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INTENSITY OF PROTEIN SYNTHESIS IN THE MICROBIAL CELL AS A
FACTOR DETERMINING THE EFFECT OF NEOMYCIN ON ITS METABOLISM

[Following is the translation of an article by Yu. O. Sazykin, Department of Chemotherapy (Head - Prof. A. M. Chernukh), Institute of Pharmacology and Chemotherapy, AMN, USSR, Moscow, published in the Russian-language periodical Antibiotiki (Antibiotics) 10(6), 1965, pages 518-22. It was submitted on 29 Apr 1964.]

It has been shown that neomycin suppresses the synthesis of protein in the microbial cell [1]. At the same time it is known that the antimicrobial activity of streptomycin, neomycin, and other antibiotics - water-soluble bases related to the conditional group of streptomycin-like substances - is clearly dependent on the degree of aeration and pH value of the medium, concentration of salts in it, phase of growth of the culture, etc. In connection with this there is interest in a study of the influence of such factors both on the intensity of protein synthesis in the microbial cell and on the inhibition of it by neomycins.

It is also known that tests for studying the effect of streptomycin-like substances on oxidizing processes often give different results: the inhibiting action of these antibiotics on respiration is revealed far from always. According to some findings it is possible to assume that disruption of respiration is related to a number of indirect manifestations of the influence of neomycins on protein synthesis [2]. In such a case the effectiveness of neomycins as inhibitors of respiration should depend on the intensity of protein synthesis in suspensions of non-multiplying cells used in gasometric tests in the Warburg variation. Synthesis of proteins in such cells takes place, though on insignificant scales, due to the stock of free amino acids and may vary to a certain degree depending on experimental conditions. In this work an attempt is made to clarify the conditions under which the inhibiting effect of neomycins on respiration is revealed.

Methods

The investigations were conducted with *E. coli* strain No 645 and preparations of colimycin and mycerin which are put out by the medical industry. Growth of the stated strain of *E. coli* was suppressed at a concentration of around 5 $\mu\text{g}/\text{ml}$ of these antibiotics in the medium. Since colimycin and mycerin did not differ in their action of biochemical processes the illustrative material

is limited to data on colimycin. Gasometric determination was performed with the help of the Warburg method in a phosphate buffer (M/30, pH 7.3). Into the main area of the vessels we added 1 ml of a suspension of cells (3.5 billion/ml), 0.1 ml of a solution of glucose - up to an end 0.025 M concentration, and 0.1 ml of a solution of colimycin (or distilled water). For preparation of the suspension we used cells which were cultivated in meat-peptone broth at 37° under various conditions: with shaking for 4 hours in flasks on a rocking device with the addition of glucose (0.025 M) or without the addition and during cultivation for 18 hours under stationary conditions in test tubes with a high layer (around 8 cm) of medium, i.e., under conditions of very weak aeration.

The method of working with labeled compounds, in particular with S³⁵-methionine, was described in a previous report [3]. Concrete conditions of the tests are given in the explanations to the tables.

Results and Discussion

1. The influence of neomycins on oxidizing processes in cells which were preliminarily cultivated under various conditions.

During the testing of the effect of streptomycin on oxidizing processes in microorganisms the results obtained by different investigators were often far from being the same even in that case when the tests were set up with the same oxidized substrate and the cells belonged to the same species. Attempts to explain this by peculiarities in manner of oxidation of metabolites in individual strains did not lead to the establishment of any general regularities. As regards the neomycins, then the degree of their inhibiting effect on oxidizing processes can also vary strongly. According to data obtained by us earlier [2], suppression of the oxidation of glucose and other substrates set in under the action of neomycins only in the event that the antibiotic and oxidizable substrate were introduced into the suspension of bacteria simultaneously.

On the basis of the presently established capacity of neomycins to rapidly suppress protein synthesis [1] it is possible to make the proposal that the inhibiting effect of neomycins on respiration is not direct and is manifested only in that case when synthesis of respiratory enzymes takes place in the cell, which is fully possible for the first time after transfer of cells into a medium with another oxidizable metabolite or after transformation of the degree of aeration of the medium (in the case of facultative anaerobes). Depending on the conditions of preliminary cultivation of cells, used then in gasometric experiments, after transfer into vibrating Warburg vessels a greater or lesser synthesis of respiratory systems takes place in them. In spite of the fact that

in media used for the study of respiration sources of nitrogen are usually lacking, a certain degree of formation of enzymes can take place here due to the background of free amino acids in the cell. The presence in the new medium of an antibiotic - an inhibitor of protein synthesis - should suppress the synthesis of respiratory enzymes, and in comparison with control cells lower the intensity of respiration. This will be stronger, the greater the new conditions of cultivation differ from the old.

