STUDIES OF PYRIMIDINE BIOSYNTHESIS BY *NEUROSPORA CRASSA* 1298

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

Olga Vital Miller

1959

STUDIES OF PYRIMIDINE BIOSYNTHESIS BY *NEUROSPORA CRASSA* 1298

By

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A THESIS

Submitted to the College of Science and Arts of Michigan State University of Agriculture and Applied Science in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Department of Chemistry

1959

Olga Vital Miller

ABSTRACT

To study the pathway postulated (6) for pyrimidine biosynthesis by the mutant Neurospora crassa 1298, dihydrouridine and ureidopropionic riboside were prepared and their effect upon the growth of the mold observed. Neither of these two compounds supported the growth of the mold when they were used as sole supplements to the basal nutrient medium.

Ureidopropionic acid riboside had no effect upon the growth of the mold on uridine or on aminobutyric acid, but dihydrouridine caused greater than additive effect upon the growth of the mold on these compounds.

These experiments do not provide evidence in support of the pathway; however, the possibility exists, that the mold was not able to phosphorylate dihydrouridine and ureidopropionic riboside, therefore the results cannot be taken as conclusive evidence against the pathway.

Several compounds which were found to be non-supporters of growth of the mold in the normal basal medium (pH of 5.6) were reinvestigated again in basal media having the pH of 4 and 3. Of these compounds succinic acid and glycine supported growth at the pH of 4 and alpha-ketobutyric acid supported growth at the pH of 3. Lowering of the pH had no effect upon the growth supporting ability of acetic acid, pyruvic acid, beta-alanine, DL-aspartic acid, gamma-aminobutyric acid, DL-alpha-amino isobutyric acid and ornithine.

The finding that alpha-ketobutyric acid supports the growth of the mold leads to the suggestion that alpha-aminobutyric acid might be able

to convert to propionic acid through the intermediate alpha-ketobutyric acid, without necessarily passing through homoserine and beta-hydroxy propionic acids as intermediates.

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ACKNOWLEDGMENT

The author wishes to express her appreciation to Dr. James L. Fairley for his interest, encouragement and guidance in completing this project.

She also wishes to express her gratitude to the Atomic Energy Commission for a grant supporting this work.

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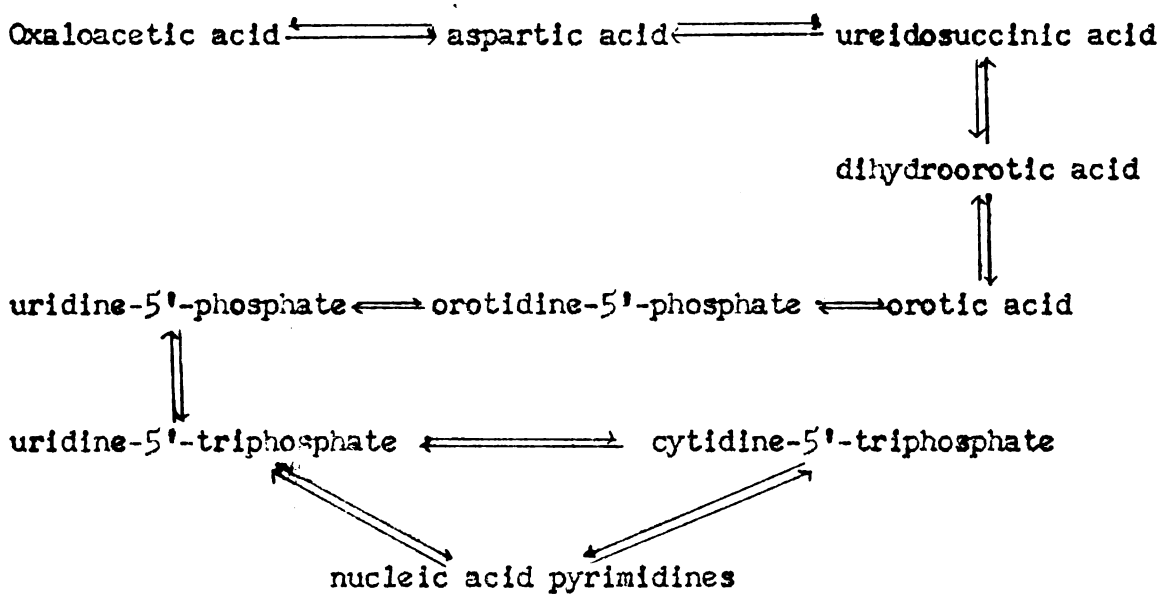
INTRODUCTION

