THE INFLUENCE OF DONOR — SPECIFIC BACTERIOPHAGES f_2 AND M 13 on the CONJUGATION TRANSFER OF MULTIPLE DRUG RESISTANCE BETWEEN SUBSTRAINS OF E. COLI K-12 WITH REPRESSED AND DEREPRESSED R-FACTOR

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R-factors which condition multiple drug resistance in bacteria, regardless of their resemblance to the F-factor, are characterized in addition by a number of peculiarities. One of them is the fact that mechanical removal of sex pili in R^+-bacteria does not account for complete discontinuqtion of the transfer of R-factors (Brinton — 3,4; Datta — 6; Watanabe — 11; Marinova and Pereverzev — 1; Brinton and Beer — 5).

With the purpose to study the role of sex pili in conjugational transfer of multiple drug resistance, experiments were conducted on the transfer of R-factors in the presence of donor-specific bacteriophages. It has been established that the latter undergo adsorption over sex pili only which, in the opinion of many investigators (Brinton — 3, Ippen and Valentine — 7), function as a conjugational bridge in the transfer of genetic material in bacteria.

Material and methods

— Bacterial strains. In the experiments described substrains of E. col K-12 were used in their capacity of donors, as follows: $J_{5,3}$ Sm, Cm, Km, Lac^+ pro^-, met^-, a carrier of mutant derepressed R-factor, and CSH-2(222) Sm, Cm, Tc, Su, lac^+, thr^-, leu^-, pro^-, B_1^-, prosessing repressed R-factor. P 678 lac^-, thr^-, leu^-, B_1^- with mutational resistance against streptomycin was used as a recipient in either of the donor strains. R-factors transfer was effected after the method of Watanabe (10). In the control experiments (without phages), the frequency of multiple drug resistance transfer in the donor strain with derepressed R-factor is 384 times higher than the frequency of transfer in the donor strain with repressed R-factor.

— Bacteriophages. The folowing donor-specific bacteriphages were used: f_2 (RNA), undergoing adsorption along the sex pili (Novotny et al — 8), and M 13 (DNA) undergoing adsorption at their free end (Tzagoloff and Platt — 9). Both phages were applied at titer 9.0×10^{10}. Addition of phages was effected in one of two ways: to donor bacteria — 7 min before the formation of conjugational mixture, or at the time of mixing donor and recipient bacteria.

Electron microscope study of sex pili in the donor bacteria, and of bacteriphages adsorbed over them, was conducted after the method of negative contrast staining, described by Brenner and Horne (2).

Results and Discussion

The results of the experiments on R-factors transfer upon bacteriophages f_2 and M 13 adsoprtion over sex pili of donor bacteria are illustrated in the table enclosed.

It is obvious from the presented data that both bacteriophages inhibit the transfer of multiple drug resistance, and in a considerably higher degree whenever $J_{5,3}$ (with derepressed R-factor) is used as a donor strain. Upon adding phages to the donors, seven minutes before its mixture with the recipient, the quantity of resistant recipient bacteria falls to 6.3 per cent relative to the control in phage f_2, and to 4.5 per cent in phage M 13. Phage addition at the moment of mixing donor and recipient bacteria exerts a weaker inhibitory effect on the transfer of multiple drug resistance (up to 7.2 per cent relative to controls for phage f_2, and up to 11.5 per cent — for phage M 13).

Under analogical conditions the inhibitory action of donor-specific bacteriophages on the donor function of strain CSH-2 (222) with repressed R-factor is much less pronounced: the transfer of R-factors falls to 50.1 per cent of the control when phage f_2 is used, and to 34.0 per cent when phage M 13 is used, provided the phages are added prior to formation of the conjugational mixture; after phage addition to the conjugational mixture, the transfer of R-factors is lowered to 50.9 per cent of the control for phage f_2, and to 54.5 per cent for phage M 13 (see table).

The weaker inhibitory effect of phages on the conjugational transfer of R-factors in donor strain CSH-2(222) with repressed R-factor may be attributed to the following causes: 1) inability of some of the sex pili to absorb donor-specific phages, 2) participation of other bacterial structures in the conjugational process. The same causes may explain also the absence of full ihibition in the transfer of multiple drug resistance in the donor strain with derepressed R-factor.

A difference exists in the degree of inhibition of R-factors transfer, exerted by RNA- and DNA-containing bacteriophages: phage f_2 (RNA) inhibits almost in equal degree the transfer both upon beforehand treatment of the donor, and upon addition of the phage at the moment of mixing donor with recipient, whereas phage M 13 inhibition is manifested more strongly upon preliminary treatment of the donor. Such a difference in the degree of inhibition is probably attributable to the different adsorptional zones of the two phages, and to the ensuing unequal effect on the transport of genetic material: phage f_2, undergoing adsorption along the sex pili, inhibits both the formation of conjugal pairs, and the transfer of genetic material, whereas phage M 13, undergoing adsorption at the free end of sex pili, inhibits the formation of conjugal pairs but exerts a weaker effect on the transport of genetic material.

