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OTS: 60-31,120

JPRS: 3149

7 April 1960

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(Price: $0.50)

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NEW YORK 17, N. Y.

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JPRS: 3149
CSO: 3373-D

A STUDY OF THE ROLE OF AMINO ACIDS IN STREPTOMYCIN FORMATION

Voprosy meditsinskoy khimii
[Problems of Medical Chemistry]
Vol 5, No 6, November-December 1959, Moscow
Pages 448-457
Russian, per

V. A. Severina,
S. V. Gorskaya,
I. V. Gracheva

The purpose of the present work is to study the effect of amino acids on the biosynthesis of streptomycin and the part they play in this process. Investigations conducted earlier into the overall role of amino nitrogen in the cultivation of the actinomyces which form streptomycin permitted the determination of a number of facts characterizing the rate of formation, accumulation and characteristics of distribution of amino nitrogen in the various fractions of fungus during fermentation in the process of assimilation of inorganic nitrogen from the culture medium. It was also possible to show certain regular relationships between the dynamics of conversion in amino nitrogen and the biosynthesis of streptomycin at various stages of the growth and development of the organism [1].

In studying the effect of various amino acids on the formation of streptomycin, attention was first of all directed to those amino acids which had been mentioned in this connection in the literature.

During the period in which the production of streptomycin was being mastered in the Soviet Union, V. N. Shaposhnikov suggested that the favorable effect of a culture medium containing corn extract on the formation of streptomycin, to a considerable degree depends on the presence of hexone bases in the extract. This suggestion was confirmed by F. Yegorov in his research on the effect of arginine on the yield of streptomycin by fermentation, and also by V. N. Shaposhnikov, P. A. Agatov and others, who demonstrated the stimulating effect of corn extract fractions containing arginine, histidine and lysine. Analogous data were obtained by us in acute experiments with mycelium freshly grown, washed, and separated from the culture liquid. Dulaney's work (1948) [2] showing the stimulating effect of L - proline on streptomycin yields, is known. Eiser and McFarlane in 1948 [3] established that a number of media which contain various mixtures of amino acids as a nitrogen source are favorable to the growth of the organism and

formation of streptomycin, though the best growth and highest yields of streptomycin take place in cultures containing histidine. The addition of valine, though not providing good growth, nonetheless facilitated synthesis of the antibiotic. This provided the basis for evaluating valine as a substance playing a specific role in streptomycin formation. In later works [4] there is evidence that glycine, when added to a synthetic medium, provides a higher yield of streptomycin. However, it was not subsequently possible to detect preferential incorporation of C^{14}-glycine, versus C^{14}-acetate, into the streptomycin molecule. There are indications in Hunter's works [5] with labled carbon dioxide that when unlabeled arginine is simultaneously introduced, depression of labeled carbon dioxide introduction into the antibiotic molecule is observed, and with this an increase in streptomycin yields. The author explained this phenomenon by the competitive action of the arginine.

As can be seen from the adduced data, toward the beginning of our work we disposed of the material which threw insufficient light on the part played by various amino acids in the biosyntheses of streptomycin. The purpose of the present work was to investigate this problem, using the new, highly active strain LS-1, and also to study the conversion of those amino acids which proved to be stimulants in the biosynthesis of the antibiotic.

