Review Letter

TRANSMEMBRANE ELECTROCHEMICAL H^+-POTENTIAL AS A CONVERTIBLE ENERGY SOURCE FOR THE LIVING CELL

Vladimir P. SKULACHEV

Department of Bioenergetics, Laboratory of Bioorganic Chemistry, Moscow State University, Moscow 117234, USSR

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1. Introduction

In 1941 Lipmann [1] put forward the idea that ATP occupies the point of intersection of biological energy transformation pathways. In the following 35 years, comprehensive experimental proof of the validity of this postulate was furnished by a great many independent lines of study. The impressive success of the ATP concept gave rise to the opinion that the system of high-energy compounds is the only convertible and transportable form of energy in the living cell. The latter conclusion was, certainly, no more than a speculation requiring deeper insight into bioenergetic mechanisms to be confirmed or rejected.

The study of one of these mechanisms, namely oxidative phosphorylation, resulted in several pieces of evidence being accumulated which indicated that the cell possesses another form of unified energy, besides ATP and related chemical substances.

2. 'Non-phosphorylated intermediate' and H^+ potential

2.1. *'Non-phosphorylated intermediate' of energy coupling ($X \sim Y$)*

The first group of facts which were indicative of the storage of oxidation-released energy in a form other than ATP, was obtained in experiments with mitochondria oxidizing substrates in the absence of ADP and phosphate. Such studies prompted Slater [2] to suggest a 'non-phosphorylated intermediate of oxidative phosphorylation'. He believed this to be an unknown high-energy chemical compound ($X \sim Y$) e.g., a thioester, functioning as an ATP-precursor in oxidative phosphorylation. Lardy et al. [3] found that the antibiotic oligomycin prevents energy transfer between $X \sim Y$ and ATP. In the presence of oligomycin, $X \sim Y$ produced by the second and third energy coupling sites of the respiratory chain was shown to be utilized to support reverse electron-transfer via the first energy coupling site. '$X \sim Y$' was postulated to be discharged by uncouplers of oxidative phosphorylation added to mitochondria in vitro [3] or formed in vivo, e.g., in response to exposure of warm-blooded animals to cold.

Further investigations revealed that $X \sim Y$ energy can be used to actuate Ca^{2+}-uptake by mitochondria [5] and energy-linked reduction of $NADP^+$ by NADH [6]. All these observations pointed to $X \sim Y$ being a polyfunctional energy source in mitochondria.

2.2. *Electrochemical H^+-potential ($\Delta\bar{\mu}H$)*

In 1961–1966 Mitchell [7,8] developed a new ('chemiosmotic') theory of biological energy coupling, postulating the existence of a gradient of electrochemical-potential of hydrogen ions ($\Delta\bar{\mu}H$) across membranes competent in respiratory or photosynthetic phosphorylation.

According to Mitchell the role of respiratory and photosynthetic redox-chains in ATP-synthesis is confined to the formation of $\Delta\bar{\mu}H$. The latter, it is suggested, is used to reverse the H^+–ATPase reaction. For example, the mitochondrial respiratory-chain would pump H^+ from the mitochondrion to the cytoplasm, creating $\Delta\bar{\mu}H$ across the inner mitochondrial membrane (negative and alkaline in the mitochondrial matrix). Then H^+-ions go back to the matrix down $\Delta\bar{\mu}H$ through an H^+–ATPase (ATP-synthetase) system

localized in the same membrane and ATP is formed from ADP and P_i [7,8,8a,b].

The electrochemical-potential of H^+-ions consists of an electrical component (transmembrane electric potential difference, $\Delta\psi$) and a chemical component (gradient of H^+-concentration, ΔpH). Both $\Delta\psi$ and ΔpH have been demonstrated across coupling membranes of many types (inner mitochondrial membranes, chloroplast thylakoid membranes and bacterial membranes). A method of universal applicability for the detection of $\Delta\psi$ in intact organelles (mitochondria and chloroplasts) and bacterial cells or their particles has been developed by our group [9,10]. It was suggested by Dr E. A. Liberman and consists of measuring the transmembrane distribution of synthetic penetrating ions.

