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The Process of Thermodialysis and Some of its Possible Consequences in Biology *

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Summary

The flow of thermal energy in liquids is associated with the development of ponderomotive forces. In non-isothermal ionic or molecular solutions a drift of the disperse phase is thus produced (thermal diffusion).

It is discussed here how thermal diffusion may have enriched or, respectively, depleted the primeval soup in some of the various materials originally present in the oceans. In particular life would, accordingly, have been reared in a potassium-enriched, sodium-depleted cradle.

The flow of heat across permeable partitions also causes processes of active transport of matter, which turn out to be closely related to thermal diffusion. These phenomena have been labelled by us with the name of "Thermodialysis".

It is further suggested here that the flux of metabolic heat through biological membranes produces active transport in living systems in a way analogous to the one which we have demonstrated to work in artificial porous septa. Localized sources of heat consisting in sites of enzymatic activity situated near to or in the cellular membrane itself, could in turn contribute intense pulsed fluxes of heat right at the site of the hypothetical membrane "pores".

Some probable characteristics of such biological "pores" are also briefly discussed.

1) Radiation-pressure associated with the flow of heat in liquids

By 1964 the spontaneous existence of high-frequency elastic waves in liquids had been unambiguously established. These waves spread over a spectrum up to frequencies of the order of 10^{12} Hz [1–6]. Not generated by any external source, they constitute the physical form taken by thermal agitation in the condensed phases. This discovery, reached by the study of the scattering of coherent laser light, confirmed an old idea of P. Debye [7] further developed by L. Brillouin [8,9] concerning the nature of thermal excitations in liquids. Accordingly, the flow of thermal energy in a liquid, such as is produced by the application of a temperature gradient, would consist of a flux of hyperacoustic elastic waves. On the other hand, various authors, among them Lord Rayleigh [10, 11] and L. Brillouin [12–15] have demonstrated that both the emission and the absorption of acoustic energy entails the development of a radiation pressure on the surface from which elastic waves are emitted or absorbed.

Tentatively, we generalyzed these ideas of Rayleigh and Brillouin by stating that, in a liquid, a momentum flow is always associated to the flow of acoustic energy [16, 17].

The density of the momentum flux associated with a flux of acoustic energy, of density $\Phi(\mathrm{E})$ would accordingly be given by

$$\Phi(\mathrm{P}) = \frac{\Phi(\mathrm{E})}{v} \qquad 1$$

where v is the phase velocity of propagation of the elastic waves in the medium.

If thermal energy in liquids consists of high-frequency elastic waves, then $\Phi(\mathrm{E}) \equiv \Phi(Q) = K\frac{\mathrm{d}T}{\mathrm{d}x}$, where K is the thermal conductivity of the medium and $\frac{\mathrm{d}T}{\mathrm{d}x}$ the temperature gradient which produces the heat flow. From eq. (1) then it follows:

$$\Phi(\mathrm{P}) = \frac{K}{v}\frac{\mathrm{d}T}{\mathrm{d}x} \qquad 2$$

Either member of eq. (2) has the dimensions of a pressure. This means that whenever, for any reason, the density of the flow of momentum associated with the flow of heat is altered, there will appear a radiation-pressure produced by the thermal waves, equal, in the proper units, to the variation of $\Phi(\mathrm{P})$.

2) Experimental evidence in support of the radiation-pressure theory of thermal waves

Having advanced the unconventional theory sketched above, we set out to deduce from it various consequences and to seek experimental verification of theoretical predictions.

In the first place, we demonstrated, that upon a slab of solid material immersed in a non-isothermal liquid, the radiation-pressure produced by heat flowing through the solid and the surrounding liquid will produce a net non-vanishing ponderomotive force [18].

The sense of such force is the same as that of the temperature gradient, or opposite to it, depending on whether the momentum conductivity of the liquid is bigger or smaller than the same quantity in the solid. Also the intensities of the measured forces agree well with the theoretical predictions, and the important quantities determining the behaviour of the system

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turn out to be—as foreseen—the $\frac{K}{\nu}$ ratios of both the solid material and the surrounding liquid phase.

In the case of suspensions of microscopic particles in a liquid, and of solutions—ionic or molecular—the radiation-pressure developed by the flow of thermal waves produces a drift of the disperse particles in the liquid. Again this drift takes place, as we have shown, in the sense of the temperature gradient or opposite to it, according to the relative values of the momentum conductivites $\frac{K}{\nu}$ characteristic of the disperse and of the dispersing phase [19-24].

On this basis we could establish a molecular theory of the hitherto obscure phenomenology of thermal diffusion in the condensed phases; furthermore a method of physical characterization of macromolecules in solution has been developed [17, 22, 23]. The dependence of thermal diffusion effects on the nature of dispersing and disperse phase, and on the molecular mass and steric conformation of the macromolecular components has been fully clarified in the radiation-pressure theory of thermal waves.

