

On Accumulation and Active Transport in Biological Systems

I. Thermodynamic Considerations *

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The extensive experimental studies on the phenomena associated in biological literature with the concepts of accumulation and active transport give evidence of the significance attached to this problem. As regards the literature on the subject reference shall be made to Höber's thorough treatise¹ and moreover to several monographs and congress reports on the problem of membrane diffusion²⁻⁶. The processes in question include for example absorption processes in intestine and kidney, various kinds of secretion processes, *e. g.* those involved in the formation of gastric juice, absorption of inorganic salts by plant cells, and the unequal distribution of alkali ions between extracellular and intracellular fluids.

Active transport may generally be characterised as a transfer of matter which for energetic reasons cannot take place spontaneously, its performance requiring the coupling to compensating processes. In accordance with this we shall define active transport as a transfer of chemical matter from a lower to a higher chemical (in case of charged components: electrochemical) potential.

The terminology generally employed is rather heterogeneous on this point, a fact which has possibly contributed to make the theoretical treatment of the subject somewhat casual. Thus two of the terms most frequently used: free energy and osmotic work cannot be used as a matter of course in the definition of active transport. With regard to the former term it is not as a rule feasible to define the system in such a way that the change in free energy can be taken as a measure for the extent of the active transport; furthermore it may be mentioned that the concept: supply of free energy to a system is

* The viewpoints of this paper were presented in a lecture at Wenner-Gren's Institute for Experimental Biology, Stockholm, in February 1947.

not included in the definition of this term. The term: osmotic work is often employed with different meanings. As a rule, however, it indicates the work associated with the movement of a semipermeable membrane in a system in osmotic equilibrium, a process characterised by potential equality of the substances transported through the membrane. Finally the definition of active transport as a transfer of matter depending on metabolic activity is inconsistent as the concept of metabolic activity is not well defined and can hardly be defined in such a way that it does not include all kinds of biological transports.

Active transport is generally assumed to take place by a coupling to intracellular chemical processes. It has not, however, been possible in any case to clear up the mechanism of such couplings. One of the main difficulties of the problem is presumably the fact that an identification and isolation of the compensating processes concerned cannot be achieved on account of our incomplete knowledge of the processes which take place interdependently or independently in these complicated systems. The question of the so-called «driving forces» is of a more general nature. What sort of a coupling with a chemical reaction is able to force upon a chemical substance a direction of movement opposite to its natural diffusion tendency? For the elucidation of this question it will generally be necessary to study model systems, and a number of such investigations which aim at and in certain cases do achieve accumulation effects similar to those of living organisms have been made.

It is the object of the present paper to contribute to the elucidation of the energetic mechanism in processes of this kind by means of certain basic model conceptions. The means used for this purpose consist in generally recognised fundamental principles and facts, and consequently it does not lead to the recognition of new principles. If this paper thus contains some commonplaces, several points may be alleged in excuse hereof: First that there exists no analysis of the present problems on the basis of basic thermodynamic principles; secondly that on the whole we have no explanations of the capacity of cells to accomplish such energy transformations. Or to quote one of the recent discussions on the subject⁷, «One of the most important unsolved problems of physiology is how the cell does work — work of any kind. The problem of heat production has been extensively investigated and is understood; but how the body transforms chemical energy into mechanical or into osmotic work is a complete blank.» Finally the present treatise on the subject, which deviates somewhat from the ordinary thermodynamic treatments, being based on Brønsted's⁸ energetic formulations of the principles of thermodynamics, seems to favour a better understanding of the very mechanism of energy transformations in general.

FORMULATION OF THE PROBLEM

We shall consider transfer of chemical matter in defined model systems. In our investigation of the conditions which determine the direction of transference we shall prefer to consider the transport of infinitesimal quantities of matter. In this way the interdependence between the process in question and the other properties of the system cannot be obscured by the secondary changes caused by the transport proper.

A chemical substance A is assumed to be localised in two phases (i) and (o) of given compositions which do not vary in time. As a rule we shall assume homogeneity with respect to temperature and pressure. Through a connecting link, M , the properties of which shall be further defined for each individual system, the possibility of communication of A between the two phases is maintained. The object is to examine the conditions associated with the transport of an infinitesimal quantity dn_A through M .

We shall distinguish between three essentially different possibilities by which the stability in such a system may be secured. The three states we propose to consider are termed total equilibrium, partial equilibrium, and stationary states respectively. Particularly the latter two are of interest in the present context.

BASIC ENERGETIC PROCESSES

Transport in total equilibrium

Here a brief summing-up of the terminology used shall be given: temperature (T), pressure with negative sign ($-p$) and the chemical potentials ($\mu_A, \mu_B, \dots \mu_K$) are designated as energetic potentials. Corresponding to each of them we have an energetic quantity: entropy (S), volume (v), and the chemical components ($A, B, \dots K$) respectively. Analogous extensions are made if there is a possibility of electric or gravitational processes in the system considered, each of these processes involving a potential and a quantity. A basic energetic process consists in transport of an energetic quantity between two potentials the values of which express the tendency of the transport concerned, transport from higher to lower potential indicating a positive tendency. Two different transport tendencies may balance each other, and a reversible total process consists of at least two balanced basic processes if the potentials involved have different values. A neutral basic process is a transport of quantity between identical potential values, and such a transfer may take place isolatedly without other energetic changes.

