TRANSLATION NO. 637

DATE: 1 July 1968

DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

Misc Tr
437

UNITED STATES ARMY
CHEMICAL CORPS BIOLOGICAL LABORATORIES
Fort Detrick, Maryland

Phage Reproduction in Penicillin Treated Enteric Bacilli Cells

by I. A. Bass and B. N. Il'yashenko

Translated from Mikrobiologiia (USSR), 28: 5: 730-735, 1959

In recent years, in the investigations of microbiologists, biochemists, and geneticists, they are more and more frequently using bacterial protoplasts, that is, cells that have been deprived of their cellular walls as a result of lysozyme treatment. The metabolism of the lysozyme protoplasts differs little from that of normal bacterial cells (Kalina, 1958; Oparin, 1957; McQuillen, 1955).

Under certain conditions they retain their capacity to synthesize proteins (Oparin et al., 1957; McQuillen, 1955 a, b) and ribonucleic acid (McQuillen, 1955 a) and also to support the reproduction of bacteriophage (Brenner, Stent, 1955; Fraser, Mahler, 1957; Mahler, Fraser, 1956; Salton and McQuillen, 1955).

The protoplast-like forms that are obtained through the use of penicillin, glycine, and bacteriophage are morphologically similar to the lysozyme protoplasts (Brenner et al., 1958). Little is known of these forms of bacteria, but while differing from the lysozyme protoplasts in their generation mechanism they evidently differ also in their properties. The protoplasts and the protoplast-like formations represent very convenient models for the experimental solution of many questions, because through the lack of or damage to the cell wall they possess an increased permeability for macromolecular substances. This has made the study of the effect of enzymes on the intracellular phase of phage development possible (Fraser and Mahler, 1957), and recently enabled the infection of protoplasts by damaged phage incapable of infecting normal cells (Fraser et al., 1957; Spizizen, 1957). The protoplasts that are produced by lysozyme treatment, which are useful in the majority of investigations, cannot be infected by a whole phage inasmuch as the receptors of the cellular membrane are required for the adsorption of the phage. The virus can be developed within the protoplasts only if the infection of the bacteria is carried out prior to the lysozyme treatment. Therefore the first phase of the phage's development in the protoplasts escapes the observance of the investigators. In this regard the protoplast-like forms of bacteria that originate from penicillin treatment can present a definite advantage over the lysozyme protoplasts (Chargaff, 1957; Hahn and Ciak, 1957; Lederberg, 1956; Adams, 1950). They evidently have a reduced membrane (Hahn, Ciak, 1957) that can stipulate a successful adsorption of the phage, and at the same time possesses an increased

permeability for macromolecules, particularly desoxyribonucleic acids (Chargaff, 1957). In addition, other advantages of these protoplast-like forms are their capacity for reversion and restoration of propagation, and simple method of their production, particularly in the strains resistant to lysozyme. The metabolism of these forms, however, is much more inadequately investigated than the lysozyme protoplasts.

Inasmuch as the phage's development is an indication of a system's capacity for extremely complex synthetic processes it seemed to us expedient to study the possibility of phage reproduction in the protoplast-like formations produced from enteric bacilli with the action of penicillin.

A freshly isolated culture of E. coli No. 600, which was received from the Moscow City Sanitary-Epidemic Station, and was typical by all characteristics, and an enteric phage F-1, which was given by A. S. Kriviskiy, served as the objects of the investigation. To produce the protoplast-like forms we used the method proposed by Lederberg (1956). A four-hour-old broth culture was diluted five times with broth containing 0.5 M sucrose, 1 % $MgSO_4$, and 1,000 units/ml of penicillin and incubated at 37°C. After 1.5 hours an absolute majority of the cells was transformed into spherical formations similar to the "large bodies" of the L-forms, and only 0.1 % of the cells retained their original morphology and capacity to form colonies with the usual seeding conditions.

