Specificities of the Mechanism of Bacterial Development during the Lag Phase

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The mechanisms of *E. coli* development during the lag phase are described on the basis of biophysical characteristics of the bacterial cell. A bioelectromagnetic model closely approximates the experimental parameters of the time course of *E. coli* and *E. aerogenes* development in the mono- and mixed culture.

Key Words: *bacteria; intercellular relationships; lag phase; bioelectronic properties*

The lag phase is a complicated period in the life of microorganisms, when the main features of a bacterial population are formed in response to given definite conditions [1]. When carried out by traditional methods, studies of the microorganisms' vital activity during the lag phase yield few data and provide only integral information about the terminal processes in the bacterial population, which stem from the primary processes initiated in the lag phase. This may be explained, primarily, by a high inertia of the traditional measurements, which take a much longer time than not only the time spent by the microprocesses in the lag phase, but, sometimes, also the lag phase per se. In addition, there is no model for a study of bacterial vital activity, parameters of which would make it possible to elucidate the mechanisms of primary microprocesses and to predict the results of experimental investigations from the data on the terminal processes in the bacterial population.

A method for a study of the vital activity of bacteria, yielding experimental results every 5 sec (which is, according to Zhdan-Pushkina [1], far shorter than the duration of the microprocesses in the bacterial population during its development) has been described previously [2]. In the same study a bioelectromagnetic model of the time course of bacterial development was presented, demonstrating a highly reliable correlation between its parameters and the parameters obtained by photoelectrocolorimetry.

These findings not only have offered an explanation of the vital processes in bacteria described in the literature, but have also made it possible to study the mechanism of initiation of the intercellular biophysical microprocesses and the pattern of their changes in the bacterial population, as well as to predict the results of experimental investigations.

In this connection the aim of the present study was to investigate, on the basis of a bioelectromagnetic model, the mechanisms of intercellular biophysical microprocesses in the bacterial population during the lag phase.

MATERIALS AND METHODS

Reference cultures of *Escherichia coli* and *Enterobacter aerogenes* were used in the study. In studies of the microprocesses the reproducibility of experimental results, which is determined by the reproducibility of the initial parameters of the test cultures, is of great importance.

Since photoelectrocolorimetry is highly sensitive to the slightest changes in the initial parameters of the test cultures (the age of the inoculum,

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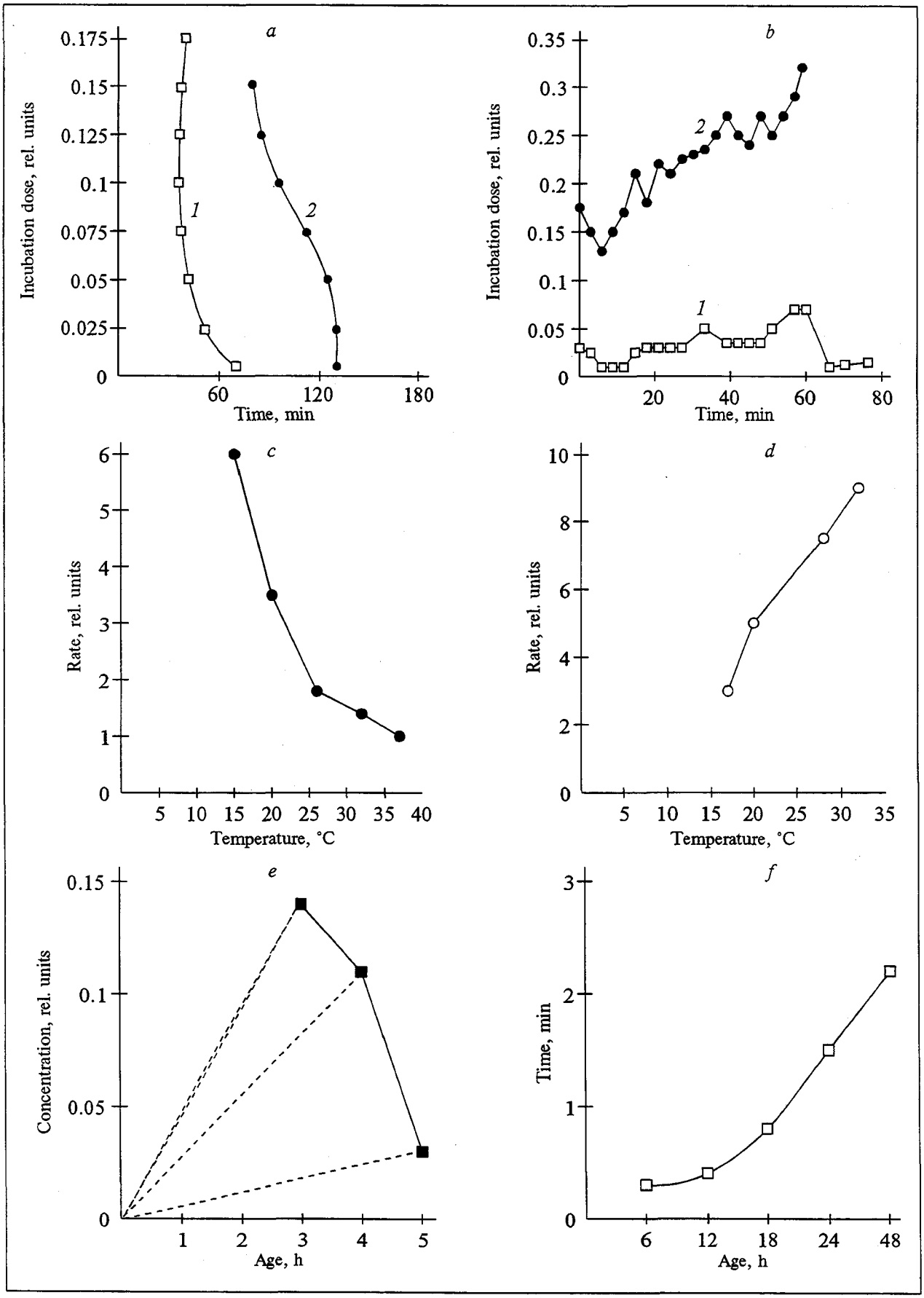
a
Incubation dose, rel. units
0.175
0.15
0.125
0.1
0.075
0.05
0.025
0
1
2
60
120
180
Time, min
b
Incubation dose, rel. units
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
2
1
20
40
60
80
Time, min
c
Rate, rel. units
6
5
4
3
2
1
0
5 10 15 20 25 30 35 40
Temperature, °C
d
Rate, rel. units
10
8
6
4
2
0
5 10 15 20 25 30 35
Temperature, °C
e
Concentration, rel. units
0.15
0.1
0.05
0
1 2 3 4 5
Age, h
f
Time, min
3
2
1
0
6 12 18 24 48
Age, h