Investigation Methods and Results

The strain LS-1 was used in the work. Cultivation was carried out on a simple synthetic medium of the following composition: $(NH_4)_2SO_4$ - 0.4%, glucose - 2-2.5%, KH_2PO_4 - 0.05%, $ZnSO_4$ - 0.001%, $FeSO_4$ - 0.005%, $MgSO_4$ - 0.01%, $MnSO_4$ - 0.005%, lactic acid - 0.6% (calculated on 100% concentrated acid) [6]; pH before sterilization was brought to 7.2 - 7.3 by adding alkali. Fermentation was conducted in 750 ml Erlenmeyer flasks to which were charged 100 ml of culture medium. Sterilization was accomplished over a period of 30 minutes at 0.5 atmospheres. After sterilization, pH was 6.9 - 7.0. Amino acids, sterilized by filtering through a Zeitz filter, were added to the medium in concentrations of 0.1% before inoculation. A sample which contained no amino acid served as control. A two-day culture, grown in a soybean medium or in a synthetic medium of the composition stated above, served as the inoculum: 2-2.5% of the culture (of the volume of the culture medium) were introduced into a flask, after which the controls and experimental sample were put on a shaker, rotating at 200 rpm a minute, at a temperature of 26° C. Tests to determine activity were taken from the third day of fermentation. Activity of the culture liquid was determined by the biological agar-plate diffusion method with the test-microbe B. mycoides.

Table 1

Amount of streptomycin formed by fermentation in the presence of arginine and histidine

Experiment No.	Amount of streptomycin formed (in μg per 1 ml)				
	control	in the presence of Arginine	stimulation (in %)	in the presence of histidine	stimulation (in %)
1	540	1,100	103	850	57
2	270	520	93	540	100
3	390	725	86	800	106
4	453	750	65	750	65
5	503	900	79	700	39

From Table 1 it can be seen that with the low level of activity in the control, the presence of arginine or histidine increases the yields of streptomycin by 50-100%.

In order to explain the dynamics of streptomycin accumulation, a number of experiments were conducted to determine the content of streptomycin in the culture liquid during the whole process of fermentation. The average data from 17 experiments are given in Table 2.

Table 2

Amount of streptomycin formed by fermentation in the presence of Arginine (in μg/1 ml)

Sample	Fermentation period									
	24 hrs	stimulation in %	48 hrs	stimulation in %	72 hrs	stimulation in %	96 hrs	stimulation in %	120 hrs	stimulation in %
Control	31	-	346	-	677	-	862	-	914	-
Arginine added	32	-	411	19	1,000	48	1,190	38	1,230	34

From Table 2 it can be seen that the stimulating effect of arginine on the formation of streptomycin is most pronounced (about 50%) at 72 - 96 hours of fermentation; however it also takes place in other periods of incubation (with the exception of the first 24 hours of fungus growth).

Data on the effect of histidine on streptomycin biosynthesis are given in Table 3 (average data from 12 experiments).

Table 3

Amount of streptomycin formed by fermentation in the presence of histidine (in μg/1 ml)

Sample	Fermentation period									
	24 hrs	stimulation in %	48 hrs	stimulation in %	72 hrs	stimulation in %	96 hrs	stimulation in %	120 hrs	stimulation in %
Control	29	-	259	-	607	-	736	-	861	-
Histidine added	27	-	366	40	809	33	966	31	1,041	12

As can be seen from Table 3, an increase in streptomycin yield of approximately the same order as in the case of arginine is also observed in the presence of histidine.

The question of whether this effect is specific or whether it depends on a large accumulation of the biological mass in the presence of an amino acid can be resolved at the time of the determination of the quantity of streptomycin units formed per unit of time by one milligram of mycelium.

Table 4

Yield of mycelium (in μg/1 mg of dry weight mycelium) per unit of time (24 hours) in the presence of arginine and histidine.

Sample	Fermentation period									
	0-24 hrs	stimulation (in %)	24-48 hrs	stimulation (in %)	48-72 hrs	stimulation (in %)	72-96 hrs	stimulation (in %)	96-120 hrs	stimulation (in %)
Control	24	-	90	-	60	-	19	-	3	-
Arginine added	25	-	100	11	83	38	23	21	0	-
Control	24	-	60	-	75	-	20	-	9	-
Histidine added	21	-	62	3	90	20	35	75	6	-

Data from suitable experiments are given in Table 4 (average values from 10 experiments for arginine and 8 for histidine).

