STUDY OF THE EFFECT OF PROTEIN SYNTHESIS INHIBITORS ON GROWING *ESCHERICHIA COLI* BACTERIA USING ELECTROCHEMICAL SENSORS

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ABSTRACT

Background. *The study of the mechanisms of action of antibiotics requires the integrated use of traditional microbiological and physicochemical methods.*
The aim. *To study the response of Escherichia coli bacteria to the action of four antibiotics, inhibitors of protein synthesis, using combined approach.*
Methods. *Bacteria were grown under aerobic conditions on minimal M9 medium with glucose. Tetracycline, kanamycin, streptomycin and chloramphenicol have been tested. The effect of antibiotics on survival (CFU) and growth rate was determined. Respiratory activity, sulfide production, extracellular potassium, as well as pH and Eh of the medium were measured using electrochemical sensors directly in a growing culture in the "real time", membrane potential was measured using a DiBAC dye and a Leica DM2000 fluorescent microscope.*
Results. *The tested antibiotics were divided into two groups according to their properties. Tetracycline and chloramphenicol showed a pronounced bacteriostatic effect, growth inhibition began immediately after the addition of antibiotics and occurred at a high rate. Both antibiotics inhibited respiration, stimulated sulfide production and an Eh jump. Bacteria treated with tetracycline and chloramphenicol retained the ability to maintain membrane potential and intracellular potassium better. Inhibition of respiration led to a decrease in glucose catabolism, as evidenced by a lower rate of acidification of the medium compared to the control. Growth inhibition with streptomycin and kanamycin was initiated with a 30-minute delay. Both antibiotics showed a bactericidal effect, did not stimulate sulfide production and Eh jump, did not inhibit respiration, but caused a drop in membrane potential and intracellular potassium. High respiratory activity promoted glucose catabolism, as evidenced by the rapid acidification of the medium. Of interest is the detection of kanamycin-induced sulfide production during E. coli growth on MOPS medium.*
Conclusion. *An analysis of the data obtained indicates that the use of electrochemical sensors in combination with traditional methods is a promising approach to studying the mechanisms of action of antibiotics.*

Key words: *Escherichia coli, antibiotics, electrochemical sensors, sulfide, dO_2, pH, growth rate*

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ИССЛЕДОВАНИЕ ДЕЙСТВИЯ ИНГИБИТОРОВ СИНТЕЗА БЕЛКА НА РАСТУЩИЕ БАКТЕРИИ *ESCHERICHIA COLI* С ПОМОЩЬЮ ЭЛЕКТРОХИМИЧЕСКИХ СЕНСОРОВ

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РЕЗЮМЕ

Обоснование. *Изучение механизмов действия антибиотиков требует комплексного использования традиционных микробиологических и физико-химических методов.*

Цель исследования. *Изучить ответ бактерий Escherichia coli на действие четырёх антибиотиков, ингибиторов синтеза белка, используя комплексный подход.*

Методы. *Бактерии выращивались в аэробных условиях на минимальной среде M9 с глюкозой. Испытывалось действие тетрациклина, канамицина, стрептомицина и хлорамфеникола. Определяли влияние антибиотиков на выживаемость (CFU) и скорость роста. Дыхательную активность, продукцию сульфида, экстраклеточный калий, а также pH и Eh среды измеряли, используя электрохимические сенсоры, непосредственно в растущей культуре в режиме «real time», мембранный потенциал – с помощью красителя DiBAC и флуоресцентного микроскопа Leica DM2000.*

Результаты*. Испытуемые антибиотики по своим свойствам разделяются на две группы. Тетрациклин и хлорамфеникол обладали выраженным бактериостатическим действием, ингибирование роста начиналось сразу после добавления антибиотиков и происходило с высокой скоростью. Оба антибиотика ингибировали дыхание, стимулировали продукцию сульфида и скачок Eh. Бактерии, обработанные тетрациклином и хлорамфениколом, лучше сохраняли способность к поддержанию мембранного потенциала и удержанию внутриклеточного калия. Ингибирование дыхания приводило к снижению катаболизма глюкозы, о чём свидетельствовала более низкая скорость закисления среды по сравнению с контролем. Ингибирование роста стрептомицином и канамицином начиналось с 30-минутной задержкой. Оба антибиотика оказывали бактерицидное действие, не стимулировали продукцию сульфида и скачок Eh, не ингибировали дыхание, но стимулировали падение мембранного потенциала и внутриклеточного калия. Высокая дыхательная активность способствовала катаболизму глюкозы, о чём свидетельствовало быстрое закисление среды. Интерес представляет обнаружение индуцируемой канамицином продукции сульфида при росте E. coli на среде MOPS.*

