STUDIES ON TRANSCARBAMYLASE ENZYMES OF NEUROSPORA CRASSA 1298

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY

D. Gene Wampler

1961

ABSTRACT

STUDIES ON TRANSCARBAMYLASE ENZYMES OF NEUROSPORA CRASSA 1298

by D. Gene Wampler

The presence and activity of some transcarbamylase enzymes in the pyrimidineless mold, Neurospora crassa 1298, were studied. These enzymes catalyse the general reaction:

$$R\text{-}NH_2 + NH_2\text{-}CO\text{-}O\text{-}PO_3^{=} \longrightarrow R\text{-}NH\text{-}CO\text{-}NH_2 + HPO_4^{=}$$

The presence of transcarbamylase activity in water soluble extracts of mold mycelia was detected by the Koritz-Cohen colorimetric test for the ureido group formed. The amount of protein in the enzyme preparation was determined colorimetrically by its reaction with Biuret reagent. Enzyme activity was then expressed in μmoles of product formed per milligram of protein per hour of reaction time.

It was found that when the mutant is grown on uridine the initial level of aspartic transcarbamylase activity is very low, and as the organism grows, this level of activity rises. When the organism is grown in α-aminobutyrate, aspartic transcarbamylase activity remains at a rather high level.

The level of ornithine transcarbamylase activity appears to be unaffected by either the type of nutrient supplied or the amount of growth. The activity of this enzyme is, however, affected by arginine and several aliphatic acids, including some which are known to promote growth of the organism in the absence of pyrimidines.

STUDIES ON TRANSCARBAMYLASE ENZYMES OF NEUROSPORA CRASSA 1298

By

D. Gene Wampler

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Chemistry

1961

ACKNOWLEDGMENTS

The author expresses his thanks and appreciation to Dr. James L. Fairley for his guidance and assistance in making this thesis possible. Appreciation is also expressed to Dr. Roland H. Davis for supplying cultures of the pyrimidineless mutants 45502, 63902, and 68902, and for his help in making the method of analysis workable.

Gratitude is also due the National Institutes of Health for financial assistance during this project.

D. G. W.

****

To Theresa

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Neurospora has been a familiar pest to bakers for well over a hundred years as a red mold growing on bread. Around the turn of the century a Dutch botanist, Professor F. A. F. C. Went (1), found the natives of Java fermenting peanut meal with Neurospora to form a delicacy which they called "ontjom". Professor Went took the mold back to Holland and worked with it in his laboratory. In 1927 Shear and Dodge (2) published work they had done with the organism and proposed the names N. sitophilia, N. crassa, and N. tetraspora for the three species.

In 1941 Beadle and Tatum (3) reported the production of mutant strains of N. crassa by treatment of the mold with X-rays. Mutant strains were identified by their inability to grow on a minimal medium containing only inorganic salts, an inorganic nitrogen source, biotin, and a simple organic carbon source such as sucrose, and their ability to grow when more complex organic molecules, such as pyrimidines or amino acids, were added. Since 1941, mutations have been produced by treatment with X-rays, ultraviolet radiation, and neutron bombardment. Over 80,000 single spore strains have been isolated, of which some 500 are nutritional mutants.

About 43 of these mutants required the pyrimidine, uracil, for growth and were named the pyrimidineless mutants. Genetic studies have made it possible to arrange these mutants into groups pyr 1, pyr2, pyr3, etc. The pyr 3 group contains a number of allelic mutants designated pyr 3a, 3b, 3c, and 3d. N. crassa 1298 (often referred to as 1298) is one of these pyr 3 mutants. There is some evidence that 1298 is a representative of the pyr 3a type, but such a position in the genetic map has not been definitely established.

Normally a nutritional requirement is explained by assuming that some step along the biosynthetic pathway of this nutrient is blocked or deficient. The pathway of pyrimidine synthesis (Fig. I) which is generally accepted for Neurospora and which has been demonstrated to be present in numberous other organisms, was proposed by Lieberman and Kornberg (4).