It was found that penetrating cations and anions differing strongly in their structures move through coupling membranes in opposite directions. This process, defined as transmembrane electrophoresis [11], was found to be supported by electron-transfer via any of four energy coupling sites of the respiratory chain as well as by ATP-hydrolysis. Similar relationships were revealed in experiments with chloroplast and bacterial membrane systems. In chloroplasts and photosynthetic bacteria, $\Delta\psi$ was detected also by measuring electrochromic shifts in absorption spectra of carotenoids and chlorophyll [12–14]. A light-dependent $\Delta\psi$ was observed by means of a micro-electrode inserted into a chloroplast in vivo [15].

To exclude any possibility of the electrical events across mitochondrial, chloroplast and bacterial membranes being a secondary consequence of the energization of a complex system like the cell or organelle, we attempted to demonstrate the electrogenic activity of purified lipoproteins incorporated into an artificial membrane [16–20]. The lipoprotein was incorporated into spherical phospholipid membrane vesicles and the resulting proteoliposomes were associated with a planar phospholipid membrane through Ca^{2+}-ions. Addition of an appropriate energy source utilizable by the lipoprotein was shown to induce generation of both an electrical potential difference and a current across the planar membrane. These were measured with a conventional electrometer and macro-electrodes immersed in the electrolyte solutions on both sides of the planar membrane. A similar procedure allowed electrical generation, by intact bacterial chromatophores associated with a planar membrane, to be measured. Maximal values of $\Delta\psi$ found were 240 mV for bacteriorhodopsin from *Halobacterium halobium*, 215 mV for the light-dependent system of cyclic electron-transport from *Rhodospirillum rubrum*, 100 mV for cytochrome oxidase from beef heart mitochondria, 70 mV for *R. rubum* pyrophosphatase and about 40 mV for mitochondrial and *R. rubrum* H^+–ATPases (at currents of 1×10^{-11}–1×10^{-12} A).

It was shown in several laboratories that electrophoretic movement of any penetrating ion across a coupling membrane, down an electrical potential gradient, results in the conversion of $\Delta\psi$ to ΔpH (for reviews, see refs. [8,21,22]). The pH outside the vesicles was measured with a sensitive pH-electrode or using pH-dependent changes in the light absorption or fluorescence of some non-penetrating dyes. Penetrating pH-indicators and detergent treatment were used to determine the pH inside the vesicles.

2.3. *Identity of 'non-phosphorylated intermediate' and H^+-potential ($X \sim Y = \Delta\bar{\mu}H$)*

In the first detailed presentation of the chemiosmotic theory, Mitchell [8] considered the following 'energy transfer chain': e^- transfer via the redox-chain

$$\text{coupling site} \rightarrow \Delta\bar{\mu}H \rightarrow X \sim Y \rightarrow \text{ATP}.$$

However, the inclusion of $X \sim Y$ in this pathway was no more than a tribute to the traditional view of the mechanism of energy coupling. Later Mitchell [23] and Glagolev [24] put forward schemes not involving $X \sim Y$. Indeed, there are no experimental observations indicating that 'non-phosphorylated intermediate' is a high-energy chemical compound. Any attempts to identify $X \sim Y$ with known high-energy substances failed. Moreover, several pieces of evidence were obtained suggesting ATP to be the only covalent high-energy compound in the 'energy transfer chain' (for review, see refs [25,25(a)]). On the other hand, $\Delta\bar{\mu}H$ seems to meet requirements for the component whose place had previously been occupied by $X \sim Y$, because:

(i) $\Delta\bar{\mu}H$ can be formed by the redox-chain in the absence of ADP and P_i, the process being resistant to oligomycin.

(ii) Interconversion between $\Delta\bar{\mu}H$ and ATP is sensitive to oligomycin.

(iii) $\Delta\bar{\mu}H$ is a common product of the forward electron-transfers via the four energy coupling sites and can be responsible for energy-exchange between these sites; it can be involved in the respiration-supported reverse electron-transfer in an oligomycin-resistant fashion.

(iv) $\Delta\bar{\mu}H$ supports ion-transport through coupling membranes.

(v) $\Delta\bar{\mu}H$ runs down in the presence of protonophorous uncouplers [22].