From the material it can be seen that the mycelium grown in the presence of arginine, in comparison with the control, is characterized by more intense formation of streptomycin, chiefly between 48 and 72 hours of fermentation. In the presence of histidine the mycelium yield is also higher, with this difference only, that the synthesis of the antibiotic is stretched over a longer period. The capability for streptomycin formation in both cases practically disappears toward the fifth day of fermentation.

In spite of the fact that the mechanism of the stimulating effect of hexonic bases on the biosynthesis of streptomycin remains yet unclear, the effect of arginine can be explained by the importance of the guanidine groups, present in arginine, for the synthesis of the streptomycin molecule. It was found, however, that α-alanine also exerts a stimulating effect on streptomycin synthesis (Table 5). In only 2 of 11 experiments was a result obtained. The greatest increase in streptomycin yields was observed after 70 hours of fermentation, i.e., in the period of fungus growth completion and the beginning of its autolysis.

These data necessitated analogous experiments with other amino acids, which, just as the α-alanine, were also added before inoculation in a concentration of 0.01 M. Average data from 4 experiments are presented in Figure 1. The amino acids which increased streptomycin yield in all experiments are placed to the right of the control; to the left are the amino acids which in no experiment provided an increase in activity in comparison with the control. Some of them (cystine and typtophan) in all instances showed a depressing effect on streptomycin formation.

Table 5

Quantity of streptomycin formed by fermentation in the presence of α-alanine (in μg/1 ml)

Sample	Fermentation period									
	24 hrs	stimulation (in %)	48 hrs	stimulation (in %)	72 hrs	stimulation (in %)	96 hrs	stimulation (in %)	120 hrs	stimulation (in %)
Control	34	-	398	-	746	-	733	-	946	-
α-alanine added	23	-	444	11	900	20	1,218	66	1,172	24

We then turned our attention to the importance of the time of addition of the amino acids which stimulate streptomycin biosynthesis. We found that the addition of amino acids after 48 hours of mycelium growth reduces their effectiveness. Thus, the introduction of the amino acids into the medium before inoculation was obligatory (Table 6).

The study of the stimulating effect of any substance on the biosynthesis of the antibiotic under ordinary fermentation conditions cannot provide data for characterizing their participation in the synthesis of the streptomycin molecule. Such substances as amino acids can facilitate the synthesis of the antibiotic by serving as the nitrogen source and intensifying the growth and development of the organism, i.e., assure good accumulation of the biological mass. In this connection, the effect of amino acids on the biosynthesis of the antibiotic under conditions where the growth phase is separated from the antibiotic formation phase was studied, i.e., under conditions of pre-grown mycelium. For this purpose, the mycelium, grown on the two-day synthetic medium described above, was separated from the culture liquid by centrifuging and washed three times with a phosphate buffer (pH 7), after which the thick mass of mycelium obtained was transferred in a fixed amount

Table 6

QUANTITY OF STREPTOMYCIN FORMED IN THE PRESENCE OF AMINO ACID ADDED APPROXIMATELY 48 HOURS AFTER THE BEGINNING OF FERMENTATION (in γ g/ml)

Experiment Conditions	Fermentation period							
	48 hrs	stimulation (in %)	72 hrs	stimulation (in %)	96 hrs	stimulation (in %)	120 hrs	stimulation (in %)
Control	317	-	649	-	789	-	825	-
In the presence of Arginine	300	-	588	-	946	20	925	12
In the presence of Histidine	294	-	691	-	973	23	920	11
In the presence of Lipine	280	-	601	-	828	5	811	-
In the presence of α alanine	304	-	620	-	906	14	920	12

to a fresh medium containing only a phosphate buffer and glucose (2%). Amino acids were added to the suspension of two-day mycelium in a concentration of 0.01 M. The results obtained are presented in Figure 2 as the average data on maximum activity from 4 experiments. The amino acids stimulating streptomycin biosynthesis are placed to the right of the control, and the amino acids which provided no stimulating effect in any experiment are to the left. Cystine and tryptophan, just as in the conditions of ordinary fermentation, inhibited streptomycin formation.