The biosynthesis of the nucleic acid pyrimidines has been studied by many investigators for a number of years, and while much experimental evidence has been collected, there remain many unanswered questions. One proposed route of pyrimidine biosynthesis, the Liebermann-Kornberg (1) scheme, is accepted by many as a pathway both for pyrimidine formation and degradation.

The sequence of compounds in this scheme is the following:



This is just one possible pathway; under different conditions, and in other organisms other pathways might assume importance. Fink and her coworkers (2) demonstrated an enzyme system in rat liver which degrades thymine to beta-aminoisobutyric acid. The conversion of beta-ureidoisobutyric acid to beta-aminoisobutyric acid appeared to be irreversible,

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but dihydrothymine and beta-ureidoisobutyric acid were interconvertible.

Canellakis (3) carried out similar experiments with uracil, dihydrouracil and ureido-propionic acid. The results were similar to those of the previous investigators, except that the overall conversion of uracil to bets-alanine was not demonstrated. The rate determining step, the slow step both with uracil and thymine, was the first step-the conversion of the pyrimidine to dihydrouracil or to dihydrothymine, while the rate of degradation of dihydrouracil and ureido-propionic acid was found to be much higher. It seems possible that these reactions can be reversed under appropriate conditions to lead to the synthesis of pyrimidine compounds.

Fairley (4) demonstrated the growth of pyrimidineless N. crassa mutants on threonine and alpha-aminobutyrate, and showed the more than additive effect of the presence of alpha-aminobutyrate on the growth of the mold with uracil and uridine. Threonine had the same stimulatory effect, while aspartic acid had no effect. There was no stimulatory effect by any of these compounds on the wild-type strain of N. crassa. This phenomenon appears to be associated with yet another mechanism for pyrimidine formation, a mechanism distinctly different from the Liebermann-Kornberg proposal.

Herrmann and Fairley (5) used aminobutyrate-3-C (14) in the basal growth medium and found that the labeling of the pyrimidines produced by the mold was much greater than any of the other constituents. The results were not completely conclusive as far as the utilization of the number 3 carbon, as the compounds isolated were only 1/10 and 1/15 as active as the administered aminobutyric acid. However, the aminobutyric acid reisolated from the mycelium also showed great dilution

of the isotopic carbon, indicating that while the mold did require aminobutyric acid for growth, during the growth it produced more aminobutyric acid or a closely related derivative.

Boyd tested a great variety of compounds in search for ones which are utilized for pyrimidine biosynthesis. She divided these compounds into four groups: the uracil group which contains compounds which were known to be intermediates or could be intermediates in the uracil and thymine degradation; the aspartic acid group, which are intermediates or could be intermediates in the Liebermann-Kornberg scheme; the alpha-aminobutyric acid group; and the propionic acid group.

In the uracil group, uracil, dihydrouracil and ureidopropionic acid supported growth. There was a time lag in the growth curve of dihydrouracil and an even greater lag for ureidopropionate, leading to the conclusion that these substances are not direct intermediates in the synthesis of nucleic acid pyrimidines, but that they can be converted to such intermediates.

The compounds in the aspartic acid group did not support the growth of the mold, which again confirmed previous experimental results that the mold uses a different route from the one outlined in the Liebermann-Kornberg scheme. Only alpha-aminobutyric acid, homoserine and threonine were used by the mold from the aminobutyric acid family. Alpha-ketobutyric acid and alpha-hydroxybutyric acid did not support growth. Propionic acid, beta-hydroxypropionic acid and methylmalonic acid did support growth while other acids in the propionic acid family, succinic acid, pyruvic acid, and acetic acid proved to be inactive.

