Hypothesis

MEMBRANE-LINKED ENERGY BUFFERING AS THE BIOLOGICAL FUNCTION OF Na^+/K^+ GRADIENT

Vladimir P. SKULACHEV

Department of Bioenergetics, A.N. Belozersky Laboratory of Molecular Biology and Bioorganic Chemistry, Moscow State University, Moscow 117234, USSR

Received 8 December 1977

1. Introduction

Development of the Mitchellian chemiosmotic concept of bioenergetics [1–4] resulted in the very important feature of the cell economy being revealed. It was disclosed that there is not one but two convertible forms of energy in the cell. One of them is the well known family of the high-energy compounds headed by ATP, and the other is transmembrane electrochemical potential of H^+ ions ($\Delta\bar{\mu}H^+$) [5]. It was shown that in a coupling membrane there are several types of the $\Delta\bar{\mu}H^+$ generators and several ways to utilize generated $\Delta\bar{\mu}H^+$. To perform such a function, energy equivalents of $\Delta\bar{\mu}H^+$ per se and/or of component(s) equilibrated with $\Delta\bar{\mu}H^+$ must be present in the amount sufficiently large to buffer the fluctuations of rates of the $\Delta\bar{\mu}H^+$-producing and $\Delta\bar{\mu}H^+$-consuming processes. Below, a hypothesis will be put forward, according to which transmembrane gradients of K^+ and Na^+ function as energy buffers.

2. Bacterial systems

2.1. *Why must $\Delta\bar{\mu}H^+$ be buffered? Ionic gradients as $\Delta\psi$ buffers*

Transmembrane uphill H^+ transport results in formation of $\Delta\bar{\mu}H^+$ composed of electrical ($\Delta\psi$) and chemical (ΔpH) constituents. As calculations show, the primary form of energy stored by a $\Delta\bar{\mu}H^+$ generator must be $\Delta\psi$. Indeed, the membrane capacitance ($\sim 1\ \mu F/cm^2$) is too low for the transported H^+ ions, required for capacitance charging, to shift pH in the solutions on either side of the membrane. The amount required is as low as 1 μmol H^+ ions/mg protein, which is commensurable with the amount of enzymes in the coupling membrane [3,4]. To store membrane-linked energy in a 'substrate', rather than in a 'catalytic' quantity, one must discharge the membrane by means of electrophoretic transmembrane movement of any charged species but H^+.

Flow of a charged penetrant through the membrane down electric gradient discharges the membrane and hence allows an additional portion of H^+ ions to be translocated by $\Delta\bar{\mu}H^+$ generators. If the amount of the penetrant is sufficiently large, H^+ concentrations in the membrane-separated compartments change, and ΔpH forms. The appearance of an H^+ concentration gradient accompanies that of a gradient of the penetrant. As a result, the energy is stored as ΔpH and $\Delta\log$[penetrant].

Now the quantity of the stored energy depends on:

(i) pH buffers capacity of the system.

(ii) Amount of the penetrant in the compartment which the penetrant leaves to be transported electrophoretically.

For a bacterial cell, the limiting factor should be pH buffer capacity (which is much higher than the electric capacity of the membrane) if the penetrant is a common cation. The cation in question, according to the hypothesis presented below, is potassium.

It is postulated that initial steps of the membrane-linked energy storage in bacteria can be described by eq. (1):

$$\text{energy sources} \xrightarrow{\text{uphill } H^+ \text{ transport}} \Delta\psi \xrightarrow{\text{electrophoretic } K^+ \text{ transport}} \Delta\text{pH}, \Delta p\mathit{K} \quad (1)$$

K^+ extrusion from the cell down K^+ concentration gradient should prevent $\Delta\psi$ from being lowered when $\Delta\bar{\mu}H^+$ generators are switched off for some time. So, ΔpK can function as a $\Delta\psi$ buffer. However, the cell solving one of its problems in this way, will be confronted with two new problems.

1. Since the intracellular volume is negligible compared to the volume of the environment, a ΔpH formation across bacterial membrane means alkalization of the cytoplasms at a practically constant pH level outside the cell. If $\Delta\psi$ formed by $\Delta\bar{\mu}H^+$ generators is completely transduced to ΔpH, the pH level inside the cell (pH_i) must be as high as 11 with the pH outside being neutral. Even partial $\Delta\psi \rightarrow \Delta pH$ transduction results in pH_i shifts which can be unfavourable for metabolism. So, stabilizing the membrane-linked energy level we destabilize such an important parameter of cytoplasm as pH. To store energy at low pH changes, high capacity of intracellular pH buffers is needed. In this case, K^+ accumulation should entail an increase in the concentration of deprotonated forms of pH buffers rather than H^+ concentration decrease. However, this hardly solves the problem as pH buffers are enzymes, metabolites etc. Deprotonation of these compounds may result in unfavourable changes of their properties important for their biological functions. To overcome this difficulty, substances possessing no other functions but pH buffering should exist.