Заключение*. Анализ полученных данных свидетельствует о том, что применение электрохимических сенсоров в сочетании с традиционными методами является перспективным подходом к изучению механизмов действия антибиотиков.*

Ключевые слова: *Escherichia coli, антибиотики, электрохимические сенсоры сульфид, dO_2, pH, скорость роста*

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The abrupt changes in the oxidation-reduction potential to negative values (Eh jumps) measured by the platinum electrode in aerobic cultures of *Escherichia coli* and other bacteria, growing in a minimal medium were previously detected when growth ceased due various stresses [1]. Later it was shown that these jumps are associated with a reversible increase in the level of sulfide or cysteine in the medium [2]. We also observed the accumulation of sulfide and sulfide-mediated Eh jumps during exposure of *E. coli* to certain antibiotics, including chloramphenicol, tetracycline and high doses of ciprofloxacin, while the other antibiotics, such as ampicillin, cefotaxime, kanamycin and streptomycin did not stimulate the formation of sulfide [1–3]. It was of interest to investigate the reason for the different response of bacteria to the action of various antibiotics. It was suggested that differences in the response of bacteria to treatment with different antibiotics may be related to the traits of their effect on growth and metabolic activity. To test this hypothesis, we examined the response of growing *E. coli* to three antibiotics, including tetracycline, kanamycin and streptomycin, known to inhibit protein synthesis in bacteria. For comparison, the list of antibiotics under study included the well-studied chloramphenicol, which has the ability to induce sulfide release [3].

To solve the problems, a combined approach was applied, including the measurement of growth parameters (growth rate and the ability to form colonies, CFU), and continuous in vivo real-time registration of a number of important physiological parameters using electrochemical sensors.

MATERIALS AND METHODS OF RESEARCH

The object of study was the gram-negative bacteria *Escherichia coli* BW25113 (wt) and the knockout mutant JW2663 (Δ*gshA*), deficient in synthesis of glutathione (GSH), both from the Keio international collection. Bacteria were cultured on M9 minimal medium [4] with glucose as a source of carbon and energy under aerobic conditions at 37 °C in a thermostatically controlled orbital shaker at 150 rpm. To determine the levels of extracellular potassium, bacteria were grown on M9 medium with a low content of potassium. Where indicated, MOPS (3-[N-morpholine]propanesulfonic acid) medium was used. Colony formation capacity (CFU) and the specific growth rate (μ) were determined in a conventional manner.

Measurements using electrochemical ion-selective sensors were carried out in flasks with aerobically growing *E. coli* cultures. All parameters were continuously and synchronously processed in real time using a hardware-software complex, including various registration blocks. The partial pressure of oxygen (dO_2) was measured using an InPro 6800 Clark electrode (Mettler Toledo, USA) on a modified BioFlo 110 controller (New Brunswick Scientific Co., USA). The concentration of extracellular sulfide ion was determined using a sulfide-specific ion-selective XC-S2-001 electrode (Sensor Systems Company, Russian Federation), the redox potential (Eh, ORP) was recorded with an ERP-105 platinum electrode (Measuring Technologies, Russian Federation), the level of extracellular potassium ions (K^+) was recorded with a K^+-selective electrode ELIS-121K (Measuring Technologies, RF). Results were expressed in mV (1 mV = 15 μM K+). Potentiometric data from these electrodes were processed with cpX-2 digital pX meters (IBP, Pushchino, Russian Federation). Synchronization of the primary data received from these sensors was carried out using the RS-232 and Modbus protocols. Graphical visualization of real time monitoring of dO_2, Eh, S^{2-} and K^+ ions is presented in the form of typical curves from a series of experiments.

Changes in the membrane potential (MP) were detected using the penetrating fluorescent dye DiBAC ([DiBAC4(3)], bis-(1,3-dibutylbarbituric acid)-trimethinoxonol) and Leica DM2000 fluorescent microscope [5]. An increase in the proportion of fluorescent cells corresponds to a decrease in MP.