In the important case of ionic solutions, surprisingly enough, the same concepts still apply. Difficulties were foreseen in the extension of the theory to particles as small as a solvated ion [16] but it looks now that initially we underestimated the range of validity of our theoretical approach. The thermodiffusive behaviour of ionic solutions indeed is also found to follow the laws deduced from the radiation-pressure hypothesis. A very interesting case [24] implying an inversion in the sense of the thermodiffusive drift of ionic species is the one provided by aqueous solutions of NaCl and KCl: in coincidence with the physiological concentration range the K^+ and the Na^+ ions migrate in opposite senses within the temperature gradient, Na^+ drifting along with the flow of heat, from the warm to the cold solution and K^+ in the opposite sense. On one hand this behaviour can be understood on the basis of the radiation-pressure theory of thermal diffusion, on the other its striking analogy with the so called "sodium-potassium pump" opens unprecedented vistas in biology.

To conclude this introduction, it can be said the consideration of the flow of momentum associated with the flow of thermal energy has allowed to develop a satisfactory molecular theory of the ponderomotive effects produced by the flow of heat in liquids.

In particular, thermal diffusion of ions and molecules has been shown to produce drift motion directed either in the sense in which heat is transported, or in the opposite sense, depending on the relative values of the "momentum conductivities" of the dispersing and of the disperse phase.

These studies have also led to the discovery of a physical analogue of the fundamental and unsufficiently explained biological phenomenon commonly called the sodium-potassium pump.

3) Some hints on a possible role of thermal diffusion in biogenesis

It is generally accepted that the evolution of living systems on the Earth was preceded by a long period of chemical evolution. The simple compounds constituing the primitive, reducing atmosphere were subjected to ionizing radiation of many types. The radiations effected the breaking of simple molecules into charged parts, with subsequent recombination of these pieces to more complex compounds. In this way some sugars, many amino acids, some organic bases and other common components known to occur in organisms were synthetized. In the absence of living consumers these materials became always more concentrated in the primeval oceans into which they were constantly washed down by the rain.

Eventually a jelly-like phase containing most of the complex organic molecules, was segregated in the form of small droplets (the Oparin coacervates [25]) or as thin layers spread over rocks or sterile muds in shallow water basins (the sub-vital areas of J.D. Bernal [26]). In both cases it is easily conceived that this primeval jelly was often subjected to the action of temperature gradients. Any geothermal phenomenon heating the rocks supporting the layered jelly or simply the effect of sunlight absorbed by the rocks—and not by the overlying water—would generate such gradients. In the case of the Oparin coacervates the accidental inclusion of a grain of mineral dust, or volcancic ashes in the droplets would lead to absorption of heat from the incident solar radiation, thus creating a radial temperature gradient from the central light-absorbing particle into the surrounding transparent medium.

In these conditions thermal diffusion would necessarily operate, and—according to our findings—it would produce the following effects:

i) Some materials were selectively enriched in the primeval jelly, while others were carried along with the flow of thermal energy and diluted in the huge mass of the surrounding water.

ii) In the case of sodium and potassium—certainly present in the primeval oceans as dissolved chlorides—potassium was concentrated in the jelly and sodium pushed out of it, so that pre-biological evolution took place in a potassium-enriched, sodium-depleted habitat, right from the start.

iii) Finally, the various organic compounds, once synthetized, were layered at different depths into the jelly, according to the differences in chemical nature and molecular mass, which conferred them different thermodiffusive properties.

It can be objected that all this is just wild speculation on an hypothetical course of long-past events, irreproducible at present.

One interesting aspect of this speculation, anyway, can be subjected to a sort of experimental verification "a posteriori" as we shall see.

It has been pointed out that the chemical composition of present-day organisms must be a function of the chemical conditions confronted by its early ancestors. The abundances of the elements in the living matter should reflect those of the earth's crust, or more specially those of oceanic waters. Crick and Orgel [27] put in evidence the great discrepancies existing between the average abundances of various atomic species in living organisms and those which would alternatively be expected on the grounds of the above reasoning. Along these lines of thought they went as far as to support the "directed panspermia" concept, in opposition to Earth-bound processes of chemical evolution, to explain the origin of life. Chappel et al. [28], Jukes [29] and Banin [30] have rebutted the arguments advanced by Crick and Orgel and discussed various doubtful points. In particular, they evidenced the great importance of the different solubilities of the various materials in determining the relative abundances of the same substances present in the oceans. In this way a general similarity between the observed relative concentrations of the elements in the oceans and in the four phyla of organisms is indeed found. Still, many discrepancies exist between the elemental abundances which would be expected and those actually found in living systems.

