THE DISTURBANCE OF PERMEABILITY IN SPHEROPLASTS OBTAINED BY MEANS OF TREATMENT WITH "GHOSTS" OF T2-PHAGE

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It was reported recently that spheroplasts, obtained by means of treatment with "ghosts" of T-even phages were an unfavourable medium for the reproduction of phages (Goldfarb et al., 1962). It was also established that the incompleteness of the "ghost" spheroplasts was conditioned by the inhibition of the intercellular phase of phage development and not by the blockage of the initial stages of the infection (adsorption).

A number of hypotheses were made explaining this phenomenon, among them one of suggesting that the "ghost" spheroplasts have a defect in the system of permeability. Such a suggestion was justified by the results of Herriott and Barlow (1958) and Puck and Lee (1955) demonstrating that the contact of bacteria with the "ghosts" of T2-phage or the native phages leads to the leakage of some of the components of the cell into the surrounding medium.

For the investigation of the possibility of injuring the system of permeability in the "ghost" spheroplasts some indexes of the oxidative and carbohydrate metabolism were taken as a model.

The "ghosts" of T2-phage were obtained by means of Herriott and Barlow's (1957) method somewhat modified. The four

hour culture of E. coli B grown on meat-peptone broth was used in these experiments. Bacterial spheroplasts were obtained by the method of Carey and others (1957), lysozyme protoplasts by the method of Fraser and Mahler (1957). Aldolase activity was determined by Tovarnitsky and Voluiskaya (1955) technique, hexokinase by the method proposed by Long (1952), glucose-6-phosphate dehydrogenase by the method of Kornberg and Horecker (1955), lactic acid dehydrogenase by the Kornberg method (1955). The concentration of glucose was studied by the method of Nelson (1944), of lactic acid by that of Barker and Summerson (1941).

By using the method of phase contrast microscopy and turbidimetry it was possible to establish the absence of lysis of the spheroplasts and protoplasts during centrifugation and incubation in the Warburg apparatus.

Table 1 shows that in the "ghost" spheroplasts(in the contrary to the lysozyme protoplasts) the phenomena connected with the utilization of glucose are disturbed.

The rate of the utilization of glucose by "ghost" spheroplasts in the oxidative and glycolytic pathways was not reducted neither by single or simultaneous addition of DPN, DPN-H, ATP, ADP, FMN, FAD or SH-gluthation into the incubation medium. Hence we could suppose that the disturbances in the energetic metabolism of "ghost" spheroplasts were due to more deep changes than that of the leakage of cofactors, particularly by release of the enzymes involved in the utilization of glucose.

The data of Table 2 show that aldolase, hexokinase, glucose-6-phosphate dehydrogenase in the E. coli B cells are nearly all localized in the soluble fraction that does not

sediment at 6 500 g; the lactic acid dehydrogenase is to a large degree connected with subcellular particles.

TABLE I

CONSUMPTION OF O_2, DIMINUTION OF GLUCOSE AND ACCUMULATION OF LACTIC ACID BY CELLS, "GHOST" SPHEROPLASTS AND LYSOZYME PROTOPLASTS OF E. COLI B

Incubation period 1 hour at 37° in the Warburg apparatus in a hypertonic medium containing 0,2 % $MgSO_4$, 4% albumin and 0,03M Na-phosphate pH 7,35. Concentration of glucose in the vessel 10 mg/3 ml. All calculations are for 10^9 cells. The "ghosts" were irradiated by ultraviolet light. Multiplicity - 150-1000

Consumption of O_2 in samples without glucose μl	Consumption of O_2 in samples with glucose μl	Diminution of glucose μg	Accumulation of lactic acid μg
	Cells*		
59	203	1940	410
	Lysozyme protoplasts**		
58	197	2035	327
	"Ghost" spheroplasts***		
32	50	186	26

* average of 2 experiments
** average of 3 experiments
*** average of 8 experiments

The lysozyme protoplasts did not release enzymes involved in the carbohydrate metabolism into the incubation medium. The "ghost" spheroplasts released into the S1 fraction: 32-48 % of aldolase, 100 % of hexokinase, 77-95 % of glucose-6-phosphate dehydrogenase and 0-18 % of lactic acid dehydroge-

TABLE 2

ACTIVITY OF ENZYMES OF THE SYSTEM OF UTILIZATION OF GLUCOSE IN FRACTIONS OF SPHEROPLASTS AND PROTOPLASTS OF E. COLI B

"Ghost" spheroplasts or lysozyme protoplasts were removed from the incubation medium by centrifugation at 6 500 g (supernatant - fraction S_1). The sediment of spheroplasts or protoplasts was subjected to lysis in the initial volume of distilled water and centrifugated at 6 500 g (supernatant - fraction S_2). The sediment was resuspended in the initial volume of distilled water (fraction P). Number of "ghosts" per bacteria - 500-1000. All calculations of activity were computed on the total volume of fraction (4 ml). Concentration of protoplasts or spheroplasts $3 - 5 \times 10^9$ ml. The period of contact of "ghosts" with bacteria - 20 min.

Aldolase *			Lactic acid dehydrogenase**			Glucose-6-phosphate dehydrogenase**			Hexokinase***		
S_1	S_2	P	S_1	S_2	P	S_1	S_2	P	S_1	S_2	P
				"Ghost" spheroplasts							
422	615	102	0	300	120	420	120	0	186	0	0
635	710	166	130	430	130	350	20	20	-	-	-
409	770	102	220	500	500	510	110	30	217	0	0
				Lysozyme protoplasts							
0	1618	122	0	270	210	0	400	60	0	203	0
0	1280	174	0	300	240	0	360	40	0	308	0

* units of activity; **units of optical density for 1min. $\times 10^4$; *** μg diminution of glucose

nase (0-30 % of the total quantity of the soluble enzyme). It is evident that lysis could not be accompanied by such a selective leakage of enzymatic activities. The electivity of release is probably due to many factors, not connected with lysis: the size of the molecules of the enzymes, the degree of disturbance of the permeability, rate of diffusion, etc.