In a system in total equilibrium all energetic potentials are constant in the whole system. It appears from this that here transports of chemical components can never be active. If the two phases have different properties, the state corresponds to an ordinary phase equilibrium, *e. g.* between two solutions. Here the stability requires no connecting link with special properties. That these systems are mentioned at all in the present connection, is because it is often difficult in actual practice to ascertain whether a given system is in total equilibrium. The first indication of an active transport is as a rule that a substance accumulates in certain places. If different solvents are involved, such differences in concentration do not indicate corresponding differences in the chemical potentials. In living organisms this applies particularly to solvents of a lipid character, and to phase boundaries the solvent properties of which usually differ greatly from the properties of the adjacent macrophases and where accumulation takes place according to Gibbs' adsorption equation. If on the other hand the two phases only differ slightly in composition, an unequal distribution of a substance may be regarded as indicative of an active transport. In this connection it is worth mentioning that in special cases the equilibrium distribution of a substance between two phases may be one-sided even if the properties of the phases only differ slightly in other respects. Such conditions are associated with the distribution of large molecules, and extreme distribution ratios between two phases of very nearly the same composition have been observed in some colloids⁹.

Transport in partial equilibrium

If individual potentials have different values in the two phases considered, this is generally an expression of a reaction tendency in the total system which without special arrangements will result in the occurrence of spontaneous processes. It is well known that in many cases it is possible by special constructions of the connecting link M to prevent the occurrence of such processes, and thus establish a state of partial equilibrium. Osmotic equilibria, including Donnan equilibria, certain reversible galvanic cells etc. belong to such systems, which may in general be regarded as membrane equilibria. As there may be a possibility of transport of energetic quantities contrary to their natural diffusion tendency in these systems, a thermodynamic description of the energetic mechanism will also throw light upon the present problem.

If the system is not in total equilibrium, an elementary thermodynamic consideration will show that at least two kinds of energetic potentials must have different values in the two phases. This appears for instance from the basic equation for the changes of potentials:

$$SdT - vdp + n_A d\mu_A + n_B d\mu_B + \dots + n_K d\mu_K = 0 \quad (1)$$

which applies to arbitrary infinitesimal changes in a homogeneous phase. The equation shows that two infinitesimally different phases which differ with regard to one potential will necessarily differ with regard to another or several other potentials. So the same must be valid if the difference between the two phases is finite.

We shall assume that A 's chemical potential is different in (o) and (i), *e. g.* $\mu_{A(i)} > \mu_{A(o)}$, and that the maintenance of this difference is not due to the fact that M is impermeable to A in any form. The latter condition can as a rule be proved experimentally by means of radioactive or other isotopes. If on these assumptions a transport of A shall be practicable in equilibrium, it follows as a condition of equilibrium that the transport must be coupled to another process with an opposite tendency so that the two tendencies exactly balance each other. In the particular system of two homogeneous phases this is only possible if the transfer of A , *e. g.* from (o) to (i), is coupled the transport of another energetic quantity K' , which thereby goes from a higher to a lower potential. What is transported between the two phases is consequently a complex of A and K' , possibly together with other quantities, which in case of potential equality will not yield any energetic contribution to the transport. Having regard to the rôle of the connecting link in such systems, we can formulate the result in the following way: The maintenance of equilibrium requires that M permits the passage of a complex of A and K' and does not permit the passage of A or K' in any other form. Purely energetic considerations thus involve the condition that the connecting link between the two phases shall be impermeable to certain quantities and permeable to others.

We shall as a, somewhat unbiological, example consider two different dilute solutions of Pb in Hg, *i. e.* two amalgams, which are separated from each other by an aqueous $\text{Pb}(\text{NO}_3)_2$ -solution. In this system, which is known as a particularly simple reversible galvanic cell, we shall notice the following properties:

1. By the use of radioactive lead it will be proved that there is a possibility of communication for Pb between the two amalgams.
2. No spontaneous equalisation will take place between the two solutions.
3. The maintenance of the difference in concentration does not require the occurrence of energy-supplying »metabolic» reactions.
4. A transport of lead from the weaker to the stronger solution may be established if the weaker solution is combined with an electron acceptor and the stronger with an electron donator. This can for instance be effected by connecting the two solutions with the two poles of a condenser.

5. By such a transport chemical work is gained, and an equal amount of electric work is lost. The process can be described as a transformation of electric to chemical energy.

