JOURNAL OF BACTERIOLOGY, Feb., 1966
Vol. 91, No. 2

Genetic Control of Amino Acid Permeability in *Neurospora crassa*

GABRIEL LESTER
Department of Biology, Reed College, Portland, Oregon

Received for publication 24 August 1965

ABSTRACT

LESTER, GABRIEL (Reed College, Portland, Ore.). Genetic control of amino acid permeability in *Neurospora crassa*. J. Bacteriol. **91**:677–684. 1966.—Strains of *Neurospora crassa* resistant to 4-methyltryptophan (4-MT) were isolated from populations of conidia exposed to ultraviolet light. In genetic crosses, 4-MT resistance behaved as a single-gene difference. Resistance to 4-MT could not be attributed to a relaxation of control of the formation or the activity of the enzymes of tryptophan biosynthesis. Growth studies involving tryptophan auxotrophs carrying the aberrant *mt* gene and uptake studies with normal and 4-MT-resistant strains showed that 4-MT resistance could be attributed to an inability of 4-MT-resistant strains to take up tryptophan and its methyl analogues. The *mt* gene is not specific for tryptophan; strains resistant to 4-MT are also resistant to ethionine, and they have a markedly reduced ability to take up serine, leucine, and α-aminoisobutyric acid. No difference was observed between strains carrying either *mt* allele in their ability to take up glucose; also, the uptake of anthranilic acid or of indole was not sufficiently impaired by the aberrant *mt* gene to prevent these tryptophan precursors from satisfying the nutritional requirement of certain tryptophan auxotrophs. The role of the *mt* gene in determining the permeability of *N. crassa* to amino acids is discussed.

The biosynthesis of tryptophan in *Neurospora crassa* appears to be regulated by repression and end-product inhibition (5, 6, 7). The levels of tryptophan synthetase activity, indole-synthesizing activity, and anthranilate-synthesizing activity formed are inversely related to the concentration of L-tryptophan in the culture medium, indicating a repressive effect of L-tryptophan on several enzymes in the sequence. Once formed, indole- and anthranilate-synthesizing activities, but not tryptophan synthetase, are markedly inhibited by L-tryptophan, suggesting an early step(s) in tryptophan synthesis as the main site(s) of end-product inhibition. In these respects, then, the regulation of tryptophan synthesis in *N. crassa* resembles the regulation of several biosynthetic pathways in bacteria.

Certain methyl derivatives of tryptophan are potent growth inhibitors for *Escherichia coli* because of their inhibition of the formation and activity of enzymes concerned with the biosynthesis of tryptophan (10, 12, 15). Consequently, strains of *E. coli* resistant to methyltryptophans with respect to growth are often markedly less sensitive to the action of tryptophan as a repressor or inhibitor of enzyme formation or activity (4, 12). An examination of the specificity of L-tryptophan showed that certain methyltryptophans inhibited tryptophan biosynthesis in *Neurospora* (6, 7), presenting the possibility of using these compounds to select for regulation mutants. The present report describes the isolation and the genetic and physiological properties of mutants of *N. crassa* selected for resistance to 4-methyltryptophan (4-MT). These mutants were not directly altered in their regulation of tryptophan biosynthesis, but they exhibited abnormal permeability to amino acids with concomitant secondary consequences affecting resistance to amino acid analogues and sensitivity to feedback inhibition of tryptophan biosynthesis by tryptophan and related compounds.

MATERIALS AND METHODS

Organisms. The present studies were initiated with the wild-type strain of *N. crassa*, 74A. In addition, the following tryptophan auxotrophs were employed in crosses: strain 10575(*tryp-1*)A which can use either indole or tryptophan for growth, and strain td_2-23(*tryp-3*)a which can grow only on tryptophan.

Media. For studies of growth, selection of mutants, and germination of conidia, Fries minimal medium (1) appropriately supplemented for the particular purpose or strain was used. Stock cultures were maintained on medium N (Vogel, *unpublished data*) containing 1% sucrose and appropriate supplements. Crosses were made on slants of a synthetic crossing medium (17) at 25 C; the medium was supplemented with the nutrients required by the strains crossed.

Biochemical activities. Tryptophan synthetase activity was estimated from the uptake of indole by germinated conidia suspended in buffer containing serine; the correspondence of this assay to that of tryptophan synthetase in cell-free extracts has been established (5). The specific activity of tryptophan synthetase is designated as millimicromoles of indole taken up per milligram (dry weight) of cells per 3.0 hr at 30 C.

