

entially depresses bacterial messenger RNA synthesis⁴, but permits phage messenger RNA and protein but not DNA to be synthesized⁵, and fully inhibits recombination. Cyanide depresses gross metabolism of phage host complexes, but does not fully or significantly block phage recombination. It seems likely that phage recombination requires a product of bacterial origin—perhaps bacterial messenger RNA or enzyme. Studies on phage recombination in recombination deficient mutants of *E. coli*⁷ would be of interest.

This work was supported by a research grant from the U.S. National Science Foundation, the East West Center, and the Microbiology Department, University of Hawaii.

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New Type of Immunity to Virulent T-phages in *Escherichia coli*

HEREDITARY immunity manifests itself in *Escherichia coli* treated with a cell free medium obtained from a stationary phase culture inoculated with a virulent phage. Immune bacteria have certain features which cannot be explained by selective or mutational processes. They become resistant to other related and unrelated virulent phages of the T-series as well as to the phage present in the cell free medium (infecting phage).

In the first experiment a stationary phase culture of *E. coli* B was inoculated with bacteriophage T₃ and then incubated at 37° C for 48 h. The bacteria were removed by centrifugation followed by filtration, and the cell free medium was concentrated fifty-fold by osmosis with polyethylene glycol, 4,000 ('Carbowax', Union Carbide Co., New York). This preparation contained 5 × 10⁸ P.F.U./ml. phage. 0.2 ml. of this concentrate was diluted serially to 10⁻⁹ in ten-fold steps, with nutrient broth. To 1.8 ml. of each dilution 0.2 ml. of a fresh culture of *E. coli* B Sr (streptomycin resistant) containing 10⁸ bacteria/ml. was added. After overnight incubation at 37° C the tubes were scored for turbidity. It was found that the tubes containing the 10⁻¹ and 10⁻² dilutions of the concentrate were turbid, those containing 10⁻³ to 10⁻⁷ dilutions were clear, and those with 10⁻⁸ and 10⁻⁹ dilutions were turbid. The turbid tubes with 10⁻¹ and 10⁻² dilutions contained infective phage shown by plating out on *E. coli* B indicator cells, whereas the high dilution tubes were phage free. Thus, large amounts of bacteria were actively growing in the presence of at least 10⁷ PFU/ml. of phage. Samples from the turbid tubes were streaked on agar plates and tested for sensitivity to homologous (T₃) and heterologous (T₂) phages by cross streaking with phage suspensions. The bacteria from the low dilution tubes were resistant to both phages while cells from the high dilution tubes were sensitive to them. Experiments in which different preparations were used also gave similar results. Stationary phase cultures inoculated with T₂ also yielded a cell free medium which exhibited the same immunity producing property.

To investigate this property the following experiment was carried out. A cell free concentrate prepared with T₂r (containing 5 × 10⁸ P.F.U./ml.) was applied in three-fold dilutions to exponentially growing *E. coli* B Sr. Marked

bacterial growth was observed in tubes containing low dilutions of the concentrate, that is large amounts of T₂r. Bacteria from the tubes showing turbidity were plated for the isolation of single colonies. The colonies obtained were phage free, as shown by plating on indicator bacteria. They were tested for resistance or sensitivity to T₂r, T₃, T₄rII and T₇ by cross streaking the different phage suspensions with colony suspensions on agar plates. The plates were incubated overnight, and the results are recorded in Table 1.

Table 1. PHAGE RESISTANCE OR SENSITIVITY OF *E. coli* B TREATED WITH A CELL FREE MEDIUM FROM A T₂ INFECTED STATIONARY CULTURE

Sensitivity (s)/resistance (r) of the bacteria tested				No. of colonies scored
T ₂ s	T ₃ s	T ₄ s	T ₇ s	0
T ₂ r	T ₃ r	T ₄ r	T ₇ r	15
T ₂ r	T ₃ r	T ₄ r	T ₇ r	8
T ₂ r	T ₃ s	T ₄ r	T ₇ s	3
T ₂ r	T ₃ s	T ₄ r	T ₇ s	3
T ₂ r	T ₃ r	T ₄ r	T ₇ s	1
T ₂ s	T ₃ r	T ₄ r	T ₇ r	2
Total colonies tested				32

Approximately half the examined colonies were resistant to T₂ only. The others showed varied resistance, ranging from resistance to all four types (8 colonies) to resistance to all but the original phage. All colonies were found to breed true.

Conjoint resistance to T₃, T₄ and T₇ which is known to occur as a single mutational step¹ can also be excluded by the fact that 26 colonies (of the 32 included in Table 1) tested against T₁ were all found to be resistant to this phage. In addition, certain data on the velocity at which heterologous resistance is attained indicate that the T₂ resistant colonies appear 20 min after the introduction of T₃ phage. The occurrence of such varied resistant patterns excludes a straightforward "mutation and selection" explanation of this phenomenon¹⁻². Preliminary observations indicate that phage is necessary for the initiation of this phenomenon. We propose the term immunity, rather than resistance, for this phenomenon, and "factor of bacterial immunity" for the factor responsible for it.

I thank Drs. N. Goldblum and J. Mager for assistance and Dr. W. Hayes, M.R.C., London, for his criticism and comments.

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Experiments in Bacterial Adaptation

RECENTLY, I found that bacteria have definite preferences in utilizing different types of hydrocarbon as sole carbonaceous nutrient and in adapting from one type to another¹. I now wish to report the results of further experiments with two of the organisms which show that this ability to ferment hydrocarbon is retained after the organisms have been grown on glucose as carbon source for a considerable period.

The organisms, *Pseudomonas fluorescens* and a species of *Corynebacterium*, were subcultured every third day for 117 days into conical flasks containing 100 ml. sterile mineral salt solution (1.6 g potassium dihydrogen phosphate; 0.8 g disodium hydrogen phosphate; 0.5 g MgSO₄·7H₂O; 0.5 g sodium chloride; 5 g ammonium chloride in 1 l. distilled water) and 1 per cent glucose. The flasks were then kept stationary at 30° C. At each transfer from one glucose medium to another, a sterile mineral salt solution containing in place of the glucose 1 per cent dodecane for the *Pseudomonas* and 1 per cent decane for the *Corynebacterium*, respectively,