The energetic mechanism which is responsible for the stability of the system and for the coupling can be described in the following way: The communication between the two amalgams is maintained exclusively by the ion Pb^{++} which from a macroenergetic point of view can be regarded as a complex of Pb and positive charge. The diffusion tendency of this complex is determined by the chemical potential difference of lead between the two amalgams and the electric potential difference (or more precisely: the electrochemical potential difference of the electron) between them. At equilibrium the actions of these potential differences on the complex compensate each other. In this system the $\text{Pb}(\text{NO}_3)_2$ -solution thus acts as an ideal semipermeable membrane which is impermeable to Pb and »free» electrons and permeable to Pb^{++} . In such a system it is usual to operate with the potential concept of the actual complex which is transferable. Thus a state of equilibrium is due to potential equality of all energetic quantities and complexes for which transport possibilities exist. Accordingly, equilibrium in the system of two amalgams is based on the fact that the electrochemical potential of Pb^{++} is constant in the entire system. In the two amalgam phases this potential is defined by Pb's chemical potential and the electrochemical potential of the electron.

Analogous systems in which the complex transferred consists of a compound of different chemical substances have not been made the subject of investigation and description.* This may be mainly due to the fact that the conditions required with regard to the permeability of the connecting link are far more difficult to realise experimentally than in the case of the corresponding electrochemical coupling. However, as such systems may be used as a basis for considering the coupling between transport of a chemical substance from a lower to a higher potential and other chemical processes, we shall deal with them in some detail.

The two phases (i) and (o) are assumed to contain the two substances *A* and *B* where $\mu_{A(i)} > \mu_{A(o)}$ and $\mu_{B(o)} > \mu_{B(i)}$. The connecting link *M* is assumed to be impermeable to *A* and *B* and permeable to a compound (*AB*) of the two substances. At equilibrium μ_{AB} is constant in the whole system, and if the compound is assumed to consist of 1 mole *A* and 1 mole *B*, the relation:

$$\mu_{A(i)} - \mu_{A(o)} = \mu_{B(o)} - \mu_{B(i)} \quad (2)$$

* Compare, however, the »purely chemical cells» introduced by E. A. Guggenheim (*Modern Thermodynamics* London (1933) 143).

is valid. At the neutral transport of the compound between (o) and (i) thus equal molar amounts of A and B are transferred through numerically identical potential differences with opposite signs. Already at an early date it was recognised that such a process, which can be taken as a mutual transformation of chemical energies, is associated with stoichiometrical relations. Thus Ostwald¹⁰ writes, »Eine unmittelbare gegenseitige Umwandlung chemischer Energien ist also nur insofern möglich, als chemische Energien miteinander in Beziehung gesetzt werden können, d. h. *innerhalb solcher Vorgänge, die durch eine stöchiometrische Gleichung dargestellt werden können.*» It shall be shown later that such a limitation of the possibilities is not generally valid. On the other hand the principle holds for the systems considered here, the equilibrium depending on a constant stoichiometrical ratio between the two coupled transported quantities A and B . Supposing that besides AB a complex AB_2 could be transported through M , we find that the equilibrium condition for this process would be:

$$\mu_{AB_2(i)} = \mu_{AB_2(o)} \quad (3)$$

or:

$$\mu_{A(i)} - \mu_{A(o)} = 2\mu_{B(o)} - 2\mu_{B(i)} \quad (4)$$

(4) and (2) can only be valid simultaneously when $\mu_{A(i)} = \mu_{A(o)}$ and $\mu_{B(i)} = \mu_{B(o)}$. At possibility for simultaneous transport of the two complexes of different composition the transported quantities of A and B are not stoichiometrically related, and consequently it will result in a potential equalisation between the two phases. Corresponding conditions apply to the analogous electrochemical coupling.

If M is selectively permeable to AB , an infinitesimal transport of the complex in one direction will cause an infinitesimal tendency towards transport in the opposite direction at equilibrium. A onesided transport of major quantities therefore cannot take place without co-operation with the surroundings. Also this is quite analogous to the electrochemical coupling. If the phase (o) is connected to a B -donator and the phase (i) to a B -acceptor, finite quantities of the complex can be transported from (o) to (i), this transport resulting in an accumulation of A in (i) opposite to its chemical potential gradient. This may for example take place if the two phases are in communication with two B -reservoirs in the surroundings. In biological systems the most frequent case is that a certain accumulation is supposed to be associated with a chemical reaction in the phase (i). It is obvious that such a coupling can be established in the present case if this reaction works as a B -acceptor, *i. e.* if B is consumed during the reaction. Suppose that in phase (i) B reacts with a non-diffusible substance C , which results in the formation of the compound

BC , and that this reaction takes place in equilibrium. In this way the coupling with a chemical reaction sought for is established. Besides equation (2) the relation:

$$\mu_{B(i)} + \mu_{C(i)} = \mu_{BC(i)} \quad (5)$$

holds in this system. By combining (5) with (2) we obtain:

$$\mu_{A(i)} - \mu_{A(o)} = \mu_{B(o)} + \mu_{C(i)} - \mu_{BC(i)} \quad (6)$$

From (6) it appears that the chemical energy necessary for the active transport of A originates from the chemical reaction:



in which neither the complex AB nor the substance A in any other form are included. Hereby an obscuration of the transport mechanism may arise, but the mechanism is also in this case based on the selective permeability of the connecting link to AB . Moreover it is seen from (6) that the energy transformation is complete, the energetic efficiency being 100 %. This condition holds in general for systems in partial equilibrium.

