THEORETICAL ANALYSIS OF TRANSPORT PROCESSES IN LIVING SYSTEMS*

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The ability of living cells to create and maintain differences in their composition from that of their environment must in many cases depend on the existence of specific mechanisms for the selective transport of certain molecular species as opposed to others. Neither differences in permeability to various species migrating at random nor alterations in structure of the intracellular medium are sufficient to explain all the observations made to date. Such specific processes peculiar to living systems are intuitively understood under the term active transport.

In recent years appreciable effort has been made to so refine the concept of active transport as to be able to ascertain experimentally (1) whether in a given system a given molecular component has been transported by a specific mechanism and (2) if so, what fraction of the total amount transported can be accounted for in terms of transport by this mechanism.1-4 Although the proposed definitions of active transport have served well to guide experimentation, none of them are satisfactory in themselves inasmuch as they are either not sufficiently inclusive or sufficiently rigorous. The various formulations and the specific ambiguities associated with them have been discussed in several earlier papers.5-10

It is our purpose in this paper to present the concepts essential to a systematic description of transport processes in living systems and to show that the types of transport phenomena generally considered to be peculiar to such systems can be formulated in rigorous terms. In approaching the problem of describing any phenomena occurring in living systems, one must bear in mind the inherent complexity of such systems. This complexity will unavoidably be reflected in their description in mathematical terms. Thus the cell contains somewhere between 10^3 and 10^6 distinguishable molecular species, and probably as many transport or transformation processes, all occurring simultaneously. Even though the physical laws governing each individual process are as simple as they would be, were the process to be observed in isolation, their interdependence must be explicitly taken into account. For this purpose we have utilized in the following treatment the notation current in thermodynamics of irreversible processes. It should be emphasized, however, that the adoption of this convenient notation does not constitute an application to this problem of the thermodynamic theory of irreversible processes as such. In particular, the validity of the treatment does not depend either on an assumption of a linear relationship between fluxes and forces or on the Onsager reciprocal relations.

23 Mahler, G. R. (private communication).
Mathematical Theory.—We choose for analysis a representative living system in contact with and open to its environment. All processes occurring within it and across its boundaries may be completely described by sets of equations of the form:\textsuperscript{11} \textsuperscript{12}

\[ \begin{align*}
\overrightarrow{J}_i &= \sum_j^n \lambda_{ij} \vec{X}_j \quad (i = 1, 2, \ldots n) \\
J_\alpha &= \sum_\beta \lambda_{\alpha \beta} A_\beta \\
\end{align*} \]

where \( \overrightarrow{J}_i \) is the vectorial flux of quantity \( i \), \( X_j \) is the \( j \)th thermodynamic force and \( \lambda_{ij} \) is the coefficient relating the two. In general, \( \lambda_{ij} \) is a function of \( \vec{X}_j \). \( J_\alpha \) is the rate of the \( \alpha \)th reaction, defined as

\[ J_\alpha v_{\alpha i} = \frac{1}{V} \frac{d_n i}{dt} \]

where \( v_{\alpha i} \) is the stoichiometric coefficient for the \( i \)th constituent in the \( \alpha \)th reaction, \( V \) the volume of the region in which the reaction is occurring, and \( d_n i / dt \) is the rate of change of the number of moles of constituent \( i \) due to the \( \alpha \)th reaction. \( A_\beta \) is the thermodynamic force, driving the \( \beta \) reaction, the so-called affinity, and \( \lambda_{\alpha \beta} \) is a coefficient which in general is a function of \( A_\beta \). The two separate sets of equations, (1) and (2), are used to describe transport and transformation phenomena, respectively, because it is necessary to distinguish the different tensorial character of the thermodynamic forces involved, inasmuch as Curie’s theorem states that it is impossible for a force of a given tensorial order to be associated with a flow of a higher tensorial order.\textsuperscript{13}

It follows, therefore, that the one and only way to effect a transport process by the scalar forces of chemical reactions is to have a source and a sink set up by such reactions in distinct but contiguous regions of space. One may thus visualize the production of a constituent by a chemical reaction in one region of space and its destruction in another region, giving rise to a thermodynamic vectorial force and a concomitant flow.

Without loss of generality and for purposes of simplification, we shall consider in the following formulations two adjacent regions of space separated by a boundary and in which \( \rho \) and \( \rho' \) reactions respectively may occur. The contribution of a chemical reaction to the transport of any constituent \( i \) may be taken as the effect of a change in the difference of its chemical potential between the two regions resulting from this reaction.\textsuperscript{14} We are thus interested in evaluating this contribution in terms of the reaction rate.