3. $\Delta\bar{\mu}H$-Supported chemical work

This includes synthesis of high-energy phosphates (ATP and PP_i) and reduction of compounds of negative redox-potential by means of the reverse electron-transfer in the redox chains. It was shown that systems of ATP-production and reversal of electron-transfer can utilize $\Delta\bar{\mu}H$ generated by (i) forward electron-transfer and (ii) addition of penetrating ions and/or changing pH outside the vesicles (for review, see ref. [26]). Non-specificity of the source of $\Delta\bar{\mu}H$ in the reaction of ATP-formation was clearly shown by Racker and Stoeckenius [26(a)] who demonstrated light-dependent ADP phosphorylation in proteoliposomes made of beef heart H^+–ATPase, *H. halobium* bacteriorhodopsin and soya-bean phospholipids.

It was observed [10,22,27] that NADPH oxidation by NAD^+ in submitochondrial particles and bacterial chromatophores produces $\Delta\psi$ of the same direction as the redox-chain and H^+–ATPase, whereas NADH oxidation by $NADP^+$ forms $\Delta\psi$ of the opposite direction. This observation allowed the energy-linked ($NADH \rightarrow NADP^+$) transhydrogenase reaction to be explained as the $\Delta\bar{\mu}H$-supported reverse electron-transfer via an additional (fourth) energy coupling site [8,22].

Reversible interconversion of $\Delta\bar{\mu}H$ and PP_i energy is of especial interest. The rate of this process, e.g., in chromatophores, is very high (a rather surprising fact since pathways of PP_i energy utilization in metabolism are not numerous). Such a relationship may be accounted for if we assume that PP_i behaves as a '$\Delta\bar{\mu}H$-buffer'. Indeed, the electrical capacity of coupling membranes is too low to store a large amount of energy as $\Delta\psi$. pH-Buffers can be used to store $\Delta\bar{\mu}H$-energy only if $\Delta\psi$ is first converted to ΔpH. It seems to be obvious that the level of $\Delta\bar{\mu}H$, when it is mainly in the form of $\Delta\psi$, requires some additional stabilizing mechanism when the activities of $\Delta\bar{\mu}H$-producing and $\Delta\bar{\mu}H$-consuming pathways are changing. In these conditions, $\Delta\bar{\mu}H$-supported conversion of P_i to PP_i, which can be easily converted back to P_i and $\Delta\bar{\mu}H$, might function as the $\Delta\bar{\mu}H$-stabilizing mechanism.

4. Osmotic work by $\Delta\bar{\mu}H$

Conversion of $\Delta\bar{\mu}H$ to concentration-gradients of compounds penetrating membranes can also act as a $\Delta\bar{\mu}H$-buffer. If a decrease in activity of $\Delta\bar{\mu}H$-generating enzymes takes place, efflux of penetrating cations from mitochondria, accumulated earlier in the matrix down an electrical gradient, should prevent $\Delta\bar{\mu}H$ from being lowered for some time. However, this is hardly the main function of the $\Delta\bar{\mu}H$-supported ion-gradients. Osmotic work by $\Delta\bar{\mu}H$ is of great importance for any living cell being responsible for the unequal distribution of many substances between intra- and extracellular spaces (microorganisms) or between intra- and extraorganelle compartments of protoplasm (plant and animal cells). At present, all known processes of uphill transport of ionized and non-ionized substances through mitochondrial and chloroplast membranes have been shown to be supported by $\Delta\bar{\mu}H$ or by one of its constituents, $\Delta\psi$ or ΔpH. It is clear now that $\Delta\bar{\mu}H$ is the driving force for many transport processes in bacteria (for review, see ref. [28]). Such a relationship seems to be specific for coupling membranes. Other membrane structures utilize ATP-energy directly to perform osmotic work, e.g., Ca^{2+}-transport into sarcoplasmic reticulum, or phosphorylation-linked transport of sugars or Na^+/K^+-antiport across animal-cell outer membranes. In the latter case, a number of compounds can be accumulated in the cell down ΔpNa by symport with Na^+ [21,22].

