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TRANSFORMATION OF STAPHYLOCOCCUS UNDER THE INFLUENCE OF KILLED CULTURES OF SALMONELLA TYPHIMURIUM AND LISTERIA MONOCYTOGENES, RESISTANT TO 75,000 UNITS/ML OF STREPTOMYCIN

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BIOLOGICAL LABORATORIES
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TRANSFORMATION OF STAPHYLOCOCCUS UNDER THE INFLUENCE OF KILLED CULTURES OF SALMONELLA TYPHIMURIUM AND LISTERIA MONOCYTOGENES, RESISTANT TO 75,000 UNITS/ML OF STREPTOMYCIN

[Following is the translation of an article by V. N. Polshkova, Microbiological Laboratories of the Scientific-Research Institute for Ear, Throat and Nose (Scientific Director -- Prof. P. P. Sakharov) of the RSFSR Ministry of Public Health, Moscow, published in the Russian-language periodical Byulleten' Eksperimental'noy Biologii i Meditsiny (Bulletin of Experimental Biology and Medicine), No 4, 1963, pp. 66-70. It was received on 12 Nov 1961 and presented by Active Member of the USSR Academy of Medical Sciences N. N. Zhukov-Verezhnikov. Translation performed by Sp/6 Charles T. Ostertag Jr.]

The peculiarities have been investigated of changes in the recipient Staphylococcus aureus under the influence of heat-killed microbial cells of cultures from the donors Salmonella typhimurium and Listeria monocytogenes which are resistant to streptomycin.

This work is a continuation of the investigations by Prof. P. P. Sakharov et al., [9, 10].

A number of other works by Soviet microbiologists and biochemists have dealt with the study of transformation of microorganisms [1-6, 11].

G. V. Levitskaya [6], utilizing the property of the natural resistance of intestinal bacilli to penicillin, cultivated Staphylococcus aureus in the presence of extracts obtained from B. coli. She succeeded in obtaining Staphylococcus aureus which in the presence of extracts of B. coli began to develop under increased penicillin concentrations.

A. A. Imshenetskiy and K. Z. Perova [5], while growing a strain of Staphylococcus aureus, sensitive to streptomycin, on extracts of a streptomycin resistant strain of Staphylococcus aureus, detected that the resistance of the first one grew and became several times higher than in the original one. These investigations confirmed that cell-free extracts have a transforming effect.

Methods of Investigation

A strain of Staphylococcus aureus freshly isolated from the organism of a sick man was used in the work.

Our mission was to obtain strains of Salmonella typhimurium and Listeria monocytogenes which were resistant to streptomycin (the increase of resistance was carried out up to 75,000 active units/1 ml) and the subsequent growing of Staphylococcus aureus in media containing heat-killed cultures of Salmonella and Listeria.

The sensitivity of the microbes to antibiotics was determined by the method of serial dilutions in common meat peptone broth. For obtaining highly resistant cultures the method was used of passaging on media containing increasing concentrations of antibiotics.

Salmonella typhimurium, which is resistant to 75,000 active units/1 ml was killed in a water bath at 75° for one hour; Listeria monocytogenes, which possesses the same resistance to streptomycin, was killed in an autoclave at 1.5 atmospheres for 20 minutes.

In the beginning of the experiment a control culture of Staphylococcus aureus was sown in order to obtain isolated colonies. Then 50 individual colonies were seeded in test tubes containing meat peptone broth with various concentrations of streptomycin (650, 1000, 2000, 3000, 4000, 5000, and 6000 active units/1 ml).

Results of the Investigation

We established the extreme individual resistance of the initial culture of staphylococcus to streptomycin as equal to 650 active units/1 ml, and to penicillin -- 900 active units/1 ml. It must be noted that the strain of staphylococcus was isolated from a patient who had received penicillin and streptomycin.

An analogous "sowing" with test cultures of Staphylococcus aureus, after they had been through 35 passages in media containing killed cultures of Salmonella typhimurium and Listeria monocytogenes resistant to 75,000 units in 1 ml of streptomycin, didn't disclose great deviations in their resistance which was equal on an average to 5000 units in 1 ml.

During growth on a killed culture of Salmonella typhimurium resistant to 75,000 units of streptomycin in 1 ml, the resistance of Staphylococcus aureus to this antibiotic gradually increased. By the 10th passage the

resistance to streptomycin increased almost twice in comparison with the control. On the 20th passage the bacteriostatic concentration of streptomycin equaled 2000 units in 1 ml, on the 30th passage -- 3500 units, and on the 35th passage -- 5000 units in 1 ml (figure 1).

