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REVIEW

Redox Potential Changes in Bacterial Cultures under Stress Conditions

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Abstract—Oxidation/reduction potential (ORP, redox potential, or Eh) is one of the physicochemical parameters characterizing the state of microbial cultures. Changes in pH and concentration of the redox-active gases (O_2, H_2, and H_2S) in the cultivation medium are assumed to be the major factors of redox potential changes in the cultures of aerobic microorganisms. In the review, results of the studies of redox potential changes in various bacterial cultures under various stress conditions are summarized. The characteristic feature of these stress factors is the absence of direct correlation between the redox potential, on one hand, and partial oxygen pressure and pH, on the other. Extracellular low-molecular weight thiols (LWT) were demonstrated to be the major contributors to such changes in the redox potential. The possible role of the changes in LWT concentrations inside and outside the cells in the processes of signal transduction and redox regulation of cellular functions is discussed.

Keywords: redox potential, bacteria, stress factors, low-molecular weight thiols.

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The process of monitoring the growth and development of microorganisms in biotechnology and scientific experiment includes measurement and registration of the parameters characterizing the state of microbial cultures. Oxidation/reduction potential (ORP, redox potential, or Eh) is one of these parameters. Redox potential has been studied in microbiology for over a century, and a large amount of data has been accumulated on its behavior in microbial cultures and practical application [1–4]. Despite the long history of redox potential exploration and its extensive application, little is known of the physicochemical reactions underlying the redox potential changes during growth and development of microbial cultures, as well as of the molecular mechanisms of the Eh affecting microbial growth and development.

In relatively simple systems, ORP theory and methods of measurement have been well developed and now provide the basis for understanding numerous processes in bioenergetics and electrochemistry. The same is not true for the ORP investigation in complex biological systems, particularly in microbial cultures. Analysis of the results previously obtained indicates that the situations when redox potential changes are caused by the changes in the medium pH or the concentration of the redox-active gases like O_2, H_2, and H_2S are studied the best. The nature of other endogenous redox agents that may be secreted into the medium and affect its redox potential is less studied [1–3].

An important role in investigation of the nature of the redox potential belongs to the development of techniques of oxygen measurement in aqueous solutions. Simultaneous measurement of redox potential, pH, and partial oxygen pressure (pO_2) suggested a conclusion that oxygen content was the major factor determining the Eh value in the cultures of aerobic microorganisms [3]. This conclusion also had somewhat negative consequences, resulting in a considerable decrease in interest in the investigation of the redox potential in microbial cultures [5].

It should be noted that in most works investigating the redox potential, the results were obtained for Eh changes in batch cultures where the temperature, stirring rate, and oxygen supply rate in the bioreactor maintained constant throughout the cultivation, while oxygen concentration, pH, and composition of the medium changed gradually in the course of the culture development as a result of its activity. In natural environments, however, the microorganisms grow under constantly changing parameters, which often exceed the optimal range. Thus, in the life cycle of enterobacteria, extremely low pH values, sunlight irradiation, sharp changes in osmotic pressure, temperature, etc. may occur sequentially.

In the review, the results of investigation of Eh variations in a number of bacterial cultures under various stress conditions are described. Under a stress impact,

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the absence of direct correlations between the changes in Eh and in pH and partial oxygen pressure was the characteristic feature which drew the attention of researchers.

STARVATION STRESS

Registration of the redox potential in aerobic cultures of *Escherichia coli* M17 and K12 grown in M9 medium revealed stepwise changes in Eh in the region of negative (reductive) values, which were not associated directly with the changes in pH and partial oxygen pressure (pO_2) in the medium [6–8]. These Eh leaps were registered upon bacterial growth cessation upon exhaustion of glucose, lactose, or glycerol as the limiting substrates and sole sources of carbon and energy in the medium. The first rapid phase started immediately after the growth stopped and lasted for 10–25 min. If the limiting substrate was not added into the medium after cessation of growth, the second phase lasting 40–150 min started when Eh value returned linearly to the initial level. If the limiting substrate was added at any phase of the leap, Eh value rapidly returned to the initial level and bacterial growth was resumed. The maximum amplitude of the leap ($\Delta Eh = 150$ mV) was registered in *E. coli* K12 culture. The Eh drop to negative values upon cessation of growth due to exhaustion of glucose was also registered in a culture of *E. coli* B [9].

The profile of the leap (amplitude and duration of the phases) depended upon the cultivation conditions, strain, and surface properties [7, 10]. Changes in the redox potential were associated both with the emergence of the soluble redox-active compounds in the medium and the interaction of the platinum electrode with cell surfaces [11, 12]. In anaerobic cultures, Eh leap upon growth cessation was not observed [7]. The amplitude of the leap decreased sharply upon decrease in K^+ concentration in the medium below 2×10 μM. Decrease in the concentrations of Ca^{++} and Na^+ to trace amounts did not affect the ability of the cells to generate the Eh leap [10, 11].