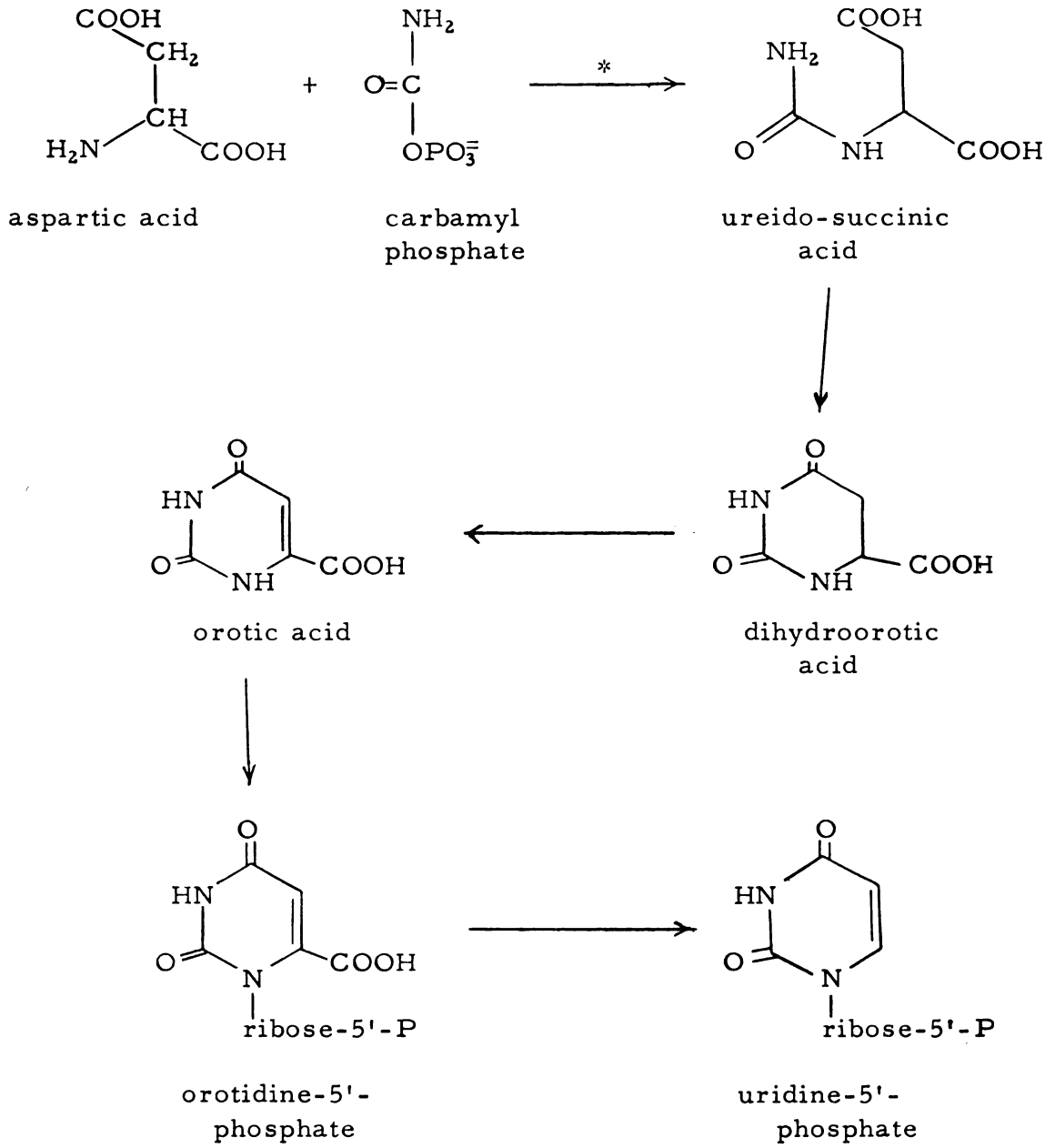
Early work with pyrimidineless mutants, therefore, centered around attempts to find intermediates of the Lieberman-Kornberg pathway which would support growth. No such intermediates were found. However, Loring and Pierce (5) did find that the pyrimidine nucleosides, such as uridine or cytidine, were much more effective in promoting growth than the free bases; Mitchell and Houlahan (6) found that the mutants will grow, although very poorly, on a few aliphatic acids such as oxalactic acid and aminofumaric acid.

Fairley (7) has found that the mutant 1298 will grow on several simple aliphatic compounds, including α-aminobutyrate and sodium propionate. Furthermore, he and Boyd (8) have found that carbon-14 labeled α-aminobutyrate and propionate are incorporated rather specifically into the pyrimidines of the mycelial ribonucleic acids, although with considerable dilution; that when labeled uracil is fed to the mutant the radioactivity is appreciably diluted; that when grown on uracil there is a considerable lag period before growth begins. On the basis of this and other evidence they have proposed an alternate pathway of pyrimidine synthesis (Fig. II).

One striking feature about the growth of 1298 on α-aminobutyrate is that this growth is arrested by extremely small quantities of arginine. Fairley and Adams (9) have found that as little as 0.04 μg of arginine per ml of nutrient will prevent growth.

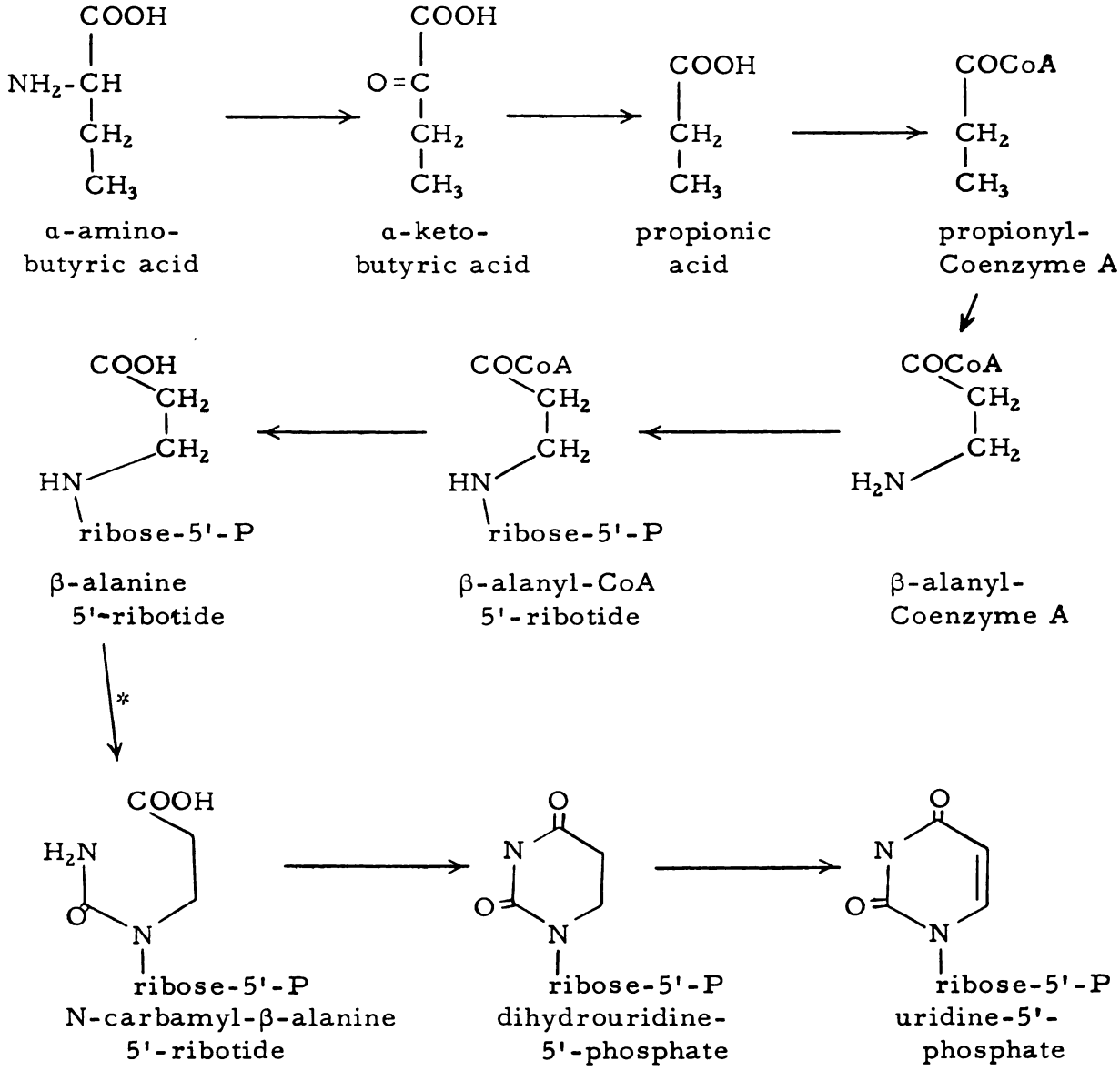
A similar case of arginine affecting the growth of a pyrimidineless mutant has been reported by Houlahan and Mitchell (10). They found that

