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New supplements for the format mutant
of Neurospora. A possible role for
ascorbic acid?

Recently we found what appeared to be a new auxotroph in one of our stocks. It was eventually traced to the formate (for) locus. Later we found that it was not a new allele, but was the original C24 for allele which had gotten into the stock by an ancient error in stockkeeping.

In the course of determining the auxotroph's growth requirements, we found that it responded strongly to three combinations of supplements not previously reported for for. The for mutant had previously been reported to grow weakly on adenine alone, strongly on adenine plus methionine, and strongly on formate or formaldehyde. On our auxanograms the response to adenine alone is very weak, but the response to adenine combined with histidine, tryptophan or ascorbic acid is very strong. Neither histidine nor tryptophan is effective without adenine. Ascorbic acid gives a definite response without adenine but gives a stronger response when adenine is present.

All tests were done auxanographically. The supposed new auxotroph and a standard stock of for (an f1 of FGSC 133) behaved identically in all tests, and both grew well on formate.

The for mutant lacks cytosolic serine hydroxymethyl transferase, which catalyzes the reaction $\text{serine} + \text{tetrahydrofolate} \rightleftharpoons \text{glycine} + \text{methylene tetrahydrofolate}$ (see Cossins and Pang 1980 *Experientia* 36:289-290). This is the chief reaction that generates the transferrable C1 units of the various folate coenzymes which are needed for the synthesis of purines, methionine, thymidylate, etc. Formate supports growth of the mutant by the formation of formyl tetrahydrofolate, which can be converted to the other folate coenzymes.

We can only speculate about how the new supplements work. Tryptophan degradation is known to produce formate, Histidine is known to stimulate the growth of post-purple adenine in the presence of adenine (M. Case, cited in Perkins et al. 1982 *Microbiol. Rev.* 46:426-570), presumably because of the connections between histidine and adenine synthesis, but the response in our case is so great as to suggest that there may be an alternate explanation. Possibly histidine is degraded to yield 5-formiminotetrahydrofolate as in mammals, but to the best of our knowledge only the first step of this pathway has so far been demonstrated in *Neurospora*.

Ascorbic acid does not work simply by lowering the pH, because a neutralized solution of ascorbic acid was also effective. Several oxidation/reduction reactions occur in the synthesis of the various folate coenzymes, but none of them is a hydroxylation of the type that ascorbic acid is known to catalyze. Conceivably ascorbate is degraded to generate a formyl group. Two possible mechanisms for this have been suggested.

First, C¹⁴ ascorbate produces C¹⁴ oxalate in man (E.L. Smith et al. 1983 *Principles of Biochemistry-Mammalian Biochemistry*, 7th ed., p. 667), and pea-seeds have an enzyme system that converts oxalate to formate (Giovanelli and Tobin 1964 *Plant Physiol.* 39:139-145). We therefore tested oxalate on for. In single auxanograms on plates of minimal with and without adenine, each of two for isolates gave definite growth responses to potassium oxalate, although only in the presence of adenine. These responses might suggest that oxalate is converted to formate, although we have no other evidence for it. nor any evidence that *Neurospora* converts ascorbate to oxalate.

Second, Dumbrava and Pall (1987 *Biochim. Biophys. Acta* 996:331-338) have found that *Neurospora* lacks detectable ascorbic acid but has a pool of erythroascorbic acid. Erythroascorbic acid resembles ascorbic in structure except that it is one methylene group smaller. Conceivably ascorbic is converted to erythroascorbic, producing a formyl group in the process.

If *Neurospora* contains no ascorbic acid when grown in its absence, any physiological role for ascorbic must be limited to situations in which exogenous ascorbic is present. This may frequently may be the case in nature, since *Neurospora* normally grows on plant materials.

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