2. A large increase in the concentration of a univalent cation inside the cell is another consequence of operation of the above postulated mechanism. This change in intracellular conditions, like changes in pH and degree of protonation of cytoplasmic pH buffers of the cell interior, cannot be without consequences for cell functioning.

2.2. *Cation/proton exchange as a ΔpH buffer*

Both problems mentioned may be solved if ΔpH, formed due to K^+ accumulation inside the cell, is used to extrude some other cation, say Na^+, by a nigericin-like mechanism [6] of the cation/H^+ antiport (eq. 2):

$$\Delta pH \xrightarrow{Na^+_{in}/H^+_{out}\ \text{antiport}} \Delta pNa \qquad (2)$$

This system:

(i) Decreases alkalization of the cell interior.

(ii) Results in lowering the intracellular concentration of a univalent cation (Na^+) which compensates for an increase in that of another univalent cation (K^+).

Under conditions unfavourable for $\Delta\bar{\mu}H^+$ generator activity, downhill Na^+ influx accompanied by H^+ efflux might temporary prevent ΔpH disappearance.

ΔpH buffering by Na^+/H^+ exchange system has an obvious advantage over non-specialized intracellular pH buffers since the effect is achieved without any changes in protonation and hence in the properties of important intracellular compounds.

Interplay of a K^+ transport system as a $\Delta\psi$ buffer and an Na^+/H^+ exchange mechanism as a ΔpH buffer can greatly facilitate utilization of the energy accumulated in gradients of each of these ions. Let us consider conditions when $\Delta\psi$ is formed due to efflux of pre-accumulated K^+. In this case, performance of $\Delta\bar{\mu}H^+$-dependent work, coupled with an H^+ translocation into the cytoplasm down $\Delta\psi$ must result in acidification of the cell interior, and hence in $\Delta\bar{\mu}H^+$ decrease. This does not occur if H^+ ions transported into the cell when work is performed, are pumped out of the cell in exchange for Na^+_{out}.

Respectively, a work supported by Na^+ influx without K^+ efflux must produce positive charging of the cell interior due to electrogenic H^+ uptake down pH gradient formed by means of Na^+_{out}/H^+_{in} antiport. Downhill K^+ extrusion can compensate for this effect, preventing $\Delta\bar{\mu}H^+$ lowering.

2.3. *Why K^+/Na^+ system was chosen as a specialized $\Delta\bar{\mu}H^+$ buffer?*

In principle, the role of a $\Delta\bar{\mu}H^+$ buffer could be performed by a gradient of any metabolite transported down $\Delta\psi$ and/or ΔpH, e.g., a gradient of lactose which is known to accumulate in bacteria by means of symport with H^+. However, such mechanisms are hardly effective for a large-scale $\Delta\bar{\mu}H^+$ buffering. In fact, $\Delta\bar{\mu}H^+$ buffer in the cell must perform $\Delta\bar{\mu}H^+$-consuming functions independent of fluctuation of the $\Delta\bar{\mu}H^+$ production and utilization processes.

If it were lactose gradient that mainly contributes to the $\Delta\bar{\mu}H^+$ buffering, the work of, e.g., the $\Delta\bar{\mu}H^+$-supported flagellar motor of a bacterium should result in a loss of pre-accumulated lactose in response to a decrease of the $\Delta\bar{\mu}H^+$ generator activity. It

would be dangerous since lactose is an energy source for the cell.

So, $\Delta\bar{\mu}H^+$ buffering should be carried out by compounds specialized in this role, that are not directly involved in metabolism. Ions of univalent metals seem to meet this requirement. Among them, K^+ accumulation in the cytoplasm and Na^+ extrusion to extracellular space seem to be the most convenient system. It is easy to obtain a large Na^+ gradient extruding Na^+ from the cell. To do so, it is sufficient to decrease Na^+ concentration in the cell interior occupying a very small portion of the medium. As to extracellular Na^+ concentration, it is sufficiently high since Na^+ is the most common univalent cation in the environment. On the other hand, K^+, the second widespread univalent cation, may be used as a component accumulated inside the cell. Its concentration outside is not sufficiently low to hinder the search for this cation in the environment. On the other hand, it is not too high to induce a large osmotic unbalance across the cytoplasmic membrane if K^+ is accumulated in the cell down $\Delta\psi$ and Na^+ is extruded down ΔpH.

