Phospholipid Variations in Mutant Strains of
*Neurospora crassa*  

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The present studies were undertaken to determine the extent to which the phospholipid composition of *Neurospora crassa* can be altered by mutations studied in conjunction with controlled supplementation. Previous work in this laboratory has shown that a mutation involving an enzyme concerned with lecithin synthesis can result in a lecithin-deficient strain (1, 2). The methylation of phosphatidylethanolamine appears to be the major metabolic source of lecithin when *N. crassa* is grown on minimal medium (3). Two mutant strains, 34486 and 47904, are known with aberrations concerning one or more of the three methylations involved in this metabolic sequence leading to lecithin synthesis (4, 5). These lecithin-deficient organisms show a growth response to exogenous supplements of either choline or dimethylethanolamine, and strain 34486 also utilizes monomethylethanolamine for growth. These growth response experiments indicate that *N. crassa* incorporates the three methylated ethanolamines into phospholipids by pathways analogous to those known in other species, involving the cytidine diphosphate derivatives of these ethanolamines as obligatory intermediates (6-8).

Since *N. crassa* has two independent metabolic pathways leading to lecithin formation, its phospholipid composition may be altered by controlling the relative contribution of these two synthetic systems. Two mutations were used to alter the substrate turnover by the phospholipid-methylating enzymes, and the contribution of the alternate routes, presumably the cytidine nucleotide pathways, to the synthesis of phospholipids was influenced by supplementation of cultures with methylated ethanolamines.

**EXPERIMENTAL PROCEDURE**

*Strains of N. crassa—* A “wild-type” strain 1A was employed in these studies as a basis for comparison with aberrant strains 34486 and 47904. *N. crassa* strain 47904 has been described in the literature (1-5, 9). This organism has a single gene involvement which prevents the conversion of phosphatidylmonomethylethanolamine to lecithin at a normal rate. As a result of this genetic aberration, strain 47904 substitutes phosphatidylmonomethylethanolamine and phosphatidyltrimethylethanolamine for much of the lecithin normally found in *N. crassa*. The slow growth of this organism can be increased to a level approaching that of a normal wild-type strain by supplementation with either dimethylethanolamine or choline. These substances serve as an exogenous source of those phospholipid bases which normal strains produce in sufficient quantity for optimum growth by methylation of phosphatidylmonomethylethanolamine. The alternate cytidine nucleotide pathway is probably utilized in the conversion of the two supplemental amines into phospholipids.

Strain 34486 will not grow on minimal medium as the result of a mutation at a single genetic locus (4, 5). This organism grows when supplemented with monomethylethanolamine, dimethylethanolamine, or choline. The conversion of these bases to phospholipids, presumably in the cytidine nucleotide pathway, negates the deficiency imposed by mutation. Since ethanolamine does not stimulate the growth of strain 34486, it is thought that this organism can make phosphatidylethanolamine from either endogenous precursors or from supplemental ethanolamine, but it cannot carry out the first step in the progressive methylation of phosphatidylethanolamine to lecithin. Although recent work has shown that glycerophosphorylcholine also stimulates the growth of this strain (10), the metabolic fate of this growth substance after entrance into the cell is not known. The phospholipids of strain 34486 have not been the subject of previous studies.

*Growth and Extraction of Mold—* Aerated cultures of *N. crassa* strains 1A, 34486, and 47904 were grown in a highly buffered citrate medium (11) at pH 5.6. The mycelium employed for the lipid analyses was obtained from 3-liter cultures grown at 25° under forced aeration at a rate of 1.6 liters of air per minute in 3-liter Florence flasks. The media were sterilized by autoclaving and inoculated with an aqueous suspension of 1 × 10⁸ conidia per culture. Strain 1A was grown on unsupplemented minimal medium, and strain 34486 was grown on medium supplemented with either 158 μmoles of monomethylethanolamine or 25.8 μmoles of choline chloride per culture. Strain 47904 was grown on either the minimal medium or minimal medium containing 25.8 μmoles of choline chloride per culture. The levels of the two supplemental amines employed in these studies were just adequate to produce an optimal growth response in the two mutant strains (4).

The lipids in mature mycelium from 5-day cultures were compared with the lipids found in the youngest cultures that could be harvested in sufficient quantity for lipid analyses. Rapidly growing mycelium, rich in young hyphal tips, was obtained from strains 1A and 34486 after a 2-day incubation period. Comparable tissue was obtained from 3-day cultures of strain 47904 because this organism has an initial growth lag of about 1 day.
The mycelium from each culture was harvested by filtration and then heated in boiling water for 1 minute to destroy enzymatic activity. After removal of excess water from the boiled tissue by filtration, it was extracted twice with 5 volumes (w/v) of methanol. The partially extracted tissue was then suspended in 3 parts of a chloroform-methanol solution (2:1, v/v) and dispersed by blending with a VirTis homogenizer. The resulting mycelial pulp was ground in a TenBroeck homogenizer and centrifuged, the supernatant solution was decanted, and the residue was washed three times with an equal volume of chloroform-methanol (2:1, v/v). The combined lipid extracts were taken to dryness on a rotary evaporator at reduced pressure.