Each result is indicated as the mean value of at least three independent experiments ± the standard error of the mean (SEM). Significant difference was analyzed by Student's t-test. A *p*-value of 0.05 was used as the cut-off for statistical significance. The results were analyzed by means of Statistica 6 (StatSoft Inc., USA).

RESULTS

Determination of growth parameters under the action of antibiotics

All studies were performed using cultures in the middle of the logarithmic phase of growth, in the range of optical density of the culture (OD_{600}) 0.4–0.8. During the growth of bacteria cultured in the absence of any treatments (control), the specific growth rate (μ) gradually decreased from the maximum value (0.71 ± 0.01) to 0.43 ± 0.02 h^{-1} (Fig. 1). In preliminary experiments, the concentration of the antibiotic was selected so that its effect was manifested in the region of the optimal bactericidal concentration, with the exception of tetracycline. The effect of this antibiotic was studied at different concentrations.

In the response of bacteria to the action of aminoglycosides kanamycin (30 μg/ml) and streptomycin (30 μg/ml), three phases were distinguished. The first phase, during which there was no statistically significant growth inhibition, lasted for the first 30 minutes after the addition of the antibiotic. In the second phase, which lasted for 15 min, a rapid drop in μ to about 0.2 h^{-1} was observed. Then the growth rate slowly dropped to zero. Under the action of tetracycline (30 μg/ml) and chloramphenicol (25 μg/ml), the phase of a rapid decrease in the growth rate was observed, during which, 15 min after the addition of antibiotics, μ decreased to 0.3 h^{-1}, and the phase of a slow decrease in the growth rate continued until to a complete stop (Fig. 1).

In experiments with tetracycline, the dependence of the effect on the concentration of the antibiotic was studied. During the first 45 min after the addi-

tion of 2 and 30 μg/ml of tetracycline, growth was inhibited in the same way, but upon treatment with 30 μg/ml, the growth stopped by the end of cultivation, while at 2 μg/ml, growth continued at a rate of 0.2 h^{-1}. Interestingly, under the action of a low dose of tetracycline (0.1 μg/ml) for 105 min of cultivation, the growth rate did not differ from that observed in the control; however, at the end of cultivation, the μ value was the same as after treatment with 2 mg/ml.

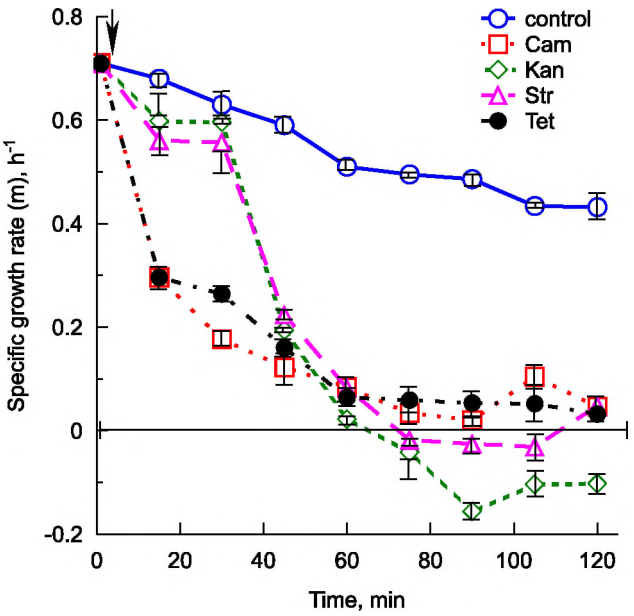

FIG. 1.

Effect of kanamycin (Kan) (30 μg/ml), streptomycin (Str) (30 μg/ml), tetracycline (Tet) (30 μg/ml) and chloramphenicol (Cam) (25 μg/ml) on the specific growth rate (μ) of E. coli (wt) growing on M9 medium with glucose. The arrow indicates the time when antibiotic was added

Data characterizing the colony-forming capacity under the action of antibiotics are presented as the difference between the logarithms of CFU in samples taken during cultivation and the values at the beginning of cultivation (ΔlogCFU) (Fig. 2). In the absence of any treatments, an increase in the number of CFU was observed as biomass accumulated. Chloramphenicol, exhibiting bacteriostatic properties, did not cause significant cell death. Tetracycline (30 μg/ml) had a significant bacteriostatic effect and a weak bactericidal effect. The value of CFU by the end of incubation in the presence of tetracycline decreased by about two times. Kanamycin and streptomycin at a concentration of 30 μg/ml had a pronounced bactericidal effect, reducing the number of CFU by 5.9×10^3 and 0.9×10^3 times, respectively. An inverse relationship was found between the rate of growth inhibition (the rate of decrease in μ) and the bactericidal action of antibiotics. Thus, kanamycin and streptomycin had the most pronounced bactericidal effect, stopping growth 45 minutes after the addition of the antibiotic. On the contrary, tetracycline and, in particular, chloramphenicol, which sharply inhibited the growth of bacteria as early as 15 min after their introduction, had rather a bacteriostatic effect.