Figure I. Lieberman-Kornberg Pathway for Pyrimidine Synthesis



*Indicates transcarbamylation step.

Figure II. Fairley-Boyd Pathway for Pyrimidine Snythesis in N. crassa 1298



*Indicates transcarbamylation step.

pyr 3a (37301) will take a second mutation, s (suppressor), and that the resulting double mutant, pyr 3a-s will grow without pyrimidines. Addition of 0.5 μmoles of arginine per ml of medium will restore the pyrimidine requirement.

Although no evidence has been presented that arginine directly affects enzymes of pyrimidine synthesis, Gorini and Maas (11) have shown that when Escherichia coli is cultured in the presence of arginine, the level of ornithine transcarbamylase is lower than normal.

There are several other reasons to suspect a relationship among arginine, transcarbamylase reactions, and pyrimidine biosynthesis in 1298. Davis (12) has demonstrated that pyr 3d (45502) lacks the enzyme aspartic transcarbamylase. If the pyr 3 mutants are true alleles, this evidence would indicate that the pyrimidine requirement is at least related to aspartic transcarbamylase in all of the pyr 3 mutants. This observation would then relate aspartic transcarbamylase to the arginine effect and therefore to ornithine transcarbamylase. Of course, aspartic transcarbamylase and ornithine transcarbamylase are already related through the common substrate, carbamyl phosphate. A further interrelationship can be found in the data of Mitchell and Mitchell (13) which imply that the synthesis of arginine and pyrimidines is somewhat competitive.

With the above information at hand it seemed advisable to study the quantitative relationship of aspartic transcarbamylase and ornithine transcarbamylase from Neurospora crassa 1298, and the possibility that some of the compounds which affect growth of the organism also affect these enzymes.

MATERIALS AND METHODS

Organisms

Mutant strains of Neurospora crassa used in this study were produced by Beadle and Tatum (3).

1298 was part of the collection here at Michigan State University. It had been kept in the laboratory here for a number of years and had acquired the ability to grow slightly in basal medium[1] after a long induction period. Toward the end of the project a new culture was received from R. L. Herrmann who had received it from the American Type Culture Collection in Washington. This new strain showed no growth after 20 days on basal medium and has been designated 1298H to avoid confusion with the original 1298.

45502, 63902, and 68902 were received from R. H. Davis from his collection at the University of Michigan.

Growth of Organisms

The organisms were maintained on 2% agar slants containing a supplement of 80 μg uridine per ml of agar. In order to keep growth of the organisms fresh, they were transplanted to new slants at least once a month.

The molds were grown in 125 ml Erlenmeyer flasks stoppered with cotton plugs. Each flask contained 25 ml of basal medium and was supplemented with 2 mg of uridine (80 μg/ml) or with 5 mg of α-amino-butyric acid (200 μg/ml). The three media (basal, uridine, and α-amino-butyric acid) were made up in large quantities, autoclaved, and siphoned

---

[1]See Appendix for preparation of all reagents.

out just before using. Flasks and contents were sterilized after filling, and allowed to cool before being incoculated.

To inoculate the medium, a spore suspension was prepared by gathering a mass of mycelia and spores on an inoculating needle and suspending the spores in 2-5 ml of sterile water. Each flask was inoculated with from 1-3 drops of the cloudy spore suspension, the number of drops depending on the number of flasks to be inoculated. The mold was then allowed to grow at room temperature in the darkness of a desk drawer.

Preparation of Acetone Powder

Mycelial pads were harvested by filtering the contents of the flasks through a Buchner funnel, washing quickly with about 20 ml of water and then immediately with several 20 ml portions of room-temperature acetone which had been dried over Na_2SO_4. The water-free mycelial pads were then homogenized in cold (-5°) dry acetone in a Vertis homogenizer. This suspension was again filtered through a Buchner funnel, the powder cake allowed to air dry and stored at room temperature. The acetone powder remained stable for at least a month.

Preparation of Enzyme

Throughout the following preparation, care was taken to keep materials at a temperature of 0° or colder. Most of the work was done in an ice-salt bath having a temperature of about -3°.

About 100 mg of acetone powder was extracted with 6 ml of 0.02 M tris-acetate buffer with the use of a small Potter tissue homogenizer. The suspension was centrifuged in a cold clinical centrifuge. The precipitate was resuspended in an additional 3 ml of buffer and reprecipitated in the cold centrifuge. The combined supernates were then centrifuged in a refrigerated ultracentrifuge at 13,000 r.p.m. (20,000xG) for 2-4 minutes.

