Table 1. Effect of arginine on the growth of *pyr 3a-s*. Minimal medium (25 ml) was inoculated with conidia of *pyr 3a-s*. Various amounts of arginine were added at the times indicated, and dry weights were measured subsequently.

Arginine		Dry weight (mg) at		
Amount (μg/ml of medium)	Time (hr)	37 hr	61 hr	85 hr
0.0		1.5	24.0	46.2
0.5	0	0.0	0.0	0.0
1.0	6			18.2
10.0	37	1.5	21.1	51.0
20.0	37	1.5	9.6	20.0
50.0	37	1.5	5.8	8.5
100.0	37	1.5	7.2	9.3

pyr 3a phenotype is a sensitivity of an early step in pyrimidine synthesis to endogenously produced arginine or arginine derivative. The suppressor, by reducing OTC activity, would presumably reduce the concentration of the inhibitory substance. This interpretation is further supported by the observation that certain arginine mutations also suppress *pyr 3a* when double mutants are grown in limiting arginine (*3*).

The postulated arginine sensitive step in pyrimidine synthesis in *pyr 3a* has not been identified. Experiments have shown that arginine has no apparent effect on the production or in vitro activity of aspartic transcarbamylase from this strain. That *pyr 3a* does not suffer a block in the utilization of pyrimidines is indicated by the findings that no pyrimidine precursors accumulate in limiting concentrations of supplement and that *pyr 3a* will grow on orotic acid (*6*).

The sensitivity of *pyr 3a-s* to arginine is most extreme in the conidial stage. While 0.5 μg of arginine per milliliter of medium completely inhibits the growth of conidial inocula for at least 7 days, much higher concentrations (20 μg/ml or more) are required to delay growth if the same inocula are allowed to develop for 37 hours before arginine is added (Table 1). In other experiments, 50 to 100 μg of arginine per milliliter of medium were required to inhibit growth of media containing extremely limiting concentrations of uridine (5 to 10 μg/ml). The data indicate that growing mycelia, as opposed to conidia, may dispose of arginine by utilization or destruction, or both, rather quickly.

These findings may well be related to those of Fairley (*7*). His observations that a different *pyr 3* mutant (1298) will grow on α-amino butyrate or propionate, and that this growth is inhibited by arginine, suggest that the stimulatory compounds, like the suppressor, may interfere with the synthesis (or the inhibitory action) of arginine, allowing the normal route of pyrimidine synthesis to operate. Alternatively, as he suggests, these compounds may be substrates of an alternate route of pyrimidine synthesis, also arginine sensitive. If the latter interpretation is correct, there is no obvious reason why the suppressed mutant, *pyr 3a-s*, could not synthesize pyrimidines by an alternate, unrecognized, and arginine-sensitive route. The data of Fairley, showing arginine inhibition on the "utilization" of the aliphatic acids in tests comparing conidial and mycelial inocula, are compatible with the similar inhibition of the suppressed mutant described here.

Enzyme assays of mixed extracts of *s* and s^+ mycelia have shown that the low OTC activity of *s* is not due to the production of an unbound inhibitor, the lack of a cofactor, or a competitive reaction. It is entirely possible that the *s* locus determines the primary structure of ornithine transcarbamylase; this hypothesis is reinforced by the finding that mutants at the arg *2* and arg *3* loci, partially blocked between ornithine and citrulline on nutritional criteria (*8*), both have normal or high OTC activities (*9*).

Other effects of the *s* mutation, notably its ability to suppress several proline-requiring mutants (*3*), and its ability to block the utilization of ornithine by ornithine-requiring mutants (*3*), are now more understandable in terms of a greatly reduced conversion of exogenous or endogenous ornithine to citrulline (*10, 11*).

Note added in proof. Subsequent experiments have shown that the *pyr 3* mutant 1298, used by Fairley for the study of growth on α-amino butyrate and propionate, closely resembles *pyr 3a* in the presence and specific activity of aspartic transcarbamylase under different nutritional conditions. This finding was made independently in Fairley's laboratory by Eugene Wampler. The finding suggests again the common basis of the action of the *s* gene and the effect of α-amino butyrate and propionate.

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11. Part of this work was done while I was a National Science Foundation postdoctoral fellow at the California Institute of Technology, 1958–60. It was supported also in part by an institutional research grant to the University of Michigan from the American Cancer Society. A more detailed report of this work is in preparation.

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Arginine and Pyrimidine Biogenesis in Neurospora

Abstract. The growth-promoting activity of propionic acid and related compounds for the pyrimidine-less strain, *Neurospora crassa* 1298, is markedly inhibited by arginine. The data suggest that arginine exerts an inhibitory effect upon or represses the synthesis of an enzyme involved in pyrimidine formation.

There are numerous indications of a relation between the biosynthetic pathways leading on one hand to arginine and on the other to pyrimidine nucleotides (*1*). A new system for the examination of this relationship has been provided with the finding that several pyrimidine-less mutants of the mold, *Neurospora crassa*, are capable of growth in minimal medium supplemented only with propionate, α-aminobutyrate, or certain related substances (*2, 3*). The present report (*4*) is concerned with the discovery that arginine is a potent inhibitor of the growth-promoting effects of these compounds.

In the experiments described, the test organism was *N. crassa* 1298. Similar results were obtained with strain 37815, a strain which is pyrimidine-deficient only at temperatures above 32°C. The minimal medium and the methods used for the culture and the harvest of the mycelia have been described (*2*).