The estimation of anthranilate-synthesizing activity has been described previously (7); the assay of this activity involves the determination of fluorescence (excitation at 315 mμ, emission at 400 mμ) in filtrates from suspensions of germinated conidia in half-strength Fries salts solution containing 1.5% sucrose. With anthranilic acid as a standard, the specific activity is designated as millimicromoles of anthranilate produced per milligram of cell dry weight per hour at 30 C.

Uptake studies. The uptake of amino acids and of glucose was determined with suspensions of germinated conidia; the production of conidia and the conditions of their germination have been described elsewhere (5). Briefly, conidia were germinated in minimal medium, and were washed with and resuspended in 0.02 M NaH_2PO_4 in 0.05 M NaCl (*p*H 5.8); this buffer was used as the medium for uptake experiments. Suspensions of germinated conidia containing amino acids or glucose were incubated at 30 C with agitation in a reciprocating water bath; usually the volume of the uptake mixture was 25 to 40 ml, in a 125-ml Erlenmeyer flask. At intervals, samples were removed and filtered through paper; the cells were dried for weight determinations and the clear filtrates were analyzed for amino acids or glucose. The uptake of these compounds by germinated conidia was estimated from the decrease in their concentration in the filtrates. In some cases, uptake was determined more directly by examining hot-water extracts of washed cells for the substance whose uptake was being studied.

Assays. Leucine-*l*-C^{14}, serine-*l*-C^{14}, and α-aminoisobutyric acid-*l*-C^{14} (New England Nuclear Corp., Boston, Mass.) were estimated by measurements of radioactivity. Portions (0.1 ml) of filtrates or extracts were spread over an area of about 3 cm^2 on aluminum planchets and were dried; all samples were prepared in duplicate. Radioactivity was determined with a thin-window counter. The concentration of radioactive materials used was such that zero-time samples would give about 20,000 counts per min per ml of medium. Each planchet was counted twice and long enough to yield at least 6,000 counts. The decrease of radioactivity in the filtrates was assumed to represent the uptake by germinated conidia of the compounds being examined.

Tryptophan was estimated by bioassay with the use of a tryptophan auxotroph of *E. coli*, strain T-3, A-11 (supplied by C. Yanofsky). The assay was carried out with medium E (16) supplemented with 0.25% glucose and 0.05% Casamino Acids (Difco). After 18 hr of incubation at 37 C with agitation, the growth was estimated turbidimetrically and converted to units of tryptophan from a standard curve. Also, tryptophan and methyl analogues of tryptophan were estimated by colorimetric assay (18) of the corresponding indolyl compounds produced after incubation with a partially purified preparation of tryptophanase from *E. coli*.

Glucose was measured by assay with Glucostat (Worthington Biochemical Corp., Freehold, N.J.; reagent made up according to manufacturer's "micromethod"). The details of this assay have been described elsewhere (8).

RESULTS

Selection of strains resistant to 4-MT. A preliminary examination of the effects of 4-, 5-, and 6-methyltryptophan on the growth of *N. crassa* indicated that only 4-MT was sufficiently inhibitory to permit the possibility of detecting strains resistant to this tryptophan analogue on plates. A clear-cut, persistent inhibition of growth could be obtained by avoiding the overcrowding of plates and by plating conidia in the agar medium rather than by spreading them on the surface.

Washed conidia of *N. crassa* 74A were suspended in water at a concentration of 10^7 per milliliter and were irradiated with ultraviolet light until the viable count was reduced by 98 to 99%. The irradiated suspension was centrifuged, and the conidia were suspended in water. The conidia were distributed in a warm (40 to 42 C) molten medium consisting of Fries salts, 1.0% sorbose, 0.1% sucrose, 1.5% agar, and 0.5 μmole of 4-methyl-DL-tryptophan per ml. On plates with a base layer of 10 to 12 ml of the same solidified medium was layered 4 ml of molten medium containing 0.5–1 × 10^5 viable conidia. The plates were incubated at 30 C for 3 to 4 days, and then were examined for the formation of distinct colonies. Colonies appeared on plates inoculated with irradiated conidia, whereas plates inoculated with unirradiated conidia showed no colonies.