The resulting residue, containing the total extractable lipids, was freed from nonlipid contaminants by the method of Folch et al. (12). Whenever feasible, lipids were extracted under an atmosphere of nitrogen.

**Analysis of Lipid Hydrolyzates**—The total lipids from *N. crassa* mycelium were hydrolyzed by the method of Horowitz and Beadle (13). The serine, ethanolamine, monomethylethanolamine, dimethylethanolamine, and choline released from phospholipids by this hydrolysis were separated by chromatography on a Dowex 50 cation exchange column previously employed for the separation of the three methylated ethanolamines (14). Serine and ethanolamine, which can also be readily isolated by employing the same column conditions, have elution peaks at eluate volumes of approximately 160 and 300 ml, respectively. These two substances were quantitatively determined in the chromatographic eluates by the method of Hayashi, Miyaki, and Unemoto (15). *N. crassa* strain 34486 was used for the quantitative assay of the three methylated ethanolamines (13).

**RESULTS**

Preliminary experiments revealed that large changes can be produced in the phospholipid compositions of strains 34486 and 47904 by varying the culture conditions under which they are grown. The two experimental variables used here to illustrate such changes are the age of the cultures and the supplemental conditions used are those which are expected to yield optimal differences in the phospholipid bases. The amount of lipid-bound bases was determined by measuring the free bases released by hydrolysis. The data in Table I show the amounts of the phospholipid bases released from the mycelial lipids of unsupplemented wild-type cultures and serve as an index of the range of values obtained with the normal strain. The data (Tables II and III) obtained from similar studies with strains 34486 and 47904 are representative of the variations in the phospholipid bases which are tolerated by growing *N. crassa*. These aberrant strains grow rapidly with normal morphology when cultured on choline-supplemented media. However, on media lacking choline, both grow with an altered, dense growth pattern (16). Cultures 1 and 2 in Table II as well as Cultures 1 and 2 in Table III exhibited this colonial growth.

The cultures of strain 34486, when grown on either monomethylethanolamine or choline (Table II), have a higher ratio of ethanolamine to choline in the lipid hydrolysates than is found in the normal strain (Table I). Two-day cultures of strain 34486 grown on monomethyllethanolamine supplements contain small amounts of this base and also some dimethylethanolamine in the phospholipids. These phospholipids disappear in the older 5-day cultures, presumably by conversion to lecithin. The young cultures of strain 34486 tend to have high values for lipid-bound ethanolamine and serine, as might be expected in an

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### Table I

<table>
<thead>
<tr>
<th>Bases</th>
<th>1 (0.54 g)*</th>
<th>2 (5.52 g)</th>
<th>3 (1.79 g)</th>
<th>4 (6.51 g)</th>
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<tr>
<td>Serine</td>
<td>2.9</td>
<td>1.4</td>
<td>2.4</td>
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<tr>
<td>Ethanolamine</td>
<td>13.7</td>
<td>15.1</td>
<td>15.8</td>
<td>11.1</td>
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<tr>
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<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Dimethylethanolamine</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Choline</td>
<td>27.8</td>
<td>21.6</td>
<td>30.8</td>
<td>25.5</td>
</tr>
</tbody>
</table>

* Experiment number and dry weight of tissue from a 3-liter culture.

### Table II

<table>
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<tr>
<th>Bases</th>
<th>Monomethylethanolamine-supplemented</th>
<th>Choline-supplemented</th>
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<tbody>
<tr>
<td></td>
<td>µmoles base/g dry tissue</td>
<td>µmoles base/g dry tissue</td>
</tr>
<tr>
<td>Serine</td>
<td>7.1</td>
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<td>Ethanolamine</td>
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<tr>
<td>Dimethylethanolamine</td>
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<tr>
<td>Choline</td>
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<td>21.0</td>
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</table>

* Experiment number and dry weight of tissue from a 3-liter culture.

### Table III

<table>
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<tr>
<th>Bases</th>
<th>Unsupplemented</th>
<th>Choline-supplemented</th>
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<tr>
<td></td>
<td>µmoles base/g dry tissue</td>
<td>µmoles base/g dry tissue</td>
</tr>
<tr>
<td>Serine</td>
<td>1.1</td>
<td>2.9</td>
</tr>
<tr>
<td>Ethanolamine</td>
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</tr>
<tr>
<td>Choline</td>
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<td>9.5</td>
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</tbody>
</table>

* Experiment number and dry weight of tissue from a 3-liter culture.
organism with a genetic block preventing the normal conversion of phosphatidylethanolamine to phosphatidylmonomethylethanolamine.