It is our opinion that a fractionation process based on the action of thermal diffusion on the primeval jelly could hypothetically explain such discrepancies in terms of thermodiffusive drifts leading to specific enrichment or depletion of the elements in the subvital areas. The few cases experimentally investigated by us so far have yielded results consistent with the hypothesis of the action of a thermal-diffusion driven mechanism of fractionation. In particular the behaviour of Na^+ and K^+ ions in aqueous solutions fully agrees with the fact that potassium is more abundant than sodium in the endocellular fluid of all the four major groups of existing organisms, notwithstanding the fact that in seawater sodium, instead, is much more abundant than potassium.

4) Matter transport induced by heat flow across permeable partitions: The process of thermodialysis

At this point one is naturally led to enquire whether in present-day organisms some processes of matter transport driven by the flow of thermal energy still exist.

Of course, heat production is ubiquitously present in living systems. Practically every metabolic activity is associated with liberation of energy and this energy will finally decay locally to thermal agitation; temperature gradients will be produced and fluxes of heat away from the energy source will ensue.

Anyway, very marked differences generally exist between the situations usually met in living organisms and those in which thermal diffusion is observed. Transport by thermal diffusion takes place in the homogeneous liquid phase, while in living systems both heat transport and transport of matter occur across complex membranes whose very peculiar properties strongly influence these phenomena of transport.

Thermal diffusion can hardly be expected to play any role in the various body fluid compartments, with the possible exception of the sap of trees, whose roots are warmer than the external part of the trunk during winter and colder during summer. Small temperature gradients are thus produced, which, being established for a long time, may cause significant selective molecular transport of substances such as diffusible salts, nutrients or plant hormones. Thus, in plants thermal diffusion may play a role in the regulation of various seasonal physiological processes.

Apart from such very special cases, the flow of heat will cause significant effects of transport of ions and molecules in living systems only if a process akin to thermal diffusion is produced also when heat flows across permeable membranes rather than through a homogeneous liquid phase.

It is necessary, therefore, to find an answer to the following question: What transport of solvent and/or solute (if any) takes place across a permeable partition when this partition separates two liquid mixtures having different temperatures? The answer should ideally be given both on theoretical and experimental grounds and, at the experimental level, should be sought in artificial systems, in non-living systems of biological nature, and "in vivo".

a) Theoretical considerations

Our problem can be schematically represented as follows: Let's have two adjacent containers separated by a porous partition (Fig. 1); the same solution or the

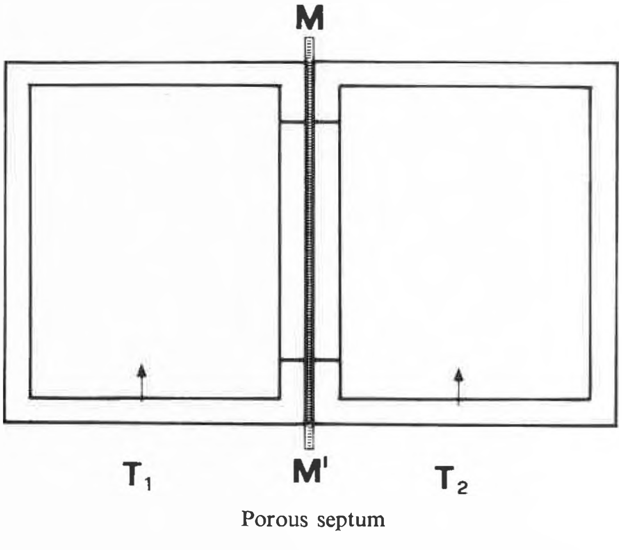

Fig. 1: Schematic representation of a porous septum MM′ separating two liquid solutions which—by suitable means—are maintained at different temperatures T_1 and T_2.

same pure liquid substance is put in both containers, then one side is heated and the other cooled. What effects will be observed?

The "classical" answer is the following: The electrochemical potential of each component, being temperature-dependent, will be different on the two sides of the partition; therefore, migration of the various components will eventually be observed. The fluxes of matter will take place "downhill" of the relative gradients of electrochemical potential. The partition by itself may interfere in various ways with the kinetics of this form of transport—preventing it altogether when the partition is impermeable. Anyway, the final steady-state distribution will be determined solely by the differences of electrochemical potential caused by the temperature difference. This is the tenet of the classical theoretical framework, within which the phenomenon of non-isothermal osmosis (thermoosmosis) has been studied until now.

A fundamentally different set of conclusions can be reached if the phenomenon is analyzed within the framework of the radiation-pressure theory of ponderomotive effects caused by a flux of thermal energy. Within this alternative approach, it can be easily shown, indeed, that a kind of thermal diffusion should take place within the liquid contained in the pores of the partition.

