Analysis of the Time Course of *E. coli* Development

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Many scientists attribute a variety of clinical manifestations of infections (latent and silent forms), pathogen carriership, and polymorphism of pathological changes in the body to the variability of infectious disease agents under the influence of various environmental factors [4,6,7]. The knowledge of microorganism vital activity mechanisms is very important for rational and effective therapy of infectious diseases, for this knowledge can be used to predict the results of cell exposure to different factors, including drugs. Model descriptions of individual mechanisms of bacterial vital activity have been presented [3,5]. However, an integral model, based on which one may explain all the known characteristics of bacteria and predict experimental results, is still to be created. The development of such a model was the aim of the present study.

MATERIALS AND METHODS

A reference strain of *Escherichia coli* was used in the study. The culture was grown in 5 ml of meat-peptone broth at pH 7.3 for 24 h at 37°C. The cells were sedimented by centrifugation and washed with physiological saline, pH 7.3, to separate vital activity products. A suspension in 5 ml of normal saline was made from the washed culture.

The cell membrane of *E. coli* is known to be approximately 0.01 μ thick [1,8]. With such a membrane thickness the frequency of electromagnetic oscillations (EMO) produced by the cells is 0.5×10^{11} Hz. There are reports about optimal interactions of EMO of such frequency with luminous flux EMO whose wavelength is within the 440-700 nm range [2]. Photocolorimetry was therefore used in the studies of *E. coli* development; a KFK-2MP device was employed with the luminous flux wavelength in the 315-980 nm band.

Cuvettes and light filters were selected before the investigation. Control and experimental cuvettes (working length 30 mm, volume 14 ml) were filled with meat-peptone broth up to the mark on the side, placed in the cuvette compartment of the photocolorimeter, and kept there closed for 1 h. Such an exposure is necessary for the temperature in the cuvette compartment to attain its equlibrium value, 37°C. After one hour, the washed suspension of *E. coli* 24 h broth culture in normal saline (o.2 ml) was layered with a micropipette onto the nutrient medium surface without mixing in the experimental cuvette and o.2 ml of normal saline was added to the control cuvette. The optical density of the solution was assessed every 5 sec during 5-6 h at luminous flux wavelength 590 nm. The cuvettes were sterilized with Nikiforov's mixture for 30 min directly before being filled with meat-peptone broth and thoroughly wiped with a sterile tampon to remove the remainder of the mixture.

RESULTS

According to thermodynamic laws, every bacterial cell in any environment exists in a certain well-defined zone (area) whose parameters are described by many authors [1,2] as EMO parameters. The authors claim

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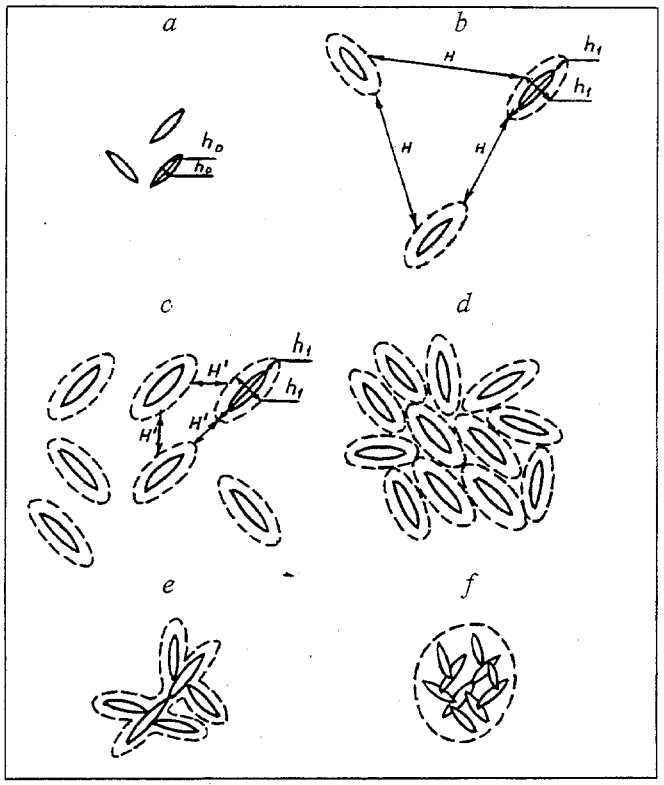

Fig. 1. Model representation of *E. coli* development stages. *a*) bacterial cell at the moment of its embedding in nutrient substrate; *b*) onset of oscillating system formation in bacterial population; c) common oscillating system of bacterial population (onset of logarithmic growth phase); d) bacterial population at end of logarithmic growth phase; e) onset of disturbances in bacterial population oscillating system; *f*) bacterial cell deformation.

that every live object, including bacteria, emits EMO of strictly defined intensity and frequency. As for the oscillation phase, it varies and depends on the developmental stage of each cell in the population.