Transport in stationary systems

As shown above the maintenance of a state of partial equilibrium is associated with very stringent requirements on the permeability of the connecting link. In such systems the latter may be regarded as a phase in the sense of the phase rule. Impermeability to certain substances is based on their absolute insolubility in the connecting phase. The system represents an ideal limiting case which is practicable in certain cases with a very great approximation. Thus it holds for the lead amalgam cell considered that the insignificant solubility of lead metal in the $Pb(NO_3)_2$ -solution and its consequent irreversible diffusion will not result in recognisable changes for very long periods. We shall now pass to systems in which corresponding processes occur to such an extent that they can no more be ignored when compared to the coupled processes.

In such systems the reaction tendency of the total system will manifest itself in the occurrence of spontaneous processes, and a system isolated from the surroundings will finally attain a state of equilibrium. On the other hand it is possible to sustain states of potential inequality in the phases (o) and (i) by co-operation with the surroundings. A potential gradient for a quantity

can be established by supplying the latter from the surroundings to one phase at a higher potential and removing the same quantity from the other phase at a lower potential. Hereby a flow of this quantity through the system is produced which will necessarily bring about and maintain other potential differences. We shall assume that the phases (i) and (o) are kept homogeneous, and that the potential gradients are limited to the connecting link. The term: stationary state shall be used for such a system if its properties are not changed in time.

The question to what a degree such systems are accessible to a thermodynamic analysis is discussed by Brønsted¹¹ in a recently published paper. Here it is shown that in stationary systems reversible and irreversible elements may be separated, and that also here the conception of energetic transport complexes can be used to describe the transformation of energy. Quantitative deductions are made for certain systems with thermoelectric, electrochemical, and thermochemical coupling.

As to the present problem we can assume that temperature gradients and pressure gradients will rarely play a major part in active transports. As a rule the mechanism has to be sought in the coupling of different chemical processes, and the problem is to explain an unequal distribution by maintenance of chemical potential gradients in the connecting link and flow of matter through it. Consequently we shall also in the following considerations assume homogeneity of temperature and pressure in the entire system.

The tendency of a mixture of chemical substances to be transferred between two phases, is expressed by the work gained in the surroundings by the reversible transfer of a conventionally fixed standard quantity of the mixture. If a given quantity of a mixture I containing n_A moles of the substance A and n_B moles of the substance B is transferred to a mixture II of the same substances, this work is given by:

$$W = n_A (\mu_{A(I)} - \mu_{A(II)}) + n_B (\mu_{B(I)} - \mu_{B(II)}) \quad (8)$$

provided that the potentials in II only change infinitesimally during the process. If the two mixtures only differ infinitesimally, we obtain from (8), using (1):

$$dW = n_A d\mu_A + n_B d\mu_B = 0 \quad (9)$$

Consequently the mixture as such has no transference tendency here, the opposite tendencies of A and B neutralising each other. We shall regard a stationary system involving various potential gradients as thermodynamically equivalent to a series of phases of infinitesimally varying composition. In

each of these phases the components of the mixture: A and B are not in equilibrium with the neighbouring phases, while on the other hand the mixture as such is in equilibrium with them. An infinitesimal transport of the mixture, which consequently may be regarded as a reversible process, represents a coupling of a positive basic process for one component with a negative basic process or active transport for the other component. There are some points of resemblance between the reversible transport of such a mixture and the corresponding transport of a chemical compound in a system in equilibrium. The fundamental difference is that the composition of the compound is constant in the connecting link, whereas the mixtures which in different places are in equilibrium with the neighbouring phases differ in composition. The complex which can be transported reversibly in such systems through finite potential differences, therefore, changes its composition continuously. A continuous flow of matter through the system is required to maintain the stationary state, *i. e.* an irreversible process which is the contribution of the surroundings towards stabilisation of the system. According to our basic presuppositions this process and its extent are not, however, included in our considerations of the reversible movements of the complex. Equation (9), or in its extended form equation (1), may thus be considered the thermodynamic basis of the coupling mechanism in active transport both at equilibrium and in stationary systems.

Under the transport of a chemical compound in partial equilibrium its components are held together by chemical forces which do not permit a dissociation of the complex in the connecting link. Different chemical components may, however, also be held together in other and looser ways than in a chemical compound. In a solution of electrolytes electrostatic forces will counteract an isolation of individual ions. Even if there exists no mutual chemical interaction of the components as for instance in mixtures of ideal gases, they will oppose an isolation, and the movement of one component will produce a diffusion tendency in and unequal distribution of the other components. Thus it is plausible that a stationary diffusion of one substance will result in an unequal distribution of other nondiffusing substances. This effect was investigated by Thovert¹² for salt solutions and later described by the same author¹³ for solutions of non-electrolytes.