The leakage of aldolase depended on the quantity of "ghosts" that the bacteria was treated by (fig. 1).

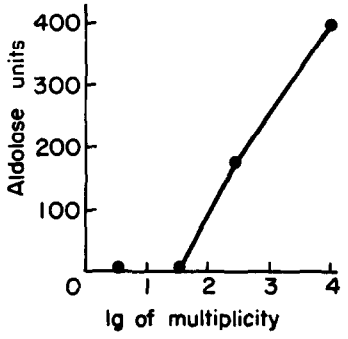

Fig 1. Release of aldolase from cells and spheroplasts of E. coli B depending on the quantity of "ghosts" of T2-phage

In the experiment fraction S_1 is used. The number of unviable cells at a multiplicity of 1800 and 180 - 98 %; at a multiplicity of 18 and 1,8 - 0 %. Medium containing 0,2% Mg^{2+}. Period of incubation of "ghosts" with bacteria - 20 min.

Cells treated by "ghosts" in quantity up to 18 did not transform into spheroplasts, and did not release enzymes in the incubation medium; the yield of viable cells was the same as in the culture untreated by "ghosts" (absence of "killing effect").

The maximal rate of release of aldolase from "ghost" spheroplast took place during the first minutes of the contact of "ghosts" with the cell, e.g. before the forming of spheroplasts began (fig. 2).

The aforementioned data show that "ghost" spheroplasts are a system with drastic damages of the barrier of permeability. It is important to note that spheroplasts' forming is the final process of interaction of "ghosts" of T2-phage with bacteria. The process of damage of the permeability begins before

the formation of spheroplasts. Probably the factor of damage of permeability plays an important role in the mechanism of the reactions in which a great quantity of phage particles are involved (lysis from without), and also in the "killing effect" of "ghosts" on the bacteria.

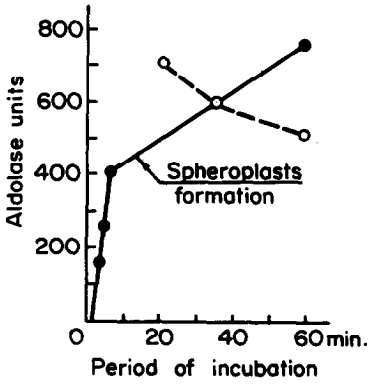

Fig. 2. Release of aldolase from cells and spheroplasts of E. coli B depending on the period of contact with "ghosts" of T2-phage

Number of "ghosts" per bacteria - 2 770; ——— fraction S_1; - - - - - lysates of spheroplasts in distilled water after removal of fraction S_1. Quantity of unviable cells after 5 min. - 96 /, after 60 min. - 98 /. Medium containing 0,2 /Mg^{2+} "Ghosts" are irradiated by ultraviolet light.

Taking into consideration the fact that "ghost" spheroplasts do not possess an adequate system of glucose utilization and that the damage of permeability does not eliminate the leakage into the incubation medium of other substances with catalytic activity and substrates involved in the synthesis of proteins and nuclein acids, it is possible to conclude that the blockage of phage reproduction in the above mentioned subcellular structures is conditioned to a great degree by the incompleteness of their energetic and plastic metabolism.

Probably in many cases the reduction of the rate of the metabolic reactions of bacteria, treated by "ghosts" of T2-phages (French and Siminovitch, 1955; Goldfarb et. al., 1962; Herriott and Barlow, 1957; Khesin et al., 1962) is not connected with the direct inhibition by phantoms (or their components) of some aspects of metabolism but are due to their ability to damage the cytoplasmatic membrane and provocate the leakage of catalytically active substances and substrates involved in the synthetic processes.

References

1. Barker S.B., Summerson W.H. J.Biol.Chem. 138, 535, 1941
2. Carey W., Spilman W., Baron L. J.Bact. 74, 543, 1957
3. Fraser D., Mahler H.R. Arch.Biochem.Bioph. 69, 166, 1957
4. French R.G., Siminovitch L. Canad.J.Microbiol. I, 757, 1955
5. Goldfarb D.M., Avdeeva A.V., Borisova N.B. Nature 195, 1203, 1962; Microbiologia 31, 869, 1962
6. Herriott R.M., Barlow J. J.Gen.Physiol. 41, 306, 1957
7. Khesin R.B., Shemyakin M.F., Gorlenko J.M., Bogdanova S.L., Afanasyeva T.P. Biochimia 27, 1092, 1962
8. Kornberg A. Methods in Enzymol. I, 441, 1955;Acad. Press, N.Y.
9. Kornberg A., Horecker B.L. Methods in Enzymol. I, 323, 1955; Acad. Press, N.Y.
10. Lehman I.R., Herriott R.M. J.Gen.Physiol. 45, 1067, 1958
11. Long C. Bioch.J. 50,407, 1952
12. Nelson N.A. J.Biol.Chem. 153, 357, 1944
13. Puck T., Lee H. J.Exp.Med. 101, 151, 1955
14. Tovarnitsky, V.I., Voluyskaya E.K. Laboratornoye delo, 6,7, 1955