

Wootton, J. C. Short-term changer in the amino acid pool of *Neurospora* mycelium.

The free amino acid pool of exponentially-growing mycelium can show marked but transient changes in composition when the mycelium is subjected to a brief change of conditions. This was observed during experiments of which the following is typical.

A vigorously-aerated liquid culture, inoculated with conidia of wild type STA4, was grown in modified Fries minimal medium (1966 *Neurospora Newsl.* 10: 34) in which ammonium tartrate and  $\text{NH}_4\text{NO}_3$  were omitted and replaced by 3 g/l  $\text{KN}_3$  and 20 g/l glucose as nitrogen and carbon sources. At 21°C exponential growth was maintained for over 24 hr., with a dry weight doubling time of 365 min. At 16 hr., when the density was 40.6 mg. dry weight/ 10 ml of culture, the mycelium was rapidly but carefully filtered off and transferred to medium of the same chemical composition (but  $^{15}\text{N}$  labelled for other purposes) approximating in  $\text{K}^{15}\text{NO}_3$  and glucose concentration to the  $^{14}\text{N}$  medium at the time of transfer. The mycelium was not allowed to dry during filtration and transfer, and this operation took 10 seconds in all. Frequent dry weight measurements before and after transfer showed that exponential growth continued without a measurable break, although a lag of 5 min. or less would not have been detected by this method.

Samples for pool analysis were filtered rapidly under suction onto glass fiber paper discs (Whatman GF/A) and plunged into 60% ethanol in water for fixation. It is unlikely that significant changes in pool composition occurred before fixation because the mycelium was immersed in ethanol within 5 seconds of filtration. Pools were extracted in 3 changer of 60% ethanol at 21°C, this mild method being chosen to avoid hydrolysis of the glutamine and asparagine amide groups. Adequate extraction was shown by the fact that only 3% further ninhydrin-positive material could be extracted with hot 0.1 N HCl.

The analyses (Table 1) demonstrate transient changes of up to nearly 4-fold in the pool sizes of some amino acids, although the total amino acid pool size remained constant. The changes occurred in a period of not more than 7 min. during and immediately after the transfer operation, and a stable, regulated pool composition was re-established 20 to 40 minutes later.

These exponential-phase pools are considerably smaller than and of different composition from the consistent and stable pools of 3-day-old mycelium reported by DeBusk and DeBusk (1967 *Neurospora Newsl.* 11: 3). In contrast to the stationary-phase pool, the pool of exponential-phase mycelium has been found to differ in size and composition in cultures of STA4 grown in the same medium but at different temperatures. Moreover, in any single culture the pool size and composition showed gradual, well-regulated changes throughout the growth cycle. Pool analyses of conidia agreed closely with those of DeBusk and DeBusk.

The technical details of amino acid analyses and experiments involving pool analyses of amination-deficient mutants are being reported in a paper (in manuscript). Workers analysing the pools of growing mycelium should note that rapid fixation is necessary. - - - Department of Genetics, The University, Leeds 2, England.

Table 1. Pool composition ( $\mu\text{M/g}$  dry weight)

Component	Minutes before					Minutes after transfer				
	transfer: 20					5	10	20	40	
Total ninhydrin-positive material	286.1	289.5	288.5	293.9	282.8					
Glutamate	41.2	42.5	36.8	38.4	40.7					
Aspartate	4.6	4.9	5.1	5.0	4.9					
Glutamine	53.0	25.4	40.5	44.2	47.8					
Asparagine	7.0	1.8	4.5	6.9	6.6					
Glycine	22.7	54.9	24.6	18.7	19.8					
Alanine	31.5	46.8	33.4	34.3	33.5					
Ammonia	1.5	1.9	1.5	1.5	1.5					
Other amino acids	124.6	111.3	142.1	144.9	127.6					