A STUDY OF THE TRANSFORMING ACTIVITY OF STREPTOMYCIN-RESISTANCE IN THE PATHOGENIC MICROORGANISMS

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Among the most important questions of modern microbial genetics, the problem of transformation deserves particular attention. As it is known, the transforming effect was first demonstrated in experiments with the pneumococcus, in which it was found that DNA extracted from type III pneumococci determines the conversion of not capsulated type II pneumococci into capsulated, pathogenic, type III pneumococci [18]. Later, similar facts were demonstrated also in relation to the activity of DNA extracted from penicillin and streptomycin resistant microorganisms, as a result of which action antibiotic-sensitive microorganisms were transformed into resistant forms [10, 21, 22, 25]. It was established that resistance to penicillin develops step-wise and, to streptomycin, rapidly, intermittently [19, 21, 22, 25, 26].

Many related questions about variability of microorganisms under the influence of antibiotics [1, 3, 4, 5, 9, 11-15], as well as about the significance of DNA, in particular, in the transfer of streptomycin resistance [2, 7, 8, 10] were reported in Soviet literature.

However, the problem of microbial transformation and, in particular, the transformation of resistance to antibiotics demands additional concentrated study and refining of a series of particularly important questions.

The purpose of the present study was the investigation of the following questions: is the transforming factor acquired by or native to microbial cells; is it specific for a particular species of microbes or does it have universal specificity (for microbes of different families); is the transforming activity preserved in the presence of "regressive" heredity (otherwise known as "prolonged modification"); is the transforming principle destroyed by boiling destructive to DNA.

Cultures of the following organisms were used in this study: Listeria monocytogenes (strains #52/1, 8-2a, M-9), Salmonella typhi-murium (Danich strain), Streptococcus hemolyticus (strain Pr.), and Staphylococcus pyogenes aureus (strain #209).

The cultures were grown in media with increasing concentrations of streptomycin after determination of the threshold dose, which allowed the growth of the original culture.

After the cultures of the above-mentioned microbes began to grow in large concentrations of streptomycin in the range of 50,000 - 100,000 units per ml., they were inoculated in meat-peptone broth, the usual liquid nutrient medium, without antibiotics, then killed by boiling for 10, 30, and 60 minutes, as well as by autoclaving (20 min. at $1\frac{1}{2}$ atmospheres) and, after checking for sterility, we grew, from the same original cultures, microbes sensitive to streptomycin in the media containing the killed microbial cells of antibiotic-resistant culture, systematically checking their morphological, biochemical, and serological properties.

EXPERIMENTAL RESULTS

The first series of experiments was carried out with a culture of L. monocytogenes. In studying the development of resistance of listeria to streptomycin it became clear that listeria begin to develop relatively rapidly in the increasing concentrations of this antibiotic. The original sensitivity of listeria of strain #52/1 to streptomycin was equal to 0.1 units/ml. In the course of the 8-10th transfer they acquired resistance to 25,000-50,000 units/ml of streptomycin in the medium, and beginning with the 20th transfer they were able to grow in a medium containing

100,000 units/ml. Cultivation of streptomycin-resistant strains from 10-12th passage, with acquired resistance to 25,000 (strain #8-2a) - 50,000 (strain #52/1) units/ml of streptomycin, on media without antibiotics, demonstrated that their resistance to streptomycin was not only maintained after 3, 6, 9, and 12 months, but, in some cells, continued to increase (they are able to grow in media containing 75,000-100,000 units/ml of streptomycin in meat-peptone broth), and in other cells of the same population one finds regressive heredity of the prolonged modification type. However, the latter does not bring about a complete loss of the newly acquired property of resistance to streptomycin and microbes retain for a long time the ability to develop in nutrient medium containing 20,000 units/ml of streptomycin [13].

Experimental cultures of listeria, which acquired resistance to 50,000-100,000 units/ml of streptomycin (from the 40th transfer of strain #52/40), grown on beef-peptone broth without antibiotic, were killed by heating, boiling, and autoclaving. In this medium containing heat-killed listeria, we cultured the original streptomycin-sensitive listeria (strain #52/1). In the fifth transfer, the latter already grew in 20,000 units/ml (donor killed by boiling) and in 15,000 units/ml (donor killed by autoclaving) of streptomycin. After 11 transfers the resistance of the recipient culture reached 50,000 units/ml (donor strain killed by boiling), and 40,000 units/ml (donor killed by autoclaving) of streptomycin.