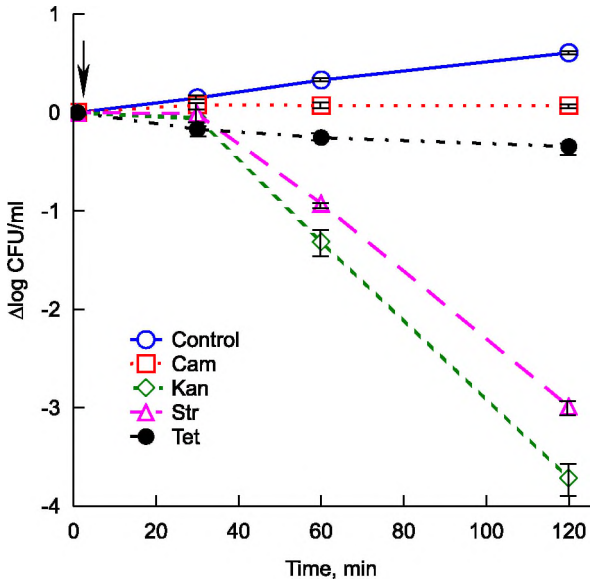

FIG. 2.

Effect of antibiotics on the ability of E. coli (wt) to form colonies (CFU). Growth medium, symbols and concentrations of antibiotics are shown in Fig. 1. The arrow indicates the time when antibiotic was added

Respiratory activity of bacteria and pH changes

Under our conditions, despite intensive agitation of the culture medium, as the biomass accumulated, the respiratory activity of growing cells led to a gradual decrease in the oxygen concentration in the medium to complete and irreversible exhaustion (Fig. 3). The addition of tetracycline or chloramphenicol to a growing culture of *E. coli* caused a rapid inhibition of respiration, most pronounced in the culture treated with 30 μg/ml tetracycline. After 120 min of incubation with the antibiotic, an increase in dO_2 in the medium was observed almost to the baseline level. Treatment with 0.1 μg/ml tetracycline caused a gradual decrease in O_2 consumption, the effect of 2 μg/ml was similar to 30 μg/ml, but less pronounced (Fig. 3).

Unlike chloramphenicol and tetracycline, cells treated with aminoglycosides continued to show high respiratory activity during the first 60–80 minutes. Notably, the rate of oxygen uptake by kanamycin was even higher than in culture untreated with the antibiotic, and in the case of streptomycin, the same as in untreated cells. In the second phase of the response to the action of aminoglycosides, respiration inhibition was observed, which was absent in cells not treated with antibiotics (Fig. 3).

During experiments, we found that the effect of kanamycin on the respiratory activity of growing *E. coli* is highly dependent on the composition of culture medium. In this series of experiments, *E. coli* BW25113 was cultured on minimal synthetic MOPS medium with glucose as a car-

bon source. Under these conditions, treatment with kanamycin caused irreversible inhibition of respiration as early as 10 min after its addition; the bacteria in this case behaved in the same way as when treated with a high concentration of tetracycline.

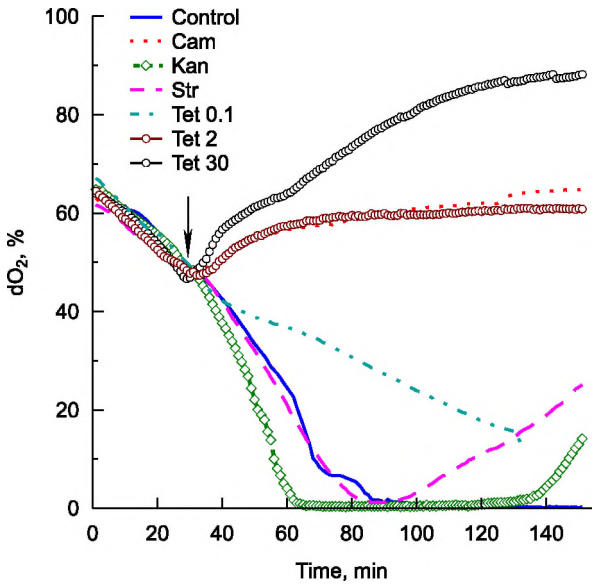

FIG. 3.