Results which typify the effect of arginine upon the growth of the mold in the presence of various growth-promoters are presented in Table 1. It may be noted that arginine in concentrations of 10 μg (about 0.006 μmole) per 25 ml of medium completely prevented growth in the presence of 50 μmole of either propionate or aminobutyrate. The molar ratio of arginine to these

Table 1. Effect of arginine on growth of *N. crassa* 1298. The values are averages of triplicate assays, each with 25 ml of minimal medium supplemented with either 4 μmole of uridine or 50 μmole of sodium propionate or aminobutyric acid. The growth periods were 3 days with uridine and 5 days with the other compounds.

L-arginine (mg/25 ml)	Dry mycelia (mg)
Uridine (4 μmole)	
0	45
10.0	47
DL-α-*amino*-n-*butyric acid (50 μmole)*	
0	25
0.001	20
.005	13
.010	0
Sodium propionate (50 μmole)	
0	17
0.001	8
.005	3
.010	0

growth-promoters necessary for 50 percent inhibition was of the order of 1:4000. On the other hand, arginine in concentrations as high as 10 mg/25 ml did not affect growth of the mutant in the presence of uridine. The arginine effect, therefore, must involve a relatively early stage of pyrimidine nucleotide formation.

The use of mycelial fragments for the inoculation process gave results similar to those described above for the usual conidial inoculum. When 1 mg of arginine was added to flasks which had been growing with propionate for 4 days (26 mg of mycelia), the growth in a subsequent 2-day period was only 10 mg of mycelia as compared with 16 mg for the controls. Also, experiments with mixtures of uridine and propionate or aminobutyrate demonstrated that the presence of 10 mg of arginine depressed growth to that obtained with the uridine alone. These results indicate that the arginine effect is not restricted to the germination process, although this may be the most susceptible stage. Higher concentrations of arginine are needed to inhibit growing mycelia, probably because the compound is removed rather rapidly by metabolic processes. With time, growth appears in inhibited cultures concomitantly with the disappearance of arginine from the medium.

Table 2. Comparison of the effect of various amino acids on growth of *N. crassa* 1298 in the presence of 50 μmole of DL-α-amino-*n*-butyric acid per 25 ml of minimal medium. The incubation period was 5 days.

Supplementary compound	Concn. of supplement (μmole/25 ml)	Wt. of dry mycelia (mg)
None		39
Arginine	0.03	11
Ornithine	0.6	17
Citrulline	6.0	30
Aspartic acid	37.0	22
Valine	43.0	12
Isoleucine	38.0	10

Experiments with the other common aliphatic α-amino acids demonstrated that only ornithine, citrulline, aspartic acid, valine, and isoleucine had inhibitory effects in this system (Table 2), although all to a lesser degree than arginine. Ornithine was much more effective than citrulline, contrary to expectation if the action of these compounds required their conversion to arginine. This may reflect differences in membrane permeability or may indicate that ornithine affects growth in a manner different from arginine.

Attempts at this time to explain these effects must be regarded as speculation, for the primary metabolic defect in *N. crassa* 1298 is of unknown nature and the mechanisms by which propionate and aminobutyrate support growth remain to be elucidated. The available evidence suggests (5) that these compounds are pyrimidine precursors and that their use for pyrimidine synthesis involves adaptive mechanisms different from those of the common aspartate-orotidylic acid pathway. It seems likely that any such utilization of these aliphatic acids requires the participation of coenzyme A at an early stage. The inhibitory action of valine and isoleucine may simply be the result of competition of their degradation products for this coenzyme.

Should the use of propionate for pyrimidine synthesis require a new transcarbamylation reaction, as is quite possible, then obvious opportunities would exist for aspartic acid and ornithine to inhibit the new transcarbamylase or to compete for the necessary carbamyl phosphate.

The mechanism of the striking inhibitory action of arginine also remains unknown. This amino acid certainly could be simply a powerful inhibitor of some enzyme. However, it may well be that the observed phenomenon is the result of the repression by arginine of the synthesis of an enzyme necessary for the utilization of propionate and aminobutyrate for pyrimidine formation. This mechanism may be of general importance in the metabolic control of pyrimidine biogenesis.

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Antifungal Agent

Abstract. From the soil of the San Joaquin Valley a fungus has been isolated, an extract of which inhibits the growth of *Coccidioides immitis* on Sabouraud's medium. An acute toxicity study in mice indicates a certain tolerance of the extract. Tentative identification indicates that the fungus is a penicillium.

Early in 1958 certain attributes of a green fungus seen occasionally in the course of our soil survey study of *Coccidioides immitis* caused one of us (M.C.E.) to isolate it and study its effect upon other fungi. It was found to overgrow and displace *C. immitis* on modified Sabouraud's medium.

It was planted on 3-day-old cultures of *C. immitis* and 1 week later washings of this combined growth were injected intraperitoneally into 12 mice. At the same time equal amounts of a much lighter suspension of pure *C. immitis* were injected intraperitoneally into 12 other mice. All of the mice that received the pure *C. immitis* culture died of coccidioidomycosis within 3 months. Only three of the mice that received the combined suspensions died.

Further studies pointed to the need for making an extract of the fungus for determining its effect upon *C. immitis*. The 8-day-old cultures of fungus grown on modified Sabouraud's medium were heated in the autoclave at 250°F for 5 min, and the mat and substrate were extracted with chloroform. The chloroform was boiled off under distilled water, and when only a tarry residue remained at the bottom, the water with

Table 1. Results of acute toxicity study.

Mice (No.)	Amount of extract (ml)	Deaths (No.)
10	0.1	none
21	0.2	3
19	0.3	18
10	0.5	10