On the 20th transfer the resistance of the recipient reached 100,000 units/ml (donor killed by boiling) and 70,000 units/ml (donor killed by autoclaving) of streptomycin. Finally, in the 30th transfer the resistance of the recipient culture to streptomycin rose to 200,000 units/ml (strain killed by boiling) and 90,000 units/ml (strain killed by autoclaving) of streptomycin.

It should be pointed out that development of resistance to streptomycin by contact of the sensitive strain of listeria with heat-killed microbial donor cells resistant to streptomycin, occurred faster than in active development

Alteration of Serological Properties in Streptomycin-Resistant Strains of Listeria
(Maximum Titers)

Antigens	Serum of Rabbits Immunized to					
	original strains			streptomycin-resistant strains		
	strain 8-2a, serotype I	strain M-9, serotype II	strain and transfer 52/1 serotype IV	strain and transfer 8-2a/20	strain and transfer M-9/25	strain and transfer 52/20
Strain and transfer 8-2a, serotype I	3200	800	400	400	1600	200
" " " 8-2a/20 " I	1600	1600	200	1600	3200	800
" " " 8-2a/37 " I	1600	1600	200	1600	3200	800
" " " M-9 " II	800	6400	800	800	3200	200
" " " M-9/25 " II	1600	3200	400	1600	6400	800
" " " M-9/33 " II	1600	3200	400	1600	6400	800
" " " 52/1 " IVa	400	400	1600	200	800	400
" " " 52/20 " IVa	800	800	400	1600	3200	1600
" " " 52/39 " IVa	800	1600	800	1600	3200	1600

of resistance in media containing increasing concentrations of streptomycin. This is apparent from the fact that during active development of streptomycin resistance in the 5th transfer of the same strain #52/1 listeria grew in 50 units/ml streptomycin, in the 20th transfer in 80,000 units/ml, and in the 30th transfer in 100,000 units/ml.

It was shown that the donor culture, resistant to 50,000 - 100,000 units/ml streptomycin, induced resistance in listerial recipients to 200,000 units/ml of streptomycin.

On the other hand, cultivation of streptomycin-sensitive staphylococci in killed streptomycin-resistant culture of listeria, brought about the development of resistance to 5,000 units/ml in the 35th transfer and to 70,000 units/ml of streptomycin in the 70th transfer.

A culture of Salmonella typhi-murium (Danich's strain) was used in the second series of experiments. The original sensitivity of the experimental strain of S. typhi-murium to streptomycin was equal to 5 units/ml of this

antibiotic in beef-peptone broth. After 96 transfers on media containing increasing concentrations of streptomycin, bacteria began to grow in 96,000 units/ml of streptomycin in the same nutrient medium.

This culture, having acquired resistance to streptomycin and grown on beef-peptone broth without antibiotic, was killed by boiling (for 10 minutes), and after testing for sterility we began to grow in the same medium initially sensitive culture of salmonella and streptomycin-sensitive culture of staphylococci. Resistance of salmonella to streptomycin began to develop rapidly in these experiments from transfer to transfer and after 25 transfers the paratyphoid organisms began to grow in 200,000 units/ml of streptomycin in MPB. The resistance of staphylococci developed, at first, very slowly; but after 35 transfers they were able to grow at 5,000 units/ml; and after 70 transfers staphylococci grew in 60,000 units/ml of streptomycin.

The resistance of streptomycin of the sensitive culture of salmonella, grown in a medium containing killed streptomycin-resistant microbial culture, was double that (200,000 units/ml) of the resistance of the donor culture (96,000 units/ml). On the other hand, salmonella resistant to streptomycin induced (as did the listeria) similar resistance in staphylococci, i. e. microbes belonging to another family than salmonella and listeria.

However, of greatest interest is that, using a similar experimental procedure, staphylococci, having acquired resistance to streptomycin (without influence of streptomycin) in turn transferred this resistance to a sensitive strain of salmonella and a sensitive strain of staphylococci.

And finally, the same results were obtained also with cultures of B-hemolytic streptococci Streptococcus hemolyticus (third series of experiments). Streptococcus proved to be a microorganism also rapidly developing resistance to streptomycin. In the course of 12 transfers in culturing in media containing antibiotics, its resistance reached 10,000 units/ml of streptomycin. A culture of streptococci killed by boiling (for 10 minutes) proved to be equally capable of inducing resistance in the originally sensitive strain of streptococci, grown in beef-peptone broth without streptomycin. In the course of 15 transfers streptomycin-sensitive streptococci, subjected to the conditions of the last experiment, acquired resistance to 20,800 units/ml of streptomycin in beef-peptone broth.