Table 1

The effect of colimycin on oxidation under similar conditions of glucose by cells of *E. coli* cultivated under various conditions

(a) Условия предвари- тельного выращи- вания клеток	(b) Концен- трация колимици- на (в мкг/мл)	(c) Поглощение O ₂ (в мкл)					
		(d) Время после начала отсчета (в мин.)					
		30	50	90	120	150	180
(e) При сильной аэра- ции в присутствии глюкозы	0	68	73	74	70	70	72
	10	66	67	68	66	65	68
	100	60	54	50	47	46	46
(f) При сильной аэра- ции в отсутствие глюкозы	0	47	59	62	60	58	54
	10	48	43	45	46	48	43
	100	40	36	32	30	29	28
(g) При слабой аэра- ции в отсутствие глюкозы	0	36	46	54	57	56	59
	10	30	31	30	33	33	36
	100	28	24	19	17	14	13

(a) Key: Conditions of preliminary cultivation of cells; (b) Concentration of colimycin (in $\mu\text{g/ml}$); (c) Absorption of O₂ (in μl); (d) time after onset of count (in min.); (e) During strong aeration in the presence of glucose; (f) During strong aeration in the absence of glucose; (g) During weak aeration in the absence of glucose.

It is clear from the data in Table 1 that at small concentrations of colimycin the conditions of preliminary cultivation of the culture determine the degree of its inhibiting action on the absorption of O₂ by a suspension of cells during gasometric tests.

It is necessary to note especially that everything stated concerns the oxidation of metabolite, not related to those which are oxidized by adaptive enzyme systems: in the given case glucose was used as the oxidizable substrate. Its oxidation, in

contrast to the oxidation of lactose for example, began immediately, but a certain intensification of this process took place, and it turned out to be expressly sensitive to colimycin. If the cells were preliminarily cultivated during weak aeration (stationary culture and thick layer of medium) the inhibiting effect of colimycin in a dose of 10 $\mu\text{g/ml}$ on these cells, transferred to an aerated medium, was quite great. In the control the absorption of O_2 during the first 90 minutes increased noticeably, in vessels with colimycin this did not take place, i.e., reduced absorption of O_2 was preserved for the entire period of the experiment.

Increase of O_2 absorption was quite weak when in the gasometric tests cells were used which were cultivated earlier under strong aeration and with glucose in the medium. The effect of colimycin (10 $\mu\text{g/ml}$) turned out to be correspondingly less noticeable. Apparently in this case the synthesis of a "supplementary amount" of respiratory enzymes following transfer from one medium to another hardly takes place at all and the capacity of colimycin to suppress protein synthesis turned out to be unrealized.

The absence of glucose during growth in an aerated medium somewhat increases the difference in absorption of O_2 by the suspension in the beginning and end of the experiment and intensifies the effect of colimycin.

It is also clear from the data in Table 1 that a difference exists in the effect on respiration by colimycin in concentrations of 10 and 100 $\mu\text{g/ml}$. The greater concentration of antibiotic caused a noticeable drop in O_2 absorption regardless of the conditions of preliminary cultivation of cells, which is apparently connected with the capacity of the antibiotic in high concentrations to disrupt not only protein synthesis, but also other biochemical processes.

If an attempt is made to compare the diverse conditions, described in the literature, of gasometric experiments which were set up with streptomycin-like antibiotics, mainly with streptomycin, then it is possible to note that preliminary cultivation of the culture of bacteria was carried out differently: with strong aeration, under stationary conditions - sometimes with a very weak admission of O_2 into the medium, finally on solid medium, etc. [2, 4, 8]. All of this is explained to some degree by the fact that gasometric tests with relatively small concentrations of streptomycin-like substances in various laboratories frequently produced different results even when the same type of bacteria was used.

Table 2

Influence of conditions of incubation on the capacity of colimycin to inhibit the incorporation of S³⁵-methionine in protein of non-multiplying cells of *E. coli* (amino acid and antibiotic added to the bacterial cells simultaneously)

(a) Условия инкубации клеток с антибиотиком	(b) Концентрация колимицина (в $\mu\text{г}/\text{мл}$)	(c) Число имп/мин	(d) Подавление включения (в %)
Встряхиваемая среда, 0.01 M концентрация глюкозы	0	885	0
	10	312	65
	100	149	83
Встряхиваемая среда, глюкоза отсутствует	0	203	0
	10	141	30
	100	105	49
Встряхиваемая среда, 0.01 M концентрация глюкозы, 0.01 M концентрация малоната натрия	0	390	0
	10	242	38
	100	217	45
Стационарная среда, 0.01 M концентрация глюкозы	0	439	0
	10	383	13
	100	278	37
Стационарная среда, глюкоза отсутствует	0	154	0
	10	141	9
	100	111	28