It is difficult to think of an ion pair other than K^+ and Na^+, specialized in $\Delta\bar{\mu}H^+$ buffering in bacteria. For example, Ca^{2+}/Mg^{2+} antiport can hardly perform this function because the affinities of these cations to intracellular substances are vastly different. Besides, accumulation of a bivalent cation inside the cell up to 0.1 M concentrations, as is the case with K^+, seems to be impossible without dramatic changes in the state of cytoplasm. Cl^-/F^- or Cl^-/Br^- exchanges are not expedient due to extremely low $[F^-]$ and $[Br^-]$ in the environment. Cl^-/SO_4^{2-} pair seems to be bad as many important properties of these two anions are too different.

It may be noted that to some degree electrophoresis of K^+ ions per se is sufficient to buffer $\Delta\bar{\mu}H^+$ without serious unfavourable consequences if the amount of transported K^+ is not too high to entail large-scale alkalinization of cytoplasm and deprotonation of the intracellular pH buffers.

The rate of K^+ transport may be low, which results in deduction of a small (but permanent!) percentage of the produced energy for a ΔpH generation and deprotonation of pH buffers. ΔpH, when formed, may compensate for some time for a sudden lowering in $\Delta\bar{\mu}H^+$ production rate, even if K^+ efflux is too slow to support a sufficient rate of $\Delta\psi$ generation.

Supplementing such a system with Na^+/H^+ antiport, the cell can greatly decrease $[H^+]_i$ changes. Again, as in the case of K^+ transport, Na^+ gradient may be slowly formed under the conditions of an excess of energy sources. The rate of this process should be limited by K^+ influx producing ΔpH, or by Na^+/H^+ antiporter activity. When the energy sources are exhausted, ΔpK and ΔpNa can be utilized to form $\Delta\bar{\mu}H^+$ if K^+ permeability of the membrane (and Na^+/H^+ antiporter activity, if it was low under energy excess conditions) can be increased. On the other hand, K^+ permeability cannot be high any time, otherwise K^+ electrophoresis would compete with other $\Delta\bar{\mu}H^+$-supported functions of the cell.

It would be hardy effective to buffer $\Delta\bar{\mu}H^+$ at the expense of ATP or any other high-energy compound. In fact, ATP is equilibrated with $\Delta\bar{\mu}H^+$ only at high $\Delta\bar{\mu}H^+$ levels. For example, below 180 mV, $\Delta\bar{\mu}H^+$ was found to be insufficient to support ATP formation in *Escherichia coli* [16] and *Streptococcus lactis* [6a]. At the same time, such important $\Delta\bar{\mu}H^+$ functions as any types of osmotic work, rotation of flagella, reverse electron transfer in transhydrogenase energy coupling site have no threshold $\Delta\bar{\mu}H^+$ level (reviewed [5,7]).

An interesting possibility is existence of a system specialized in $\Delta\bar{\mu}H^+$ buffering at a high (phosphorylating) level. This may be H^+-inorganic pyrophosphatase [5].

$\Delta\bar{\mu}H^+$ formation by membrane pyrophosphatase was first described in *Rhodospirillum rubrum* chromatophores [8]. Such system catalyzing reversible $PP_i \longleftrightarrow \Delta\bar{\mu}H^+$ energy transduction proves very active in this bacterium. In fact, it is more active than H^+-ATPase. As to other systems utilizing PP_i energy to perform work, they are certainly much less numerous and active than those utilizing ATP.

3. Some predictions following from the hypothesis

The above hypothesis predicts several features of membrane energetics, which must be universal for the kingdom of bacteria.

(1) Carrier(s) or channel(s) responsible for electrophoretic K^+ efflux and electrogenic K^+ efflux

must exist in the cytoplasmic membrane of bacteria.

(2) In the same membrane, Na^+/H^+ antiporter(s) must be localized.

(3) Activity of systems (1) and (2) must be regulated in such a way that K^+/Na^+ exchange across the bacterial membrane, which is slow under energy excess, should be strongly stimulated when $\Delta\bar{\mu}H^+$ tends to decrease.

(4) Different $\Delta\bar{\mu}H^+$-linked functions of the bacterial cell can be supported by K^+ efflux and Na^+ influx under conditions favourable for stimulation of systems (1) and (2).

4. Observations supporting the hypothesis

To testify this hypothesis in toto, special studies of bacterial energetics are required. Yet, some relevant observations can be quoted.

There are several lines of evidence of an electrophoretic K^+ transport through the bacterial cytoplasmic membrane (reviewed [9]). Among them the data of Epstein's group are especially interesting [10]. It was found that in *E. coli* there is an electrophoretic system of K^+ accumulation (the so called TrkF system), which is characterized by a relatively low rate and unsaturable dependence of this rate upon the outer K^+ concentration. It is the TrkF system that is responsible for K^+ transport if:

(i) Outer K^+ level is not too low.

(ii) ATP and/or $\Delta\bar{\mu}H^+$ levels are below a certain critical value.