A series of experiments on a more detailed study of mycelium productivity was then conducted. In as much as a nitrogen source in the form of an amino acid was contained in the experimental samples, an additional control, (apart from the usual control), with ammonium sulfate, (also in concentrations of 0.01 M), was used in these experiments. The average data from the experiments are presented in Table 7.

Table 7

Productivity of mycelium (in μg/1 mg of dry weight) per unit of time (24 hours) in the presence of amino acids.

Added substrate	Time (in hours) after mycelium transfer				Stimulation (in %)
	0-24	24-48	48-72	0-72	
Control	20	19	14	53	-
-alanine	23	28	20	71	34
Glycine	27	25	17	69	30
Valine	22	33	19	74	40
Arginine	25	27	20	72	36
Histidine	29	18	16	63	20
Lysine	20	23	22	65	23
Isoleucine	25	35	18	78	47
Phenyl alanine	22	21	20	63	20
Ammonium sulfate	15	16	14	45	-

With very few exceptions, mycelium productivity in the presence of the indicated amino acids was higher than in the control. In isolated instances, streptomycin yields in the experimental samples was 70-80% higher than in the control. The samples with ammonium sulfate added did not differ from the control, and in a number of instances, productivity values were even lower.

Thus, it was possible to assume that the regular increase in yields of streptomycin, in conditions where mycelium growth is absent, depends on their specific influence on the formation of the antibiotic. Lysine, methionine, serine, theonine, thyrosine and dicarboxylic amino acids (glutanic and aspartic), which do not show a stimulating effect in relation to the biosynthesis of streptomycin under ordinary fermentation (Figure 1), were also indifferent under conditions involving pregrown mycelium.

When analyzing the data obtained, the question arose as to whether the mechanism of the stimulating effect of amino acids (which are very dissimilar in chemical structure and properties) is common to all, or whether it is distinguished, if not for each, then possibly for individual amino acids, more or less similar in structure and composition. The increase in streptomycin yield under the influence of the amino acids tested is most understandable in the presence of arginine, as it has guanidine groups which are capable of participating in the synthesis of the streptomycin molecule. The mechanism of the effect of such amino acids as α-alanine, glycine, valine, and some others still remains absolutely unclear. In this connection, it was of interest to trace the paths of conversion, if only of some of the amino acids, during one process of their assimilation by the fungus. First of all, it was established by paper chromatography that upon adding arginine, histidine, gylcine and α-alanine, their disappearance from the cultured liquid was observed toward the end of the second day. Taking into account the possibility of deamination of the amino acids, we conducted a series of experiments to calculate the amount of ammonia formed and the amount of amino nitrogen loss.

The mycelium, as usual, was cultivated on a synthetic medium. A specific amount of the cultural liquid was removed from the fermentation flask every 24 hours. The culture liquid was separated from the mycelium, and a moist weighed portion of the mycelium, pressed out in a Buchner funnel, was diluted in a specific amount of buffer. A control sample and an experimental sample with an added amino acid in a final concentration of 0.01 M were charged from the suspension obtained. The flasks were placed in a thermostat on a shaker at 27-28° C and, after 4 and 24 hours incubation, the samples were removed to calculate the nitrogen ammonia and amino nitrogen.

The data obtained for arginine are presented in Table 8 and Figure 3. From the table it can be seen that after 4 hours, insignificant amounts of NH_3-N are separated out; after 24 hours incubation the amounts of NH_3-N fluctuate within the limits of 15-20 mg/equiv. The loss of NH_2-N after four hours is also insignificant. After 24 hours incubation the amino nitrogen of the added arginine almost completely disappears. The amount of nitrogen ammonia formed during this period surpasses the amount of amino nitrogen by an average 3 1/2 times. On the basis of the data in Table 8, it was possible to assume that the arginine at first breaks down under the influence of arginase to form ornithine and urea, and then under the influence of urease, ammonia is formed from the urea. The results of analogous experiments (Figure 4), in which urea instead of arginine was added, confirms this.

