THE TRANSFORMATION OF STAPHYLOCOCCI BY KILLED CULTURES OF Salmonella typhimurium AND Listeria monocytogenes RESISTANT TO 75,000 UNITS/ML OF STREPTOMYCIN

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The peculiarities of transformation in recipient Staphylococcus aureus has been investigated by studying the effect of heat-killed cultures of Salmonella typhimurium and Listeria monocytogenes which are resistant to streptomycin.

The present investigation is a continuation of the investigations of Professor P. P. Sakharov and his co-workers [9, 10].

A number of Soviet microbiologists and biochemists have been concerned with the study of microbiological transformation [1-6, 11].

G. V. Levitskaya [6], using the property of natural resistance of E. coli to penicillin, grew Staphylococcus aureus in the presence of extracts of E. coli. She succeeded in obtaining Staphylococcus aureus which in the presence of E. coli extracts were able to grow in the presence of increased concentrations of penicillin.

A. A. Imshenetskii and K. Z. Perova [5] grew a strain of Staphylococcus aureus sensitive to streptomycin on extracts of a streptomycin-resistant strain of Staphylococcus aureus, and observed that resistance of the former increased several-fold over the original strain. These investigations confirm the fact that a cellular extract possesses transforming power.

EXPERIMENTAL METHOD

A freshly isolated strain of Staphylococcus aureus from a patient was used in this investigation.

Our purpose was to obtain a streptomycin resistant strain of Salmonella typhimurium and Listeria monocytogenes and to subsequently grow Staphylococcus aureus on media containing heat-killed cultures of Salmonella and Listeria.

The microbial sensitivity to the antibiotics was determined by the method of serial dilutions in the customary meat peptone broth. Serial passage on media containing ever increasing concentrations of antibiotic was used to obtain resistant cultures.

Salmonella typhimurium resistant to 75,000 units/ml were killed in a water bath at 75° for one hour; Listeria monocytogenes possessing similar resistance to streptomycin were killed in an autoclave at 1.5 atmospheres for 20 min.

At the beginning of the experiment a control culture of Staphylococcus aureus was cultured to obtain isolated colonies. The 50 isolated colonies were grown in flasks containing meat peptone broth with varying concentrations of streptomycin (650, 1000, 2000, 3000, 4000, 5000, 6000 units/ml).

EXPERIMENTAL RESULTS

We found that the original culture of staphylococcus was resistant to streptomycin at a level of 650 units/ml and to penicillin at 900 units/ml. It should be pointed out that the staphylococcal strain was isolated from a patient who had received penicillin and streptomycin.

The experimental cultures of Staphylococcus aureus, after 35 passages on media containing killed Staphylococcus aureus and Listeria monocytogenes resistant to 75,000 units/ml of streptomycin, became resistant to no more than 5000 units/ml.

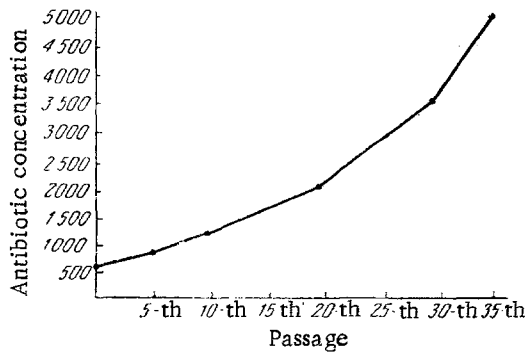

Fig. 1. Increase in resistance to streptomycin in Staphylococcus aureus grown on killed Salmonella typhimurium cultures resistant to 75,000 units streptomycin per ml.

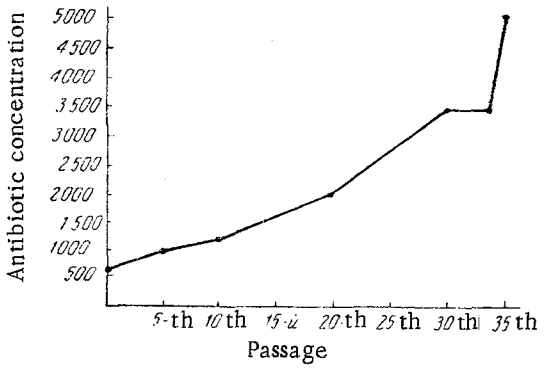

Fig. 2. Increase in resistance to streptomycin and Staphylococcus aureus grown on a killed culture of Listeria monocytogenes resistant to 75,000 units of streptomycin per ml.

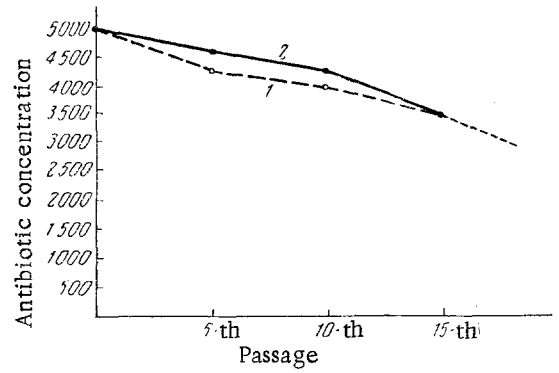

Fig. 3. Decrease in resistance to streptomycin among staphylococci during passage on media containing no killed cells of Listeria monocytogenes and Salmonella typhimurium. 1) Streptomycin-resistant staphylococci grown on killed Salmonella typhimurium. 2) Streptomycin-resistant staphylococci grown on killed Listeria monocytogenes.