Small sections of the colonies were placed on similar plates containing media with and without 4-MT, and those few colonies whose growth was inhibited by 4-MT were discarded. Those colonies showing resistance to 4-MT were cultured on slants of medium N containing 0.5 μmole of 4-MT per ml. Conidia from these cultures were streaked on the Fries-sorbose-4-MT medium described above, and isolated colonies were cultured on slants of medium N containing 4-

MT. The purified cultures were tested for 4-MT resistance in liquid medium (Fries with 2% sucrose) with or without 1.0 μmole of 4-MT per ml; the medium was distributed in 3-ml volumes in test tubes. After 40 to 48 hr of incubation at 30 C, it was easy to distinguish between strains sensitive or resistant to 4-MT. With longer periods of incubation, sensitive strains began to grow in the presence of 4-MT; this growth was not due to a selection of resistant types, since retesting of these cultures showed them to be still sensitive to 4-MT. Fourteen independent isolates showing resistance to 4-MT were produced initially. These were designated as strains *mt-1*, *mt-2*, etc., and the parent, sensitive strain was designated mt^+. Stock cultures were maintained on slants of medium N with 1.0% sucrose, 1.5% agar, and 0.5 μmole of 4-MT per ml.

Some physiological consequences of the mt mutation. Previous studies (6, 7) have shown that 4-MT inhibits preformed anthranilate-synthesizing and indole-synthesizing activities of certain tryptophan auxotrophs of *N. crassa*, but that 4-MT does not affect the formation of these activities. Also, germinated conidia of strain 74A appear to contain a maximally repressed level of tryptophan synthetase (5), presumably owing to the endogenous formation of excess tryptophan (11). If 4-MT acts by inhibiting tryptophan biosynthesis, the presence of sublethal amounts of 4-MT in the growth medium should cause a reduction of endogenous tryptophan synthesis in strain 74A, and a consequent reduction of repression should result in increased levels of tryptophan synthetase. If 4-MT resistance in *mt* strains is due to an inability of 4-MT to affect tryptophan biosynthesis, then the presence of 4-MT in the growth medium should not inhibit tryptophan synthesis in *mt* strains, and tryptophan synthetase formation should be unaffected.

Conidia were germinated in the presence and absence of 4-MT, and the germinated conidia were assayed for tryptophan synthetase activity (Table 1). The growth of strain 74A was inhibited by 4-MT, but, as expected, no significant effect of 4-MT on the growth of *mt* strains was observed. However, 74A cells grown in the presence of 4-MT showed about twice as much tryptophan synthetase activity as did cells grown in the absence of 4-MT. The higher activity approximates that observed previously (5) in a tryptophan auxotroph grown on low levels of tryptophan. Thus, it appears that 4-MT reduces the endogenous synthesis of tryptophan in strain 74A(mt^+). On the other hand, 4-MT did not significantly influence the formation of tryptophan synthetase activity in *mt* strains. Moreover, the tryptophan synthetase activity of the *mt* strains is similar to that of strain 74A, indicating that a decreased repressibility of the formation of tryptophan synthetase is not associated with resistance to 4-MT. These results are consistent with the idea that resistance to 4-MT reflects an inability of 4-MT to affect the biosynthesis of tryptophan. However, these results do not distinguish between a loss of sensitivity of early reactions in tryptophan biosynthesis and an inaccessibility of such reactions to 4-MT.

TABLE 1. *Effect of 4-methyl-DL-tryptophan (4-MT) on the formation of tryptophan synthetase activity in strain 74A(mt^+) and mt strains*

Strain	Culture		Tryptophan synthetase (specific activity)
	4-MT (0.5 μmole/ml)	Germinated conidia	
		mg/ml	
74A(mt^+)	−	3.1	122
	+	1.4	250
mt-2A	−	3.3	124
	+	3.3	130
mt-6A	−	3.3	102
	+	3.0	104
mt-8A	−	3.0	94
	+	3.1	99
mt-10A	−	3.0	96
	+	3.0	107
mt-12A	−	3.2	102
	+	3.2	97

The possibility of altered regulation by end-product inhibition was examined by incorporating the *mt* gene into a tryptophan auxotroph and then determining the effects of normally inhibitory compounds on the preformed ability to synthesize characteristic intermediates in tryptophan biosynthesis. From the cross *mt-6A* × *tryp-1A*, tryptophan-requiring progeny which should carry the *mt* gene were obtained from one ascus, since the growth of tryptophan-independent ascospores from this ascus were inhibited by 4-MT; this was verified by the recovery of the *mt* character in the tryptophan-independent progeny obtained from a cross between the putative *tryp-1*, *mt* strain and a wild-type strain. One of the ditype progeny, *tryp-1*, *mt-6*, was examined for anthranilate-synthesizing activity.