In accordance with the basic task of the investigation we first of all explored the possibility of infecting the protoplast-like forms with the F-1 phage. For this purpose a suspension of these forms in a 0.5-percent agar was mixed with the phage and placed into a vonBruhn oil chamber. The multiplicity of infection in the different experiments ranged from 1 to 10. With a visual observation in a phase-contrast microscope at 37°C it was observed that of the 34 spherical bodies that were in contact with the phage 26 were lysed within 30-50 minutes, whereas in the control (without the phage) only one of 32 was lysed. These findings indicated, evidently, a possibility of infection and lysis of the protoplast-like forms by the F-1 phage, although it has still not been proved that phage reproduction with the formation of mature viral particles occurs within them. To find the capacity of the phage to reproduce within the protoplast-like forms we determined the phage production at different time intervals after infection of both the spherical bodies and normal cells. It is seen from the resultant data (table 1) that the F-1 phage reproduces in the protoplast-like forms, although the number of phage particles is 10-11 times less than in the whole cells. It was possible to explain such a large disparity in the quantity of phage by one of two reasons: either the spherical forms present a less favorable biological system for phage reproduction than do the normal cells, or the phage development in them is repressed by the penicillin.

Therefore in the next experiment the development conditions for the phage in the bacilli and in the spherical bodies were balanced by adding penicillin to the tests with the normal cells. As seen in table 2, in a 60-minute incubation under these conditions the number of phage particles increased 68 times at the expense of the bacterial cells, and in the test with the protoplast-like forms it increased only 13 times. The low phage production in the latter case cannot be explained by the reproduction attributed exclusively to the remaining cells,

however, because calculation will show that with such an assumption the phage production from one cell would exceed 5,000 corpuscles, which is completely unrealistic and, as will be seen later, contradicts the results of the experiments. Thus, one should recognize that the phage production data resulting from the tests with the protoplast-like forms are limited chiefly to its reproduction in the spherical bodies alone. Consequently, it can be considered as established that the protoplast-like forms that are produced by the effects of penicillin are not only infected by the phage, but are also capable of securing its reproduction.

For a more detailed account of the penicillin's effect, experiments were conducted in three parallel tests: the first consisted of cells with sucrose and $MgSO_4$, the second of protoplast-like forms in the same medium with 50 units per ml of penicillin, and the third of protoplast-like forms with 1,000 units/ml of penicillin. As seen from table 3 the phage reproduction in the spherical bodies is significantly lower than in the cells, but the increase of the concentration of penicillin lowers their phage production still more.

The effect of the antibiotic could be attributed either to a suppression of the phage's adsorption to the protoplast-like forms, or to a suppression of the phage's processes of intracellular development. The next series of experiments were conducted to resolve this question. The protoplast-like forms were washed from the penicillin by a single centrifugation, and were resuspended in a hypertonic medium without the antibiotic. To one portion of them 1,000 units/ml of penicillin was added directly prior to the infection, another portion was infected without the antibiotic, and to a third portion the penicillin was added 5 minutes after the infection with the phage in order to exclude the antibiotic's effect on adsorption. The resultant data (table 4) shows that in an absence of penicillin the number of phage is increased 90 times during a 50-minute incubation, whereas, in its presence the quantity increases only 3-4 times, whereupon the second and third tests were practically identical. From this it is possible to conclude that the penicillin, in decreasing the phage production in the protoplast-like forms, affects the intracellular phase of its development rather than its adsorption.

Taking this into consideration, in the next experiments, which were undertaken to make the productivity of the phage in the spherical bodies more clear, we washed the spherical bodies from the penicillin. In these experiments we determined the phage production from single spherical bodies and from single normal cells by the Barnet method (Adams, 1950). As seen from table 5 the number of the F-1 phage particles in a separate bacterial cell reaches an average 660, whereas the lysis of a single spherical body releases an average of 60 corpuscles. The difference in the number of phage particles in the normal cells and in the protoplast-like forms is approximately the same as in the case of the lysozyme protoplasts (Mahler and Fraser, 1956). By analogy with the latter it was possible to suppose that because of the damage to the cell-wall structure lysis of the spherical bodies occurs before the phage is able to reproduce in them. For a check of this hypothesis we staged experiments in which we determined the duration of the phage development in the period of two consecutive generations (by a type of two-phased curve) in the protoplast-like forms and in normal cells. These experiments had to simultaneously give a

