The Influence of Folic Acid, Threonine and Glycine on Serine Synthesis in Tetrahymena

BY VIRGINIA C. DEWEY AND G. W. KIDDER

Biological Laboratory, Amherst College, Amherst, Massachusetts, U.S.A.

SUMMARY: Only one of sixteen strains of Tetrahymena, studied, was found to be completely dependent on an exogenous source of serine for growth. A second strain appears to lack threonine aldolase, as it can synthesize serine from added glycine but not from added threonine. The remaining fourteen strains will utilize either glycine or threonine (as a source of glycine) for serine synthesis provided the folic acid concentration in the medium is high.

Early observations on the growth of Tetrahymena pyriformis (Kidder & Dewey, 1945a) indicated that this ciliate is capable of growth in the absence of serine. In later attempts to repeat this observation (Kidder & Dewey, 1947), using a medium of known chemical composition, growth failed when serine was omitted. It appeared possible that the more nearly defined medium lacked some factor(s) present in crude material which would permit synthesis of serine by the ciliate. A system for the synthesis of serine from glycine under the influence of folic acid (or one of its derivatives) is of widespread occurrence (Plaut, Betheil & Lardy, 1950; Siegel & Lafaye, 1950; Totter, Kelley, Day & Edwards, 1955; Kisliuk & Sakami, 1935; Blakley, 1954; Lascelles & Woods, 1954). Therefore an investigation of the influence of glycine and folic acid on serine synthesis by the ciliate was undertaken (Kidder & Dewey, 1958). Although both compounds are present in the completely defined medium used, it was found that considerably higher levels of each must be present when serine is omitted. Addition of higher levels of folic acid alone made possible a certain amount of growth, indicating that there might be an endogenous source of glycine. In the absence of serine the most probable source of glycine is threonine, via threonine aldolase (Lin & Greenberg, 1954).

Of 14 strains of Tetrahymena pyriformis and two of T. vorax tested, all but one were capable of growth on glycine with high levels of folic acid and all but two would grow on increased concentrations of threonine in place of glycine or serine, indicating the presence of threonine aldolase in 14 of the strains (Dewey & Kidder, 1955).

METHODS

Fourteen strains of Tetrahymena pyriformis which have been maintained in axenic culture for varying periods were used in this study. Twelve of these strains have been designated H, TP, T, W, GHH, GP, GC, S, E, GL, G1-R and Ch-S, and Corliss (1952) has given their origin and history. Strain WH6 was isolated by Elliott & Hayes (1958) and strain CM was obtained from
Dr H. G. Kimpel of the Carnation Company. Two strains of *T. vorax*, PP and V (Kidder & Dewey, 1945b), were also used.

The minimal medium consisted of the following amino acids (µg./ml.): L-arginine hydrochloride, 86; L-aspartic acid, 122; L-glutamic acid, 288; L-lysine hydrochloride, 122; L-histidine hydrochloride hydrate, 42; DL-isoleucine, 126; L-leucine, 194; DL-methionine, 68; L-phenylalanine, 100; L-proline, 175; L-tryptophan, 24; DL-valine, 132. To this mixture were added vitamins, salts, nucleic acid derivatives and Tween 80 as described for Medium A (Dewey, Parks & Kidder, 1950). DL-Serine, DL-threonine, glycine or glycine peptides, glucose or sodium acetate were added to the minimal medium as indicated below. In some cases additional amounts of DL-thioctic acid and/or folic acid were also used.

Cultures were incubated for 4 days in a slanted position at 25°C. Growth was measured turbidimetrically using a Lumetron photoelectric colorimeter with the 650 filter. In some cases growth was measured daily in order to follow growth rates.

Aside from the evidence for the presence of threonine aldolase obtained from growth studies, further indication of its presence was obtained by aerating suspensions of frozen and thawed cells with and without threonine as a substrate. The air was then passed through a solution of semicarbazide (Lin & Greenberg, 1954).