Table 8

Accretion of nitrogen ammonia (NH_3-N) and loss of amino nitrogen (NH_2-N) (in mg/equiv. per 1 gm of dry weight mycelium) in the presence of arginine.

Experiment No.	Mycelium growth (in hours)	Streptomycin formation (in g/ml)	NH_3-N			NH_2-N		
			Original amount	After 4 hrs incubation	After 24 hrs incubation	Original amount	After 4 hrs incubation	After 24 hrs incubation
1	41	370	0	0.1	15.0	5.3	0.5	5.0
	65	690	0	0.6	15.0	5.7	2.2	5.7
	89	975	0	0.9	27.0	6.5	0.9	6.5
2	16	12	0	0.3	27.0	7.0	0.3	7.0
	40	385	0	0.3	20.0	5.5	1.4	5.5
	64	920	0	0.6	26.0	5.5	1.0	5.4
	88	1,150	0	0.7	20.0	6.3	0.9	6.1
3	18	48	0	0.4	15.0	4.3	0.4	4.3
	42	560	0	-	15.0	5.2	-	5.2
	66	1,169	0	0.5	14.6	5.1	1.6	5.1
	90	1,015	0	0.7	14.6	4.0	0.6	4.0
4	19	-	0	0.1	17.6	5.0	0.0	5.0
	43	-	0	0.2	16.0	4.0	1.5	4.0
	67	-	0	1.7	18.0	4.5	0.4	4.0
	90	-	0	0.1	20.0	6.0	0.3	5.4

It is obvious from Figure 4 that after 24 hours of incubation in the presence of urea, approximately the same picture as with the addition of arginine is observed. The considerably greater amounts of NH_3-N formed in the samples with urea after 4 hours of incubation, in comparison with the data obtained from arginine, can be explained in that with the addition of urea, there is already a prepared substrate for the action of the urease and the subsequent separation out of the ammonia, while in the presence of arginine, a somewhat longer period of time must elapse for the formation of ammonia from urea. The results of these investigations are in complete agreement with data obtained earlier from chromatographic analysis which show the loss of arginine from the cultured liquid toward the end of the second day of fermentation.

Since it is known that active synthesis of streptomycin begins only after 48 hours of fungus growth, the assumption concerning the substantial value of the arginine metabolism products or their derivatives in the biosynthesis of streptomycin is not without foundation.

A natural continuation of these experiments was the study of the effect of guanidine and urea on the formation of the antibiotic. The substrates mentioned, added in concentrations of 0.01 M, were tested both under ordinary fermentation conditions and in acute experiments with pre-grown mycelium, but no stimulating effect whatever was shown in streptomycin synthesis.

In as much as the role of arginine in the biosynthesis of the antibiotic is apparently connected with its participation in the formation of the streptidine fraction of streptomycin, it was of interest to examine the effect of streptidine itself on the synthesis of the antibiotic (Table 9).

Table 9

Effect of Streptidine on Streptomycin Biosynthesis

Experiment No.	Maximum streptomycin content (in μg/1 ml)			
	in fermentation		in acute experiments	
	control	streptidine added	control	streptidine added
1	1,180	1,875	867	1,850
2	1,188	1,405	860	912
3	1,264	1,070	460	845
4	2,000	3,050	645	881
5	1,628	1,492	233	654
6	1,750	1,788	501	1,231
7	-	-	519	934
Average	1,502	1,780	584	1,043

As was expected, streptidine stimulates the synthesis of streptomycin in ordinary fermentation, but proved to be considerably more effective in experiments with pregrown mycelium, i.e., under conditions most favorable for the introduction of a prepared fragment into the streptomycin molecule.