the inoculation dose, the culture temperature, etc.), the present investigation called for additional studies of the effect of these parameters on the reproducibility of the time course of development of the bacterial population. The results of these studies are presented in Fig. 1.

Analysis of the effects presented in Fig. 1, *a* and *b* attests to definite relationships between the inoculation dose and the time course of the bacterial population. On the one hand, an increase in the inoculation dose reduces the duration of the lag phase (Fig. 1, *a*, curve *1*) and of the phase of logarithmic growth (Fig. 1, *a*, curve *2*). On the other hand, in the case of a relatively high inoculation dose, which in our study corresponds to an initial optical density of the working solution of 0.175 (Fig. 1, *b*, curve *2*), it is not possible to detect any regularity in the development of the bacteria during the lag phase or to determine the time of onset of development of the population, i.e., the genesis of the intercellular biophysical relationships.

When the inoculation dose corresponds to an initial optical density of the working solution of 0.025-0.050, the duration of the lag phase and of the logarithmic growth phase increases by 10-12 and 60 min, respectively (Fig. 1, *a*). It is also to be noted that for this inoculation dose the bacterial population exhibits a stable course of development (Fig. 1, *b*, curve *1*).

Thus, in studies of bacterial vital activity the optimal inoculation dose is a dose corresponding to an initial optical density of the working solution from 0.025 to 0.050.

Curves showing the rate of development of the bacterial population as a function of the culture temperature are presented in Fig. 1, *c* and *d*: the duration of the lag phase depends exponentially on the culture temperature (Fig. 1, *c*). In the stage of logarithmic growth, however, the temperature dependance of the rate of bacterial development approaches a linear one (Fig. 1, *d*). The same pattern of bacterial development is observed during the lag phase if culturing is performed at a temperature from 31 to 37°C (Fig. 1, *c*). Thus, between 31 and 37°C the development of the bacterial population stabilizes and depends little on the culture temperature.

Hence, a temperature from 31 to 37°C is the optimal culture temperature for studies of the time course of bacterial development.

Figure 1, *e* presents the concentration of bacteria in the daughter culture as a function of the age of the inoculum. At each specified time (in this case, 2 h postinoculation), depending on the age of the inoculum, the concentration of daughter cells in the nutrient medium may be different for the same inoculation dose, this being reflected in the time course of intercellular biophysical processes.