This final supernate was used directly for the aspartic transcarbamylase assay, or diluted with 2 volumes of 0.02 M tris-acetate buffer for the ornithine transcarbamylase assay.

Enzyme Assay

Developing an acceptable method of assaying for the presence of a transcarbamylase enzyme was a major problem. The method of Koritz and Cohen (14), used by Davis for his studies of transcarbamylase activity, was avoided at first because arginine was known to interfere, and one of the studies planned was to test aspartic transcarbamylase activity in the presence of arginine. As it turned out, passing the reaction product through a short column of Dowex-50W removed the arginine. In the case of ornithine transcarbamylase, a blank containing arginine was also run and the amount of interfering color subtracted. Before this method was developed, an attempt was made to follow the reaction by assaying for the inorganic phosphate liberated, using the method of Lowry and Lopez (15). After several months of trying, without success, to adapt this method to the assay at hand it was abandoned and the Koritz-Cohen method was again employed. One of the reasons that the Lowry-Lopez method did not work may be that carbamyl phosphate is rather unstable, and, therefore, liberated a large background of inorganic phosphate.

Returning to the Koritz-Cohen method did not immediately solve all of the problems. Several "tricks" in the method had to be mastered before the results were reproducible. Right up to this writing minor changes have been made in the procedure. The method to be described on pages 9-10 is not the method by which all of the assays were run, but it is the method which seems now to work best. The more recent changes were, however, mostly fine points and should not make a significant difference in the results. For instance, in the first part of the work

aspartic acid was neutralized against a pH meter in 25 ml quantities and kept for up to 10 days. Toward the end of the work, the acid was neutralized in 5-10 ml quantities just before using and the pH adjusted against pH paper. The most serious change was the change in the 1298 mutant, which has already been mentioned. However, data received from the two mutants did not differ significantly.

For the aspartic transcarbamylase assay, the following reagents were mixed in an 8 inch test tube, in the order given, and allowed to react for 15 minutes at room temperature:

0.25 ml of 1.0 M glycine-NaOH buffer pH 9.1
1.0 ml of potassium aspartate solution pH 9
1.0 ml of enzyme preparation
1.0 ml of carbamyl phosphate solution

At the end of 15 minutes the reaction was stopped by the addition of 0.5 ml of 2 N perchloric acid. The denatured protein was allowed to coagulate for a few minutes and then precipitated in a clinical centrifuge. A 3 ml portion of the supernate was decanted and allowed to pass through a column containing 1 ml of Dowex-50W-hydrogen form. The column was washed with a 2 ml and then a 1 ml portion of water, giving a final volume of 6 ml.

To 2 ml of this final solution was added 4 ml of a 50% solution of sulfuric acid, 0.2 ml of diphenylamine-p-sulfonate, and 0.2 ml of diacetylmonoxime. The tube was capped, heated in a boiling water bath for 10 minutes and then cooled rapidly to room temperature. To the cooled solution was added 0.2 ml of potassium persulfate and the solution returned for exactly one minute to the boiling water. The solution was then cooled once again and the color allowed to develop for 10 minutes away from direct sunlight.

After 10 minutes the optical density was read on a Klett-Summerson Photoelectric Colorimeter, using a green (540 mμ) filter. The readings, kept in the range of 100-300 units, were converted into concentration of product by reference to a standard curve. The standard curve was prepared by adding known amounts of urido-succinate to water blanks and running them through the assay.

At the same time that the assay for product was run, a 1 or 2 ml sample of enzyme was diluted with water to 5 ml, and 5 ml of Biuret reagent was added. After 20 to 40 minutes, the colorimeter was zeroed on a solution containing 5 ml of Biuret reagent and 5 ml of water. Using the same green filter, the optical density of the protein-Biuret solution was read. This reading was converted to mg of protein by reference to a standard curve prepared with known amounts of egg albumin.

For ornithine transcarbamylase, the following reagents were mixed in the order given:

0.25 ml of 1.0 M tris-acetate buffer pH 9.1
1.0 ml of ornithine solution pH 9
0.2 ml of enzyme preparation
0.8 ml of inhibitor or water
1.0 ml of carbamyl phosphate solution

After 15 minutes the reaction was stopped by the addition of 0.5 ml of 2 N perchloric acid. Since so little enzyme was used, the mixture was not centrifuged. Since the Dowex 50W column removes citrulline, the chromotagraphy step was omitted. Instead, 3 ml of the reaction mixture was taken and added directly to 3 ml of water. A 2 ml portion of this diluted reaction mixture was then assayed as for aspartic transcarbamylase. In this case, however, the reference standard was citrulline rather than ureido-succinate.

When β-methyl aspartic acid and β-alanine were used as possible substrates for the reaction, the assay for aspartic transcarbamylase was used.

Figure III shows a typical assay for transcarbamylase activity. Controls which were a standard part of the assay were:

Tube 1 Water blank used to set the base reading of the instrument.

Tube 2 "Time O" blank, giving the amount of color produced in the absence of a reaction. This tube was particularly important in the arginine studies.

Tube 10 Reference standard containing a known amount of the product.