5. $\Delta\bar{\mu}H$-Supported motility of bacteria

It has been unambiguously established that mechanical work performed by animal systems can be supported by contractile protein-mediated ATP-

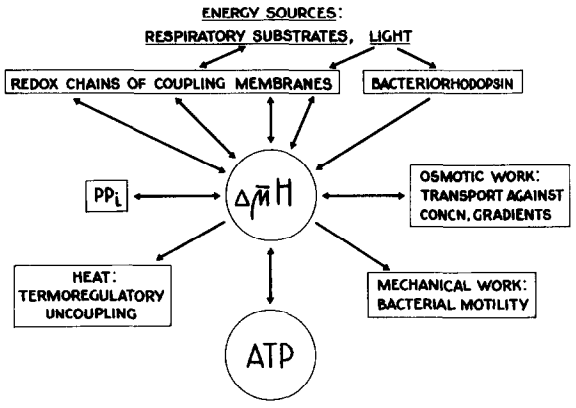

Fig.1. The central place of the transmembrane electrochemical-potential of hydrogen ions ($\Delta\bar{\mu}H$) in the system of the membrane-linked energy conversions.

hydrolysis. This was shown, in particular, for motility of spermatozoa and unicellular animals. One might think that motility of bacteria by means of flagella might be explained in the same way. However, the structures of the bacterial flagellum and, for example, that of the tail of the spermatozoon are quite different, the first being much smaller and simpler. The bacterial flagellum is made of many identical protein subunits (flagellin) demonstrating no ATPase (or any other enzymatic) activity. Mitchell [29,30] put forward a hypothesis that motility of bacteria by means of flagella is due to direct utilization of $\Delta\bar{\mu}H$ generated across the bacterial membrane. Accordingly, H^+-ions, pumped out from bacteria by the redox-chain or H^+–ATPase, return back to the cytoplasm via a flagellum which is postulated to be giant ionophore. Since H^+-ions are extruded through all the surface of the bacterial cell and return down $\Delta\bar{\mu}H$ via a single flagellum only, an ion-current appears along the cell surface which can, according to the hypothesis, cause movement of the bacterium along the long flagellum axis.

In 1974 Larsen et al. [31] and independently Thipayathasana and Valentine [32] demonstrated that mutants of *Escherichia coli* and *Salmonella typhimurium* possessing no H^+–ATPase and, hence, no oxidative phosphorylation, can support their motility by respiration or nitrate reduction. Glycolysis was not an energy source for motility in these mutants. Additions of arsenate decreased the intracellular ATP level to $< 0.3\%$ of normal but had no effect on motility. On the other hand, addition of a protonophore, *m*-chlorocarbonylcyanide phenylhydrazone (CCCP), induced immediate and complete inhibition of motility, although the ATP-level remained unaffected [31]. These data were confirmed by an observation on flagellum rotation: D-lactate oxidation was found to support rotation even in the presence of arsenate-induced ATP-exhaustion. CCCP switched off the rotation (see [31]). The authors concluded that 'an intermediate of oxidative phosphorylation', rather than ATP per se, is the energy source for bacterial motility [31] and the 'energy-transducing ATPase' is required for anaerobic but not for aerobic motility [32]. If $\Delta\bar{\mu}H$ is the oxidative phosphorylation intermediate and H^+–ATPase is involved in ATP → $\Delta\bar{\mu}H$ energy transformation, we can conclude that the bacterial motility studied was supported by $\Delta\bar{\mu}H$. However, it remained obscure whether this conclusion is of general importance or it is only true for the above mutants of two heterotrophic bacteria.

Recently, Dr A. N. Glagolev in this laboratory has studied the effects of inhibitors on the motility of *Rhodospirillum rubrum* wild strain. It was found that *R. rubrum* can obtain energy for motility by the four following means:

(i) Light-dependent (photosynthetic electron transfer)

(ii) Rotenone-sensitive (respiration)

(iii) Oligomycin-sensitive (glycolysis + H^+–ATPase)

(iv) Fluoride-sensitive (glycolysis + H^+–PPase).