conclusive answer to yet another question: are the protoplast-like forms that are produced by the effect of penicillin actually distinguished from the lysozyme protoplasts by a capability to adsorb phage, or can they only sustain the reproduction of phage that has been adsorbed earlier (table 5). Actually, one would think that after 1.5 hours of the penicillin's action a portion of the cells would still retain a comparatively slightly altered cellular membrane with the receptors to which the phage is adsorbed. If the phage adsorption is stipulated only by these cells, then they should be lysed by the first generation of phage and there should be no second cycle. As seen from the drawing two cycles of phage reproduction are observed in the normal cells and in the protoplast-like forms, whereupon the duration of the first and second generations are practically identical both for the normal cells and for the spherical bodies. Consequently, the small quantity of phage particles in the spherical bodies cannot be explained by their premature lysis. These tests also allow one to consider as conclusively proved that the protoplast-like forms received by penicillin treatment have the capacity to adsorb phage, in distinction from the lysozyme protoplasts. Their adsorption of phage attains 50-80 %, whereas for normal cells it amounts to 85-90 %.

From here it follows that the protoplast-like forms retain certain surface structural components that participate in the process of phage adsorption. And, indeed, agglutination experiments on the spherical bodies with specific immune serum, which was produced by immunization with bacterial cells, showed that they are agglutinated as high as a titer and, consequently, contain certain surface antigens. These facts came to light in a cytomorphological investigation of the spherical bodies by the Knaysi method for the development of the membrane (Knaysi, 1941). We discovered in them the presence of both a cytoplasmic membrane and also a wall, though possibly in a reduced condition.

The results of all of the described experiments give a basis to conclude that the protoplast-like forms produced through the use of penicillin present a sufficiently suitable system capable of securing a full-value development of phage. Moreover we have shown, jointly with Goldfarb, Gorlenko, Nankinaya, and Hesin, that the protoplast-like forms, like the lysozyme protoplasts (Fraser, 1957), can be infected by preparations of disintegrated phage that are completely inactive in relation to normalcells. This phenomenon has been reproduced both on the intestinal phage T-4 and on several other entero-dysenteric phages, whereupon the"penicillin" protoplast-like forms of phage-sensitive and phage-resistant strains of enteric and dysenteric bacteria can serve as the recipients.

Conclusions

1. The protoplast-like forms produced from enteric bacilli by the effect of penicillin are capable of adsorbing phage, and are thus distinguished from the protoplasts produced through the use of lysozyme.

2. The protoplast-like forms are capable of securing the reproduction of phage, although the phage production is ten times less than that from normal cells.

3. The penicillin does not affect the amount of phage adsorption, but

suppresses the intracellular phase of the phage's development in the spherical bodies. Therefore, in a study of the given process it is necessary to remove the penicillin from the medium in which they are situated.

4. Inasmuch as the protoplast-like formations are capable of securing phage development and at the same time, evidently, possess an increased permeability for the macromolecular substrates, their use seems expedient for the study of the reproduction mechanism of bacterial viruses.

Received 14 Aug 1958

Institute of Biophysics of the Academy of Sciences of the USSR, Institute of Epidemiology and Microbiology im. (in name of) N. F. Gamaleya, Academy of Medical Sciences of the USSR.