Figure III. Typical Assay for Transcarbamylase Activity

α-Aminobutyric acid inhibition of Ornithine Transcarbamylase activity from 1298H grown on α-aminobutyric acid for 241 hours

	Tube Number									
	1	2	3	4	5	6	7	8	9	10
Reagents										
ml H_2O	3	0	0	.2	0	.2	.4	.6	.8	2.8
0.25 ml buffer	x	x	x	x	x	x	x	x	x	x
1 ml 0.04 M ornithine	0	x	x	x	x	x	x	x	x	0
0.2 ml 1 mM citrulline	0	0	0	0	0	0	0	0	0	x
ml α-aminobutyric acid										
0.48 M	0	.8	.8	.6	0	0	0	0	0	0
0.24 M	0	0	0	0	.8	.6	.4	.2	0	0
0.2 ml enzyme[1]	0	x	x	x	x	x	x	x	x	0
0.5 ml $HClO_4$	0	x	0	0	0	0	0	0	0	0
1 ml carbamyl phosphate	0	x	x	x	x	x	x	x	x	0
Reaction time 15 min.										
0.5 ml $HClO_4$	x	0	x	x	x	x	x	x	x	x
Koritz-Cohen Assay	x	x	x	x	x	x	x	x	x	x
Klett Readings[2]										
8-12 minutes	0	0	87	102	122	143	180	218	277	87
12-15 minutes	0	1		101		139		218		92
10 min. (calculated)	0	1	87	102	122	143	181	218	279	94
Corrected for 0 time			86	101	121	142	180	217	298	94
Calculated Results										
μmoles citrulline formed			.19	.22	.26	.30	.38	.46	.59	.20
mmoles of inhibitor			.38	.29	.19	.14	.10	.05	.00	0
Per cent inhibition[3]			68	63	56	49	36	22	0	

[1]A Biuret reaction with 2 ml of the enzyme preparation gave a reading of 43 Klett units. This corresponds to 3.8 mg of protein, or 0.38 mg per tube.

[2]The minutes refer to the time interval during which the readings were taken. These minutes are measured from the time that the last heating step in the assay was completed.

[3]Since Klett readings increase in a linear fashion with increasing concentration of citrulline, the percent inhibition figures were calculated directly from the Klett readings.

RESULTS

Both ornithine transcarbamylase and aspartic transcarbamylase activities were found in Neurospora crassa 1298. When the organism was grown on α-aminobutyric acid, the level of aspartic transcarbamylase activity remained consistently high, but when grown on uridine the level of activity started low and rose as the mold grew. The level of ornithine transcarbamylase activity seemed to be independent of the age of the mold. These data are given in Table I and are represented graphically in Figure IV.

A preliminary study was made to determine whether or not arginine affected the activity of aspartic transcarbamylase. No effect was found. However, the concentration of arginine used in the study (2.1 μmoles per tube) was much lower than that used to produce inhibition of ornithine transcarbamylase (190 μmoles per tube).

It was found that arginine slightly suppressed the formation of ureido groups by ornithine transcarbamylase. It was also found that a number of aliphatic acids, some of which were known to promote growth of the mutant, suppressed the apparent activity of ornithine transcarbamylase. One such compound raised the apparent activity. These data are recorded in Table II and in Figure V. Figure V emphasizes interesting relationships among the seven compounds tested. They seem to fall into three rather distinct groups; two inhibit rather strongly, four rather weakly, and one increases the apparent activity.

When β-methylaspartic acid and β-alanine were supplied as possible substrates for transcarbamylation, no reactions were detected.

Mutants 45502, 63902, and 68902 did not grow on minimal medium supplemented with α-aminobutyric acid.

Table I. Aspartic Transcarbamylase and Ornithine Transcarbamylase Activities from N. crassa 1298

Growth Medium	Mutant	Age in Hours	Weight in mgs.	% Maximum Growth	ATC Activity*
α-Aminobutyric acid	1298	66	4	11	5.5
"	1298	133	25	71	4.0
"	1298	133	25	71	3.9
"	1298	207	34	97	5.0
"	1298H	113	11	22	5.8
"	1298H	113	11	22	6.4
Uridine	1298	43	10	21	1.0
"	1298	43	10	21	2.6
"	1298	66	37	75	3.3
"	1298	66	37	75	4.0
"	1298	133	46	92	2.9
"	1298	133	46	92	3.0
"	1298	207	44	89	3.8
Uridine	1298H	27	2.8	3.7	0.27
"	1298H	40	20	27	1.0
					OTC Activity*
Uridine	1298H	40	20	27	5.8
α-Aminobutyric acid	1298H	241		100	6.0

ATO = Aspartic Transcarbamylase
OTC = Ornithine Transcarbamylase

*Activity is expressed in μmoles of product formed per mg of protein per hour of reaction time.

Table II. Effect of Several Aliphatic Acids on Ornithine Transcarbamylase from N. crassa 1298

