



WET-AND-MURBURN-MEMBRANE-MODEL

PART I: SOLUTE DISTRIBUTION, TRANSPORT, VOLUME CONTROL AND REDOX METABOLISM

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*Cell membranes are 'said' to consist of membrane proteins and lipids, and to execute the following functions: (1) maintenance and control of internal ion and solute composition by means of ion pumping establishing a steady-state non-equilibrium bioenergetics, (2) trans-membrane transport (passive and active, the latter by ion pumping and coupled transport), (3) generation of a membrane potential by electrogenic ion pumping, (4) cell volume control through van 't Hoff osmosis, (5) chemiosmosis in prokaryotic, inner mitochondrial and thylacoid membranes, (6) signal recognition and transduction by receptors, associated enzymes and elements from cytoskeleton and extracellular matrix/cell wall, (7) functional attachment of these and other structures, (8) exocytosis and endocytosis. The general physiological model underlying functions 1 to 5, known as 'membrane-pump theory' (MPT) is unequivocally falsified and must be replaced by other fundamentals. The primary roles of two 'forgotten' substances, water and O₂, disclose an entirely new understanding of membranes and of the fluid they encapsulate. The proclaimed membrane functions 1, 2 and 4 appear to be mainly executed by the colloid structure of the macromolecule-matrix inside. Functions 2, 3 and 5 involve an interaction of the cell's reducing interior with O₂ entering the cell and starting a radical-based 'burning process' with physiological functions for diffusible ROS at low concentrations (murburn concept: MURed BURNing), which energize the membrane. **Biomed Rev 2025; 36: 45-84***

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Abbreviations: WMMM: wet & murburn membrane model; MPT: membrane-pump theory; AIH: association-induction hypothesis; GSH: gel-sol hypothesis; ETC-CRAS: electron-transport chain & chemiosmotic rotational ATP synthesis; POM: polarized oriented multi-layer; DROS: diffusible reactive oxygen species; DRS: diffusible reactive species; ADR(O)S: anions of DR(O)S radical cascade; A-state: action state; R-state: resting state; A-to-R: action to rest; R-to-A: rest-to-action; NKA: Na/K-ATPase; GHK: Goldman-Hodgkin-Katz; pmf: proton-motive force.

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INTRODUCTION

This treatise does not deny the basic achievements of the Singer and Nicholson fluid-mosaic model [1] and its modern extensions/adaptations [2,3], though the role of lipids remains under-emphasized [4,5]. Among Nicholson's 2014-extensions/adaptations are the transient formation of rafts, other lateral aggregations, complex-formation with peripheral proteins, and the existence of phase transitions. The latter even become more central than ever before, as will be demonstrated. This treatise follows a functional approach and deals with 5 aspects of membranes, which are intimately interconnected, both in the generally agreed view of 'membrane-pump theory' (MPT) and its steady-state non-equilibrium thermodynamics dependent on ion pumping, and in the alternative view presented here. The latter is based on overlooked changes of the thermodynamic state of water and an unexpected role of oxygen in bioenergetics. The traditional treatment of membrane functioning and its detailed mechanisms as described in modern textbooks on membranes [3] on the one hand presents valuable detailed molecular structural data, which are not contested *a priori*, on the other hand are wrongly and unilaterally interpreted within the context of an outdated MPT background. It will be argued that MPT is meanwhile fully falsified. MPT only *appears* comprehensible because it is instructed worldwide and because most bio-scientists follow it since more than half a century – it became common knowledge, it is familiar. However, in this dominating position it systematically ignores the existence of valuable alternatives (a common practice of almost all textbooks and most university courses) and contains many unnoticed internal contradictions. For instance, crowding of the intracellular fluid is well acknowledged while at the same time the physics of ideal diluted solutions is applied to calculate ion and solute gradients. Anomalies in the present-day theorization must urgently be stopped. If there are theoretically and experimentally well-underpinned views which compete with MPT for the same data and can explain numerous additional but largely unmentioned data that cannot be fitted into the MPT paradigm, and which also take away all these anomalies, honesty dictates to present MPT and the competing ideas both with their main arguments. This will be done here.

1. INTERCONNECTED MEMBRANE FUNCTIONALITIES THAT MUST BE REINTERPRETED

The 5 interrelated aspects of membrane function (ion and

solute distribution, transport, cell potential, cell volume, and chemiosmosis) as understood by MPT (which on first look *appears* to follow a strong logic) all depend on the correctness of the (largely ignored) very first premise, where the error arose. This goes back to the conclusions drawn from an experiment by the famous physiologist and Noble prize winner Archibald Hill executed in 1930 [6]. Before there was much dispute among cell physiologists between two schools of thought. The '*membrane school*' saw the cell as delineated by a plasma membrane that enveloped the internal solution and was supposed to be responsible for the maintenance and control of the unusual concentration of ions and solutes on both sides of the plasma membrane, most remarkably those of K^+ and Na^+ . The real existence of a plasma membrane was not certain those days, whereas there were quite direct observations of protoplasm behaving as a colloid. So, the '*colloid school*' tried to explain these '*concentration differentials*' (the new term adopted here) as due to the *observed* colloid (better coacervate, which is a complex colloid) nature of protoplasm (meanwhile defined as cytoplasm plus cell membrane). Hill wanted to terminate this ongoing dispute. He experimentally determined the equilibrium distribution (partition) coefficient of the test-substance urea between resting muscle cells and the surrounding Ringer, and this was close to one. From this *too-limited experiment* he derived the *too-extrapolating conclusions* that: (1) inside and outside (Ringer) are the same phase corresponding to that of *normal* water, (2) hence the cell is not a colloid and has no colloid osmotic pressure, (3) hence the quantitatively by far most abundant solute, K^+ , must be free in solution in order to explain the measured total osmotic pressure of the cell. The consequences of this experiment are huge and influence thinking up to now, 95 years later. Indeed, the data *seemed* to permit application of the laws of *ideal* diluted solutions to calculate (a) ion and neutral solute *concentration gradients* according to the laws of Nernst and Fick respectively, and (b) cell volume by van 't Hoff's law. Hill's lecture at the 1930 physiological congress was so impressive that it convinced most adherents of the colloid view, who came to adopt membrane theory instead. When in 1941 radioactively labeled Na^+ became available so that previously contested permeability of cell membrane for Na^+ became established, Dean [7] calculated the Na^+ concentration gradient using Nernst's law and found that the obtained value did not correspond with the actual Na^+ differential; therefor he proposed the existence of a Na^+ pump in the plasma membrane compensating for the difference between theory

(his calculation) and measurement. Later Skou [8] found that a membrane-located ATPase obligatorily dependent for its activity on the presence of Na^+ and K^+ , a Na^+/K^+ -ATPase (NKA), had similar properties (K_M , inactivity with ITP, ouabain sensitivity) than the physiological measurements of Na^+/K^+ -pumping. Before that time Hodgkin, Huxley and Katz [9] already interpreted their quantitative data on cell potential within the context of the emerging MPT. Their work gave an immense boost to the further development of MPT with particular emphasis on the role of membrane permeability that was seen as selective for different ions and could temporarily increase upon surpassing threshold potential.

During the further development of thoughts NKA and some other 'pumps' became to be seen as responsible for the above mentioned 5 interrelated functions: (1) maintenance and control of ion and solute distribution between the cell/organelle interior and the external milieu thanks to the activity of ion pumps, which were seen as responsible for a steady-state non-equilibrium bioenergetics – they should pump *continuously* to compensate *uninterrupted* passive leaks; (2) trans-membrane ion and solute transport in the so-called *active* direction, executed directly by pumps or indirectly by coupled transport; (3) cell/organelle potential *thought* to be generated by electrogenic ion pumping (for instance NKA was *thought* to pump 3 Na^+ outwards in exchange for only 2 K^+ inwards; redox complexes of so-called '*electron transport chains*' (ETC's) were *thought* to only pump protons out without an electrical balancing co-transport; (4) cell/organelle volume control by simple van 't Hoff's osmosis, *thought* to be operative because despite membrane permeability for ions and many simple solutes, pumps and coupled transport systems were *thought* to actively keep concentration balance, which would make the membrane *virtually impermeable* for them, while being permeable for the solvent; (5) chemiosmosis which is based on two concepts: (a) the MPT-idea of electrogenic proton pumping, in this case not energized by ATP but by redox activity setting up an electrogenic potential and pH-gradient, together called proton-motive force (pmf) that in turn is *thought* to drive rotatory ATP synthesis, and (b) the concept of a *serially* organized electron transport *chain* (ETC), a concept introduced by Keilin and Hartree in 1939 [10] that was applied to oxidative and photo-phosphorylation. Though there were vigilant discussions about the mechanism by which the ETC would produce pmf (even up to now), to much surprise a consensus view was reached (despite ongoing opposition) taking the existence of an ETC containing redox-driven proton

pumps and the establishing of a pmf for granted, which lead next to broad acceptance of Boyer's 'Chemiosmotic Rotational ATP Synthesis model for F_0F_1 -ATP synthase (together 'ETC-CRAS model') [11], though discussion about the details remained.

The experiment of Hill was later repeated, and this confirmed his results on urea. However, 31 other non-ionic test-substances were investigated with the same experimental setup, and this revealed that equilibrium distribution (partition) coefficients of substances with at least 3 carbon-atoms are below unity, roughly the lower the larger the molecular size with only 0.227 for D-mannitol, 0.132 for sucrose and 0.1 for raffinose ([12,13]; all data recently reviewed: [14]). These experiments unequivocally settle: (1) that cytoplasm is a colloid (coacervate), (2) hence that cell water is largely '*bound*' to a macromolecular matrix (has low water activity) as it is the case in a colloid, (3) the high colloid osmotic pressure typical for every colloid then implies that most of intracellular K^+ must be in an adsorbed state to account for measured total osmotic pressure.

These more elaborate measurements make the entire MPT logic to shrivel and necessitate that all ideas with respect to the above 5 membrane functionalities, whereby *assumed* (meanwhile disproved) ion pumping (whether based on ATP or ETC) is *thought* to be the energizing principle, must be replaced by a new understanding of bioenergetics. Fortunately, the latter already exists in the form of several new hypotheses which are largely complementary, hence need to be integrated. Also, the primary premise of a *serial* ETC (not a *parallel* system) as essential part of functionality 5 must be reconsidered. The latter premise goes back to a quite logical *assumption* made by Keilin and Hartree [10]. After they had revealed the different cytochromes, they reasoned that electron transport must be organized as a *serial* system (a *chain*) with oxygen serving as terminal electron acceptor at cytochrome oxidase (still the classical view), since the other theoretical possibility, a *parallel* system whereby each individual complex would react directly with oxygen, would generate harmful reactive oxygen species or ROS (well formulated in the textbook of Lehninger [15]). Yet, it is meanwhile generally known that ROS are formed at each of the redox complexes, even the more at higher respiratory rate, which demonstrates a kinetic relationship [16]. This suggests that apart from their harmful and signaling functions, ROS may execute a more penetrating constitutive physiological function at physiological (low) concentrations. These normal physiological functions were recently discovered

by Manoj [17-25] and described in his ‘*murburn concept*’, a major breakthrough in bioenergetics. ‘Murburn’ is an acronym for MURed BURNing, a radical-based burning process in a ‘*mured*’ somehow delineated volume. This concept is meanwhile theoretically and experimentally well-developed. Its deeply penetrating effects are described with much detail for many different 1-electron transfer processes (survey [22-25]). With respect to respiration and photosynthesis arguments are collected for a parallel system of redox complexes instead of an ETC.

2. ARGUMENTS DISQUALIFYING MPT AND ETC-CRAS

1. The measurements of equilibrium distribution coefficients of many non-ionic solutes mentioned above actually suffice to disprove MPT, but much can be learned from other data falsifying MPT.

2. Not long after Hill’s argumentation Schrödinger [26] argued that, if a cell would work according to the random character of free diffusion (the heart of MPT), its accuracy to regulate its functioning would have a lower limit dictated by ‘ kT ’. This is an insurmountable argument. He brings into remembrance that the ‘ $n^{1/2}$ law’, which applies to a diffusive mechanism, means that for 100 particles of a given kind being present, the accuracy to control them is only 10%, for 1000 it is 1%, for 10.000 it is 0.1%. He mentions genes which usually occur only as a single copy per cell. But this law is also particularly restrictive for small cells (prokaryotes) and organelles such as mitochondria and chloroplasts. For instance, the number of protons in a small Cyanobacterium of about 10^{-16} L at a pH of 8 is about 1. In an averagely sized mitochondrial-matrix it is about 6. How to control proton concentration on behalf of statistical laws in which a diffusion parameter is taken up as done in Mitchell’s chemiosmotic theory [27,28]? Can a single proton serve thousands of redox complexes at the same moment as necessitated at high respiratory rate? Can these few protons build a proton-motive force (pmf) of sufficient extent to drive F_0F_1 -ATP synthase on behalf of the Boyer mechanism [18,25,29]? Undeniably, the whole thing must work differently. If the number of protons would have been 1000, implying a cell/organelle volume of 10^{-13} L, the accuracy to control them, if based on a diffusion mechanism (Fick, Nernst), would still have a relative error of 1%. This makes a chemiosmotic mechanism for prokaryotic cell membrane, mitochondrial inner membrane and thylacoid membrane unworkable. In 1944 Schrödinger could only

imagine that mechanisms of quantum coherence (but then at ambient temperature!) would be sufficiently accurate to regulate cells. This was a bold idea at that time, and probably the reason that his idea was not picked up. But meanwhile long-term quantum coherence at life temperature is experimentally corroborated, explicitly for the case of the microtubule-water system [30,31] and indicated for many other cellular systems (more details: point 7).

3. Already in 1951 Ling [32] reasoned that, if a cell would use membrane-based *active transport*, then bringing metabolism to a halt, should strongly reduce Na^+ efflux. He used resting muscle cells at $0^\circ C$, hold under 99,99% N_2 and in the presence of 1mM iodoacetate and 1mM NaCN to make active transport impossible. The very small amount of lactate formed was taken up in the calculation, and he also measured total heat output in order not to miss an eventual unknown energy-delivering mechanism. He found that, if MPT should be correct in applying its Nernst-based calculation method, the *assumed pumps* under this stringent experimental setup would consume between 15 to 30 times more energy than the cell was found to deliver. Minkoff and Damadian [33] came later to the same conclusion. They measured insufficient energy delivery in experiments with *E. coli* whereby they measured 6 different so-called ‘*active*’ ion/solute fluxes. Such energy shortage needs an explanation, which will be given in argument 6. In addition, experiments conducted by Keynes and Maisel in 1954 [34], despite they strongly advocated MPT, revealed that both 0.2mM 2,4-dinitrophenol and a combination of iodoacetate with 3mM cyanide (pH 7.6) failed to reduce Na^+ efflux rate. They proposed the existence of a Na^+/Na^+ exchange diffusion, but later they found using a Na^+ -free medium that this amounts to only 50% and fades away below $10^\circ C$. Hence this cannot explain Ling’s $0^\circ C$ experiment. All these findings prove that the MPT-view on bioenergetics CANNOT WORK on behalf of thermodynamic measurements.

4. As early as 1949 Henniker [35] had already reviewed more than hundred publications containing data that indicated that cell water is not normal water and exhibits long-range order. Much later an astonishing number of data obtained by means of many different modern techniques revealed that averagely cell water is **Polarized** and **Oriented** into **Multi-layers** (POM-water) [36-39] (other data about cell water: [40-50]; with respect to DNA [48]; for a general physical derivation of the many different thermodynamic states of fluids and solids, with particular emphasis on water and cell water: [50]. All these studies invalidate the most primary conclusion

of Hill's experiment (and of MPT), namely the *assumption* that cell water is normal water. Direct *in vivo* observations of the polarization of entire tissues (sometimes even the whole body) of different microscopically small animals by means of polarization microscopy defeat the *supposedly* (MPT) non-polarized state of the main cellular component, cell water [46].

The finding that cell water is largely POM-water, which has low solvency for most substances with more than 3 carbon atoms [13,14], and for hydrated Na^+ [51] make that the way how concentration gradients are calculated by followers of MPT is *not permitted*. Hence the term '*concentration gradient(s)*' should no longer be used either, because it is inseparably related to a wrong calculation method. It is suggested here to replace it with the new neutral term '*ion/solute differential(s)*'. Moreover, Ling [38,39,52] demonstrated that the structure of water changes when a cell-system is activated from rest-to-action (for instance initiation of an action potential) and during the recovery phase from action-to-rest. This totally invalidates using the concept of concentration as such; It should be multiplied by an activity coefficient, and the latter may change according to the physiological state of a cell-system. Hereby, physiological changes in adsorption and in the solvency of water during these phase transitions should be accounted for.

Delalande *et al.* [53] scrutinized the applicability margins of Nernst equations, demonstrating undeniably that it is not allowed to use these equations to complex systems such as cells, as first done by Bernstein [54] in 1902 and by most others afterwards. Several conditions are not met. If the calculation method is not allowed, it follows that also the concept of ion pumps must be questioned. The alternative mechanisms described here do not deny the existence of proteins called '*ion pump*' but show that these proteins do not work according to the principles of a pump, but act by means of a combination of phase transitions and changes in redox state (section 4.3 and 5.2.a). The extensive evidence in favor of such new understanding of the mechanism of those membrane proteins challenges '*established truth*' with repercussions to almost all real-time functions of cells, those of membranes but also those of the cell-matrix which governs internal fluid state.