The presence of the partition, however, will alter the ordinary behaviour of thermal diffusion characteristic of liquids in bulk in various ways:

1) The value of the temperature gradient will be increased very much if the partition is very thin. The whole temperature difference, indeed, will be confined to the thickness of the partition—if vigorous stirring is provided. Correspondingly the effect of thermal diffusion (which is proportional to the temperature gradient) will be enhanced.

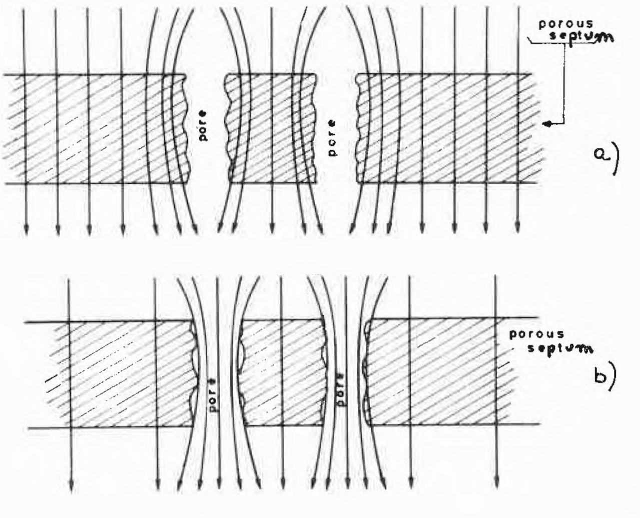

Fig. 2: Schematic representation of the flow of heat through a porous septum:
a) The material of which the semptum is made is a better thermal conductor than the liquid contained in its pores.
b) The liquid permeating the pores is a better conductor of heat than the material of the septum.

2) The flux of thermal energy will be either concentrated in the liquid medium contained within the pores, or in the surrounding solid material of which the porous partition is made, according to the circumstances (Fig. 2): When the liquid is a better thermal conductor than the partition material, the flux of heat will be concentrated in the liquid (Fig. 2b). The effect of thermal diffusion will be enhanced by this fact. In the opposite case heat will flow in the solid material around the pores, and little or no heat transfer will take place within them (Fig. 2a). Correspondingly little or no thermal diffusion will be observed.

3) The well-known effect of epitaxis will alter the physical properties of the liquid contained within a narrow pore, and the corresponding variation of the value of the momentum conductivity of the dispersing phase will change its thermodiffusive properties. By the name "epitaxis" one indicates the modification of the degree of order and hence of density, thermal conductivity etc. induced in a liquid—through the action of the adhesion forces—on the layers of fluid laying in close proximity of a solid surface wetted by the liquid.

While the first two factors discussed above modify only quantitatively the process of thermal diffusion, the effect of epitaxis modifies in a much subtler way the transport of energy and matter. The modifications brought about can be either quantitative or qualitative. Even inversions of the sense of matter transport can be produced, as we shall see in the following.

Thermal diffusion across a permeable partition is therefore expected to exhibit a much more complex behaviour than in the liquid in bulk. These phenomena of matter transport induced by the flow of heat should also evidence aspects not foreseen by the classical theory of thermoosmosis. For these reasons the new name of "thermodialysis" given by us to the process of thermal diffusion across porous partitions, appears justified.

A detailed, analytical theory of the process of thermodialysis has been developed by us [31]. It would be out of place to discuss here its formal aspects. We shall only briefly discuss the main predictions of the theory and describe the experimental supporting evidence which we have gathered so far [32].

Before doing so, we would like to dwell for a moment on one point: In current theories of thermoosmosis the effect of epitaxis is not considered. Even if it would, the conclusions arrived at should still be quite different from the ones reached in the theory of thermodialysis. The fundamental difference indeed resides in the fact that in the "classical" framework the variation of the physical properties of the liquid contained in the pores of the partition will influence only the kinetics of the phenomenon of transport: For instance, a variation of the viscosity of the epitaxed liquid relative to the liquid in bulk will facilitate or impede the drift motions and the fluxes of solvent and solute in the pores. The properties of the warm and the cold liquid on the two sides of the partition, however, will stay unaffected; therefore, the driving force—that is the difference of electrochemical potential—will remain the same, being independent from the change of properties of the intermediate portion of liquid. The final, steady-state distribution predicted by thermoosmosis consequently will be unaffected by epitaxis and only the time required to reach it will be modified.

