Weston, N. J. and A. G. DeBusk. Amino acid transport in poky (mi) mutants of Neurospora crassa.

Woodward and Munkres (1966 Proc. Natl. Acad. Sci., U.S. 55: 872) have shown an amino acid substitution to occur in the mitochondrial structural protein (MSP) in certain poky mutants. It is clear that enzymes attached to membranes containing an altered MSP may show decreased affinity for substrates, as was demonstrated in the case of malate dehydrogenase (Munkres and Woodward 1966 Proc. Natl. Acad. Sci. U. S. 55: 1217). Furthermore, not only ore membranes of mitochondria altered in &mutants, but the same abnormal structural protein is found in other membranes of the cell as well (Woodward, personal communication).

We have attempted to test the hypothesis that a permease system which is part of or attached to a membrane may have an altered activity when associated with such an amino acid substituted structural protein. Table I comparer conidial phenylalanine transport in two wild type strains and several mi mutants. Incubations were carried out in the absence of a carbon source, employing techniques similar to those previously described (DeBusk and DeBusk 1965 Biochim. Biophyr. Act. 104: 139). Transport by mycelial pads of wild type and mi-1 are also compared, since mi-1 fails to conidiate. (Although sometimes revealing, mycelial experiments are far more difficult to do with precision.) The poky strains failed to show a decreased transport rate when compared with wild type strains. Surprisingly, in one instance (mi-4 ) there war a marked increase in both the rate of transport and capacity of conidia for phenylalanine. However, segregants of this strain show normal transport rates. The studier with phenylalanine reported here and additional studier with other amino acids indicate that the &phenotype has little effect on amino acid transport.

Table 1. Phenylalanine transport in mi strains.

| Time (min) | 15 | 30 | 45 | 60 | 75 |
|---|---|---|---|---|---|
| Strain | | | | | |
| 74A | 280 | 473 | 513 | 650 | 680 |
| SY7A | 253 | 460 | 578 | 615 | 6% |
| mi-2 | 342 | 617 | 758 | 833 | 092 |
| mi-3 | 307 | 540 | 583 | 621 | 608 |
| mi-4 | 456 | 978 | 1186 | 1227 | 1242 |
| 74A* | 123 | 206 | 241 | 505 | 460 |
| mi-1* | 87 | 198 | 351 | 383 | 454 |

Valuer represent total phenylalanine uptake in the absence of a carbon source expressed as CPM/O.5 mg (dry weight) conidia; saturating concentrations of L-phenylalanine were employed.

Table 2. Phenylalanine transport in wild type and mi strains in the presence of metabolic uncoupling agents.

| Time (min) | 15 | 30 | 45 | 60 | 75 |
|---|---|---|---|---|---|
| Strain | | | | | |
| 74A (control) | 265 | 335 | 490 | 530 | 560 |
| 74A ($NaN_3$) | 80 | 140 | 220 | 215 | 160 |
| 74A (Antimycin A) | 79 | 100 | 180 | 185 | 240 |
| 74A (DNP) | 145 | 250 | 305 | 300 | 375 |
| mi-1 (control) | 184 | 400 | 435 | 415 | 460 |
| mi-1 | 60 | 85 | 110 | 100 | a5 |
| mi-1 (A($NaN_3$) A) | 45 | 50 | | 54 | 55 |
| mi-T (DNP) | 100 | 110 | 60 | 97 | 80 |
| | | | 85 | | |

Valuer represent total phenylalanine uptake with mycelial discs in the absence of a carbon source expressed as CPM/mg (dry weight) mycelia.

Tissieres et al. ( 1953 J. Biol. them. 205: 423) have shown that the respiration of mi-1 (,poky ) is insensitive to sodium azide and approximately one-third that of wild type. Preliminary experiments have shown that while respiration of poky is insensitive to both azide (0.5 mM) and antimycin A (0.025 mg/ml), the uncoupling agent DNP reduces respiration by approximately 50%. Also, as shown in Table II, uptake of $^{14}C$ phenylalanine by poky decreased when the cells were incubated with the above-mentioned inhibitors. These data strongly suggest that the energy coupling system for active transport is not dependent on the cytochrome terminal oxidase system.

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