Table 2 compares the effects of L-tryptophan and related compounds on the anthranilate-synthesizing activities of *tryp-1*, mt^+ and of *tryp-1*, *mt* strains. As previously observed (7), the preformed anthranilate-synthesizing activity of strain *tryp-1* is markedly inhibited by L-tryptophan and indole, 4-methyl- and 6-methyltryptophan are moderately inhibitory, and 5-methyltryptophan has hardly any effect. The preformed

TABLE 2. *Effect of tryptophan and related compounds on preformed anthranilate-synthesizing activity in strains carrying mt+ and mt genes*

Strain*	Compound added†	Amt	Anthranilate synthesis (specific activity)
		μmoles/ml	
tryp-1, mt+	None	—	70
	L-Tryptophan	0.1	2
	4-MT	0.5	41
	5-MT	0.5	65
	6-MT	0.5	44
	Indole	0.2	2
tryp-1, mt	None	—	59
	L-Tryptophan	1.0	59
	4-MT	1.0	60
	5-MT	1.0	61
	6-MT	1.0	60
	Indole	0.5	1

* The germination medium for *tryp-1, mt+* contained 0.04 μmole of L-tryptophan + 0.5 μmole of 4-MT per ml. The germination medium for *tryp-1, mt* contained 0.05 μmole of L-tryptophan per ml.

† MT = methyl-DL-tryptophan.

activity in strain *tryp-1, mt-6* is not affected at all by tryptophan or its methyl analogues. Of particular interest, however, is the observation that indole does inhibit preformed anthranilate-synthesizing activity in both strains. This suggests that the *mt* locus is not directly concerned with the sensitivity of early reactions in tryptophan biosynthesis to feedback inhibition, but rather with the accessibility of these reactions to exogenously supplied tryptophan and its analogues. Thus, the above results are consistent with the idea that 4-MT resistance is associated with a defect in the permeability of *mt* strains to tryptophan and methyltryptophans.

The notion that 4-MT resistance represents impermeability to tryptophan is supported by the observation that the presence of the *mt* gene restricts the ability of *tryp-1* and *tryp-3* strains to utilize an exogenous source of tryptophan. From the cross *mt-6a* × 10575(*tryp-1*)A, ascospores were recovered which grew poorly on tryptophan, but grew well when the medium was supplemented with indole. The cross *mt-6A* × td_2-23(*tryp-3*)a yielded asci which contained wild-type spores, but the other spores in these asci (presumably *tryp-3, mt* ditypes) grew very slowly, if at all, on tryptophan. These results indicate that the presence of the *mt* gene renders the cells impermeable to tryptophan.

Other evidence for the relationship of *mt* to a permeability defect comes from an examination of the ability of tryptophan and its precursors to support the growth of *tryp-1* and *tryp-2* strains carrying the *mt* gene (these *tryp-1, mt* and *tryp-2, mt* ditypes were prepared by D. R. Stadler). The results are given in Table 3. With the *tryp-1* strain, either indole or tryptophan supported growth; the slight growth on anthranilic acid alone probably was due to the leakiness of this strain (2). In the presence of the *mt* gene, significant growth occurred only with indole. Similar results were obtained with the *tryp-2* strain; tryptophan supported growth only when the *mt+* allele was present, whereas anthranilic acid or indole supported growth with either the *mt+* or the *mt* allele.

Uptake of tryptophan and methyl-tryptophans. The preceding observations suggest that the *mt* mutation affects the permeability of *N. crassa* to tryptophan. A direct examination of this possibility was made by comparing the abilities of strains *74A* and *mt-6A* to take up tryptophan and some of its methyl analogues. Germinated conidia of both strains were incubated in buffer containing one of the tryptophan compounds examined. Samples were taken at 0 and 90 min and filtered, the filtrates were removed, and the cells were rapidly rinsed with water. The cells were resuspended in water, heated for 3 min in a boiling-water bath, and then cooled and filtered. The filtrates of the original suspensions and the hot-water extracts were analyzed for tryptophan compounds with tryptophanase.

Table 4 shows that strain 74A is capable of taking up tryptophan and certain of its methyl analogues to varying extents. L-Tryptophan is

TABLE 3. *Influence of mt gene on the ability of tryptophan auxotrophs to utilize tryptophan or its precursors for growth*

Addition to medium*	Growth†			
	tryp-1		tryp-2	
	mt+	mt	mt+	mt
Anthranilic acid	5	5	54	65
Indole	84	79	42	60
L-Tryptophan	90	10	67	6

* Basal medium: Fries + 2% sucrose, 20 ml per 125-ml Erlenmeyer flask. Additions were in amounts of 0.25 μmole/ml.