We shall now see how much at variance with these conclusions are those reached by the radiation-pressure theory of thermodialysis and to what extent experimental results support the theoretical predictions.

b) Experimental verification of the theory of thermodialysis

The fundamental conclusion of the theory of thermodialysis is that the phenomenon is an active form of transport, capable of proceeding "uphill", that is against gradients of electrochemical potential and of pressure. This owing to the fact that the flow of heat generates ponderomotive forces, whose rate of working can be used to increase the potential energy of the system.

We have found convincing experimental verifications of this fundamental theoretical prediction.

A very simple example is the following: A porous septum of glass fibre separates two containers such as those of Fig. 1, one being filled with an aqueous solution of methyl violet at a concentration 10 mg/l, and the other containing pure water. The septum is completely permeable to both solvent and solute, so that a gradual mixing would normally be observed.

If, however, the temperature of the water is lowered and the solution is heated, no diffusion of methyl violet is observed as long as the temperature difference is maintained. More striking is the fact that water is transported through the septum, from the warm into the cold container, against the osmotic pressure due to the concentration gradient and against the hydrostatic pressure produced by the increase of the level of water in the cold container.

Even more striking is the behaviour of an aqueous solution (40 mg/l) of fluorescein and (10 mg/l) of brilliant green, mixed together. This solution is put in both containers and a glass fibre porous septum (Millipore AP-20) is interposed between the containers. One container is heated and the other cooled. After a few hours the fluorescein becomes concentrated in the warm container and the brilliant green in the cold one, against very steep concentration gradients which are gradually formed.

Similar phenomena of active transport can be observed also with other solutions, ionic, molecular and macromolecular, and quite different septa and membranes, as long as these are permeable to both the dispersing and the disperse phase. In the case of an ionic solution also the simultaneous creation of electric potential differences is observed.

Another clear-cut prediction of our theory is that the rate of transport and the magnitude of the final, steady-state separations produced, should be proportional—everything else being the same—to the temperature gradient across the partition rather than to the temperature difference between the two adjacent solutions. Unambiguous evidence proving our hypothesis has been obtained employing similar septa of different thickness [32]. We have even found that other researchers [33] had already obtained somewhat similar results, but the circumstance had passed unnoticed, even by themhelves, probably owing to the lack of a theory stating the importance of temperature gradients in membrane transport. In the radiation-pressure theory, of course, the ponderomotive forces, being proportional to the density of flux of momentum, turn out to be also proportional to the temperature gradient which is the "generalized force" producing the heat flow and the consequent ponderomotive effects.

Another distinctive feature of the proposed theory stems from the supposed role of epitaxis in thermodialysis. We have been able to demonstrate various experimental consequences of epitaxis in non-isothermal membrane transport [32]. We shall mention here just one simple but direct verification of the influence of epitaxis: Polyvinylpyrrolidone K-25 of 24,000 a.m.u., when dissolved in n-butanol is found to thermodiffuse from cold to warm [22, 23] in the bulk liquid. The momentum conductivity of n-butanol is 0.1210 $\frac{\text{ergs}}{\text{cm}^2 \cdot \text{K}}$ at room temperature, and that of the polyvinylpyrrolidone particles is found to be 0.1370 in the same units [22]. When thermodialysis of Poly K-25 in n-butanol is studied employing "Nuclepore" membranes of various pore sizes, the following results are obtained: With pore sizes of 80,000 Å the polymer is transported from the cold into the warm solution at a very fast rate. The phenomenon is precisely what is expected on the basis of the results of thermal diffusion. When the pore size decreases, however, the effect of epitaxis starts to modify a progressively greater part, and finally all of the liquid contained in the pores. The average momentum conductivity of the partially epitaxed butanol in the pores increases, until it eventually becomes greater than that of the polyvinylpyrrolidone particles. Correspondingly the absolute value of the separations obtained first decreases, until there is an inversion in the sense of transport of the macromolecular component in coincidence with the point at which the momentum conductivity of the liquid becomes equal to that characteristic of the polymer particles. Then, decreasing the pore radius still further to values of 3,000 Å and 1000 Å and 150 Å respectively, the polyvinylpyrrolidone K-25 particles drift from the warm into the cold solution, at a rate which increases with decreasing pore radius.

In our opinion, experiments such as these, provide direct and unambiguous evidence in support of the role attributed by us to epitaxis in the phenomenon of thermodialysis.

5) Membrane transport of ions and molecules induced by the flow of heat in living organisms: A plausible hypothesis?

Thermodialysis is an active process of transport; it is also sufficiently versatile to account, in principle, for the many aspects of transport of matter through permeable membranes and porous partitions.

Nature therefore might have harnessed the flow of heat emerging from the cells—last decay product of every matabolic activity—to transport ions and molecules through the cellular membrane. A principle of continuity would have guided it to proceed from thermal diffusion to thermodialysis, as soon as the privemal sub-vital lumps of jelly became compartimentalyzed and developed a limiting membrane.