Time course studies by Boyd (6) showed that the biosynthesis from propionic acid and alpha-aminobutyric acid follow different paths from

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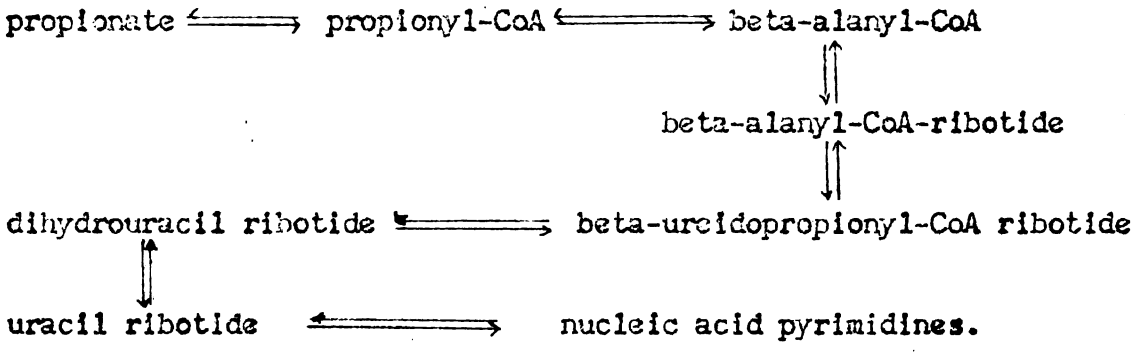
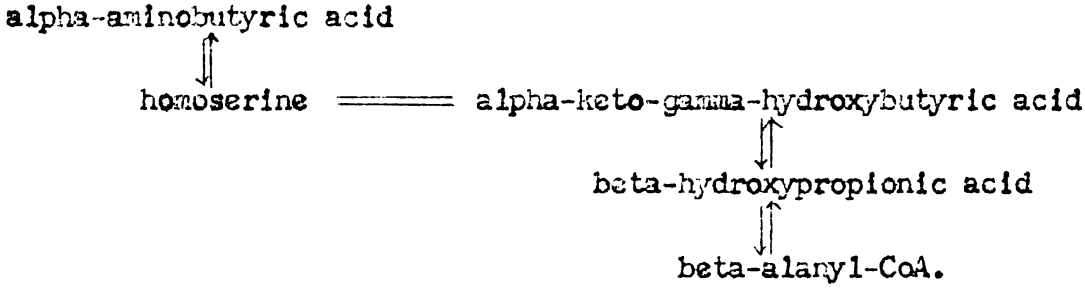
that used by the uracil group. The similarity of the growth pattern with aminobutyric acid and propionic acid and also their similar inhibition by arginine as shown by Fairley (4), led to the suggestion by Boyd (6) that they are utilized by the same route, with a common intermediate. Aminobutyric acid, homoserine and threonine all support growth in similar manner, as was shown by Fairley (4). Homoserine and threonine are possibly interconvertible through a vinylglycine intermediate (7) which would also give alpha-aminobutyric acid upon hydrogenation (8).

The pathway proposed by Boyd (6) for pyrimidine synthesis in the mutant, Neurospora crassa 1298 is:



Boyd's scheme also proposed the following introduction of alpha-aminobutyric acid into this scheme:



The reason for this suggested mechanism, rather than the simpler production of propionyl-CoA from aminobutyrate by deamination and decarboxylation, was the experimental evidence that alpha-ketobutyric acid did not support the growth of the mold, while the fact that hydroxypropionic acid supported growth of the mold made the hypothesis a more likely one.

Mokrasch and Grisolia (9) have reported that in rat liver preparations, uridylic acid, dihydrouracil ribotide and ureidopropionic acid ribotide are utilized for nucleic acid pyrimidine formation, but uridine and dihydrouridine are not used as readily. The phosphorylating mechanism therefore appears to be absent in the rat liver homogenate.

The experiments described in this thesis were initiated to provide further evidence concerning the route of pyrimidine formation in the mutant organism, Neurospora crassa 1298, with emphasis on two major aspects. It seemed possible to test the hypothesis of Boyd for the biosynthetic route by preparing several of the suggested intermediate compounds and testing them for growth-supporting abilities. Compounds with readily ionizable groups, such as the phosphate groups of the ribotide intermediates, will not pass through the mycelial membrane. These compounds could not be tested directly. However, in comparison with uridine, it might be expected that the mold could absorb the ribosides and phosphorylate them inside the mycelium. Accordingly it was decided to prepare two of the ribosidic compounds, dihydrouridine and ureidopropionic acid riboside, corresponding to two of the ribotides of Boyd's scheme and test them for activity with the mold.