That this »diffusion rétrograde» can serve as a coupling mechanism in active transport is seen most immediately when the components considered are ideal gases. We shall consider a system with the phases (i) and (o) containing the two gases A and B , and with a equal pressure all over, which is secured by the use of a wide tube as connecting link. By communication with the surroundings through semipermeable walls or the like B 's partial pressure

is kept higher in (o) than in (i) so that a constant diffusion of B will take place through the system from (o) to (i). In this stationary state the non-diffusing component A can be transported actively from (o) to (i), *e. g.* by an infinitesimal increase of A 's or B 's partial pressure in (o).

Phenomena of the same kind may occur in liquid mixtures. In a mixture of acetone and water which is kept in a stationary state by a constant diffusion of acetone the chemical potential of water is increasing from places with a higher to places with a lower concentration of acetone. If a third non-diffusing substance is dissolved, no general predictions can be made with regard to sign and magnitude of its potential gradient in the stationary state, the substance being influenced by the potential gradients of both solvent components. It has been claimed¹⁴ that such a substance will be distributed according to its solubility so that its chemical potential assumes the same value in the whole system. According to the present considerations such a distribution is not, however, more probable than other distributions. Apart from the fact that an equipotential distribution is unlikely for symmetrical reasons, cases can easily be pointed out in which no such distribution occurs. If for instance deuterium oxide is dissolved in the mixture considered, its distribution will be nearly the same as that of the ordinary water, and consequently it will also assume the same potential gradient.

The distribution which takes place in a dilute electrolyte solution when a stationary flow of a salt passes through it is one of the few cases of stationary systems of which a quantitative treatment has been given¹⁵. An electric potential gradient which arises on account of different mobilities of the diffusing anions and cations will affect the non-diffusing ions in a way solely determined by their charge (Teorell's diffusion effect). The distribution arising hereby is analogous to the corresponding equilibrium system where one of the ions cannot diffuse at all (Donnan equilibrium). The calculations are based upon the assumption that a non-diffusing ion has the same electrochemical potential in the whole system. In concentrated solutions with great potential gradients and also in solvents with a small dielectric constant great individual deviations from such a distribution may be expected.

Active transport caused by the different diffusion effects discussed will as a rule be characterised by similar distributions of substances with similar chemical or electrochemical properties. It is very probable that such effects occur to a large extent in biological systems. This will, however, render it difficult to explain the specific active transports which for various reasons are the most interesting. Cells which transport potassium from and sodium to a solvent poor in K^+ and rich in Na^+ , other membranes which prefer hexoses to pentoses require mechanisms working more specifically. The substances

which form the components of the transported complex must be more specifically attached to each other than it is the case in the »diffusion complexes» mentioned above. If a substance A is transported actively through a connecting link through which a chemically similar substance, A_1 , cannot be transported actively, this is tantamount to the two substances being affected by different forces or potential gradients in the connecting link. For the purpose of explaining such active transports it will be difficult to get round the assumption that chemical compounds in which the actively transported substances are included as components participate to a great extent in the transport between the two phases. Under the treatment of transport in partial equilibrium the reversible limiting case for such couplings, which is characterised by equilibrium with regard to a compound AB , was discussed. In a stationary system the movement of such a compound through M may be influenced with regard to rate and direction by other simultaneously diffusing substances. Special conditions exist when there is also a possibility of diffusion for the substances A and B besides for AB . Simultaneous diffusion of other substances which have no chemical relation to the compound AB may be assumed not to affect the latter specifically and consequently shall be ignored in the following discussion.

We shall assume that the phases (o) and (i) are two homogeneous solutions of A and B in the same solvent, and that potential gradients are maintained in the connecting link by the supply from outside of B to (o) and removal of B from (i). On the other hand such possibilities of communication are not assumed to exist for A . If $\mu_{B(o)}$ in this system is greater than $\mu_{B(i)}$, and if M is permeable to a compound AB , a potential difference $\mu_{A(i)} - \mu_{A(o)} > 0$ will be maintained by B 's flow from (o) to (i). The stationary state involves that equal quantities of A as such or in the form of AB pass through each section of the system in opposite directions. By an infinitesimal increase of A 's transference tendency in the direction (o) to (i) this substance is transported actively. But while the efficiency was 100 % in the equilibrium system, it is always less in the stationary system.