The five phospholipid bases found in strain 47904 (Table III) show a greater departure from the wild-type pattern than was observed with the 34486 cultures. Monomethylethanolamine remains the major phospholipid base in strain 47904, even when the mold is cultured in the presence of exogenous choline for the attainment of optimum growth, and its lecithin content, based on choline recovery, tends to be lower than that of either strain 1A or 34486. Phosphatidyl(dimethylethanolamine is present in large amounts in strain 47904, especially in the unsupplemented cultures (3). A choline-supplemented culture of strain 47904 has more lecithin after 3 days of growth than an unsupplemented culture of the same age. This indicates that this organism incorporates exogenous choline into lecithin by a route analogous to the cytidine nucleotide pathway described by Kennedy (7) and Kennedy and Weiss (6, 8).

**DISCUSSION**

Two mutant strains of *N. crassa* with abnormalities in their phospholipid metabolism were studied to determine the extent to which the bases released from their phospholipids can differ from the normal strain. These studies were based on a previous observation that one of these mutants (strain 47904) accumulated phosphatidyl(monomethylethanolamine and phosphatidyl(dimethylethanolamine, two lecithin precursors not present in wild-type *N. crassa* (1, 2). There are at least two ways in which lecithin can be synthesized. The stepwise methylation of the nitrogen of phosphatidylethanolamine has been demonstrated in liver preparations (17-19), and this route for lecithin synthesis is also found in *N. crassa* (2, 3). The mutation in *N. crassa* strain 34486 has apparently blocked the first methylation in this sequence, and in strain 47904 the accumulation of lecithin precursors is the result of a genetic aberration involving the second and third methylation. The specific enzyme involvements associated with these two mutations have not been characterized.

An alternate pathway for the synthesis of lecithin was established by Kennedy (7) and Kennedy and Weiss (6, 8), in which cytidine diphosphate choline and an α,β-diglyceride react to yield lecithin and cytidine monophosphate. Analogous pathways are also known for the incorporation of dimethylthanolamine (20, 21) and ethanolamine (8) into the correspondent phosphatidyl derivatives. *N. crassa* apparently can also incorporate monomethylethanolamine into phospholipids by a similar cytidine nucleotide pathway, and the presence of monomethylthanolamine in the lipids of a 2-day 34486 culture (Table II) is evidence in support of this conversion. The concurrent participation of the phospholipid-methylation system can transform this phosphatidyl(monomethylthanolamine to lecithin. Phosphatidyl(dimethylethanolamine, an obligatory intermediate in this conversion, is also found in a young, fast growing culture, and after 5 days of growth only the completely methylated choline occurs in the lipids of monomethylthanolamine-supplemented cultures of strain 34486. This strain does not accumulate detectable amounts of the two partially methylated precursors of lecithin when it is grown on choline. The genetic block, which negates the initial methylation of phosphatidylethanolamine in this organism, probably makes the mold entirely dependent on the nucleotide route for lecithin synthesis when choline is the only available supplement in the basal medium.

Previous studies have indicated that the stepwise methylation of phosphatidylethanolamine is the major pathway for the synthesis de novo of lecithin in normal unsupplemented *N. crassa* (2, 3). An alternate route, presumably the cytidine nucleotide system, provides a means for the utilization of phospholipid bases released by tissues. The free bases, as well as their phosphate esters, are found in aging cultures of *N. crassa* (4, 5, 9, 14). When a genetic block prevents the methylation of phosphatidylethanolamine as in strain 34486, the cytidine nucleotide pathway becomes the dominant synthetic system dependent on an exogenous source of methylated ethanolamines, without which this mold will not grow. The phosphatidylethanolamine, which strain 34486 apparently can make, does not fulfill the growth requirements of the mold in the absence of supplements which permit the concurrent synthesis of lecithin through cytidine nucleotide intermediates. Normal *N. crassa* grows without an exogenous source of phospholipid bases, and presumably can synthesize phosphatidylserine and convert it to phosphatidylethanolamine in a manner analogous to that demonstrated in other species (22-25). Young, rapidly growing cultures of strain 34486 have elevated levels of lipid-bound serine and ethanolamine (Table II) because the genetic block probably prevents the normal participation of these basic moieties as precursors of lecithin.