To achieve this qualitative leap forward the evolutive pressure must have acted mainly in the direction of the development of a suitable architecture of the "pores" of pre-biological membranes. These channels, indeed, needed to be the right dimensions and had to be endowed with the appropriate thermal conductivities of the channel itself (high) and of the membrane material (low). Finally the walls had to develop a molecular architecture and chemical composition such as to be capable of inducing in the aqueous phase contained inside their cavities the right amount of ordering, through the effect of epitaxis. Also, a strategical distribution of localized heat sources (constituted by sites of exoergonic chemical reactions) adjacent to the pores would have been a primary goal for the build-up of an efficient and flexible system of transport.

Nature doubtlessly has the ingenuity needed for this task, and has also had at its disposition a sufficiently long stretch of time to develop the necessary tricks. Of course, the big question is: Did the evolution of biological membrane transport system actually follow this path? Let us try and see whether this hypothesis is plausible enough to justify investigations directed to the aim of checking it.

It is unfortunately impossible to study at present the conditions met by eobionts during their evolution; an alternative question which can and must be answered anyway is the following: Are the densities of the fluxes of heat generally met at present in living organisms sufficient to produce appreciable effects of transport? Obviously if this would not be the case the hypothesis would lose much of its interest.

Taking the data of calorimetric measurements made on the various kinds of organisms, and knowing the number and dimensions of the cells employed in the experiments, one can easily calculate the value of the density of the heat flow leaving the cell surface. In this way it is easy to conclude that in the case of actively metabolyzing cells the density of the flux of thermal energy through the cellular outer membrane is comparable, and in many cases higher, than those which we usually have in our apparatuses for the study of thermodialysis. Therefore, transport effects of similar or greater magnitude than the ones observed in vitro by us, can be produced in vivo.

Of course, the above calculation gives only a minimal estimate of the density of the heat current. If indeed the cell membrane is made of a material which is a poor thermal conductor (the lipid bi-layer for instance) and if in this membrane—here and there—exist "channels" filled with an aqueous medium having the relatively high thermal conductivity of water, then the flux of thermal energy will be channelled within the pores. Local densities of heat flux many times higher than the ones obtained in the case of a uniform distribution over the whole surface of the cell will therefore be encountered in the pores.

The next problem, however, arises directly from the kind of answer which we have just given to the preceding question. Does indeed the living membrane have "pores"? This is a crucial point, because if there are no pores the molecules and ions to be transported should each dig a "hole" for itself in the membrane, a process this one, which would require a greater force than the radiation-pressure may reasonably provide.

Even if many researchers claim that the plasma membrane indeed has holes and that channels have been observed in it, there is no general agreement on this point, and furthermore, two very serious objections to the existence of pores can be raised. In the first place, holes larger than 8 ÷ 10 Angstroms should be seen by high-resolution electron microscopy, and in the second place, they would be incompatible with the survival of the cell. Its vital sap, indeed, would leak out through such pores, and osmotic pressure gradients would provoke bursting or intollerable shrinkage of the cell. The second objection can be answered at once: in the presence of a flow of heat a "hole" is no longer an open gate through which any particle can enter or leave, as long as it is smaller than the gate itself. Our experiments with porous septa have definitely proved that a hole in the presence of a flux of thermal energy is a highly selective and directional passage through which only some materials can enter while others can only come out. If it is further supposed that the pores can close whenever metabolic activity is halted, the intergrity of the cell, also in absence of metabolism, would be quite well compatible with the existence of even relatively large pores.

The closure of the pores in coincidence with the halting of the metabolic activity might also quite well explain why such channels are not seen by electron microscopy. Anyway, some more serious objections can be raised on very different grounds. It has been proved beyond doubt that enzymatic activity is closely connected with various kinds of membrane transport in vivo. Carrier enzymes, "permeases", have been identified, and genetic defects leading to malfunction or absence of specific forms of transport are currently studied [34, 35, 36, 37, 38]. Moreover, it is well known that cellular membrane transport can be altered or stopped by introducing traces of substances which inhibit enzymatic activity or induce conformational transformations in proteins. All this evidence appearently shows that biological evolution early chose to rely on the sophisticated—and highly selective—systems of enzymatic transport, rather than on comparatively less specific physical phenomena. Thermodialysis could therefore have been adopted by emerging life as a primeval tool soon to be superseded by specific sequences of chemical reactions. The main trend of biological evolution, based on the development of the versatile architecture of proteins with their almost unbelievable ability to adapt to every task, would accordingly have prevailed also in the development of biological transport systems. At present transport based on physical phenomena powered by the flow of thermal energy, at most would still survive in very peculiar cases as "fossil" processes, and only by an accurate search or by sheer chance one might discover some of these cases.