If both [ATP] and $\Delta\psi$ are sufficiently high, another K^+ transport system (TrkA) is actuated which directly utilizes the energy of ATP, and probably $\Delta\psi$ energy too. This system is saturable (K_m 1.5 mM) and its activity is very high (V_{max} 550 μg ions K^+/g min). The TrkF and TrkA systems are constitutive. Growth under K^+-deficient conditions induces formation of a third (Kdp) system of a very high affinity to K^+ (K_m 0.002 mM, V_{max} 150 μg ions K^+/g min). This system uses ATP, requires a periplasmic protein and is operative even in the absence of $\Delta\bar{\mu}H^+$.

In terms of the above concept, the TrkF system is necessary to accumulate K^+ and transduce a part of $\Delta\psi$ to ΔpH at a usual $[K^+]_{out}$ and a suboptimal level of energy production in the cell. Since this system is of low rate and affinity to K^+, it cannot compete with other energy-consuming processes, so that only a small portion of the energy produced is utilized for the uphill K^+ influx. Cessation of $\Delta\bar{\mu}H^+$-generating systems may induce efflux of the pre-accumulated K^+ and hence $\Delta\psi$ buffering.

When the activity of the energy-producing mechanism exceeds that of the energy-consuming one, ATP and $\Delta\bar{\mu}H^+$-levels are maximal and TrkA system switches on to transport K^+ much faster and up to a higher gradient than TrkF. As a result, larger amount of energy can be stored as ΔpK. The $\Delta\psi$-generating efflux of K^+ in this case, as well as in the case of Kdp system, might occur via TrkF system, since $[K^+]_{in}$ is high, and this is favourable for TrkF having an unsaturable $[K^+]$ dependence.

Another example of the above type of regulation was demonstrated by Harold in *Streptococcus faecalis* [9,11–13]. K^+ accumulation in the studied *S. faecalis* stain was found to be electrophoretic, being supported by $\Delta\psi$ formed by H^+-ATPase utilizing glycolytic ATP. It was shown that not only K^+ accumulation but also $^{42}K^+/K^+$ exchange requires glycolysis, and this effect is not abolished by an H^+-ATPase inhibitor, DCCD. The simplest explanation for this fact is that ATP is an allosteric activator of the K^+ carrier. When the ATP level is low, large amounts of energy cannot be spent on K^+ transport since the K^+ carrier is inactive. Only if there is an excess of energy, K^+ transport is actuated, and a large-scale $\Delta\bar{\mu}H^+$-buffering takes place.

Harold mentioned [9] that Na^+/H^+ antiport in *S. faecalis* seems to require also ATP as an allosteric activator. As to energy source for this exchange, when it occurs against Na^+ concentration, it is ΔpH, so that Na^+ flux is directed from the cell interior to the extracellular medium [9,12].

There are several observations indicating ΔpK-supported ATP formation in bacteria loaded with K^+ and then suspended in K^+-free medium containing valinomycin (reviewed [9]). The only question is whether this effect takes place without valinomycin. A precedent of this kind was apparently furnished by Wagner and Oesterhelt [14] who noted that a downhill K^+ efflux stabilizes the ATP level in *Halobacterium*

halobium cells without valinomycin. The authors concluded that the K^+ gradient in *H. halobium* can be an energy reserve for ATP synthesis. However, it is difficult to exclude that this property is a feature specific for extremal halophilic bacteria.

In the same paper [14], capacities of ΔpK and ATP as energy buffers were compared. It was found that switching on the light, actuating light-dependent bacteriorhodopsin $\Delta\bar{\mu}H^+$-generators, in addition to H^+ pumps of the respiratory chain and ATPase operating in the dark, gives rise to an increase in:

(i) $\Delta\psi$ by about 20 mV.
(ii) ΔpH by 0.5 unit.
(iii) ATP by 2.8 mmol/kg cell water.
(iv) $[K^+]_{in}$ by 1500 mmol/kg cell water.

Assuming H^+-ATPase to transport $2H^+$/ATP, we may conclude that the ratio of the capacity of the ΔpK energy to that of the ATP energy is 750:2.8, or approx. 250:1. Again, such a high ratio may be specific for halobacteria containing about 4 M KCl solution inside the cell. However, the above change in $\Delta\psi$ was only about 20%. Higher $\Delta\psi$ changes must induce much higher changes in $[K^+]_{in}$ which exponentially depends upon $\Delta\psi$ (e.g., in the above experiment, 20% increase in $\Delta\psi$ from the original level of about 100 mV resulted in doubling $[K^+]_{in}$).

Wagner and Oesterhelt [14] have also measured the $\Delta\psi$ value across *H. halobium* membrane as a function of $[K^+]_{out}$. The data confirm the conclusion that K^+ movement is electrogenic. Very high ion specificity of K^+ transport system was demonstrated. Again, as in the cases of *E. coli* and *S. faecalis*, fast K^+ uptake could be observed only when the energy sources were in excess. Namely, the K^+ influx reached its maximum at much higher light intensities than ATP synthesis did [15].

