rapidly and at a significantly lower percent than in the 120-day groups ($P < .01$). Thus it may be concluded that although the home cage concentration of alcohol solution prior to testing did not seem to be significant in these tests, two important factors did influence the change in preference for alcohol: (i) the amount of time spent drinking prior to testing, and (ii) occurrence of an interval when water was ingested while alcohol was in the gustatorily noxious range.

Finally, in view of these and other data, it is apparent that the arbitrary selection of a predetermined alcohol solution, such as the commonly used 10-percent concentration (*6*), is a questionable procedure for studying those experimental variables affecting the preference of this substance. Attributing animals' refusal of 10-percent solution to some physiological condition or alteration may be entirely erroneous, since this concentration simply could be above the normal organism's maximum preference level (refer again to Figs. 1 and 2).

In investigations which utilize preference for alcohol (and probably other substances) as a main experimental effect, the following factors must be considered: (i) acclimation period or prior exposure to the substance; (ii) the preference threshold for the specific genetic strain of individual animals under investigation (*7*); (iii) the nutritional and metabolic states of the organisms (*8*); and (iv) technical details,

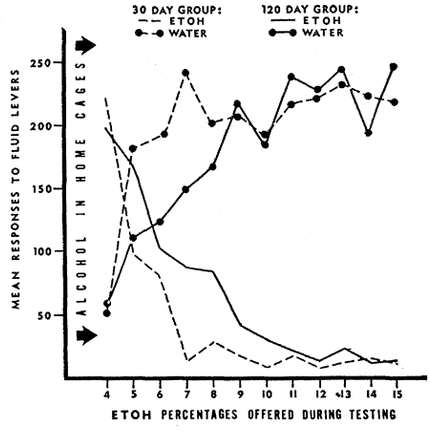

Fig. 2. Mean daily preferences for alcohol and water in rats whose fluid intakes in their home cages were restricted to 5-percent or 20-percent alcohol for 30 days and 120 days. These plots are based upon combined data of increasing-decreasing order groups and the 5- and 20-percent groups, and represent the mean fluid intakes on each 1-hour consecutive daily test session.

including cage position of the available substance and possible stress conditions (*9*). Neglect of any or all of these factors could seriously affect the validity of the research (*10*).

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5. Volumetrically prepared by mixing U.S.P. ethyl alcohol with tap water.
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Suppressor of Pyrimidine 3 Mutants of Neurospora and Its Relation to Arginine Synthesis

Abstract. The basis of the mutant phenotype of the *pyr 3a* strain of *Neurospora* appears to be the arginine sensitivity of an early step in pyrimidine synthesis. The effect of a suppressor mutation which renders *pyr 3a* pyrimidine-independent is to reduce arginine levels in the mycelium by its effect on ornithine transcarbamylase.

The *pyr 3* locus of *Neurospora* is represented by a number of independent mutations which impose a pyrimidine requirement upon the organism. It has been found that a group of mutants represented by *pyr 3d* (45502) lacks the enzyme aspartic transcarbamylase (ATC) (*1*) and does not respond to the presence in the same genome of an unlinked suppressor mutation, *s* (*2*). The mutants represented by *pyr 3a* (37301), on the other hand, display normal aspartic transcarbamylase activity (*1*), and the pyrimidine requirement is entirely or almost entirely eliminated in the presence of *s* (*2–4*). Previous work indicates that the *pyr 3a* mutation affects a step in the synthesis of pyrimidines which lies prior to the appearance of the product of the ATC reaction, ureidosuccinic acid (US) (*1*). Such steps may be the ATC reaction itself, or the availability of its substrates, carbamyl phosphate (CAP) or aspartic acid.

Direct attempts to identify the reaction affected in *pyr 3a* have been unrewarding. A study of the action of the suppressor, *s*, was undertaken, therefore, because it reverses the effect of the *pyr 3a* mutation. Very small concentrations of arginine (0.05 μg/ml medium) have been shown to restore a pyrimidine requirement to the suppressed mutant (*pyr 3a-s*) (*4*), indicating an inhibition of pyrimidine synthesis by arginine. Because ornithine transcarbamylase (OTC), catalyzing the formation of citrulline from carbamyl phosphate and ornithine, could well regulate arginine production, the effect of the suppressor gene upon this enzyme was investigated.

Growth conditions have been described previously (*1*). Acetone powder extracts were assayed for ornithine transcarbamylase activity by measuring the appearance of citrulline colorimetrically (*5*) in the following reaction mixture: 20 μmole of ornithine, 20 μmole of carbamyl phosphate, 250 μmole of tris acetate buffer, *p*H 9.0, and an aliquot of the extract; total volume, 3.25 ml; final *p*H, 8.7.

It was found that, under the conditions of the experiments, extracts of wild type, *pyr 3a* and *pyr 3d* mycelia at similar stages of growth displayed an ornithine transcarbamylase activity of 15 to 20 μmole of citrulline per milligram of protein per hour, while similar extracts of *pyr 3a-s* displayed activities of 0.2 to 0.6 μmole/mg per hour. Ascus analysis of a cross of *pyr 3a-s* to wild type showed that low OTC activity segregated regularly with suppressor action, and, where *s* was expected in otherwise wild type genomes, OTC activity was also low.

Strains carrying *s* (without *pyr 3a*) grew normally on minimal medium, despite the great reduction of OTC activity. Such strains were stimulated, if at all, by only 6 percent in linear growth rate when arginine was added to the medium, while all wild type strains were unaffected by the addition of arginine. This finding indicates that growth was limited only slightly by the lowered OTC activity, and that the inhibition of *pyr 3a-s* by arginine is a function of the *pyr 3a* mutation rather than of *s*. Most important, the data strongly suggest that the basis of the

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