Stepwise fall of Eh values was observed upon *E. coli* growth cessation caused by exhaustion of ammonium or after treatment of the cells with chloramphenicol, tetracycline, or valine [7, 13]. The latter one stopped growth causing isoleucine starvation [14]. In case of *E. coli* diauxic growth on a mixture of glucose and lactose, two sequential Eh leaps were observed, each one corresponding to exhaustion of one of the substrates [11].

No decrease in the redox potential was observed upon exhaustion of glucose (or another source of carbon and energy) in aerobic cultures of auxotrophic *E. coli* strains grown in complex media containing meat-peptone broth or protein hydrolysates. Under these conditions, the observed Eh changes reflected mainly the changes in oxygen concentration. However, when these strains were grown in glucose–mineral medium supplemented with essential amino acids, an Eh leap was also observed upon exhaustion of the growth-limiting amino acid.

Apart from *E. coli*, a reversible Eh leap under starvation stress was revealed in aerobic cultures of another gram-negative bacterium, *Serratia marcescens* [15]. Eh changes in mixed cultures of *E. coli* and *S. marcescens* are of particular interest. Under prolonged co-culturing of the bacteria, growth cessation caused by glucose exhaustion resulted in Eh increase, in contrast to its decrease in pure cultures. This inversion of the Eh leap was observed even when the share of *S. marcescens* in the mixed population did not exceed 1% [15]. The phenomenon makes it possible to use Eh registration in bacterial cultures for early detection of contamination.

In gram-positive bacteria *Bacillus subtilis* and *B. megaterium*, a characteristic Eh fall to negative values upon growth cessation after glucose exhaustion was also observed [12]. Under these conditions, in gram-positive bacteria, unlike *E. coli*, in the second phase of the leap was absent, i.e., the Eh drop was irreversible until the growth was resumed with glucose supply renewed. This observation suggests that the leap profile may be not only strain-specific, but also species-specific.

The differences in the redox potential profile under starvation stress in gram-positive and gram-negative bacteria may be explained by their different behavior during transition to the stationary phase. In aerobic cultures of *E. coli* upon glucose exhaustion, the cells start to utilize organic acids (acetate, succinate, and lactate) accumulated in the process of glucose metabolism [16]. When glucose was added, bacterial growth resumed rapidly. This ability to resume growth upon glucose addition to aerated *E. coli* cultures is retained during long periods of the stationary phase. In contrast to *E. coli*, in the cultures of *B. subtilis* and *B. megaterium* glucose exhaustion stimulated sporulation and metabolism rearrangements required for it [17]. This fact probably explains the absence of reversibility of the Eh leap upon glucose exhaustion in gram-positive bacteria.

The redox potential behavior in *B. subtilis* cultures under starvation stress caused by ammonium exhaustion against excess glucose is also of interest (Fig. 1) [12]. This situation is known not to promote sporulation. Growth cessation in this case was also accompanied by a drop in redox potential. If the bacteria had been in a stationary phase long enough, after the drop, Eh returned gradually to the initial values. Upon addition of NH_4Cl, the bacteria resumed growth and 10–25 min later, two progressive rises in Eh were registered. Apparently, this was a consequence of synchronous cell division attained by the cells upon long ammonia starvation since both the growth rate and oxygen consumption decreased during these latter Eh leaps.

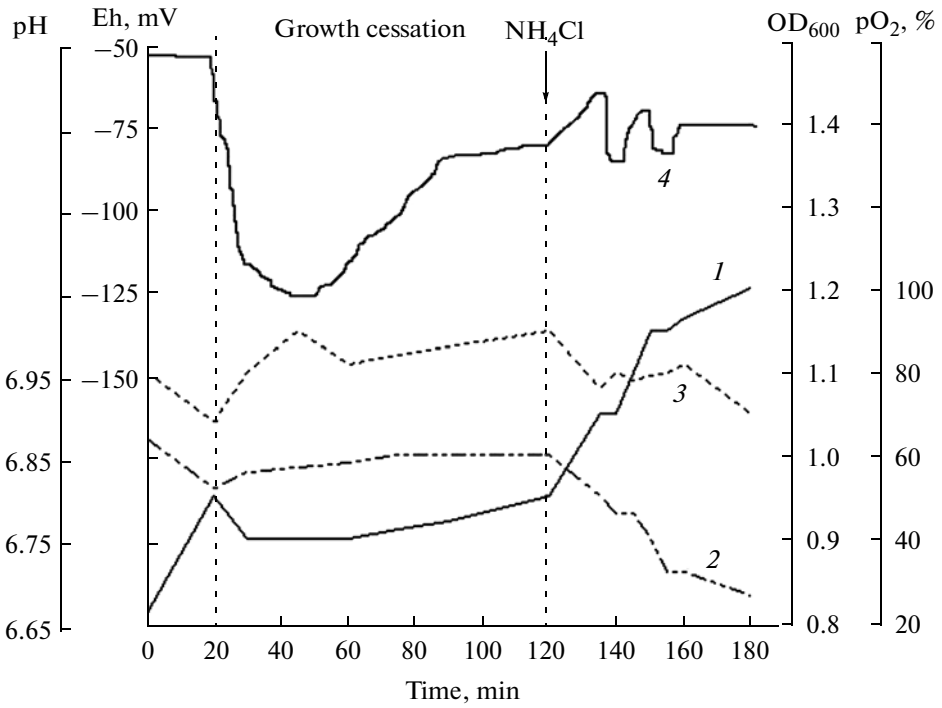

Fig. 1. Parameters of *B. subtilis* VKM428 aerobic culture during starvation stress induced by ammonium exhaustion. Optical density (OD_{600}) (*1*), pH (*2*), pO_2 (*3*), and Eh (*4*). See text for explanation. Adapted from [12].