Boyd's conclusions concerning some of the early reactions of her proposed mechanism were based solely on the results of growth tests at pH 5.6. It seemed possible that permeability considerations, particularly with respect to some of the more acidic compounds tested, could have led to erroneous conclusions in that some of the compounds may not have been able to pass through the mycelial membrane barrier in the ionic form. This possibility has been tested in the present work.

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EXPERIMENTS AND RESULTS

Effects of dihydrouridine and ureidopropionic acid riboside on the growth of N. crassa 1298.

Preparation of Dihydrouridine

Dihydrouridine was synthesized from uridine by a slight modification of the procedure of Green and Cohen (12). An all glass semimicro hydrogenation apparatus was used. The burette was filled with hydrogen from a reservoir after repeated flushing of the apparatus with hydrogen. The pressure of the system was atmospheric. The boat containing 90 mg. of a rhodium on alumina catalyst with 250 mg. of uridine in 40 ml. of water was shaken by an electric shaking device during the hydrogenation. The catalyst was first saturated with hydrogen, the uridine was then added and the hydrogenation was continued until the hydrogen uptake ceased, a period of about 50 minutes. The hydrogen uptake corresponded to the theoretical 100 percent hydrogenation.

Levene (13) reported the production of dihydrouridine as an oil, but Cohen (12) obtained it as an amorphous compound after repeated evaporation of the hydrogenated compound from absolute alcohol and precipitation of the solid from the alcohol solution by absolute ether.

Most of the growth studies of the present report were made by using dihydrouridine as an oil, assuming approximately 100 percent yield from uridine. Dihydrouridine, however, was finally obtained as a white crystalline solid after several attempts. Some of the previous growth experiments were then repeated and gave results identical to those obtained by the use of the oil.

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Demonstration of Purity of Dihydrouridine

That the oil obtained in most experiments was pure dihydrouridine was demonstrated by showing that no uridine remained, that no free sugar was present, that no free ureido group was present, that a group was present which was converted to the ureido group upon treatment with alkali and that no free dihydrouracil was present. The same methods were used to show the purity of the crystalline dihydrouridine.

To show the absence of uridine from the hydrogenated product a Beckmann Spectrophotometer was used. At a wave length of 260 mμ uridine absorbs radiation. The molar extinction coefficient is 10,000. Fifteen mg. of dihydrouridine dissolved in 3 ml. of water gave an absorbtion of 0.402. Therefore the maximum amount of uridine was less than 0.01 mg/ml., or less than 0.2 percent of the starting amount. Both the oil and the crystalline preparation of the dihydrouridine gave identical results concerning the presence of the maximum amount of uridine.

To show the absence of free ribose in the preparation aniline hydrogen phthalate color reagent (14) was used. The reagent was prepared by dissolving 0.93 g. aniline and 1.66 g. phthalic acid in 100 ml. of n-butyl alcohol saturated with water. Fifty micrograms of the dihydrouridine preparation was placed on Whatman No. 1 paper with micropipette, allowed to dry, and then sprayed with the aniline hydrogen phthalate reagent. The paper was then placed in a ventilated oven at 105°C. for five minutes. No color developed. The same procedure was repeated with different portions of ribose solutions and

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even one microgram ribose produced pink color with aniline hydrogen phthalate. Therefore, the ribose was still in the glycosidic form.