According to my hypothesis, existence of a Na^+ gradient directed from outside should be favourable for ΔpK-supported performance of a work. This may explain why ATP synthesis coupled with K^+ efflux from *E. coli* cells in the presence of valinomycin is well demonstrated in a Na^+ containing medium [16,17].

With *H. halobium* it was shown [18–21] that the ΔpNa energy can be used for the uphill transport of many amino acids into the cell, which seems to be coupled with downhill Na^+ influx. In the same bacterium, Na^+/H^+ antiport has been directly demonstrated [22,23].

5. Applicability of the hypothesis to eukaryotes

5.1. *$\Delta\bar{\mu}H^+$ buffering in cytoplasmic membranes of fungi and plants and in chloroplasts*

There are some indications of $\Delta\bar{\mu}H^+$ production across cytoplasmic membrane of fungi and plants (this process was studied mainly in unicellular organisms; reviewed [24]).

There is no reason or evidence now why we cannot extend the $\Delta\bar{\mu}H^+$ buffering hypothesis to cytoplasmic membrane of these organisms. On the other hand, a mechanism of an animal type (see below) is not excluded either.

As to the thylakoid membrane of chloroplasts, it is well known that $\Delta\bar{\mu}H^+$ exists mainly in the form of ΔpH. The $\Delta\psi \longrightarrow \Delta pH$ energy transduction is due to extrusion of Mg^{2+} and, to a lesser extent, of K^+ from the intrathylakoid space, charging positively under energized conditions [25,26]. Cl^- movement in the opposite direction was also revealed [27–29]. Apparently, Mg^{2+} cannot cross the outer chloroplast membrane, so that the $\Delta\psi$-driven Mg^{2+} redistribution between intra- and extrathylakoid compartments of the chloroplast does not essentially change the Mg^{2+} level in the cytoplasm outside chloroplasts.

ΔpH formed across the thylakoid membrane should chiefly be due to acidification of the intrathylakoid space, rather than to alkalinization of the extrathylakoid space of the chloroplasts, since the former is much smaller. It is important that almost all enzymatic processes of chloroplasts are localized outside thylakoids or on their outer surface. Therefore, large-scale pH changes inside thylakoids hardly affect the metabolic pattern of chloroplast. Maybe, this is the reason why the energetics of the chloroplast, unlike that of bacterial cells, is not complicated with $\Delta pH \longrightarrow \Delta pNa$ energy transduction. Another reason may be that conditions in which the organelle exists in the cytoplasm, are certainly much more constant than those of the bacterium.

5.2. *Ion transport in mitochondria*

Energy supply of mitochondria must be even more constant, than that of chloroplasts which entirely depends upon illumination. This is probably why in mitochondria, $\Delta\psi$ comprises a larger portion of $\Delta\bar{\mu}H^+$ compared to chloroplasts. Nevertheless, some ΔpH is always present in the energized mitochondria [4]. It

is most probable that electrophoretic movement of K^+ and Ca^{2+} into mitochondria (negative inside) mainly contributes to $\Delta\psi \longrightarrow \Delta pH$ transition.

It is not clear whether mitochondria need a specialized K^+ carrier (or channel) for electrophoretic K^+ movement. Maybe, at $\Delta\psi$ and $[K^+]_{out}$ as high as 0.2 V and 0.1 M, respectively, leakage of K^+ through the mitochondrial membrane proves sufficient to provide the necessary $\Delta\psi \longrightarrow \Delta pH$ transition.

According to Mitchell [2], mitochondria usually face the problem of preventing electrophoretic K^+ influx since K^+ equilibration with $\Delta\psi$ must result in K^+ concentration in the matrix up to 10 M or even higher. Certainly, this would be catastrophic for mitochondria. Mitchell postulated an K^+/H^+ antiporter removing K^+ from matrix down pH gradient [2] and experimental indications of activation of such process at acidic pH were reported [3].

Above I have dealt with a favourable consequence of formation of K^+ gradient across mitochondria membrane, and as this may have a functional importance it will be not surprising if there is a K^+ carrier in mitochondria catalysing the process. If it were the case, a possibility of regulation of K^+ flux would arise. An indication of this kind was recently obtained by Évtodienko et al. [30] who succeeded in isolating a component of the phospholipid nature, inducing electrogenic K^+ transport across bilayer phospholipid membrane. The concentration of this component was found to increase in mitochondria under the conditions when their K^+ conductivity is elevated. Apparently, reversible formation and decomposition of the above K^+ carrier are involved in steady oscillations of mitochondrial functions [30].