The following data evidence participation of the extracellular low-molecular weight thiols (LWT) in the generation of Eh leaps under starvation stress:

1. In M9 medium, magnesium sulfate is the sole source of sulfur for thiol synthesis. Under decrease of sulfate concentration below 0.1 mM, an inversion in the Eh leap was observed; that is, glucose exhaustion in the medium was accompanied not by a drop in Eh values but by its increase [18].

2. Addition of SH reagents such as *N*-ethylmaleimide (NEM), *p*-chloromercuribenzoate (pCMB), or dithionitrobenzoate (DTNB) in the concentrations of 0.1–1.0 mM at any growth phase stimulated recovery of the initial Eh value. Notably, addition of DTNB led to the relief of redox potential alterations at any pH, including pH 8.0. Since DNTB at pH 8.0 acts as a cell-impermeable SH reagent [19], this observation evidences that an increase in the extracellular thiols causes an Eh leap.

3. Close correlation between Eh changes and the number of available SH groups outside the cell was revealed in *E. coli* and *B. subtilis* cultures upon exhaustion of glucose or ammonium [13, 18, 20]. This correlation was observed in both the cultures of *E. coli* and *B. subtilis* and in the filtrates obtained form these cultures [18].

EFFECTS OF CHANGES IN $\Delta\mu H^+$ AND INTRACELLULAR pH

The transmembrane difference in the electrochemical potentials of hydrogen ions ($\Delta\mu H^+$) and its constituents, namely, the transmembrane difference in hydrogen ion concentration (ΔpH) and the transmembrane difference in electric potentials ($\Delta\Psi$), play an important role in the interrelated processes of energy generation, transport, and intracellular pH homeostasis [21–24]. Observation of redox potential leaps upon treatment of growing *E. coli* cells with sodium acetate or propionate (Fig. 2) is therefore of particular interest. Eh changes induced by acetate correlated with SH group concentration in the medium, were prevented by sulfhydryl reagents, and were absent at pH above 7.5 [13, 20, 25].

Being a weak, penetrating acid, acetate may be distributed between the medium and the cell at acidic pH according to the chemical gradient of protons (ΔpH). It crosses the membrane in an undissociated form and, after it reaches cytoplasm, it releases a proton thus decreasing the intracellular pH (pH_{in}) and ΔpH [26]. At $pH_{out} \sim 7.5$, ΔpH is close to 0 and $\Delta\mu H^+$ is represented by the difference in electric potential values [27, 28]. Under these conditions, acetate does not influence the ΔpH and $\Delta\Psi$ values and does not affect the intracellular pH. At pH above 7.5, ΔpH direction prevents acetate entry into the cell in amounts required for changes in pH_i, ΔpH, and $\Delta\Psi$. These data imply that the reason for the acetate-induced Eh leap