5. Using many modern techniques and different cell types (mainly resting muscle cells, neurons, erythrocytes and *E. coli*) it has been shown that in a resting cell-systems such as relaxed muscle cells and axons below threshold intracellular K^+ exists largely in an adsorbed state [47,55-66]. The results of Bogner and his collaborators [66] are particularly important

because they studied erythrocytes of several races of dog and sheep, which differ substantially in intracellular K^+/Na^+ ratio, and exceptional situations are very powerful to verify a hypothesis. When a cell-system in the resting state (R-state) is stimulated, adsorbed K^+ is desorbed, resulting in an increase of K^+ activity and this is involved in power output, such as for instance in muscle contraction [52] or action potential generation (section 5.3).

6. Cells without a complete cell membrane can keep internal K^+ and Na^+ concentrations at the physiological level over prolonged periods: at least 2 days for transversally cut relaxed muscle cells inserted with their open end in a Ringer solution provided with radioactive Na^+ and K^+ for measuring fluxes [67]; experiments of Ling repeated over 70 times! The explanation given is that resting muscle cells keep their low internal Na^+ concentration because POM-water associated with the cell-matrix has low solvency for hydrated Na^+ [37,51], whereas high internal K^+ is explained by adsorption to fixed negatively charged sites of protein carboxyl groups of the cell-matrix [55-69], but other minor types of adsorption sites exist too: phosphate groups for instance in *E. coli* [61] or carbonyl groups such as in valinomycin and possibly in some ion channels. These experiments undeniably prove that the R-state is a '*long-term meta-stable high-energy low-entropy excited coherent state*', whereby order and coherence are due to POM-water and adsorption of K^+ . The results may *appear* unrealistic, because for many cell types perforating their cell membrane results in leakage of ions and solutes, but this is only because most cell types always have several regions in the A-state. Typical of resting muscle cells is that most parts of cytoplasm are in the R-state. This is also the case for sub-threshold axons. Several other cells with incomplete membrane were shown to keep ion differentials [41]. Dormant *Artemia* cysts are an extreme case. Here the entire system is at rest. It presents the ideal system to get undeniable proof of the meta-stable high-energy low-entropy R-state. Clegg [70] kept dormant *Artemia* cysts for 4 years under complete anoxia without measurable metabolism and still they were able to hatch. Clearly, in the R-state ion fluxes are passive in both directions, and maintenance of ion and solute distribution is a function of cytoplasm, not of the cell membrane as incorrectly *thought* by MPT! This fundamentally disproves MPT's '*steady-state non-equilibrium bioenergetics maintained by uninterrupted ion pumping*'. It implies that cell systems that occur in R-state do not consume energy for maintaining this state over prolonged periods (if not activated by stimuli,

eventually years), i.e., that cell systems, even entire cells can stay ON or OFF (the case of *Artemia* cysts). These experiments explain why so-called membrane-located proteins incorrectly called ‘*pumps*’ use more energy than the cell can deliver, if calculated in the MPT-way using the non-permitted Nernst or Fick laws to calculate concentration gradients. In contrast, they demonstrate that the R-state does not consume energy (figure 1).

Only when this state is disturbed due to a sufficiently strong activating stimulus that surpasses a threshold, the action state (A-state) is initiated, net ion fluxes in one direction may occur powered from the stored low-entropy of the R-state (R-to-A phase transition), but direct redox energy may be involved too [25]. When after power output the lower-energy higher-entropy de-cohered action-end state (for muscle cells resembling *rigor mortis*) is reached, ATP adsorption (not hydrolysis!!) [60,71,72] upon the involved protein system is needed to recover again the excited high-energetic equilibrium R-state, as evidenced by the need of ATP to relieve *rigor mortis* [73]. This means that the A-to-R phase transition needs input of metabolic energy for the synthesis of new ATP. The binding

of ATP to so-called ‘*cardinal adsorption sites*’ of the protein system generates a Lewis inductive effect that is the opposite of that generated by the stimulus. The Lewis inductive energy input of cardinal adsorbents such as ATP and different stimuli reaches so-called ‘*regular adsorption sites*’ (executive sites) involved in water polarization/depolarization, K^+ adsorption versus Na^+/H^+ or another protein/macromolecule.

7. The possibility of a long-term meta-stable high-energy low-entropy coherent excited state at body temperature was derived by Ling by means of statistical mechanics applied to non-independent (not diluted) particles whereby adsorption is included [71]. It could also be derived in independent ways from the primary principles of several other physical theories. Fröhlich [74-79] using non-linear electrodynamics derived that excited polarized (coherent) water in association with water-polarizing proteins (and B-DNA [49] and membranes [52]), if sufficiently excited (ATP adsorption suffices), can reach long-lived quantum coherence at ambient temperature by means of strong polarization (low entropy), as proposed by Schrödinger. Hereby all components (macromolecules and all associated POM-water molecules vibrate at a common frequency and

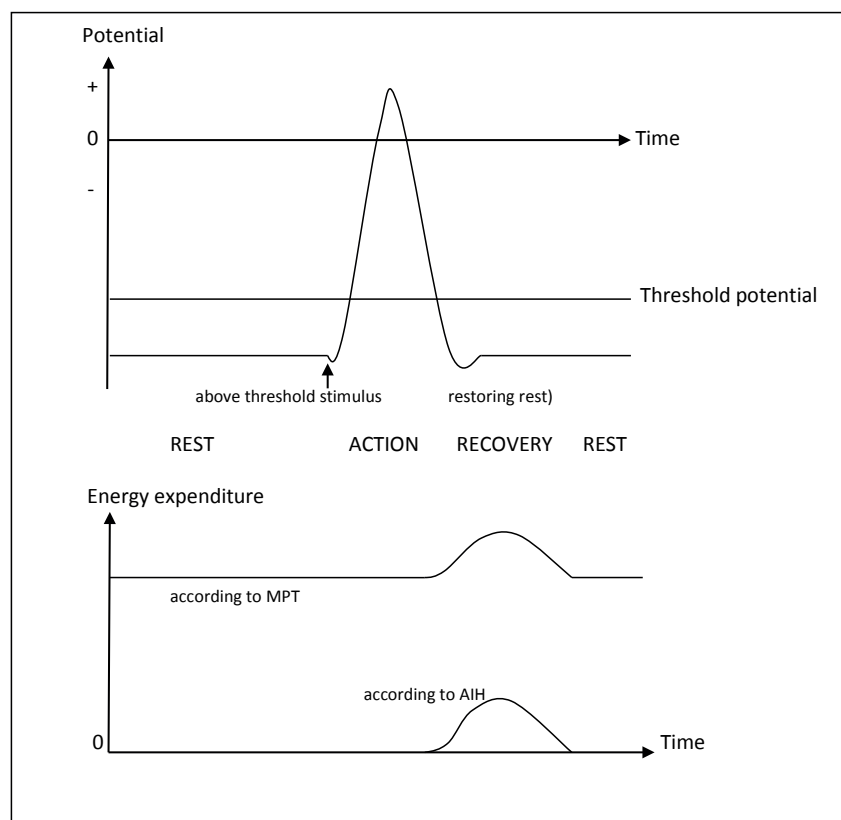


Figure 1. Comparison of the energy expenditure to complete an action potential supposed MPT would be correct versus according to AIH. The difference is massive because according to AIH the below-threshold state does not consume.

its harmonics. Such specific frequencies could be calculated for different cell systems and were then corroborated by experiment, either by measuring the real occurrence of the calculated frequencies in cellular systems, or by irradiation of cell systems with the appropriate calculated coherent frequency at extremely low doses and observing the effects [74-77] (with respect to cancer [76,77]. The group of Del Giudice used non-linear quantum field theory taking care of the omnipresence of the quantum vacuum (absolutely necessary, but often omitted) [80-84]. Unification of these physical theories with Ling's physiological AIH was obtained by Jaeken [29] by extending the interpretation already obtained partially by Tuszyński *et al.* [84] of the coherent solutions of a non-linear Schrödinger equation developed by them and presenting another independent physical approach in agreement with the coacervate-coherence nature of life [29]. Such derivations from *ab initio* physical principles are not proof *per se* but indicate a mechanism's feasibility. A fourth independent approach is from non-linear optics and involves direct observations of coherent or quasi-coherent electromagnetic emission, including the photonic range (IR, VIS, UV, particularly around 270 nm) [86-89] and the decrease of coherence in case of tumor tissue [87]. The data may be linked to observations of Damadian from 1971 [40] using chemical NMR that water in healthy cells is highly ordered, and that in tumor tissue it is much less, observations which directly stood at the origin of the development by Damadian in collaboration with others of the MRI apparatus. The latter in fact presents a direct application of the ideas presented in Ling's AIH. The possibility of the presence of cell water that is polarized to different degrees is also underpinned by the *ab initio* derivations of the possibility of a large number of different thermodynamic states of the liquid and solid aggregation states and their application to cell science by Kaivarainen [50]. End then there is the crucial extension of Prigogine's theory of non-linear thermodynamics far from equilibrium [91]. Prokhorenko and Matveev derived the previously not considered possibility of the existence of non-linear systems far from equilibrium, which nevertheless exhibit *long-term meta-stability*, and this is an absolute requisite to explain evolution [29;93,94]. It allows that during evolution a new level of coherence can be added to an already existing one without losing the latter because of its longevity [22,25,29,93]. Moreover, Prokhorenko and Matveev were able to apply their theoretical derivations directly to Ling's AIH.

Together these 7 different *ab initio* physical approaches all theoretically underpin the observed coacervate-coherence

nature of life such as described in the AIH and a number of additional coacervate-based physiological theories [29], whereas at once disproving MPT, because coherence is the opposite of randomness, hence is absolutely incompatible with MPT. An important aspect of life is missing in those theories, namely how redox activity works. Ling [95] gave many strong arguments that Mitchell's chemiosmosis could not work, and in 1991 Mitchell himself admitted that his proposal could not work, but these opinions did not reach many adherents. Arguing against is not the same than having a valuable alternative (the one of Ling dealing with redox activity [95] is not very convincing). One had to wait until the revelation of the murburn concept of Manoj [18-20] discussed in section 4.1.

It might be important in general to understand that the quantum approach of Fröhlich [74-79] and the group of Del Giudice [80-84] and the direct observations of quantum coherence on isolated microtubules [30,31] goes about the concept of resonance. This central mechanism of life was also treated by Cosic [97] in her '*Resonant Recognition Model*' (RRM). Each amino acid can be characterized by the average energy state of its valence electrons, known as the electron-ion interaction potential. Using these values, an amino acid sequence can be transformed into a numerical series of their average energy states, which can be transformed using fast Fourier transformation in a frequency series in the region between 10^{13} to 10^{15} Hz including IR, VIS and UV. From this frequency spectrum information can be extracted regarding the biological function of a given protein. It appeared that within a group of proteins of similar function, some common frequencies are generated that are absent in other proteins, and that proteins with different functions never have a common frequency of that type; they have another typical frequency. Moreover, proteins and their targets possess a common frequency. It was therefore concluded that: (1) the typical RRM-frequencies characterize a particular function, and (2) that these RRM-frequencies are also involved in the attraction and reciprocal recognition between a protein and its target, a quantum property of proteins (likely of excited biological macromolecules in general) that was also previously derived for enzymes by Fröhlich [78]. These quantum coherent properties of excited bio-systems challenge the usually applied '*random collision model*' as being inappropriate. Specificity of the interaction of a protein with its target is due to resonant electromagnetic energy transfer of a specific frequency typical for each function. The model was tested on different protein groups. It fits with the quantum behaviour of living cells. The

RRM mechanism and quantum coherence in general do not mean that all processes are quantum coherent. Two examples. The action-end state of a process is decohered and at a given moment many systems in a cell may occur in that state, and at another moment some other systems might be active. Secondly, de-coherence exists in tumour tissue. Thirdly, murburn processes described in section 4.1 are demonstrably stochastic.

8. Red cell ghosts, *said* to pump K^+ and Na^+ upon addition of ATP [98] – an experiment *thought* to be in strong support of MPT – are filled with lots of cytoplasmic material as seen on electron micrographs [99]. Hence, the effects of ATP on K^+ and Na^+ fluxes could as well be due to cytoplasmic activity (as also revealed by the open-ended resting muscle cells that keep ion differentials for 2 days [67]). Moreover, better rinsed red cell ghosts, shown to be free of cytoplasmic material on electron micrographs, do not show additional K^+ accumulation and Na^+ exclusion upon ATP supply [100, 99]. That cytoplasm is responsible for keeping ion differentials and controlling them (as advocated by Ling [101]) was recently further corroborated by the *in vivo* fluorescence spectroscopy and fluorescence microscopic studies of Olsen and his collaborators [47]. Using an oscillating yeast suspension they showed that the time sequence, but also the spatial distribution of the oscillations coincides with fluctuations in K^+ activity (reversed relationship), with the degree of cytoplasmic water polarization (direct relationship), and with oscillations of ATP/ADP and NADH/NAD⁺ ratios (confirming a basic tenet of Ling's AIH). It was also demonstrated that these concentration fluctuations are governed by cytoplasmic actin, which was shown to be ouabain-sensitive. Insofar ouabain-sensitivity (Skou's argument) can no longer be taken as an argument for a membrane-dependent process. Obtained data are direct evidence that cytoplasm regulates intracellular ion concentrations. In the textbook of Alberts *et al.* ([102] Skou's argument is used to conclude that cell volume control is exercised by Na/K-ATPase (NKA) (see also point 11 below).

9. The kinetic data of the experiment of Freedman [98] were also important for proponents of MPT, since the ratio of the efflux rate of Na^+ versus K^+ influx rate upon addition of ATP was 3 Na^+ outwards for 2 K^+ inwards, which *seemed* to corroborate the idea of electrogenic ion pumping. However, in this experiment only Na^+ and K^+ were measured as electric charges, denying the existence of so many other ionic species (mineral and organic), which may be involved upon adding ATP, particularly since it is now clear that the observed fluxes are due to a cytoplasmic mechanism, which is so complex

that it hides many details. In addition, the 3:2 ratio may be questioned. First, the data points fall in 2 clusters, one for Na^+ and one for K^+ , which though presenting a broad distribution, could be linearized statistically. Secondly, in an attempt to confirm Skou's conclusion that NKA activity and pumping activity are due to the same system Bonting [103] compared cation fluxes and activities of NKA in six different tissues, whereby the lowest flux (human erythrocytes) is only 3.87×10^{-14} mole.cm².sec and NKA activity 1.38×10^{-14} mole.cm².sec (ratio 2.80) and the highest flux is $86,100 \times 10^{-14}$ mole.cm².sec and NKA activity is $38,800 \times 10^{-14}$ mole.cm².sec (ratio 2.22) (non-innervated Sachs organ of the electric eel). Lowest and highest ratios among the six tissues are respectively 1.86 (frog toe muscle) and 3.00 (squid giant axon), and the mean is 2.56 ± 0.19 . These statistically relevant data firmly establish absence of whole number stoichiometry and at once disprove the deterministic mechanism of the system. The consequences of these early data remained unnoticed until the development of the murburn concept. Similarly, with respect to oxidative phosphorylation stoichiometric problems in P/O and P/H⁺ ratios were noted by Hinkle [104], a fact observable in almost every experiment as these ratios are used as a control to judge the fitness of a mitochondrial fraction. This anomaly between measurement and theory has never been really solved, whereas the deterministic nature of the traditional model demands strict non-decimal ratios. The *supposed* slippery nature of systems is taken as an excuse. In contrast, non-stoichiometry is *the* hallmark of the murburn concept (section 4.1).

10. It is shown in many ways that cellular physiology is governed by observed changes of the coacervate state corresponding to phase transitions [29,38,52,60,69,74,75, 80,105-111]. Adaptable phase boundaries are most obvious at the surface of membrane-less organelles [111] but can also be observed and characterized when one opens a hen's egg [112]. This directly falsifies the '*always one phase principle*' of MPT. Threshold potential in axons also marks a phase transition [108,113], an aspect not accounted for in Hodgkin-Huxley-Katz (HHK) theory, where it is masked by the proposal of the Goldman-Hodgkin cycle. In HHK only a permeability change is taken into account, whereas Heimbarg [113] calculated that propagation of an action potential spike (duration about 1 msec) along an axon involves a moving region with a length between front and rear end of about 10cm, propagating at a (maximal) velocity of about 100m/sec, whereby the membrane is slightly thickened and longitudinally contracted in comparison to the sub-threshold state. Such giant phase transition exhibiting

cooperativity over such large region is spectacular, and not only involves membrane proteins, but the entire protein-lipid-water-oxygen system as a single dynamically coherent system (a main tenet of this review). This demonstrates that not only ion fluxes, but large-scale mechanical aspects are involved too. Such combination of electrical and mechanical effects strongly suggests that *electron currents* coherently linked to propagating *phonons* (vibrations of the molecular structure/lattice) must be the ultimate mechanism responsible for action potential propagation. Ion currents *per se* would not reach a propagation velocity of 100m/sec and electron conduction on its own would be faster. Intimate and precise linkage between electron conduction and phonon conduction, i.e., coherent electron-phonon coupling, is known to reach propagation velocities in the range of 100 m/sec. Such couplings are called '*polarons*'. They can reach loss-less (dispersion-less) propagation, one of the hallmarks of action potentials [114,115].

