

Nelson, N. Cross-feeding experiments to test for biological activity.

These cross-feeding experiments were originally performed to determine whether any of the 11 groups of adenine mutants induced in 74A and differentiated on the basis of heterocaryon tests would accumulate a diffusible precursor capable of supporting the growth of any of the other groups. Petri plates of Fries minimal agar plus 5 gamma/ml. adenine sulfate (20 ml. medium/petri plate) were inoculated with testers from each of the 11 heterocaryon groups. After 24 hours growth, cores were cut with a 1 cm. stainless steel cork borer from the growing hyphae and one core/plate was placed in a hole of identical size in the middle of a petri plate of unsupplemented Fries minimal agar (20 ml./plate). A circle of sterile dialyzer tubing, 3 inches in diameter, was used as the diffusible membrane. These circles were laid over the transplanted core. A core of the second tester strain was placed directly above the first core with the mycelial surfaces facing each other but separated by the membrane layer. If the top core showed stimulation, mycelial growth was on the surface of the membrane; while if the lower core grew, hyphae penetrated the agar. Tests were made in all combinations in this manner, and controls showed that the residual growth from the cores was negligible. In these experiments only the ad-8 mutants (adenine specific) produced a marked stimulatory effect. This stimulation was true for all groups except the ad-4 mutants which are also adenine specific. The ad-8 mutants are blocked in the adenine pathway between inosine monophosphate and adenosine monophosphate succinate and accumulate hypoxanthine which can feed all adenine mutants prior to this step. The same type of procedure might prove to be useful in other biochemical pathways to determine whether a given mutant accumulates a compound that can be used to feed mutants blocked earlier in the sequence. (These experiments were done while the author was at Yale University, New Haven, Connecticut, U.S.A., Edited by M. Case).