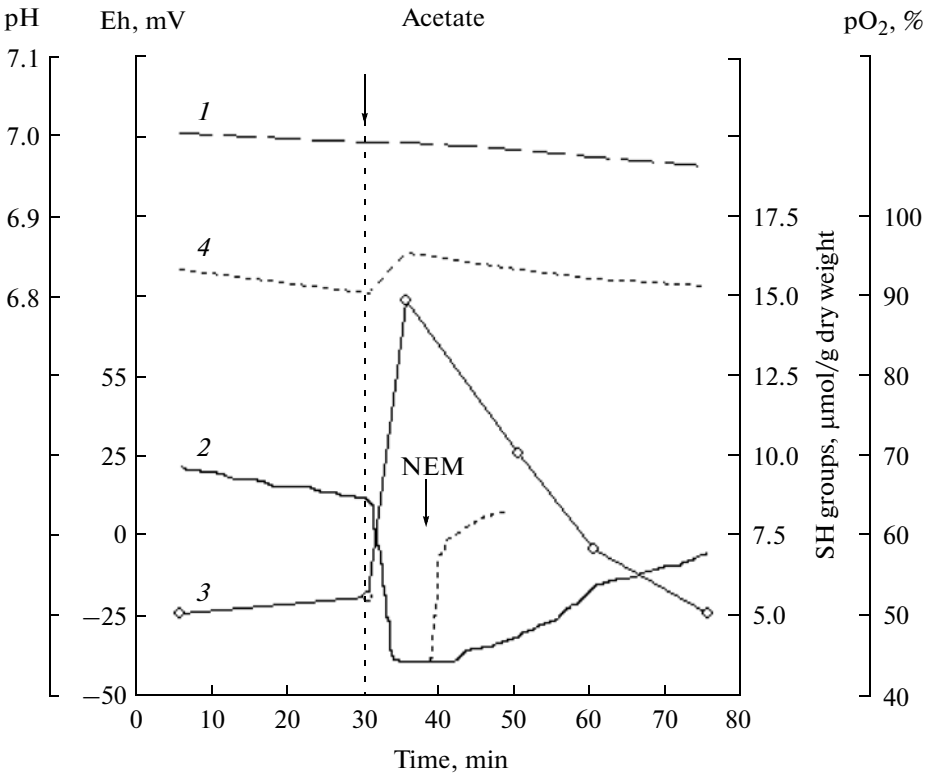

Fig. 2. Parameters of *E. coli* K12 growing culture upon addition of acetate: pH (*1*), Eh (*2*), number of available SH groups (*3*), and pO_2 (*4*). Acetate (50 mM) addition is indicated by an arrow. Bacteria were grown in M9 medium supplemented with glucose, pH 7.0. Adapted from [20].

may be the change in ΔpH and ΔΨ and/or acidification of the cytoplasm. *E. coli* cells are characterized by high sensitivity to the changes in intracellular pH and their growth is completely inhibited at pH_{in} 6.6–6.8 [24]. It should be noted that treatment with acetate or other weak organic acids is a strong stress for bacteria which induces growth inhibition and activates expression of a number of genes, as well as metabolic rearrangements [16, 29–33].

In a growing culture of *E. coli*, a protonophore carbonyl cyanide *m*-chlorophenyl hydrazone (CCCH) at pH 7.0, similar to acetate, induced an Eh drop to negative values which was in turn inhibited by NEM. In the medium with pH 7.5–7.6 no Eh change was registered in response to CCCH; in other words, at pH_{out} below 7.6 the protonophore acted the same way as acetate. At pH_{out} 8.0, addition of CCCH to the growing culture resulted in an Eh increase [20]. Protonophores like CCCH at pH of 7.0 induced partial or complete relief of both ΔpH and ΔΨ and therefore cytoplasm acidification [27]. At pH_{out} 8.0, the protonophore induces elimination of ΔpH and ΔΨ and therefore, cytoplasm alkalinization. Therefore, the protonophore induced an Eh drop upon cytoplasm acidification and, on the contrary, its rise under alkalinization. No noticeable changes in pH or pO_2 which would have been capable of the adequate Eh changes were observed upon treatment with CCCH. It should be noted that an ATPase inhibitor dicyclohexylcarbodiimide (DCCD) at pH below 7.3 also induced an Eh drop to negative values [34].

Some data indicate that changes in $\Delta\mu H^+$ and intracellular pH may be associated directly or indirectly with the Eh drop upon starvation stress. Magnitude of the Eh leap upon *E. coli* cell transition to the stationary phase after glucose exhaustion depended on the pH of the medium and therefore the ΔEh value was minimal at pH of ~7.5, that is, when ΔpH = 0 [27, 28]. Decrease and increase in pH_{out} resulted in an increase of ΔEh. In another experiment, it was found that in *E. coli* culture treated with CCCH at pH 7.0 glucose, exhaustion led to Eh rise; that is, the platinum electrode response was opposite to that in normal bacterial cultures not treated with the protonophore [18]. As was mentioned above, Eh changes described in this section correlated with the changes in extracellular LWT. Subsequent direct measurements demonstrated that changes in the concentration of the tripeptide glutathione may be the main reason for Eh changes in *E. coli* under factors affecting the intracellular pH and $\Delta\mu H^+$ [35].