Let us examine *E. coli* development from the moment of inoculation to the end of the logarithmic growth phase.

At the moment of inoculation of the bacterial suspension the fluctuating systems of microorganisms become disordered when they start interacting with fresh nutrient medium. The bacterial cells "instinctively" cease oscillatory movements and stop dead, as it were (collapse), trying to preserve their appearance. The relationship between transparency and time during this period should remain unchanged all the time that this state persists. In fact, optical density values are indeed the same for 15-20 sec. Being collapsed and having infinitely small (in relation to the nutrient medium volume) geometric parameters (h_o) delimiting the volume taken up directly by the bacterial cell, the bacteria represent at this time independent (autonomic) particles (Fig. 1, *a*). The particles start diffusing 15-20 sec later, thermally interacting with the molecules of the nutrient medium and hence causing a transparency increase. This process lasts for t_o=9-10 min and is expressed on the curve presented in Fig. 2, *b* by an optical density drop. After adaptation (t_1=2-3 min) (Fig. 2, *b*) the microorganisms resume oscillations of a certain frequency and amplitude (Fig. 1, *b*). The distance between individual cells decreases by $h=h_1-h_o$, this being associated with an increase of optical density. Simultaneously with EMO recovery the bacterial cells start dividing, thus contributing to a still more marked optical density increase. As the areas of microorganisms draw closer to each other due to multiplication processes, there comes a moment when the distance between individual cells attains a certain critical value H' (Fig. 1, *c*), signifying direct interactions of cells with

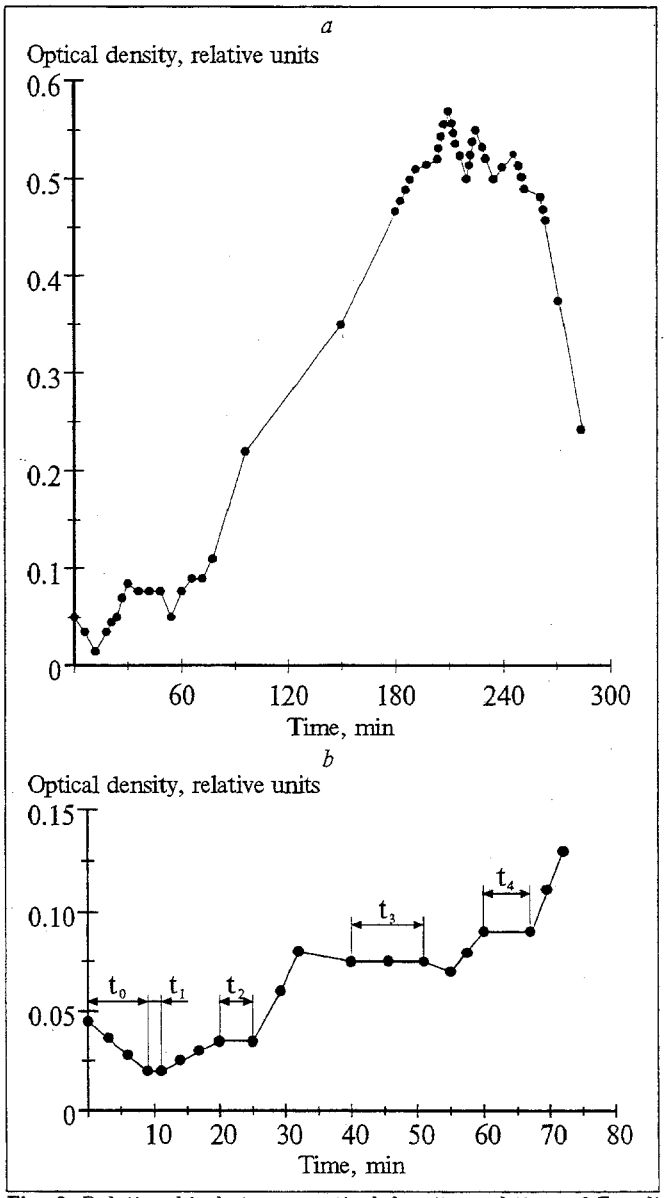

Fig. 2. Relationship between optical density and time of *E. coli* development. *a*) *E. coli* development curve; *b*) lag phase curve portion.