11. Both cells with and without complete cell membrane can swell and shrink. Colloid osmotic pressure, which is independent from the presence of a membrane, must be the major mechanism, but some other minor mechanisms contribute too. Curves of total osmotic pressure in function of a wide range of external concentrations of different salts do not follow a straight line (particularly at more extreme values, which were not investigated prior to Ling's in dept analysis from 1984 [52]). Moreover, also different cation-anion combinations appear to differ in effect. All this contradicts the '*only van 't Hoff mechanism*' of MPT. Moreover, when each contributing mechanism is quantitatively studied separately and the curves are mathematically summated, the curve of measured total osmotic pressure is matched [52]. These experiments corroborate the coacervate nature of cells in general and the main tenets of Ling's AIH in particular. The data prove that function 4 (maintaining and controlling cell volume) is not primarily a function of cell membrane (MPT), but one of the intracellular cell-matrix.

12. Schrödinger's argument (point 2), particularly its application to the insufficient number of protons in small prokaryotes or mitochondria, suffices to disqualify chemiosmosis [29, 116]. In this and next points some other arguments will be given against chemiosmosis. First, there is no reason to think that ROS are only harmful unwanted side-products of normal metabolism, generating a signal to the cell to take measures when they reach a too high concentration. It is now demonstrated beyond any doubt that at lower

physiological concentrations ROS exert obligatory constitutive central functions in normal metabolism [18-25]. Charts of metabolism (such as the well-known Boehringer maps) will need profound adaptation. Manoj uses the abbreviation '*DROS*' whereby '*D*' stands for '*diffusible*' (to discriminate from bound ROS existing too) – one should indeed remember that it goes about reactants in solution! Formation of DROS is inescapable both thermodynamically and kinetically because cells are a reduced system into which oxygen enters from outside and single-electron reduction of oxygen is very fast and spontaneous ($\Delta G = -250$ kJ/mol) while oxygen is also highly mobile (range of $\sim 10^2$ Å/ μ s). Insofar DROS formation is done already before the much slower inter-molecular deterministic e-transfers across several Angstroms between different redox complexes, as in the hypothesis of a *serial* ETC, could take place. Things happen even before superoxide dismutase and catalase can intervene – the latter only start to intervene when DROS reach a higher concentration and for instance start leaking from mitochondria. The direct observation of very fast DROS formation at every redox complex is in favor of a primarily *parallel system of redox complexes*, with eventual lateral interactions being secondary [117,118]. Note that textbooks present only quite weak arguments in favor of a serial ETC: some redox potential values, and some inhibitor results, which both can be interpreted by the murburn mechanism as well. The serial ETC is a perpetuated fairy tale based on weak grounds, which could survive because before Manoj's discoveries no one saw the obviousness, even inescapability of a radical mechanism such as it occurs in burning wood (500-1000°C) as being feasible on a nano scale in the living organism at body temperature. According to the murburn concept the latter is possible thanks to the huge numbers (trillions and trillions) of extremely small relatively '*mured*' burning chambers with electron *injection* (allowing primary control) combined with direct energy conversion in chemical coupling and electrical and mechanical power output and by way of the high degree of energy recuperation of the emitted electromagnetic radiation in driving indirectly metabolic and electro-mechanical processes at a distance.

13. Ling [95] calculated that in a steady-state non-equilibrium theory (MPT) with a '*single ion electrogenic delocalized pump*' (chemiosmosis) there is a huge violation of the law of electro-neutrality. Since the potential (ψ) over the surface of a sphere with radius $r=10^{-6}$ m (taken slightly too large) and loaded with a charge (Q) of 10^{-3} gram ions or one milli-equivalent, as proposed by Mitchell [119], is given

by $\psi = Q\epsilon/\epsilon_0 r$ with $\epsilon_0 = 1.11 \times 10^{-10}$ coulombs/volt.m and with a dielectric constant for an isolated mitochondrion of about $\epsilon = 100$, this would give $\psi = 10^{-3} \times 0.96 \times 10^5 / 1.11 \times 10^{-10} \times 10^{-2} = 10^{16}$ Volt: definitely impossible, and only the consequence of the electrogenic principle! Nath [120-122] presented a 2-ion model (H^+ with another cation such as K^+ in counter-transport, or H^+ together with an organic acid in symport). Whereas this solves the question of electro-neutrality, it does not take away that of the non-availability of protons [18,123].

14. To allow a chemiosmotic process the 'energetic' membrane needs to be intact. Komai *et al.* [124] experimentally demonstrated that oxidative phosphorylation could be obtained in mitoplasts from which the membrane has been severely disrupted with lyssolecithin (in line with the experiments of point 6). Also, chemiosmotic theory *assumed* that the membrane has very low permeability towards protons (except at the sites of electrogenic proton transport and at the F_0F_1 -ATPase) and to other ions (particularly problematic for K^+ , which is highly soluble in POM-water and in non-POM-water too!), otherwise a proton gradient cannot be built. To guarantee these requirements a high specific electrical resistance of $10^9 \Omega cm^2$ was *assumed*. Hereby the calculation method only considered protons, neglecting the undeniable contribution of the many other ions. Direct micro-electrode measurements on giant mitoplasts, came at a value of 1-6 Ωcm^2 , a value over 10^8 times lower and in the same range as that of other membranes [125,126].

15. Azzone *et al* [127] argued that below a level of medium respiratory rate Mitchell's delocalized gradient would be too weak to even get ATP synthesis started, whereas ATP synthesis is observed at all respiratory rates. Note that this argument does not hold for localized chemiosmosis [128,129]. Mitchell [96] has always been critical to his own model. He did not trust it with respect to complex IV, and he lately admitted that it could not work at all.

16. If cells should be governed by MPT and chemiosmotic principles, they could never have originated by stepwise evolution, since too many necessary membrane constituents should work together with high precision before the cell could start to work according to these principles, particularly in the case of phosphorylating membranes. Though the first cells must have been simple, according to common thinking they would need a phosphorylating membrane to make ATP. How such membrane could have come to existence is still an unsolved question, a real nightmare? In contrast, it was shown recently by Manoj and Jaeken [22] that simple coacervates

resembling Fox microspheres during the early pre-biotic era, long before there were membranes (even existence of primitive enzymes is not a necessity) could already perform all basic *instant* functions of later cells by just physical-chemical means: (1) growth (by accretion/adsorption of new oligomers), (2) physical-chemical budding (without genetic control), (3) extensive accumulation of K^+ by adsorption [130], (4) exclusion of Na^+ and some other solutes through low solvency of the POM-water in the coacervate interior (without pumps; just a thermodynamic equilibrium), (5) execution of a simple circadian Fe^{2+} -UV/ Fe^{3+} driven anabolic/catabolic chemical metabolism, (6) whereby DROS could chemically and *in fluid* phosphorylate adenine, AMP and ADP leading to ATP in an exergonic way according to the *neglected* radical reaction $ADP + Pi + DROS \rightarrow ATP + H_2O + H_2O_2 + \text{heat}$ (the reaction from ADP, Pi and K_2O was performed chemically *in vitro*, and needs low water activity that could be found inside early coacervates!), whereby UV-generated Fe^{3+} during the day could generate DROS from water [22,25], (7) reach some homeostasis through the interconnectedness of all equilibria involved [22,25], (8) exhibit (quantum) coherence and connection with the quantum vacuum [81-84,131] due to the order of POM-water, of K^+ being largely adsorbed and of the interlinked but adaptable structure of the oligomer matrix that operates as a single unit, (9) go through volume changes that influence all chemical and physical-chemical equilibria (cytotonus regulation, i.e. volume-dependent regulation [132]), (10) possess a surface boundary potential without membrane [133], (11) be sensitive to external and internal influences that give rise to often distributed (acting as a single unit) and appropriate reactivity/adaptation [69], and produce an all-coordinating electromagnetic matrix [90,134,135].

The entire working is equilibrium-based and obtains its high-energy long-term meta-stability from physical forces opposing each other quantitatively, i.e., they equilibrate each other. Because of their simplicity, reliability, robustness, and ability to slowly evolve the coacervate structure persisted up to now with its most *basal* level of homeostasis and coherence through the interconnectedness of all equilibria and by the concerted effects of cytotonus regulation, typical of every colloid. Yet, it is an excited system with long-term meta-stable low-entropy and exhibiting several forms of coherence: behavior as a single whole as well as quantum coherence with POM-water connected to the quantum vacuum [74-76,78-84,131]. This insight is so important that it deserves the new denomination '*basal homeostasis*'. Later other more complex

homeostatic mechanisms (allosteric, genetic, epigenetic) were added on top of this '*basal regulatory mechanism*' [20, 23-25].

3. GENERAL REASON TO PROPOSE A 'WET AND MURBURN MEMBRANE MODEL' (WMMM)

Could there still be any doubt that MPT must be urgently abandoned in view of the number and the strength of these undeniable disqualifying arguments (many more, particularly detailed ones, to be found in the appropriate references)? There is now sufficient evidence to replace it by a physiology based on the murburn concept of Manoj [20,23-25] for redox metabolism, the coacervation-coherence properties for its structure build-up [38,109,29] and the concept of life's electromagnetic matrix [134] bringing overall unity in the behavior of organisms. *Together* these principles and properties can produce a valuable alternative because of the individual arguments given above, but particularly because of the last argument 16, namely that cooperation of these principles/properties already worked well during the early pre-biotic era, when pre-biotic coacervates resembling Fox-microspheres entered the stage, i.e. before there were membranes. It can be said that a coacervate or a simple colloid such as a gelatin gel has a volume, but also a surface that stays in contact with the environment. Both volume and surface have POM-water as most abundant compound. When at a later pre-biotic stage membranes came to exist, they were inserted somewhere at the surface of the overall POM-water phase [133]. Their introduction must have separated the overall POM-water phase into an outer so-called 'exclusion zone' (EZ) [42], since then produced by the membrane, and an internal POM-water phase that remained associated with the original coacervate oligomer matrix and by the inner face of the membrane. There must have been limited amounts of POM-water inside the membrane itself to allow exchanges of hydrophilic substances between interior and exterior prior to the existence of specific transporters. Hence, water must be seen as an essential part of a '*wet membrane*' [52] Though the early pre-biotic era was anaerobic, the circadian Fe^{2+} -UV/ Fe^{3+} cycle must have allowed synthesis of DROS from water and DROS in turn allowed the synthesis of oxygen-containing substances of primary biological importance: hydroxy-acids, keto-acids, carboxylic acids and amino acids (detailed reactions found in [22]). DROS got functionally involved later when the first coacervates were formed executing DROS-dependent exergonic chemical reactions including that of *in fluid* ATP synthesis [22]. These

functions remained when membranes originated and then cells. Hereby DR(O)S kept their central metabolic and homeostatic importance up to now at the most basal level of metabolism, because these reactions are thermodynamically inescapable, no other system is faster, and it worked to produce ATP and other important substances. In systems with a delineating membrane and a reducing interior, wherein O_2 enters from outside, DROS production must at least take place in the region of the cell membrane (though this does not exclude other locations). Clearly, a '*Wet and Murburn Membrane Model*' (WMMM) is needed. Water and O_2 /DROS are the forgotten components for understanding membranes.

4. ALTERNATIVES INVOLVED IN THE REPLACEMENT OF MPT

4.1. Murburn Concept

Redox activity is the primary mechanism for energizing cells (photosynthesis, respiration, NADPH from pentose phosphate pathway, ...). It is responsible for the synthesis of life's monomer building blocks and for energy liberation during their breakdown. Together biological organic matter and O_2 can be seen as the poles of a battery spanning 0.8mV from about +0.4mV to -0.4mV redox potential. Some redox pathways power the synthesis of ATP, the secondary energy currency, which in turn is responsible for synthesis of life's macromolecules and its coacervate properties which will be dealt with in section 4.2.

Murburn concept has been consolidated in about 60 articles and reviews in peer-reviewed journals without much arguable opposition (rebuttal to remarks: [21]. Although it still awaits general recognition, theoretical and experimental arguments are very abundant (those not presented here can be found in the references) and suffice to consider murburn concept as a valuable alternative for the falsified classic mechanism of ETC-CRAS. The latter was shown to be untenable on behalf of 30 slashing arguments [18,19,116,123], the most important ones given in sections 2 and 3.

The murburn concept was developed from studying xenobiotic reactions by chloro-peroxidase [17] and thereafter cytochrome-P450 oxidoreductase [137,138]. It was then applied to oxidative phosphorylation [18-21,116-118,139-141], thermogenesis [118] and photosynthesis [142,143]. It explains LDH's oxidative direction from lactate to pyruvate with DROS as electron donor, overcoming an unsurmountable thermodynamic problem if NAD^+ would be the sole electron acceptor as generally *believed* [144]. It

explains how haemoglobin could produce additional ATP necessary to supplement the otherwise unfulfilled energetic demands of erythrocytes [145]. It reinterprets membrane potential and homeostasis [146-149], signal transduction in vision with rhodopsin as a DROS producer and transducin as a DROS-dependent GTP synthase, a '*murzyme*' [149]. It revealed the DROS-dependent mechanism of Na^+/K^+ -ATPase [150], hydraulic bacterial flagella movement by means of inherent water production during murburn processes [151] and some (not all) posttranslational protein modifications [152]. Importantly, it can explain the early pre-biotic steps towards life including early ATP synthesis, and further evolution in small gradual steps, whereas a *supposed* serial ETC working in a chemiosmotic way cannot [22,25].

The first new insight is that in all these life systems the mechanism is essentially a **BURNing** process involving radicals. When O_2 attacks organic matter in bio-systems, as it is the case in an open fire or a combustion engine, similar radical reactions take place in an unrestricted way. They are vigorous, fast and strongly exergonic, and that might be the reason why a burning process was not previously considered as physiologically significant in normal cellular redox metabolism: too hazardous, too wild, too uncontrollable. However, during evolution life developed ways to get some control over this type of reactions governed by the 1-electron's magnetic field of radicals: (i) by making the *burning chambers* very small (nano-sized) and '**MURed**' (rather delineated), for instance in fluid-filled cavities and spongy regions inside an enzyme-complex (redox complexes) or free space between the subunits of multimeric enzymes (receptor-trimeric G-protein complexes, LDH, cytochrome P_{450} oxidoreductase, etc.); (ii) by its dependence on the moment and site of initial 1e^- donation as O_2 *per se* is quite unreactive. As in a combustion engine, ignition of fuel needs a spark; (iv) by regulating a rate-limiting reactant, in this case not oxygen as in a furnace, but protons, the regulation of which is executed in case of coupling membranes by F_0F_1 -ATPase (a new understanding of this protein [140] – combustible compounds that are eventually present (metabolites, ions, toxins, medicinal compounds) in the reaction mixture may influence reaction rate in a hormetic way [20,150] – ; (v) the thermodynamic state of water has a profound effect as demonstrated by the *in vitro* exergonic chemical radical-based ADP phosphorylation reaction (see below) that only proceeds at low water activity, *in vivo* this means in POM-water; (vi) by the development during evolution of a system of redox enzymes (superoxide dismutase,

catalase, etc.) setting upper limits to DROS concentrations, and at a later evolutionary time of DROS-dependent signalling systems that allowed genetic and epigenetic adaptation when needed.

The Second new insight came from demonstrating the *in vitro* and *in fluid* exergonic chemical synthesis of ATP from ADP and P_i in the presence of the DROS-generating compound K_2O (reaction called '*murburn phosphorylation*'):



A detailed set of reactions involved in the radical cascade can be found in reference [123]. There are indications for its occurrence *in vivo* [20,123].

Thirdly, the occurrence of such radical cascade is supported structurally. Docking studies revealed that the well-known ADP-binding sites present on the redox complexes of coupling membranes (and some other proteins) but from which the function remained unknown are located close to the 1-electron redox centres of these complexes in a mured environment (known intra-protein cavities and spongy regions with previously unknown reason of existence) in which O_2 may accept the donated electron generating $\text{O}_2^{\cdot-}$ which initiates the radical cascade interacting with ADP. These ADP-binding sites are unusual in that they bind ADP by its adenine moiety, presenting the terminal phosphate group into the surrounding fluid. Therefor they are called '*ADP presenting sites*' in analogy to '*antigen presenting sites*' to discriminate them from nucleotide binding Walker sites, which can only catalyse ATP hydrolysis. In the case of respiratory complexes 4 ADP binding sites exist on complex I, 2 on complex III and 1 on complex IV [123]. All data (thermodynamic, kinetic, structural and probabilistic) make redox complexes the primary ATP-synthesises. Many other proteins with ADP presented or liberated could be ATP-synthesises (or NTP-synthesises); 2 cases worked out already are haemoglobin [145] and transducin [149]. Each of the 4 redox complexes reacts with O_2 in an independent way; a serial ETC is not required, solving the great mystery of how the process of oxidative and photo-phosphorylation could ever have come into existence. Murburn concept challenges the interpretations and most of the conclusions derived from existing experimental data obtained in numerous experiments with isolated entire mitochondria, mitoplasts and reconstituted systems. One can no longer be sure that observations of ATP production in such systems are due to complex V, which was previously *thought* to be the *exclusive* ATP-production machine of respiration and photosynthesis in prokaryotes and in eukaryote organelles; redox complexes at least may produce

a substantial amount of ATP. Even the famous experiment of Racker and Stoeckenius [153] taken as the most direct evidence for chemiosmosis, needs re-interpretation, particularly when one looks to the experimental details. In this experiment, when F_0F_1 -ATP synthase was not previously provided with the OSCP coupling subunit, not the expected zero ATP, but 18,6% of the ATP output of the OSCP-coupled situation was found. In view of the murburn concept this 18,6% might be attributed to DROS production by the 1-electron donor property of bacteriorhodopsin in the same way as rhodopsin does [149], which leads to murburn phosphorylation of added ADP. The 5 times higher ATP output in the OSCP-coupled situation can be explained upon *assuming* that OSCP-coupling generates a favourable conformation for DROS-dependent phosphorylation of the newly discovered ADP-presenting sites on F_1 driven by DROS produced by bacteriorhodopsin. F_0F_1 -ATP synthase and NKA are good examples of proteins which do not contain a typical redox centre of their own, but are located in a murzone enabling ATP synthesis at high DROS synthesis rate.

