Table 1. PHAGE TITRES PER ML. ON THE GIVEN INDICATORS

Phage	98	Indicators 8354	8354/8426
8426 <i>P</i>	10 ¹⁰	10 ¹⁰	10 ⁸
8354 <i>P</i>	10 ⁸	0	10 ⁸
60 <i>P</i>	10 ⁸	0	10 ⁸
105 <i>P</i>	10 ⁸	0	10 ⁸

and 105 *P*. The first-named being its own phage, the problem arose of the stability of the system. It was found that 8354/8426 appeared to contain no 8354 *P* as judged by two methods. First, indicators for 8354 *P* showed no plaques when spotted with the supernatant of 8354/8426. Secondly, the supernatant did not plaque on 8354/8426, which would be expected if any 8354 *P* was present. It appears, therefore, that when 8426 *P* infects 8354 it displaces the prophage of 8354 *P*⁵ and as a result 8354 is now sensitive to its own phage and in fact to other phages. This would have the effect of changing the typing pattern of the culture in an unexpected way. This work is being extended by testing other systems made with 8426 *P*.

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Artificial Elimination of the *F* Factor in *Bact. coli* K-12

A FEW years ago, Lederberg and his co-workers^{1,2} found that in *Bact. coli* K-12 there was a difference in sex-compatibility between the two mating cells, one having a transmissible agent, *F*, and the other lacking this agent. Hayes³ demonstrated that these mating types were very stable heritable characters; but the accidental disappearance of the *F* factor was found by Lederberg *et al.*¹, and by Hayes⁴. The study reported here reveals that it is possible to obtain *F*⁻ cells at will from *F*⁺ cells under certain environmental conditions. This phenomenon may be called '*F* elimination'. The environmental factor responsible for *F* elimination is the cobalt or nickel concentration in the medium (Co(NO₃)₂, CoCl₂, etc.; Ni(NO₃)₂, NiSO₄, etc.).

The first method of artificial elimination of *F* factor is called the 'citrate method'. A culture of *F*⁺ cells was inoculated into peptone medium (peptone, 10 gm., and glucose, 2 gm., in 1 litre distilled water), and incubated overnight at 37° C. After 24 hr., 0.15 ml. of 0.4 *M* cobalt solution was added to 3 ml. of the culture (the final concentration was 20 mM), which was incubated for 24 hr. more. This treated culture was then spread on nutrient agar containing 40 mM of sodium citrate, which was used for detoxification of cobalt, and single colonies grown on the medium were isolated at random. By this procedure, it was found that many colonies were converted into *F*⁻. For example, in the mutant K-12 strain 58-161 and the derived strains from 58-161, 10-30 per cent of the colonies were converted into *F*⁻. However, all the *F*⁻ were sensitive to cobalt. The frequency of conversion varies according to the strain of K-12 used.

The second method is called 'resistant isolation', in which cobalt-resistant colonies are selected by serial transfer in peptone medium containing cobalt in increasing concentrations. A slow and smooth increase of resistance was observed. Starting from a concentration of 0.5 mM, in which strains derived from the original K-12 are able to grow, resistant strains tolerating even 20 mM were obtained. These resistant strains were plated on nutrient agar containing 20 mM of cobalt, and ten well-grown colonies were isolated at random. In mutant strains 58-161 and Y-40, for example, all ten colonies were *F*⁻, whereas in the W-1485 strain, only four colonies were *F*⁻. However, drug resistance and the *F*⁻ character of these strains could be separated; cobalt-sensitive strains derived from the resistant were *F*⁻ as before, and cobalt-resistant strains receiving the *F* factor from *F*⁺ bacteria became *F*⁺ themselves.

The mating behaviour of the *F*⁻ strains corresponded to that of the *F*⁻ strain described by Hayes^{3,6} and by Lederberg *et al.*¹. By the cobalt treatment, other characteristics, for example, nutritional requirements, sugar fermentation, phage-resistance, lysogenicity, indol formation, shape, Gram staining, and antibiotic resistance were not changed. The *F*⁺ trait is very stable in successive serial passages in ordinary media such as broth, peptone and minimal medium. Therefore, in the *F*⁺ clone, the replication of the *F* factor may be closely concerned with bacterial reproduction.

This hereditary conversion of mating type within a clone gives rise to the question of the basis of the change and the mechanism by which the *F*⁻ strains occurred. There are two well-recognized possibilities: (a) *F* elimination may be due to the selection of spontaneous gene mutation; (b) it may be induced by the nickel or cobalt in the medium. From the experimental data, the following may be concluded: (1) *F* elimination occurs even in the absence of cell division (in the first method); (2) the occurrence of *F* elimination is about 100,000 times more frequent than that of other bacterial mutants; (3) *F*⁺ and *F*⁻ strains are equally susceptible to the killing action of cobalt or nickel as judged by viable counts, and *F*⁻ trait is not linked with cobalt or nickel resistance. These conclusions seem to exclude possibility (a). The conjecture used for explaining the contradiction in the stability and instability of the *F* factor is that it is a non-lytic symbiotic agent, as reported by Cavalli *et al.*² and Hayes⁴. It may behave as a cytoplasmic factor and/or as extruded state on to the surface of the *F*⁺ cells at a certain stage of its life-cycle. In this state, the sensitivity of the *F* factor to cobalt or nickel may be greater than that of other cytoplasmic factors or of nuclear genes.

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