But is this really so? Or the two appearently mutually exclusive classes of phenomena in truth do not compel to make an either-or choice?

Let us consider the current model of the structure of the plasma membrane [39]. In this model the liquid bi-layer contains, embedded into it, proteins, some of them stretching across the whole thickness of the membrane.

There is a rather general consensus on the close association of the ensymes involved in the transport with the membrane.

The simplest hypothesis consistent with this opinion and with the current membrane model, would be that the integral proteins extending through the entire thickness of the membrane are themselves the transport enzymes.

The effect of thermodialysis together with this last observation allows an unified hypothetical working model of the unit membrane to be formulated. In Fig. 3 we have schematically represented this model; as one can see this is an almost perfect copy of the picture of the liquid mosaic membrane of Singer and Nicholson [39]. Only we have represented the conformation of some of the integral proteins in such a way as to constitute a kind of "pore". Namely, the tertiary conformation of these proteins should be such that a funnel-like cavity across the membrane thickness results. The enzymatic active site should be located at one end of the pore—in the figure we have drawn a section through one such enzyme and the active site is schematically represented at the inner end of the channel. The active site could quite well be the "gate" which opens or closes according to circumstances.

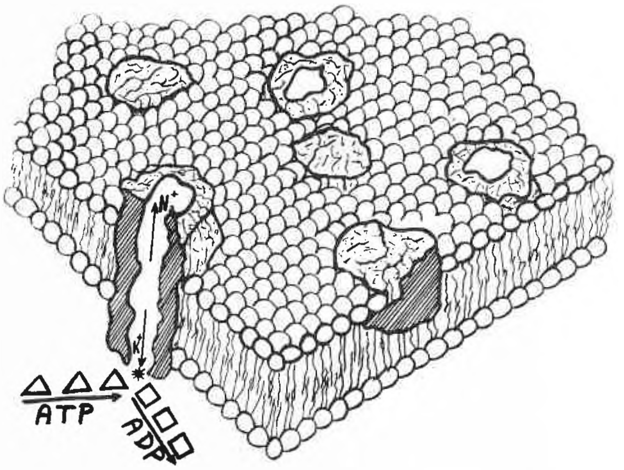

Fig. 3: Schematic representation of our proposed membrane model. The original liquid mosaic model of Singer and Nicolson [39] is slightly modified. Some of the integral proteins are drawn here as funnel-shaped architectures with an enzymatic site at one end of the channel. In the figure the enzymatic site is marked by an asterisk and is supposed to be located on the "inner" surface of the membrane.

Anyway, this gating capacity would not be the most important function of the enzymatic activity. Far more meaningful for cellular transport would be the energy liberated in the course of the reaction with the substrate. In the figure the "modus operandi" of an ATP-ase, according to our model, is schematically represented. The energy released in the reaction would in part be converted "in loco" into molecular motion, i.e. it would quickly decay into heat. The heat generated by these events would be channelled into the pores filled with an aqueous medium of relatively high thermal conductivity. The intense, local, pulsed heat source would therefore sum its discrete, quantified contribution to the continuous flow of heat produced by the metabolic activity of the whole cell. Our results with thermal diffusion and thermodialysis in artificial membranes do prove that in such conditions the Na^+ ions present in the pore would be pushed out of the cell (or mitochondrion) and that K^+ ions would be pulled in. The effects would probably exhibit a rather precise stoichiometry, owing to the well defined volume of the pore and to the quantified energy of the heat pulse. The repetition rate of the process would be the one characteristic of the enzyme. This circumstance leads to interesting and multifarious regulatory possibilities of the level of Na-K pumping.

It is easy to conceive variations of this scheme by which practically every kind of ion or molecule can be carried in or out of cells, mitochondria or tissues.

One last observation: Considerations of thermodynamic stability show that in such a conformation as that of the figure, the lipophilic groups of the protein will be preferentially in contact with the surrounding lipid bi-layer, while the inside of the channels should contain most of the hydrophilic groups. Therefore, the channels themselves would exhibit hydrophilic properties. It is quite conceivable that hydrophobic channels can eventually be formed by proteins containing a very great percentage of hydrophobic groups. More complicate structures in which "pores" are defined by clusters of proteins are also conceivable.

It would be out of place to develop further this model here. In our opinion the interesting part of the model resides in its simplicity and in its versatility. Minor changes, implying only a few mutations in the genome, can deeply modify the transport properties of such a kind of pore, and will enable thereby a ready adaptation to the most varied needs and environments. The possibility to reconcile within this model all the existing knowledge on the biochemistry of biological transport with our recent findings on molecular processes of transport mediated by the flow of heat, seems to make a further study of the model and a search for supporting evidence in vivo well worth trying.