A fourth discovery is that with the above description one expects a different kinetics than that of usual enzymes. The latter exhibit stoichiometric binding of reactants to their active centre, leading to a deterministic outcome. In contrast, all aspects of the *out of active centre in fluid* reactivity are stochastic, and the eventual presence of compounds (from cellular origin or from outside (including poisons and medicinal drugs), which can be captured by some radicals, will interfere in a stochastic manner with the process. This leads to: (i) a typical *hormetic* kinetics, whereby at lower concentrations of reactants (μ Molar) or interfering substances the reaction rate increases with increasing concentration, but at high concentrations (mMolar) it decreases again, (ii) and *idiosyncratic* reactivity whereby effects can be individually different [20,154]. This not previously described kinetics constitutes a strong diagnostic tool for identifying murburn processes. Experimental data which clearly fit these kinetics are abundant in literature. Many studies of the Manoj group give striking examples of this [20,150]. Many poisons among which DNP [155], valinomycin [156], rotenone, [157] and medicinal compounds such as metformin [158] and others, well-known inhibitors of respiration at mMolar concentrations, are activators at μ Molar concentrations. Explanations based on adaptation are not denied, but such mechanism would need time to get induced and therefore cannot explain the many cases where effects are instant, a characteristic of murburn

processes. For instance, direct intervention of cyanide in the radical mix is the *only* explanation that can explain the ultra-fast fatal effect at such low dose, a very strong argument in favour of the murburn concept [141]. As to Eukaryotic cell membranes the usually inhibitory influences of trans-dermal peptide, cardiotonic steroids, lithium ion, volatile anaesthetics, dinitrophenolics, oleate/oleic acid, arginine, oximes, and N-/O-heteroatom alkyl substituted compounds upon NKA activity also follow a non-stoichiometric, fast and hormetic kinetics pointing to a murburn mechanism [150].

Manoj's discovery of the murburn mechanism entails that classification of bio-catalysts must now be rearranged into 2 main groups: (1) *murzymes* promoting murburn external *in fluid* chemical conversions with a stochastic, hormetic and idiosyncratic kinetics, and (2) *enzymes with an active centre* follow the better-known deterministic and stoichiometric kinetics.

A fifth insight is that formation of $O_2^{\bullet-}$ and of secondary radical and non-radical anions (for instance OH^-) produced at one surface of a membrane due to the specific location of the involved redox centre donating a single electron to O_2 results in '*effective charge separation*' (ECS) characterized by membrane hyperpolarisation at that side. This kind of potential is essentially an electrode potential located at that membrane surface. The hyperpolarizing effect due to the synthesis of radical and non-radical anions was experimentally indicated by measuring the resting potential of quail eggs under air in comparison to being held under N_2 whereby the potential difference decreased by two thirds [149]. It goes about unfertilized eggs (R-state) and since maintaining resting potential does not consume energy (section 2, point 6), the role of O_2 cannot be due to incapacitated mitochondria when kept under N_2 . It can be concluded that similarly to mitochondrial inner membranes and prokaryotic cell membranes, also the resting potential of eukaryotic cell membrane is generated primarily by redox activity. NAD(P)H oxidases are indicated as DROS production sites, but other redox enzymes can be involved too. The redox-driven hyperpolarization potential (slightly lower than resting potential) is installed very fast following DROS production (as DROS reactions are very fast) and might be subsequently modified slightly reaching resting potential after some time interval by means of other and slower equilibrating mechanisms. For instance, this may happen via neutralisation of some anionic radicals by protons (in mitochondria and chloroplasts) or by intracellular K^+ (forming K_2O at the inner face of the eukaryotic cell membrane), or

by other effects. Insofar cell membranes of prokaryotes, mitochondrial inner membranes and thylacoid membranes on the one hand and eukaryotic cell membranes on the other hand are not so fundamentally different than was previously thought. In eukaryote cell membranes redox enzymes (NAD(P)H oxidases or others) must be seen as the primary and overlooked protein components that establish resting potential. Also, in both cases no electrogenic ion pumping (an endergonic step) is involved; instead, all reactions are exergonic and fast, hence inevitable. Similarities also include some other membranes: rod cell disc-membranes [149], ER-membranes with P₄₅₀ oxidoreductase in hepatocytes [138], SR-membranes with NADH oxidase [159], and other membrane types may follow.

A sixth aspect of DROS metabolism according to murburn concept is that the involved reactions emit high frequency radiation in the IR, VIS and UV region, the energy of which can be recuperated by resonance bringing about excitation of other components in cells and tissues leading to overall coordination, i.e., to achieve that living organisms work as a single whole [25] (see also section 4.3).

It can be concluded that the murburn concept is the only valuable alternative for the untenable ETC-CRAS model. After over 60 years of endless discussion (often marginalized) about non-solved anomalies in the mechanism of oxidative and photophosphorylation a non-chemiosmotic mechanism for membrane functionality which does not require ion pumping is finally revealed in all its simplicity, robustness, and the ease to come into existence and to further develop through numerous very gradual evolutionary steps.

4.2. Association-Induction Hypothesis

Ling's 'association-induction hypothesis' (AIH) deals with the directly observed coacervate nature of living organisms, which is required to achieve coherence [38,52,57,60]. The term 'association' stands for ensembles of one or more macromolecules with intimately associated POM-water that can be depolarized, and with adsorbed ions (particularly K⁺ in the R-state) from which the selectivity can be switched (for instance when A-state is initiated). The R-state is a long-term meta-stable high-energy low-entropy excited coherent state acting as a single whole. The high-energy is stored in the form of the low-entropy of POM-water and of adsorbed K⁺. The A-state is initiated by a small amount of activation-energy delivered by the binding of a stimulus external to the system allowing it to leave the R-state's high-energy basin, so that stored high entropy can be converted into useful

work. It starts with the hydrolysis of bound ATP and goes along with POM-water depolarization and K⁺ desorption and after power output terminating in a lower-energy higher-entropy de-cohered action-end state. Subsequent reinstalling of the R-state needs input of the high adsorption energy (not hydrolysis energy!) of the binding of ATP(NTP) to the protein system to overcome an energy threshold and to reach again the high energy R-state that exhibits static coherence (long-lived, at rest, no energy consumption once installed). Examples of cellular systems in the R-state are relaxed muscle cells, below-threshold membrane parts at resting potential or generator potential, dormant cysts and spores, unfertilized eggs, inactive protein systems and cascades, B-DNA, etc. As stated, using non-linear electrodynamics Fröhlich [79] derived that in such (R-state) systems all components, including the many water molecules, vibrate at a common frequency, and this was later experimentally corroborated [74-77].

The term '*induction*' stands for Lewis-induction, which is seen as the mechanism that brings about changes from R-to-A and A-to-R [71]. '*Cardinal adsorbents*' is the term used by Ling to indicate the regulatory ligands that adsorb to such systems at a '*cardinal adsorption site*'. In doing so they generate the necessary Lewis-inductive energy that flows through the molecular system and reaches different '*regular adsorption sites*', where some physiological output is brought about through phase transitions. For instance: water depolarization liberates binding sites for adsorption of another macromolecule (for instance actin to myosin, some IDP's to DNA/RNA) or for similar macromolecules (actin and tubulin polymerization) or for another part of the macromolecule's own structure as in the case of unfolded polypeptide stretches folding to an α -helix conformation in IDP's with dynamical α -helices, or desorption of K⁺ allowing the binding of another ion (Na⁺, H⁺, or exchange of phospholipid-bound Mg²⁺ for Ca²⁺ during rest-to-action phase transition of membrane regions) or desorption of mineral ions in exchange for binding a macromolecule (desorption of K⁺ from myosin heads in exchange of actin binding). Ling's view is that, when one wants to understand physiological power output, one must '*follow the electrons*' (involved in Lewis inductive shifts), a view also adopted by Manoj (but then concerning more strong induction leading to electron transfer). This electron-centered view is adopted in this review.

Hereby a long-standing misconception in biochemistry must be halted, namely the idea that energy is injected into an ATP-dependent system by means of ATP hydrolysis. This

would imply that the initial stages of strong actin-myosin binding would be more energetic than dissociated actin and myosin filaments in the relaxed state. Yet, to dissociate actomyosin at the end-action state (comparable to *rigor mortis*) ATP must be added [73]. ATP adsorbs; the adsorption energy lifts the system in its long-term meta-stable high-energy low-entropy excited coherent R-state, but bound ATP is not immediately hydrolyzed [72]. Indeed, the free energy of ATP adsorption upon actomyosin is -39,7 kJ/mol [160] with an equilibrium adsorption constant of 9.84×10^6 [72], and it is used to bring again order after reaching the action-end state. Stimulating cardinal adsorbents such as Ca^{2+} deliver the opposite inductive energy than ATP/NTP converting the energy stored in the R-state into power output which starts with hydrolysis of bound ATP, a reaction which in many cases is near equilibrium.

Ling [60,71,] argues that the backbone polypeptide chain of proteins behaves as an '*inductive highway*'. The '*Yang and Ling cooperative adsorption isotherm*' (see section 5.1) is a potent diagnostic tool to calculate the energetic interaction between cooperatively-linked binding sites of a given type on a single molecule or on larger complexes, for instance between adjacent peptide groups on a polypeptide, oxygen-binding sites on hemoglobin, propagated pulses along microfilaments and microtubules and propagation of action potentials along membranes.

The Lewis-inductive effect generated by binding of the cardinal adsorbent ATP to ATP-binding proteins in many cases leads to (i) preferential binding of K^+ on protein carboxyl-groups and (ii) to POM-water formation on water-exposed peptide backbone groups (often following unfolding of dynamic α -helices or exposure of backbone polypeptide stretches by other means, for instance due to dissociation of a macromolecule bound to it). Activating stimuli such as Ca^{2+} usually elicit the opposite inductive effect leading to folding of water-exposed polypeptide stretches and changes in binding affinity of protein carboxyl groups (for instance: $\text{myosin} \cdot \text{xK}^+ + \text{actin} \rightarrow \text{actomyosin} + \text{xK}^+$). Yet, Ling [52] warns that this rule is not absolute, since transfer of inductive effect through macromolecules can be complex, particularly in the case of membrane proteins, which stay in contact with two different fluids to which they react differently (see section 5.2.a).

Under the influence of MPT K^+ is not generally considered as an important allosteric regulator [161], since its activity is considered to be always high. Ling [162] however argued that, since K^+ activity is very different between R-state (very

low) and A-state (high), K^+ becomes a non-appreciated, yet primary allosteric modulator. Some enzyme systems are completely inactive at the low K^+ activity of the R-state: glycolysis, respiration, protein synthesis, etc., in agreement with halted metabolic activity in cells which are entirely at rest such as *Artemia* cysts [70]. In contrast, onset of the A-state liberates bound K^+ . This automatically leads to activation of those metabolic processes. Together with ADP and P_i liberated during the A-state and high Ca^{2+} activity this leads to synthesis of ATP which immediately adsorbs to nearby systems occurring in action-end state, and this restores the R-state. In this way K^+ activity regulates the (local) ON/OFF state of many central systems of metabolism.

Ling's AIH is experimentally corroborated mainly by studies on muscle cells, axons, eggs and epithelia [52]. It is extended by Pollack [69] in his '*gel-sol hypothesis*' (GSH) in which processes not dealt with in the AIH are detailed: amoeboid movement, ciliary beating, mitotic spindle behaviour, cell division, and the mechanism of secretion. Pollack made an in dept study of the properties of simpler synthetic colloids. From this he adopted the terms 'gel' and 'sol'. It is important to realize that Ling's concept of R-state and A-state is not directly related to Pollack's gel-state and sol-state. R- and A-states can exist at different hierarchic levels of organisation, but this was not yet understood in the review of Jaeken [163], which despite a wrong correlation between the AIH and Pollack's GSH, achieved the unification of Ling's AIH with the physical derivations and theories mentioned in section 2, point 7. Correction of the mistake was made later [29] by means of the insight formulated in his '*hierarchical hypothesis of R- and A-states*'. The latter hypothesis is crucial to understand the bioenergetics of different states of cell systems including some pathological ones as it explains why ultimately degenerated sclerotic states can be produced. A brief example: F-actin is the A-state with respect to G-actin but presents as an R-state with respect to the contraction process. In terms of Pollack's GSH G-actin is a sol state (emulsion) with the highest amount of POM-water bound to actin (hence the lowest entropy), F-actin is a weak gel-state with less POM-water bound (more entropy), and actomyosin is the final strong gel-state (with still higher entropy, alike to *rigor mortis*). Membranes can also go through phase transitions taking place between their '*liquid state*' (R-state) and a '*quasi-solid*' or better '*glassy state*', also denominated as gel-state (A-state). The connotations of the AIH remain applicable at every separate level, giving insight into the energetic aspects, each

R-state being a higher energy state relative to its corresponding A-state due to adsorption of a larger amount of POM-water; but A-states are not per definition free of POM-water [43,44].

Some molecular structures of membranes are particularly important in producing POM-water during the membrane's R-state (sub-threshold state in case of excitable membranes), but this does not imply that the A-state is completely free of POM-water. For instance, Kabir *et al.* [44] found that F-actin is partially associated with POM-water and partially with hyper-fluid water (HF-water). This finding marks an important shift in thinking relative to the understanding of that of Ling, who sees the local state of water in a region in the A-state as (averagely) de-polarized, which might give the impression that it conforms to bulk water. It is more complex. It may be a mixture of some remaining POM-water, some amounts of HF-water, eventually some clathrate water at more hydrophobic surfaces in contact with water. Even a globular protein such as lysozyme appears to be covered by different forms of water [164]. That quite large amounts of POM-water remain during the A-state is demonstrated specifically for the case of F-actin [44] and as the overall average for an active cell by the NMR study of Wheatley *et al.* on HeLa cells [43]. Yet, the entropy of this complex mixture during the A-state is higher than during the corresponding R-state [52].

Membranes in R-state (for excitable membranes this means below threshold potential) are bordered by POM-water on both sides with some POM-water within that connects the two sides [29,45,52,166,167]. Similarly to the situation in cytoplasm (near F-actin and perhaps elsewhere, membrane regions in A-state may locally contain HF-water [29] (see also section 5.2.d). It is proposed here that the local fixed charge and partial charge distribution of the involved macromolecular surfaces is crucial. Several membrane molecular structures are able to produce highly excited POM-water, for instance water-exposed random coil loops of membrane proteins on both sides of the membrane, phospholipid phosphate groups, which are at a correct distance from each other in their Mg-bound R-state, a distance which disappears by exchange of bound Mg^{2+} in favour of Ca^{2+} upon increasing Ca^{2+} activity (A-state), peptide groups of sphingolipids and of N-acetylated glycosyl groups of glycolipids and glycoproteins, the glycosyl-structure properly and eventually sulphate groups of certain glycosyl chains. Exposed hydrophobic parts will polarize water in the form of less excited clathrate water, and hydrophilic surfaces with an incorrect (partial) charge distribution (depending on the degree of mismatch) will de-structure water clusters

eventually down to a cluster size of possibly only a few water molecules producing HF-water. Because it is unknown if this de-structuring goes down to water-monomers, i.e., if super-fluid water exists in cells, the term 'hyper-fluid' water is more appropriate. The whole issue of water structure near bio-molecular structures discloses a high need to study (partial) charge distribution of macromolecular surfaces by developing some facile computer models for surface charge analysis. For *ab initio simulation* programs developers may consider Kanarev's new and simple Newtonian(!), non-probabilistic(!) atomic theory that originated after he discovered some fatal flaws in existing orbital theory [168].

4.3. A new bioenergetic replacing MPT's steady state non-equilibrium

Although murburn concept deals with 1-electron transfers, in a broader perspective the Manoj-group acknowledges the importance of coacervation, and much effort is taken to study the reciprocal relationships between the structure of water and O_2 /ROS chemistry [22]. Combining a murburn approach with coacervation allowed to propose a new model of life's bioenergetic based on 3 interdependent and collaborating principles [25] (figure 2 and 3):

(1) Murburn concept describes the primary energizing principle dealing with redox metabolism. Its roots go back to the earliest pre-biotic era in the form of radical mechanisms which stay at the origin of the synthesis of oxygen-containing organic precursors for life: amino-, hydroxy-, keto- and carboxylic acids, and later of murburn phosphorylation of adenosine, AMP and ADP (which needs low water activity, POM-water). The mechanism does not involve ion-pumping, but some present-day systems are membrane-linked/located [22,25].

(2) The coacervate-coherence properties of life are based on an interconnected lacework of biomolecules (coacervate, and membranes) able to produce excited and coherent POM-water, which is able to extract energy from the quantum vacuum [81-84,131] for maintaining its meta-stable high-energy low-entropy excited coherent state (R-state) over prolonged periods of time without metabolic energy input (if not disturbed). When disturbed through an appropriate stimulus, its low-entropy can be transformed into physiological power output, but this may happen in conjunction with a direct energy input from redox (murburn) activity turned ON during the A-state (example: NKA described in section 5.2.a). Since coacervation-dependent processes need ATP (NTP) adsorption to recover their R-state after power output, an indirect input

of energy comes from murburn redox activity used to produce the necessary ATP during the A-state.