† The tryp-1 strains were incubated at 30 C for 72 hr; the tryp-2 strains (also carrying co-4) were incubated at 25 C for 96 hr. The values given are the average dry weights (milligrams) of mycelia from duplicate cultures.

TABLE 4. *Uptake of tryptophan and related compounds by strain 74A(mt+)*

Compound*	Initial concn	Uptake†	
		From medium	Intracellular
	μmoles/ml		
L-Tryptophan	1.0	98	90
DL-Tryptophan	2.0	148	118
4-MT	2.0	43	44
5-MT	2.0	7	Nil
6-MT	2.0	58	45

* MT = methyl-DL-tryptophan.

† The values given are millimicromoles per milligram (dry weight) per 90 min of incubation at 30 C. The concentration of germinated conidia was 3.6 mg (dry weight) per ml for strain 74A. Tryptophan and the methyltryptophans were estimated by enzymatic assay with tryptophanase.

taken up most effectively, and its uptake appears to be stimulated by the presence of D-tryptophan (note that the tryptophanase assay is specific for L-tryptophan). The reason for this stimulation of uptake is not clear, but it may be noted that a smaller proportion of the tryptophan taken up is recovered from the cells exposed to DL-tryptophan than to L-tryptophan. This might suggest that D-tryptophan stimulates the metabolism of L-tryptophan. 4-Methyl- and 6-methyltryptophan are taken up to similar extents, but none of the 4-methyl compound is metabolized, whereas only about 75 to 80% of the 6-methyltryptophan taken up is recovered from the cells. Only a slight uptake of 5-methyltryptophan was observed, which probably accounts for its relative ineffectiveness as an inhibitor of growth or of tryptophan biosynthesis.

Of primary interest is the observation that the *mt* strain did not take up detectable amounts of tryptophan. The inability of this strain to take up tryptophan compounds accounts for its resistance to 4-MT, and for the inability of tryptophan auxotrophs carrying the *mt* allele to use tryptophan effectively for growth. These results appear to establish as the basis for 4-MT resistance a defect in the permeability of *N. crassa* to tryptophan compounds.

Effect of ethionine on the growth of strains 74A and mt-6A. It would appear that the *mt* locus is somewhat specific for tryptophan, insofar as the entry of anthranilic acid or of indole is not sufficiently blocked in an *mt* strain to prevent their utilization for growth (see Table 3). However, to determine the extent of *mt* specificity, the effect of another inhibitor, the methionine analogue ethionine, was examined for its effect on growth. Table 5 shows that both ethionine and 4-MT are effective inhibitors of the growth of strain 74A, and that their inhibitory effects are reversed by methionine and tryptophan, respectively. The growth of strain *mt-6A* was not affected by either inhibitor, suggesting that the *mt* locus is not specific for tryptophan permeability. Thus, it appears that the *mt* gene determines the permeability of methionine as well as that of tryptophan.

Uptake of serine, leucine, and tryptophan. The lack of specificity of the *mt* locus is further evidenced by Fig. 1, which shows that the uptake of serine and leucine, as well as tryptophan, is markedly restricted in the *mt* strain relative to the uptake of these amino acids by strain 74A. Hot-water extracts of cells harvested at the end of the experiment (150 min) contained about 80% of the radioactivity (serine and leucine) which disappeared from the suspending medium. A similar recovery was made in the case of tryptophan.

Assuming that the intracellular radioactivity represented intact serine and leucine, it was possible to calculate the intracellular concentrations of these amino acids and of tryptophan. Based on previous estimates (8) of the relationship of cell water to dry weight [1.0 mg of dry weight equals 2.45 mg (μliters) of intracellular water], the internal concentrations of the amino acids were calculated. Table 6 shows that strain 74A is able to accumulate these amino acids intracellularly against a concentration gradient of about 80 to 140, depending on the particular

TABLE 5. *Effects of L-ethionine and 4-methyl-DL-tryptophan on the growth of strains 74A(mt+) and mt-6A*

Addition to medium*	Amt added	Growth†	
		74A(*mt*+)	*mt-6A*
	μmoles/ml		
None	—	49	42
L-Ethionine	1.0	3	46
L-Methionine	1.0	41	45
L-Ethionine	1.0		
+ L-Methionine	1.0	45	48
4-Methyl-DL-tryptophan	0.5	7	45
L-Tryptophan	0.5	51	40
4-Methyl-DL-tryptophan	0.5		
+ L-tryptophan	0.5	44	43

* Basal medium: Fries + 2% sucrose, 10 ml per 50-ml Erlenmeyer flask.