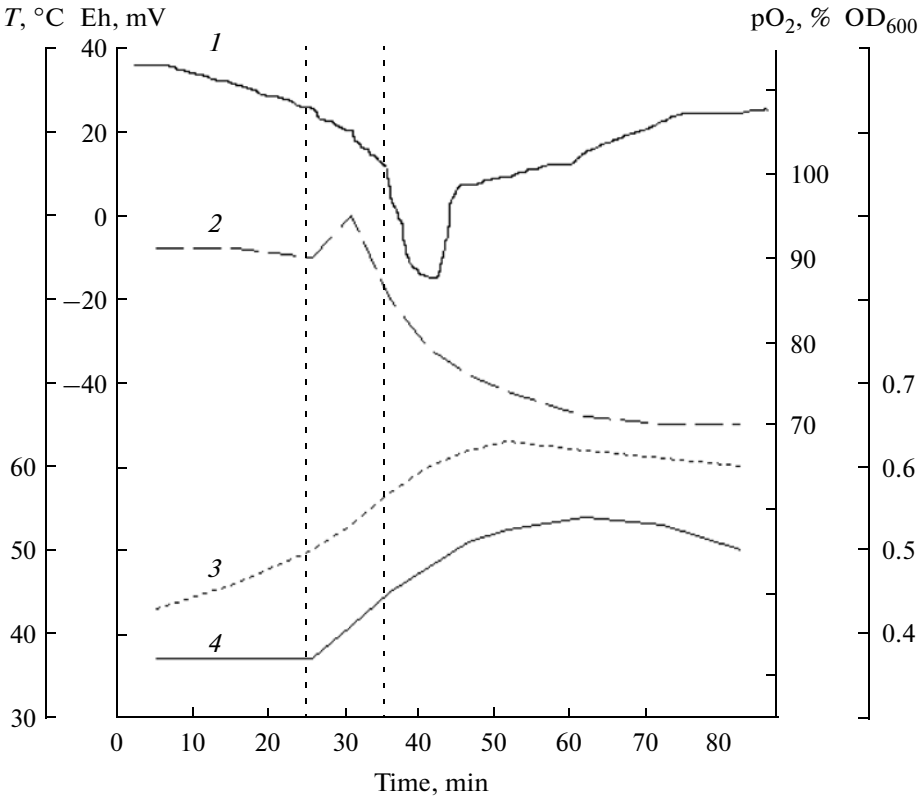

Fig. 3. Effect of heat shock on *E. coli* K12 culture grown aerobically [36]: Eh (*1*), pO_2 (*2*), OD_{600} (*3*), and temperature, °C (*4*).

HEAT SHOCK

Growth slowdown accompanied by a characteristic Eh fall to negative values was observed in an aerobic culture of *E. coli* heated gradually from 37 to 46°C (Fig. 3) [36]. Analysis of the culture parameters demonstrated that the Eh drop was not directly associated with the changes in pO_2 or pH of the medium. Heating of a stationary *E. coli* culture above 37°C resulted in significant Eh decrease only at 55°C. A sharp decrease in optical density was observed simultaneously, probably due to osmotic barrier dysfunction upon cytoplasm membrane damage followed by cell lysis. Sulfhydryl reagents added at any phase of the leap caused rapid restoration of the initial Eh value, while pretreatment of the cells with SH reagents aborted the leap generation. These data indicated the possible connection between the Eh leap in the growing *E. coli* cells under heat shock with the increasing concentration of low-molecular weight thiols outside the cells. Subsequent direct measurements demonstrated that extracellular GSH concentration increased outside and decreased inside *E. coli* cells under heat shock [37]. Interestingly, in *E. coli* cultures adapted to increased temperatures, elevated extracellular GSH was also observed [38].

Eh changes in *E. coli* upon heat shock probably reflect mainly the processes occurring upon membrane damage. It is known that the outer and cytoplasm membranes of *E. coli* cells collected at the logarithmic and stationary phases differ significantly in their physicochemical properties, which affects their physiological properties [39]. This may explain the Eh behavior under heat shock in growing and stationary cultures. The stepwise nature of Eh changes in growing cells indicates the possibility of the membrane structural rearrangement through cooperative transition from one conformational state to another. Interestingly, the temperature of the Eh leap generation in growing *E. coli* cultures (46°C) coincides precisely with the upper temperature growth limit for these bacteria that had been determined previously by measurements of bacterial growth rate at different temperatures [40]. This is another indication of the importance of continuous monitoring of Eh as a critical parameter of microbial activity.

OSMOTIC SHOCK

As it was mentioned above, Eh drop under starvation stress may be reversed by treatment of *E. coli* cells with sulfhydryl reagents. It was found that in aerobic *E. coli* cultures, the same effect is produced by hyperosmotic shock. Hyperosmotic shock also reversed the Eh drop induced by treatment of bacteria with sodium acetate, tetracycline, chloramphenicol, or valine (Fig. 4) [13]. Remarkably, osmoprotectants betaine

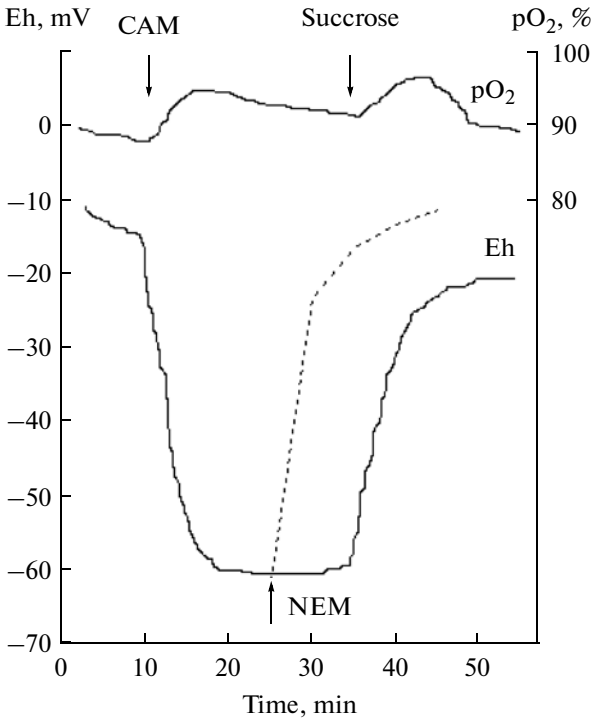

Fig. 4. Effect of osmotic shock on *E. coli* K12 growing culture pretreated with chloramphenicol (25 μg/mL; CAM). Osmotic shock was induced by addition of 0.44 M succrose or 0.3 M NaCl. Adapted from [13].

and proline induced a stepwise Eh drop in *E. coli* cultures subjected to hyperosmotic stress [13].