A given reaction, \( \alpha \), occurring in one of the specified regions will produce a change in the quantity of constituent \( i \) according to equation (3). The total change in the quantity of the constituent \( i \) is given by

\[ \frac{dn_i}{dt} = \frac{d^\rho n_i}{dt} + \frac{d^\tau n_i}{dt} = V \sum_\alpha \lambda_{\alpha i} v_{\alpha i} - \vec{S} \cdot \overrightarrow{J}_i \quad (i = 1, 2, \ldots n) \]

where the superscripts \( P \) and \( T \) denote the total contributions of reactions and tran-
port respectively, and $\vec{S}$ is the vector area defined as $\int_s \vec{r} \cdot d\vec{S}$ where $\vec{r}$ is the unit vector perpendicular to an element of the surface, $d\vec{S}$.

The relation between the change in chemical potential of $i$ resulting from changes in the quantities of constituents, $j$, due to the $\alpha^{th}$ reaction is given by

$$
\frac{d_n\mu_i}{dt} = \sum_j \frac{\partial \mu_i}{\partial n_j} \frac{dn_j}{dt} \quad \{i = 1, 2, \ldots n\} \quad \{\alpha = 1, 2, \ldots \rho\} \tag{5}
$$

where $g_{ij} = \left(\frac{dn_i}{dn_j}\right)_{nk}$.

If we now define for a given region

$$
\eta_{ai} = \int_0^\tau \frac{d_n\mu_i}{dt} dt \quad \{i = 1, 2, \ldots n\} \quad \{\alpha = 1, 2, \ldots \rho\} \tag{6}
$$

where $\tau$ denotes an arbitrary period of observation, then $\eta_i$, the total contribution of chemical reactions to the chemical potential of constituent $i$ for the interval $\tau$ in a region is given by

$$
\eta_i = \sum_\alpha \eta_{ai} \quad (i = 1, 2 \ldots n) \tag{7}
$$

and may be evaluated in terms of the reaction velocities with the use of equations (3), (5), and (6). For the two-compartment system under consideration the vector forces in the flux equations (1) may thus be expressed as

$$
\vec{X}_i = -(\mu_i' - \mu_i) = -(\xi_i' - \xi_i + \eta_i - \eta_i) = -(\Delta\xi_i + \Delta\eta_i) \quad (i = 1, 2 \ldots n) \tag{8}
$$

where

$$
\xi_i = \xi_{i0} + \int_0^\tau \frac{d\mu_i}{dt} dt = \xi_{i0} + \int_0^\tau \sum_j g_{ij} \vec{S} \cdot \vec{j}_d dt \quad (i = 1, 2 \ldots n). \tag{9}
$$

It is evident that $\xi_i$ is the sum of the initial chemical potential, $\xi_{i0}$, and the change in $\mu_i$ brought about by transport processes in the period $\tau$. From equations (1) and (8) it follows that the free flux of constituent $i$ is

$$
\vec{J}_i = -\sum_j \lambda_{ij}\Delta\xi_j - \sum_j \lambda_{ij}\Delta\eta_j = \vec{J}^F_i + \vec{J}^R_i \tag{10}
$$

where the first term $\vec{J}^F_i$ is that resulting from forces to which there has been no contribution from chemical reactions and the second, $\vec{J}^R_i$ accounted for exclusively in terms of sources and sinks resulting from reactions.

In a system in which the molecular structure of constituent $i$ can be altered by addition or removal of a chemical group or by a change in conformation, to yield constituents $k$, transported in this form, and reconstituted to form constituent $i$ upon completion of transport, the total resulting flux of $i$ will be

$$
\vec{J}_{i\tau} = \vec{J}_i + \sum_{k(i)} r_{ik} \vec{J}_{ik} \quad (k = 1, 2 \ldots m). \tag{11}
$$
The symbol, \( k(i) \), implies that \( k \) is derived from \( i \), and \( \nu_{ik} \) is the number of moles of \( i \) yielding one mole of \( k \).

In addition, \( i \) may be either incorporated into or released from other constituents, designated \( b \), which are not subject to directed translational movement. Thus we must distinguish between two types of reactions both of which by involving constituent \( i \) contribute to the forces involved in its transport but in fundamentally different ways. In the former, the transport is effected by the flux of constituents \( k \) derived from \( i \), whereas in the latter it results from an alteration of the chemical potential difference of constituent \( i \) itself, by direct reaction. There remains then only the contribution of those reactions which do not directly involve constituent \( i \). Accordingly, we may divide the contribution resulting from chemical reactions into these categories:

\[
\Delta \eta_i = \sum k \Delta \eta_{ki} = \sum \Delta \eta_{ki} + \sum \Delta \eta_{bi} + \sum \Delta \eta_{ji}
\]

where \( k \) are reactions leading to the formation or decomposition of \( k \) derived from \( i \) for which \( J_k \neq 0 \); \( \beta \), the reactions leading to the formation or decomposition of \( b \) containing \( i \), for which \( J_b \equiv 0 \); and \( \gamma \), the remaining reactions not directly involving \( i \).

