all the carriers operate at the same time. Sometimes, a given ligand is charged by only one carrier, but most often several carriers charge it. In any case, the various carriers have in common the H^+ ligand whose transport affects the electrostatic potential which can influence all the other transports.

In general it is not possible to perform more than one measurement on the same preparation: titration measurements require pure membranes with good phase equalization on both sides while flux measurements require intact cells or vesicles within which the chemical potential of the ligands is difficult to determine with any precision. These difficulties will be progressively overcome, notably by means of artificial membranes. This does not mean that the theoretician must remain idle in the meantime.

It will be necessary to take into consideration the structure of the membrane and refine the carrier movement by introducing intermediates which present certain aspects of a two-dimensional phase.

Another crucial area is the theory of coupling of transport and chemical reactions via the carrier. The difficulty here is caused not so much by the intervention of the Curie principle, which can be applied to discontinuous systems, as by the complexity introduced in the detailed balances and by the abandoning of the local equilibrium hypothesis.

Finally, it will be necessary to study transport systems arranged in series as they operate in the intestinal mucosa or kidney.

APPENDIX

Correspondence with previous work

The work most like this study is that of Heinz et al. [3]. Three ligands are bound on the carrier: a substrate, b agonist, c antagonist. Each one is bound at most once, and b and c cannot be attached together. The principle of detailed balances is taken into consideration for the fixation states. Heinz's principal formulas (Numbers 26–29) can be deduced from the present formalization by making the following changes in notation.

	Present paper	Heinz et al. [3]
Phase notation	α, β	', ''
Transit rate constant	k	p
Partition function	ϕ_α ϕ_β	U', U''

In addition, V' and V'' in Heinz are functions of ψ type and the value R corresponds to $(\phi_\alpha\psi(\beta) - \phi_\beta\psi(\alpha))/X_1$

The correspondence with the models summarized by Schultz and Curran in ref. 1 is easy to establish, at least for "affinity" type models whose formulas can be recalculated by setting $\phi_\alpha = SNa + K_3Na + K_2S + K_1K_2$ and $kk'/k - k' = P$. Correspondence is not so satisfactory for the general cases, since only the analytical form is found and coincidence is obtained only for symmetrical membranes ($k_i = k'_i$, $\alpha_{i\alpha} = \alpha_{i\beta}$, etc.).

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