In the study of the conversion paths of glycine and α-alanine, it was found that these amino acids disappear from the culture liquid toward the end of the second day of fermentation and are subjected to deamination. The data obtained are presented in Figures 5 and 6.

The correspondence of the amount of nitrogen ammonia increase and the loss of amino nitrogen in the balance experiments convincingly enough points out the presence of an oxidative deamination reaction, the deaminase activity being equally expressed in the new and old mycelium.

As can be seen from Table 10, in the experiments with histidine the increase of ammonia after 24 hours surpasses by approximately 1 1/2 times the decrease of amino nitrogen. This indicates that the ammonia is formed partially at the expense of the nitrogen from the imidazole ring of histidine.

Table 10

Increase of nitrogen ammonia and decrease of amino nitrogen (in mg/equiv. to 1 gm dry weight mycelium) in the presence of histidine and lysine.

Mycelium growth (in hours)	In the presence of histidine						In the presence of lysine					
	NH_3 - N			NH_2 - N			NH_3 - N			NH_2 - N		
	Original amount	4 hrs	24 hrs	Original amount	4 hrs	24 hrs	Original amount	4 hrs	24 hrs	Original amount	4 hrs	24 hrs
20	0	1.0	13.4	9.9	4.0	9.8	0	0.1	12.0	14.5	0.0	14.5
44	0	0.5	14.2	9.3	2.0	9.6	0	0.0	7.1	9.0	0.0	7.1
72	0	0.0	15.0	9.3	3.6	9.0	0	0.2	7.0	9.6	0.0	7.0
96	0	0.2	19.0	12.0	3.8	11.7	0	0.0	7.0	7.2	2.1	6.5

The complete correspondence of the amounts of nitrogen ammonia increase and of amino nitrogen decrease in the presence of lysine and also the correspondence of the amount of amino nitrogen in the original sample to the amount of its decrease after 24 hours incubation indicates that lysine is subjected to deamination in the α-amino group, with the preservation, apparently, at a given stage, of the metabolism of the amino group separated from the carboxyl.

The regularities found give some idea of the original paths of conversion of amino acids. These data, along with the above established fact of the increased yields of streptomycin in the presence of the indicated amino acids, are the basis for further study of the paths of their conversion, with the purpose of clarifying the importance and roles of both amino acids and the products of their metabolism in the biosynthesis of streptomycin.

Conclusions

1. The stimulating effect of arginine, histidine, lysine, glycine, α-alanine, valine, phenylalanine and isoleucine on streptomycin biosynthesis under conditions of ordinary fermentation and in acute experiments with pregrown mycelium was established.

2. It was shown that aspartic and glutamic acids, serine, theonine, methionine, tyrosine and leucine, under the conditions indicated above, do not stimulate streptomycin formation. Cystine and tryptophan inhibit the synthesis of the antibiotic.

3. It was established that in the primary paths of metabolism, glycine and -alanine are subjected to oxidative deamination. The regularities found in balanced experiments with arginine indicate the presence in a given actinomyces of active fermentation systems of arginase and urease activity. With respect to the α-amino group, arginine is subjected to deamination.

4. It was established that guanidine and urea do not stimulate streptomycin synthesis in ordinary fermentation and in acute experiments.

5. Streptidine added to the medium increases the yields of streptomycin; this effect is most clearly evident in acute experiments with pregrown mycelium.

Literature

1. Severina, V. A., Gracheva, I. U., Voprosy meditsinskoy khimii [Problems of Medical Chemistry], Vol 4, No 6, 1958 p 455.