Changes in the level of dissolved oxygen in the culture of E. coli (wt) treated with antibiotics. Growth medium, symbols and concentrations of antibiotics are shown in Fig. 1. The arrow indicates the time when antibiotic was added

During aerobic growth of *E. coli* on the M9 minimal medium containing glucose as the only source of carbon and energy, a gradual decrease in pH is observed due to the accumulation of products of incomplete glucose oxidation [6]. The degree of change in the pH of the culture medium during continuous monitoring can indirectly indicate the rate of carbohydrate metabolism in bacteria and serve as an additional integral indicator of the state of the culture. The choice of the pH sensor and the operating mode of the controller made it possible to achieve high sensitivity and stability of indications. The measurement results were expressed as the difference between the values at the time of antibiotic addition and in the final (ΔpH). Under our conditions, during normal growth, a gradual decrease in the pH of the medium from 6.86 to 6.66 (ΔpH = 0.2) was recorded with a noticeable acceleration after the 90th minute of incubation. This coincides with the time of complete depletion of dissolved oxygen in the medium and the transition from aerobic to anaerobic catabolism.

Under the action of tetracycline and chloramphenicol, only a slight decrease in pH was noted (ΔpH = 0.04 and ΔpH = 0.07, respectively). On the contrary, treatment with aminoglycosides was accompanied by a decrease in pH, comparable to that observed in culture without treatment, and even greater in the case of kanamycin. In general, in aerobic cultures, observed changes in pH correlate well with changes in respiratory activity. Higher respiratory activity promoted a higher rate of glucose utilization and the accumulation of acidifying metabolites, which is recorded as a greater decrease in pH.

Redox status of the medium (Eh) and sulfide production

In the absence of treatments, Eh gradually changed from +190 mV (at the point corresponding to the time of addition of antibiotics) to –150 mV at the end of the experiment. The transition of Eh to negative values is associated with the gradual depletion of oxygen in the medium, which confirms the role of O_2 as one of the main redox-active components in aerobic bacterial cultures. In the antibiotic-free medium, sulfide production was maintained at a constant low level (Fig. 4a).

Tetracycline and chloramphenicol, when added to *E. coli* culture, induced a jump in Eh to negative values with amplitudes of 101.5 ± 6 and 133.8 ± 7.5 mV, respectively. The addition of tetracycline in an amount of 0.1 μg/ml did not significantly affect the redox potential of the medium, while the addition of 2 μg/ml caused the Eh jump with amplitude of 82 ± 4 mV. Simultaneously, tetracycline and chloramphenicol caused a sharp, short-term increase in endogenous sulfide production with amplitudes of 15 ± 2.5 mV and 36.6 ± 2.7 mV, respectively (Fig. 4a). Chloramphenicol stimulated the largest drop in the potential of the sulfide electrode in terms of amplitude and duration among all tested antibiotics. A dose-dependent pattern of action of tetracycline was revealed (Fig. 4a). A close relationship was noted between the degree of inhibition of the specific growth rate and respiratory activity, and the production of sulfide under the action of the tested antibiotics.

Treatment of growing bacteria with aminoglycosides (kanamycin and streptomycin) did not cause an abrupt change in the potential of the Eh electrode, however, it significantly accelerated its decrease (compared to the control), during the first 60 minutes for kanamycin and 80 minutes for streptomycin, after which gradual return of the potential to the base value was recorded. The observed changes in Eh under these conditions can be associated with changes in dO_2. There were also no jumps in the potential of the sulfide electrode under the action of both antibiotics on the cells of the parental strain *E. coli* BW25113.

We have previously shown that stress-induced production of extracellular sulfide in some cases can be significantly increased in *E. coli* mutants deficient in the synthesis of glutathione (GSH) [2]. To test this effect, in the case of kanamycin, we used the knockout mutant *E. coli* JW2663 Δ*gshA*, deficient in glutathione synthesis. When grown on M9 medium, this mutant, like the parental strain, did not produce sulfide under the action of 30 μg/ml kanamycin. In the mutant (but not in the parent) growing on MOPS medium with glucose, kanamycin-induced jumps in the potential of the sulfide electrode were recorded (Fig. 4b). Their amplitude was about 100 mV, which was significantly higher than in the other examined above cases.