(3) Emission of non-coherent high-quality photons (near-IR, VIS, UV) from stochastic murburn reactions are absorbed by surrounding coherent POM-water and re-emitted from POM-water as coherent photons, called '*biophotons*'. The latter are defined as measurable photons produced by living organism, which are no longer produced at death [86-89]. In turn these biophotons are resonantly captured by other cell systems exerting a coordinating function (cell-matrix, cytoskeleton including centrioles, membranes, DNA, nucleosomes, extracellular matrix, enzymes, etc.) which in this way reach an excited state that allows specific RRM-based (quantum) functioning. Supra-molecular and supra-cellular structures can get excited by longer wavelengths and emit a characteristic EM-field. Maxwell's law implies that the electric field of moving charges (charges are everywhere in cells and everything moves!) generates an accompanying magnetic field, together producing an EM-field. Biological EM-frequencies cover the entire range from photonic to micrometer, millimeter, ELF waves and longer), a range spanning about 75 octaves [46]. This EM-field accompanying life was called the '*electromagnetic matrix*' (EM-matrix) by Szent-Györgyi [134]. In healthy organisms it was shown to be

a quite coherent field [76,87] orchestrating all life processes, its metabolism, structure, morphogenesis, development, mental and emotional issues [46]. It makes living organisms behave as a single unit. High frequencies act on short distances at the molecular level within cells, lower frequencies at the tissue, organ and entire body level. Disturbance of this EM-matrix can lead to degenerative diseases. In tumor cells and tissues the EM-matrix has lost much of its coherences and produces its own distinct frequencies [76,77]. Importantly, observable EM-matrix alterations precede oncological mutations [169].

5. REINTERPRETATION OF THE CONTESTED FUNCTIONALITIES 1 TO 4

The arguments given in section 4.1 in favour of the murburn concept together with the arguments given in section 2 against MPT and chemiosmosis (and many more in the references) suffice to replace the MPT-based mechanisms of respiration and photosynthesis including oxidative and photophosphorylation, which were indicated in the introduction as the 5th functionality of membrane phenomena in need of reinterpretation. Remains to discuss the other 4 functionalities.

5.1. Ion and solute distribution

This functionality is already sufficiently discussed in previous

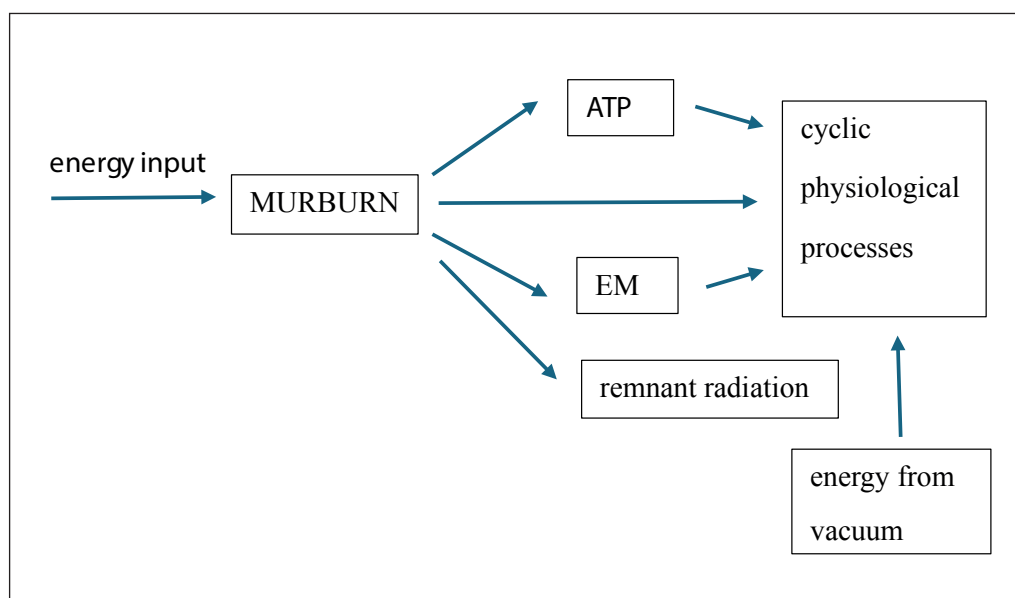


Figure 2. General energy diagram as proposed by Jaeken and Manoj [25]. Primary energy input by murburn mechanisms and glycolysis. Dual secondary energizing system through ATP adsorption and direct resonant EM energy transfer (as proposed by Cilento [90]). Besides these flows there is also resonant energy input from the quantum vacuum (or aether EM-field) involved in keeping low-entropy of the long-term meta-stable high-energy excited coherent R-state, as proposed by the group of Del Giudice [81-84].

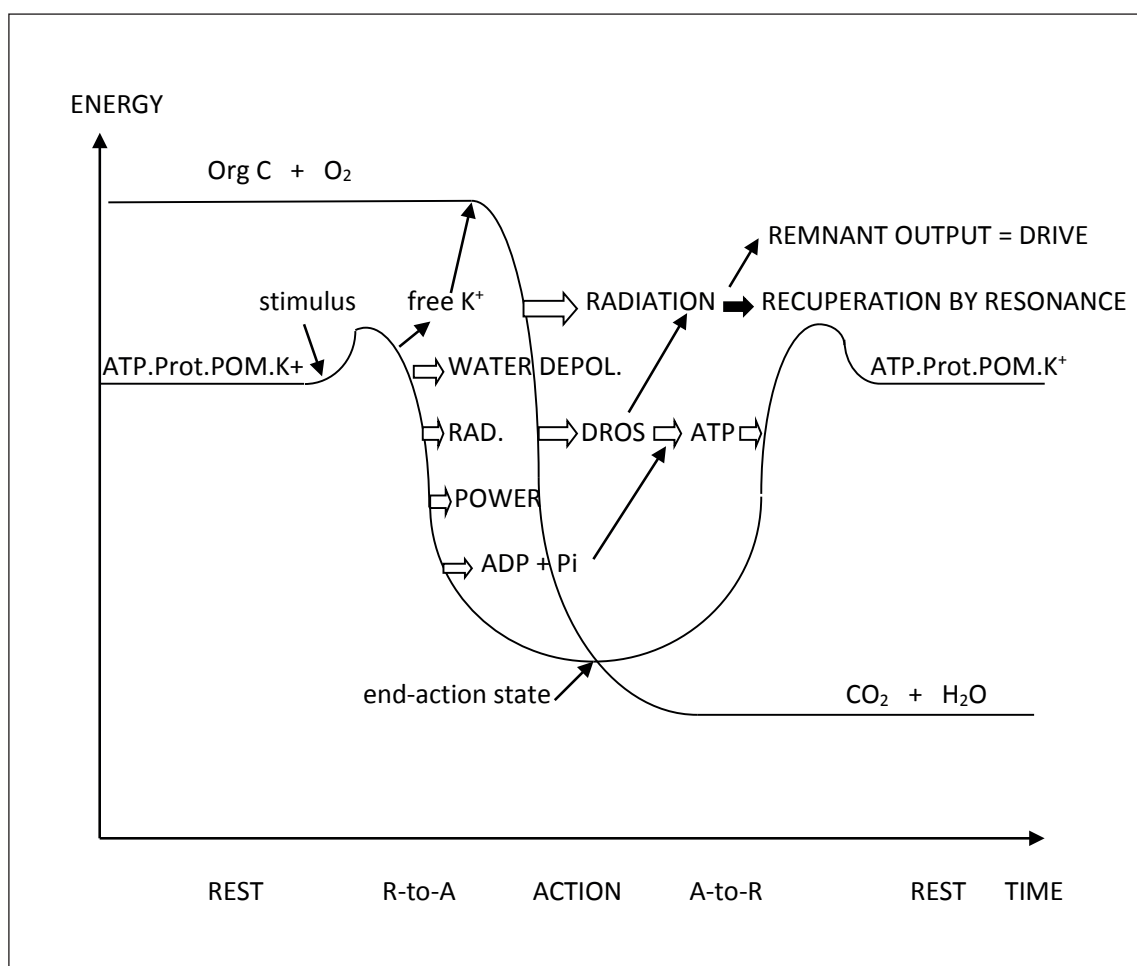


Figure 3. Cyclic processes such as muscle relaxation-contraction-relaxation or rest-action-rest potential are iso-entropic. They need energy input (often murburn activity) to achieve recovery of the resting state.

sections. Here only some important equations will be given. Remark that MPT even does not have an equation describing ion and solute distribution. It also does not give a mechanism of how internal and external concentrations are measured, which is an absolute requisite for any regulatory system to allow control of these concentrations. Clearly, control and homeostasis of the composition of the internal solution as a primary function of ion pumps and their associated coupled transport systems cannot be true. Ion and solute distribution are determined by a cytoplasmic mechanism (at the thermodynamic level), with the membrane being only important for the fluxes (kinetics) through the cell's surface, which are adapted to those of cytoplasm.

Troshin [12] and Ling [71] each developed a 2-term equation for ion and solute distribution consisting of a term dealing with the fraction in solution plus a term for the adsorbed fraction. In the Troshin equation, which is the basic

one, the second term is given by the Langmuir adsorption isotherm.

Troshin equation: $C_c = C_s K [1 + A_\infty / (C_s K + a)]$ (eq.1) with C_c = concentration of the solute in the coacervate; C_s = concentration of the solute dissolved in the water of the coacervate, K = the coefficient of proportionality characteristic of the aqueous phase of the coacervate as a solvent, A = the amount of adsorbed solute, A_∞ = the limit of adsorption, and a = a constant.

Similarly, in Ling's equation the internal concentration of solute $[p_i]_{in}$ (suffix $i = i^{th}$ solute) is given by the sum of the concentration of the solute dissolved within cell water (subscript 'cw') plus the concentration of the adsorbed fraction (subscript 'ad'), with external concentration $[p_i]_{ex}$ given in mmoles/liter of water in the external medium, with α being the water content in liters/kilogram fresh cells, and q_i as the equilibrium distribution coefficient of the i^{th} solute in cell

water, whereby it is understood that cell water may not be uniform in its solvent properties (which is the case) and that the parameters $[p_i]$ and q_i are hence weighted averages. In the absence of adsorption:

$$[p_i]_{cw} = \alpha q_i [p_i]_{ex} \quad (\text{eq.2})$$

In contrast to the Troshin equation Yang and Ling [71] worked out the second term for the many biologically relevant cases whereby auto- or hetero-cooperativity is involved between adjacent adsorption sites for the same solute, and which are expressed in the *Yang and Ling cooperative adsorption isotherm*. In case of non-cooperativity this term reduces to the Langmuir adsorption isotherm used by Troshin. In the development of the Yang and Ling isotherm also the possibility that 2 solutes (i and j) competing for the same adsorption sites is taken into account, whereby the ratio of $[p_i]/[p_j]$ is more important than their absolute concentrations, a crucial aspect which is not always considered when experiments are designed, though ratios are the crux of physiology. The product ξ^{ij} of this ratio $[p_i]/[p_j]$ with the equilibrium constant of exchange of j for i , ($K^{00}_{j \rightarrow i}$), is given by the term

$$\xi^{ij} = [p_i]/[p_j] \cdot K^{00}_{j \rightarrow i} \quad (\text{eq.3})$$

A further interesting aspect is that the Yang and Ling cooperative adsorption isotherm can use experimental adsorption data for the calculation of the Lewis-inductive energy transfer between cooperatively linked nearest neighbor adsorption sites. Since the dimensional content of the concept of energy contains time, the isotherm presents not only a state but tells something about the process. The nearest-neighbor-interaction energy with one neighbor is given as $-\gamma/2$. The 2 in the denominator arises from the fact that in a series of adsorption sites there are often 2 neighbors, one behind, one in front. $-\gamma/2$ is given by:

$$-\gamma/2 = \tau(-\Delta F^{00}_{j \rightarrow i}) \quad (\text{eq.4})$$

with τ = transmissivity constant of Lewis induction and $\Delta F^{00}_{j \rightarrow i}$ = free energy of exchange adsorption (i replacing j).

The general equation for the adsorbed solute $[p_i]_{ad}$ in competition with p_j with $[f]$ as the concentration of adsorption sites, all expressed in mmoles/kg fresh cells, is:

$$[p_i]_{ad} = ([f]/2) / [1 + (\xi^{ij} - 1) / [(\xi^{ij} - 1)^2 + 4 \xi^{ij} \exp(\gamma^{ij}/RT)]^{1/2}] \quad (\text{eq.5})$$

The equation is broader than the calculation method for the Hill coefficient (used for auto-cooperativity of oxygen binding in hemoglobin) and includes the possibility to calculate that coefficient. Ling [52] presents many data, including *in vivo* measurements, illustrating the strength of the new

equation. Thoke *et al.* [170] studied the ATP handling enzymes hexokinase and pyruvate kinase in a crowded environment by adding different amounts of water polarizing polyethylene glycol and found that under crowded conditions the above equation better fits the experimental data than Michaelis-Menten or Monod-Wyman-Changeux models based on mass action. The latter are successful for diluted situations. The above equation also allowed the authors to describe the obtained curves of glycolytic oscillations in yeasts. Said once more: in contrast to this important work, MPT does not even have an equation describing solute distribution.

5.2. Trans membrane transport

a. NKA

The very fact that theories will be applied which contradict the idea of ion-pumps might upset many scientists. They believe that there are too many data ascertaining their existence. Yet consider that the proteins called ‘*pumps*’ according to MPT exist indeed but work in a different way than generally *believed*. Several data *thought* to support ion pumping and underpinning the Post and Alberts model for NKA [102,171-174] remain, some need adjustment (for instance the stoichiometry), some ideas (not data) must just be discarded, for instance, MPT’s strict idea of ‘*valves that open and close*’, and many additional data that are generally ignored, will and must be addressed to reach a better understanding of how these proteins work. The effects of O_2 and DROS, of the adaptable structure of water, and adsorption processes must be accounted for. These are effects of central importance about which MPT theorization and the Post and Alberts model remain completely silent. However, also consider that the Post-Alberts model is much more flexible than generally explained, details which are also omitted here. For instance, Post and Klodos [175] discuss experimental data that point towards phase behaviour. Yet, an attempt is undertaken to incorporate at least the most important missing data into a new model of what might be the basic working mechanism of NKA.

The MPT view about NKA (and channels) is characterized by a quite mechanical idea of ‘*valves that open and close*’. But that is not the way how proteins function. Arguments are the following (Manoj *et al.* [147,148]).

(i) If pumps or channels conduct $\sim 10^6$ – 10^7 ions/s by literally opening and closing a gate each time that an ion passes, the protein would need to move parts at supra-MHz speeds with atomic precision, which is impossible for a flexible, breathing macromolecule in a viscous environment of water/lipid at

300 K. They would need to cycle a million times per second, much faster than any known protein conformational change ($\sim 10^3\text{--}10^5\text{ s}^{-1}$), whereas according to textbook information (Alberts *et al.*, 1989, p. 305 [102]) NKA is known to hydrolyze only about 100 ATP/sec.

(ii) Cryo-EM and X-ray structures show something that resembles ‘open’ and ‘closed’ states, but these are but *static averages*, and what is visualized are not perfectly discrete conformations but rather a continuum of states [176]. MD simulations consistently reveal that ions and water molecules are in constant motion within these protein ‘pores’ whereby ions are not handed from one specific site to the next in a mechanical ratchet but are diffusing through a dynamic, hydrated pore whose energy landscape is shaped by the protein [177]. This ‘wet and fluid’ picture fits with the murburn concept of stochastic ion movements governed by local phase-dependent (almost non-calculable) electrochemical gradients, whereby function emerges from shifting local equilibria, a central tenet of the murburn concept that challenges MPT’s determinism.

(iii) The idea of a ‘gate’ assumes sequential, clocklike action, whereas experiments show noisy, fluctuating currents, consistent with probabilistic assisted permeation rather than rigid valve-like gating.

(iv) The kinetics of many diverse pharmacological blockers and modulators acting upon NKA was shown not to be stoichiometric (as should be expected from a deterministic open-close mechanism – they don’t act like ‘stoppers in a valve’ – but their stochastic and in particular hormetic kinetics indicate that they alter local electrostatics, hydration, solvency and redox equilibria, as advocated by murburn concept [24].

(v) Classical gating models struggle to explain why mutations far from the pore change conductance or kinetics; but if the protein just modulates a diffusive ionic/electrostatic pathway, this makes sense.

Following this less idealized more realistic way of functioning of NKA (and channels) a model will be proposed in which the number of ions transported will not be indicated with 3Na^+ and 2K^+ but as $x\text{Na}^+$ and $y\text{K}^+$ whereby x and y are fluctuating decimal numbers. Also, there is more than one sites to which ATP can bind, which can lead to different effects that likely are not fully deterministic and whereby stochastic effects due to the availability of ATP may play a role.

