Resume of discussion with Hirota and Kikkawa, 9/13/56

F+ and F- are equally killed by cobalt, though the tests were made with separate cultures and I don't think he did a reconstruction experiment (I had trouble with communication on this point, and don't believe he ever understood my questions or my reasons for emphasizing this point)

Cobalt-Resistance is closely linked to methionine locus.

When F- developed with cobalt reverts to cobalt-S it remains F-

1. Direct method (This works only for 58-161)
Bacteria grown in glucose (2g/l), peptone (10g/l), distilled water with pH adjusted to 7.2 by HCl addition. After 24 hours growth, 20 milimole cobalt (nitrate or chloride) made up by addition to the same tube. This was left for 24 hours, now reduced to 3. Then a sample was spread on a plate. With a 24 hour treatment citrate was always added to the plate, but with 3 hour could be omitted. Citrate conc. 40 milimole/l With 3 hour treatment survival 80-100% (but I failed to get the details of how the count was made, or what precautions were taken).
The colonies were picked after 24 hours and 10-20% were F-.

2. Resistant isolation method
This worked for 1023, PC, Y40, W1485, and K12 which could not be converted by the direct method.

Glucose peptone medium with .5 to 1 milimolar concentration of cobalt inoculated. Then serial cultures were made with gradually increasing cobalt concentrations. Final concentration 20 milimolar, attained after 25-30 transfers. Then plated as before.

F+
Co-R/strains obtained in this manner cannot be converted to F- by direct method (except 58-161)

An F- strain derived from PC by the second method was then infected (?) with F+, from 58-161. But this does not convert directly to F-, so the rapid convertability property belongs to the genotype and not the source of the F+. (The complementary experiment wasn't done, i.e. F+ from another source in 58-161 cells)

JF Crow