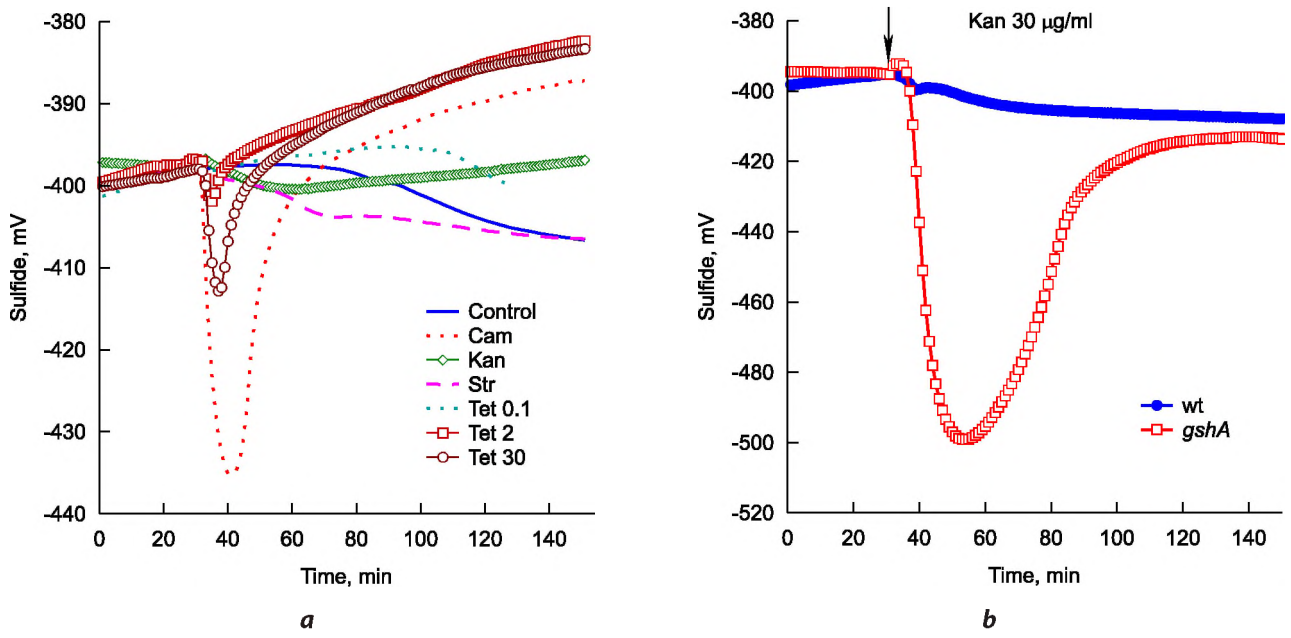

FIG. 4.

a *– sulfide production by E. coli (wt) treated with antibiotics. Bacteria were grown on M9 medium with glucose. Symbols and concentrations of antibiotics are shown in Fig. 1.* ***b*** *– sulfide production in E. coli gsh knockout mutant deficient in glutathione synthesis and in the parent strain (wt). Bacteria growing on the MOPS medium with glucose were treated with kanamycin (30 µg/ml). The arrow indicates the time when antibiotic was added*

Changes in membrane potential and extracellular K^+ levels

In the absence of antibiotics, the growth of *E. coli* was accompanied by an uptake of potassium from the medium in an amount proportional to the number of cells, which was recorded as a gradual decrease in the potential of the potassium-specific electrode. Under these conditions, bacteria maintained the membrane potential at a constant level, and the number of stained cells did not exceed 1 %. Treatment with chloramphenicol or tetracycline caused a decrease in membrane potential in no more than 6 % of cells.

After treatment with chloramphenicol, the bacteria continued to uptake potassium at a rate close to that observed in the absence of the antibiotic. 30 min after the addition of chloramphenicol, a slowdown in potassium uptake was observed which corresponded in time to the end of the phase of rapid growth inhibition and a decrease in respiratory activity. Tetracycline affected potassium uptake in a dose-dependent manner. At a concentration of 0.1 µg/ml, there was no change in the rate of potassium uptake within 60 minutes after treatment (compared to the control), while at 2 µg/ml of antibiotic a significant slowdown in potassium absorption was noted already after 30 minutes. The addition of 30 µg/ml tetracycline completely stopped the entry of K^+ into the cells.