In *E. coli* under hypoosmotic stress, the same Eh fall to negative values eliminated by NEM was observed as under starvation stress and treatment with sodium acetate or protein synthesis inhibitors. Therefore, the Eh changes under hyper- or hypoosmotic stresses were oppositely directed. All tested compounds as such had no significant effect on the platinum electrode potential.

The following data evidence that Eh shift to positive (oxidative) values under hyperosmotic shock may be in connection with the decrease in extracellular LWTs:

1. Hyperosmotic shock reversed the Eh leap induced by starvation stress, treatment with acetate, or protein synthesis inhibitors in the same manner as thiol reagents did.

2. Any changes in Eh under osmotic shock were absent if the cells were pretreated with NEM.

3. Increase in osmotic pressure in a culture of glucose-starved *E. coli* cells caused a significant decrease in the extracellular GSH and the GSH/GSSG ratio [13]. Simultaneous decrease in the extracellular glutathione and its increase in the cytoplasm in response to hyperosmotic shock were noticed in growing *E. coli* cells [41]. It was demonstrated that together with K^+ and glutamate accumulation, increase in intracellular glutathione is characteristic of the initial stage of *E. coli* response to osmotic shock [42–44].

PHYSIOLOGICAL IMPORTANCE OF THE EXTRACELLULAR LOW-MOLECULAR WEIGHT THIOLS

Utilization of the inhibitors of SH groups and direct chemical analysis demonstrated that Eh changes observed under stress conditions reflected the changes in extracellular low-molecular weight thiols dissolved in the cultural liquid and associated with the outer surface of the cells. The data available on LWT metabolism in bacteria indicate that in tested bacterial cultures, two thiols, glutathione and cysteine, may be responsible for the major contribution to the generation of Eh leaps.

Glutathione (L-γ-glutamyl-L-cysteine-glycine, GSH) is the most abundant low-molecular weight thiol in most living organisms from bacteria to mammals. It is known that in *E. coli* GSH plays an important role in protection against electrophiles and heavy metals [45, 46], as well as in adaptation to ethanol, osmotic, and temperature stresses [36, 43, 47, 48]; however, for many of the situations it is still unclear how the tripeptide function is realized at the molecular level. Still less is known about the functions of extracellular glutathione, although the ability of *E. coli* and *S. typhimurium* to accumulate glutathione in the medium was established quite long ago [49, 50].

During the recent years, the authors carried out extensive investigation of glutathione status inside and outside the cells under various stress conditions, including those described in this review [35, 51]. Altogether, the data evidences that, in growing aerobic cultures of *E. coli*, reduced glutathione is subjected to constant transmembrane circulation between the cells and the medium. As a result of dynamic equilibrium between GSH escape and its consumption, constant GSH concentration per unit of biomass is maintained in the medium. The exact value of this concentration depends strictly on the external pH. GSH circulation occurs only in respiring cells and is hampered by the factors affecting $\Delta\mu H^+$ and ATP levels. GSH export is modified by phosphate shortage in the medium. Mutations in thiol redox systems of the cytoplasm and periplasm considerably affect the glutathione levels and its redox status in the medium and in the cells.

In *E. coli*, a number of systems has been described which could be responsible for GSH circulation. It was demonstrated that GSH transport to the medium is performed by an ATP-binding cassette transporter CydDC. Along with CydDC, an unidentified transport system, sensitive to the presence of oxygen and redox-active compounds, takes part in GSH export [52]. Import of the extracellular GSH may be performed by a γ-glutamyl transpeptidase (GGT) localized in the periplasm, as well as by the transporter which imports GSH as a whole molecule inside *E. coli*

cells. This transporter, as well as GGT, plays an important role in utilization of glutathione as a sole source of sulfur [50, 53].

A situation in bacterial life cycle is known when export of glutathione may be vital for bacteria. It was demonstrated that, upon increase in the intracellular cysteine concentration, it reduces free iron in the Fenton reaction, enhancing the production of hydroxyl radicals and inducing oxidative DNA damage [54]. Intracellular concentration of cysteine is normally maintained at a very low level; therefore, cysteine inclusion in GSH, which reduces free iron much less actively than cysteine, may be one of the mechanisms of protection against oxidative stress. Transmembrane circulation of GSH in a growing *E. coli* culture may be an element of cysteine homeostasis. Various stress stimuli may induce complete or partial inhibition of constructive metabolism and thus lead to a temporary increase in cysteine levels and, therefore, production of reactive oxygen species (ROS). Under these conditions, elimination of excesses of the potentially toxic cysteine via its incorporation in GSH and further export of the tripeptide into the medium may play an important role in cells' adaptation to stress. This process may be the cause of the Eh leaps observed upon bacteria growth cessation due to exhaustion of the growth-limiting substrate or effects of antibiotics.