ΔpH formed across the mitochondrial membrane due to electrophoretic transport of K^+ as well as Ca^{2+} and some other cationic penetrants of cytosol, is stabilized by pH buffers of both cytosol and mitochondrial matrix.

In a tissue working in a fluctuating regime, i.e., in skeletal muscle, specialized pH buffers, carnosine and anserine, seem to be involved in stabilizing the intracellular H^+ concentration.

5.3. *Specialized pH buffers of the cell – carnosine and anserine*

In skeletal muscles of various animals there are dipeptides, β-alanyl histidine (carnosine) and β-alanyl methylhistidine (anserine) whose concentration can be as high as 0.1 M. The biological function of these compounds is still obscure, although the former was discovered by Gulevitch and Amiradzhibi 77 years ago [31]. In 1953 Severin et al. [32] described the phenomenon of carnosine stimulation of isolated frog muscle contraction. It was found that carnosine addition to Ringer solution surrounding the muscle, initiates the contractions which disappeared due to fatigue. Contractile activity was found to be maintained by carnosine for a long time. Analysis of metabolites in the control and carnosine-treated muscles revealed only one striking difference: in the samples with carnosine, lactate concentration was almost one order higher, whereas ATP, ADP, phosphate and creatine phosphate levels were of the same order of magnitude as in the control. The carnosine effect could be demonstrated both with direct and indirect muscle irritations. A special study showed that the carnosine action cannot be explained by any immediate specific effects on glycolytic enzymes [33].

So, it seems that carnosine simply allows muscle to accumulate lactate up to very high concentrations (about 0.1 M). The mechanism can be explained if we consider that one carnosine pK value is 6.85 [34,35] and it can simply neutralize H^+ ions produced due to acidic dissociation of lactate (pK 3.85). pK of anserine is 7.05 [34,35]. Carnosine + anserine buffer capacity at neutral pH comprises about 40% total pH buffering in the muscle [35].

Consider:

(1) A very high concentration of carnosine and anserine in muscle.
(2) Their pK values.
(3) The above effect on lactate accumulation.
(4) The absence of any metabolic or regulatory functions of these compounds, which could explain such a large content of dipeptides.

We can conclude that, most probably, carnosine and anserine are specialized pH buffers of the muscle tissue, as was postulated by Bate Smith [35] and Davey [36]. It is to be asked whether there are other compounds specialized in this function.

5.4. *Na^+/K^+ gradient across outer membrane of animal cell*

In animal cells, $\Delta\bar{\mu}H^+$ generation is inherent in only one type of membrane structure, namely inner

membrane of mitochondria, whereas other membranes are specialized in functions other than energy production. In particular, the outer cell membrane performs the role of the system responsible for:

(i) Osmotic work of the cell.

(ii) Regulation of the cell activity by extracellular factors.

It was found (reviewed [37]) that one type of mechanism of osmotic work in this membrane utilizes $\Delta\bar{\mu}Na^+$ as the driving force. Usually, an electroneutral metabolite is translocated together with Na^+ across the outer cell membrane into cytosol, moving down electrical gradient (minus inside the cell) and Na^+ concentration gradient $[Na^+]_{out} > [Na^+]_{in}$). $\Delta\bar{\mu}Na^+$ is generated by Na^+, K^+-ATPase carrying out $3Na^+_{in}/2K^+_{out}$ antiport per 1 ATP hydrolyzed. Most probably, the energy storage in this system, occurs in the following way. $\Delta\bar{\mu}Na^+$ is primarily formed as $\Delta\psi$ due to the electrogenic character of the $3Na^+/2K^+$ antiport (eq. (3)). Then $\Delta\psi$ is converted to ΔpNa and ΔpK, mainly because of the high K^+ permeability of the outer cell membrane (electrophoresis of K^+ ions to the cell interior, eq. (4)). Thus K^+ transport seems to play the same role of the $\Delta\psi$-utilizing system in outer membrane of animal cells as that postulated above for the coupling membranes.

$$ATP \xrightarrow{\text{uphill } 3Na^+_{in}/2K^+_{out} \text{ antiport}} \Delta\psi \qquad (3)$$

$$\Delta\psi \xrightarrow{K^+_{out} \text{ electrophoresis}} \Delta pNa, \Delta pK \qquad (4)$$

It is to be questioned why Na^+:K^+ stoichiometry of Na^+, K^+-ATPase is not 1, so that electrophoresis of K^+ ions via some additional channel (or carrier) is necessary to store energy in 'substrate' amounts. A possible answer is that such mechanism allows to work in two different regimes, i.e., the first, when the K^+ channel is closed and the membrane becomes ready to act immediately after energy is furnished, and the second, when the K^+ channel is open so that a lag phase, required for ΔpNa to be formed, appears between the energy source addition and the work. The first regime is fast but unstable, the second slow but protected against fluctuations.