Analysis of the lag phase duration as a function of the age of the inoculum showed that the older the maternal culture, the longer the lag phase (Fig. 1, *f*). If inoculation is performed with bacteria from 6-, 12-, 18-, 24-, and 48-h cultures, the multiplication curves will be different. Therefore, in order to obtain reproducible parameters of vital activity, it is important that the age of the maternal culture be taken into account. Our experiments showed that the age of the inoculum for which a high reproducibility of the time course of development is observed is 24 h.

Thus, if the initial parameters of the test cultures are in agreement with the above-mentioned values, the reproducibility of the experimental results with an error of not more than 5-7% can be guaranteed.

These conditions were achieved as follows. The test culture was grown in 5 ml of meat-peptone broth (pH 7.3) at 37°C for 24 h. The cells were separated from the vital activity products by centrifugation in physiological saline (pH 7.3). The washed culture was suspended in 5 ml of physiological saline. When suspension of the test culture in a volume of 0.2 ml was placed in a photoelectrocolorimetric cuvette (effective length 30 mm, volume 14 ml) containing meat-peptone broth, the initial optical density of the working solution constituted 0.025-0.050. The cuvettes with meat-peptone broth (control and experimental) were preliminarily placed in the cuvette compartment of the photoelectrocolorimeter and kept there closed for the time required for a temperature of 31°C to be attained in the compartment. The optical density of the test solution was measured as described previously [2].

RESULTS

The results of a study of the time course of the test cultures are presented in Fig. 2.

Fig. 1. Effect of initial culturing parameters on the reproducibility of the time course of a bacterial population. *a* and *b*) duration of lag phase (curve *1*) and logarithmic growth phase (curve *2*) duration and time course of bacterial development, respectively, as functions of inoculation dose; *c* and *d*) duration of lag phase and rate of bacterial development, respectively, as functions of culture temperature; *e*) concentration of bacteria in daughter culture 2 h after inoculation as a function of age of inoculum; *f*) duration of lag phase as a function of age of inoculum.