E. coli possesses a multicomponent molecular mechanism which may potentially provide the transmembrane circulation of cysteine not involving glutathione. This system includes a number of transporters exporting cysteine to the periplasm and the medium, and a transporter importing cysteine from the periplasm to the cytoplasm after it has been oxidized [55, 56]. One of the transporters is induced by peroxide stress and is involved in protection against exogenous H_2O_2 [56]. These data evidence that under certain circumstances extracellular cysteine may contribute to Eh leaps in the medium upon stresses. Figure 5 demonstrates the proposed scheme of LWT circulation by means of GSH and cysteine and its effects on the redox potential in *E. coli* cultures.

It is assumed that one of the important functions of the extracellular GSH is cell protection against potentially toxic electrophiles at distant frontiers [57]. Extracellular GSH may act as a reducing agent when *E. coli* cells utilize exogenous cystine [58]. A number of observations evidenced the possible physiological role of glutathione in the periplasm as a regulator of redox reactions and an antioxidant [59, 60]. Continuous transmembrane circulation of GSH may be involved in the antioxidant protection system of the periplasm.

Another function of glutathione may be associated with its participation in control over K^+ exit channels KefB and KefC [61, 62]. In the medium of high osmolarity, mutants *kefB*, and *kefC* are characterized by lower capacity for osmoadaptation. It was noticed that positive effects of glutathione in osmoadaptation are not directly linked to its effects on K^+ retention [44]. However, a certain link between potassium flow and glutathione status may be followed. Under starvation stress, pH shifts, hyperosmotic shock, and in the presence of betaine, changes in the intracellular pools of potassium ions and LWT were demonstrated to be synchronous and co-oriented. The most profound effects were observed during the first stage of response to stresses [63, 64].

In natural environment, rapid changes in the redox potential caused by increase in LWT levels may act as a nonspecific factor of intercellular signaling [65], promoting the adaptation of the cell population to stress. In this connection, the hypothesis is of interest, which proposed to explain the role of extracellular glutathione by the example of human carcinoma cells. It is assumed that degradation of the extracellular glutathione involving γ-glutamyl transferase leads to formation of small amounts of peroxide, which is a proliferative and anti-apoptotic signal ("life signal") [66]. It was demonstrated that extracellular thiol–disulfide status considerably influences the proliferation rate in human tissue cultures [67].

Changes in Eh observed in bacterial cultures under stresses may be significant. Are these changes able to affect bacterial metabolism and behavior? The possibility of Eh role in the regulation of cellular functions has been discussed in earlier papers [1–3]. Achievements of molecular biology confirm the idea and permit a new view on the problem. For example, it was suggested that activity of some transport systems in *E. coli* may be regulated by the proton motive force via affecting the redox potential of the medium or the membrane, which involves glutathione and thioredoxin [68]. It was found that *E. coli* cells exhibited redox taxis, which included monitoring of the medium redox status and migration to the regions of optimal potential. According to the model proposed by the authors, a redox-active molecule (effector) interacts with the electron transport system (ETS) and an unknown membrane sensor detects the changes in the proton motive force or redox status of the ETS and generates the signal for the taxis [69]. In another work, it was demonstrated that the redox potential of the medium determined, to a considerable extent, the pH homeostasis and the value of proton motive force in non-growing *E. coli* cells [70]. It is assumed that the effect of Eh on the proton motive force may be mediated through modification of SH groups in energy-dependent membrane transport systems [68]. Another possible way is the difference in activities of glutathione-gated K^+ exit channels [71] that potentially may modify pH homeostasis.

In [72], it was demonstrated that a Eh decrease induced in *E. coli* culture by treatment with dithiothreitol (DTT) led to an increase in the lag phase duration and decrease in the growth rate. DTT also induced a decrease in the membrane potential ($\Delta\Psi$)