It is important that $\Delta\bar{\mu}Na^+$ can be used as a transportable form of energy transmitted along the outer membrane of the cell and even from one cell to another via the so-called gap junctions of the cell membranes which were found to be highly permeable to low molecular weight compounds, in particular to K^+ and Na^+ ions [38,39].

It is also well known, that in excitable tissues, ΔpNa plays the key role in the process of excitation transmission. Excitation was shown to arise due to large increase in the Na^+ permeability of the cell membrane resulting in Na^+ influx which entails the membrane depolarization.

In fact, the function of Na^+/K^+ gradient in nerve excitation was understood much earlier than their role in the energy supply of the osmotic work of the animal cell. This is probably, why the biological significance of unequal cation distribution across the cell membrane is traditionally believed to be associated with such functions as regulatory, signalling and so forth, rather than with energetics.

However, the large energy expenditures required for the Na^+/K^+ gradient formation (as high as 1/3 ATP utilized by an average 'resting' cell [40]) if connected solely with a regulatory or signalling process, might only be understandable for a cell specialized, e.g., in signal transmission. It is obvious that Na^+/K^+ gradient is a feature inherent in any living cell rather than in a cell adapted to some specific type of work. Therefore it seems reasonable to suggest that the role of Na^+/K^+ gradient in the excitation process is secondary, and there is another, basic function accounting for this universal biological phenomenon. The function in question, I suppose, is energy buffering.

One might think that Na^+ ions are incompatible with life and this is the reason why K^+ is substituted for Na^+ in the cell interior. Apparently, it is not the case as, e.g., in halophilic bacteria $[Na^+]_{in}$ can reach 2 M [41]. The very fact that some enzyme systems work better in the presence of K^+ than of Na^+, may be considered as a secondary adaptation of enzymes to the K^+-rich and Na^+-poor conditions in the cytosol [40]. Besides, it would have been dangerous to couple any work performance with Na^+ influx to the cytoplasm if Na^+ were a cell poison.

6. Conclusion

The hypothesis described above postulates Na^+/K^+

gradient across cellular membrane to be a component required to buffer the membrane-linked energy produced primarily in the electrical form.

1. In the absence of an electrophoretic transmembrane flow of a charged species, very small (catalytic) amount of an energy source proves sufficient to charge the membrane capacity and form $\Delta\psi$ by means of an H^+ extrusion from the cell. The process is catalyzed by $\Delta\bar{\mu}H^+$ generators in cytoplasmic membrane of bacteria as well as, perhaps, of fungi and plant cells.

2. Electrophoretic K^+ influx (down $\Delta\psi$) results in $\Delta\psi$ being converted to ΔpH. This is a large-scale process since the capacity of the cell pH buffers is many orders higher than the electric capacity of the membrane. In this case, high ('substrate') amounts of the energy source should be used to change the total $\Delta\bar{\mu}H^+$ capacity of the system. As a result, the system becomes more resistant to short-term fluctuations in the activities of energy-producing and energy-consuming mechanisms.

3. ΔpH, when formed, can be converted to ΔpNa by means of a Na^+/H^+ antiporter extruding Na^+ from the microbial cell in exchange for H^+. Thus:
   (i) Further enlargement of the $\Delta\bar{\mu}H^+$ capacity takes place.
   (ii) An increase in the intracellular concentration of a univalent cation (K^+) proves compensated to some degree by decrease in the concentration of another univalent cation (Na^+).

4. In the outer animal cell membrane, the membrane-linked energy buffering is achieved by operation of the Na^+, K^+-ATPase-mediated $3Na^+/2K^+$ antiport followed by electrophoresis of K^+ ions.

The formed ΔpNa is then used to support osmotic work of the animal cell membrane and, in excitable tissue, the excitation process.

Acknowledgements

I would like to express my gratitude to Dr A. N. Glagolev, Dr A. A. Konstantinov and Dr I. A. Kozlov for discussion, Ms N. M. Goreyshina for the help in the preparation of the manuscript and Ms T. I. Kheifets for correcting the English version of the paper.