To examine what factors are decisive for the efficiency, we shall consider two simplified limiting cases in which the concentrations of the components concerned are assumed to be so low that their diffusion takes place independently of each other: 1) Only AB and B but not A , can diffuse. As A is transported through M exclusively in the form of AB in this system, AB is in equilibrium in the whole system, and accordingly equation (2) holds here just as for the system in partial equilibrium. In contradistinction to the latter this state must be stabilised by B 's irreversible diffusion through the system. Hereby a constant quantity of B passes through each section of the system in

unit time. For the maintenance of given potential differences this quantity will be the smaller, the smaller the concentration gradient of B is in the connecting link. This means that the efficiency increases with decreasing solubility of the substance B in M . If an active transport of A is accomplished by increased supply of B from outside to (o), B will flow through M partly in the form of B as such and partly in the form of AB . Hence it appears that the efficiency increases with increasing solubility and rate of diffusion of AB in M . 2) Only AB and A but not B , can diffuse. In this system B is transported through M exclusively in the form of AB so that $\mu_{AB(o)} > \mu_{AB(i)}$, and consequently $\mu_{A(i)} - \mu_{A(o)} < \mu_{B(o)} - \mu_{B(i)}$. In the stationary state a constant amount of AB passes through each section of M in the direction (o) to (i), and a corresponding amount of A passes in the opposite direction in unit time. Moreover this amount is equal to the amount of B supplied to (o) and removed from (i). Analogous to 1) a low solubility and mobility of A and a high solubility and mobility of AB in the connecting link will tend to increase the efficiency.

It has not been attempted to make quantitative calculations by the use of hypothetical distribution ratios and insertion of hypothetical values for concentration gradients and diffusion coefficients in Fick's law of diffusion, which — as pointed out by Danielli³ — has a very limited applicability in the systems considered. Qualitatively it appears from the two cases discussed that the efficiency of the coupling is determined by the ratio of the solubilities of the compound and its components in M and by the difference between their mobilities. As mobility is mainly determined by molecular volume, which for ordinary molecules only varies within comparatively narrow limits, the solubility conditions seem to be decisive for the accumulation effect*. Thus the difference in μ_A will only assume a major value when the amounts of A and B in the connecting link are small compared to the amount of AB , in other words when the deviations from the ideal system in partial equilibrium are not too great. Such conditions can only be expected to exist if the chemical character of the connecting link as a solvent differs essentially from those of two phases.

B 's function in the systems mentioned may be taken as a carrier function, the active transport of A being induced by B 's positive transport tendency in the total transport. In our foregoing considerations the difference in μ_B is assumed to be maintained by transference processes of B between the phases

* After the manuscript of the present paper was finished, a paper by J. Franck and J. E. Mayer appeared (*Arch. Biochem.* 14 (1947) 297), in which the authors propose some models mainly based upon differences in rate of diffusion. From quantitative calculations it is derived that for the energetic efficiency such high values as 30 % can be reached.

and the surroundings. This potential difference may, however, also be maintained if the said transference processes are replaced by different chemical reactions which consume and produce B respectively.

For the further elucidation of this case we shall choose a system where the phase (i) — just as a single cell — is completely surrounded by the phase (o) and separated from the latter by the connecting link or membrane M . A potential difference $\mu_{A(i)} - \mu_{A(o)} > 0$ is assumed to be maintained by supply of the «metabolite» G from the surroundings to (o) and removal of the metabolic end product C from the same phase. As, in contradistinction to the above-mentioned systems, (i) has no possibility of direct communication with external surroundings, the removal of the carrier substance from this phase can be effected only by a chemical reaction therein. This condition is generally valid — also for the non-specific diffusion coupling — for such systems in which one phase is completely surrounded by the other: The stationary maintenance of potential gradients — including pressure and temperature gradients — for quantities which are transportable through the membrane is only possible by simultaneous chemical reactions in the inner phase.

The carrier function must be ascribed to either the substance G or an intermediary reaction product X . Particularly the latter case is of interest in connection with the mechanism in biological systems. As it is a necessary presupposition for the performance of the active transport of A that the substance X has a positive diffusion tendency from (o) to (i), it follows that the latter must be produced in the phase (o) or on the boundary between the latter and the membrane. Consequently, in this model chemical reactions in which the carrier substance participates take place on both sides of the membrane. As before, the actual coupling mechanism is due to the transfer of a chemical compound including both the carrier and the actively transported substance. But the occurrence of the carrier substance may be limited to the membrane proper, a fact which, considering the dimensions of biological membranes, will render a direct observation of its presence and transport function extremely difficult. The different localisation of chemical reactions in biological systems may be ascribed to the presence of different metabolic enzyme systems on the two sides of the membrane.

DISCUSSION

The basis of the present considerations is the conditions of stability in systems in which potential differences can exist persistently between two phases although possibilities of exchange are assumed to exist for the components not in equilibrium. In the ideal limiting case, the system in partial

equilibrium, the maintenance of this state requires no coupling to spontaneous chemical reactions. The conditions here required with regard to the selectivity of the membrane permeability cannot, however, be assumed to be complied with in biological systems. On the other hand it is not impossible that systems with a low metabolism and high chemical potential differences are not far from the equilibrium system. Such conditions may exist at the distribution of alkali ions between erythrocytes and plasma where it was shown by means of radioactive indicators^{16, 17} that a formerly presumed absolute impermeability to cations does not seem to exist. That there is an interdependence between this distribution and cell metabolism appears *i. a.* from Wilbrandt's¹⁸ results.