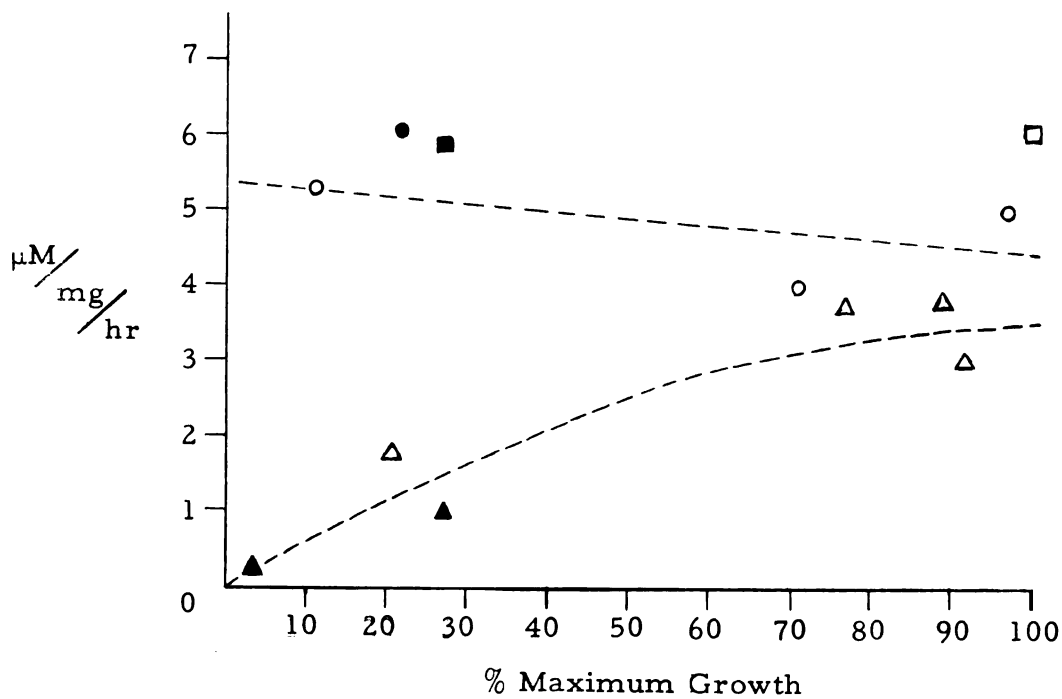
Inhibitor	mM of Inhibitor	OTC Activity*	OTC Activity* with Inhibitor	% of Control
α-Aminobutyric acid	.12	6.0	3.6	60
Arginine	.19		5.3	88
α-Aminobutyric acid	.05	6.2	4.4	78
"	.10		4.0	64
"	.14		3.2	51
"	.19		2.7	44
"	.23		2.3	37
"	.38		2.0	32
Homoserine	.32	6.7	4.75	71
"	.12		6.2	93
Threonine	.32		5.7	85
"	.12		6.1	91
2,4-Diaminobutyrate	.32		2.95	44
"	.12		4.6	69
Propionate	.04	5.3	5.5	104
"	.12		5.7	109
"	.24		6.0	115
"	.32		6.1	116
Alanine	.24		4.4	83
"	.08		5.0	94

All data taken from 1298H grown on α-aminobutyric acid for 241 hours, 100% maximum growth. Ornithine concentration is 0.04 mmoles per tube.

OTC = Ornithine Transcarbamylase

*Activity is expressed in μmoles of product formed per mg of protein per hour of reaction time.

Figure IV. Aspartic Transcarbamylase and Ornithine Transcarbamylase Activities as a Function of Per Cent Maximum Growth



Aspartic Transcarbamylase

o 1298 ● 1298H -- grown on α-aminobutyrate

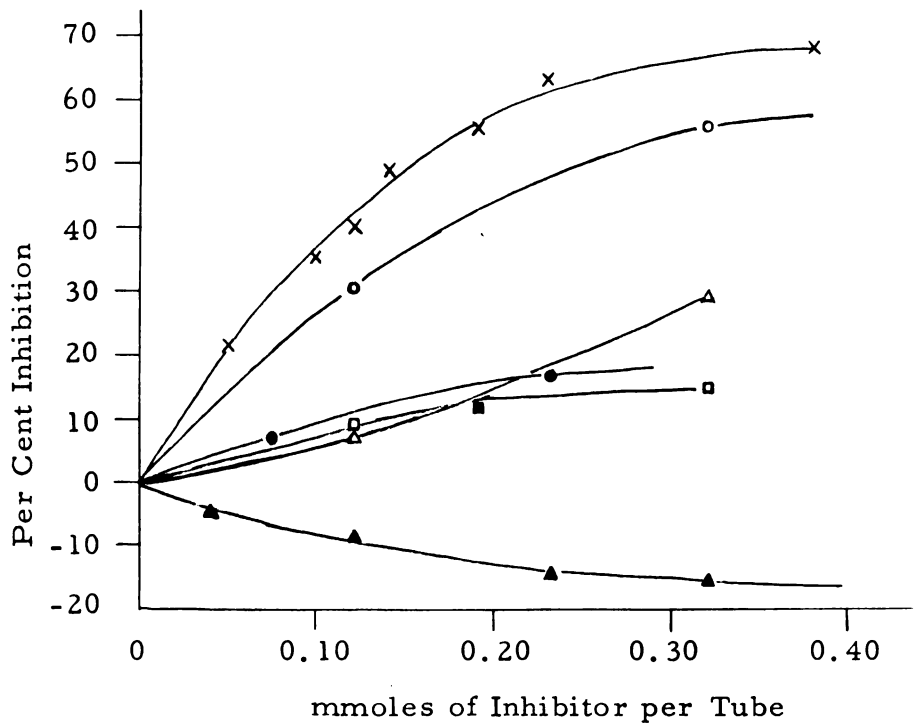
△ 1298 ▲ 1298H -- grown on uridine

Ornithine Transcarbamylase

□ 1298H grown on α-aminobutyrate

■ 1298H grown on uridine

Figure V. Apparent Inhibition of Ornithine Transcarbamylase



x = α-aminobutyrate

o = 2,4-diaminobutyrate

Δ = homoserine

□ = threonine

● = alanine

■ = propionate

▲ = arginine

Ornithine concentration is 0.04 mmoles per tube.

DISCUSSION

This study has demonstrated the presence of aspartic transcarbamylase activity in Neurospora crassa 1298, when grown on both α-aminobutyric acid and on uridine.

It has also been shown that pyr 3d (45502) and two other pyr 3 mutants (63902 and 68902) will not grow on basal medium supplemented with α-aminobutyric acid. This information, coupled with the finding of Davis (12) that the pyr 3d mutant has no aspartic transcarbamylase, suggests that 1298 is not a pyr 3d mutant. However, since 1298 is still in the pyr 3 group, the basis for the pyrimidine requirement must still be connected somehow with the aspartic transcarbamylase enzyme.

While some of the known transcarbamylase reactions were being run, it was decided to try a few compounds as possible substrates for unknown transcarbamylation reactions. One of the substrates for transcarbamylation in the Fairly-Boyd pathway is β-alanine. This compound did not produce detectable amounts of the ureido function under the conditions of the assay employed. Another compound tested was β-methylaspartate. This was tried mainly to test the specificity of aspartic transcarbamylase, but also because of the possibility that such a reaction might play a role in the biosynthesis of thymine. Like β-alanine, β-methylaspartate gave no reaction.