The obtained results corroborate the participation of sex pili in conjugational transfer of multiple drug resistance in bacteria. The incomplete inhibition of R-factors transfer upon adsorption of DNA- and RNA-containing donor-specific phages over sex pili, as well as upon mechanical removal of the latter, does not rule out the participation of other superficial bacterial cell structures in the transport of R-factors in bacteria.

The results of the electron microscope study are illustrated in Figs. 1 and 2.

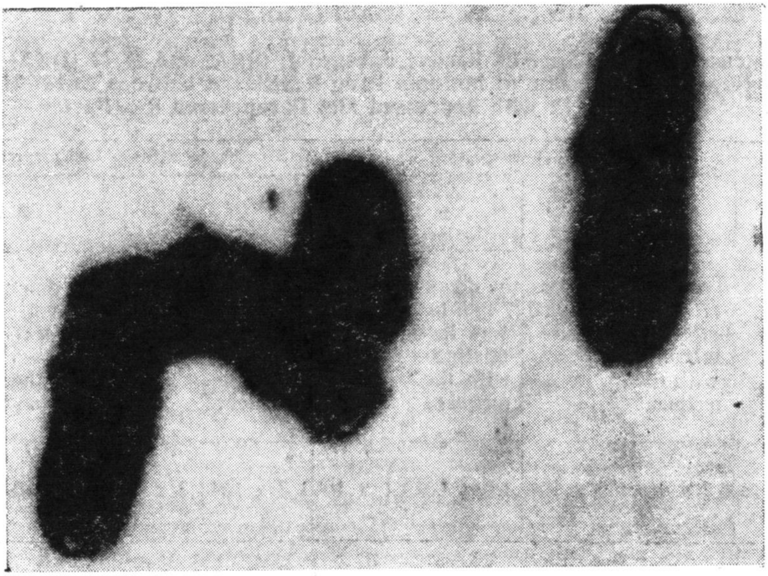

Fig. 1. Bacterial cells from conjugational mixture $J_{5,3}$xP 678. One of the bacterial cells has a sex pilus with adsorbed phage f_2 particles over it.

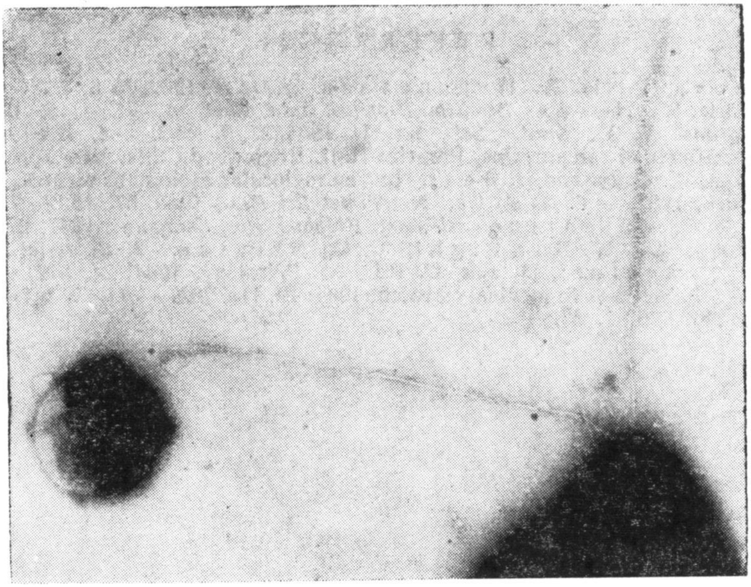

Fig. 2. Bacterial cells from conjugational mixture $J_{5,3}$xP 678, united by means of a sex pilus along the course of which phage f_2 particles are adsorbed.

Table

Influence of Donor-Specific Bacteri ophages f_2 (RNA) and M 13 (DNA) on Conjugational Transfer of Multiple Drug Resistance between Substrains of E. Coli K — 12 with Repressed and Derepressed R — Factor

Donor strains	Factor ($J_{5,3}$) derepressed R				Factor (CSH — 2/222) repressed R			
Bacteriophages	f_2		M 13		f_2		M 13	
Bacteriophage added to:	the donor (7 min before its mixture with the recipient	the conjugational mixture	the donor (7 min before its mixture with the recipient	the conjugational mixtuire	the donor (7 min before its mixture with the recipient	the conjugational mixture	the donor (7 min before its mixture with the recipient	the conjugational mixture
Resistant recipient bact/ml	6.3×10^4	6.9×10^4	$4,3 \times 10^4$	1.1×10^5	1.3×10^3	$1,3 \times 10^3$	$9,3 \times 10^2$	$1,4 \times 10^3$
% of resistant recipient bacteria from the control	6.3	7,2	4.5	11.5	50.1	50.9	34.0	54.5

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