The absence of a ureido group, but the presence of a group which can be easily converted to a ureido group was shown by the use of the characteristic color reaction of the ureido group with p-dimethyl - aminobenzaldehyde (15). The color reagent was prepared by dissolving 1 g. of p-dimethylaminobenzaldehyde in a solution of 100 ml. of ethanol and 10 ml. of concentrated hydrochloric acid. Portions of the dihydrouridine preparation corresponding to 30, 70, and 150 micrograms of dihydrouridine were applied with a micropipette attached to a hypodermic syringe to two strips of Whatman No. 1 paper 3.5 inches from the end and one dimensional paper chromatography was carried out. The paper was stretched between books and dried with a hair dryer between the addition of portions of the solutions to help maintain the size of the spots to a small circle. After complete application of the solutions, the papers were folded 1 inch from the origin, and then again 2 inches from the origin in the opposite direction. The one inch flaps were placed in a glass trough and the papers were passed up and over glass rods in such a way that the second fold coincided with the glass rod and the paper hung straight down from this point. The solvent used was a mixture of t-butyl alcohol, sec-butyl alcohol and water in the ratio of 1:5:5.6. The glass troughs were placed in a 12 X 24 inch battery jar which contained 100 ml. of solvent in the bottom, and then the jar was covered with a glass plate. After an hour's waiting period 75 ml. of solvent was added to the glass troughs through the holes of the glass plate and the system remained covered until the solvent

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almost reached the end of the paper strips. The papers were then hung to dry in the hood, the drying being facilitated by the use of an electric fan. One of the strips of paper was sprayed with 0.5 N sodium hydroxide and allowed to stand for an hour to dry. The other paper was not treated with sodium hydroxide. Both strips then were sprayed with the p-dimethylaminobenzaldehyde solution. On the paper which was treated with sodium hydroxide yellow spots developed upon standing at an R_f of 0.50, which indicates the presence of a ureido group produced by basic hydrolysis, but no colored spot developed on the other paper, which showed the absence of the free ureido group from the original dihydrouridine preparation. The R_f value is defined as the ratio of the distance of migration of the compound being chromatographed to the distance traveled by the solvent.

To show the absence of dihydrouracil from the preparation, 50 micrograms of dihydrouracil and 100 micrograms of the prepared dihydrouridine were spotted on a strip of paper and one dimensional paper chromatography was carried out as in the procedure above. The dried paper was sprayed with 0.5 N sodium hydroxide, dried again and then sprayed with the p-dimethylaminobenzaldehyde color reagent. The R_f values of dihydrouracil and dihydrouridine were 0.36 and 0.50 respectively. The dihydrouridine preparation did not produce a yellow color spot corresponding to the 0.36 value of dihydrouracil, which confirms that in the hydrogenation and isolation process the glycosidic linkage remained intact.

Preparation of Ureidopropionic Acid Riboside

Ureidopropionic acid riboside was synthesized from a portion of the dihydrouridine prepared by the procedure described above. No attempt was mde to prepare ureidopropionic riboside in crystalline form, as the glycosidic linkage is quite unstable. A procedure has been reported by Batt (16) to obtain the barium salt of ureidopropionic acid, but at the pH of 2 as the synthesis is carried out, the ribose group of the ureidopropionyl riboside would not remain attached.

Seventyfive mg. dihydrouridine in 25 ml. of water was allowed to stand a few hours with 25 ml. 0.1 N sodium hydroxide. The solution was neutralized to a pH of 7 with 0.1 N hydrochloric acid, the course of neutralization followed by a pH meter. The amount of hydrochloric acid used was 19.2 ml. and the total volume of the solution increased to 75 ml. The approximate concentration of ureidopropionic acid riboside in the solution was 1 mg/ml.

Demonstration of The Purity Of The Ureidopropionic Acid Riboside

The purity of the ureidopropionic acid riboside preparation was demonstrated by showing that no dihydrouridine was left, that no free ribose was present, and that the free ureido group was present.

To show the absence of dihydrouridine paper chromatography was used again as described previously with the same solvent. On one strip of paper dihydrouridine was spotted and on two other papers the ureidopropionic acid riboside preparation. The papers remained

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overnight in the troughs with the solvent. The lengths to which the solvent traveled was marked. After the papers dried in the hood, the paper on which the dihydrouridine was spotted and one of the papers on which the ureidopropionic acid riboside was spotted were sprayed with the 0.5 N sodium hydroxide solution and allowed to stand to dry for an hour. Then all three strips of papers were sprayed with the p-dimethylaminobenzaldehyde reagent. Both strips of paper on which the ureidopropionic riboside preparation was spotted developed only a single yellow spot upon the treatment with the p-dimethylaminobenzaldehyde. The R_f values were identical, 0.30. The R_f value of dihydrouridine was 0.50 as was determined from the third paper. The results indicate the complete conversion of dihydrouridine to ureidopropionic riboside.