Key: (a) Conditions of incubation of cells with antibiotic; (b) Concentration of colimycin (in $\mu\text{g}/\text{ml}$); (c) Number, imp/min; (d) Suppression of incorporation (in %); (e) Vibrating medium, 0.01 M concentration of glucose; (f) Vibrating medium, glucose absent; (g) Vibrating medium, 0.01 M concentration of glucose, 0.01 M concentration of sodium malonate; (h) Stationary medium, 0.01 M concentration of glucose; (i) Stationary medium, glucose absent.

II. Influence of neomycins on the incorporation of amino acids in protein under various conditions of incubation of cells

The experiments, the results of which are cited in this section, were set up for the purpose of clearing up what conditions are most favorable for the inhibiting action of neomycins on the synthesis of protein in non-multiplying cells. A culture of *E. coli* was cultivated in meat-peptone broth under stationary conditions. The cells were separated from the medium, washed, and suspended in a saline buffer solution as was described earlier [5]. Table 2 reflects the results of experiments on the incorporation of S^{35} -methionine in the protein fraction of cells of *E. coli* under various conditions of their incubation with colimycin.

The greatest rate of incorporation in the control was observed during strong aeration of the medium and in the presence of a source of energy - glucose. Under these conditions the suppression of incorporation of amino acids by colimycin (in percentage expression) was the highest - colimycin in a dose of 10 $\mu\text{g}/\text{ml}$ suppressed it by 65%, and in a dose of 100 $\mu\text{g}/\text{ml}$ - by 83%. Both factors - aeration and the presence of a source of energy in the medium - intensify the effect of colimycin.

Reduction of aeration leads to a noticeable suppression of incorporation of amino acid in the control, but does not weaken residual incorporation in the presence of colimycin (if one were to compare conditions I and IV). In comparison with conditions which are most favorable for the synthesis of protein during weak aeration the amount of label in the test increases strongly relative to the amount of label in the control.

Incubation of cells during aeration, but in the absence of a source of energy, also leads to a noticeable weakening of the effect of colimycin: suppression of incorporation in a percentage expression is lowered by approximately 2 times (conditions I and II).

If the cells are incubated under the most favorable conditions, i.e., during aeration and addition of glucose to them, but malonic acid - an inhibitor of the Krebs' cycle - is introduced into the medium, then again a relative weakening of the action of colimycin on protein synthesis is observed. Malonic acid disrupts the aerobic energy-giving reactions and as if imitates in part anaerobic conditions.

Finally, colimycin has a very weak effect if weak aeration is combined with the absence of glucose in the medium.

Results of the tests with labeled methionine, just as the gasometric tests, show that conditions which are most favorable for the synthesis of protein make the effect of colimycin (neomycins) more noticeable.

A weakening of protein synthesis in the cell correspondingly reduces the capacity of neomycins to disrupt viability, by stopping protein synthesis. This does not mean that the antibiotic effect of neomycins in the event of very weak protein synthesis is absent completely. In addition to the rapid inhibition of protein synthesis, neomycins can cause a slow, but sufficiently clearly expressed suppression of other processes of metabolism. In contrast to the effect on respiration their capacity to disrupt these processes is not connected with the stopping of protein synthesis in the microbial cell [1]. However, under ordinary conditions the influence of neomycins on the cell is connected directly with the inhibition of protein synthesis.

Conclusions

1. The inhibition of the effect of neomycins on the oxidation of glucose by non-multiplying bacterial cells is determined to a considerable degree by the conditions of their preliminary cultivation. Dependent on the latter is the synthesis of protein (respiratory enzymes) during the transfer of cells to a new medium and correspondingly the effect of neomycins, which are inhibitors of protein synthesis.

2. Under conditions which are unfavorable for the incorporation of amino acids in protein in quiescent cells - in the absence of aeration and a source of energy in the medium, the difference between indices of incorporation of labeled methionine in the control and with the addition of neomycins is relatively small. Factors which intensify the synthesis of protein: aeration and the presence of a source of energy in the medium, cause a much greater divergence between indices of incorporation in the test and in the control.

3. Results of gasometric changes and tests with the incorporation of labeled precursors in protein show that the degree of disruption of biochemical processes in the microbial cell by neomycins depends mainly on the intensity of flow of protein synthesis in it.

Literature

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