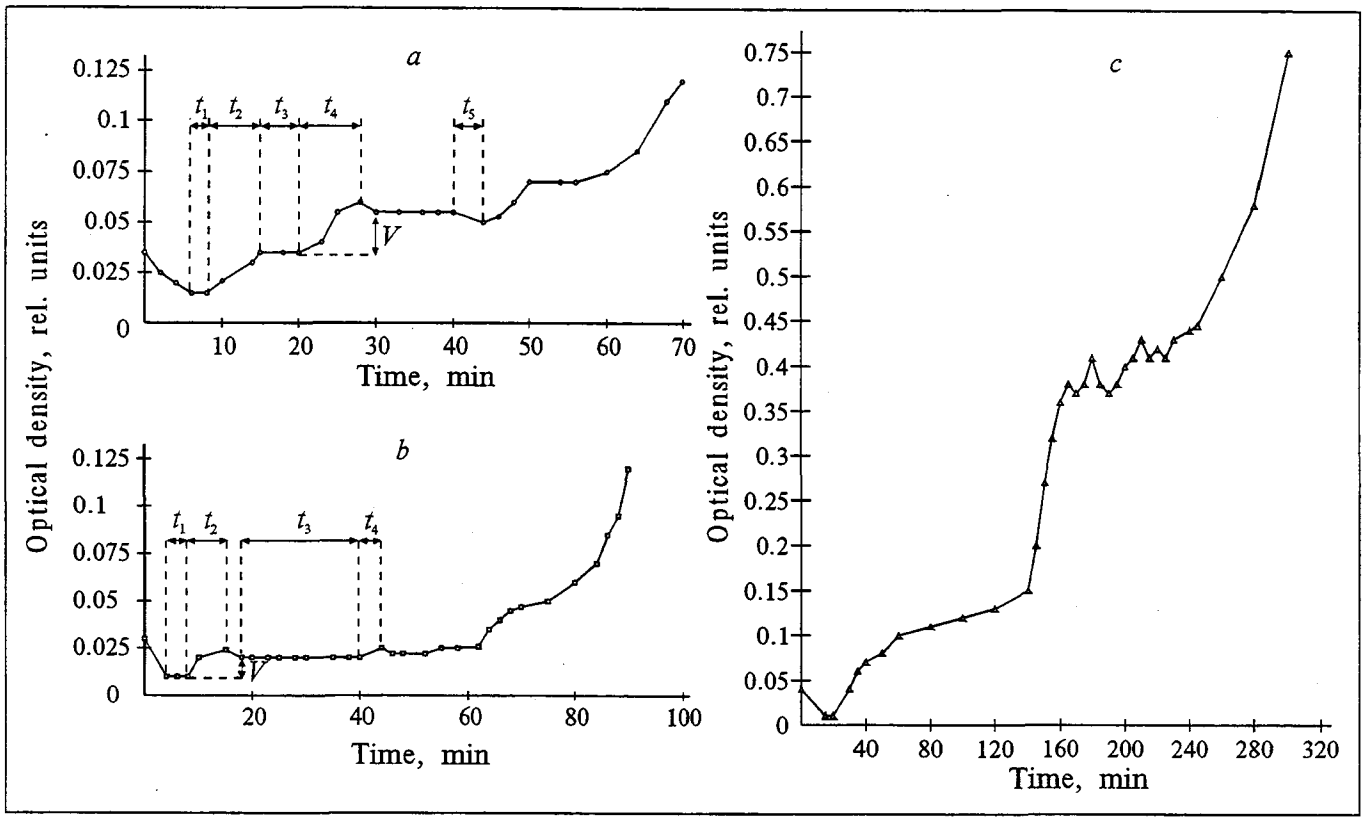

Fig. 2. Time course of development. *a*) *E. coli* in lag phase; *b*) *E. aerogenes* in lag phase; *c*) *E. coli* and *E. aerogenes* in mixed culture.

Analysis of the time course of bacterial development over the lag phase shows that the curves may be divided into several characteristic portions. During the first 3-10 min postinoculation, all the test cultures exhibit virtually the same pattern of the time course of development. In the previous study [2], according to the bioelectromagnetic model, this period was described as the period of diffuse distribution of independent particles, which is the mechanism by which equilibrium is established between the bacterial cells in the nutrient medium. In fact, portions of the curves for $0<t<9$ min (Fig. 3, *b* and *c*) are well approximated by Fick's 2nd-order equation, which is valid only if the bacterial concentration has a definite limited value (the volume of nutrient medium and the inoculation dose being constant) over this period:

$$C_x = C_0 \cdot \exp(-\frac{x^2}{4Dt}),$$

where C_0 is the concentration of bacteria in the volume of inoculum, $C_{x,t}$ is the concentration of bacteria as a function of coordinate x and time t, x is the distance from the surface of the nutrient medium traveled by the bacterial cells toward a decrease of the concentration gradient; t is the time when the concentration gradient disappears, and D is the diffusion coefficient.

The perfect similarity of the curve described by the solution of the 2nd-order Fick equation (Fig. 3, *a*) [3] to the curve showing the early stage of the lag phase ($0<t<9$ min) in the test cultures (Fig. 3, *b* and *c*) attests to the absence of intercellular relationships during this stage of bacterial development, i.e., during this period the bacterial cells are actually autonomic particles, independent of one another, which develop passively and, hence, are governed by the force of their concentration gradient.

Comparison of the portions characteristic of a diffuse distribution of bacteria in the nutrient medium demonstrates that the duration of this process is different for different types of bacteria (Fig. 3, *b* and *c*). This indicates that bacterial cells exhibit different patterns of migration in the nutrient medium, which in turn are underpinned by diverse intracellular physicochemical processes. Hence, as early as at the stage of diffuse distribution of bacteria it is possible to study and predict their vital activity.

In addition, according to fundamental physical laws, every nonliving body has a specific diffusion coefficient. The solution of the 2nd-order Fick

equation is irrespective of whether the elements are living or not, and describes processes for independent particles. Hence, it may be assumed that when autonomic bacterial cells are involved in the establishment of an equilibrium in the nutrient medium, they must also have a species-specific diffusion coefficient.

In fact, a number of investigations of the time course of development of the test cultures showed that, provided the experimental conditions are unchanged, the tangent of the slope of the curve corresponding to the diffuse distribution of bacteria, is a unique parameter characteristic of each type of bacteria, i.e., it may serve as an identification test.

Analysis of the next portion of the curve, $6<t_1<9$ min (Fig. 2, *a*), $3<t_1<9$ min (Fig. 2, *b*), shows that a certain amount of time is required to trigger the vital processes in bacteria, which, according to the bioelectromagnetic model, was described as the adaptation period in our previous study [2]. During this period, in response to the external factors rearrangement of the intracellular physicochemical processes begins, shaping the future pattern of intercellular biophysical relationships, as was described in our previous study [2] in terms of the parameters of electromagnetic oscillations (EMO): phase, frequency, and amplitude. The primary formation of intercellular biophysical relationships starts in local volumes of nutrient medium, this culminating in explosive spurts of multiplication of bacteria, which was described previously [1,2] as synchronous division (Fig. 2, *a*, portion of curve for $9<t_2<15$ min and Fig. 2, *b*, portion of curve for $9<t_2<15$ min). This suggests that the spurts of development are produced by bacteria with the same phase of EMO which randomly conglomerate into accumulations (microsystems) [2]. However, the curves in Fig. 2, *a* and *b* show that this synchronous division is limited in time and lasts in the test bacteria for not longer than 6 min, i.e., during synchronous division the number of divisions bacteria can undergo is strictly limited.