The absence of free ribose was again shown by the use of the hydrogen phthalate reagent.

Organism

Neurospora crassa 1298 was produced from a wild strain by Beadle and Tatum (10) using X-ray treatment. Loring and Pierce (11) showed that the mutant will not grow on a simple basal medium of salts, sugar, and biotin, but it will grow upon addition of pyrimidine compounds.

The mold was maintained on culture slants which were kept in a desiccator over a saturated solution of calcium nitrate to maintain the proper humidity for optimum growth. The culture slants were prepared by dissolving 2 g. of agar and 100 mg. of uracil in 100 ml. of basal medium by the use of heat. Ten ml. fractions of the solution were transferred to Pyrex culture tubes. The tubes were stoppered with

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cotton plugs and sterilized by autoclaving. The tubes were then placed on a slant and allowed to gel in that position to provide a greater surface for the mold to grow on.

TABLE I

THE COMPOSITION OF THE BASAL NUTRIENT MEDIUM

Calcium chloride	1 g.	Trace element solution	50 µl.
Ammonium tartrate	50 g.	Sodium tetraborate	8.8 g.
Ammonium nitrate	10 g.	Ammonium molybdate	6.4 g.
Potassium dihydrogen phosphate	10 g.	Ferric chloride	50.0 g.
		Zinc sulfate heptahydrate	200.0 g.
Magnesium sulfate heptahydrate	5 g.	Cupric sulfate	27.0 g.
Sodium chloride	1 g.	Manganous chloride	4.5 g.
Sucrose	101 g.	Distilled water to	500 ml.
Biotin	26 µg.	Distilled water to	10 l.

The mold was also maintained on culture slants in which 100 mg. alpha-aminobutyric acid replaced the 100 mg. uracil.

The mold was transferred from tube to tube at two week intervals using standard sterile technique.

General Growth Procedure

The mold was grown in 125 ml. Erlenmeyer flasks to which 25 ml. of basal nutrient was added. The flasks were stoppered with cotton plugs, then autoclaved for a 20 minute period. When the compound which was tested for growth was heat stable, it was added before the autoclaving procedure to the basal medium and the complete solution was autoclaved. The labile compounds, dihydrouridine and ureidopropionic acid ribbside, were not autoclaved, but the solutions of these compounds were filtered through a sterilized, sintered glass, bacteriological filter and pipetted with sterile pipettes into the autoclaved

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basal nutrient medium. The solutions in the cooled flasks were inoculated with spores of N. crassa 1293 suspended in sterile water. All solutions were run in triplicate, with a solution without any supplement to the basal medium running alongside of them as a blank. After 4 days in the incubator at 26° C, any mycelial pads formed by growth were washed with distilled water, dried overnight in an oven at 50° C, and then weighed on a torsion balance.

Results of Testing for Growth

The results of experiments in which dihydrouridine and ureidopropionic riboside were sole supplements to the basal medium are given in Table II. The result of the growth of the mold on uridine is also given for comparison.

The possible effect of dihydrouridine and of ureidopropionic riboside upon the growth of the mold in the presence of uridine was also determined. The results of these experiments are summarized in Table III.

The enzymes of N. crassa 1293 involved in the formation of pyrimidines by this new route are probably adaptive in nature. Boyd (6) showed this by demonstrating the time lag in the growth of the mold both on aminobutyric acid and on propionic acid. To see what effect the adaptive nature of the enzyme had upon the growth of the mold on dihydrouridine and on ureidopropionic acid riboside, a set of experiments was carried out in which the inocculation was done, rather than by a spore suspension, by the use of a suspension of fragments of

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TABLE II

RESULTS OF THE GRID TEST PROCEDURE FOR DIHYDROURACIL AND UREIDOPROPIONIC RIBOSIDE

Supplement	Concentration of supplement	Average weight of mycelium
no supplement		0 mg.
uridine	0.2 mg.	17 mg.
dihydrouridine	0.2 mg.	0 mg.
dihydrouridine	5.0 mg.	0 mg.
ureidopropionic riboside	0.2 mg.	0 mg.
ureidopropionic riboside	5.0 mg.	0 mg.