References

1 *J.L. Fabelinskii:* Soviet Phys. – Usp. Fiz. Nauk. *77* (1962) 649 (English translation: *5* [1963] 667).

2 *G.B. Benedek, J.B. Lastovka, K. Fritsch* and *T. Greytak:* J. Opt. Soc. Am. *54* (1964) 1284.

3 *R.Y. Chiao* and *B.P. Stoicheff:* J. Opt. Soc. Am. *54* (1964) 1286.

4 *D.J. Mash, V.S. Starunov* and *J.L. Fabelinskii:* Zh. Experim. i. Teor. Fiz. *47* (1964) 783 (English translation: Soviet Phys. – JETP *20* [1965] 523).

5 *W.J. Cowley:* Contemp. Phys. *4* (1962) 15.

6 *S.E.A. Hakim* and *W.J. Cowley:* Nature *208* (1965) 1082.

7 *P. Debye:* Ann. Phys. (Paris) XXXIX, *4* (1912) 798.

8 *L. Brillouin:* J. Phys. (Paris) VII, *8* (1936) 153.

9 *L. Brillouin:* Ann. Phys. (Paris) *17* (1922) 88.

10 *L. Rayleigh:* Phil. Mag. *3* (1902) 338.

11 *L. Rayleigh:* Phil. Mag. *10* (1905) 364.

12 *L. Brillouin:* J. Phys. Radium *6* (1925) 337.

13 *L. Brillouin:* Ann. Phys. (Paris) X, *4* (1926) 528.

14 *L. Brillouin:* Rev. Acous. *5* (1936) 99.

15 *L. Brillouin:* J. Phys. Radium *17* (1956) 379.

16 *F.S. Gaeta:* Phys. Rev. *182* (1969) 289.

17 *F.S. Gaeta* and *A. Di Chiara:* J. Poly. Sci., Poly. Phys. Ed. *13* (1975) 163.

18 *G. Brescia, E. Grossetti* and *F.S. Gaeta:* Il Nuovo Cimento, Serie XI, *83* (1972) 329.

19 *F.S. Gaeta* and *N.M. Cursio:* J. Poly. Sci., A-1, *7* (1969) 1697.

20 *F.S. Gaeta, A. Di Chiara* and *G. Perna:* Il Nuovo Cimento, Serie X, *66 B* (1970) 260.

21 *F.S. Gaeta:* J. Poly. Sci: Symp. Nº 42, Part 3 (1973) 1157.

22 *F.S. Gaeta, G. Scala, G. Brescia* and *A. Di Chiara:* J. Poly. Sci., Poly. Phys. Ed. *13* (1975) 177.

23 *F.S. Gaeta, G. Perna* and *G. Scala:* J. Poly. Sci., Poly. Phys. Ed. *13* (1975) 203.

24 *F.S. Gaeta, D.G. Mita, G. Perna* and *G. Scala:* Il Nuovo Cimento, Serie XI, *30 B* (1975) 153.

25 *A.I. Oparin:* "The Origin of Life" – Dover Publications, Inc., New York 1953.

26 *J.D. Bernal:* "The Origin of Life" – Weidenfeld and Nicolson, Inc., London 1967.

27 *F.H.C. Crick* and *L.E. Orgel:* Icarus *19* (1973) 341.

28 *W.R. Chappel, R.R. Meglen* and *D.D. Runnels:* Icarus *21* (1974) 513.

29 *T.H. Jukes:* Icarus *21* (1974) 516.

30 *A. Banin* and *J. Navrot:* Science *189* (1975) 550.

31 *F.S. Gaeta* and *D.G. Mita:* "Radiation pressure theory of thermal diffusion across permeable partitions: The process of thermodialysis" – submitted for publication.

32 *D.G. Mita, F.S. Gaeta* and *A.D'Acunto:* "Experimental investigation of the phenomenon of thermodialysis" – submitted for publication.

33 *R. Haase* and *C. Steinert:* Z. Phys. Chem. (N.F.) *21* (1959) 270.

34 *A.B. Pardee:* Science *162* (1968) 632.

35 *E.C.C. Lin:* A. Rev. Genetics *4* (1970) 225.

36 *D.L. Oxender:* A. Rev. Bioch. *41* (1972) 777.

37 *J. Guardiola, M. De Felice, T. Klopotowky* and *M. Iaccarino:* J. Bact. *117* (1974) 382.

38 *J. Guardiola, M. De Felice, T. Klopotowky* and *M. Iaccarino:* J. Bact. *117* (1974) 393.

39 *S.J. Singer* and *G.L. Nicolson:* Science *175* (1972) 720.