The limited ability of strain 47904 to methylate further phosphatidyl(monomethylthanolamine results in a slowly growing organism which contains very little lecithin. The phosphatidyl(monomethylthanolamine and phosphatidyl(dimethylethanolamine found in this mold apparently can substitute for most or all of the lecithin existing in normal strains. This is in contrast to phosphatidylethanolamine, which alone cannot replace lecithin in strain 34486. A choline-supplemented culture of 47904 utilizes the phospholipid-methylation system to make primarily phosphatidyl(monomethylthanolamine and phosphatidyl(dimethylethanolamine, and it also makes lecithin from exogenous choline by an alternate pathway. Under culture conditions when this unique metabolic situation exists, strain 47904 continues to incorporate large amounts of the two lecithin precursors into its tissues, even though the alternate route is available for lecithin synthesis from choline (Table III). The low content of lipid-bound ethanolamine in strain 47904 under all culture conditions studied may reflect some feedback effect by the accumulated lecithin precursors.

Shatkin and Tatum (16) made the observation that mutant strains of *N. crassa* bearing lesions affecting phospholipid metabolism may be subject to metabolic imbalance and accompanying morphological aberrations. As an example, it was observed that the lecithin-deficient 34486 and 47904 strains grow slowly with a dense colonial morphology on media lacking choline. Inositol-lacking mutants cultured on suboptimal inositol also show this growth pattern, and these workers proposed that the colonial morphology in inositol-lacking mutants is due to a metabolic imbalance between membrane synthesis and the synthesis of other cellular constituents (16). Shatkin and Tatum found that the growth pattern of inositol-lacking mutants is a function of "carbon and nitrogen sources, inositol levels, utilization of carbon source, ratio of concentration of sugar to inositol and size of conidial inoculum." On the basis of less extensive studies with choline-lacking mutants, these workers realized that the growth characteristics of these strains are also sensitive to changes in culture conditions.
In the present studies the growth rate, morphology, and lipid composition of strains 34486 and 47904 were affected more than those of the normal strain by changes in culture conditions. The viability and nutritional status of the conidial inoculum was a variable affecting growth and consequently the lipid composition of these molds. This variation in the germination of conidia from lecithin-deficient strains may be a general characteristic of phospholipid-aberrant molds, since the viability of conidia from inositol mutants is also abnormal. The "inositol-less death" of these conidia is used as the basis for a mutant selection technique (26). The viability of spores as a function of "unbalanced growth" has been the subject of an extensive report by Strauss (27). A systematic study of this phenomenon in strains 34486 and 47904 was not attempted here.

The data concerning the phospholipid bases of strains 34486 and 47904 (Tables II and III) are representative of the many variations that can exist in the two molds. The eventual goal in these studies will be an attempt to relate such aberrations to other measurable changes in the properties of the membranous cell constituents. Exploratory studies have shown that strains bearing the 47904 lesion have altered growth response to niacin (11) and aminopterin (28). Since it is difficult to interpret the significance of such findings with living tissues, this work will have to be extended to investigations with cell-free systems obtained from strains 1A, 34486, and 47904.

**SUMMARY**

Mutant strains of *Neurospora crassa* (strains 34486 and 47904) are available which have abnormalities in their phospholipid metabolism as a result of genetic blocks involving lecithin biosynthesis. The relative amounts of lipid-bound serine, ethanolamine, monomethylethanolamine, dimethylethanolamine, and choline in unsupplemented wild-type *N. crassa* were compared with the quantities found in the lecithin-lacking strains after culture for different time intervals on media varying with respect to choline or monomethylethanolamine supplements. The content of the phospholipid bases in the two aberrant strains can differ considerably from the pattern of the normal strain.

Monomethylethanolamine is the predominant phospholipid base in strain 47904 when it is grown on either minimal or choline-supplemented medium. This organism also contains large amounts of dimethylethanolamine and smaller amounts of choline in its lipid hydrolysates. Strain 34486 accumulates small amounts of monomethylethanolamine and dimethylethanolamine in its phospholipide during its early growth on monomethyl ethanolamine-supplemented medium. These bases are not found in the lipids of the organism when choline is the only culture supplement. The quantities of the five phospholipid bases in both mutant strains are variable and dependent on culture conditions.

These studies indicate that *N. crassa* can make lecithin by methylation of phosphatidylethanolamine and also by the direct incorporation of preformed bases, presumably through cytidine nucleotide intermediates. The relative contribution of the two pathways leading to lecithin synthesis can be influenced by mutations and culture supplementation.

**REFERENCES**

1. **HALL, M. O., and NYC, J. F., J. Am. Chem. Soc., 81, 2275 (1959).**
2. **HALL, M. O., and NYC, J. F., J. Lipid Research, 2, 321 (1961).**
3. **HALL, M. O., and NYC, J. F., Biochim. et Biophys. Acta, 66, 370 (1962).**
5. **HOROWITZ, N. H., J. Biol. Chem., 162, 413 (1946).**
7. **KENNY, E. P., J. Biol. Chem., 223, 185 (1956).**
20. **ARTOM, C., Federation Proc., 19, 233 (1960).**
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