References

[1] Mitchell, P. (1961) Nature 191, 144–148.
[2] Mitchell, P. (1966) Chemiosmotic Coupling in Oxidative and Photosynthetic Phosphorylation, Glynn Research, Bodmin.
[3] Mitchell, P. (1968) Chemiosmotic Coupling and Energy Transduction, Glynn Research, Bodmin.
[4] Mitchell, P. (1977) FEBS Lett. 78, 1–20.
[5] Skulachev, V. P. (1977) FEBS Lett. 74, 1–9.
[6] Ovchinnikov, Yu. A., Ivanov, V. T. and Shkrob, A. M. (1974) Membrane-active complexones, Elsevier, Amsterdam.
[6a] Maloney, P. C. and Wilson, T. H. (1975) J. Memb. Biol. 25, 285–310.
[7] Skulachev, V. P. (1972) Energy Transformation in Biomembranes, Nauka, Moscow.
[8] Isaev, P. I., Liberman, E. A., Samuilov, V. D., Skulachev, V. P. and Tsofina, L. M. (1970) Biochim. Biophys. Acta 216, 22–29.
[9] Harold, F. M. (1977) Curr. Top. Bioenerg. 6, 83–149.
[10] Rhoads, D. B. and Epstein, W. (1977) J. Biol. Chem. 252, 1394–1401.
[11] Harold, F. M. and Papineau, D. (1972) J. Membr. Biol. 8, 27–44.
[12] Harold, F. M. and Papineau, D. (1972) J. Membr. Biol. 8, 45–62.
[13] Harold, F. M. and Altendorf, K. H. (1974) Curr. Top. Membr. Transp. 5, 2–50.
[14] Wagner, G. and Oesterhelt, D. (1976) Ber. Deutsch. Bot. Ges. 89, 289–292.
[15] Oesterhelt, D., Gottschlich, R., Hartmann, R., Michel, H. and Wagner, G. (1977) Soc. Gen. Microbiol. Symp. 27, 333–349.
[16] Wilson, D. M., Alderate, J. F., Maloney, P. and Wilson, T. H. (1976) J. Bacteriol. 126, 327–337.
[17] Maloney, P. C., Kashket, E. R. and Wilson, T. H. (1974) Proc. Natl. Acad. Sci. USA 71, 3896–3900.
[18] Lanyi, J. K., Renthal, R. and MacDonald, R. E. (1976) Biochemistry 15, 1603–1610.
[19] Landyi, J. K., Yearwood-Drayton, V. and MacDonald, R. E. (1976) Biochemistry 15, 1595–1603.
[20] MacDonald, R. E. and Lanyi, J. K. (1975) Biochemistry 14, 2882–2889.
[21] Lanyi, J. K. and MacDonald, R. E. (1977) Biophys. J. 17, 32a.
[22] Lanyi, J. K. and MacDonald, R. E. (1976) Biochemistry 15, 4608–4614.
[23] Eisenbach, M., Cooper, S. H., Garty, H., Johnstone, R. M., Rottenber, H. and Caplan, S. R. (1977) Biochim. Biophys. Acta 465, 599–613.

[24] Harold, F. M. (1978) Ann. Rev. Microbiol. in press.
[25] Chow, W. S., Wagner, G. and Hope, A. B. (1976) Aust. J. Plant Physiol. 3, 853–861.
[26] Barber, J., Telfer, A., Mills, J. and Nicolson, J. (1974) Proc. 3rd Intern. Congr. Photosynth. (Avron, M. ed) pp. 53–63, Elsevier, Amsterdam.
[27] Rotterberg, H., Grunwald, T. and Avron, M. (1972) Eur. J. Biochem. 25, 54–63.
[28] Rotterberg, H. and Grunwald, T. (1972) Eur. J. Biochem. 25, 71–74.
[29] Hind, G., Nakatani, H. Y. and Izawa, S. (1974) Proc. Natl. Acad. Sci. USA 71, 1484–1488.
[30] Gyekhandanyan, A. V., Evtodienko, Ju. V., Zhabotinsky, A. U. and Kondrashova, M. N. (1976) FEBS Lett. 66, 44–47.
[31] Gulewitch, W. S. and Amiradzhibi, S. (1900) Ber. Deutsch. Chem. Gesellsch. 33, 1902.
[32] Severin, S. E., Kirson, M. V. and Kaftanova, T. M. (1953) Dokl. AN SSSR 91, 691–696.
[33] Qureshi, Y. and Wood, T. (1962) Biochim. Biophys. Acta 60, 190–192.
[34] Deutsch, A. and Eggleton, Ph. (1938) Biochem. J. 32, 209–211.
[35] Bate Smith, E. C. (1938) J. Physiol. 92, 336–343.
[36] Davey, C. L. (1960) Arch. Biochem. Biophys. 89, 303–308.
[37] Mitchell, P. (1970) Soc. Gen. Microbiol. Symp. 20, 121–166.
[38] Loewenstein, W. R. (1973) Fed. Proc. Fed. Am. Soc. Exp. Biol. 32, 60–64.
[39] Bennett, M. V. L. (1973) Fed. Proc. Fed. Am. Soc. Exp. Biol. 32, 65–75.
[40] De Witt, W. (1977) Biology of the Cell, W. B. Saunders Co., Philadelphia, London, Toronto.
[41] Ginzburg, M., Sachs, L. and Ginzburg, B. Z. (1970) J. Gen. Physiol. 55, 187–207.