Artificial elimination of F factor in Bact. coli K-12.

A few years ago, Lederberg and his co-workers (1952; 1953)
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.
Hayes (1953b) demonstrated that these mating types were very
stable heritable characters, but, the accidental disappearance
of the F factor was found by Lederberg et. al. (1952), and by
Hayes (1953a). 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
($Co(NO_3)_2$, $CoCl_2$, etc.; $Ni(NO_3)_2$, $NiSO_4$, etc.). The first
method of artificial eliminatin of F factor is called the
"direct" method. A culture of F^+ cells was inoculated into
peptone medium (peptone, 10g. and glucose, 2g. in 1 liter
distilled water PH.7.2), and incubated overnight at 37°C. After
24hrs., 0.15ml. of 0.4M. cobalt solution was added to 3 ml.
of the culture (the final concentration was 20mM.) which was
incubated for 24 hrs. more. This treated culture was then spread
on nutrient agar containing 40mM. 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 % of the colonies were converted into F^-. However, all of
the F^- were sensitive to cobalt. The frequency of conversion
varies according to the strain of Bact. coli K-12 used. The
second method is called "resistant isolation", in which cobalt-

medium containing cobalt in increasing concentrations. A slow
and smooth increase of resistance was observed. Starting from
a concentration of 0.5mM., in which strains derived from the
original Bact. coli K-12 are able to grow, resistant strains
tolerating even 20mM. were obtained. These resistant strains
were plated on nutrient agar containing 20mM. of cobalt, and
10 well grown colonies were isolated at random. In mutant
strains 58-161 and Y-40, for example, all 10 colonies were F^-
whereas in the W-1485 strain, only 4 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 resistants received the F factor
from F^+ bacteria to become F^+ themselves. The mating behavior
of the F^- strains corresponded to that of the F^- strain described
by Hayes (1952a;b) and by Lederberg et. al. (1952). By the
cobalt treatment, other characteristics, e.g. nutritional require-
ments, 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 concernd 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 occured. 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

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 conjection used to explain the contradiction in the stability and instability of the F factor is that it is a non-lytic symbiotic agent, as reported Cavalli et. al. (1953), and Hayes (1953a). It may behave as a cytoplamic factor (and/or as extruded state onto the surface of the F⁺ull 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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