**RESULTS**

Since threonine is an absolute growth requirement for *Tetrahymena*, it was added to the minimal medium at a barely optimal level (88 µg./ml.) in experiments on the replacement of serine by glycine. No growth occurs in a medium containing glycine instead of serine unless the folic acid concentration of the medium is also increased. Under these conditions strains Gl-R and GC of *Tetrahymena pyriformis* grow well at a folic acid concentration of 0.05 µg./ml., GP, W, GL, H, TP, T, WH6 and CM require 0.10 µg./ml. and Ch-S, E and S grow only if the concentration is raised to 1.0–10 µg./ml. (one of these, strain S, requires a longer growth period in addition). One strain, GHH, does not grow at all on glycine. The two strains of *T. vorax* resemble the majority of the strains of *T. pyriformis*.

Figure 1 shows that the effect of the increased folic acid is chiefly on the growth rate. If the growth period is sufficiently long, even the minimal amount of folic acid (0.01 µg./ml.) is sufficient for the strains with the smaller requirements. After 4 days in a medium containing only the basal amount of folic acid (0.01 µg./ml.) no amount of glycine less than a toxic level will permit half-maximal growth. As may be seen from Table 1, however, any peptide of glycine is much more active than the free amino acid and some very much more so. Glycylserine is considerably more active than serine itself or an equimolar mixture of serine and glycine. The greater activity of the peptides may possibly be explained on the basis that they increase growth rate, exhibit less competition with free amino acids (Dewey & Kidder, 1958) or are able to undergo transpeptidation reactions (Fujii & Fruton, 1958).
In the test for threonine aldolase frozen and thawed preparations of washed cells of strains W, WH6, T and GL all produced an excess of acetaldehyde semicarbazone in the presence of threonine as substrate as compared to controls without substrate. This was not the case, however, with preparations of strain CH-S.

Table 1. Response of Tetrahymena pyriformis W to peptides of serine and glycine

Medium contains alanine (110 μg./ml.); acetate (1 mg./ml.); glucose (2.5 mg./ml.) and low folic acid (0.01 μg./ml.). Figures represent the amount (μmoles) required for half maximum growth.

<table>
<thead>
<tr>
<th>Peptide</th>
<th>Amount (μmoles)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>&gt; 6.00</td>
</tr>
<tr>
<td>DL-Serine</td>
<td>0.20</td>
</tr>
<tr>
<td>Glycine + DL-serine</td>
<td>0.01</td>
</tr>
<tr>
<td>Glycine-DL-serine</td>
<td>0.06</td>
</tr>
<tr>
<td>Glycine-DL-alanine</td>
<td>0.00</td>
</tr>
<tr>
<td>Glycyglycine</td>
<td>0.80</td>
</tr>
<tr>
<td>DL-Alanylglcyline</td>
<td>0.88</td>
</tr>
<tr>
<td>L-Leucylglycine</td>
<td>1.00</td>
</tr>
<tr>
<td>Glycyl-L-tyrosine</td>
<td>2.18</td>
</tr>
<tr>
<td>Glycyl-DL-phenylalanine</td>
<td>3.00</td>
</tr>
<tr>
<td>Glycyl-L-leucine</td>
<td>&gt; 5.8</td>
</tr>
</tbody>
</table>
Fig. 2. Curves showing rates of growth of T. pyriformis E in the presence of 250 μg./ml. of DL-serine (curve 1); 250 μg./ml. of glycine and 1 μg./ml. of folic acid (curve 2); 500 μg./ml. of DL-threonine and 1 μg./ml. of folic acid (curve 3).

Most of the strains of Tetrahymena pyriformis tested and both of the strains of T. vorax contain a threonine aldolase sufficiently active to permit growth on threonine in the absence of both serine and glycine (Table 2). In a few cases (Fig. 2) growth is slow, with a more pronounced lag phase, in this medium, and in two cases, Ch-S and GHH, growth fails entirely. Since strain Ch-S

Table 2. Comparative growth of strains of Tetrahymena on serine, glycine or threonine as sources of serine and glycine