bioelectromagnetic fields. Some bacterial cell whose EMO phases coincide start synchronously dividing, thus once again increasing optical density (Fig. 2, *b*) [3]. Establishment of an equilibrium state, that is, EMO phase leveling between divided and undivided cells in a population, takes place during t_2=6 min, t_3=12 min, and t_4=6 min (Fig. 2, *b*). Moreover, the distance to H' between the remaining cells situated at distance H decreases. As soon as the majority of cells in a population start oscillating in a single O phase, the mechanism of synchronous division is triggered. The process of microorganism EMO phase leveling culminates in the formation of a single oscillating system: every cell in a population starts oscillating in a common phase with a common frequency and amplitude and therefore starts dividing at a common rate. The logarithmic growth phase starts from the moment of formation of a common oscillating system.

The distance between bacterial cells is rapidly reduced in the phase of logarithmic growth due to intensive multiplication. In other words, the increase of the cell count in a population indicates an increased number of EMO emission sources whose wavelength is comparable to the light source wavelength. An increased number of EMO emission sources results in enhanced absorption of the luminous flux formed by the photoelectrocolorimeter, this being associated with a rapid increase of optical density (Fig. 2, *a*). This process is nonlinear and is well approximated by Bouguer-Lambert's law [8]: $I=I_0\times\exp(-\mu h)$, where I_0 is the incident light intensity, I is the light intensity after passing distance h in the environment, and μ is the coefficient describing the rate of multiplication of the microorganisms.

The logarithmic growth phase lasts until the onset of the effect of short-range action between the cells (Fig. 1, *d*), resulting in a disturbance of the oscillating system. The bacterial cells start "interfering" with each other; this is paralleled by changes in both oscillation amplitude and frequency, signaling to the cell that the normal developmental conditions are disturbed (Fig. 1, *e*). To attenuate this short-range effect deforming the microorganism (Fig. 1, *f*), the cells "strive" to stop the oscillatory movements (collapse). Some of the dead cells begin to sink to the bottom. All this leads to an increase of solution permeability for the luminous flux. Since the disturbances of the intercellular bonds due to the reduced oscillation amplitude are transient, the curve shows a marked decrease of optical density (Fig. 2, *a*). The oscillatory movements cannot cease instantaneously, for this is a genetically determined property. Some bacterial cell which have not precipitated on the bottom of the cuvette rush into the free space, restoring the intercellular bonds and causing a new optical density increase. The diagram shows that this process is repeated with fading (Fig. 2, *a*). At the end of this period of microorganism activity each cell in the bacterial population again becomes an independent (autonomic) particle, thus causing an optical density reduction. Moreover, dead cells sink to the bottom of the cuvette under the force of gravity, promoting a still greater reduction of optical density (Fig. 2, *a*).

Hence, the suggested model of the time course of *E. coli* development based on biophysical characteristics of the bacterial cell offers an explanation of bacterial vital activity processes described in the literature and helps predict microorganism behavior under the influence of external factors, including drugs.

REFERENCES

1. N. D. Devyatkov and M. B. Golant, in: *Electronic Technology, UHF Electronics Series* [in Russian], Moscow (1982), Vol. 12 (348), pp. 46-49.
2. N. D. Devyatkov, *Ibid.* (1987), Vol. 9 (403), pp. 60-66.
3. S. M. Zhdan-Pushkina, *Fundamentals of Microorganism Culture Growth* [in Russian], Leningrad (1983).
4. D. G. Kudlai, *The Variability of Intestinal Bacteria* [in Russian], Moscow (1954).
5. G. M. Larionov and V. D. Belyakov, *Zh. Mikrobiol.*, No. 1, 13-17 (1990).
6. V. V. Sukhnev, *Ibid.*, **14**, No. 5, 760 (1935).
7. V. D. Timakov, *Sovetskoe Zdravookhranenie Turkmenii*, No. 3, 8 (1937).
8. B. A. Fikhman, *Microbiological Refractometry* [in Russian], Moscow (1967).