When aminoglycosides were added, a two-phase pattern of changes in the concentration of extracellular potassium was observed. In the first phase, the cells continued to uptake potassium at a rate similar to that observed in the absence of treatment. 45 min after the addition of streptomycin and 60 min after the addition of kanamycin, the second phase began, in which the concentration of potassium in the medium began to increase, indicating the loss of the ability of bacterial cells to retain K^+. An increase in the level of extracellular potassium may be associated with cell lysis or a drop in membrane potential. Indeed, after the treatment of bacteria with streptomycin, already after 30 minutes, there was a significant loss of MP in 6.3 ± 0.6 % of cells. By the end of the experiment, MP lost 19.2 ± 0.04 % of cells. The action of kanamycin caused a less pronounced effect: after 60 minutes of incubation with the antibiotic, the number of stained cells began to increase (4.65 ± 1.05 %), by the 120th minute 19.6 ± 1.6 % of the cells lost MP. In general, under the action of all tested antibiotics, the observed changes in the membrane potential were closely associated with the ability of bacterial cells to retain potassium. In addition, a correlation was found between growth inhibition and the ability of cells to maintain the membrane potential.

DISCUSSION

In most cases, the monitoring of changes in the parameters of growing bacterial cultures is carried out by microbiological and physicochemical methods in samples taken by centrifugation or filtration. In the process of sampling, cells are exposed to various influences that are stressful for them. When a sample taken from a growing aerobic culture, bacteria, being in a centrifuge tube, consume oxygen for several seconds and go into

a state characteristic of anaerobic or microaerobic growth. Conversely, under membrane filtration, cells are exposed to increased oxygen content. A similar situation is observed when cells are placed on agar surface in Petri plates. As a consequence, the obtained values of the measured parameters may not correspond to those observed in situ. This is especially true for parameters reflecting the respiratory activity and transmembrane ion fluxes, which are characterized by a rapid response to changes in environmental conditions.

A feature of the above research was the use of electrochemical ion-selective sensors installed directly in flasks with aerobically growing cultures, which allowed to simultaneously and continuously measuring several important parameters in real time. Using this approach, a comprehensive real-time monitoring of such important parameters of a growing *E. coli* culture as respiratory activity, redox potential, sulfide production, and changes in the levels of extracellular K^+ ions under the action of antibiotics that block protein synthesis was carried out, in conjunction with classical microbiological methods (determination specific growth rate and colony-forming ability, as well as membrane potential). The results obtained indicate that these parameters, although to varying degrees, are closely related (Table 1). Growth inhibition of *E. coli* by tetracycline and chloramphenicol began immediately after the addition of the antibiotic and occurred very quickly. Both antibiotics had a weaker bactericidal effect and a pronounced bacteriostatic effect. It is important that it was tetracycline and chloramphenicol that stimulated the production of sulfide and the jump in Eh. As shown earlier, the extracellular reductant sulfide, interacting with a platinum electrode, shifts its potential to negative values, which is recorded as a jump in Eh [2]. Inhibition of respiration led to a significant decrease in glucose catabolism, as evidenced by a lower rate of acidification of the medium compared to the control. At the same time, cells treated with tetracycline and chloramphenicol retained better the ability to maintain the membrane potential and intracellular potassium.

An essential characteristic of the action of streptomycin and kanamycin is the 30-minute delay in growth inhibition after they have been added to a growing culture. Both antibiotics had a bactericidal effect, did not stimulate the production of sulfide (and, accordingly, a jump in Eh), did not inhibit respiration, but stimulated a drop in the membrane potential and, accordingly, in intracellular potassium. Continued respiration in the presence of both antibiotics promoted glucose catabolism, as evidenced by acidification of the medium.

Collins et al. showed that treatment of *E. coli* with a range of bacteriostatic antibiotics that inhibit protein synthesis (including chloramphenicol and tetracycline) resulted in rapid suppression of cellular respiration. In contrast, three bactericidal antibiotics (ampicillin, gentamicin, and norfloxacin) accelerated respiration. Growth inhibition is associated with suppression of central metabolism, while killing is linked to activation of metabolism [7].