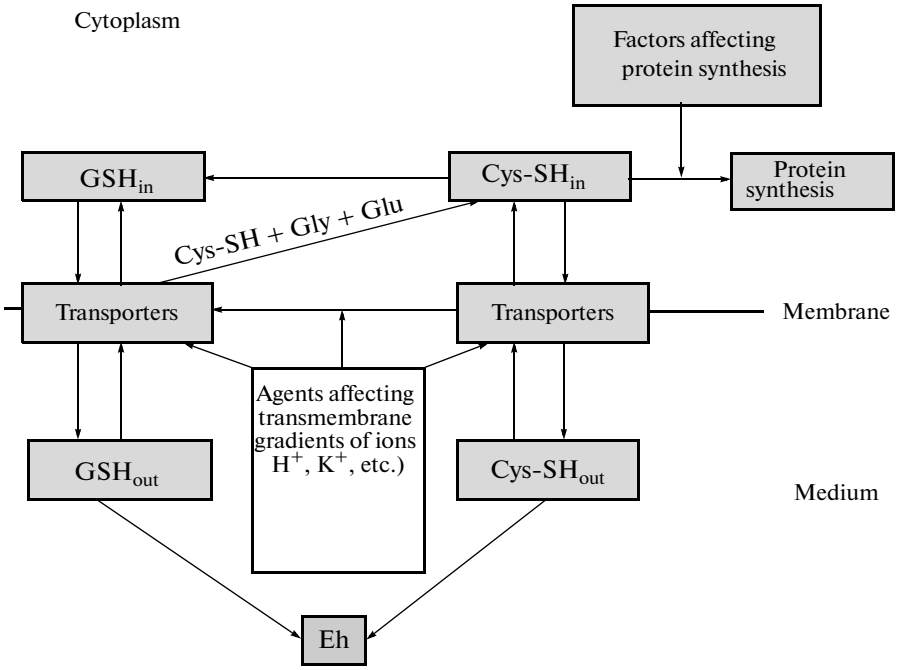

Fig. 5. Proposed scheme of the interrelation between redox potential and LWT circulation between medium and *E. coli* cells under stress. GSH_{in} and GSH_{out} are intra- and extracellular glutathione pools; Cys-SH_{in} and Cys-SH_{out} are intra- and extracellular cysteine pools; Glu, glutamate; Gly, glycine.

and inhibited K^+ accumulation. The authors assumed that thiol groups possibly played an important role in the observed effects. In *S. typhimurium*, Eh of the medium may regulate the levels of the transcription factor RpoS which controls bacterial adaptation to many stress factors [73]. It should be noted that in most papers cited above, Eh change was achieved by the addition of such redox-active compounds as DTT, ferricyanide, borohydride, etc., to the medium.

The data accumulated by molecular biology indicate that if there are molecular mechanisms of regulation of cellular activity in response to the changes in the medium redox potential, sensors capable of direct recognition of the redox changes and their transformation to regulatory signals should exist.

In *E. coli,* one of the main transcription regulators controlling the transition from aerobic to anaerobic metabolism is the cytoplasmic protein FNR comprising the sensor and DNA-binding domains. The sensor domain in the active (anaerobic) state contains a [4Fe–4S] cluster. In the presence of oxygen, FNR is inactivated due to direct interaction of the cluster with oxygen and its conversion to [2Fe–2S]. The process is sensitive to very low intracellular O_2 concentration (1 μM) [74]. Glutathione may be involved in FNR inactivation as a reducing agent [75].

Apart from FNR, a two-component system ArcAB also regulates *E. coli* switching from aerobic to anaerobic growth and is activated under low oxygen pressure. In case of oxygen shortage, the O_2-sensitive kinase ArcB is autophosphorylated and further phosphorylates ArcA, thus imparting it with the ability to bind DNA and regulate about 100 operons controlling carbon catabolism and the intracellular redox status. The sensitivity of ArcB to oxygen is related to the response to decrease in the electron flow through the respiratory chain [76]. Coupling between the respiratory chain and inhibition of the ArcB kinase activity under aerobic conditions is achieved through formation of intermolecular disulfide bonds due to specific oxidation of the two redox-active cysteine residues of the sensor by quinones [77]. Recently, it was found that ArcAB participates in RpoS regulation at the levels of transcription and proteolysis [78]. ArcB belongs to a large group of proteins containing the so-called PAS domains. As a rule, these proteins participate in signal transduction in various cell types in the course of their response to changes in the redox status, oxygen concentration, energy metabolism, etc. [79].

In prokaryotes, a number of other redox-sensitive proteins were revealed. Their oxidative modification is a signal for induction of the genes they control, or other regulatory events. The regulators of transcription OhrR, PpsR/CrtJ, and PerP, the chaperon Hsp33, and others, belong to this group. The proteins are activated by various mechanisms: the only cysteine residue of OhrR forms a stable conjugate with sulfenic acid (C_{15}–SOH); suppressors PpsR/CrtJ form a reversible disulfide bond, and Hsp33 oxidation is accompanied by formation of two disulfide bonds and

zinc release followed by the protein dimerization [80]. The transcription factor OxyR, which controls the response to peroxide, is sensitive to H_2O_2 and intracellular thiol–disulfide redox status [81]. Remarkably, activation of OxyR in *E. coli*, even by rather high H_2O_2 concentrations, is not associated with noticeable changes in intracellular glutathione and GSH/GSSG ratio [82].

Therefore, the well studied redox-sensitive regulatory systems may respond not to redox potential expressed as a ratio of all oxidized forms of the medium components to all reduced ones (red/ox), but to individual redox-active compounds or factors. As a result, high sensitivity and selectivity of the systems to their own effectors is achieved. Since a number of the regulatory systems mentioned above possess sensitive elements in both the cytoplasm and the periplasm, the Eh changes observed under stress conditions and caused by the changes in the levels and redox status of extracellular LWT may modulate the systems' response induced by specific effectors. Taking into account that the Eh and LWT level changes described in the present work are already being registered several seconds after the stress factor application, they are most likely based on physiological rather than genetic reactions. Transmembrane circulation of LWT, together with the reactions determining the direction of ion currents and intracellular pH, may comprise a considerable part of the sensor and executive mechanisms reacting rapidly to the changes in cell and medium parameters.

The results described above indicate that there is a possibility of a wider application for continuous registration of redox potential in biotechnology and scientific experiments for evaluation of the physiological state of bacteria and studies of their response to stresses.

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