We are now in the position to express the total flux of constituent \( i \) in terms of the individual contributions of different types of processes. From equations (10) and (11) we have

\[
J_{i\tau} = -\sum_j \lambda_{ij} \Delta \xi_j - \sum_j \lambda_{ij} \Delta \eta_j - \sum_{jk} \nu_{ik} \lambda_{kj} \Delta \xi_j - \sum_{jk} \nu_{ik} \lambda_{kj} \Delta \eta_j \quad (i = 1, 2 \ldots n)
\]

(13a)

wherefrom we obtain by subdividing the second term on the right in accordance with equation (12)

\[
J_{i\tau} = -\sum_j \lambda_{ij} \Delta \xi_j - \sum_{jk} \nu_{ik} \lambda_{kj} \Delta \xi_j - \lambda_i (\Delta \eta_i^K + \Delta \eta_i^B + \Delta \eta_i^\gamma)
\]

\[-\sum_j \lambda_{ij} \Delta \eta_j - \sum_{jk} \nu_{ik} \lambda_{kj} \Delta \eta_j \quad (i = 1, 2 \ldots n).
\]

(13b)

On the basis of the origin of the thermodynamic forces involved, it is thus possible to separate the total flux of any component \( i \) into four distinct terms:

\[
\tilde{J}_{ip} = -\sum_j \lambda_{ij} \Delta \xi_j - \sum_{jk} \nu_{ik} \lambda_{kj} \Delta \xi_j \quad (14a)
\]

\[
\tilde{J}_{iA} = -\sum_{jk} \nu_{ik} \lambda_{kj} \Delta \eta_j - \sum_j \lambda_{ij} \Delta \eta_j \quad (14b)
\]

\[
\tilde{J}_{iB} = \lambda_i (\Delta \eta_i^B + \Delta \eta_i^\gamma) \quad (14c)
\]

\[
\tilde{J}_{iR} = -\lambda_i \Delta \eta_i^K \quad (14d)
\]
\[ \bar{J}_{iP} = \bar{J}_{iP} + J_{iA} + J_{iB} + \bar{J}_{iR} \quad (i = 1, 2 \ldots n). \]  (14e)

In agreement with the intuitive use of the words, the above equations describe the passive (14a), the active (14b), the binding or storage (14c), and the reflux (14d) components of transport respectively. Their physical significance is discussed in the following section.

Discussion.—In the preceding section we have presented a mathematical analysis which permits a complete and unambiguous categorization of transport processes occurring in living systems. This analysis is based on a description of the processes involved which is completely general and takes into account all forms of interaction between them. Although the particular form of description is that used in thermodynamics of irreversible processes [equations (1) and (2)], it is to be re-emphasized that we have not found it necessary to impose any such restrictions as linear relationships between fluxes and forces, reciprocal relations among phenomenological coefficients (i.e., the Onsager relations), or small deviations from equilibrium; thus the validity of our results is independent of these restricting conditions.

In order to clearly delineate those transport processes intuitively termed active, we have made use of the necessary condition that the only manner in which a chemical transformation can give rise to a transport process is by creating a source or a sink for the transported species. This condition is a direct consequence of Curie’s theorem. Given the facts that the course of a chemical reaction is not associated with any direction in real space and is thus properly described by scalar quantities, while transport processes have directional properties, the applicability of the theorem and the validity of the conclusion are self-evident. It is thus possible to express the contribution of chemical reactions to the thermodynamic driving force of a given transport process in terms of the rate of the pertinent chemical reactions, as is done in equation (8).

Even though the contribution of metabolic reactions to a transport process must always take the form of a source or a sink, in a real system sources or sinks affecting the transport of a given component \( i \) may arise in more than one way and this has been explicitly taken into account. Thus, sources and sinks may influence the flux of a given component \( i \) either (1) by affecting the thermodynamic potential of \( i \) itself in unchanged chemical form or (2) by affecting the thermodynamic potential of a distinct constituent, \( k \), which is derived from \( i \) by reversible chemical alteration for purposes of transport. Several types of such alterations, reversible upon completion of transport, are conceivable for a molecular species: (a) combination with another molecule, (b) removal of a chemical group, and (c) change of configuration. The contribution of chemical reactions to the transport of species \( i \) in any of such altered forms is explicitly taken into account by equation (11). The possibility that \( i \) may be transported in more than one altered form is expressed by summation over all possible \( k \).