† The cultures were incubated at 30 C for 70 hr. The values given are the average dry weights (milligrams) of mycelia from duplicate cultures.

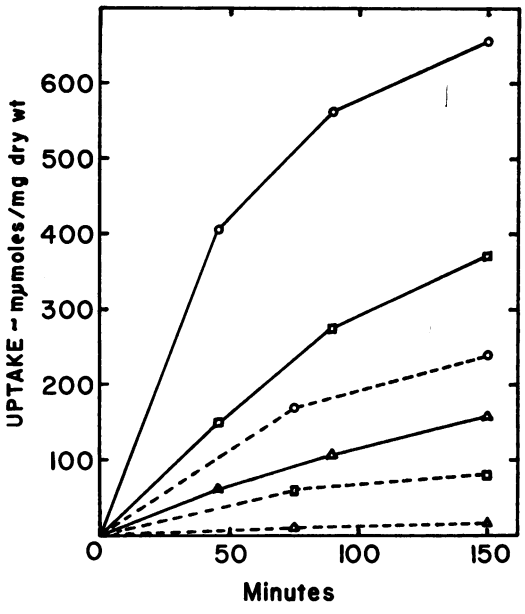

FIG. 1. *Uptake of serine* (○), *leucine* (□), *and tryptophan* (△) *by strains 74A (solid lines) and mt-6A (broken lines). The initial concentrations of* L-*serine,* L-*leucine, and* L-*tryptophan were 5.0, 2.0, and 1.0 μmoles/ml, respectively. The concentrations of germinated conidia were 3.3 and 3.5 mg (dry weight) per ml for strains 74A and mt-6A, respectively.*

amino acids involved. Strain *mt-6A* can also concentrate these amino acids, but to a lesser extent; the values for tryptophan are not significant.

Uptake of glucose and α-aminoisobutyric acid. The specificity of the *mt* locus was examined further in terms of the uptake of glucose and α-aminoisobutyric acid. The examination of glucose uptake was relevant not only to the question of specificity, but to that of energy metabolism as well. Glucose disappearing from the medium is not found in the cells (9), and, thus, glucose uptake probably represents glucose metabolism. α-Aminoisobutyric acid is not used as a carbon or nitrogen source by *N. crassa* (as determined by growth experiments), nor does it exhibit stereoisomerism, and, thus, α-aminoisobutyric acid serves as a neutral amino acid.

Figure 2 shows that strains 74A and *mt-6A* take up glucose at very similar rates. This indicates that the *mt* locus probably is not concerned with energy metabolism, and furthermore that the restriction on amino acid uptake by *mt-6A* is not due to a gross aberration of the energy supply for the active transport of amino acids. These results also indicate that the permeability restriction imposed by the *mt* gene does not include the sugar glucose. Contrariwise, the uptake of α-aminoisobutyric acid by strain *mt-6A* occurs at about one-half the rate observed with strain 74A. However, a significant intracellular accumulation of α-aminoisobutyric acid against a concentration gradient is observed in strain *mt-6A* as well as in strain 74A; at the termination of the experiment, the intracellular concentration of α-aminoisobutyric acid was 140 and 46 times as great as that in the medium for strains 74A and *mt-6A*, respectively.

DISCUSSION

To define the genetic identity and location of 4-MT resistance and to produce strains useful for the examination of the relationship of 4-MT resistance to the regulation of tryptophan biosynthesis, crosses were made between strain *mt-6A* and three tryptophan-requiring strains carrying mutations in the *tryp-1*, *tryp-2*, and *tryp-3* loci. The *tryp-4*, *pdx-1* (pyridoxine), and *co-4* (colonial) loci were also used in crosses to establish the site of the *mt* locus. A detailed description of these crosses will appear elsewhere

TABLE 6. *Intracellular accumulation of amino acids by strains 74A(mt⁺) and mt-6A**

Strain	Amino acid	Uptake from medium (μmole/mg of cells)	Intracellular concn		Concn in medium	Ratio of amt in cell water to amt in medium
			μmoles/mg of cells	μmoles/ml of cell water		
					μmoles/ml	
74A(*mt⁺*)	L-Serine	0.66	0.55	225	2.86	79
	L-Leucine	0.37	0.28	107	0.79	135
	L-Tryptophan	0.16	0.13	53	0.49	108
mt-6A	L-Serine	0.24	0.21	86	4.18	21
	L-Leucine	0.08	0.07	28	1.17	24
	L-Tryptophan	0.02	0.01	4	0.97	4

* Data taken from same experiment as described in Fig. 1; see text for details.