The first energy source was studied in detail. This process was found to be resistant to oligomycin, rotenone and fluoride and very sensitive to CCCP. Valinomycin (+ K^+) or a synthetic cationic penetrant, tetraphenyl phosphonium (TPP^+) decreased the rate of movement of the bacterium. Motility was completely inhibited by addition of a penetrating weak acid, e.g., acetate, together with valinomycin + K^+ or TPP^+. In the absence of a penetrating cation, acetate was without effect. These facts suggest that *R. rubrum* motility can be supported by both constituents of $\Delta\bar{\mu}H$, namely $\Delta\psi$ (which is discharged by cationic penetrants) and ΔpH (which is discharged by penetrating weak acids).

In agreement with such a conclusion, it was found that the rate of motility of *R. rubrum* cells closely

correlates with the level of membrane-potential. In these experiments, tetraphenyl ammonium accumulation by the bacteria was used as the $\Delta\psi$-probe, light as the energy source and *o*-phenanthroline or antimycin A as the inhibitors of the light-dependent electron transfer. No correlation of the motility-rate and the ATP-level was found. It was also shown that motility can be actuated by a rapid change in the extracellular pH (from 8.5–5.5) in the absence of any usable energy source other than artificially formed ΔpH between the medium and the cytoplasm. The motility ceased in 1–2 min, most probably due to ΔpH-dissipation.

It should be mentioned that the above data support only that part of Mitchell's hypothesis of bacterial motility which considers $\Delta\bar{\mu}H$ as the driving force. However, the intimate mechanism by which $\Delta\bar{\mu}H$ actuates motility remains obscure. It seems to me that an ion-flow along the bacterial membrane is hardly an effective mechanism. It may be more probable that motility is a result of flagellum rotation induced by a rotating movement of the basal membranous protein structure connected with the flagellum. In its turn, the rotation of the basal structure may be a result of the downhill H^+-movement, via this structure, from medium to cytoplasm.

It seems interesting to consider the possibility that a $\Delta\bar{\mu}H$-supported type of biological movement is used by systems other than bacteria, e.g., by intracellular organelles.

6. $\Delta\bar{\mu}H$ and regulatory heat production

It is known that the regulatory heat production by warm-blooded animals, induced by their exposure to cold, is due partly to actomyosin-mediated ATP hydrolysis ('shivering thermogenesis'). Other mechanisms of additional heat generation in cold conditions are defined as 'non-shivering thermogenesis' which is inherent in animals adapted to low temperatures.

In some cases non-shivering thermogenesis occurs by means of respiratory energy dissipation with no ATP involved. Beyer et al. [33] and Smith and Fairhurst [34] have described some decrease in P/O ratio of liver mitochondria of rats acclimatized to cold for several weeks. In our group [35] it was shown that 15 min cold exposure of rats adapted to cold treatments results in desorption of a portion of the cytochrome *c* pool from the inner membrane to the intermediate space of liver mitochondria. This solubilized cytochrome *c* functions as a shuttle between the NADH-specific flavoprotein–cytochrome b_5 pathway in the outer membrane and cytochrome oxidase in the inner membrane. At the same time skeletal-muscle mitochondria, having no cytochrome b_5, respond by uncoupling of the phosphorylating pathway [4,37]. A similar mechanism was revealed in brown-fat tissue which is specialized in thermoregulatory heat production [38]. The mechanism of this phenomenon, called 'thermoregulatory uncoupling' [4], includes cold-induced release of noradrenalin followed by lipolysis of fat both in brown-adipose tissue and muscles. This results in an increase in the concentration of unesterified fatty acids in both cytosol and mitochondria [22,42]. Fatty acids enhance the H^+-conductance of the mitochondrial membrane, dissipating the $\Delta\bar{\mu}H$ which is generated by the respiratory-chain. Nichols [39] obtained some indications that the H^+-conductance of brown-fat mitochondria is mediated by a special mechanism which is controlled by the level of extramitochondrial purine nucleotides. There are nucleotide binding sites on the outer surface of the inner membrane of brown-fat mitochondria. Their saturation by, e.g., GDP strongly decreases membrane H^+-conductance.

It seems possible that warm-blooded animals are not the only group of organisms using $\Delta\bar{\mu}H$-dissipation to form heat. In some plants, anthesis is accompanied by a strong increase of temperature in the inflorescence. For example, the temperature in the inflorescence of Eastern skunk cabbage (*Symplocarpus foetidus* L.) was found to be +15°C when the air temperature was −15°C [40]. Lowering of the ambient temperature induced an increased oxygen consumption by the inflorescences. This increase was the greater, the smaller the size of the inflorescence, in accord with Rubner's law which was previously believed to apply only to warm-blooded organisms.