Although neither compound gave positive results, it cannot be concluded that their transcarbamylases are not present in the mycelial extract. Possibly something in the assay system inhibits the reaction, as glycine in the glycine-NaOH buffer inhibits ornithine transcarbamylase. Or perhaps the reaction is favored only at a pH more nearly that found in living mycelia.

Yates and Pardee (16, 17) have found that pyrimidine synthesis in E. coli is normally controlled by controlling the aspartic transcarbamylast reaction. This reaction is controlled by pyrimidines. Thus pyrimidines control their own synthesis. The results obtained when 1298 was grown on uridine suggest a repression mechanism very similar to that found by Yates and Pardee in E. coli. During early stages of growth, when there is abundant uridine present, the level of aspartic transcarbamylase is very low. As the mold grows, presumably using up the fed uridine, the level of transcarbamylase rises.

These data, suggesting repression, are further evidence that the mutation in 1298 is not simply an insufficient amount of aspartic transcarbamylase. If this were the case, the mold should grow best when the aspartic transcarbamylase level is the highest, that is, on the least amount of uridine. In fact, however, increasing uridine concentration increases growth rate.

The ability of the mold to control the level of aspartic transcarbamylase appears to be normal. Of course, this does not mean that in vivo activity of the enzyme is normal. It might easily be the case that within the cell the enzyme is inhibited. There is reason to believe that arginine may be such an inhibitor.

The fact that when the organism is grown on β-aminobutyric acid the level of aspartic transcarbamylase is higher than is found (12) in the wild strain (about 4.5 μM/mg/hr as compared with 1.44 μM/mg/hr) might suggest that this acid stimulates production of the enzyme. However, it is more likely that this high level of enzyme is due to the deficiency of uridine (or other pyrimidine), since levels of aspartic transcarbamylase as high as 13 μM/mg/hr have been obtained (18) when the organism is grown on limiting concentration of uridine.

The discovery that arginine exerts a powerful inhibition on the growth of 1298 seems to be an important step in the attempt to explain

the pyrimidine requirement of this mutant. Evidence which suggests a relationship between arginine and pyrimidine synthesis was presented in the introduction. If arginine does in some way prevent the synthesis of pyrimidines, the logical way to produce growth would be to remove arginine. Since ornithine transcarbamylase is essential for the production of arginine, a comparison of the ability of a compound to inhibit ornithine transcarbamylase and its ability to promote growth might be useful. Such a comparison is given in Table III:

Table III. Relation of Growth Promoting and Inhibiting Power of Several Aliphatic Acids

Acid	Growth in mg[1]	% Inhibition[2]
α-Aminobutyric acid	43	57
Propionic acid	22	-11[3]
Homoserine	18	18
Threonine	11	13
2,4-Diaminobutyric acid	5	44
Alanine	0	14

[1]Data from Fairley, et al. (19).

[2]Data from Table II, this paper.

[3]A minus number indicates that enzyme activity was increased rather than inhibited.

Growth is expressed in dry weight of mycelia produced in 4 days when 0.05 mmoles of the acid is added to 25 ml of basal medium (19). Inhibition is the per cent decrease in activity caused by the addition of 0.2 mmoles of the acid (5 times the ornithine concentration) to the ornitine transcarbamylase assay.

The data presented in Table III does not seem to indicate a positive correlation between the ability of a compound to promote growth of 1298 and its ability to inhibit ornithine transcarbamylase. However, it must be remembered that the enzyme preparation used was only a crude extract, and many reactions may be taking place other than the one being studied. The data may reflect the ability of the "inhibitors" to undergo transcarbamylation reactions of their own. For instance, threonine might be inhibiting ornithine transcarbamylase strongly, and, at the same time be undergoing a transcarbamylation reaction of its own. The net formation of the ureido function would then be the sum of these two opposing factors. It is also possible that a compound added as an inhibitor might be rapidly converted into something else which could undergo a transcarbamylation reaction. For instance, propionate, which cannot undergo a "propionic transcarbamylation" reaction, might be converted into something else which could act as a carbamyl acceptor.

If, however, the data are taken at face value it appears that the α-amino acids generally act as inhibitors and that similarity of structure to ornithine is an important requirement. On the other hand, if structural similarity is the key, it is difficult to understand why α-aminobutyrate is a better inhibitor than 2,4-diaminobutyrate.

The data presented in this study, together with previous work on the ability of arginine to suppress growth of the mutant studied, suggest the possibility that the pyrimidine requirement in 1298 is closely connected with the presence of arginine. They suggest further that the removal of this requirement by α-aminobutyric acid and other aliphatic acids may be effected by limiting the amount of arginine present, through controlling the activity of ornithine transcarbamylase.

SUMMARY

The levels of aspartic transcarbamylase and ornithine transcarbamylase activities in Neurospora crassa 1298 were studied. The presence of these enzymes was detected by allowing aqueous extracts of mycelia to catalyze the production of ureido-compounds. The product of this reaction was detected colorimetrically by the method of Koritz and Cohen.

It was found that the level of aspartic transcarbamylase activity varied with the medium in which the organism was grown and in some cases with the stage of growth.

Ornithine transcarbamylase was also found to be present in 1298. A number of aliphatic acids were found with the ability to inhibit this enzyme. One acid was found which apparently increased its activity.

Attempts to identify other transcarbamylase enzymes in the organism were unsuccessful. The mutants 45502, 63902, and 68902 were found to be unable to grow on basal medium supplemented with α-aminobutyric acid.

The implications of these findings as they relate to pyrimidine synthesis in 1298 are discussed.