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TABLE III

EFFECT OF DIHYDROURIDINE AND UREIDOPROPIONIC RIBOSIDE UPON THE GROWTH OF THE MOLD ON URIDINE

Supplements	0.1 mg. uridine	0.2 mg. uridine	
		Exp. 1	Exp. 2
No supplement	5.9 mg.	17.8 mg.	13.0 mg.
Dihydrouridine 0.2 mg.	7.3 mg.	14.8 mg.	13.6 mg.
Dihydrouridine 0.5 mg.	21.1 mg.	46.5 mg.	31.1 mg.
Ureidopropionic riboside, 0.2 mg.	5.8 mg.	12.5 mg.	11.1 mg.
Ureidopropionic riboside, 5.0 mg.	5.6 mg.	11.6 mg.	11.2 mg.

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mycelium which had previously been grown on aminobutyrate. The results of these experiments are given in Table IV.

The mycelium was grown in flasks containing 25 ml. of basal medium with 10 mg. of aminobutyric acid as the supplement. After five days of growth the mycelium was homogenized with an autoclaved Waring Blendor and 0.5 ml. of the homogenate was used to inocculate the solutions. Since the homogenate contains some aminobutyric acid, greater variation was expected in the growth of the mycelium, and therefore the experiment was carries out in quadruplicate.

Effect of pH on Growth

The optimum pH for the growth of Neurospora crassa 1298 is the pH of the basal medium as given in Table I, a pH of 5.6. Nucleotides and other strongly acidic compounds are not able to permeate the membrane of the mold at this pH. Some of the compounds which were found to be non-supporters of the growth of the mold at the normal pH were tested again at a pH of 4 and a pH of 3 to test the possibility that this negative result was due to the impermeability of the membrane to the compound.

The inocculation and the procedure for growth was the same as previously described under the general procedure. At highly acidic conditions the growth of the mold is poor, and the mycelium difficult to collect and weigh. The amount of growth with different supplements was only estimated relative to each other by visual comparisons of the amounts of mycelium produced.

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TABLE IV

GROWTH OF THE MOLD N. CRASSA 1298 ON DIHYDROURIDINE AND UREIDOPROPIONIC RIBOSIDE WHEN THE INOCCULATION IS BY MYCELIAL FRAGMENTS

Supplement	Concentration of Supplement	Average weight of Mycelium
no supplement		3.9 mg.
aminobutyric acid	1.5 mg.	12.7 mg.
dihydrouridine	3.0 mg.	19.4 mg.
ureidopropionic riboside	3.0 mg.	3.4 mg.

The compounds tested were acetic acid, pyruvic acid, glycine, aspartic acid, beta-alanine, gamma-aminobutyric acid, alpha-aminoisobutyric acid, beta-aminoisobutyric acid, ornithine, alpha-ketobutyric acid, succinic acid, dihydrouracil, propionic acid and for comparison uracil.

The compounds which did not support growth of the mold under any of these conditions were acetic acid, aspartic acid, beta-alanine, gamma-aminobutyric acid, alpha-amino-isobutyric acid, beta-aminoisobutyric acid and ornithine.

The remaining compounds are arranged in Table V according to their decreasing ability to support the growth of N. crassa 1298.

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TABLE V

RELATIVE RATE OF GROWTH OF THE MOLD AT DIFFERENT pH VALUES WITH VARIOUS SUPPLEMENTS

pH 5.6	pH 4	pH 3
uracil	uracil	uracil ≃ alpha-ketobutyric acid
propionic acid	propionic acid ≃ succinic acid	propionic acid
	glycine	
dihydrouracil	dihydrouracil ≃ pyruvic acid	dihydrouracil
glycine ≃ succinic acid ≃ alpha-ketobutyric acid (no growth)	alpha-ketobutyric acid (no growth)	glycine ≃ succinic acid (no growth)

DISCUSSION

The mutant, N. crassa 1298, synthesizes the pyrimidines of the nucleic acids by a route other than the Liebermann-Kornberg scheme. Boyd did suggest that this route involves the conversion of propionate through beta-alanyl-CoA, beta-ureidopropionyl-CoA ribotide, and through dihydrouracil ribotide to uridine-5'-phosphate. The present work involves the testing of this scheme by synthesizing compounds related to intermediates of this proposal, and observing their effect upon the growth of the mold. This result does not support the existence of this pathway.