Simultaneously it was established that microbial cultures acquiring resistance to streptomycin undergo changes in morphological, cultural, biochemical and serological properties. This process was most clearly demonstrated in listeria and B-hemolytic streptococci. Streptomycin-resistant listeria and streptococci lost their type specific properties, and their agglutination reactions with rabbit antibodies, immunized with the original cultures of listeria and streptococci, were weaker. At the same time it was shown that streptomycin-resistant cultures of listeria acquire new group antigens (see table).

Analogous, but less strongly expressed, results were demonstrated by salmonella and staphylococci that acquired resistance to streptomycin.

In this manner, the above indicated results show that the factor of streptomycin resistance, acquired in the course of cultivation in media with increasing concentrations of streptomycin, possesses a strong transforming property. As a result of this transforming property, there appears in listeria and salmonella resistance to 200,000 units/ml of this antibiotic. It should be pointed out that in training in media containing streptomycin it was not possible to obtain salmonella resistant to more than 96,000 units/ml. From this it can be seen that the action of the transforming factor is more pronounced than independent acquisition of resistance by microbes in media containing streptomycin.

One of the most important problems consists in the proof that the factor of resistance to streptomycin is acquired and is not pre-existent in microbial population as, for example, described by Welsh [26]. The latter hypothesis is proven by our special investigations which clarified that the maximum deviation of streptomycin resistance in a microbial population of listeria, salmonella and staphylococci did not extend beyond 50 units per 1 ml and that 1, 2, and 3 hours after transfer to a medium containing streptomycin there was observed extensive growth of colonies (or confluent growth) on solid nutrient medium, which speaks either of the presence of orthogenesis according to Eimer [20], or of ortho-selection according to Plate [23]; the appearance of individual colonies, whose resistance to streptomycin proved to be significantly higher than that of microbes from the original colonial growth, was observed only in infrequent cases in the course of transfer in increasing concentrations of streptomycin. These cases we evaluate as directed mutations, according to Jollos [22].

In addition, the following facts confirm that resistance to streptomycin is a newly acquired property: salmonella having acquired resistance to streptomycin lose their native resistance to penicillin; streptomycin-resistant streptococci lose their native type specific antigens and become greening from B-hemolytic, and streptomycin resistant

listeria of different serotypes acquire serological similarity. Consequently, along with the appearance of resistance to streptomycin, there occur in different microbes significant changes in metabolic activities which characterize the newly acquired properties.

Of great significance, from our point of view, is also the fact that even in the presence of regressive form of inheritance, noticed in experiments with cultures of listeria, salmonella, and streptococci, the acquired resistance to streptomycin possessed almost equal transforming activity on sensitive strains of these microbes and staphylococci. From this it can be seen that regressive inheritance, otherwise designated as "prolonged modification" is actually nothing but one of the methods of inheritance inasmuch as the acquired property, transmitted to the following generations in the prolonged modification manner, possesses an expression of transforming activity.

And finally, we consider it important that the transforming factor turned out to be thermostable, retaining the activity after boiling and autoclaving of the culture.

The data in the literature point out that high temperature breaks down DNA [6] or brings about structural changes [18, 25, 26] removing its biological activity.

Enzymes are also not thermostable agents. Therefore, it is hardly possible to regard streptomycinase also as an inherited factor. Evidently, there occur certain interchangeable structural regroupings of amino acids which are common to many microorganisms in development of streptomycin resistance.

SUMMARY

Evidence is produced that the streptomycin-resistance acquired by listeria, salmonella, streptococci and other microorganisms is capable of being transmitted by heredity, in one set of cases by the type of progressive, and in the other, of regressive, heredity. Irrespective of the form of the hereditary transmission, the streptomycin resistance produce a transforming effect of the sensitive strains of the microbes. The transforming effect of streptomycin resistance proved to be common for various species, genera and families of the microbes possessing no species' specificity. The transforming factor of streptomycin-resistance proved to be thermostable. The presence of transforming action of the acquired streptomycin - resistance confirms the fact that the latter is transmitted to the subsequent generations according to the law of the acquired properties' inheritance.

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