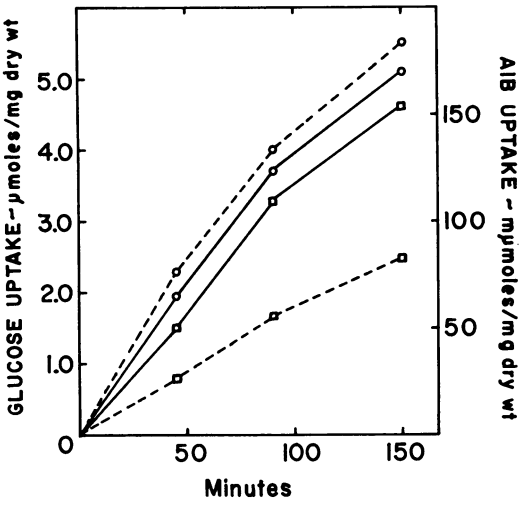

FIG. 2. *Uptake of glucose* (○) *and α-aminoisobutyric acid (AIB)* (□) *by strains 74A (solid lines) and mt-6A (broken lines). The initial concentrations of glucose and AIB were 20 and 1.0 μmoles/ml, respectively. The concentrations of germinated conidia were 3.6 and 3.3 mg (dry weight) per ml for strains 74A and mt-6A, respectively.*

(D. R. Stadler, *in preparation*). The salient conclusions of the genetic analyses are as follows: (i) the *mt* character behaves in crosses as a single gene; (ii) the *mt* gene is located in linkage group IV, close to the *co-4* and *pdx-1* loci; (iii) the *mt* gene is not associated with any of the loci determining the enzymes concerned with tryptophan biosynthesis; (iv) in heterocaryons *mt* is recessive to mt^+, in terms of sensitivity to 4-MT.

Together with the above observations, the data presented here demonstrate that the ability of *N. crassa* to take up any one of a variety of amino acids can be determined by a single gene. The *mt* locus does not appear to be unique in this respect, since it has been shown (13) that the initial rate of the uptake of certain amino acids and other metabolites (indole, dipeptides) is affected by mutation at a locus (*mod-5*) situated in a different linkage group than the *mt* locus. However, the consequences of the observed mutations at these loci are quite different. The *mod-5* mutation results in an *initial increase* in the rate of uptake of certain metabolites, but subsequently (after a lag of 30 to 60 min) similar uptake rates are observed in both normal and mutant strains. In contradistinction, the *mt* mutation results in a persistently decreased ability to take up amino acids. The evidence available at present (although not overwhelming) suggests that the *mt* locus is more specifically concerned with amino acid uptake, whereas the *mod-5* locus appears to have a more generalized function. It is noteworthy that, in heterocaryons of *mod-5* or *mt* with their corresponding normal alleles, both mutations are recessive with respect to sensitivity to 4-MT. It would be of interest to determine the characteristics of the double mutant *mod-5, mt* to elucidate the dominance relationships of these genes, and, perhaps, to determine whether either gene is uniquely or directly concerned with amino acid permeability.

The *mt* locus appears to be specific for amino acid permeability as compared with glucose uptake or the utilization of anthranilic acid or indole. It would appear that of the amino acids examined the uptake of tryptophan is the most severely affected by the *mt* mutation. However, since the rate of uptake might reflect the rate of metabolism of an amino acid, as well as the operation of a permeability system *per se*, it is difficult to assign comparative values for the effect of the *mt* mutation on amino acid uptake without exact knowledge of the metabolic fate of the amino acids examined. In the case of α-aminoisobutyric acid, the uptake studies are less equivocal since α-aminoisobutyric acid is not metabolized; these studies demonstrate that the *mt* locus is concerned with the permeability of *N. crassa* to amino acids.

The generalized effect of the *mt* mutation on amino acid transport suggests the operation of a single system for the uptake of several amino acids. The likelihood of such a unitary system appears to be borne out by the competitive effects which have been frequently observed in the uptake of amino acids or in their utilization for growth (e.g., 3, 13, 14). Also, all the strains resistant to 4-MT examined thus far are closely related, genetically. In view of the present observations, the original meaning of *mt*, methyltryptophan, might be subverted here to "amino acid transport."