The agglutination titer of the sera from a rabbit immunized with an antigen of the original strain of staphylococcus, which reached a titer of 1:3200 (+++) with the original culture, produced titers with test cultures equal to only 1:100 (++++) and 1:800 (+++).

Simultaneously in the test staphylococcus, the capability appeared to become agglutinated by the blood sera of a rabbit immunized with streptomycin resistant salmonella, in the extracts of which it was incubated for 35 passages. The degree of expressedness of this agglutination reached a titer of 1:200 (+++). We also detected a similar phenomenon during the incubation of staphylococcus in media containing an ordinary killed culture of salmonella. The result of this experiment was that staphylococcus started to agglutinate in a titer of 1:400 with antisalmonella serum.

It was also ascertained that adaptation to the metabolic products of streptomycin resistant salmonella was accompanied by a change in a number of properties of the staphylococcus. Changes in the morphological features of the microbial cells were observed already by the 5th passage. The cocci lost the characteristic grouping by clusters or small heaps. Many individual cells were encountered, sometimes arranged by twos or threes. By the 10th passage increases and decreases in the sizes of the cocci began to appear. Some were very small and others were two or three times larger than the original ones. By the 21st passage the number of morphologically changed cells had grown considerably, and in the 35th passage normal sized staphylococcal cells had disappeared.

Beginning with the 15th passage, microbial cells began to appear which took the Gram stain in a lignter tone in comparison with the controls. With each subsequent passage the number of such cells increased. The size of the colonies of the staphylococcus strain on agar after 35 test passages changed: The test strain started to produce growth in the form of small colonies with a diameter of 0.58 mm, the control colonies had an average diameter of 1.56 mm.

An analysis of the biochemical activity showed that on the 35th passage the test culture of staphylococcus, in comparison with the control, began to ferment sorbitol and ceased decomposing mannitol.

In studying the pathogenicity of the test culture it was ascertained that mice, infected with a control culture of Staphylococcus aureus, died in

the course of the first days following inoculation of the culture in the foot muscle, while mice infected with staphylococcus incubated on streptomycin resistant salmonella easily endured the disease and didn't perish.

During the cultivation of Staphylococcus aureus on a killed culture of Listeria monocytogenes, resistant to 75,000 units/ml of streptomycin, the increase of resistance of the staphylococcus to this antibiotic proceeded with the same regularity as in experiments with staphylococcus raised on a killed culture of salmonella. The process of adaptation of the test strain of staphylococcus to metabolic products of listeria was also accompanied by changes of cell morphology, colony form, biochemical activity, antigenic structure, etc. On the 10th passage the resistance to streptomycin increased to 1250 units/1 ml, on the 20th passage -- 2000 units, and on the 30th passage -- 3500 units/ 1 ml. On the 35th passage the test staphylococcus was transferred from the meat peptone broth containing the killed culture of Listeria monocytogenes, to ordinary meat peptone agar which didn't contain a killed culture of listeria. After several days growth on solid nutrient media the test culture increased its resistance to streptomycin up to 5000 units/ 1 ml.

A similar occurrence was demonstrated in the experiments of V. Iollos [12, 13], conducted on Paramecium caudatum and Drosophila melanogaster, and also in the works of P. P. Sakharov et al., [9, 10, 11], from the point of view of the so-called progressive heredity.

The resistance of this culture to penicillin during the course of the entire experiment continued to remain at a level not exceeding 900 units/ ml (figure 2).

In the experimental staphylococcus the ability emerged to agglutinate with the blood serum from a rabbit immunized with streptomycin resistant listeria in the titer limits of 1:20 (++++) to 1:100 (+++).

We attempted to ascertain if the property of streptomycin resistance acquired by the transformation from streptomycin resistant culture-donors of Salmonella typhimurium and Listeria monocytogenes is hereditary. After the 35th passage on media containing the metabolic products of listeria and salmonella, the test strains were transferred to meat peptone broth which did not contain killed microbial cells from a culture of streptomycin resistant donors for the period of 15 passages with reseedings after two days in the third.

A lowering was apparent in the threshold of resistance of the staphylococcus up to 5000 units of streptomycin in 1 ml. In the 5th passage it lowered to 4400 - 4600 units, in the 10th passage to 4000 units, and in the 15th passage to 3500 units of streptomycin in 1 ml. The lowering of resistance to streptomycin of the staphylococcus cultures, raised earlier on streptomycin

resistant salmonella and listeria, proceeded almost parallel (figure 3). This testifies to the typical "prolonged modification" feature acquired by means of transformation.