Similarly, the reactions affecting the thermodynamic potential of \( i \) itself may be further categorized into those in which (1) \( i \) is either put into or released from storage by a direct reaction, the product \( b \) not being transported, (2) \( i \) is altered to form a transportable species \( k \) and (3) \( i \) itself is not altered but its thermodynamic potential is affected by the production or removal of other constituents in the system. This categorization is made explicit by equation (12).
The total flux of any constituent \( i \) must of course include all contributions, whether produced by metabolism or not, as given by equations (13a) and (13b). These equations thus allow an unmistakable distinction between metabolically dependent and metabolically independent terms of the total flux. It is a matter of arbitrary convention whether the entire group of metabolically dependent terms, or only part of it is designated as "active." It appears to us, however, that a specific nomenclature for the individual flux terms separable on the basis of the origin of the thermodynamic force has the advantage of conceptual clarity. Accordingly, we have distinguished four terms: (1) the passive flux term (14a) which arises from forces to which metabolic processes make no direct contribution, (2) the active term (14b) describing the flux of \( i \) either in a chemically altered form or under the effect of metabolically dependent forces other than its own thermodynamic potential gradient, (3) the binding or storage term (14c) describing the flux of \( i \) resulting from the metabolically produced component of its own thermodynamic gradient, and (4) the reflux term (14d) which results from sources and sinks arising from the formation or decomposition of \( k \), an altered transportable form of \( i \). This last term represents in effect the flow of \( i \) in a direction opposite to its active flux in so far as this results from the changes of the chemical potential of \( i \) which accompany its transformation for purposes of transport.

Equations (14a) to (14e) provide the necessary framework within which the transport phenomena occurring in living systems may be completely described. However, to obtain their solution from experimental data one must have a sufficient number of simultaneous measurements of all the fluxes and forces occurring in the system to establish the functional relations between each of the coefficients and their forces. The number of required observations may of course be reduced by introducing simplifying assumptions whenever such may be applicable. For instance, an assumption that the coefficients \( \lambda_{ij} \) or \( \lambda_{ae} \) are constant will reduce the number of separate simultaneous observations of all fluxes and forces to \( n + \rho \). Further simplifications result when it may be assumed that some of the \( \lambda_{ij} \)'s or \( \lambda_{ae} \)'s vanish or that relations of the type \( \lambda_{ij} = \lambda_{ji} \) and \( \lambda_{ae} = \lambda_{ea} \) apply.

The above analysis indicates quite clearly that the question whether a given component \( i \) is transported actively or passively and to what extent cannot be given a precise and unique answer unless the system is completely described. The task of obtaining the answer is a formidable one and will require an approach by successive approximation.

Summary.—A general and inclusive formulation of transport processes in living systems is given and the conditions for the coupling of transport processes to chemical reactions are defined. It is shown that the contribution of metabolic reactions to the thermodynamic forces of transport can be described in terms of the reaction rates and that several different categories of transport processes may be distinguished on the basis of the origin of the thermodynamic forces involved. This allows a new and rigorous definition of active transport which is free from the ambiguities inherent in earlier attempts at its definition.

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ENZYMIC MECHANISM OF INCREASED UTILIZATION OF GLUCOSE DURING VIRUS MULTIPLICATION IN THE CHORIOALLANTOIC MEMBRANE OF THE CHICK EMBRYO

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The chorioallantoic membrane of the chick embryo offers distinct advantages over other tissues for the study of multienzyme systems. Since it is composed of only three layers of cells, limiting factors of diffusion hardly interfere with measurements of the metabolism of intact cells in vitro. For these reasons we have undertaken the study of complex biochemical processes with the aid of excised chorioallantoic membranes. The influence of virus multiplication on the metabolism of the host tissue was one of the problems under investigation.

Earlier metabolic studies on the excised membrane revealed that during multiplication of myxoma,1 fibroma, herpes simplex, vaccinia, Rous sarcoma, swine influenza, and Newcastle viruses,2 the rate of lactic acid formation from glucose was markedly elevated. It was also shown that this increase in the accumulation of lactate was not due to inefficient transfer of electrons to O₂, an effect which might be assumed to occur as a consequence of "pathological changes caused by viruses," but was the result of an increased amount of glycolytic enzymes in cells where the multiplication of viruses took place. Aldolase and the glycolytic enzymes, catalyzing the formation of 3-phosphoglycerate from fructose 1-6-diphosphate were earlier identified1,2 and the increase of their concentration in infected membranes determined. It was found, furthermore, that the chorioallantoic membrane contains glucose-6-phosphate dehydrogenase.3 This was believed to indicate that the dual