Literature

Kalina, G. P.: Zhurnal Mikrobiol., Epidemiol. i Immunobiol, 1: 136, 1958
Oparin, A. I.: The outbreak of life on the Earth (Symposium), The biochemical processes in the simplest structures, 1957
Oparin, A. I.; Gel'man, N. S.; and Zhukova, I. G.: Biokhimiya, 22: 1-2: 399, 1957
Adams, M. H.: Methods in medical research, 2, 1950.
Brenner, S., and Stent, G. S.: Biochim. biophys. acta, 17: 4: 473, 1955
Brenner, S.; Dark, F. A.; Gerhardt, P.; Jeynes, M. H.; Kandler, O.; Kellenberger, E.; Klieneberger-Nobel, E.; McQuillen, K.; Rubio-Huertos, M.; Salton, M. R. J.; Strange, R. E.; Tonicsik, J.; and Weibuel, C.: Nature, 181: 4625: 1713, 1958
Chargaff, E.; Schulman, H. M.; and Shapiro, H. S.: Nature, 180: 4591: 851, 1957
Fraser, D., and Mahler, H. R.: Arch. Biochem. Biophys., 69: 166, 1957
Fraser, D.; Mahler, H. R.; Shug, A. L.; and Thomas, C. A.: Proc. Nat. Acad. Sci., 43: 11: 939, 1957
Hahn, F. E., and Ciak, J.: Science, 125: 3238: 119, 1957
Knaysi,G.: J. Bact., 41: 2: 141. 1941
Lederberg, J.: Proc. Nat. Acad. Sci., 42: 9: 574, 1956
Lederberg, J., and Clair, J. S.: J. Bact., 75: 2: 143, 1958
Mahler, H. R., and Fraser, D.: Biochim. Biophys. Acta, 22: 1: 197, 1956
McQuillen, K.: Biochim. biophys. acta, 17: 3: 382, 1955a
McQuillen, K.: J. Gen. Microbiol., 13: 1: IV, 1955b
Salton, M. R. F., and McQuillen, K.: Biochim. biophys. acta, 17: 4: 465, 1955
Spizizen, J.: Proc. Nat. Acad. Sci., 43: 8: 694, 1957

Table 1

The pattern of phage production from normal cells and from the protoplast-like forms.

Test	The phage titer after (minutes)					Phage production (No of particles per cell)
	0	10	30	45	60	
Normal cells	4.8×10^6	7.4×10^5	-	4.0×10^8	8.0×10^8	196
Protoplast-like forms	4.8×10^6	2.5×10^6	2.8×10^6	1.3×10^7	4.1×10^7	17.8

Table 2

Phage production from normal cells and the protoplast-like forms in the presence of 500 units/ml of penicillin.

Test	No. of viable cells in a ml	Phage titer after (minutes)		Phage production (No of particles per cell)
		0	50	
Normal cells	3.3×10^8	4.8×10^8	3.2×10^{10}	66.7
Protoplast-like forms	1.0×10^6	4.8×10^8	6.3×10^9	13.1

Table 3

The effect of the concentration of penicillin on the reproduction of phage in the protoplast-like forms.

Test	Penicillin concentration in units/ml	Phage titer after (minutes)		Phage production (No of particles per cell)
		0	50	
Normal cells	-	1.0×10^7	8.6×10^8	86
Protoplast-like forms	50	1.0×10^7	5.5×10^7	5.5
	1000	1.0×10^7	1.1×10^7	1.1

Table 4

The effect of penicillin of the adsorption and reproduction of phage in the protoplast-like forms.

When the penicillin was added	Phage titer after (minutes) 0	10	50	Phage production (No of particles per cell)
Not added	8.8×10^6	3.4×10^6	4.9×10^8	90
prior to infection	8.8×10^6	5.7×10^6	9.5×10^6	3.1
5 minutes after infection	8.8×10^6	5.0×10^6	1.6×10^7	4.2

Table 5

The number of F-1 phage particles in the normal cells and in the protoplast-like forms (the single cell method).

Test	Number of Tests	Quantity of phage	Average
Normal cells	32	21,190	662
Protoplast-like forms	11	633	57.5

Phage reproduction rates in whole cells and in the protoplast-like forms (in percentages from the maximums).
1 - Normal cells; 2 - The protoplast-like forms.