Following the synchronous division, the period of adaptation or leveling starts again, in this case involving the microsystems [2]. Evidence of this is the increased duration of the adaptation period ($15<t_3<21$, Fig. 2, *a*), which is associated with the fact that the inertia of the microsystems is higher than that of the individual cells.

In the portion $21<t_4<27$ min (Fig. 2, *a*) the enlarged microsystems start synchronously dividing, a process which, as in the first case, goes on for at most 6 min. A subsequent drop of optical density can be explained by a reduction of the distance between individual cells to subcritical values [2], owing to an increase in the concentration of bacteria during the synchronous division. As a result, the intercellular relationships weaken, and some bacterial cells collapse and become caught up in thermal diffusion.

However, the microsystem does not entirely dissociate, as is demonstrated by the V value (Fig. 2, *a* and *b*). In the event of complete disintegration of the microsystem, all its individual components would be uniformly distributed over the entire volume of nutrient medium, which would result in an increase of the optical density of the test solution by the value of V. A change of optical density by the value of V is indicative of the presence of accumulations in the path of the light flux.

During adaptation of enlarged microsystems, it is possible for one of the microsystems not to coincide in phase with the others. This is attended by disintegration of this system followed by the involvement of its components in thermal diffusion. As a result, the optical density drops (Fig. 2, *a*, portion of curve for $42<t_5<45$ min).

The formation of intercellular biophysical relationships continues until an integrated system with uniform intercellular relationships is formed. The bacterial population then enters the next stage of its development: the stage of logarithmic growth (Fig. 2, *a*, portion of curve for $t>60$ min and Fig. 2, *b*, portion of curve for $t>75$ min).

A comparative analysis of the time course of development showed that the duration of the adaptation period (phase leveling) is different in *E. coli* and *E. aerogenes*, due to different physicochemical properties of these bacteria and to their different type of intercellular relationships. In ad-

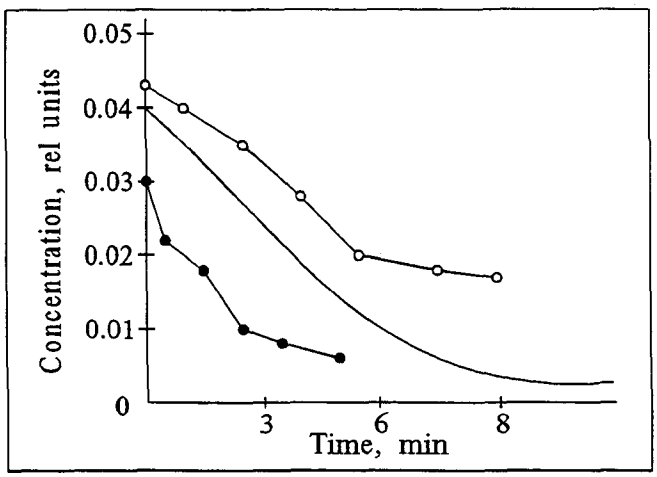

Fig. 3. Changes in concentration of bacteria for diffuse mechanism of their migration in meat–peptone broth. *a*) theoretical; *b*) *E. coli*; *c*) *E. aerogenes*.

dition, many more individuals are involved in the synchronous division in *E. coli* than in *E. aerogenes*. On the whole, these findings show that the mechanisms of adaptation to external conditions are diverse, suggesting that *E. coli* will suppress the development of *E. aerogenes* in a combined culture.

Indeed, the experimental findings did show *E. coli* to have inhibitory properties. The results of studies of the time course of *E. coli* and *E. aerogenes* development in a mixed culture are presented in Fig. 2, *c*. The experimental curve may be divided into two portions. The first portion of the curve is characteristic of the time course of the bacterial type with a higher rate of multiplication, i.e., of *E. coli*. After the development of *E. coli* is completed, the development of *E. aerogenes* begins (Fig. 2, *c*, second portion of curve). Such a long persistence of *E. aerogenes* in the lag phase (for more than 3 h) attests to the inhibitory effect of *E. coli*.

Thus, our findings demonstrate that the time course of bacterial development in the lag phase has a spurt-type pattern; the results of studies can be predicted from the pattern of formation of the intercellular biophysical relationships, and the period of diffuse distribution of bacteria may serve as the test for their identification.

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