2. Dulaney, E. L., Journal of Bacteriology, Vol 56, 1948, p 305.

3. Eiser, N. M., McFarlane, W. D., Canadian Journal of Research, Vol 26, 1948, p 164.

4. Numerof, P., Gordon, M., Virgona, A., and others, Journal of the American Chemical Society, Vol 76, 1954, p 1341.

5. Hunter, G. D., Herbert, M., Hockenhull, D., Biochemical Journal, Vol 58, 1954, p 249.

6. Severina, V. A., Gorskaya, S. V., Antibiotika [Antibiotics], Vol 2, No 2, 1957, p 26.

FIGURE APPENDIX

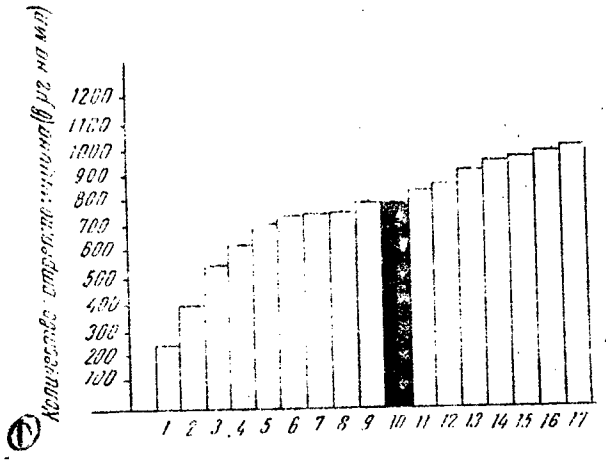

Figure 1. Effect of various amino acids on streptomycin formation by fermentation.

1 - tryptophan; 2 - cystine; 3 - methionine; 4 - phenylalanine; 5 - tyrosine; 6 - serine; 7 - aspartic acid; 8 - threonine; 9 - leucine; 10 - control; 11 - glycine; 12 - valine; 13 - lysine; 14 - ornithine; 15 - arginine; 16 - a-alanine; 17 - histidine.

Legend: (1) Amount of streptomycin (in μg/1 ml)

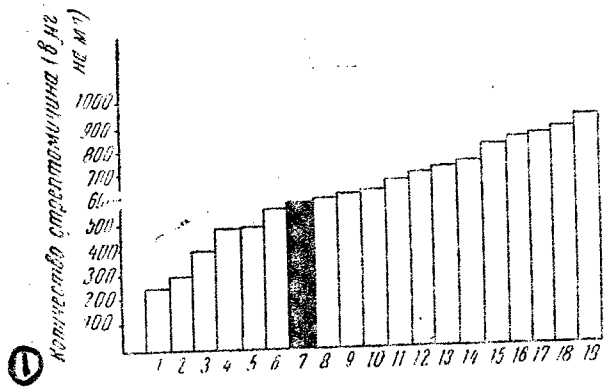

Figure 2. Effect of various amino acids on streptomycin formation (see experimental conditions in text).

1 - cystine; 2 - methionine; 3 - tryptophan; 4 - aspartic acid; 5 - leucine; 6 - glutamic acid; 7 - control; 8 - serine; 9 - ornithine; 10 - tyrosine; 11 - α-alanine; 15 - lysine; 16 - histidine; 17 - valine; 18 - arginine; 19 - glycine.

Legend: (1) Amount of streptomycin (in μg/1 ml)

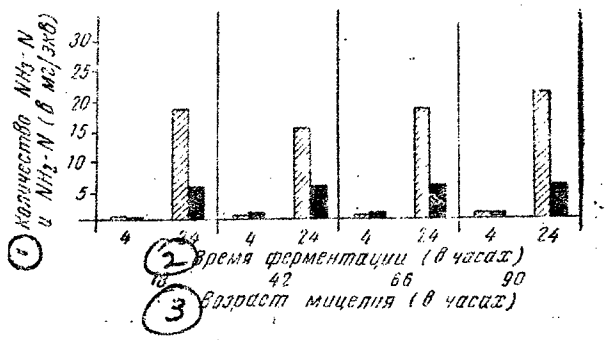

Figure 3. Accretion of nitrogen ammonia (NH_3 - N) and decrease of amino nitrogen (NH_2 - N) in mg/equiv. to 1 g dry weight mycelium in the presence of arginine. Slashed columns -- NH_3 - N accretion; dark columns -- decrease of NH_2 - N.