It is interesting that the property of streptomycin resistance, transferred to staphylococcus by means of transformation, increased similarly in cultures raised on streptomycin resistant salmonella and listeria.

This effect did not depend on the degree of heating of the latter. The first were killed at 75° for one hour, and the second by autoclaving (1.5 atm) for 30 minutes. Consequently, autoclaving did not destroy the transforming action of the streptomycin resistance factor which in our experiments proved to be thermostable.

Thus streptomycin resistance is established as a thermostable, specific biochemical complex which is common for salmonella, listeria, and staphylococcus.

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[The following English summary appears with the original article.]

The work shows that killed autoclaved cultures of Listeria monocytogenes, resistant to 75,000 Units/ml of streptomycin, used in the capacity of a donor, transforms streptomycin resistance in the Staphylococcus pyogenes aureus recipient, analogous to the transforming action of the Salmonella typhimurium culture resistant to 75,000 Units/ml and killed at 75° C. The rise of streptomycin resistence to staphylococcus cultivated in the autoclaved Listeria culture ran a parallel course with the rise of the resistance of staphylococci reared in a heated salmonella culture. The fact of transmission of streptomycin resistance to staphylococcus by microorganisms of two different genera points to the thermostability and similarity of factors of streptomycin resistance for staphylococci, listeria and salmonella.

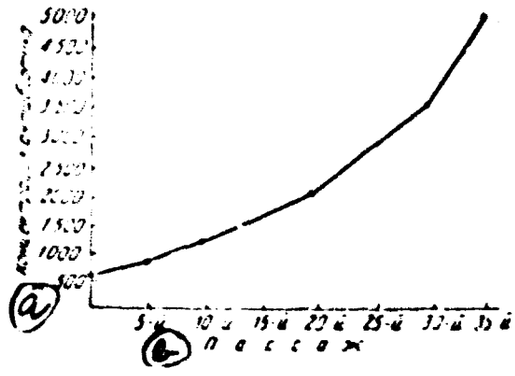

Figure 1. Increase of resistance to streptomycin in Staphylococcus aureus incubated on a killed culture of Salmonella typhimurium resistant to 75,000 units/ml of streptomycin.

a. Concentration of antibiotic
b. Passage

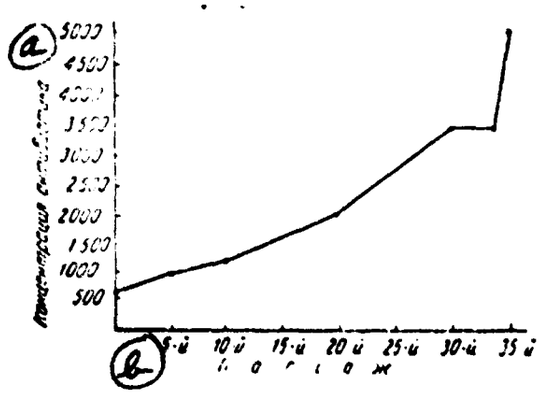

Figure 2. Increase of resistance to streptomycin in Staphylococcus aureus incubated on a killed culture of Listeria monocytogenes resistant to 75,000 units/ml of streptomycin.

a. Concentration of antibiotic
b. Passage

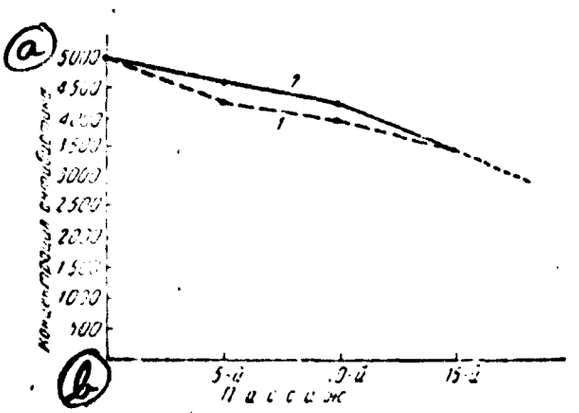

Figure 3. Lowering of resistance to streptomycin in staphylococcus following passages in a medium without any killed cultures of Listeria monocytogenes and Salmonella typhimurium.

a. Concentration of antibiotic
b. Passage

1. Streptomycin resistant staphylococcus raised on a killed culture of Salmonella typhimurium
2. Streptomycin resistant staphylococcus raised on a killed culture of Listeria monocytogenes