When cultivated in a killed culture of Salmonella typhimurium, resistant to streptomycin in a concentration of 75,000 units/ml, the resistance of Staphylococcus aureus to this antibiotic gradually increased. At the 10th passage the resistance to streptomycin was almost double that observed in the controls. At the 20th passage the bacteriostatic concentration of streptomycin was 2000 units/ml, at the 30th passage – 3500 units/ml, and at the 35th passage – 5000 units/ml (Fig. 1).

Throughout the investigation the experimental Staphylococcus aureus maintained its threshold of resistance to penicillin, equal to 900 units/ml.

At the same time the experimental staphylococci could be agglutinated with rabbit serum immunized with salmonella which were streptomycin-resistant, the extracts of which were used during the 35 passages. The degree of expressivity of this agglutination rose to a titer of 1:200 (+++). We observed similar phenomena during growth of staphylococci on media containing killed salmonella. Staphylococci could be agglutinated in a titer of 1:400 by the anti-salmonella sera as a result of this experiment. Thus it appeared that adaptation to the products of viable salmonella which were resistant to streptomycin was accompanied by changes in a number of properties of the staphylococci. Morphologic changes in the cells were already observed by the fifth passage. The cocci lost their characteristic appearance. Many single cells were found as well as groups of two and three. By the 10th passage increase and decrease of the size of the cocci was apparent: some became very small, and others 2 to 3 times bigger than the original. By the 21st passage, the number of morphologically altered cells was significantly increased, and by the 35th passage the normal staphylococci had disappeared.

Starting with the 15th passage the microbial cells stained lighter with Gram's stain in comparison with the control. With each subsequent passage the number of such cells increased. The size of the colonies on agar after the 35th passage were altered: the experimental strain gave very tiny colonies measuring 0.58 mm in diameter whereas the controls had an average titer of 1.56 mm.

Biochemical analysis showed that by the 35th passage the experimental cultures in contrast to the control could metabolize sorbitol and ceased to split mannose.

In studying the virulence of the experimental culture, it was shown that mice injected with the control culture died within the first few days after innoculation of the cultures into leg muscles whereas mice injected with staphylococci grown on streptomycin-resistant salmonella readily withstood the injection and did not die.

Upon growing the staphylococci on the killed culture of Listeria monocytogenes resistant to 75,000 units per ml of streptomycin, the increase in staphylococcal resistance to this antibiotic occurred in the same fashion as in the experiments

with staphylococci grown on killed cultures of salmonella. The process of adaptation of the experimental strain to the products of viable listeria also was accompanied by morphological changes of the cells, colonial forms, biochemical activity, antigenic structure, etc. By the 10th passage streptomycin resistance increased to 1250 units per ml, by the 20th passage to 2000 units and by the 30th to 3500 units per ml. On the 35th passage the experimental staphylococci were transferred to meat peptone broth containing no killed Listeria monocytogenes. After several days growth on the solid media the experimental culture increased its resistance to streptomycin to 5000 units per ml.

A similar phenomena was shown in the experiments of V. Iollos [12, 13] carried out with Paramecium caudatum and Drosophila melanogaster as well as in the work of P. P. Sakharov and his co-workers [9, 10, 11] from the point of view of so-called progressive inheritance.

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

In the experimental staphylococci the ability to be agglutinated by rabbit sera immunized with streptomycin-resistant listeria ranged from a titer of 1-20 (++++) to 1:100 (+++).

We tried to find out whether streptomycin resistance was acquired by transformation from streptomycin-resistant cultures of the donor Salmonella typhimurium and Listeria monocytogenes. Experimental strains after 35 passages on media containing products of viable listeria and salmonella were transferred to meat peptone broth containing no killed microbial cells of the streptomycin-resistant donors, for 15 passages with transfer every third day.

A decrease in the threshold of staphylococcus resistant to 5000 units of streptomycin/ml was shown. By the fifth passage it decreased to 4600 units, by the 10th passage to 4000 units, and by the 15th to 3500 units of streptomycin/ml with a decrease in resistance to streptomycin of the staphylococci previously grown on streptomycin-resistant salmonella and listeria which was almost parallel (Fig. 3). This verifies typical "linear modification" of a characteristic acquired by means of transformation.

It is interesting that the property of streptomycin resistance transmitted to staphylococci by means of transformation increases in parallel with growth on streptomycin-resistant salmonella and listeria.

This phenomenon does not depend on the degree of heat of the latter. The first culture was killed at 75° for one hour, the second in an autoclave at 1.5 atmospheres for 30 min. Thus autoclaving does not destroy the transforming property of streptomycin resistance, which appears to be heat stable in our experiments. Thus, streptomycin resistance is determined by a heat stable factor of salmonella, listeria, and staphylococci which is a single specific biochemical complex.

SUMMARY

The work shows that killed autoclaved culture 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 resistance to staphylococcus cultivated in the autoclaved listeria culture ran a parallel course with the rise of the reistance 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.

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