Legend: (1) Amount of NH_3 - N and NH_2 - N (in mg/equiv)
(2) Fermentation period (in hours)
(3) Mycelium growth (in hours)

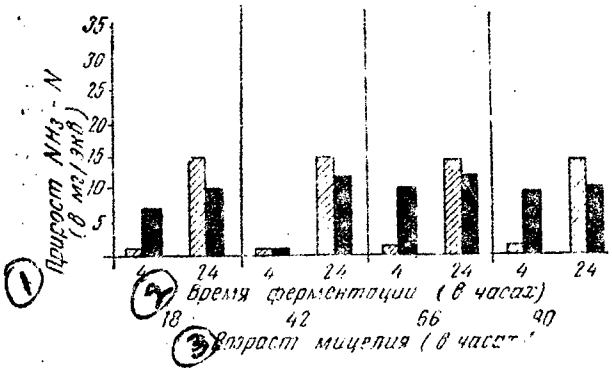

Figure 4. Nitrogen ammonia (in mg/equiv to 1 g dry weight mycelium) in the presence of arginine and urea.

Slashed columns - arginine added; dark columns - urea added.

Legend: (1) NH_3 - N accretion (in mg/equiv.)
(2) Fermentation period (in hours)
(3) Mycelium growth (in hours)

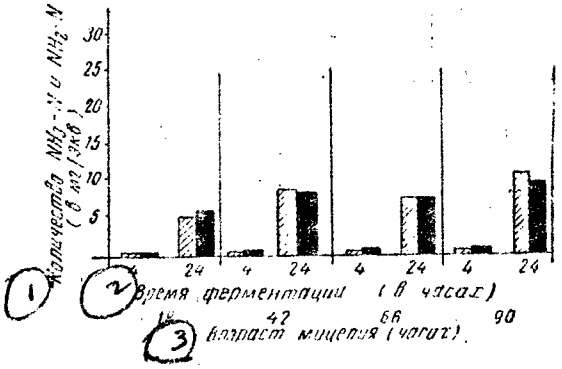

Figure 5. Accretion of nitrogen ammonia and decrease in amino nitrogen in mg/equiv. to 1 g dry weight mycelium in the presence of glycine.

Slashed columns - nitrogen ammonia accretion; dark columns - decrease in amino nitrogen.

Legend: (1) Amount of NH_3 - N and NH_2 - N (in mg/equiv)
(2) Fermentation period (in hours)
(3) Mycelium growth (in hours)

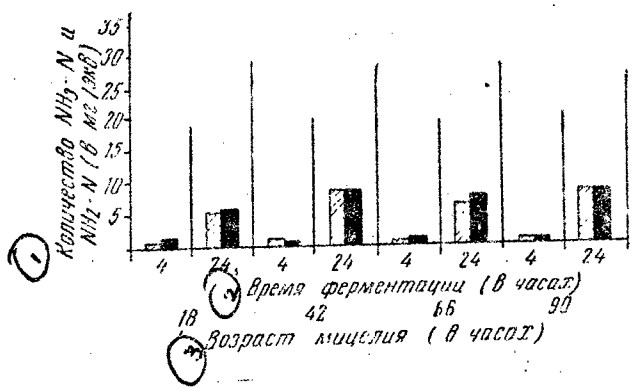

Figure 6. Accretion of nitrogen ammonia and decrease in amino nitrogen (in mg/equiv to 1 g dry weight mycelium) in the presence of α-alanine.

Slashed columns - nitrogen ammonia accretion; dark columns - decrease in amino nitrogen.

Legend: (1) Amount of NH_3 - N and NH_2 - N (in mg/equiv)
(2) Fermentation period (in hours)
(3) Mycelium growth (in hours)

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