In a stationary system for the maintenance of which spontaneous processes are required a substance A which is not in equilibrium must be influenced by other forces besides its chemical potential gradient in the connecting link between the two phases. Such forces can generally be identified with a comparatively small number of energetic potential gradients, including the gradients for temperature and pressure. Here the limitation is the same as the limitation of the different forms of energy possible which is used in Gibbs' differential equation for the total energy of a system. The condition that A is influenced by other energetic potential gradients must be due to the fact that the system considered evidently does not permit an isolated transport of A between the two phases, each infinitesimal transfer of this component being coupled to the transfer of one or possibly several other components which are not in equilibrium either. This qualitative description of the energetic mechanism is unambiguous and exhaustive for systems in which the connecting link is of macroscopic dimensions. The question may, however, be raised whether the use of concepts with a statistical basis such as temperature, pressure, chemical potential etc. is permissible for biological membranes the thicknesses of which frequently correspond to a few molecules only. As regards this question it can be stated that even if the fluctuations about the probable values will naturally increase with decreasing dimensions, the conception of a continuity of these quantities will justify their application to the present problem. As mentioned subsequently, diffusion through cell membranes seems to be mainly determined by a kind of membrane solubility, a relation which may be taken as an experimental confirmation of the applicability of chemical potentials to express diffusion tendencies.

The same mechanism which is responsible for the maintenance of the stationary state of the system can in all cases be employed for the performance of active transport. An infinitesimal relative increase of the forces which act on A in the opposite direction of its chemical potential gradient in the station-

ary state will thus result in the transfer of A from a lower to a higher chemical potential. Here we have distinguished between unspecific and specific active transports. It is characteristic for the former that chemically similar substances are affected similarly. In such a diffusion complex the binding between actively and passively transported components of the complex is analogous. Thus the effect of electric potential gradients on Na^+ and K^+ is the same — *e. g.* in Teorell's diffusion effect — because the ratio between charge and chemical matter is identical for the two components. A diffusion coupling may be assumed in many cases to contribute towards the maintenance of non-equilibria in living organisms. A special case of this kind is active transfer of water which is of a more special character on account of the rôle played by water as a solvent. A model which can reproduce such transfers by the maintenance of other potential gradients is described by Hartley¹⁴.

In specific active transports the composition of the transport complexes is quite different for components with similar chemical properties. This can be due to differences in chemical affinities or — particularly in biological systems — to the participation of specific enzymes in the formation of the complex, in which chemical compounds consequently must be included. The coupling efficiency is mainly determined by the difference in the distribution ratios of the compound and its components between phases and membrane. In order to attain major effects the membrane must have solvent properties quite different from those of the phases. In living systems the latter consist of aqueous solutions, and therefore substances of a nonpolar or a lipid character will from a purely chemical point of view be considered best suited as structural constituents of such membranes. This point of view is in accordance with the obtaining views concerning the character of the plasma membrane which were originally set forth by Overton¹⁹, and which have been verified subsequently by numerous results. The theory of the lipid character of plasma membranes is mainly based on the close parallelism which is found between the distribution ratios of chemical substances between water and solvents as ether or olive oil and their rate of diffusion through cell membranes. Numerous attempts have been made at a close reproduction of the solvent properties of the cell membrane by model studies, and for this purpose both pure substances of a lipid character and mixtures of such have been used, without particularly satisfactory results. From many studies on permeability, especially by Collander²⁰ and his school, it appears that the solvent properties of the cell membrane may vary within very wide limits for different species, and that besides solubility also a pore mechanism may possibly be of importance to the rate of diffusion. But moreover it must be assumed that the particular orientation which is supposed to exist in the cell membrane, and

which may be paralleled with liquid crystals, will result in solvent properties which cannot be reproduced completely in a macrophase.

With these modifications active transports can thus be ascribed to the lipid solubility or «membrane solubility» of the transport complex which must be much higher than that of the components. In many papers Osterhout ²¹ has emphasised similar views with regard to active salt transport, which is assumed to take place in undissociated form. In numerous models the existence of analogies between cell membranes and guajacol solutions is shown, particularly with respect to the diffusion of Na^+ and K^+ . These views are consistent with those stated in this paper; it must, however, be observed that the lipid solubility of an undissociated salt is not sufficient by itself for the explanation of the mechanism of active transport, but for energetic reasons it is required that the diffusion tendency of one ion shall be overcompensated by the opposite tendency of the other ion in the total transport. Some conditions involved in the transport of alkali ions, and in this connection the models of Osterhout, will be further discussed in a following paper.

The component the diffusion tendency of which determines the transport direction of the complex may be described as an energetic carrier. Various results in literature seem to affirm the existence of substances or systems with such a function. Especially some findings may be mentioned by which it is demonstrated that a given form of transport can have a maximum capacity so that an increase in concentration exceeding a certain limiting value does not increase the amount of matter transported hereby. Such results are particularly apparent in the case of glucose absorption in the intestine and glucose re-absorption in the kidney tubules where different transport mechanisms can also be distinguished by means of metabolic poisons ²²⁻²⁴.