It is somewhat premature to speculate on the nature or mechanism of the amino acid permeability system(s) in *N. crassa* on the basis of the data presently available. It is necessary, at least, to describe more definitively the specificity relationships of the genes examined to amino acid permeability before attempting to designate the functions of these genes. Also, it is possible that the observed aberrations of amino acid permeability are secondary consequences of alterations of systems such as those concerned with the permeability and accumulation of cations like potassium or ammonium ions.

ACKNOWLEDGMENTS

The excellent technical assistance of P. A. Michaud and A. J. Byers is gratefully acknowledged. Deep

thanks are due M. Bonner for an isolation, in order, of ascospores from the crosses *mt-6A* × td_2-23(*tryp-3*)a and *mt-6a* × 10575(*tryp-1*)A.

This investigation was supported by Public Health Service grant CA-06073 from the National Cancer Institute.

LITERATURE CITED

1. BEADLE, G. W., AND E. L. TATUM. 1945. *Neurospora*. II. Methods of producing and detecting mutations concerned with nutritional requirements. Am. J. Botany **32**:678–686.
2. BONNER, D. M., C. YANOFSKY, AND C. W. H. PARTRIDGE. 1952. Incomplete genetic blocks in biochemical mutants of *Neurospora*. Proc. Natl. Acad. Sci. U.S. **88**:25–34.
3. BROCKMAN, H. E. 1964. Effects of amino acids on the utilization of tryptophan and indole for growth by a mutant of *Neurospora crassa*. J. Gen Microbiol. **34**:31–41.
4. COHEN, G., AND F. JACOB. 1959. Sur la répression de la synthese des enzymes intervenant dans la formation du tryptophan chez *Escherichia coli*. Compt. Rend. **248**:3490–3492.
5. LESTER, G. 1961. Some aspects of tryptophan synthetase formation in *Neurospora crassa*. J. Bacteriol. **81**:964–973.
6. LESTER, G. 1961. Repression and inhibition of indole-synthesizing activity in *Neurospora crassa*. J. Bacteriol. **82**:215–223.
7. LESTER, G. 1963. Regulation of early reactions in the biosynthesis of tryptophan in *Neurospora crassa*. J. Bacteriol. **85**:468–475.
8. LESTER, G., D. AZZENA, AND O. HECHTER. 1962. Permeability and metabolism of lactose in *Neurospora crassa*. J. Bacteriol. **84**:217–227.
9. LESTER, G., D. STONE, AND O. HECHTER. 1958. The effects of deoxycorticosterone and other steroids on *Neurospora crassa*. Arch. Biochem. Biophys. **75**:196–214.
10. LESTER, G., AND C. YANOFSKY. 1961. Influence of 3-methylanthranilic and anthranilic acids on the formation of tryptophan synthetase in *Escherichia coli*. J. Bacteriol. **81**:81–90.
11. MATCHETT, W. H., AND J. A. DEMOSS. 1962. Factors affecting increased production of tryptophan synthetase by a TD mutant of *Neurospora crassa*. J. Bacteriol. **83**:1294–1300.
12. MOYED, H. S. 1960. False feedback inhibition: inhibition of tryptophan biosynthesis by 5-methyltryptophan. J. Biol. Chem. **235**:1098–1102.
13. ST. LAWRENCE, P., B. D. MALING, L. ALTWERGER, AND M. RACHMELER. 1964. Mutational alteration of permeability in Neurospora: effects on growth and the uptake of certain amino acids and related compounds. Genetics **50**:1383–1402.
14. SOBOREN, J., AND J. F. NYC. 1961. Amino acid interactions in *Neurospora crassa*. J. Bacteriol. **81**:20–25.
15. TRUDINGER, P. A., AND G. N. COHEN. 1956. The effect of 4-methyltryptophan on growth and enzyme systems of *Escherichia coli*. Biochem. J. **62**:488–491.
16. VOGEL, H. J., AND D. M. BONNER. 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97–106.
17. WESTERGAARD, M., AND H. K. MITCHELL. 1947. *Neurospora* V. A synthetic medium favoring sexual reproduction. Am. J. Botany **34**:573–577.
18. YANOFSKY, C. 1955. Tryptophan synthetase from *Neurospora*, p. 233–238. *In* S. P. Colowick and N. O. Kaplan [ed.], Methods in enzymology, vol. 2. Academic Press, Inc., New York.