Geng, A. M. and A. M. Srb. Phenocopies of Neurospora mutants induced by biotin deficiency.

A number of oscospore shops mutants hove been described in N. crassa ond N. tetrasperma. Among these ore indurated ascus (Dodge 1934 Mycology 26: 360), rand spore (Novak and Srb 1973 Con. J. Genet. Cytol. 15: 685), and triangle spore (Srb et al. 1973 J. Hered. 64: 242). Although several of these mutants have been studied cytologically and/or biochemically, we have, as yet, little information regarding the biochemical processes which ultimately leod to these alterations in spore shape. Using backcrossed isolates of a wild strain of N. tetrasperma (Dunnellon 1b-P542), we hove observed thot when a wild-type cross is mode on purified agar (Difco) -- with no nutrients added -- indurated asci, round spores ond triangle spores, as well as wild-type spores, ore produced. When such aberrant spores are isolated ond allowed to cross, all Progeny spores ore wild type in shape. This result, togetherwith the fact that such deviant spore shops con be induced at will by altering the crossing medium, indicates that the aberrantly shaped spores are phenocopies rather than actual mutants.

In order to determine whether the presence of phenocopies could be attributed to the lock of a specific component of the standard crossing medium (Westergaard's, 1/2% sucrose), media were prepared with contained individual components or groups of components of Westergaard's medium. For this purpose, the components of the standard medium were divided into five groups: a) Westergaard's Salts -- 1 g KN03, 1 g KH_2PO_4, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.1 g $CaCl_2$, 0.1 g NaCl, 1000 ml distilled water; b) trace elements -- as described by Beadle and Tatum, 1945, Am. J. Bot. 32: 678; c) biotin -- 5 micrograms/liter; d) sucrose -- 1/2% (w/v); e) agar -- 1% Difco purified agar. Wild-type crosses were mode on the following media (all solidified with 1% purified agar): (I) standard Westergaard's (i.e. salts, trace elements, biotin, sucrose), (2) Westergaard's salts alone, (3) trace elements alone, (4) biotin alone, (5) sucrose alone, (6) purified agar, (7) Westergaard's salts + sucrose, (8) trace elements + sucrose, (9) biotin + sucrose, (10) Westergaard's minus biotin (i.e. salts, trace elements, sucrose).

All medio were capable of supporting perithecial formation. Although the number of perithecia ond the proportionate number of phenocopies present varied depending on the components of the medium, phenocopies were produced only on those media which lacked biotin. All crosses mode on media containing biotin resulted in the production of standard shaped spores, as seen in a normal wild type cross. This was true even when biotin was the only nutrient added to the purified agar.

Similar, although less extensive, results hove been obtained with N. tetrasperma strain T-220. Attempts to repeat the experiments just described but using N. crassa hove bean hampered by the fact that N. crsa crosses very poorly, if at all, on the various deficiency media listed above.

It should be noted that the above results do not imply that N. tetrasperma, as opposed to N. crassa, can grow in the absence of externally available biotin. Since biotin is required only in extremely small amounts and since no precautionary steps were token to rid glassware, etc., of contaminating biotin, the growth observed in the absence of added biotin may well be due to the traces of biotin on glassware and/or in the components of the media.

In relation to the above results, Barnett and Lilly (1947 Am. J. Bot. 34: 196), while studying the effects of biotin deficiency on the crossing of S. fimicolo, also observed indurated asci in crosses on biotin-deficient media. Thus, the effects of biotin deficiency an ascospore formation do not seem to be limited to the genus Neurospora and the results may imply that one or more biotin-dependent steps are essential for the proper determination of spore shape. - - - Section of Genetics, Development and Physiology, Cornell University, Ithaca, New York 14853.