Our data largely confirm the data discussed above. However, there are differences. Of the two bactericidal antibiotics, kanamycin stimulated respiratory activity and, judging by the pH change, showed a higher metabolic activity, while streptomycin did not have these effects. Notably, when *E. coli* was cultured on the MOPS medium kanamycin caused irreversible inhibition of respiration.

We assume that, in our case, the key event determining the differences in the action of bacteriostatic and bactericidal antibiotics may be the presence of the delay between the addition of the antibiotic and the onset of growth inhibition in the first case and its absence in the second case. It is possible that, in turn, this is a consequence of the different rates of penetration of antibiotics into cells. ppGpp, the signaling molecule of the stringent response, which is the master regulators of the bacterial response to various stresses, may play an important role. It is known that the activities associated with the action of ppGpp are largely determined by the growth rate of bacteria at the time of stress exposure [8], including the action of antibiotics [9]. However, it should be noted that chloramphenicol, which does not have a bactericidal effect, inhibits the synthesis of ppGpp [10].

The scientific literature concerning studies of the action of antibiotics contains a number of conflicting data. One of the reasons for this is the use of different culture conditions by different authors. In this regard, it is of interest to detect the production of sulfide under the action of kanamycin on the *E. coli* knockout mutant, when the growth medium is changed from M9 to MOPS medium. These results indicate that the observed effects can largely depend not only on the concentration of the active substance, but also on the composition of the me-

TABLE 1

ACTION OF PROTEIN SYNTHESIS INHIBITORS ON GROWING *ESCHERICHIA COLI*

Antibiotics	Growth inhibition	BS/BC	RA	H2S	MP	pH
Chloramphenicol	+	BS	↓	↑	–	0.07
Tetracycline	+	BS	↓	↑	–	0.04
Kanamycin	delay	BC	↑	–	↓	0.23
Streptomycin	delay	BC	–	–	↓	0.17

Note. Growth inhibition – growth inhibition starts immediately after antibiotic addition (+); BS – bacteriostatic action; BC – bactericidal action; RA – respiratory activity in the first phase of response to antibiotic action, inhibition (↓), stimulation (↑), no effect (–); H_2S – presence (↑) or absence (–) of production; MP – decreased membrane potential (↓), no effect (–); pH – decrease of by the end of cultivation.

dium, which is important to consider when interpreting the results.

In *E. coli*, the release of sulfide into the medium is part of the process aimed at maintaining cysteine homeostasis during stress-induced arrest of protein synthesis. An excess of cysteine in this situation can lead to stimulation of the Fenton reaction, during which hydrogen peroxide oxidizes the free divalent iron ion with the formation of a highly toxic hydroxyl radical that can damage DNA, proteins and lipids. Cysteine in this situation can act as a reductant that reduces oxidized iron [11]. To keep cysteine at a safe level, bacteria incorporate some of the cysteine into glutathione and some of it is excreted into the environment. In addition, part of cysteine is cleaved to form sulfide, which is released into the environment and under our conditions can be detected by a sulfide-specific electrode [2]. Based on the presented scheme, the production of sulfide by kanamycin, observed by us in the MOPS medium, can be associated with the stimulation of the Fenton reaction due to the presence in this medium (in contrast to the M9 medium) of an increased amount of iron. At the same time, these data confirm the important role of glutathione in maintaining cysteine homeostasis in *E. coli* under stress. It should be noted that endogenously produced H_2S renders multiple bacterial species highly resistant to oxidative stress and various classes of antibiotics [12, 13].

CONCLUSION

The results obtained in this work not only confirm our previous data, but also provide a deeper understanding of the mechanisms of bacterial response to the action of antibiotics, inhibitors of protein synthesis. Although the studied antibiotics inhibit the same function in *E. coli*, two of them are bacteriostatic and two are bactericidal. This is accompanied by significant differences not only in such general physiological parameters as growth rate and survival, but also in respiratory activity, the rate of utilization of the carbon and energy source, stress-induced sulfide production, and the ability to maintain membrane potential and intracellular potassium. The increase in the number of pathogenic microorganisms resistant to antibiotics, observed in recent years, requires the improvement of approaches and methods for the development of new antimicrobial drugs and increasing the effectiveness of widely used ones. The data obtained in this work indicate the promise of using electrochemical sensors for screening chemical compounds for antimicrobial activity.

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Conflict of interest

The authors of the article have no conflict of interest to disclose.

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