<table>
<thead>
<tr>
<th>Strain</th>
<th>Serine, 250 μg./ml.</th>
<th>Glycine, 250 μg./ml.</th>
<th>Threonine, 500 μg./ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Med. 1</td>
<td>Med. 2</td>
<td>Med. 3</td>
</tr>
<tr>
<td>T*</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>W†</td>
<td>100</td>
<td>100</td>
<td>85-90</td>
</tr>
<tr>
<td>Gl-R‡</td>
<td>100</td>
<td>65-80</td>
<td>45-65</td>
</tr>
<tr>
<td>Gl</td>
<td>100</td>
<td>290</td>
<td>310</td>
</tr>
<tr>
<td>Ch-S</td>
<td>100</td>
<td>72</td>
<td>10</td>
</tr>
<tr>
<td>GHH</td>
<td>100</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Percentage growth referred to serine

Med. 1 and 2 contain DL-threonine at 88 μg./ml. Med. 2 and 3 contain folic acid at 1 μg./ml.

* Also strains WH9, CM and TP
† Also strains H, GC, GP, T. vorax PP and V
‡ Also strains S and E.
is capable of growth on glycine, it is apparent that it is a lack of threonine aldolase which prevents its growth on threonine. The situation is not so clear in the case of strain GHH. Since it fails to grow on glycine, growth would not be expected on threonine even if the aldolase were present (Dewey & Kidder, 1955).

The behaviour of strain GL is somewhat anomalous. Growth of GL is very poor on serine, better on glycine and best on threonine (Table 2). In the presence of glucose the growth of this strain is perfectly normal with any one of the three substrates. The effect of glucose on serine synthesis will be considered in another paper.

The specificity of the threonine aldolase of the ciliates appears to resemble that of the mammalian enzyme (Lin & Greenberg, 1954). D-threonine is not utilized for the synthesis of glycine or serine, nor can it replace the L-threonine requirement. L-Allothreonine is at least as active as L-threonine in replacing glycine or serine, although it cannot take the place of the threonine required as such. Figure 8 shows the sparing of L-threonine by L-allothreonine.

![Graph](image)

**Fig. 8. Dose response of T. pyriformis W to L-threonine alone (curve 1) and with L-allothreonine at 50 μg./ml. (curve 2), 100 μg./ml. (curve 3) and 200 μg./ml. (curve 4). Medium contains acetate (1 mg./ml.), glucose (2.5 mg./ml.) and folic acid (0.1 μg./ml.).**

**DISCUSSION**

It appears that the failure to obtain growth in the absence of serine in media containing no crude materials was due to inadequate supplies of both glycine and folic acid. Both of these constituents of the medium were reduced in concentration in modifications made in the media between 1945 and 1947. The growth failure in the absence of glycine (serine present) reported (Kidder & Dewey, 1945a) was, in all likelihood, the result of amino acid imbalance as suggested later (Kidder & Dewey, 1947; Dewey & Kidder, 1958).

Since all of the strains tested have a similar quantitative requirement for
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folic acid (less than 0.002 µg./ml. for half-maximal growth) in the presence of serine, it is not possible to decide whether or not the differences in the folic acid requirements for serine synthesis represent a deficiency in the ability to form the necessary coenzyme from folic acid or a deficiency in the apoenzymes involved.

The source of the 'one-carbon' fragment necessary for the conversion of glycine to serine is not known in the case of Tetrahymena. Addition of formate to cultures containing glycine and folic acid does not stimulate growth. Both formaldehyde and methanol are unsuitable for growth experiments because of toxicity. The ciliates are capable of producing formate (unpublished experiments), but its source is not known. It is possible that glycine itself serves as the 'one-carbon' donor and that serine is the source of the formate found in cultures, since serine was included in the medium in these experiments.

There have been reported (Elliott & Clark, 1958) a number of clones (variety nine) of Tetrahymena pyriformis which exhibit no serine requirement in a medium containing no glycine and only a low concentration of folic acid (0.01 µg./ml.). Since synthesis of serine is the general rule among the strains studied in this laboratory, with only quantitative differences between strains, it is possible that these strains of Elliott & Clark are merely still less demanding in regard to folic acid level.

In addition to the conversion of threonine to glycine and, hence, to serine, propionate and β-hydroxybutyrate may be formed in the catabolism of threonine. Utilization of propionate for serine synthesis will be discussed in a subsequent communication.

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REFERENCES


Studies on the biochemistry of Tetrahymena. III. Strain differences. Physiol. Zool. 18, 186.


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