In *Sauromatum* spadix, this effect was found to be associated with the loss of respiratory control in mitochondria isolated from inflorescences during the day of anthesis [41]. A day before and a day after anthesis, coupling was at its usual high level.

The fact that in such diverse objects as muscle and

brown-adipose tissues of animals and flowers of the above mentioned plants, the same type of mechanism of urgent heat production is used, i.e., $\Delta\bar{\mu}H$-dissipation or a bypass of the $\Delta\bar{\mu}H$-generating steps of respiratory-chain, may suggest that this phenomenon is of common biological significance.

7. $\Delta\bar{\mu}H$ as a transport form of energy in the cell

Consideration of possible biological functions of membrane-potential suggested to me the idea that it may play the role of a unified transportable physical form of energy in the cell, just as ATP (or more generally, $\sim P$) functions as a chemical energy carrier [42,43]. In fact, if $\sim P$ is used as the only transportable form of energy, the energy transport can be impeded by intracellular membrane systems and the high viscosity of cytoplasm. Membrane-potential spreading rapidly along the membrane may create, together with ATP-diffusion over short intermembrane distances, a united energy service in the cell.

Without question, $\Delta\bar{\mu}H$ can spread along the membrane of a single mitochondrion. One would think that if $\Delta\bar{\mu}H$-transport is limited to a single mitochondrion, it cannot be used to transfer energy over distances comparable with those existing in cells, since mitochondria are believed to be small, spherical corpuscules dispersed in the cytoplasm. This assumption is based on electron micrographs of random sections across cells and tissues. However, analysis of serial sections of some unicellular organisms has given evidence of the existence of quite another type of mitochondrion, a giant branched membranous structure of a size comparable to that of the cell itself. In particular one of the most fascinating recent publications presents a picture of a single mitochondrion reconstituted from serial sections of a flagellate (*Polytomella agilis*) cell [44]. In one of the cells studied, the authors observed a single mitochondrion which looks like a hollow sphere with many apertures, arranged at a small distance from the outer cell membrane.

The view that giant mitochondria are monsters, with which one may meet, only in quite peculiar forms of cells, was recently shaken by the discovery of such structures in a common yeast, *Saccharomyces cerevisiae* [45] and in rat liver cells [46].

The best object in which to study giant mitochondria, if they are the usual form of this organelle, might be giant cells and among them the muscle-cell is of special interest. Inside the very large cells that form muscle tissue, gradients of available energy should exist, since:

(i) Actomyosin fibrils being the main intracellular energy consumers fill the major part of the cell volume

(ii) Energy sources come from the cell periphery (from capillaries and intercellular spaces)

(iii) The distance between the edge of the cell and its core is much greater than in cells of other tissues.

To organize the $\Delta\bar{\mu}H$-conducting pathways in muscle-cell, 'super-giant' mitochondria would be required. Indeed, structures of this type have been described in diaphragm [47,48] and skeletal-muscle [49]. Mitochondrial material in the red-fibres of these tissues was found to be organized as structures of three types:

(i) Thin transverse tubules forming networks in isotropic regions of the muscle-cell, oriented parallel to the Z discs.

(ii) Longitudinal tubules crossing two or three Z discs.

(iii) Large spherical bodies, localized close to the cell edges, with branches leading to the cell core.

It was suggested that all these structures are connected, thereby organizing the united mitochondrial system of the muscle-cell [47].