In addition, the activities in which ATP (and ADP) react are usually executed in complex with Mg ion. It is known that the isotopic mixture on earth consists of 79% ^{24}Mg ,

10% ^{25}Mg and 11% ^{26}Mg , from which only the $^{25}\text{Mg}^+$ has net magnetic spin with a magnetic moment of +0.85 Bohr magneton making it more reactive than the other isotopes [178–181]. These studies evoke many questions. Are cells or certain cellular processes isotope-specific in general? Or does the mechanism only work better with a 10% chance of catching a $^{25}\text{Mg}^+$ ion? Do some NTPases (and enzymes generating ATP: creatine phosphorylation, glycolytic ATP synthesis) work with a radical mechanism (redox complexes) and others with an ionic mechanism? Or, do they possess a dual mechanism (as ATP-synthase/ATPase does): ionic and magnetic? Or do some magnetic Effects from the EM-matrix specifically guide $^{25}\text{Mg}^+$ to some systems, and perhaps not to others? Fact is that some reactions are just too fast to be based on an ionic mechanism: microtubule polymerization, DNA and RNA polymerization, codon-anticodon recognition [179]. DROS reactions taking place at redox complexes are extremely fast too. Is there a preference for a particular Mg-isotope? The authors find indications that ATPases may possess a dual reaction mechanism, a more usual and a radical mechanism based on magnetic force. The arguments in favor of a murburn DROS-dependent mechanism anyhow are magnetic.

Another issue is that pumps and channels are redox-active proteins. They occur in a murzone where 1-electron donors (NADPH oxidase) and O_2 inevitably produce DROS [150]. Cell membrane does possess redox activity. And, though NKA (and channels) do not possess redox centres containing transition metals, they possess Cys, Met, Tyr and Trp residues that can bring about electron transfers (expected to occur upon passing threshold), while regions rich in Arg are already understood as being redox sensors. Fluorescence and EPR spectroscopic studies have detected changes in the electronic state of Trp and Tyr residues during its reaction cycle [182]. In many redox active membrane proteins such residues exert key roles in redox catalysis, some understood as ‘ROS protection’, or is it obligatory ‘ROS involvement’?. It is well-known that NKA’s activity is regulated by the local redox state [183]. These authors remark: “*Na,K-ATPase activity is a complex process that cannot be explained only by oxidative damage to the protein. Na,K-ATPase activity is maximal at physiological oxygen concentrations and decreases by both hypoxia and hyperoxia, as well as due to decrease or increase of intracellular glutathione concentrations*”. At present there are no indications that NKA possesses ADP-presenting sites, but during NKA’s catalytic cycle ADP and Pi are liberated near the inner face of the membrane where DROS is produced. Hence, local murburn

phosphorylation cannot be excluded. The possibility that NKA is a redox enzyme will be further explored in section 5.3.

NKA (and channels) also act by means of phase transitions, as proposed in the AIH. Arguments were given already for the following statements: (1) in membrane regions below threshold (R-state) NKA must be inactive (the only possible conclusion from EMOC experiments), (2) below threshold the membrane is lined by POM-water on both sides and staying in continuity with some POM-water inside, (3) POM-water has good solvency for K^+ , but not for Na^+ and H^+ , i.e., POM-water constitutes the real permeability barrier for the latter ions and many other solutes, (4) comparable to other ATPases NKA works by means of phase transitions when threshold is surpassed, leading to temporary NKA activation for R-to-A, inactivation for A-to-R; this phase behaviour which is also important to understand action potentials, (5) given these phase transitions, although A-to-R must be metabolically energized, ion transports *properly* are always passive in both directions as they depend on physical equilibria (changes in solvency and adsorption), (6) ATP and protons act as cardinal adsorbents, which by means of Lewis induction (assisted by mechanical lattice vibrations or phonons) generate the phase transitions of respectively R-to-A and A-to-R, (7) upon surpassing threshold water de-polarisation/de-structuring (R-to-A) and re-polarisation (A-to-R) may be expected, and because NKA is a trans-membrane protein the front of change is expected to proceed in a peculiar direction with respect to membrane surfaces (detailed further), (8) the necessary protein and lipid surface structures for water polarisation are abundantly present on both sides (listed before, but NKA's outside-located β -subunit rich in exposed backbone peptide groups may require special attention), (9) NKA functionally interacts with associated membrane lipids, which undergo a collective phase transition from sub-threshold non-bilayer lipid (with associated POM-water) to the above-threshold bilayer structure, whereby also redox interactions are involved [150], (10) for trans-membrane proteins such as NKA and channels effects of Lewis induction due to the binding of cardinal adsorbents may differ from AIH's more common effects as described in section 4.2, because trans-membrane proteins stay in contact with different fluids on both sides of the membrane.

Any model for the mechanism of NKA must integrate the data about its redox activity and its phase-dependent properties (point 2). Omitting them will lead *de facto* to an incomplete model. Integrating them, as will be done below, should be considered an attempt to come to a better understanding

of this system within its milieu consisting of water, lipids, other proteins, electron donors, O_2 and DROS. A tempting model for NKA's working mechanism is proposed, whereby something of two major but flexible conformations remain part of the model (say E_1 and E_2), yet the precise number of adsorbed K^+ and Na^+ is not fixed [103, 151] in agreement with (i) observations of the non-deterministic (stochastic) and non-stoichiometric interactions between NKA's ATP-cycle and Na^+ and K^+ transport, and (ii) ion transport being semi-conductive comprising also non-occupied holes, the number of which is non-determined [107, 184, 185], (iii) solubility properties (also stochastic) of these ions in different states of water together with the redox state of the protein and changes of the overall electromagnetic field are involved (iv) adsorption opposes diffusion implying that the stochastic nature of diffusion is part of the adsorption equilibrium. For these reasons the number of bound Na^+ ions will be indicated here with the coefficient 'x' for Na^+ and 'y' for K^+ which can be decimal numbers.

It is conjectured that the main cardinal adsorbents, ATP and H^+ , affect and change the preferential binding for either Na^+ or K^+ on appropriate cation binding sites, but also influence the structure of water in a different way on the two sides of the membrane. All these data are captured in a model with 6 main states (figure 4: first state on top left).

1. POM-water is proposed to be present at the external membrane surface and non-polarized water at the cytoplasmic side from the moment on that ATP binds to end-A-state NKA (*de-phosphorylated* $E_2.xK^+.H^+$ conformation) producing the high-energy $E_1.ATP.xK^+.H^+$ conformation. Typical for membrane transporters might be that formation of POM-water at the outer membrane surface is not synchronized with its formation at the inner surface. The consequence of this is that the R-state may not consist of a single conformation. The common aspect of the R-state conformations 1, 2 and 3 consists of the protein's E_1 conformation, supposed to be linked to POM-water association at the outer membrane surface. It will be proposed that POM-water formation at the inner membrane side might be linked to the phosphorylated enzyme conformation (steps 3, 4 and 5: see further). This might be the reason why there is not only an ATP adsorption step, but also an enzyme phosphorylation step.

2. Lewis induction from previous ATP binding brings about A-to-R phase transition by changing ion-binding selectivity at the inner side with liberation of K^+ and the adsorbed proton in favour of Na^+ binding producing $E_1.ATP.yNa^+$. The liberation of K^+ increases local K^+ activity in the nearby cytoplasm,

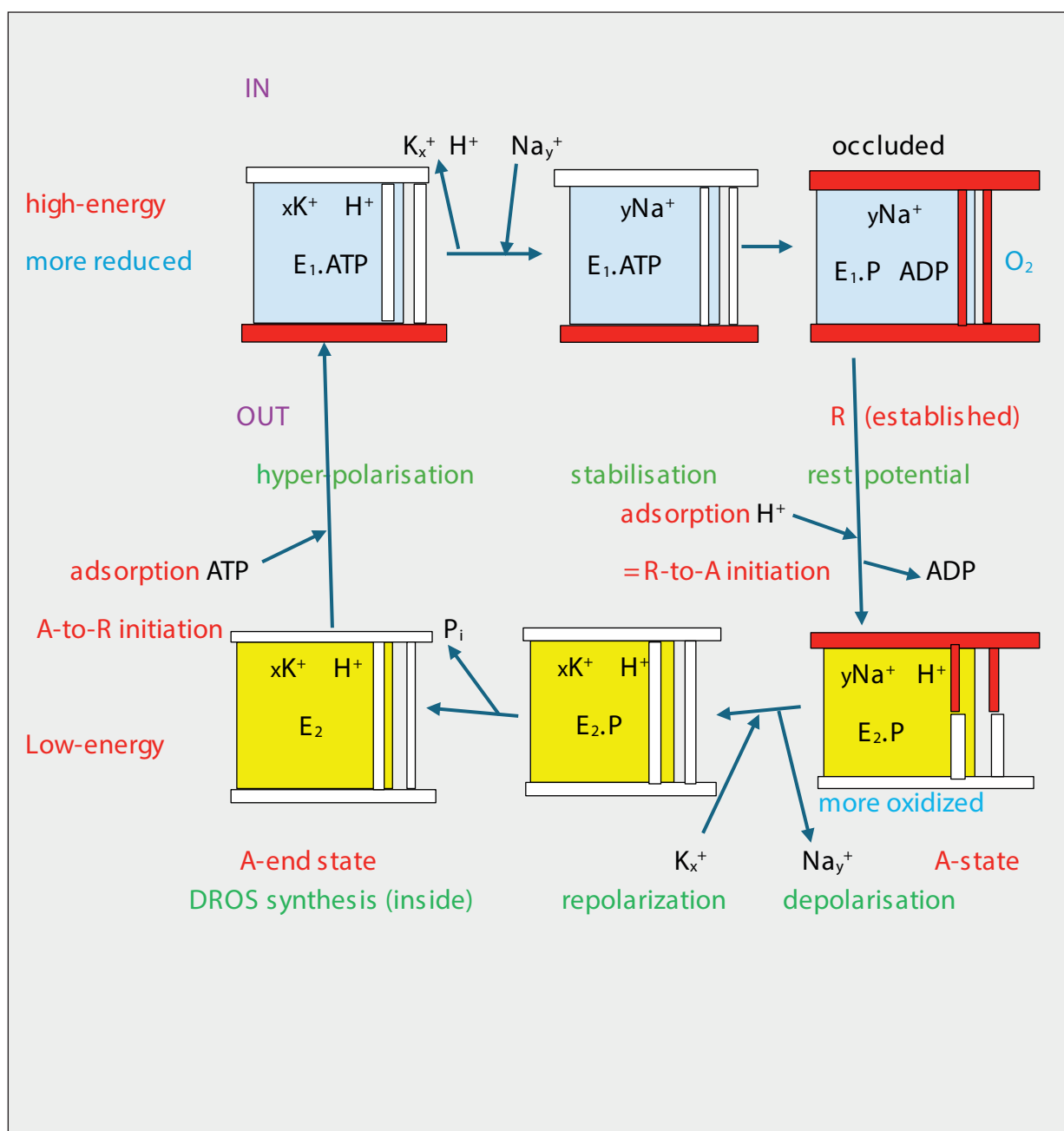


Figure 4. Proposal of a model for NKA in which AIH and murburn aspects are included (see text). Blue: E_1 conformation; yellow: E_2 conformation; white: membrane side in A-state; vertical white bars: conductive transmembrane conducts, vertical red bars: non-conductive transmembrane conducts; red: membrane side in R-state; red text: energy aspects, high-energetic is more reduced, low energetic is more oxidized; green text: consecutive sub-states.

which then occurs in A-state. K^+ desorption is also driven by the local negative potential at the inner side at that moment. Na^+ adsorbs due to an increase in affinity, but also because of increased local Na^+ activity due to local water depolarisation near the inner surface (cytoskeletal elements collaborating with NKA might be involved in these local cytoplasmic

conformational changes).

3. It is known that Na^+ is required for protein phosphorylation. So, once Na^+ is bound, ATP is hydrolysed, P_i liberated inside the enzyme where it auto-phosphorylates NKA, and ADP remains bound [174]. Besides the main phosphorylation site some secondary phosphorylation sites exist making

ATP binding and phosphorylation non- deterministic too. Enzyme phosphorylation is *assumed* to be responsible for formation of POM-water at the inside so that bound Na^+ ions are locked inside the protein by POM-water on both sides of the membrane. The mechanism of this encapsulating of Na^+ is just phase behaviour; hence no deterministic stoichiometry is expected, as indeed observed. NKA has now reached its (meta)-stable high-energy low-entropy full-resting state associated with non-bilayer lipid ($E_1\text{-P.yNa}^+.\text{non-bilayer-lipid}$ conformation), which constitutes the inactive (OFF) R-state allowing only very limited exchanges of Na^+ , H^+ , Ca^{2+} and most solutes between the fluids on both sides of the membrane due to their low solubility in POM-water. Membrane associated POM-water on both membrane sides not only occludes NKA, but also the associated lipid region with which NKA forms a functional entity. Passive flux of K^+ in both directions can still take place as K^+ is well soluble in POM-water which is present within NKA and inside the non-bilayer lipid system and other pores [45]. It was found that two transmembrane helices unfold in the intra-membrane region of NKA [175], which forms a lattice for POM-water formation inside. A membrane lined with POM-water on both sides may also encapsulate O_2 within the apolar regions of the membrane as O_2 is quite apolar, whereas POM-water is highly polar.

4. The R-to-A phase transition is initiated by the binding of a proton as cardinal adsorbent from the inside leading to ADP liberation to the inside, in agreement with the Post and Alberts model. Protons might reach the inner surface of NKA by means of a Grotthuss mechanism (allowed by POM-water), but they cannot pass the membrane at the half-way membrane location because during the membrane's R-state POM-water on both sides of the membrane is oppositely polarized [45]. There might be a connection between the moment that protons reach NKA molecules and local cytoplasmic activity, since local cytoplasmic power output implies the hydrolytic reaction $\text{ATP}^{4-} + \text{H}_2\text{O} \rightarrow \text{ADP}^{3-} + \text{Pi}^{2-} + \text{H}^+$ (usually inaccurately written without proton liberation) and H^+ is the internal stimulus to activate NKA. H^+ transforms R-state $E_1\text{P.ADP.yNa}^+.\text{non-bilayer-lipid.POM(both sides)}$ into a $\text{H}^+.\text{E}_2\text{P.yNa}^+.\text{bilayer-lipid}$ A-state conformation, which goes along with an affinity change of ion-binding sites in favour of K^+ instead of Na^+ . Dissociation of Na^+ works out towards the outside. This observation might be explained by means of a directional propagation of POM-water de-polarization/de-structuring. Na^+ desorption and efflux is preceding K^+ influx and adsorption. This may explain the inwards direction of the K^+ flux insofar

that Na^+ efflux locally upsets electroneutrality outside generating a primarily electrical drive for K^+ influx. At a later moment (stage 6-1) this tendency might be attenuated by ADRS production at the inner membrane face [151]. Propagated de-polarisation/de-structuring of external POM-water could be due to rearrangement of the topological distribution of fixed charges at the exterior macromolecular surface of the NKA-lipid structure which includes transformation of non-bilayer lipid into bilayer lipid. There are arguments that a redox effect is involved [151]. Lewis inductive conduction through the external $\beta 1$ -subunit may play a primary role in directional EZ-water de-polarisation as well as re-polarisation at a later moment.

5. Bound K^+ is required for de-phosphorylation of the phosphoenzyme. Desorbed Pi is released to the inside leaving the system in its $\text{E}_2.\text{xK}^+.\text{H}^+$ action-end state.

6. The latter conformation is free to adsorb ATP as soon as it is synthesized. The ATP adsorption energy brings about A-to-R transformation with POM-water induction at the outside, rendering the system again to step 1 (E_2 -to- E_1 transformation).

It is *assumed* that the de-phosphorylated enzyme (steps 6, 1 and 2) and its associated membrane components are lined with non-polarized/de-structured water at the inner membrane face, allowing O_2 to enter the reduced milieu of the cell interior. It can enter from the environment during steps 4, 5 and 6 when the outer surface is not lined by POM-water. 1-electron donation to O_2 reaching the inside generates a DROS cascade, which eventually can synthesize ATP from meanwhile liberated ADP (step 3-to-4) and Pi (step 5-to-6), but ATP might be obtained from other nearby sources. The ADROS produced generate ECS characterized by maximal hyperpolarization (during steps 6 and 1). The subsequent stabilization of the potential during step 2 is due to equilibration of several chemical and physical equilibriums and reaches a stable R-state, eventually for a long period. The interaction between released K^+ to the inside (step 2) and its binding to meanwhile produced O_2^{*-} may produce some amounts of non-dissociated K_2O that might be crucial in keeping the R-state stable, i.e., to install the overall sub-threshold equilibrium (and corresponding resting potential) whereby solvency, adsorption and reaction equilibriums are involved. Some amounts of O_2 may become sequestered inside the apolar membrane during the occluded-R state, which may be kept for a long period of time.

