Hanson, B. A. and S. Brody. The utilization of purines and other nitrogen compounds nit and ty-1 mutants.

We have studied the utilization of different nitrogenous compounds as sole nitrogen sources for the growth of the nitrate reductase (nit) ond tyrorinare (ty-1) mutants of N. crassa.

Seven strains were tested: nit-I, nit-2, nit-3, ty-1 and wild types RL3-8, CR851 and OR1 16. Mycelial fragments from seven-day slant cultures were used to inoculate synthetic crossing medium containing the appropriate nitrogen sources in 15 x 100 mm petri dishes. The surfacer of the agar plater were covered with circles of *sterile dialysis* tubing in order to facilitate the harvesting of the *mycelia*. After *inoculation*, the *plates were incubated* in an upright position at 23° C in constant light for six days and were harvested by scraping the mycelium from the dialysis tubing. The mycelial samples were rapidly frozen, lyophilized overnight, and weighed. The mycelial dry weight in mg/petri dish for each mutant grown on a particular nitrogen source was then calculated.

Solid media were used in the experiments instead of liquid media because of the difficulty in solubilizing several of the purine derivatives. The undissolved purine compounds could be uniformly suspended throughout solid medium. The media for all experiments were prepared from a modified Mitchell and Westergaard synthetic crossing medium (Westergaard and Mitchell 1947 Am. J. Bot. 34: 573). The control nitrogen compound for all experiments was ammonium chloride.

nit-I was unable to grow with either nitrate or hypoxanthine as its role source of growth nitrogen at either concentration tested. Growth on the other nitrogen sources was equal to or greater than the growth achieved on the control nitrogen source, ammonium chloride (see Tables 1 and 2). This nonutilization of nitrate and hypoxanthine indicates that probably only nitrate reductase and hypoxanthine oxidase are affected by the nit-I mutation. Ketchum et al. (1970 Proc. Nat. Acad. Sci. USA 66: 1016) suggested that there two enzymes share a common structural subunit in Neurospora and that this subunit is affected by the nit-I mutation. In Aspergillus, Pateman et al. (1964 Nature 201 : 58) suggested that there is a common subunit for nitrate reductase and hypoxanthine oxidase.

The nit-2 mutant was the most pleiotropic of the three" mutations studied. nit-2 did not grow on nitrate, nitrite, or hypoxanthine as sources of growth nitrogen (see Tables). In additions, the mutant did not grow well on high concentrations of xanthine or uric acid. The growth on high levels of hypoxanthine and xanthine was 0.5% and 4%, respectively, of that on ammonium chloride. The inability of the mutant to grow on there high levels of nitrogen suggests that the nit-2 mutation is a regulatory one affecting the ability of the organism to induce or derepress the synthesis of the enzymes uricase and allantoinase.

The nit-3 mutant did not appear to be pleiotropic. The only source of nitrogen that it did not utilize was nitrate (see Tables).

The effect of different nitrogen sources on growth was studied in the ty-1 mutant, a female sterile mutant, because it is known that one of the determining factors in the formation of protoperithecia is nitrogen in the form of nitrate (Hirsch 1954 Physiol. *Plantarum* 7: 72 and Horowitz et al. 1960 J. Mol. *Biol.* 2: 96). It *is* therefore *possible* that *some alteration* in nitrogen metabolism in this mutant may be related to its female sterility. It is apparent the nitrogen metabolism has been altered in the ty-1 mutant, but in a manner opposite to that of the nit mutants. ty-1 grew extremely well on nitrate, allantoin, urea, glutamine and glutamate, at both concentrations used (see Tables). The growth yield on the lower concentration of there compounds was about two timer greater than for the ammonium chloride control and it was about six timer greater on the higher concentration. This suggests that the ty-1 mutant is dereprerred for the utilization of nitrogenous compounds other than ammonium. This dereprerred growth on certain nitrogen sources appeared to be related to the ty-1 mutation and not the genetic background of the strain since it grew better than did many of the wild type strains. Both the nit mutants and wild type strains showed on inhibition of growth on higher levels of nitrogen.

The *ty-1* mutation *affects* the *regulation* of tyrorinare synthesis *and is* not *a mutation at* the *structural gene for* this enzyme. From our evidence it is apparent the ty-1 mutation which causes female sterility and affects the induction of tyrorinare may also be involved in the regulation of several nitrogen enzyme systems of Neurospora. It would be interesting to ascertain what the relationship of female sterility is to the altered nitrogen utilization of this mutant.

Table 1. Growth on purines and other sources of nitrogen at a concentration of 0.01 moles N/liter.

	Nitrogen sources									
Strains	Ammonium chloride	Potassium nitrate	Sodium nitrite[a]	Ammonium nitrate	Hypoxanthine	Xanthine	Uric acid	Allantoin[a]	Urea[a]	Glutamate[a]
nit-1	39	0.08	49	38	1	27	52	55	44	37
nit-2	41	0.05	1	46	2	13	35	37	50	55
nit-3	38	0.06	52	36	52	26	51	63	38	47
ty-1	62	130	27	147	118	72	128	137	103	154
RL3-8	29	42	35	38	16	14	27	27	22	43
CR851	70	115	86	213	151	38	65	133	32	69
OR116	74	133	95	164	98	36	83	116	65	89

[a] These compounds were filter-sterilized and then added to the autoclaved medium.
Results expressed as mg lyophilized mycelium/petri dish after 6 days' incubation at 23° C.

Table 2. Growth on purines and other sources of nitrogen at a concentration of 0.05 moles N/liter.

Strains	Nitrogen sources										
	Ammonium chloride	Potassium nitrate	Sodium nitrite[a]	Ammonium nitrate	Hypoxanthine	Xanthine	Uric acid	Allantoin[a]	Urea[a]	Glutamate[a]	Glutamine[a]
nit-1	47b	0.3	0	33	0	21	7	33	200	181	181
nit-2	59[c]	0.6	0	44	0.3	2	4	14	337	323	143
nit-3	41[d]	0	0	39	7	18	27	83	263	219	221
ty-1	61[e]	316	99	307	352	72	a7	432	464	345	366
RL3-8	27f	6	0	27	8	7	7	10	15	85	151

[a] These compounds were filter-sterilized and then added to the autoclaved medium.
[b] Standard error ± 2.
[c] Standard error ± 2.
[d] Stondord error ± 3.
[e] Standard error ± 6
[f] Stondord error ± 7

Results expressed as mg lyophilized mycelium/petri dish after 6 days' incubation at 23° C.

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