REFERENCES

1. Went, F. A. F. C., Centralb. f. Bacteriol., Abteilung 2, 7, 544 (1901).

2. Shear, C. L., and B. O. Dodge, J. Agric. Res., 34, 1019 (1927).

3. Beadle, G. W., and E. L. Tatum, Proc. Natl. Acad. Sci., 27 499 (1941).

4. Lieberman, I., and A. Kornberg, J. Biol. Chem., 212, 909 (1955).

5. Loring, H. S., and J. G. Pierce, J. Biol. Chem., 153, 61 (1944).

6. Mitchell, H. K., and Mary B. Houlahan, Federation Proc., 6, 506 (1947).

7. Fairley, J. L., J. Biol. Chem., 210, 347 (1954).

8. Boyd, J. B., and J. L. Fairley, J. Biol. Chem., 234, 3232 (1959).

9. Fairley, J. L., and A. B. Adams, Science, in press.

10. Houlahan, Mary B., and H. K. Mitchell, Proc. Natl. Acad. Sci., 33, 223 (1947).

11. Gorini, L., and W. K. Maas, Biochim. et Biophys. Acta, 25, 208 (1957).

12. Davis, R. H., Federation Proc., 46, 677 (1960).

13. Mitchell, M. B., and H. K. Mitchell, Proc. Natl. Acad. Sci., 38, 205 (1952).

14. Koritz, S. B., and P. P. Cohen, J. Biol. Chem., 209, 145 (1954).

15. Lowry, O. H., and J. A. Lopez, J. Biol. Chem., 162, 421 (1946).

16. Yates, R. A., and A. B. Pardee, J. Biol. Chem., 221, 757 (1956).

17. Yates, R. A., and A. B. Pardee, J. Biol. Chem., 227, 677 (1957).

18. Davis, R. H., Personal Communication.

19. Fairley, J. L., R. L. Herrmann, and J. M. Boyd, J. Biol. Chem., 234, 3229 (1959).

APPENDIX

PREPARATION OF SOLUTIONS

REAGENTS

General

0.02 M Tris-acetate buffer - pH 8.0

Dissolve 1.21 g of Tris (Tris (hydroxy-methyl) aminomethane) (Sigma Chemical Corp.) in 400 ml water and adjust to pH 8.0 with acetic acid. Dilute to 500 ml.

1.0 M Tris-acetate buffer - pH 9.1

Dissolve 12.114 g of Tris in 80 ml water and adjust to pH 9.1 with acetic acid. Dilute to 100 ml.

1.0 M Glycine-NaOH buffer - pH 9.1

Dissolve 7.505 g of Glycine and 5.845 g NaCl in 70 ml water and add strong (15 N) NaOH to pH 9.1. Dilute to 100 ml.

2 N Perchloric acid

Dilute from concentrated (11.8 N) perchloric acid.

Biuret Reagent

Dissolve 0.75 g copper sulfate and 3.00 g sodium potassium tartrate in 150 ml 10% sodium hydroxide and make up to 500 ml with CO_2-free water.

Dowex 50W-X12

Dowex 50W-X12 100-200 mesh (Dow Chemical Co.) is washed with water, NaOH, water till neutral, HCl, and finally with water again until neutral.

Reactants

The following reagents should be made up just before using:

0.04 M Potassium-L-aspartate

Suspend 26.6 mg of L-aspartic acid (Mann Res. Labs.) in 4 ml of water and neutralize with weak KOH to a pH of 9, as indicated by pH paper. Dilute to 5 ml.

0.04 M Ornithine

Adjust 33.7 mg DL-ornithine hydrochloride (Nutritional Biochemicals Corp., hereafter abbreviated Nut. Bio. Corp.) to pH 9 as above and make up to 5 ml.

0.04 M Potassium-DL-β-methyl aspartate

Adjust 29.4 mg of DL-β-methylaspartic acid (Nut. Bio. Corp.) to pH 9 as above and make up to 5 ml.

0.04 M β-Alanine

Adjust 17.6 mg of β-alanine (Pfanstiehl Chem. Co) to pH 9 as above and make up to 5 ml.

0.01 M Carbamyl Phosphate

Dissolve 15.3 mg of carbamyl phosphate (California Corp. for Biochemical Research) in 10 ml of water.

"Inhibitors"

All of the following solutions were made 0.4 molar and adjusted to approximately pH 9 with weak KOH.

D, L-Alanine - (Nut. Bio. Corp.) 178 mg/5 ml

D, L-α-Amino-n-butyrate - 206 mg/5 ml

L-Arginine - (Mann Res. Labs.) 348 mg/5 ml

2, 4-Diaminobutyrate - (Nut. Bio. Corp.) 168 mg/5 ml

D, L-Homoserine - (Nut. Bio. Corp.) 238 mg/5 ml

Sodium Propionate - (Prepared by A. B. Adams in this laboratory from propionic acid from Eastman Kodak Co.) 192 mg/5 ml.

D, L-Threonine - (Bios. Labs. Inc.) 238 mg/5 ml

Assay

0.4%Diphenylamine-p-sulfonate - (Eastman Kodak Co.) 20 mg/5 ml

3% Diacetylmonoxime - 150 mg/5 ml

1% Potassium Persulfate - 50 mg/5 ml

COMPOSITION OF THE BASAL MEDIUM

	Grams per Liter	
Ammonium tartrate	5.0	
Ammonium nitrate	1.0	
Potassium dihydrogen phosphate	1.0	
Magnesium sulfate	0.5	
Calcium chloride	0.1	
Sodium chloride	0.1	
Sucrose	10.0	
Biotin (μg/liter)	5.0	(Nut. Biol. Corp.)

Trace Elements	μg per liter
Sodium tetraborate	88
Magnesium II chloride	45
Ammonium molybdate	64
Copper II chloride	270
Iron III chloride	500
Zinc sulfate $\cdot 7H_2O$	2000

Supplements	μg per ml	
Uridine	80	(Schwartz Labs & Nut. Bio.)
α-Aminobutyric acid	200	