Note that this cycle follows the same sequence of events than that of the Post-Alberts model, yet is much more flexible, not just a simple open-close system by means of the

mechanics of a supposed valve. Changes of the properties of water are operative instead, and above all, the energetics are entirely different, making the new model essentially different insofar that ion movements *properly* are equilibrium-driven. However, cycling of the entire system with associated ion movements requires metabolic energy, but this is introduced by ATP adsorption (from step 6 to 1), not hydrolysis (step 2 to 3), another essential difference. The moment of energy input (step 6 to 1, A-to-R, according to AIH) is the same as in the Post-Alberts model (conformational change from E_2 to E_1). The latter model contains an internal contradiction since it takes E_2 -to- E_1 conversion as the step in which internal energy is increased, while also maintaining that energy is added to the system during ATP hydrolysis. This contradiction is due to the worldwide misunderstanding of the mechanism of ATP explained in section 4.2. The mechanism presented here is not an uninterrupted pumping and certainly not one energized by ATP hydrolysis. There are two overlooked phenomena involved: phase transitions and murburn effects. The mechanism is also non-deterministic and non-stoichiometric, explaining the hormetic effects of most substances having effect on NKA kinetics [151]. Murburn effects were not considered prior to the latter review, whereas they are also corroborated by the direct observations on quail eggs [149]. Redox effects are also involved in the description of the action potential, whereby NKA and channels are turned into their A-state (section 5.3).

b. Na^+ and K^+ channels

The above argumentation concerning the phase behaviour and murzyme properties of NKA also holds for *ion-channel-lipid-POM-water- O_2 /DROS ensembles* [147]. This would explain the analogue (not digital) integrative nature of stimuli-integration, which is the primary characteristic of all sub-threshold phenomena (generator potentials). Below threshold conditions are ruled by principles which all have a stochastic component: adsorption coefficients, distribution coefficients, murburn reactivities, i.e., equilibriums determining the direction and intensity of fluxes of ions and other solutes. Na^+ and K^+ channels locate in a murzone, whereas they are also sensitive to redox-state and can interact with changes in the redox state of associated membrane lipids with effects on cation conduction [150]. Moreover, passage of these cations through the membrane follows several paths, not only through proteins, but also through lipids. Synthetic pure lipid membranes display voltage-gated ion-conduction with

a voltage-dependence that is comparable to that of protein channels [4,5,186,187]. Details of a murburn-centred view of KcsA can be found in references [147,148,150].

c. SR- Ca^{2+} -ATPase

Ca^{2+} behavior at the sarcoplasmic reticulum (SR) is perhaps the simplest and best studied case to illustrate Ling's AIH. The SR is bordered by a membrane containing lipids, an IP_3 (stimulus)-dependent Ca^{2+} channel, SR- Ca^{2+} ATPase, an SR-membrane located NADH oxidase (different from NADPH oxidase in the cell membrane and T-tubule membrane), and the SR lumen contains calsequestrin. Adsorption of Ca^{2+} to calsequestrin in the volume of the SR is responsible for Ca^{2+} accumulation in the SR-lumen. Hence SR- Ca^{2+} ATPase does not need to be a pump. It only catalyzes net passive inward Ca^{2+} transport, provided that calsequestrin occurs in a state allowing Ca^{2+} adsorption. IP_3 -dependent passive outward Ca^{2+} transport occurs when calsequestrin desorbs Ca^{2+} . The ATPase activity is not needed for active Ca^{2+} transport as generally believed, but for depolarizing/de-structuring SR-membrane associated POM-water in a way that might compare to the NKA mechanism proposed above, a process which may go along with changes in solvency for Ca^{2+} , directed adsorption-desorption, and changes in redox state. The SR- Ca^{2+} ATPase must adsorb newly synthesized ATP (during local cytosolic A-state) to reinstall the SR-membrane's R-state (with water re-polarization). Membrane lipids might be involved in spreading water de-polarization and re-polarization from the sites of SR- Ca^{2+} ATPase properly to a larger membrane region, enhancing the extent of the Ca^{2+} fluxes (as lipids may conduct ions as well: see further). Murburn effects quite likely play an important role since the SR-membrane also contains a SR-NADH oxidase that produces more superoxide per mg protein than mitochondrial production [159]. It might be conjectured that membrane-associated potential changes due to SR-NADH oxidase-dependent ECS have direct effect on Ca^{2+} transport, but as DROS production might be expected to be formed at the cytosolic side of the SR, it might have some effect on nearby filaments of the myofibrils as well, which are redox-sensitive.

d. coupled transport

What is called '*coupled transport*' in the MPT-jargon, can take place by two different but complementary mechanisms without coupling to a pump, one discovered by Wheatley *et al* [165], the other described by Ling and Will [167]. Most experiments undertaken to study transport through membranes are biased

by the MPT doctrine. Hereby two important points may be overlooked. Is the observed transport due to only a membrane permeability change (MPT) or are factors in the membrane-delineated volume involved too by means of an adsorption process in the volume (discussed by Ling in section d2 below). But even if adsorption is considered, another unexpected aspect was discovered. Usually flux measurements miss the first 5 seconds, and they can be so important, as the study of Wheatley demonstrates (section d1 below).

d.1. Wheatley

In a clever experimental setup Wheatley *et al.* [165] were able to measure the extremely fast uptake by HeLa S-3 cells of a mixture of amino acids reaching equilibrium within 5 seconds. The uptake is into a free intracellular pool in which the external concentration ratios are maintained. This pool is shown to serve protein synthesis. Amazingly, in that short period of time inside concentrations in this pool rise above those of the incubation medium, demonstrating accumulation, and this not only at 37°C, but also at 4°C when active transport should certainly be incapacitated. This directly disproves the concept of trans-membrane active transport by means of coupled transport (The term '*active transport*' may not be used for transport across a single membrane, but only for metabolism-dependent trans-epithelial and trans-cellular ion/solute transport [52]. Adsorption of amino acids to the cell-matrix (Ling's mechanism: section d2) takes place also, but much later after protein synthesis is served. Ultra-fast accumulation is slightly higher at 37°C as compared to 4°C, indicative of a metabolism-dependent aspect at physiological temperature attributed to a cytoplasmic gel-sol-driven hydraulic perfusion mechanism acting on this free pool, and demonstrable with laser-Doppler microscopy. Protoplasmic perfusion is a neglected mechanism of metabolic regulation [188,189]. HeLa cells are quite active cells. Concerning the A-state of cell systems Ling [38] argues that during R-to-A transition (i) POM-water is depolarised leading to increased Na⁺ solvency, hence a Na⁺ flux towards the active region, and (ii) adsorbed K⁺ is dissociated leading to a K⁺ flux away from the active region. The question is: What is the state of water in the A-state? According to Ling water is de-polarized in A-state regions, but to what extent? Applying proton-NMR to HeLa cells in varying hypotonic milieus down to 30 mosmolar revealed that in these active cells T₁ relaxation times did not rise in proportion to the influx of water (when expressed under the assumption that it would be bulk water), and that they did not shorten in hypertonic

milieus up to 1500 mosmolar in proportion to that of a loss of supposed bulk water [43]. These results indicate that in these active cells both under swelling and shrinking conditions the cell-matrix is still able to polarize almost all cell-water. This means that non-treated cells with a normal volume exhibit excess colloid osmotic pressure that must be compensated by the tensegrity properties of the cytoskeleton [190-192]. The latter put cellular water under hydrostatic/turgor pressure (in plants and bacteria the cell-wall is involved too).

According to Jaeken [29] this conclusion must be linked to the spectroscopic data of Kabir *et al.* [44], who discovered that F-actin (A-state relative to G-actin, the latter with more POM-water) is partially associated with POM-water, partially with HF-water. Jaeken argued that the ultra-fast accumulative amino acid uptake data could only be explained by *assuming* the existence of a pool of HF-water. Chaplin [193,194] showed that water clusters which are de-structured to tetrameric clusters, possibly a major cluster size in HF-water, can exhibit two different conformations, non-polarized and polarized. Logically the non-polarized form might be found in narrow clefts between regions with oppositely polarized POM-water. The possibility of this situation was physically derived as one of the coherent solutions from Tuszynski's non-linear Schrödinger equation describing protein-water systems, and interpreted as such by Jaeken [29]. The other possibility is that two regions with POM-water polarized in the same direction are interrupted by an introvert disruptive region of the protein surface. To reach high solvency for amino acids, which are polarized molecules, the second situation should be considered.

The extraordinary velocity of the amino acid uptake implies that such pool with polarized HF-water is not only present in cytoplasm, but also exists in the cell membrane (in an A-state region), as the latter does not seem to restrict the high-velocity influx. Aquaporins [45] must be considered. and other large-sized transmembrane conduction structures (in A-state) for water and a large set of solutes. During R-state these structures appear even impermeable for protons [45]. Such mechanism may explain non-specific permeation. Of course, this does not deny the existence of more specific trans-membrane transport systems for some selected physiological solutes (see section d2).

d.2.Ling.

Ling [52] recapitulates the early history of the idea of semi-permeability. In 1867 Traube [195] discovered that an artificial membrane made of copper ferrocyanide *gel*

allows passage of water but not of larger-sized solutes and hypothesized that this membrane would contain pores that are so small that only water could pass through. In 1877 Pfeffer [196] applied this idea to cells and founded membrane theory. However, later X-ray and electron diffraction data revealed that the pore size in membranes from copper ferrocyanide *gel*, cellulose acetate and some other polymers and gels is larger than the molecular size of sucrose, while being impermeable. Ling explains these data by the presence of POM-water in the pores. Unfortunately, his idea and the X-ray and electron diffraction data did not reach large dissemination, and so the idea of protein-based rigid pores or channels (making use of conformational changes) combined with Overton's idea of membrane lipids as an essential permeability barrier received more credit. Increasingly more details got interpreted in this overall context. In contrast, Ling's '*Wet Membrane Model*' [197] is based on the observation that the permeability properties of cellulose acetate membranes (with large POM-water filled pores) towards water and 9 hydroxylic non-electrolytes is identical ($r = \pm 0.96$) to those of living tissue (reversed frog skin). His model of membrane permeability and structure holds that the membrane is in essence a two-dimensional replica of the cell-matrix in cytoplasm. Membranes are lined with and contain quite large interstices filled with POM-water that constitutes the real permeability barrier, which can be relieved during POM-water depolarisation/de-structuring in membrane regions in A-state. Passage of solutes by means of this mechanism is not specific, not saturable and gives a rectilinear flux-concentration curve. The membrane also contains adsorption-desorption sites that can be alternately exposed to the external and internal milieu. This mechanism is rather specific, saturable, characterized by a hyperbolic curve and prone to regulatory control and influences from added activators and inhibitors. Both mechanisms are passive. For solute accumulation Ling only considers solute adsorption to an intracellular macromolecule (as for K^+ to myosin, actinin and some other proteins, or Ca^{2+} to calsequestrin in the SR). Other examples will be given below.

The continuity of POM-water from the outside (EZ) through the membrane into cytoplasm was confirmed by observations that in frog ovarian eggs [198] and giant barnacle single muscle fibres [199] cell membrane has no greater resistance to the diffusion of 3H -labelled water than cytoplasm. These observations agree with the spin echo NMR studies of Mild *et al.* [200]) and the pulse gradient spin echo

NMR studies of Cooper *et al.* [201]. Confirmation of POM-water being the common permeability barrier was obtained from studies of the permeability of numerous hydrophilic solutes. For instance, when the permeability (of the membrane, but also of cytoplasm) for Na^+ increases in animal cells, the permeability of 11 hydroxylic non-electrolytes (from water to sucrose) also increases [52]. The simplest explanation is that exclusion of many solutes by POM-water in the outer EZ, in the membrane and in cytoplasm disappears when it is depolarised so that Na^+ and other excluded solutes can now permeate together.

Ling [52] also attacks the classic interpretation of the electron-microscopic observation of the tri-lamellar aspect of cell membrane *thought* to present the bilayer structure. It figures on the first page of the textbook '*Membrane Structural Biology*' of Luckey [3]. Ling ([52] figure 12.3) presents an electron micrograph of de-lipidized mitochondrial inner membranes, that keep this tri-laminar structure. Conversely, structural continuity of the aqueous phase from mitochondrial matrix to intermembrane space is clearly visible on electron micrographs from sections obtained with artefact-minimizing methods preserving the watery nature of cells and contrasted with water soluble negative staining methods (Edelmann [166] figure 4, insert).

Ling's dual mechanism for solute transport through membranes is captured in his two-terms containing equation below. The non-saturable fraction deals with solute transport through the liquid with limited specificity dictated only by the solute's molecular size and the degree of solvent structuring. It gives a rectilinear dependence of transport rate in function of solute concentration. The saturable fraction makes use of an adsorption-desorption process, exhibits specificity and competitive behaviour, it is hyperbolic and may be subjected to regulatory control. The rate of entry of the i th solute (V_i) is given by his two-term equation:

$$V_i = A[p_i]_{ex} + (V_{i,max} \cdot K_i[p_i]_{ex}) / (1 + K_i[p_i]_{ex} + K_j[p_j]_{ex}) \quad (\text{eq. 6})$$

where i and j are solutes competing for the same adsorption sites, A is a constant, K_i and K_j are the respective adsorption constants of the competing solutes, $[p_i]_{ex}$ and $[p_j]_{ex}$ are their external concentrations and $V_{i,max}$ is the maximal rate of entry of solute i .

Often, influx measurements do not discriminate between passage through the cell membrane (surface) and further transport through cytoplasm, and this has resulted in many misinterpretations. Ling's observations of the rather equal

rate of diffusion of water through the cell surface layer and through cytoplasm of ovarian eggs and barnacle muscle cells suggest a basic similarity of the properties of cell surface and cell interior [52]. Hereby he did not foresee the presence of HF-water existing along with POM-water. Insofar, the transport rate through the fluid fraction (first term of equation 6) should be seen as an average, which results from likely a small fraction of HF-water with very high transport rate for certain substances (not all), but with variable volume depending on overall cellular activity (percentage of the intracellular fluid in A-state) and a large fraction of POM-water with quite low transport rate but a large volume, hence a relatively constant volume. It should still be investigated, but probably cell membrane regions in A-state, similarly to intracellular fluid, may still contain much POM-water.

The strongest argument against the classic mechanism of coupled transport *thought* to be energized for instance by a Na^+ gradient, is that Na^+ and K^+ activities are in equilibrium below threshold [67], hence their fluxes are equilibrium-driven. They cannot be active transport. There is no gradient as defined by MPT, though there can be an ion/solute differential due to solvency and adsorption effects. If in an experiment external Na^+ is added (for instance to study the uptake of D-glucose), this brings the physiological equilibrium out of equilibrium creating a tendency to reinstall the equilibrium. This goes along with locally and temporarily altered fluxes, giving a false impression of coupled transport. There is direct experimental evidence falsifying accumulation by coupled transport. Three examples will be given.

Ling's study on D-glucose [167,202] makes use of reveals the two mechanisms found in his equation (and as such corroborate it): a non-saturable in the absence of insulin (that compares to that of Na^+ transport), and an additional saturable in the presence of insulin (that compares to that of K^+ transport). Both mechanisms are passive. Glucose dissolves in cell water and this depends on the distribution coefficient (0.25 ± 0.01 indicative of POM-water), which results in a rectilinear relationship between internal and external water activity (the first term in equation 6). In the presence of insulin the former mechanism persists, but another fraction of D-glucose is adsorbed on intracellular protein sites controlled by insulin acting as a cardinal adsorbent and establishing extensive accumulation (the second term of equation 6). Muscle cells pre-incubated at 25°C , in the presence or absence of insulin, were subsequently incubated at 0°C , and whilst they stayed alive at this temperature, ATP production

and glucose metabolism were halted, yet D-glucose transport continues as can be followed by adding labelled D-glucose. In the absence of insulin in the pre-incubation medium a rectilinear curve between transport rate and external D-glucose concentration is obtained. Presence of competing unlabelled D-glucose makes no difference. The system is non-saturable. Typical for this fraction is that intracellular concentrations always remain below external concentrations. This indicates that D-glucose transport does not follow the same mechanism than that of ultra-fast amino acid uptake [165]. Likely D-glucose does not dissolve in polarized HF-water (as amino acids are supposed to do [29]), but in POM-water, whilst non-polarized HF-water is likely not present in sufficient quantities to explain accumulation by means of the average solubility in a mixture of POM-water and non-polarized HF-water. When insulin is present in the pre-incubation medium, a curve is obtained that is the sum of the above rectilinear curve and a hyperbolic one. The latter fraction is saturable, which is indicative of adsorption. Intracellular concentrations of about 100 times the extracellular concentration can be obtained. At 0° incubation this cannot be the result of active transport.

The other example goes about the study of Eddy *et al.* [203] that stood at the origin of the concept of coupled transport. They investigated the effect of external Na^+ and K^+ concentration on the rate of glycine uptake by Ehrlich ascites cells. They postulated the presence in the cell membrane of a carrier molecule with two kinds of binding sites, one for glycine and one able to bind either Na^+ or K^+ , whereby the Na^+ gradient was *thought* to energise the accumulation of glycine. Theoretically there can be 7 different equilibrium dissociation constants, which were also experimentally determined. Ling [52] converted these constants into their reciprocals, 7 adsorption constants. This reveals that the previous binding of Na^+ appears to increase amino acid adsorption strength by a factor 2, and the previous binding of K^+ to reduce amino acid adsorption strength by a factor of 14. Glycine accumulation shows a good correlation with intracellular K^+ concentration [204] and appears inversely correlated with intracellular Na^+ concentration.

Ling [52] then postulated a 3-step mechanism for the transporter. During the adsorption phase the transporter preferentially binds Na^+ over K^+ leading to highest affinity binding of the amino acid. This binding is favoured because the external Na^+ concentration is much higher than that of K^+ . During the libration-desorption phase binding sites that were

exposed to the outer side are now exposed to the cytoplasmic side. Na^+ dissociates and K^+ binds, whereby the chance of dissociation of the adsorbed amino acid is greatly enhanced. When by chance the desorbed radio-labelled glycine returns to the outside, no change is measured. But, if by chance it desorbs in the opposite direction, its uptake is registered as a Na^+ -dependent glycine uptake. This mechanism does not depend on a Na^+ and K^+ gradient generated by a pump, but on adsorption effects, a hallmark of AIH. The third phase, accumulation, is a cytoplasmic one. According to AIH, accumulation of a solute in a cell is always due to adsorption. The experimental observation of glycine accumulation may then be explained by intracellular proteins, which differ from those at the cell surface in that they bind amino acid more strongly when in the K^+ -adsorbed state rather than the Na^+ -adsorbed state. This will discourage desorption of bound amino acids in an intracellular region in R-state and deliver it in a region in A-state where they are needed. Intracellular accumulation by means of adsorption is corroborated by the data of Wheatley *et al.* [165] on the slower mechanism involved in amino acid storage after protein synthesis is served via the fast uptake mechanism.