In a discussion on the character of the binding to the carrier system Höber ²⁵ proposes three different possibilities: 1) The carrier operates as a solvent, 2) as an adsorbent, and 3) it combines chemically with the actively transported substances. According to Höber's own results ²⁶ with active transport of certain dyestuffs in kidney and liver a nonpolar-polar molecular structure favours the possibility of active transport simultaneously with favouring the adsorption on the membrane boundary according to well known laws. Thus it is assumed that adsorption can possibly be conceived as the first stage in active transport although adsorption as such does not require an energetic coupling. On the basis of the conception proposed here, the said results, *e. g.* with azo dyestuffs, might be explained by the assumption that the system is able to block enzymatically the hydrophilic sulphonate groups which are situated on the same side of the molecule and thereby make this intermediately lipid-soluble, whereas a corresponding blocking in molecules

with many and fairly equally distributed sulphonate groups should not be possible. The effective molecular orientation in such a blocking process may be assumed to be contrary to the normal, the hydrophilic groups being directed towards the membrane. The transport direction of the whole complex would be determined by the blocking part, which according to the above suppositions must include components with a positive diffusion tendency in the transport direction. Such a mechanism would be most consistent with the third of the above alternatives. It is, however, rather doubtful whether there exists a possibility of distinguishing sharply between these alternatives in the systems considered.

A consequence of the present views on the nature of the transport mechanism is that compounds, which are per se strongly lipid soluble, cannot be accumulated by active transport. This also is in full accordance with Höber's observations on the transport of dyestuffs.

On the other hand it is a necessary condition, irrespective of the character of binding, that the carrier passes the membrane just like the actively transported substance. If this movement has the direction from the outer phase into a single cell, the carrier must be transformed in the latter by a chemical reaction in order that the diffusion tendency should continue to be maintained. In case of transport in the opposite direction the analogous condition will involve that the carrier must be formed by a chemical reaction within the cell. By these conditions a dependence is established between active transport and metabolism, two functions closely connected in living systems. The said continuous reactions in the cell must be regarded as a part of metabolism so that the carrier can be considered an intermediary product thereof. The connection between active transport and metabolism appears most clearly from systems in which the normal concentration of an actively transported substance A is varied. When the carrier does transport A through the membrane at all, it is due to an existing restraint on the isolated transport of the carrier, *e. g.* its slight membrane solubility. If A is removed from the system, this restraint will manifest itself more clearly and in accordance with the above retard the metabolic reactions, a phenomenon frequently observed. Conversely, if the outer concentration of A is increased, there is a possibility of an increased transport of the carrier and thereby an acceleration of metabolism. This case seems to be realised in Steward's²⁷ results with potato discs where an increased concentration of potassium results in an increase of both carbohydrate and nitrogen metabolism.

While the association of intracellular enzymes with active transport is immediately plausible, this is perhaps less so with regard to the enzyme action on the outer surface of the membrane. This problem can be elucidated by the

consideration of systems to which no substances which can act as carriers are supplied from outside. Many plant cells are able to accumulate salts, especially potassium salts, from very dilute salt solutions which are supplied with oxygen²⁸. As no other metabolites are supplied from outside, the particular carrier must be delivered from the cell where the increased metabolism manifests itself in *i. a.* mobilisation of the carbohydrate stores. However, a substance produced inside the cell will have an outwardly directed diffusion tendency and, accordingly, will not be able to act as a carrier in this case. Therefore, the most plausible explanation of this function is obtained on the assumption that the carrier in question is formed from another intermediary product on the outer side of the membrane, a reaction which may be assumed to be controlled enzymatically. A few observations of a more direct character support the assumption of the enzyme localisation on the outer surface of the membrane. A lactase is presumably present on the outside of yeast cells²⁹, and an enzyme which phosphorylates glucose seems to act on the outside of muscle cells in fasting animals^{30, 31}. The presence of proteins in general on the outer membrane surface seems to be a well established fact.

The picture of a living mechanism given above on a thermodynamic basis naturally can only be very rough and schematic. However, in connection with biological facts some more concrete details have been recognised. As a main result a general and simple mechanism is proposed, based upon the normal permeability and solvent properties of the cell membrane combined with the action of metabolic enzyme systems which are involved in the building up and breaking down of transport complexes on both sides of the membrane. Such membrane soluble complexes may include both intermediary metabolic products and normal membrane components as steroids or phospholipids. Some models on this basis concerning the active transport of glucose and of alkali ions will be presented in following papers. With respect to model studies in general it may be concluded that it will not be possible to realise a close reproduction of active transports in living systems without the use of metabolic enzymes. Finally it must be assumed that an analogous mechanism also acts in many cases of biological transport which are not included in the definition of active transport.

SUMMARY

The thermodynamics of active transport of chemical matter in equilibrium and in stationary systems are discussed. A general mechanism for active transport is proposed on the basis of the thermodynamic results combined with known properties of living membranes and other characteristics related to the problem in biological systems.

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