While these experimental results do not provide supporting evidence for the proposal of Boyd, these negative results cannot be taken as conclusive evidence against the proposed pathway. Although the mold is able to phosphorylate uridine it may not possess the specific enzymes needed to catalyze the phosphorylation of dihydrouridine and of ureidopropionic riboside. Mokrasch and Grisolia (9) demonstrated the absence of such phosphorylating mechanisms in rat liver homogenate. The phosphorylated compounds, as has been noted, cannot be used in growth experiments due to the impermeability of the cell membrane toward them. The final answer to the question must await enzymatic experiments in which mycelial barriers are eliminated.

Dihydrouridine enhanced considerably the growth of the mold in the presence of small amounts of uridine and alpha-aminobutyric acid, while ureidopropionic acid riboside had no effect. The explanation for the great increase of growth in the presence of a small amount of

uridine or alpha-aminobutyric acid is not obvious. One possibility is that the compound is hydrolyzed by the mold to dihydrouracil in the course of the growth on the uridine or aminobutyrate. Dihydrouracil (6) is known to stimulate the growth of the mold in a fashion similar to that found here for dihydrouridine. Another possible explanation is that once the growth is established on aminobutyric acid or on propionic acid, the mold may be able to produce in adaptive fashion an enzyme capable of phosphorylating dihydrouridine.

Alpha-aminobutyric acid and propionic acid probably follow the same pathway in nucleic acid pyrimidine biosynthesis. This explains the identical lag phase in the growth of the mold on these compounds (6) and the similar inhibition effect of arginine upon these compounds.(4) The obvious method of conversion of alpha-aminobutyric acid to propionic acid would be the deamination of the amino acid, followed by oxidation, then decarboxylation. Alpha-ketobutyric acid would be an intermediate in this conversion. The finding that alpha-ketobutyric acid does support the growth of the mold at the pH of 3 while it did not promote the growth at the pH of 4 and of 5.6, indicates that alpha-ketobutyric acid is not able to penetrate through the cell membrane except in highly acidic medium. The conclusion of Boyd that the keto-acid is not an intermediate in aminobutyrate utilization is therefore not necessarily valid. This evidence makes it likely that alpha-aminobutyric acid can be converted to propionic acid without passing through the intermediates homoserine and beta-hydroxypropionic acid.

Succinic acid and glycine were the other two compounds which were affected in their growth promoting action by the change of the pH of the medium. The pK values of propionic acid and succinic acid are 4.87

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and 4.19 respectively, therefore no great differences in the permeability would be expected on the basis of the pH of these compounds. The similarity in the growth promoting action at the pH of 4 of these compounds and the complete lack of growth-promoting ability of succinic acid at the normal pH would indicate that succinic acid is probably farther away from the nucleic acid pyrimidines in the biosynthetic pathway; therefore the presence of greater concentration of succinic acid in the cell would be required before effective growth could take place. The growth with glycine cannot be explained at the present time. Presumably it can be converted relatively directly to a compound of the biosynthetic path leading toward pyrimidines.

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SUMMARY

1. Experiments were carried out to obtain evidence concerning the nature of the alternate route for nucleic acid pyrimidine biosynthesis used by the mutant N. crassa 1298. Dihydrouridine and ureidopropionic acid riboside were synthesized. The mold was not able to utilize either of these compounds for growth when they were used as sole supplements to the basal nutrient medium.

2. Dihydrouridine greatly enhanced the growth of the mold on uridine and on aminobutyric acid. Ureido-propionic acid riboside had no effect upon the growth of the mold with these compounds.

3. These experiments do not provide evidence in support of the biosynthetic pathway postulated (6) for nucleic acid pyrimidine biosynthesis from propionic acid. The possibility exists, however, that the mold is not able to phosphorylate the nucleosides.

4. Succinic acid and glycine were utilized at a pH of 4, but were not utilized at the normal pH of 5.6 or at a lower pH.

5. Alpha-ketobutyric acid at a pH of 3 was utilized by the mold as well as was propionic acid, but at a pH of 4 and at a pH of 5.6 did not support the growth of the mold.

6. The conversion of alpha-aminobutyric acid to propionic acid through the intermediate alpha-ketobutyric acid is suggested.

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