The third case deals with some remarkable data gathered by Ling [52] on the induction of lactose transport in *E. coli* (lac-operon). The inducible transporter for lactose (and other β -galactosides), namely lactose permease, appears to elicit over 100-fold accumulation of lactose in the bacterial cytoplasm. In terms of MPT this should represent cell-membrane-bound coupled transport. However, 6 other so-called active transport systems (when calculated in the MPT-way) already require more energy than *E. coli* cells can deliver [33]. Secondly, there is the study of Kolber and Stein from 1966 on the proteins executing the functions coded by the lac-operon [205]. They state that there is no convincing evidence that lactose permease is membrane-bound. They used DEAE-cellulose chromatography to isolate the 3 proteins induced by stimulation of the lac-operon. They compared the ^{14}C -labeled protein content of induced cells with the ^3H -labeled protein content of non-induced cells. The chromatogram clearly showed 3 well-defined extra peaks for the induced sample, 2 of which could be identified as the 2 induced enzymes by way of their enzymatic activity. The third peak is not enzymatically active and must be the permease. But the data show that it locates in the soluble fraction, and thorough extraction of the membrane fraction by vigorous methods failed to yield any enrichment. This indicates that the accumulating activity is

present in cytoplasm in a way similar to the accumulation of K^+ , the bound fraction of amino acids, D-glucose and Ca^{2+} in the SR as described above. When a transporter trans-locates a hydrophilic substance through a membrane, it is called either a 'carrier' or a 'channel', but when an apolar substance must be trans-located through the aqueous cytoplasm, it is called a 'binding protein' or sometimes a 'receptor', but the mechanism is essentially the same: they both can adsorb or desorb a given substance. The protein executing β -galactoside accumulation must be a cytoplasmic binding protein that adsorbs a hydrophilic substance and stays under the inductive allosteric control of insulin. These observations do not deny that there also exists a membrane-bound protein, also called 'lactose permease', that mediates the transport of β -galactosides through the membrane, but the latter is not a coupled transport system and cannot be responsible for accumulation. The case is similar to that of the SR, where there are two different transporters in the SR-membrane responsible for Ca^{2+} influx and efflux and calsequestrin in the lumen responsible for accumulation.

More insight into the mechanism of so-called coupled transport can be obtained from considering transepithelial transport and transport between environment and tonoplast in plant cells [52,206]. In these two cases the solute must pass cytoplasm and two different parts of the cell membrane. This is the only situation where the term 'active transport' may be applied, since there is a real gradient between the two fluids separated by the cell's protoplasm. Trans-epithelium transport goes in one direction, in some cases apical to basolateral, in other cases the reverse direction is followed. MPT explains this directionality by the presence of different transporters in the apical and basolateral membrane, a so-called coupled transport system (indirectly depending on NKA) in one of them and a passive transporter in the other. Hereby cytoplasm is regarded as only a bulk solution for the passage of the solutes. The membrane with the driving machinery is *thought* to determine the direction of trans-epithelial transport. According to Ling [52,206] trans-epithelial transport cannot take place without the major contribution of intracellular cytoplasm-driven transport systems. Directional transport of a substance, for instance from the apical to the basolateral side of an epithelial cell, implies a *continuous cooperatively linked protein-system* from the apical cell membrane through cytoplasm up to the basolateral cell membrane which works with water polarisation/de-structuring and/or adsorption-desorption.

Finally, each local R-to-A-to-R phase-transition has

its corresponding osmotic effects, which are much more complicated than just van 't Hoff osmosis (section 4). The uni-directionality of the inductive pulse also leads to the unidirectionality of water transport.

e. O₂ and DROS

Two solutes are overlooked when it is about solute transport: O₂ and DROS. O₂ is poorly soluble in normal water; its limited solubility is due to the weak polarizability of the O-O bond. As POM-water is more polarized than normal water, it may be expected that it has even lower solvency towards O₂. Insofar, a membrane in R-state lined on both sides with POM-water must behave as a strong barrier for trans-membrane O₂ transport. In contrast O₂ has better solvency in more apolar solvents such as the apolar interior of membranes. Hence O₂ may spread in cells in majority through the interior of membranes and through direct membrane-membrane contacts. Myelin sheaths could provide action potential propagation, which depends on DROS synthesis for recovery, directly with O₂ present inside the membrane. During the A-state O₂ may leave the membrane interior and reach the near-membrane regions where DROS is produced. During the A-state of membrane and cytoplasm O₂ may also be distributed through the then expanded HF-water system, which may flicker between being polarized and non-polarized.

DROS, and particularly anionic DROS and other anions formed during DROS cascades, must be more soluble in POM-water and in polarized HF-water. Murburn phosphorylation needs low water activity (POM-water) together with the presence of ADP and Pi.

5.3. Potentials and effective charge separation

All the foregoing makes univocally clear that membrane potential cannot be due to electrogenic pumping, not by NKA at the eukaryotic cell membrane, not by redox complexes at prokaryotic, mitochondrial inner and thylakoid membranes. This invalidates the explanation based on MPT given by Hodgkin, Huxley and Katz [9, 207], though their data keep their value. Recently new arguments were given that fundamentally invalidate MPT-based consensus explanation including the Goldman-Hodgkin-Katz equation [5, 53, 133, 147-149, 208-2012] complementing older critiques [95, 106-108, 184, 185, 213-215]. By now the ultimate energization of prokaryotic cell membrane, mitochondrial inner and thylakoid membranes became finally understandable through data collected within the context of murburn concept, and this after so many years of

ongoing critiques to the consensus chemiosmotic explanation. Primary energization is by means of redox complexes which inescapably donate single electrons to O₂ producing O₂^{-•} that starts a DROS cascade in which anionic species are present which generate effective charge separation (ECS) with an associated electrode potential at the side of the membrane where electron donation takes place. This explanation also holds for eukaryotic cell membrane in which oxidases such as for instance NADPH oxidase and others are involved in ECS. In this explanation electron displacements/flow/transfer is the primary cause of electrophysiological behavior, ion flows being a consequence of electron behavior, hence secondary. The topic of ECS and potentials will be discussed at length in part II of this double review.

5.4. Volume control

In section 2 it was argued that colloid osmotic pressure of the cell-matrix (not to be confounded with cytoskeleton, though the latter is part of it) is the main factor controlling cell volume. The cell-matrix is defined by Ling as the ensemble of bio-molecules able to produce POM-water. Though the membrane is lined with POM-water and contains small amounts inside, the volume of cell membrane is very low in comparison to that of cytoplasm. Insofar it is fully justified to state that water-polarizing compounds present in cytoplasm are the main factors determining cell volume. This is experimentally underpinned by the observation that resting muscle cells with incomplete membrane can keep their resting state volume [52, 67]. Some cytoplasmic compounds are particularly important in determining cell volume: actin and tubulin monomers and polymers and IDP's, but all molecular structures with a correct (partial) charge distribution contribute. The adsorbed state of a large amount of K⁺ during the resting state makes that the involvement of van 't Hoff osmotic pressure is minimal. During the A-state of certain cell systems K⁺ is desorbed (often locally), introducing a (locally) increased van 't Hoff osmotic effect, which can lead to hydraulic mechanisms of power output, known for instance in case of amoeboid movement [69, 189] and proposed for muscle contraction [52]. Since there is a tendency in cells to produce more POM-water than there is space for it (which would lead to swelling) volume control is also dependent on the tensegrity effects of the cytoskeleton opposing swelling. The cytoskeleton stays under pre-stress, which can be adapted [190-192]. Insofar the ratio of monomeric over polymeric actin, tubulin and other filament-

producing molecules is very determining for cell volume in eukaryotic cells.

Something similar applies to prokaryotes and mitochondria. Under the influence of chemiosmotic theory, transport of protons and of numerous solutes by means of *assumed* coupled transport, was/is *thought* to be more intensive the higher the respiratory rate, and this was understood – without proof – to lead to van 't Hoff osmotic behavior, *thought* to be responsible for prokaryote and mitochondrial contraction at high respiratory rate. However, in 1962 Lehninger [216] was able to extract and purify an actin-like protein from swollen mitochondria. Devoid of this protein mitochondria were no longer able to contract nor to activate oxidative phosphorylation, and both abilities were restored after the actin-like protein was reintroduced into the mitochondria. Minkoff and Damadian [132] compared the properties of an isolated and purified 45kd cytoplasm-located actin-like protein from normal *E. coli* and from a K^+ transport deficient mutant and compared it with the swelling ability of the two cell cultures. The mutant strain cannot swell, and its actin-like protein cannot polymerize, whereas the wild-type *E. coli* can swell and its actin-like protein polymerizes under the same conditions as muscle actin. It was concluded that actin-like protein and its ability to polymerize is involved in cell volume changes and K^+ accumulation. From these two studies Minkoff and Damadian [132], who investigated the similarity between cells and ion-exchange resins, derived their '*cytotonus hypothesis*'. It holds that these contractile proteins quantitatively determine cell/organelle volume and in this way indirectly influence physical-chemical and chemical equilibria, effectuating almost all aspects of cell/organelle activities in a concerted way. This is particularly effective in case of small volumes (prokaryotes, cell organelles). Cytotonus regulation establishes a particular kind of coherent behavior (for different types of coherence [29,93]).

Both examples demonstrate that not membrane-dependent van 't Hoff osmotic effects, but actin-like proteins (together with the proteins with which they interact) in cytoplasm and in other intracellular compartments are obligatorily involved in volume changes in prokaryotes and mitochondria, as is actin in eukaryotes. The results of Lehninger imply that regulation of oxidative phosphorylation is not fully understood when the detailed mechanism by which prokaryotic and mitochondrial actin-like proteins bring about volume changes remains

a mystery. In prokaryotes and mitochondria hydrostatic pressure build-up due to production of large amounts of water by murburn respiration together with colloid osmotic pressure is counteracted by the force generated by their cytoskeleton consisting of actin-like proteins and the proteins with which they interact. By analogy to the *E. coli* study polymerization/depolymerization of mitochondrial actin-like protein is the likely mechanism for controlling oxidative phosphorylation, but other mechanisms cannot be excluded [29]. Question becomes to further explore the relation between murburn and actin-like protein behavior under different degrees of cytoplasmic activity.

CONCLUSIONS

A new paradigm is formulated for cellular bioenergetics [25], which is thermodynamically, kinetically, structurally and probabilistically sound and evolvable, whereas MPT is not. A limited number of undeniable falsifications of MPT and its ion-pump-dependent bioenergetics are first presented. Thereafter a brief presentation of the alternative is given. It is based on the principles and observations of: (i) the murburn concept (with a recently developed mathematical formalism for e-transfer and catalysis rate [218] and an algorithm for identification of murzymes and murburn systems [219]), (ii) the coacervate-coherence nature of life, (iii) the existence of an EM-matrix and (iv) the reciprocal interactions of these cellular properties. The alternative has all ingredients to replace the untenable, outdated MPT paradigm. It brings a totally different explanation for the same data and for numerous crucial but ignored and marginalized data which MPT cannot explain. Although it deals with both cytoplasmic and membrane features of cells, it is applied here in particular to the Eukaryotic cell membrane with some reference to the mechanism of respiration in Prokaryotic cell membrane, inner mitochondrial and photosynthetic membranes. A new functional '*Wet and Murburn Membrane Model*' (WMMM) is presented in which the predominant roles of water, O_2 and DROS are not longer neglected.

The way that membrane functionalities 1 to 5 described in the first section are traditionally described within the context of meanwhile falsified MPT, cannot be continued. The alternative WMMM model is developed from a combination of several hypotheses which do not depend on the concept of ion pumping. Each of these hypotheses (AIH, GSH, EZ-water, several physical derivations, murburn concept, cytotonus control, tensegrity, HF-water-dependent amino acid

accumulation and the existence of an EM-matrix are discussed with respect to membrane functioning.

Several functionalities traditionally *thought* to be executed by membranes are largely executed by cytoplasmic systems in the *volume* of the cell (ion and solute accumulation and exclusion, coupled transport, cell/organelle volume control) with only limited contribution of the membrane, which is the *surface* of the cell and is only implicated because some compounds must pass the cell surface. Water is common to both structures and forms an uninterrupted continuum. Its remarkable adaptable properties are involved in almost every membrane functionality. So is the presence of O₂ and DROS.

Structuring/de-structuring and polarization/depolarization of water associated with the two surfaces of a membrane and present inside membranes plays a crucial role in the functioning of membranes, reason to use the term '*wet membrane*' introduced by Ling [52].

Murburn concept deals with stochastic chemical *in fluid* (outside active center) radical reactions between the essentially reducing nature of cells and incoming O₂ which inevitably lead to DROS/ADRS formation at the inner surface of the membrane. First O₂^{-•} is produced followed by a radical cascade. Murburn mechanisms disclosed: (1) the instant constitutive metabolic and physiological roles of DROS at low concentrations as couplers (including murburn phosphorylation of ADP) and regulators of *in fluid* chemistry acting as the oldest and still primary '*basal level*' of non-active-site analogue regulation, i.e., homeostasis and coherence through the interconnectedness of all equilibriums (chemical and physical-chemical). The reactions within this unbound ion-molecule-radical mix are characterized by a new type of stochastic, hormetic and idiosyncratic kinetics. These reactions are ultra-fast and strongly exergonic, hence inevitable. They rooted in the early pre-biotic era, stood at the origin of life and remained up to now because together they formed a simple system, exhibited some basal homeostasis and allowed evolution by small incremental steps. (2) ADRS formed in the radical cascade generate ECS which is responsible for hyperpolarization, the highest energy state of the membrane; (3) murburn reactions are also responsible for the primary emission of IR, VIS and UV radiation in establishing the EM-matrix. A common point between a murburn-based and a coacervate-coherence-based physiology is that both mechanisms do not require ion pumping. The perfect going together of the two properties of life makes that Ling's '*Wet Membrane Model*' should be adapted to become a '*Wet-and-*

Murburn-Membrane-Model'.

Concerning Prokaryote cell membrane, mitochondrial inner membrane and thylacoid membrane the ideas of a serial ETC, capable of proton pumping, setting up a pmf, and using pmf for rotation-dependent ATP synthesis on Walker sites (ETC-CRAS model) must be abandoned. The murburn concept presents a much simpler entirely exergonic mechanism which is firmly founded in thermodynamics, kinetics, backed up by structural features and can evolve simply. It takes away many unsolved questions. A new type of kinetics linked to a new type of outside-active center catalysis by the new category of '*murzymes*' and a new type of simple chemistry-based basal homeostasis are presented.

Alternative explanations and equations are given for (i) ion/solute distribution, (ii) transport (including a sound explanation for phenomena wrongly called '*ion-pumping and coupled transport*'), which are in fact passive in both directions, (iii) volume control, (iv) the chemiosmotic ETC-CRAS mechanism, and (v) it explains the origine of high-energetic EM-emissions.

A tentative rather detailed model for the working of NKA is given because the reigning Post-Alberts model is based on the untenable ideas of MPT, is entirely silent about the observed central roles of water (coacervate-linked), of O₂/DROS-ADRS, cannot explain the absence of deterministic stoichiometry and hormetic kinetics, and does not see the primacy of electrons as ultimate causative agents with ion currents as a consequence (murburn-linked properties). Hence a reinterpretation is a necessity. So-called '*ion pumps*' and (some) channels are redox enzymes and can bring about phase transitions.

The primacy of the cell-matrix and of actin-like proteins in Prokaryotes and mitochondria and of actin in Eukaryotic cells in directly controlling cell/organelle volume and indirectly all chemical and physical-chemical equilibriums through a combination of cytotonus (Damadian), perfusion (Wheatley), tensegrity (Ingber) and basal homeostatic (Manoj) mechanisms of regulation is established.

The ideas brought together in the WMMM increase the similarity between prokaryote cell membrane, mitochondrial inner membrane, thylacoid membrane on the one hand and eukaryote cell membrane on the other hand.

The new list of functions of the cell membrane and possibly of every membrane consists of:

1. 1-electron redox activity with DR(O)S production (due to the influx of O₂ into the cell's reducing interior).

2. Physiological DR(O)S-dependent (murburn) processes including murburn phosphorylation.

3. ECS (and its associated potential) energizing electrophysiological processes,

4. Primary steps in the built-up of an EM-matrix (particularly the highest frequencies, including IR, VIS and (mitogenic) UV. The EM-matrix functions in the overall instant coordination making that organisms act as a single whole,

5. Trans-membrane transport of water, gases, ions and solutes, passive in both directions and controlled by the oxidative state/ECS and phase transitions determined by, the thermodynamic state of water (adaptable solvency) and changes in adsorptive properties,

6. Connection with the aether (omni-present background electromagnetic field) due to coherence of the R-state and of the R-to-A and A-to-R conversions.

7. Sensory function, coupled to signal integration and transduction (eventually long-distance signal propagation) with coupling to effector functions.

8. Functional attachment of the cytoskeleton and extracellular matrix (or cell wall).

9. exocytosis and endocytosis.

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