Reconstitution of a rat diaphragm-cell, by Dr L. E. Bakeeva and Dr Yu. S. Chentsov in this laboratory, confirmed such an idea [49(a)]. Connections between networks, longitudinal tubules and clusters of the mitochondrial material in the cell periphery were demonstrated. The system described, defined as mitochondrial reticulum [20], seems to be an association of several giant, branched mitochondria joined without fusion of inner and outer membranes. Reconstitution of one of these giant mitochondria revealed a structure crossing a muscle-cell from one edge to the other, being parallel to a Z disc. In this structure, continuity of at least the outer mitochondrial membrane was revealed. Some branches of the mitochondrion in question were found to form specially organized contacts with adjacent giant mitochondria. At the sites of the junction of two branches, four (two outer and two inner) mitochondrial membranes were situated so close to each other that extramitochondrial and intermembrane spaces could not be seen. It is not

excluded that there is electrical conductivity between the two mitochondria through the junction site and that the mitochondrial reticulum represents a united electrical system which is equivalent to a single mitochondrion, meaning the ability to transport energy in the form of $\Delta\bar{\mu}H$. (Electrical conductivity through the junctions of the outer membranes of the adjacent animal-cells is well known.) However, if such conductivity between mitochondria is low, the intermitochondrial energy-exchange may be organized as a 'mixed relay' in which the energy is transmitted over the major part of the distance as $\Delta\bar{\mu}H$ (along the membranes of giant mitochondria) whereas the short distances at junctions of adjacent mitochondria are overcome by ATP-diffusion:

respiration → $\Delta\bar{\mu}H$ → ATP → $\Delta\bar{\mu}H$ → ATP → actomyosin contraction.

An interesting possibility may arise from the coexistence of two types of mitochondria in muscle-cells: small spherical ones, near the cell nuclei and giant ones, joined as a mitochondrial reticulum penetrating the bulk of myofibrils. The small ones may give energy-support to biosyntheses and other usual cellular functions whereas the giant mitochondria may be specialized in providing energy for muscle-contraction.

Summarizing the discussion on the energy-transport function of $\Delta\bar{\mu}H$, I should like to stress that there is a precedent for the involvement of such a system in cellular energetics. This is photophosphorylation in *H. halobium* [50–52]. This bacterium converts light-energy into $\Delta\bar{\mu}H$ by means of bacteriorhodopsin, localized in a membrane region, which contains no other proteins (purple-sheets). The light-generated $\Delta\bar{\mu}H$ is utilized for ATP-synthesis by an ATP-synthetase which must be localized in a membrane region other than the purple-sheets. Connection between sheets and ATP-synthetase-containing membrane areas can occur via $\Delta\bar{\mu}H$-transfer along the membrane over distances of thousands of Å.

8. Conclusion

The ways by which $\Delta\bar{\mu}H$ is formed and utilized are summarized in fig.1. Energy from external sources (light or respiratory substrates) may be converted to $\Delta\bar{\mu}H$. This process is supported by reducing equivalent transfer down a redox potential gradient via energy coupling sites of the photosynthetic- and respiratory-chains. In the case of bacteriorhodopsin, $\Delta\bar{\mu}H$-formation is apparently coupled to the light-induced conformation change of retinal.

$\Delta\bar{\mu}H$, when formed, can be used to support:
(i) ATP-synthesis
(ii) PP_i synthesis
(iii) Uphill transport of ions and non-ionised compounds
(iv) Cell motility (bacteria)
(v) Heat production in cold conditions

Besides this, $\Delta\bar{\mu}H$ can be utilized to move reducing equivalents against a redox-gradient (reverse electron-transfer via coupling sites) which results in NAD^+ and $NADP^+$ reduction.

To perform all these functions, energy in the form of $\Delta\bar{\mu}H$ must be transported for some distance along the coupling membrane. This distance is limited by the size of the coupling membrane vesicle, e.g., the mitochondrion. In cells possessing large mitochondria, $\Delta\bar{\mu}H$ might be transported for distances comparable to the cell size.

At present, discussions concerning membrane-potential and energy coupling problems are focused mainly on the questions:

(1) How is $\Delta\bar{\mu}H$ formed by the redox-chain?
(2) How is $\Delta\bar{\mu}H$ utilized by H^+–ATP synthetase?
(3) Is the $\Delta\bar{\mu}H$-route the only possibility for coupling electron-transfer and ADP phosphorylation?

In this paper I have tried to demonstrate another aspect of the problem, namely that whatever the results of the studies to answer the three questions above, one can already conclude that the transmembranous electrochemical-gradient of hydrogen ions is, as well as ATP, the convertible and transportable form of energy in the living cell.

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